Appendix D. Individual Acute, 8-Hour, and Chronic Reference Exposure Level Summaries

D.1 Summaries using this version of the Hot Spots Risk Assessment guidelines

D.2 Acute RELs and toxicity summaries using the previous version of the Hot Spots Risk Assessment guidelines (OEHHA 1999)

D.3 Chronic RELs and toxicity summaries using the previous version of the Hot Spots Risk Assessment guidelines (OEHHA 2000)

(Revised July 2014 to reflect adoption of additional new or revised RELs)

Appendix D.1 Summaries using this version of the Hot Spots Risk Assessment guidelines

Substance	Inhalation REL ($\mu g/m^3$)		Oral REL (µg/kg BW-day)	Hazard Index Target Organs	Species
Acetaldehyde (75-07-0)	**A**	470		Eyes; respiratory system (sensory irritation)	H
	8	300		Respiratory system	R
	C	140		Respiratory system	R
Acrolein (107-02-8)	**A**	2.5		Eyes; respiratory system (sensory irritation)	H
	8	0.7		Respiratory system	R
	C	0.35		Respiratory system	R
Arsenic (7440-38-2) **& inorganic arsenic compounds (including arsine)**	**A**	0.20		Development; cardiovascular system; nervous system	M
	8	0.015		Development; cardiovascular system; nervous system; respiratory system; skin	H
	C	0.015	0.0035	*Inhalation and Oral:* Development; cardiovascular system; nervous system; respiratory system; skin	H
Benzene (71-43-2)	**A**	27		Developmental; Immune System; Hematologic System	M
	8	3		Hematologic System	H
	C	3		Hematologic System	H
Butadiene (106-99-0)	**A**	660		Development	M
	8	9		Reproductive system	M
	C	2		Reproductive system	M
Caprolactam (105-60-2)	**A**	50		Eyes (sensory irritation)	H
	8	7		Respiratory system	R
	C	2.2		Respiratory system	R
Formaldehyde (50-00-0)	**A**	55		Sensory irritation; eyes	H
	8	9		Respiratory system	H
	C	9		Respiratory system	H
Manganese (7439-96-5) **& manganese compounds**	**A**	--			
	8	0.17		Nervous system	H
	C	0.09		Nervous system	H
Mercury (7439-97-6) **& inorganic mercury compounds**	**A**	0.6		Nervous system, development	R
	8	0.06		Nervous system	H
	C	0.03	0.16	Nervous system	H
Nickel & nickel compounds (except nickel oxide for chronic inhalation exposures) (Inhalation concentrations as µg Ni/m^3: oral dose as µg Ni/kg-day)	**A**	0.2		Immune system	M
	8	0.06		Respiratorysystem, immune system	R
	C	0.014	11	*Inhalation:* Respiratory system; hematologic system	R
				Oral: Development	R

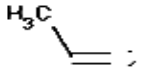
Substance	Inhalation REL ($\mu g/m^3$)		Oral REL (µg/kg BW-day)	Hazard Index Target Organs	Species
Nickel oxide (1313-99-1) (Inhalation concentration as µg Ni/m^3: oral dose as µg Ni/kg-day)	**C**	0.02	11	*Inhalation:* Respiratory system	M
				Oral: Development	R

Species: H = Human M = Mouse R = Rat

Acetaldehyde Reference Exposure Levels

(ethanal; acetic aldehyde; acetylaldehyde; ethylaldehyde; diethylacetyl)

CAS: 75-07-0



1. Summary

Based on acute and chronic inhalation studies conducted mostly in experimental animals, the target tissue for acetaldehyde has consistently been at the portal of entry with effects occurring primarily in the upper respiratory tract at lowest concentrations. The major noncancer health effects of acute exposure in humans to acetaldehyde vapors consist of irritation to the eyes, skin, and respiratory tract. Low to moderate air concentrations (25 ppm to 200 ppm) cause eye and upper respiratory tract irritation. Moderate concentrations (~ 300 ppm or greater) also cause bronchoconstriction in asthmatics as measured by a greater than 20% drop in forced expiratory volume (FEV_1). Signs of acute toxicity in animals at high concentrations (~10,000 ppm) include labored respiration, mouth breathing, weight loss, and liver damage. The studies described in this document include those published through the Spring of 2008.

OEHHA used the critical effect of bronchoconstriction in asthmatics as the basis for determination of the acute Reference Exposure Level (REL).

Subchronic and chronic exposure to acetaldehyde causes inflammation and injury to the respiratory tract (e.g. lesions including hyperplasia and metaplasia of the olfactory mucosa). Exposure to acetaldehyde, as seen in experimental animal studies, causes histopathological changes in the nose, larynx, and trachea including degeneration, hyperplasia, and metaplasia. Chronic toxicity to rats and hamsters following inhalation exposure to acetaldehyde includes increased mortality and growth retardation. OEHHA used degenerative, inflammatory and hyperplastic changes of the nasal mucosa in rats as the basis for the 8-hour and chronic REL.

Children, especially those with diagnosed asthma, may be more likely to show impaired pulmonary function and symptoms of asthma than are adults following exposure to acetaldehyde. Acetaldehyde is identified as a Toxic Air Contaminant (TAC); this report presents evidence that it should also be listed as having the potential to differentially impact infants and children due to its effects as a respiratory irritant and possible exacerbation of asthma. In addition, acetaldehyde has high California Hot Spots and mobile source emissions, and secondary formation in the atmosphere (OEHHA, 2001).

1.1 Acetaldehyde Acute REL

Reference Exposure Level	**470 µg/m^3 (260 ppb)**
Critical effect(s)	Sensory irritation, broncoconstriction, eye redness and swelling
Hazard index target(s)	Bronchi, eyes, nose, throat

1.2 Acetaldehyde 8-Hour REL

Reference Exposure Level	**300 µg/m^3 (160 ppb)**
Critical effect(s)	Degeneration of olfactory nasal epithelium
Hazard index target(s)	Respiratory system

1.3 Acetaldehyde Chronic REL

Reference Exposure Level	**140 µg/m^3 (80 ppb)**
Critical effect(s)	Degenerative, inflammatory and hyperplastic changes of the nasal mucosa in animals
Hazard index target(s)	Respiratory system

2. Physical & Chemical Properties

Description	Colorless liquid or gas (above 21°C)
Molecular formula	C_2H_4O
Molecular weight	44.05 g/mol
Density	0.79 g/cm^3
Boiling point	21 °C
Melting point	-123.5 °C
Vapor pressure	755 mm Hg @ 20°C
Odor threshold	0.09 mg/m^3
Solubility	Miscible in all proportions with water and the most common organic solvents.
Conversion factor	1.8 mg/m^3 = 1 ppm @ 25°C

3. Occurrence and Major Uses

Acetaldehyde is used as an intermediate for the manufacture of a number of other chemicals, including acetic acid, acetic anhydride, ethyl acetate, peracetic acid, pentaerythritol, chloral, alkylamines, and pyridines (HSDB, 2004). Sources of acetaldehyde emissions include interior finish materials such as sheet vinyl flooring and carpets, and wood-based building products such as fiberboard and particleboard. Some consumer products also emit acetaldehyde, including adhesives and glues, coatings, lubricants, inks, nail polish removers, liquid wax for wood preservation, detergent and cleansers, deodorants, fuels, and mold inhibitors (Beall and Ulsamer, 1981; CARB, 1993). Emissions of acetaldehyde also occur during combustion processes such as cigarette smoking, automobile exhaust, and use of fireplaces and woodstoves, although long-term indoor concentrations tend to be dominated by non-combustion sources.

An emissions study of new building materials found that samples of carpet, fiberboard, particleboard, and non-rubber resilient flooring emitted acetaldehyde (Burt et al., 1996; IWMB, 2003). Air concentrations based on the acetaldehyde emission rates from these various building products, when modeled to standard State office and classroom dimensions, ranged from 4.6 to 26 μg/m^3 (2.6 to 14 ppb).

Indoor concentrations of acetaldehyde often greatly exceed outdoor levels and appear to dictate personal exposures, which is consistent with the more significant and widespread indoor sources of this aldehyde. In 2002, the annual average outdoor concentration of acetaldehyde in the South Coast Air Basin was 2.5 μg/m^3 (1.4 ppb). In Brazil, which has a high usage of ethanol as a transportation fuel, outdoor acetaldehyde concentrations have been measured as high as 63 μg/m^3 (35 ppb) while a highway tunnel had measured levels of acetaldehyde of 430 μg/m^3 (240 ppb). The mean acetaldehyde concentrations in U.S. homes range from 15 to 36 μg/m^3 (8.3 to 20 ppb), but reached as high as 103 μg/m^3 (57.2 ppb) in newly manufactured homes (Zweidinger et al., 1990; Lindstrom et al., 1995; Hodgson et al., 2002; Kinney et al., 2002). Acetaldehyde concentrations measured in Southern California portable classrooms ranged from 5.7 to 12.8 μg/m^3 (3.2 to 7.1 ppb) with a mean of 9.8 μg/m^3 (5.4 ppb) (Hodgson et al., 2004) Similar concentrations were found in classrooms of the main buildings. Measured concentrations of acetaldehyde in public/office buildings range from 3 to 16 μg/m^3 (1.7 to 8.9 ppb).

Environmental tobacco smoke (ETS) has been found to be a source of environmental acetaldehyde. Although long-term acetaldehyde levels in smoking and non-smoking homes tend to be similar, acetaldehyde concentrations in homes as a result of exposure from ETS for nonsmoking Californians has been estimated at 11-15 μg/m^3 (6.1 to 8.3 ppb) (Miller et al., 1998). Concentrations of acetaldehyde measured over a 72-hour period in 57 homes ranged from 3 to 23 μg/m^3 (1.7 to 12.8 ppb). However, no significant difference was observed between the homes of smokers and nonsmokers (Brown et al., 1994). A 48-hour integrated measurement of breathing-zone concentrations revealed that people who work in garages (9 smokers and 13 nonsmokers) had significantly higher levels of breath acetaldehyde than controls (4

smokers and 11 nonsmokers), and the smokers had significantly higher levels of breath acetaldehyde than the nonsmokers.

The concentration of breath acetaldehyde (endogenous level) in non-alcoholic, non-smokers range from 0.7 to 11.0 μg/m^3(0.4 to 6.1 ppb), but can be somewhat higher in smokers (16 ± 3 μg/m^3 = 8.9ppb). The higher concentrations are seen in the breath of smokers after they ingest alcohol. With alcohol consumption, the concentrations of acetaldehyde produced vastly exceed the trace amounts generated from microorganisms or other possible endogenous substrates. When subjects with normal aldehyde dehydrogenase (ALDH) activity drink small amounts of alcohol (0.4-0.8 g/kg), the concentrations of breath acetaldehyde may reach between 200 and 2200 μg/m^3 (111 to 1222 ppb) (Shaskan and Dolinsky, 1985; Jones, 1995).

In a controlled human study, five healthy nonsmoking adults inhaled low doses of ethanol (ETOH) and concentrations of ETOH and acetaldehyde were measured in the alveolar air using only the last portion of air in the sampling bag after forced expiration through a three-way valve (Tardif et al., 2004). Exposures were for six consecutive hours to 25, 100, or 1000 ppm ETOH. After two hours of exposure at 25 ppm, acetaldehyde and ETOH were measured in the alveolar air at 0.06 and 7.5 ppm, respectively.

In Asian subjects with a genetic deficiency of the enzyme aldehyde dehydrogenase (ALDH), the concentration of acetaldehyde in the breath after drinking can reach 8.8-22 mg/m^3 (4.9 to 12.2 ppm). Higher concentrations of acetaldehyde have been shown to activate mast cells, which then induce histamine release. In one case study, a patient had a severe bronchial asthma attack after ingesting food containing small amounts of alcohol, and was found to be homozygous for the ALDH-2 mutant genotype. Both acetaldehyde and ethanol inhalation tests were performed on the patient. The ethanol inhalation test was negative, but acetaldehyde inhalation (5, 10, 20, or 40 mg/ml) decreased $FEV_{1.0}$ by 33.5% at 20 mg/ml (Saito et al., 2001).

4. Disposition

Acetaldehyde is readily absorbed through the lungs into the blood following inhalation exposure. Acetaldehyde is rapidly exchanged and equilibrated between blood entering the lungs and alveolar air. Male Sprague-Dawley rats exposed to acetaldehyde vapor concentrations in air ranging from 9 to 1000 mg/l (0.009 to 1 mg/m^3 or 500 to 555 ppb) for one hour had higher levels of acetaldehyde in the blood than liver (Watanabe et al., 1986). Levels in the arterial blood were also higher than in peripheral venous blood.

Two studies were performed using humans and dogs to determine the percent retention of inhaled acetaldehyde in the respiratory tract (Egle Jr, 1970; Egle Jr., 1972a; 1972b). In humans, the total respiratory tract retention of acetaldehyde was 45-70% when inhaled either orally or nasally (Egle Jr, 1970). Physiological respiratory total retention in multiple breath experiments was independent of tidal volume, and uptake was controlled by frequency and duration of ventilation. Total respiratory tract retention of acetaldehyde in dogs was found to be very close to human retention values and

inversely related to ventilatory rate in the same manner as humans (Egle Jr., 1972b). Uptake was also found to be higher in the upper than the lower respiratory tract and unrelated to changes in concentration inhaled or tidal volume (Egle Jr., 1972b).

Acetaldehyde deposition efficiency is strongly dependent on the inspired concentration, with deposition being less efficient at high compared to low concentrations. Species differences have been observed in uptake efficiency with uptake being significantly higher in the mouse, rat, and hamster compared to the guinea pig at 100 ppm, but at 10 ppm the rat had the lowest uptake (Morris, 1997a).

Following oral administration, acetaldehyde is readily absorbed from the gastrointestinal tract and undergoes extensive first pass metabolism in the liver; only 5% remains unchanged (Morris, 1997b).

Acetaldehyde rapidly diffuses through cellular membranes and is distributed to various organs for metabolism. The half-life in rats after inhalation of actetaldehyde was 10 minutes, and the time to total body clearance was 40 minutes (Shiohara et al., 1984). Inhaled acetaldehyde does not undergo a first pass effect and is distributed to all tissues including the liver. Inhaled acetaldehyde undergoes extrahepatic metabolism and is metabolized by aldehyde dehydrogenase in the lungs to acetate. Aldehyde dehydrogenase is found in both the cytosol and the mitochondria. Inhaled acetaldehyde undergoes extrahepatic metabolism by the respiratory-olfactory epithelium, kidneys, blood, brain, and spleen, and only small amounts reach the liver. Acetaldehyde also crosses the blood-brain barrier. Protons (H^+) are a by-product of acetaldehyde metabolism (to acetate), which under high exposure conditions, have the potential to acidify cells and cause cytotoxicity, if cellular buffering systems and proton pumps are overwhelmed (Bogdanffy et al., 2001).

Various isoenzymes of alcohol dehydrogenase transform ethanol into acetaldehyde, which in turn is rapidly oxidized by aldehyde dehydrogenase (ALDH) into acetate. Both pathways for acetaldehyde metabolism (low-affinity (cytosolic ALDH1) and high-affinity (mitochondrial ALDH2) are present and have been described in rodent nasal olfactory and respiratory tissues (Casanova-Schmitz et al., 1984; Morris, 1997a; 1997b; Bogdanffy et al., 1998).

Functional genetic polymorphisms and ethnic variation exist at various genes encoding these enzyme proteins which all act to alter the rate of synthesis of the toxic metabolite acetaldehyde, or decrease its further oxidation. About 50% of the Asian population are alcohol-sensitive, having a deficiency or low activity in aldehyde dehydrogenase enzymes that are important in ethanol metabolism. This can result in high acetaldehyde levels in blood and breath following alcohol consumption.

A small amount of acetaldehyde is produced in the body during normal intermediary metabolism and is also a product of microbial fermentation of sugars in the gut. However, based on studies in animals, the critical effects of exposure to exogenous acetaldehyde occur at the site of initial contact (i.e., the respiratory tract following inhalation).

At least two isozymes of aldehyde dehydrogenase were found in the rodent nasal mucosa, differing with respect to their apparent Vmax and Km values (Morris, 1997a). Male F344 rats were exposed to 1500 ppm acetaldehyde for 6 hours/day for 5 days. Oxidation of acetaldehyde occurred more rapidly in the homogenates of the respiratory than the olfactory mucosa (Morris, 1997a). The nasal tissue is the first to contact acetaldehyde vapors upon inhalation. The aldehyde dehydrogenase acts as a defense mechanism helping to minimize or prevent toxic injury to nasal tissues exposed to airborne compounds. Pretreatment with an ALDH inhibitor reduced nasal acetaldehyde deposition rates (Morris, 1997a).

Acetaldehyde can be eliminated unchanged in urine, expired air, and skin (Baselt and Cravey, 1989) and is metabolized by aldehyde dehydrogenase to acetate which is readily excreted in the urine.. Acetaldehyde is highly reactive and can bind to amino acids and blood and membrane proteins, and act as a hapten (Mohammad et al., 1949; Eriksson et al., 1977; Gaines et al., 1977; Donohue Jr. et al., 1983; Tuma and Sorrell, 1985; Dellarco, 1988; Hoffmann et al., 1993; Wickramasinghe et al., 1994; Tyulina et al., 2006). Antibodies against acetaldehyde conjugates have been detected in human and rabbit serum (Gaines et al., 1977). Acetaldehyde is a weak clastogen that induces sister chromatid exchanges and reacts with DNA to form DNA-protein and DNA-DNA cross-links (Dellarco, 1988). Acetaldehyde causes lipid peroxidation, which can lead to adduct formation and free radical-induced cell injury.

5. Acute Toxicity of Acetaldehyde

5.1 Acute Toxicity to Adult Humans

Several studies in human volunteers are available, including several recent studies in asthmatics where subjects inhaled aerosolized acetaldehyde. The ability to determine a one-hour reference exposure level (REL) is limited due to the extremely short exposure period of only 2-4 minutes that was used in these studies. However, inhalation experiments with human volunteers in which exposure lasted longer are old and of limited design. The major acute effects of human exposure to acetaldehyde vapors consist of irritation to the eyes, skin and respiratory tract, and bronchoconstriction in asthmatics. The key study used to determine the acute Reference Exposure Level (REL) was a study performed in human volunteers investigating bronchoconstriction in response to inhaled aerosolized acetaldehyde (Prieto et al., 2000). The Prieto et al. (2000) study determined the mean acetaldehyde concentration causing a 20% decrease in Force Expiratory Volume (FEV_1) in asthmatic human volunteers.

Silverman et al. (1946) exposed human volunteers to acetaldehyde to determine the sensory response limit for solvent concentrations when estimating ventilation requirements for comfortable working conditions (Silverman et al., 1946). The sensory limits were reported and compared to the maximum allowable concentration, which was stated as 200 ppm for acetaldehyde at the time of the study. Twelve volunteer human subjects of both sexes were used for each solvent exposure. During the 15 minute exposure period, motion pictures were shown to occupy the subjects' attention and divert their thoughts from the atmospheric exposure in the chamber. The results,

though described in a limited way, are useful because the analysis was performed in human subjects and the concentrations tested were as low as 25 ppm. At 50 ppm, the majority of subjects experienced eye irritation (number not specified). The subjects that did not report eye irritation had reddened eyelids and bloodshot eyes after exposure at 200 ppm. Several subjects reported unspecified irritation at 25 ppm and "objected strenuously." Finally, nose and throat irritation were reported as occurring at concentrations greater than 200 ppm (Silverman et al., 1946).

A second acute human study was found in the historical literature, where fourteen male subjects were exposed to 134 ppm acetaldehyde in a chamber for 30 minutes (Sim and Pattle, 1957). Subjects reported mild upper respiratory tract irritation (Sim and Pattle, 1957). However, a major confounder with this study appears in the methods section, which stated that subjects were permitted to smoke inside the "chamber" during the 30 minutes.

Acetaldehyde provocation tests have been conducted with asthmatic and non-asthmatic human subjects using aerosolized acetaldehyde solutions. As mentioned previously, aldehyde dehydrogenase plays an important part in the metabolism of ethanol in making possible the conversion of acetaldehyde (previously formed from ethanol by alcohol dehydrogenase) to acetic acid. Lower activity of aldehyde dehydrogenase leads to elevated concentrations of acetaldehyde in the blood, which in asthmatic subjects may produce bronchoconstriction. There are indications that enhanced release of histamine from pre-activated airway mast cells plays an important role (Myou et al., 1993). As a result of the polymorphism of ALDH-2, nearly half of the Japanese patients with asthma show bronchoconstriction after drinking alcohol, a phenomenon that is also known to occur in other Asian populations (Myou et al., 1993; Myou et al., 1994; Fujimura et al., 1999). In several studies in asthmatic volunteers, inhaled acetaldehyde aerosol has been tested for its bronchoconstrictive effect, first in three studies in Japanese subjects (Myou et al., 1993; Myou et al., 1994; Fujimura et al., 1999) and subsequently in several studies in Caucasian subjects (Prieto et al., 2000; Prieto et al., 2002a; Prieto et al., 2002b). In these studies, subjects inhaled aerosolized acetaldehyde for very short periods; exposure was (2-4 minutes).

Myou et al. (1993) exposed a group of nine asthmatic volunteers (age 39.2 ± 5.4 yr) and nine age- and sex- matched controls to aerosolized acetaldehyde for 2 minutes immediately followed by measurement of Force Expiratory Volume in one second (FEV_1). The solutions of 5, 10, 20, or 40 mg/ml of acetaldehyde were in saline and were inhaled from a nebulizer for 2 minutes by mouth tidal breathing wearing a noseclip. The aerosol was produced using a DeVilbiss 646 nebulizer operated by compressed air at 5 liters per minute. Nebulizer output was not reported but probably was the same as in later studies by this group, i.e. 0.14 ml/minute. No measurements of acetaldehyde concentration in air were made. The dose response study showed significant reductions in FEV_1 at all acetaldehyde test concentrations in asthmatics whereas no effect was seen in normal subjects (Myou et al., 1993).

In further experiments with the same group of volunteers, the influence of oral terfenadine, a histamine H1 blocker, was examined as was the bronchial

responsiveness to methacholine (challenge with methacholine is a common asthma identification test). The response seen after inhalation of acetaldehyde was completely suppressed by pretreatment with terfenadine, which supports the hypothesis that bronchial hyper-responsiveness is a precondition of acetaldehyde induced bronchoconstriction, which is caused indirectly via histamine release in asthmatics (Myou et al., 1993). A rough estimate from the dose response curve as presented in the paper, suggests a PC_{20} for acetaldehyde (acetaldehyde concentration producing a 20% reduction in FEV_1) of about 20 mg/ml (Myou et al., 1993). The acetaldehyde aerosol concentration as mg/m^3 in this study can be estimated as follows. The nebulizer was operated at 5 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.14 ml/minute. When given at this rate a 20 mg acetaldehyde/ml solution (the estimated PC_{20}) corresponds to a concentration in air of approximately 560 mg/m^3 (about 314 ppm).

In a subsequent acute human study, nine asthmatic subjects of Japanese origin were used to determine whether bronchial responsiveness to inhaled methacholine (STET: a standard test used to identify agents that potentially exacerbate asthma) was altered when asthmatic subjects inhaled a sub threshold concentration of aerosolized acetaldehyde which did not cause bronchoconstriction, and whether any increase in bronchial hyper-responsiveness after acetaldehyde was mediated by histamine release (Myou et al., 1994). For each subject, the concentration of acetaldehyde producing a 20% fall in FEV_1 was determined (PC_{20}) using ascending doses (5, 10, 20, or 40 mg/ml) of acetaldehyde. The mean concentration of PC_{20} for the nine subjects was 23.3 mg/ml of acetaldehyde (Myou et al., 1994). The nebulizer was operated at 5 liters air/minute for 4 minutes with an acetaldehyde solution output of 0.14 ml/minute. Therefore, a 23.3 mg acetaldehyde/ml solution corresponds to a concentration in air of approximately 652 mg/m^3 (about 362 ppm).

In part two of this study, nine subjects inhaled a sub threshold concentration of 0.8 mg/ml acetaldehyde at 0.14 ml/minute for four minutes or saline followed by provocation with a range of increasing methacholine concentrations (Myou et al., 1994). FEV_1 was measured before and after treatment. Acetaldehyde potentiated bronchial hyper-responsiveness to provocation by methacholine (Myou et al., 1994) producing a marked reduction in PC_{20}-MCH (0.48 mg/ml versus 0.85 mg/ml after saline treatment) (Myou et al., 1994).

Myou et al. (1995) examined tachyphylaxis occurring in response to repeated inhalation of histamine or acetaldehyde in nine asthmatic subjects. The mean acetaldehyde concentration causing a 20% decrease in FEV_1 increased significantly from a geometric mean of 18.4 mg/ml (with a geometic standard error (GSEM) of 0.14) to 45.2 mg/ml (GSEM 0.14) over a period of one hour ($p<0.002$). The mean histamine concentrations causing a 20% decrease in FEV_1 were no different.

In a later study in asthmatics of Japanese origin, the hypothesis was tested that asthmatics that are sensitive to alcohol (showing bronchoconstriction after drinking alcohol) also have increased airway responsiveness to inhaled acetaldehyde when compared to asthmatics not sensitive to alcohol (Fujimura et al., 1999). Ten alcohol-

sensitive asthmatics and 16 alcohol insensitive asthmatics (20-65 years) of Japanese origin inhaled acetaldehyde aerosol for 2 minutes by tidal mouth breathing and FEV_1 was measured. Increasing concentrations of acetaldehyde solutions in saline (0.04 to 80 mg acetaldehyde/ml) were inhaled until FEV_1 showed a fall of 20%. In the alcohol-sensitive group the geometric mean PC_{20} was 21.0 mg/ml (range not reported), whereas in the alcohol-insensitive group this was 31.7 mg/ml (range not reported). The difference between the groups, however, was not statistically significant (Fujimura et al., 1999). The aerosol was produced using a DeVilbiss 646 nebulizer operated by compressed air at 5 liters/minute with a nebulizer output of 0.14 ml/minute. The nebulizer was operated at 5 liters air/minute for 2 minutes with a acetaldehyde solution output of 0.14 ml/minute. At this rate, inhalation of acetaldehyde solutions of 0.04 to 80 mg/ml corresponds to concentrations in air of approximately 1.12 to 2240 mg/m^3. Similarly, the geometric mean PC_{20} in the alcohol-sensitive group corresponds to approximately 588 mg/m^3 (about 330 ppm) and the geometric mean PC_{20} in the alcohol-insensitive group to approximately 888 mg/m^3 (about 500 ppm).

In the key study (Prieto et al., 2000) used for the acute REL determination, the responses to methacholine and acetaldehyde challenges were measured in 81 non-smoking adults to determine differences in airway responsiveness between asthmatics and healthy subjects and to examine the relationship between acetaldehyde responsiveness and the variability of peak expiratory flow (PEF). Prieto et al. (2000) examined whether the bronchoconstriction seen in Japanese asthmatics after inhalation of acetaldehyde also occurred in Caucasian subjects. They exposed 61 mildly asthmatic subjects and 20 healthy subjects (control group) to aerosolized acetaldehyde (5 to 40 mg acetaldehyde/ml) for two minutes using a two-minute tidal breathing-method and FEV_1 was measured 60 to 90 seconds after inhalation of each concentration until FEV_1 dropped by more than 20%. In this study, the PC_{20} values for acetaldehyde ranged from 1.96 to 40 mg/mL with a geometric mean value of 17.55 mg/ mL and the 95% confidence interval of the geometric mean (95% CI 4.72-38.3 mg/ml). Therefore, the lower limit of the geometric 95% CI was 4.72 mg/ml (Prieto, 2008). Effectively, the data are for the geometric mean (antilog of the mean PC20 acetaldehyde) and the 95% CI is for the antilog of this value also. This is the traditional way these type of data are presented for acetaldehyde, methacholine or AMP (Prieto 2008). In the asthma group 56/61 subjects showed bronchoconstriction compared to 0/20 in the control group. Inhaled acetaldehyde was much less potent as a bronchoconstrictor than methacholine in asthmatic patients. Peak expiratory flow variation was significantly but weakly related to acetaldehyde responsiveness ($p = 0.004$). The results obtained by Prieto et al. (2000) indicate that airway hyper-responsiveness to acetaldehyde is a sensitive and specific indicator for separating normal and asthmatic subjects.

In the Prieto et al. (2000) study, aqueous solutions containing acetaldehyde were nebulized in a Hudson 1720 nebulizer operated by compressed air at 6 liters/minute with a nebulizer output of 0.18 ml/minute. Flow rates were reported in a National Advisory Committee document from the U.S. EPA, based on a personal communication from the Prieto group (NAS, 2004). The nebulizer was operated at 6 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.18 ml/minute. At this rate, inhalation of acetaldehyde solutions of 5 to 40 mg/ml corresponds to concentrations in

air of approximately 150 to 1200 mg/m^3. The observed geometric mean PC_{20} of 17.55 mg/ml corresponds to 527 mg/m^3 (about 293 ppm) and the lower 95% confidence interval of 4.72 mg/ml corresponds to approximately 142 mg/m^3 (about 79 ppm).

In a follow-up study, Prieto et al. (2002a) exposed mildly asthmatic subjects (age 18-58 years) to 2.5 to 80 mg acetaldehyde/ml using a Hudson 1720 nebulizer with an output of 5 liters/minute. In the first group, 16 subjects were measured for their response to acetaldehyde which was compared to that of methacholine and adenosine-5'-monophosphate (two bronchoconstrictive agents of known potency). In the second group of 14 subjects, repeatability and side effects of acetaldehyde inhalation were examined. For acetaldehyde the PC_{20} ranged from 8.4 to 80 mg/ml with a geometric mean of 38.9 mg/ml (geometric mean values for methacholine and AMP were 0.6 and 17.4 mg/ml, respectively). The response to acetaldehyde was found to be moderately repeatable. For the group in which repeatability was examined, for acetaldehyde concentrations producing a >20% fall in FEV_1, most subjects had cough (64%), dyspnea (57%) or throat irritation (43%) (Prieto et al., 2002a). The nebulizer was operated at 5 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.16 ml/minute. At this rate, inhalation of acetaldehyde solutions of 2.5 to 80 mg/ml corresponds to concentrations in air of approximately 80 to 2560 mg/m^3. The observed geometric mean PC_{20} of 38.9 mg/ml corresponds to approximately 1245 mg/m^3 (about 692 ppm).

In a further volunteer study, Prieto et al. (2002b) studied comparative airway responsiveness to acetaldehyde (2.5 mg to 80 mg/ml) in subjects with allergic rhinitis (n=43), asthmatics (n=16), and healthy subjects (n=19). The number of subjects with a fall in FEV_1 >20% was 8/43 in the group with allergic rhinitis, 13/16 in the asthmatic group and 0/19 in the healthy subjects group. PC_{20} values in the group with allergic rhinitis ranged from 15.5 to 80.0 mg/ml with a geometric mean of 67.7 mg/ml whereas in the asthmatic group PC_{20} ranged from 8.4 to 80.0 mg/ml with a geometric mean of 35.5 mg/ml ($p < 0.001$) (Prieto et al., 2002b). The PC_{20} values in the allergic rhinitis group were also significantly lower than in the healthy control group ($p = 0.04$) (Prieto et al., 2002b). The nebulizer was operated at 5 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.16 ml/minute. Thus, inhalation of acetaldehyde solutions (2.5 to 80 mg/ml) corresponds to concentrations in air of 80 to 2560 mg/m^3. The observed geometric mean of 67.7 mg/ml corresponds to approximately 2166 mg/m^3 (about 1210 ppm) and the geometric mean of 35.5 mg/ml to approximately 1136 mg/m^3 (about 631 ppm).

As indicated above, the provocation tests involved acetaldehyde solutions that were aerosolized, and then inhaled by mouth. Aerosolized acetaldehyde solutions have been shown to be about 265 times less potent than methacholine in constricting the airways of asthmatic subjects, with aerosolized acetaldehyde solutions of 80 mg/ml resulting in cough, dyspnea, and throat irritation in the asthmatic subjects (Myou et al., 1993). In addition, the exposure times were very short (several minutes) and the concentrations eliciting a response in FEV_1 were much higher. However, it is important to note that in the Myou et al. (1994) study, aerosolized acetaldehyde potentiated bronchial hyper-responsiveness to provocation by methacholine at concentration equivalents in the air of about 22.4 mg/m^3 (or 12.5 ppm) similar to the concentration that produced eye

irritation (25 ppm) in human volunteers as seen in the Silverman et al. (1946) study. This response is of concern and an experimental analog to asthma. This may be indicative that the same chemo-sensory response triggered both the reactiveness in the airways and eye irritation. The potentiation of methacholine-induced bronchoconstriction shows the potential of acetaldehyde at concentrations of approximately 12.5 ppm or higher to exacerbate asthma. It should be noted that the model of nebulizer used was shown to have inconsistent delivery; thus the estimate of concentration of acetaldehyde that potentiated methacholine-induced bronoconstriction is uncertain.

In summary, exposure to acetaldehyde, at concentrations as low as 25 ppm, resulted in sensory irritation in human volunteers (Silverman et al., 1946). Aerosolized acetaldehyde at concentrations equivalent to approximately 12.5 ppm potentiated bronchial hyper-responsiveness to provocation by methacholine (Myou et al., 1994). Adult asthmatics showed large inter-individual variation in PC_{20} values (59 ppm to 1200 ppm) (Prieto et al., 2000). Finally, adult asthmatics that inhaled aerosolized solutions of acetaldehyde showed increased irritation and bronchoconstriction at 293 ppm (Prieto et al., 2000). Table 5.1.1 summarizes the aerosolized acetaldehyde provocation studies in adult human volunteers.

Table 5.1.1. Summary of Aerosolized Acetaldehyde Provocation Studies in Adult Human Volunteers

Human Volunteers	Concentration in aerosol	(PC_{20})*	Reference
Japanese asthmatics	5, 10, 20, 40 mg/ml	314 ppm	Myou et al., 1993
Japanese asthmatics	5, 10, 20, or 40 mg/ml	362 ppm	Myou et al., 1994
Alcohol-sensitive Japanese asthmatics	0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, 80 mg/ml	327 ppm	Fujimura et al., 1999
Alcohol-tolerant Japanese asthmatics	0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, 80 mg/ml	500 ppm	Fujimura et al., 1999
Japanese asthmatics	0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 40, 80 mg/ml	286 ppm	Myou et al., 1995
Caucasian asthmatics	5-40 mg/ml	293 ppm	Prieto et al., 2000
Caucasian asthmatics	2.5-80 mg/ml	692 ppm	Prieto et al., 2002a
Caucasian asthmatics	2.5-80 mg/ml	631 ppm	Prieto et al., 2002b

* Values for PC_{20}, which are geometric means are converted from mg/mL of aerosolized acetaldehyde to approximate concentration in air (ppm).

5.2 Acute Toxicity to Infants and Children

No studies on the effects of acute exposure to acetaldehyde in non-adult humans were located. However, as noted above for adults, there is some evidence that following acute exposure to acetaldehyde, asthmatics are more sensitive to acetaldehyde exposure and are likely to show symptoms such as wheezing, shortness of breath, bronchoconstriction, and/or decrements in pulmonary function consistent with

immediate and/or delayed bronchoconstriction. Furthermore, some asthmatics may respond with significant reductions in lung function due to the irritant effects, sensitized or not. The potential association between acetaldehyde exposure and asthma is of special concern for children because they have higher prevalence rates of asthma than adults, and their asthma episodes can be more severe due to their smaller airways. Hospitalization rates of children for asthma, especially for the first four years of life, are higher than for other age groups (Mannino et al., 1998). In addition, infants and children may have qualitatively different responses due to different target tissue sensitivities during windows of susceptibility in the developmental process.

Findings also support the view that toxic air contaminants, such as acetaldehyde, in communities in proximity to major emission sources, including both industrial and traffic sources, have adverse effects on asthma in children (Delfino et al., 2003). The average daily residential exposure to acetaldehyde in high school students living in inner-city neighborhoods of New York City and Los Angeles and living with a smoker was evaluated. The exposure concentration range measured in juveniles living with smokers was 6.3 to 14 $\mu g/m^3$ (Nazaroff, 2004). This study estimated that approximately 16 million juveniles are exposed to environmental tobacco smoke, and hence acetaldehyde by living with smokers.

5.3 Acute Toxicity to Experimental Animals

Acetaldehyde causes sensory irritation in experimental animals. Male B6C3F1 or Swiss-Webster mice were exposed to acetaldehyde in a head-only exposure chamber for 10 minutes and sensory irritation was quantified by measuring respiratory rate depression during the exposures (Steinhagen and Barrow, 1984). The respiratory rates were recorded with a plethysmograph and the average maximum decrease in respiratory rate for one minute was computed from the response of each group of animals. Five concentrations (750 to 4200 ppm) were used to construct a concentration-response curve and the RD_{50} was calculated (the concentration eliciting a 50% decrease in respiratory rate). RD_{50} values were 2932 and 2845 ppm for B6C3F1 and Swiss-Webster mice, respectively (Steinhagen and Barrow, 1984).

In a study using young adult albino male Wistar rats, acetaldehyde (nose-only) exposure resulted in an initial rapid decrease in breathing frequency during the first minutes of exposure (Cassee et al., 1996a). The minimum decrease in respiratory rate considered significant was 12%. The animals were exposed to acetaldehyde vapors for thirty minutes. The exposure concentrations were reported as 2800, 4600, and 6500 ppm for acetaldehyde. The RD_{50} for acetaldehyde in the single-compound study was calculated to be 3046 ppm (Cassee et al., 1996a).

Similarly, male F-344 rats were exposed in a head-only inhalation chamber to acetaldehyde (approximately 800 to 10,000 ppm though exact concentrations from the graph were not provided in the paper) for 10 minutes and experienced sensory irritation as measured by reduction in respiratory rate (Babiuk et al., 1985). The RD_{50} (the level inducing a 50% reduction in respiratory breathing rate) was 2991 ppm (95% CI 2411-3825) for this study (Babiuk et al., 1985).

In addition to sensory irritation, histopathological effects have been observed after exposure to acetaldehyde. Albino, male Wistar rats, 8 weeks old, were exposed for 6 hours a day, to either one or three day exposures on consecutive days, in a nose-only inhalation chamber to acetaldehyde (750 or 1500 ppm) (Cassee et al., 1996b). Acetaldehyde exposure resulted in histopathological nasal changes with the three-day exposure group consisting of increased incidence and severity of "single-cell necrosis" in olfactory epithelium with increasing concentration. Biochemical changes consisted of concentration-dependent increases of nonprotein sulfhydryl groups in nasal respiratory epithelium with one- and three-day exposure, which was statistically significant with exposure to 1500 ppm. Activities of biotransformation enzymes (glutathione peroxidase, glutathione S-transferase, glutathione reductase, formaldehyde dehydrogenase, and nonspecific aldehyde dehydrogenase) were not affected by any of the exposures (Cassee et al., 1996b).

Acute lethality studies have also been performed with acetaldehyde. In an historical acute inhalation study in rats, groups of eight per dose were exposed to acetaldehyde vapors 7,778 to 31,667 mg/m^3 (14,000 to 57,000 ppm) for thirty minutes (Skog, 1950). The acute LD_{50} value (reported as LD_{50}) for acetaldehyde inhalation was 20,600 ppm (37,000 mg/m^3) (Skog, 1950).

Appelman et al. (1982) determined the LC_{50} for acetaldehyde using twenty male and twenty female albino Wistar rats. The animals were exposed for 4 hours in horizontally placed glass exposure cylinders with a total airflow through the cylinder of 8 l/min. Concentrations were given as the mean of 10 to 15 determinations and were as follows: 10,436, 12,673, 15,683, and 16,801 ppm. Within the first half-hour of the four-hour LC_{50} study, rats exhibited restlessness, closed eyes and labored breathing to acetaldehyde concentrations as low as 10,436 ppm. In the subacute portion of the study, rats exhibited severe dyspnoea and excitation within the first half-hour of exposure to 5000 ppm. The behavior of animals exposed to 2200 ppm or lower for six hours was unremarkable. The four-hour LC_{50} and the 95% confidence limits were calculated to be 13,300 ppm (95% CL: 11,200, 15,400) (Appelman et al., 1982).

Syrian Golden hamsters were exposed to acetaldehyde vapors for 4 hours at doses ranging from 14,450 to 17,600 ppm (26,010 to 31, 680 mg/m^3) (Kruysse et al., 1975). After one to two hours of exposure at all concentrations, the animals showed severe lacrimation, salivation, and nasal discharge. The 4-hour LC_{50} was determined to be 17,000 ppm (30,600 mg/m^3) for this study. In all exposure groups, the animals that died during exposure had convulsions. Some animals survived at all concentrations, but only after a deep narcosis and apnea (Kruysse et al., 1975).

Aldehyde dehydrogenase 2 (ALDH2) is an important enzyme that oxidizes acetaldehyde. Isse et al. (2005) compared the acute acetaldehyde toxicity between wild-type (Aldh2+/+) and Aldh2-inactive transgenic (Aldh2-/-) mice after inhalation. The null aldehyde dehydrogenase 2 (ALDH2) transgenic mice (-/-) or wild type (+/+) mice were exposed by inhalation to 5000 ppm acetaldehyde for four hours. Mice were observed at 0, 2, 20, 40, 60, 120, and 240 minutes after administration. Within the first twenty minutes, hypoactivity, crouching, bradypnea, closed eyes, and piloerection were

observed in both the wild type and the knockout mice. By one hour, the ADLH (-/-) mice were showing a staggering gait (Isse et al., 2005). This study concluded that acute acetaldehyde toxicity after inhalation is higher in aldehyde dehydrogenase 2 knockout than in wild-type mice (Isse et al., 2005).

Female CD1 mice were exposed in inhalation chambers to a target acetaldehyde exposure of 200 ppm (actual mean of 5 exposures was 180 ± 35 ppm), twice the threshold limit value, for single and multiple three-hour exposures, and then evaluated for changes in their susceptibility to experimentally induced *Streptococcus* aerosol infection and pulmonary bactericidal activity to inhaled *Klebsiella* pneumoniae after one or five days (Aranyi et al., 1986). The results showed increased pulmonary bactericidal activity in response to 200 ppm of acetaldehyde possibly by a pollutant-induced recruitment of unexposed alveolar macrophages. This study suggests that inhaled toxicants such as acetaldehyde may alter susceptibility to or severity of respiratory infection (Aranyi et al., 1986).

Table 5.3.1 summarizes the acute animal data for acetaldehyde inhalation. The data indicate that humans may be more sensitive to the acute effects of acetaldehyde than animals. For the endpoint of sensory irritation, measured as reduction in respiratory rate, the lowest RD_{50} for mice and rats were 2845 and 2991 ppm, respectively. With respect to histopathological changes, effects were observed at 1500 ppm. In the acute lethality studies, the lowest LC_{50} was 13,300 ppm in rats. In contrast, the LOAEL for human sensory irritation was reported to be 25 ppm in one historical study (Silverman et al., 1946). In addition, potentiation of methacholine-induced bronchoconstriction was shown in one study at approximately 12.5 ppm.

Table 5.3.1 Summary of Acute Studies in Experimental Animals

Endpoint	Strain/Species	Exposure	Response	Reference
Sensory irritation	B6C3F1 mice	750 to 4200 ppm for10 min	RD_{50} = 2932 ppm	(Steinhagen and Barrow 1984)
	Swiss Webster mice	750 to 4200 ppm for10 min	RD_{50} = 2845 ppm	(Steinhagen and Barrow 1984)
	F-344 rats	~800 to 10,000 ppm for 10 min	RD_{50} = 2991 ppm	Babiuk et al., 1985
	Wistar rats	2800, 4600, or 6500 ppm for 30 min	RD_{50} = 3046 ppm	Cassee et al., 1996a
Histopathological	Wistar rats	750 or 1500 ppm for 1 and 3 days	Olfactory epithelial lesions at 1500 ppm	Cassee et al., 1996b
Lethality	Rats	14,000 to 57,000 ppm for 30 min	LD_{50} = 20,600 ppm	Skog, 1950
	Wistar rats	10,436 to16,801 ppm for 4 hours	LC_{50} = 13,300 ppm	Appelman et al., 1982
	Syrian Golden hamsters	14,450 to 17,600 ppm for 4 hours	LC_{50} = 17,000 ppm	Kruysse et al., 1975
Behavioral/Other effects	Syrian Golden hamsters	14,450 for one to 2 hours	lacrimation, salivation, and nasal discharges	Kruysse et al., 1975
	Wistar rats	10,436 ppm within first 30 min	restlessness, closed eyes and labored breathing	Appelman et al., 1982
	Wistar rats	5000 ppm for 30 min	severe dyspnoea and excitation	Appelman et al., 1982
		5000 ppm for 20 minutes	crouching, brady-pnea, closed eyes, and piloerection	Isse et al., 2005a
	CD1 mice	200 ppm for 3 hours	increased pulmonary bactericidal activity	Aranyi et al., 1986

6. Chronic Toxicity of Acetaldehyde

6.1 Chronic Toxicity to Adult Humans

No studies were found for human chronic exposures. Therefore the chronic REL was based on an animal study. However, as mentioned previously, it is important to note that acetaldehyde can be produced endogenously after food intake and ethanol consumption. Therefore, certain segments of the population may be at higher risk for chronic exposure due to alcoholism or frequent drinking or smoking. Those members of the population who smoke or are consistently exposed to ETS may be at increased risk of problems related to chronic toxicity of acetaldehyde.

6.2 Chronic Toxicity to Infants and Children

No studies were found on chronic exposure of infants and children to acetaldehyde. However, we anticipate that chronic exposure to acetaldehyde may exacerbate breathing problems in infants and children with asthma.

6.3 Chronic Toxicity to Experimental Animals

Exposure to inhaled acetaldehyde produces non-carcinogenic injury including degeneration and hyperplasia in the rat respiratory tract. The nasal cavity is the primary target with nasal olfactory mucosa being more sensitive than respiratory mucosa to the effects of acetaldehyde (Morris, 1997a; b). Deposition efficiency of inhaled acetaldehyde is highly dependent on airflow rate and on the inspired concentration in rodents (Morris, 1997a; b). Pretreatment with an ALDH inhibitor reduces nasal acetaldehyde deposition rates in anesthetized rodents (Morris and Blanchard, 1992).

In a subchronic study, male and female rats were exposed to acetaldehyde (6 hr/day, 5 days/week) for four weeks to concentrations of 400, 1000, 2200, or 5000 ppm, which resulted in degeneration of olfactory nasal tissues at all concentrations. Therefore a lowest observable adverse effect level (LOAEL) for this study was 400 ppm (Table 6.3.1) (Appelman et al., 1982). Nasal respiratory tissue lesions were seen at the three highest concentrations, tracheal and laryngeal lesions were observed only at the two highest concentrations, and mild injury to the lower respiratory tract was observed only at the highest concentration. Respiratory distress (dyspnea) was noted at 5000 ppm. Subsequent 4-week exposure studies in males of the same rat species at 150 and 500 ppm, resulted in observed degeneration of olfactory nasal tissues at 500 ppm, but not in the 150 ppm exposure group (Appelman et al., 1986). Therefore, 150 ppm was designated the no observable adverse effect level (NOAEL).

Table 6.3.1: Incidence of Nasal Olfactory Tissue Effects in Rats

Degeneration of nasal olfactory epithelium	Exposure Group (ppm)						
	0	150	400	500	1000	2200	5000
Number examined	40	10	20	10	20	20	20
Number affected	2	0	16	10	20	19	20

(Appelman *et al.*, 1982)

Exposure of rats to 243 ppm (442 mg/m^3) acetaldehyde for 8 hr/day, 5 days/week for 5 weeks resulted in an "intense" nasal inflammatory reaction with olfactory epithelium hyperplasia and polymorphonuclear and mononuclear infiltration of the submucosa (Saldiva et al., 1985). Changes in pulmonary mechanics, including increased functional residual capacity, residual volume, total lung capacity, and respiratory frequency was observed, but may have been the result of mechanical damage during pulmonary function testing.

In a subchronic study, male F344 rats were exposed to acetaldehyde (6 hr/day, 5 days/week) for 13 weeks to concentrations of 0, 50, 150, 500, or 1500 ppm, which resulted in degeneration of olfactory and respiratory epithelium (Dorman et al., 2008). The lowest observable adverse effect level (LOAEL) for the endpoint of degeneration of olfactory nasal epithelium was 150 ppm for the 65-day observation (Table 6.3.2). The no observable adverse effect level (NOAEL) for degeneration of olfactory nasal epithelium was 50 ppm. For the incidence of respiratory epithelial hyperplasia (Table 6.3.3), the LOAEL was 500 ppm and the NOAEL was 150 ppm.

Table 6.3.2: Incidence of Nasal Olfactory Tissue Effects in F344 Rats at 65 Days

Degeneration of nasal olfactory epithelium	Exposure Group (ppm)				
	0	50	150	500	1500
Number examined	12	12	12	12	12
Number affected	0	0	12	12	12

From Dorman *et al.,* 2008 (supplemental data Table IV. provided by author)

Table 6.3.3. Incidence of Respiratory Epithelial Hyperplasia in F344 Rats at 65 Days

Degeneration of respiratory epithelium	Exposure Group (ppm)				
	0	50	150	500	1500
Number examined	12	12	12	12	12
Number affected	0	0	1	11	12

Dorman *et al.*, 2008 (supplemental data Table II. provided by author)

This study also examined the endpoints of incidence of respiratory epithelial inflammation and squamous metaplasia using the same dose groups and time-points (data not shown), however the degeneration of olfactory and respiratory nasal epithelia were the endpoints of interest.

In a subchronic exposure of hamsters to 0, 390, 1340, or 4560 ppm acetaldehyde 6 hr/day, 5 days/week for 90 days resulted in growth retardation, and ocular and nasal irritation in the high dose group. Histopathological changes were observed only in the respiratory tract and consisted of necrosis and inflammatory changes of the epithelium in the nasal cavity, larynx, bronchi and lungs in the high dose animals, and mild tracheal epithelial lesions in the mid-dose group. No adverse effects were observed at 390 ppm (Kruysse et al., 1975).

In a subsequent study, 36 hamsters per dose group were chronically exposed in a whole body inhalation chamber to 0, 1500, or 2500 ppm acetaldehyde for 7 hr/day, 5 days/week for 52 weeks resulting in growth retardation and hyperplasia and metaplasia of the nasal and tracheal epithelium in exposed animals (Feron et al., 1982). Rhinitis and epithelial lesions of the larynx were also noted at the highest exposure. The average concentration in the high exposure group (2500 ppm) was lowered several times during the study due to severe growth retardation to a final concentration of 1650 ppm. The authors noted that the nasal lesions were very similar to those previously seen in hamsters repeatedly exposed to 4560 ppm in the 13-week study Kruysse et al. (1975) study. Following a 26-week recovery period, the upper respiratory tract lesions were still present in high exposure animals, but were nearly or completely absent at the low exposure animals (Feron et al., 1982). However, the authors note that the acetaldehyde-induced hyperplasia and metaplasia of the nasal and laryngeal epithelium persisted and was irreversible (Feron et al., 1982).

In chronic inhalation studies, rats were exposed to 0, 750, 1500, or 3000 ppm acetaldehyde for 6 hr/day, 5 days/week for up to 28 months (Woutersen et al., 1984; Woutersen et al., 1986; Woutersen and Feron, 1987). The concentration in the high-dose group was gradually lowered over 15 months to 1000 ppm due to early mortality, respiratory distress (dyspnea) and severe growth retardation. Nasal olfactory tissue degeneration, hyperplasia, and metaplasia were seen at all exposure levels including the LOAEL of 750 ppm. A NOAEL was not determined for this study. Larynx and nasal respiratory epithelium lesions were observed at the two highest concentrations (1500 and 3000 ppm), and slight to severe rhinitis and sinusitis was observed at the highest concentration (3000 ppm). Growth retardation occurred in males of each test group and in females of the two highest concentration groups.

In a pulmonary immune response study, groups (n = 8) of non-sensitized and ovalbumin (OA)-sensitized guinea pigs were exposed to 0 or 200 ppb (360 $\mu g/m^3$) acetaldehyde or 0 or 600 ppb benzaldehyde for 6 hr/day, 5 days/week for four weeks (Lacroix et al., 2002). Two animals from each group were examined histologically and 6 animals from each group underwent bronchoalveolar lavage. Analyses of protein, PGE2 and leokotriene content, and cellularity of the BALF were reported. In sensitized animals, acetaldehyde exposure did not modify the inflammatory and allergic response to subsequent challenge

with ovalbumin (OA) aerosol relative to that induced by sensitization alone. Interestingly, benzaldehyde exposure suppressed the response of sensitized guinea pigs to OA challenge. In nonsensitized guinea pigs, acetaldehyde exposure resulted in "slight irritation" (n = 2) of the lung, trachea and nasal respiratory epithelium, and induced a significant increase in the number of alveolar macrophages, but not eosinophils or neutrophils, in bronchoalveolar lavage fluid (n = 6)(Lacroix et al., 2002). There was no increase in total protein, PGE2, or leukotriene content in the BALF. Acetaldehyde exposure did not change any of these parameters in OA-sensitized animals. Limitations of the LaCroix et al. (2000) study include a lack of quantitative data for irritation and reported large variability in the concentration of acetaldehyde in the chamber atmosphere. In addition, there was no acetaldehyde-induced exacerbation of response to OA challenge in the sensitized animals. If the slight irritation seen in non-sensitized animals was exposure related, the effect would be expected to be greater in the sensitized animals than the non-sensitized animals, but there was no increase in response in acetaldehyde-exposed sensitized animals beyond sensitization alone. Finally, human data indicate exacerbation of methacholine induced bronchoconstriction after acetaldehyde exposure, yet acetaldehyde exposure did not exacerbate OA challenge in this study. Given the lack of quantitative data on irritation, lack of exacerbation of response by acetaldehyde in OA-sensitized animals, and the inconsistency of this study with other rodent studies vis-à-vis irritation NOAELs, we decided against using this study for the 8-hour or chronic REL.

Inhaled acetaldehyde is genotoxic and is a clastogen, and induced sister chromatid exchange (Dellarco, 1988). *In vivo* and *in vitro* studies have shown that acetaldehyde can form DNA-DNA and DNA-protein crosslinks (Morris, 1997a). Acetaldehyde vapor causes chronic tissue injury and tumor formation in nasal tissues at exposure concentrations of 750 ppm or higher (Feron et al., 1982; Woutersen et al., 1986). Acetaldehyde is a Proposition 65 listed carcinogen. Carcinogenicity of acetaldehyde is discussed in the health effects assessment for identification of acetaldehyde as a Toxic Air Contaminant.

7. Developmental and Reproductive Toxicity

Both clinical and experimental studies have shown that ethyl alcohol causes developmental and reproductive toxicity. Acetaldehyde, the primary metabolite of ethyl alcohol, has been suggested as a possible etiologic agent in fetal alcohol syndrome (FAS) (Pratt, 1980; West, 1994; Eriksson, 2001). Current studies suggest that ethyl alcohol and acetaldehyde work through different mechanisms, but it is still unknown if one or both are the basis for FAS. As a small lipid soluble molecule, acetaldehyde is able to cross membranes by simple diffusion (Zorzano and Herrera, 1989a). Acetaldehyde has been shown to cross the placenta in mice and distribute to embryos (Blakley and Scott Jr., 1984b). Placental transfer occurred when acetaldehyde was administered via i.p. injection to pregnant CD-1 mice at 200 mg/kg on day 10 of gestation, and acetaldehyde was detected within the embryo within 5 minutes (Blakley and Scott Jr., 1984a; b). Maximal concentrations of acetaldehyde were also reached in the maternal blood, liver, and yolk sac in the first five minutes.

Acetaldehyde also freely crosses the placenta of Wistar rats (Zorzano and Herrera, 1989b). Following i.v. injection of acetaldehyde (10 mg/kg) to pregnant rats on gestation day 21, acetaldehyde concentrations reached peak levels within five minutes in the maternal blood, fetal blood, and amniotic fluid. Indeed, after just two minutes of maternal intravenous administration of acetaldehyde at high concentrations, it freely crosses the placenta.

Acetaldehyde has been shown to cause adverse developmental effects in some rodent species when administered in high doses via i.p. or i.v. injection. Rats were exposed 50, 75, or 100 mg/kg acetaldehyde by i.p. on gestation day 10, 11, or 12 and then sacrificed on day 21. Significant fetal resorptions and malformations were observed including: edema, microcephaly, micrognathia, micromelia, hydrocephaly, exencephaly, and hemorrhages. Somatometric measurements of fetus, crown rump length, transumbilical distance, and tail length notes severe growth retardation (Sreenathan et al., 1982). In another study in rats, after a single i.p. injection of 50, 75, or 100 mg/kg, teratogenicity, embryolethality, and growth retardation were observed (Blakley and Scott Jr., 1984a).

In vitro models have found that acetaldehyde was teratogenic to C_3H mouse embryos between 8 and 10 days of gestation after 28 hours of exposure (Thompson and Folb, 1982). Morphological parameters and DNA synthesis were measured and correlated. Eight and nine-day embryos were exposed to doses of 7.4, 19.7, or 39.4 mg/l acetaldehyde in incubation medium. The 39.4 mg/l dose group at eight days showed a significant effect on somite count, neural tube fusion, CNS development (size and symmetry), and significant reduction in DNA synthesis. The nine-day embryos at 39.4 mg/l had increased somite count, absent heart beat, and a significant increase in limb development, while the 19.7 mg/l group had significant abnormalities in development of visceral arches, CNS development, and reduction in DNA synthesis.

Acetaldehyde significantly induced cytotoxicity *in vitro* in cultured rat embryonic midbrain cells. The levels of p53, bcl-2, and 8-OHdG were also changed by acetaldehyde treatment (Lee et al., 2005). The purpose of this study was to elucidate the molecular mechanisms involved in alcohol-induced Fetal Alcohol Syndrome (FAS) during embryo and fetal development. It is not clear whether the observed toxicity associated with FAS is due to direct exposure to ethanol, to its metabolite(s) (e.g. acetaldehyde) or to both.

Both acetaldehyde and ethanol significantly inhibited the gonadotropin-stimulated biosynthesis of testosterone, and acetaldehyde and was 4,000 times more potent than ethanol *in vitro* in enzymatically dispersed cells. Testicular steroidogenesis was blocked by acetaldehyde selectively, specifically inhibiting the conversion of androstenedione to testosterone (Cicero and Bell, 1980; Cicero et al., 1980a; Cicero et al., 1980b). As little as 50 ∝M acetaldehyde was effective in suppressing testicular steroidogenesis; however, cell viability was unaffected.

8. Derivation of Reference Exposure Levels

8.1 Acetaldehyde Acute Reference Exposure Level

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 in the Technical Support Document).

Numerous studies on adult humans with and without asthma, investigated provocation with acetaldehyde solutions in saline (Table 5.1.1), which resulted in significant pulmonary decrements and more so in asthmatics. The study by Prieto et al. (2000) was selected for development of the acute REL as it investigated short-term exposure of human volunteers to aerosolized acetaldehyde solutions.

Study	Prieto et al., 2000
Study population	61 adult asthmatic human volunteers
Exposure method	Inhalation by nebulizer
Exposure continuity	
Exposure duration	2-4 minutes
Critical effects	Bronchoconstriction, PC_{20} >20% drop in FEV_1
LOAEL	142 mg/m^3 (79 ppm)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	10 (severe effect, no NOAEL)
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (inter-individual variation)
Toxicodynamic (UF_{H-d})	30 (asthma exacerbation in children, hyper-responsiveness to methacholine)
Cumulative uncertainty factor	300
Reference Exposure Level	**470 µg/m^3 (260 ppb)**

Sixty-one asthmatic subjects were used to determine the concentration of acetaldehyde producing a 20% fall (PC_{20}) in Forced Expiratory Volume in one second (FEV_1) using ascending doses (5 to 40 mg/ml) of aerosolized acetaldehyde solutions. The geometric mean concentration of PC_{20} for the sixty-one subjects was 17.55 mg/ml of acetaldehyde and the values ranged from 1.96 to 40 mg/mL (Prieto et al., 2000). The 95% confidence interval of the geometric mean was (4.72-38.3 mg/ml) (Prieto, 2008). Therefore, the

lower bound of the geometric 95% CI was 4.72 mg/ml, which was converted to ppm, and was the value used as the point of departure for the acute REL derivation (Prieto, 2008). The Hudson 1720 nebulizer was operated at 6 liters air/minute for 2 to 4 minutes with an acetaldehyde solution output of 0.18 ml/minute. Therefore, the lower 95% confidence interval of 4.72 mg/ml corresponds to approximately 142 mg/m^3 (about 79 ppm).which was used as the LOAEL for the acute REL determination.

An uncertainty factor of ten is associated with the use of a LOAEL for severe effects in the absence of a NOAEL (see Section 4.4.5 of the TSD). The key study used to determine the acute REL was a human study, therefore the interspecies uncertainty factor, toxicokinetic (UF_{A-k}) and toxicodynamic (UF_{A-d}) components were each assigned the default value of one.

For the toxicokinetic component of the intraspecies uncertainty factor (UF_{H-k}) a value of one was used since sensory irritation is not expected to involve large toxicokinetic differences among individuals, and the effects are largely confined to the site of contact, in this case, the eyes, nose, and upper respiratory tract, with negligible or no systemic effects. The deposition kinetics of reactive gases is generally thought not to be greatly different between adults and children. Because of this, a value of one is used for the kinetic component of the intraspecies uncertainty factor (UF_{H-k}), rather than a more extended values of $\sqrt{10}$ or ten used where metabolic processes also contribute to inter-individual variability.

The toxicodynamic component of the intraspecies uncertainty factor UF_{H-d} was assigned an increased value of 30 for the acute REL determination due to multiple lines of evidence. A portion of the UF_{H-d} is applied to account for the potential greater susceptibility of children. The respiratory irritant effect of acetaldehyde, with documented potential to exacerbate asthma, is an effect with the potential to differentially impact infants and children. Myou et al., 1994 demonstrated hyper-responsiveness to methacholine after provocation with a sub-threshold dose of aerosolized acetaldehyde at concentrations equivalent to approximately 12.5 ppm. Additional studies have also shown that adult asthmatics are more sensitive to the irritative properties of inhaled aerosolized acetaldehyde solutions, which significantly decreased their forced expiratory volume in one second (FEV_1) by more than 20% (Myou et al., 1993; Myou et al., 1994, Fujimura et al., 1999, Prieto et al., 2002a; Prieto et al., 2002b). Finally, alcohol sensitive asthmatics had a selective hyper-responsiveness to acetaldehyde (Myou et al., 1993; Myou et al., 1994; Fujimura et al., 1999).

Myou et al. (1994) also observed that aerosolized acetaldehyde potentiated bronchial hyper-responsiveness to provocation by methacholine at concentration equivalents in the air (22.4 mg/m^3 or 12.5 ppm) similar to the concentration that produced eye irritation (25 ppm) in human volunteers as seen in the Silverman et al. (1946) study. This response is of concern and an experimental analog to asthma. This may be indicative that the same chemo-sensory response triggered both the reactivity in the airways and eye irritation. The potentiation of methacholine-induced bronchoconstriction shows the potential of acetaldehyde at concentrations of approximately 12.5 ppm or higher to exacerbate asthma. Of note however, some uncertainty

is associated with the use of a DeVillbis nebulizer, which has been shown to have considerable variability in aerosol output and delivered dose (Hollie et al., 1991).

In conclusion, using the LOAEL of 142 mg/m^3 (79 ppm) for bronchoconstriction from Prieto et al. (2000), divided by the cumulative uncertainty factor of 300, an acute reference exposure level (REL) for acetaldehyde was determined to be 470 µg/m^3 (260 ppb). This level is considered safe for infants and children during an acute exposure period.

Strengths of the Prieto et al. (2000) study include: the study was performed in human subjects, had good experimental design, and a large sample size (n = 61 adult asthmatics) compared to the other aerosolized acetaldehyde provocation studies, and had an endpoint (bronchoconstriction) of interest and concern. Limitations of the Prieto et al. (2000) study include very short exposure periods of 2-4 minutes and the use of aerosolized acetaldehyde solutions in saline. In view of possible but unquantified differences between deposition from an aqueous aerosol and from the gas phase, and resulting differences in dose received by bronchial tissues, there is uncertainty involved in converting the concentration values from mg acetaldehyde/ml solution to an equivalent concentration in air. In provocation studies by other groups, a DeVilbiss nebulizer was used, which has been shown to have considerable variability in aerosol output (Hollie et al., 1991). However, the Prieto study used a Hudson 1720 nebulizer, which is considered to be more consistent.

In a supporting study, Silverman et al. (1946) investigated eye irritation in non-asthmatic adults after acetaldehyde whole body exposure. Upper respiratory tract, nose, throat, and bronchial irritation typically followed that effect closely. Exposure to 50 ppm for 15 minutes caused moderate eye irritation in all subjects, whereas 25 ppm caused complaints of slight eye irritation in an unspecified number of volunteers. Nose and throat irritation and transient conjunctivitis were seen at concentrations of 200 ppm or greater. The Silverman et al. (1946) study had a LOAEL of 25 ppm for slight eye irritation, but a NOAEL was not determined.

In this supporting study, the output (acetaldehyde vapor) is sent generally into an environmental chamber in an effort to mimic real-life exposures and the subject's nose, respiratory tract, eyes, and uncovered skin are concomitantly exposed to the chemical stimulus (Silverman et al., 1946). Generally speaking, the lowest concentration of an irritant that can be discerned by sniffing or by ocular exposure is considered to be the threshold for irritation (Doty et al., 2004). As a general rule, most volatile chemicals that are capable of eliciting irritative sensations (e.g., via the trigeminal nerve) can also elicit an odor (via CN I); furthermore, the odor is often evoked at concentrations one or more orders of magnitude below those that evoke irritation. For most volatile chemicals, ocular irritation is equivalent in sensitivity to nasal irritation in humans with thresholds of equivalent magnitude (Cometto-Muniz and Cain, 1995; 1998; Cometto-Muniz et al., 1998; Cometto-Muniz et al., 1999; 2001; 2002; Doty et al., 2004).

Study	Silverman et al., 1946
Study population	24 adult human volunteers
Exposure method	Whole body
Exposure continuity	
Exposure duration	15 minutes
Critical effects	Eye and upper respiratory tract irritation
LOAEL	45 mg/m^3 (25 ppm)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	not applied (sensory irritation, no Haber's Law adjustment)
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	6 (default: mild effect, no NOAEL)
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	60
Reference Exposure Level	**750 µg/m^3 (420 ppb)**

The trigeminal nerve, which gathers sensory signals from the nasal mucosa amongst several other places, appears to be the only sensory nerve pathway directly involved with the respiratory response to inhaled irritants. In rodents, a reflex decrease in respiratory rate is observed after the initial sensory irritation (Bos et al., 2002); the human response is more complex in its expression although similar in neurological mechanism.

A default uncertainty factor of six is associated with the use of a LOAEL for mild effects in the absence of a NOAEL (see Section 4.4.5 of the TSD). The study was performed in humans, therefore the interspecies uncertainty factor, toxicokinetic (UF_{A-k}) and toxicodynamic (UF_{A-d}) components were each assigned the default value of one. Eye irritancy appears to be more a function of concentration rather than duration of exposure (Yang et al., 2001), so no time correction factor was applied.

For the toxicokinetic component of the intraspecies uncertainty factor (UF_{H-k}) a value of one was used since sensory irritation is not expected to involve large toxicokinetic differences among individuals, and the effects are largely confined to the site of contact, in this case, the eyes, nose, and upper respiratory tract, with negligible or no systemic effects. The deposition kinetics of reactive gases is generally thought not to be greatly different between adults and children. Because of this, a value of one is used for the kinetic component of the intraspecies uncertainty factor (UF_{H-k}), rather than a more

extended values of √10 or ten used where metabolic processes also contribute to inter-individual variability.

A toxicodynamic uncertainty factor (UF_{H-d}) of ten was used to account for the potential greater susceptibility of children. While ocular irritation is not expected to be substantially different between children and adults, the respiratory irritant effect, with documented potential to exacerbate asthma, is clearly an effect with the potential to differentially impact infants and children. The toxicodynamic component of the intraspecies uncertainty factor UF_{H-d} is therefore assigned an increased value of ten to account for potential asthma exacerbation. As mentioned earlier, asthmatics are more sensitive to the irritative properties of inhaled aerosolized acetaldehyde solutions, which significantly decreased their forced expiratory volume in one second (FEV_1) by more than 20% (Prieto et al., 2000; Prieto et al., 2002b). And, alcohol sensitive asthmatics had a selective hyper-responsiveness to acetaldehyde (Myou et al., 1993; Myou et al., 1994; Fujimura et al., 1999). These considerations are applied equally to the acute, 8-hour and chronic REL.

Limitations with the Silverman et al. (1946) study include: small sample size, subjective and non-quantitative measure of irritation, absence of a clear description of exposure method and experimental procedure, which was further unsubstantiated by lack of a clear experimental procedure.

In conclusion, using the LOAEL of 45 mg/m^3 (25 ppm) for eye irritation from Silverman et al. (1946), divided by the cumulative uncertainty factor of 60, an acute reference exposure level (REL) for acetaldehyde was determined to be 750 $\mu g/m^3$ or 420 ppb for the endpoint of eye irritation. Therefore, the acute REL calculated using the key study of Preito et al. (2000) of 470 $\mu g/m^3$ or 260 ppb would also be protective for eye irritation.

8.2 Acetaldehyde 8-Hour Reference Exposure Level

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the Technical Support Document).

Bronchoconstriction, eye irritation and nasal mucosal histopathology are all legitimate concerns for the 8-hour REL and occur in a broadly similar concentration range over the relevant time scale. The repeated nature of an 8-hour REL makes use of the acute studies inappropriate. Therefore, the 8-hour REL was derived using the subchronic animal study (Appelman et al., 1982; 1986) in rats exposed to acetaldehyde six hours per day, five days per week for four weeks. Incidence of degeneration of nasal olfactory epithelium was the most sensitive end-point. These data are supported by Dorman et al. (2008) who reported endpoints of degeneration of the nasal olfactory and respiratory epithelia.

Study	Appelman et al., 1982; 1986
Study population	Wistar rats (10-40 animals/group)
Exposure method	Inhalation exposure to 0, 273, 728, 910, 1820, 4004, 9100 mg/m^3 (0, 150, 400, 500, 1000, 2200, or 5000 ppm)
Exposure continuity	6 hours per day, 5 days/week
Exposure duration	4 weeks
Critical effects	Degeneration of olfactory epithelium
LOAEL	720 mg/m^3 (400 ppm)
NOAEL	270 mg/m^3 (150 ppm)
Benchmark Concentration (BMC_{05}) (using continuous model)	178 mg/m^3 (99 ppm)
Human equivalent concentration	242.1 mg/ m^3 (134.6 ppm)(99 ppm* 1.36 (DAF) Teeguarden et al. (2008)
Time-adjusted exposure	86.5 mg/m^3 (48.1 ppm) = (134.6*6/24*20/10*5/7)
LOAEL uncertainty factor (UF_L)	1
Subchronic uncertainty factor (UFs)	√10 (exposure 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (interspecies PBPK model for acetaldehyde)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (inter-individual variation)
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	300
Reference Exposure Level	**300 µg/m^3 (160 ppb)**

The animal studies by Appelman et al. (1982; 1986) used subchronic exposure of Wistar rats to acetaldehyde for six hours per day, 5 days per week, for four weeks. Incidence of degeneration of nasal olfactory epithelium was the most sensitive end-point. The animal study has a histopathological endpoint for which there is a presumption of Haber's law (C x t) cumulation, at least over moderate timeframes. The time adjustment for an 8-hour REL used is 6 h/24 h x 20 m3/10 m3, rather than 6 h/8 h, because we assume that the 8 hours includes the active waking period when an adult inhales 10 m3 of air, i.e. half the daily total intake of 20 m3.

The 8-hour REL was determined using the Benchmark Dose (BMDS) program developed by the U.S. EPA (2003). The BMC_{05} is defined as the 95% lower confidence limit of the concentration expected to produce a response rate of 5%. The animal data from the Appelman et al. (1982; 1986) studies were used to develop a BMC_{05} for acetaldehyde.

The male and female data were analyzed both together and separately (Table 8.2.1). The study with exposure concentrations of 150 and 500 ppm used only males. Data on incidence of degeneration of olfactory epithelium were converted to a continuous data set ranked by severity of effect (Table 8.2.1). The means and standard deviations at each dose-group are shown, which were calculated from the severity grading of individual animals in each dose group. Each severity category had a name and a corresponding value assigned: no effect = zero, minimal = one, slight = two, moderate = three, marked = 4, moderate with hyperplasia = 5, severe with hyperplasia = 6, and very severe with hyperplasia =7. The means and standard deviations for each dose group were entered into the BMDS program using continuous modeling. The Hill and Polynomial models in the BMDS program gave the best fit to the data (Table 8.2.2). The mean of the three models that best fit the data was calculated to be 99 ± 1.20 ppm and used as the BMC_{05}.

Table 8.2.1. Incidence of Degeneration of Olfactory Epithelium using Weighted Means by Severity[1] .

	Males			**Females**[2]		
Dose (ppm)	Number	Mean	Stdev	Number	Mean	Stdev
0	30	0.07	0.25	10	0	0
150	10	0	0			
400	10	2.6	1.17	10	0.9	0.74
500	10	2.5	0.97			
1000	10	2.8	0.63	10	3.6	0.70
2200	10	5.3	2.21	10	5.1	1.91
5000	10	6.7	0.67	10	6.9	0.32

[1]Severity categories: no effect=0; minimal=1; slight=2; moderate=3; marked=4; moderate w/ hyperplasia=5; severe w/ hyperplasia=6; and very severe w/ hyperplasia=7.
[2] In the 150 and 500 ppm dose groups, only male animals were used.

Table 8.2.2. BMDS Results Modeling Incidence of Degeneration of Nasal Olfactory Epithelium Using Weighted Means by Severity in Rats Using a Continuous Model.

Method	BMC_{05}*	BMC*	P-value	AIC
Hill Model	100	205	0.07	55.96
Polynomial (2°)	101	126	0.02	56.18
Polynomial (3°)	97	165	0.03	55.95

* BMC_{05} and BMC are in units of ppm. Source data from Appelman et al. (1982; 1986)

The standard Human Equivalent Concentration (HEC) adjustment using an RGDR was not used for the dosimetric interspecies extrapolation. Instead, species information based on pharmacokinetic modeling for toxicants that result in specific nasal olfactory tissue damage was applied for interspecies extrapolation of acetaldehyde toxicity (Teeguarden et al., 2008). Dosimetry data for the nasal olfactory epithelium shows that the rat is more efficient in scrubbing organic vapors in this region of the nasal cavity than humans (Frederick et al., 1998; Frederick et al., 2001). Consequently, rats receive a similar, or greater, tissue dose of inhaled organic vapors than humans in the olfactory epithelium. Sensitivity to acetaldehyde of the rat olfactory epithelium is a major factor for olfactory tissue damage, even though the specific activity of aldehyde dehydrogenase is greater in the respiratory epithelium (Bogdanffy et al., 1998; Stanek and Morris, 1999). The interspecies adjustment also takes into account differences in the deposition of inhaled vapors and breathing rates. While rodents are obligate nose breathers, humans are not, which has implications for exposure of nasal tissues. Other factors when extrapolating toxicity findings from rodents to humans include dosimetry, nasal anatomy and airflow dynamics, target tissue metabolism, species differences in gross anatomy, distribution of nasal airway epithelia, and distribution and composition of mucous secretory products (Feron et al., 2001).

The dosimetric adjustment factor (DAF) was derived based on a physiologically based pharmacokinetic (PBPK) model of rat and human nasal tissues constructed for acetaldehyde (see Section 4.4.7.2.2 of the TSD). The rodent model was developed using published metabolic constants and calibrated using upper-respiratory-tract acetaldehyde extraction data (Teeguarden et al., 2008). The human nasal model incorporated previously published tissue volumes, blood flows, and acetaldehyde metabolic constants. The acetaldehyde upper airway PBPK model is structurally the same as the inhalation vinyl acetate model consisting of the nasal cavity, nasopharynx, and larynx (Plowchalk et al., 1997; Bogdanffy et al., 1999; Bogdanffy et al., 2001). The computational fluid dynamic model compartmentalizes the nasal cavity by specific tissue type and location. The rat nasal cavity model has five major compartments, and the human model structure has four. Equations for acetaldehyde concentration, flux, and pH in rats and humans were provided with the model (Teeguarden et al., 2008) . In addition a sensitivity analysis was performed to incorporate humans with ALDH2 polymorphisms into the model. The respiratory and olfactory epithelial tissue acetaldehyde concentrations were determined to be largely linear functions in both species. The impact of the ALDH2 polymorphisms was deemed negligible and not a

significant contributor to acetaldehyde metabolism in the nasal tissues (Teeguarden et al., 2008).

OEHHA determined the DAF using the acetaldehyde concentration metric by calculating the ratio of acetaldehyde concentration values reported for the rat (8.41) and human (6.20), which equaled 1.36. This ratio was then multiplied by the NOAEL to obtain a human equivalent concentration (HEC) (see REL summary table for calculation) (Teeguarden et al., 2008).

Since a PBPK model specifically for acetaldehyde was used, the toxicokinetic component of the interspecies uncertainty factor UF_{A-k} was assigned a value of one. In addition, since acetaldehyde exerts mainly a localized effect on nasal olfactory epithelium, toxicokinetics including distribution and metabolism play less of a key role, the extent of likely interspecies variation is likely less than the default of √10.

The LOAEL uncertainty factor (UF_L) of one was chosen, since both a LOAEL and NOAEL were determined in the key studies (Appelman et al., 1982; Appelman et al., 1986), and the benchmark approach was used to determine the 8-hour REL. In addition, the subchronic uncertainty factor (UFs) was assigned a value of √10 since the 8-hour REL is based on anticipated repeated exposures over a longer period of time than the study duration of four weeks.

The toxicodynamic portion of the interspecies uncertainty factor (UF_{A-d}) is √10 because the key studies are in non-primates and data on toxicodynamic interspecies differences are not available.

An uncertainty factor (UF_{H-k}) of √10 was used to account for intra-individual toxicokinetic variation. The intraspecies uncertainy factor was selected because acetaldehyde is a reactive substance that produces lesions at the point of contact with the tissue, therefore there would be less variability to take into account for children versus adults. However, data are not available for the impact of ALDH2 deficiency on olfactory tissue lesions. One study does indicate that in Japanese alcohol-sensitive asthmatics versus alcohol-insensitive asthmatics, PC_{20} geometric mean values were 330 ppm versus 500 ppm, respectively, but their ALDH2 status was unknown (Fujimura et al., 1999).

The toxicodynamic uncertainty factor (UF_{H-d}) of 10 was used to account for the potentially greater susceptibility of children and asthmatics. The resulting cumulative uncertainty factor was calculated as 300 and used to determine the 8-hour REL of the experimental animal study. The 8-hour REL with the endpoint of degeneration of olfactory epithelium in rats was calculated to be 300 $\mu g/m^3$ (160 ppb).

Dorman et al. (2008) conducted a 13-week study in male F344 rats (n=12 per group) with acetaldehyde exposures of 0, 50, 150, 500, or 1500 ppm. They reported degeneration of olfactory and respiratory epithelium (Dorman et al., 2008). The LOAEL and NOAEL for the endpoint of degeneration of olfactory nasal epithelium were 150 and 50 ppm, respectively for the observations at 65 days (Table 6.3.2). Benchmark concentration analysis was performed on the data and several models provided a

BMC_{05} in close agreement with the NOAEL (quantal linear BMC_{05} = 45.3 ppm and probit BMC_{05} = 48.3 ppm), but statistically were not as reliable due to the small sample size and dose spacing. Adjusting the NOAEL using the dosimetric adjustment factor (DAF) of 1.36, as described previously, based on the PBPK model for acetaldehyde (Teeguarden et al., 2008), yielded a NOAEL of 68 ppm. Thus the BMC_{05} value from the Dorman study and also the LOAEL and NOAEL values from the same study are supportive of the 8-hour REL determined from the data of Appelman et al. (1982; 1986).

For the incidence of another endpoint reported by Dorman et al. (2008), respiratory epithelial hyperplasia, benchmark concentration modeling was performed on the 65-day exposure data (Table 6.3.3). The Probit model yielded the best result with a BMC_{05} of 100 ppm, which is in good agreement with the BMC_{05} of 99 ppm from the Appelman study and is therefore also supportive of the derived 8-hour REL.

The Dorman et al (2008) study was not used for determination of the 8-hour REL due to its small sample size and the response rate rising from 0% to 100% in the olfactory epithelium data. This creates uncertainty in determination of a "true NOAEL" and an inability to use benchmark dose modeling in determination of the REL due to lack of an adequate fit of the model to the data. Another limitation of the Dorman study was the length of the study was 12.5% of the test animal's lifetime, which borders the criteria for subchronic and chronic (12% of lifetime). With the Appelman studies, not only could the benchmark dose be determined for incidence, but also the provision of severity grading data for each individual animal allowed for continuous BMDS analysis, which provided a better dose-response and low-end extrapolation of the data.

Eye irritation and nasal mucosal histopathology are both legitimate concerns for the 8-hour REL for acetaldehyde and occur in a broadly similar concentration range over the relevant time scale. However, repeated 8-hour exposures could result in tissue damage. Therefore, the REL (300 $\mu g/m^3$ (160 ppb)) using the animal study with a histopathological endpoint was used. The experimental animal study used as the basis for the 8-hour REL, with an endpoint of degeneration of nasal olfactory epithelium, would also be protective of the human sensory response since the acute REL derived from the Silverman et al. (1946) human study is higher. The animal study was chosen because it was a well-conducted study with adequate dose groups and a time-period relevant for the 8-hour REL. In addition, using benchmark dose and PBPK modeling decreased the uncertainty associated with the REL derivation compared with using the traditional NOAEL/LOAEL and HEC (with an RGDR) procedures.

8.3 Acetaldehyde Chronic Reference Exposure Level

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

Study	Appelman et al., 1982; 1986
Study population	Wistar rats (10-40 animals/group)
Exposure method	Inhalation exposure to 0, 273, 728, 910, 1820, 4004, 9100 mg/m3 (0, 150, 400, 500, 1000, 2200, or 5000 ppm)
Exposure continuity	6 hours per day, 5 days/week
Exposure duration	*4 weeks*
Critical effects	Degeneration of olfactory epithelium
LOAEL	720 mg/m^3 (400 ppm)
NOAEL	270 mg/m^3 (150 ppm)
Benchmark Concentration (BMC_{05}) (using continuous model)	178 mg/m^3 (99 ppm)
Human equivalent concentration	242.1 mg/ m^3 (134.6 ppm)(= 99 * 1.36 (DAF) Teeguarden et al. (2008)
Time-adjusted exposure	43.2 mg/m^3 (24 ppm) = (134.6*6/24*5/7)
LOAEL uncertainty factor (UF_L)	1
Subchronic uncertainty factor (UFs)	√10 (exposure 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (intraspecies PBPK model for acetaldehyde)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (inter-individual variation)
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	300
Reference Exposure Level	**140 µg/m^3 (80 ppb)**

The chronic REL was based on four-week exposure data in rats from Appelman et al., (1982, 1986), and supported by Saldiva et al., (1985); Woutersen et al., (1986, 1984); and (Woutersen and Feron, 1987), which included a 28-month chronic study in rats. Incidence of degeneration of nasal olfactory epithelium was the most sensitive end-point. The proposed chronic REL was estimated by a benchmark concentration modeling approach using the continuous polynomial and Hill models of analysis (U.S. EPA, 2003) as previously described in detail in Section 8.2. The average experimental exposure data were adjusted to reflect chronic exposure. Table 8.2.1 shows the data expressed as the mean and standard deviation of the degeneration of nasal olfactory epithelium by severity for each dose group, which were the data used for the BMDS model. As shown in Table 8.2.2, three models were selected that best fit the data and their mean and standard deviation was 99 ± 1.20 ppm and therefore used as the BMC_{05}.

As described in detail in Section 8.2, OEHHA used a dosimetric adjustment factor (DAF) for acetaldehyde of 1.36 based on the PBPK model for acetaldehyde developed by Teeguarden et al. (2008). The limited uncertainty associated with this assumption is reflected in the use of the toxicokinetic component of the interspecies uncertainty factor $UF_{A\text{-}k}$ equaling one since the model was specific for acetaldehyde.

The animal studies by Appelman et al. (1982; 1986) used subchronic exposure of Wistar rats to acetaldehyde for six hours per day, 5 days per week, for four weeks. Incidence of degeneration of nasal olfactory epithelium was the most sensitive endpoint.

The LOAEL uncertainty factor (UF_L) of one was chosen, since both a LOAEL and NOAEL were determined in the key studies (Appelman et al., 1982; Appelman et al., 1986), and the benchmark approach was used to determine the chronic REL.

The subchronic uncertainty factor (UFs) was assigned a value of $\sqrt{10}$ since the chronic REL is representative of exposures over a lifetime, and because the supporting chronic study (Woutersen et al., 1986) didn't give a dramatic increase in injury compared to the four-week studies by Appelman et al., (1982; 1986). In addition, Saldiva et al., (Saldiva et al., 1985) observed "intense" nasal lesions in rats exposed to 442 mg/m^3 (243 ppm) for slightly longer exposure durations than that used by Appelman et al., (1982; 1986).

The value of one was chosen for the toxicokinetic component of the interspecies uncertainty factor ($UF_{A\text{-}k}$) since a DAF from a PBPK model for acetaldehyde was used, which adequately incorporates the differences between humans and rodents (Teeguarden et al., 2008). The toxicodynamic portion of the interspecies uncertainty factor ($UF_{A\text{-}d}$) is $\sqrt{10}$ because the key studies are in non-primates and data on toxicodynamic interspecies differences are not available.

Intraspecies variability can be as much as a factor of 1,000-fold for VOCs measured in human subjects (Fenske and Paulson, 1999). An uncertainty factor ($UF_{H\text{-}k}$) of $\sqrt{10}$ was used to account for intra-individual toxicokinetic variation. The intraspecies uncertainty factor was selected because acetaldehyde is a reactive substance that produces lesions at the point of contact with the tissue, therefore there would be less kinetic variability to take into account for children versus adults. The toxicodynamic uncertainty factor (UF_{Hd}) of 10 was used to account for the potentially greater susceptibility of children and asthmatics.

The resulting cumulative uncertainty factor was calculated to be 300 and used to determine the chronic REL of the experimental animal study. The chronic REL with the endpoint of degeneration of olfactory epithelium in rats was calculated to be 140 µg/m^3 (80 ppb).

The current chronic RfC for acetaldehyde determined by the U.S. EPA and based on Appelman et al. (1982; 1986) is 9 µg/m^3 (5 ppb) and is within the range of normal human breath acetaldehyde concentrations of 0.7 to 11.0 µg/m^3 (0.4 to 6.1 ppb). OEHHA's proposed chronic REL of 140 µg/m^3 (76 ppb) is above the range of human breath concentrations of acetaldehyde, but is mostly exceeded when humans consume

significant amounts of alcohol, resulting in human breath concentrations ranging from 200 to 2200 μg/m^3. Thus, frequent alcohol use and abuse by humans is a major source of acetaldehyde exposure to the airway tissue that can exceed the chronic REL.

The LOAEL of 750 ppm from the chronic exposure data by Woutersen et al., (1984, 1986) and Woutersen and Feron (1987) produced similar injuries and was confined to the nasal olfactory epithelium as the LOAEL of 400 ppm from the 4-week Appelman studies. Thus, the subchronic UF was kept at √10, to account for similar findings from the chronic studies.

Analyses were also performed on the incidence of respiratory epithelial changes using the LOAEL from the chronic rat studies, although it was a less sensitive end-point (Woutersen et al., 1984, 1986; Woutersen and Feron 1987). The 100% response rate at the LOAEL combined with the lack of a NOAEL prevented the chronic studies from becoming the basis of the REL.

Significant strengths for the chronic REL include: (1) the use of a well conducted repeated exposure study with histopathological analysis and (2) independent studies demonstrating comparable key effects (nasal lesions) in experimental animals. However, major areas of uncertainty are the lack of adequate human chronic inhalation dose-response data in adults and children, and inadequate long-term inhalation animal data, therefore a subchronic animal study was used.

8.4 Acetaldehyde as a Toxic Air Contaminant

Acetaldehyde was identified by the ARB as a toxic air contaminant (TAC) in accordance with section 39657(b) of the California Health and Safety Code (Title 17, California Code of Regulations, section 93001) (CCR, 2007). In view of the potential of acetaldehyde to exacerbate asthma (Section 5.1, 5.2), and the differential impacts of asthma on children including higher prevalence rates, coupled with widespread exposure (e.g., indoors from exposure to environmental tobacco smoke, and outdoors due to numerous emissions sources), OEHHA recommends that acetaldehyde be identified as a toxic air contaminant (TAC) that may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

9. References

Appelman LM, Woutersen RA and Feron VJ (1982). Inhalation toxicity of acetaldehyde in rats. I. Acute and subacute studies. Toxicology 23(4): 293-307.

Appelman LM, Woutersen RA, Feron VJ, Hooftman RN and Notten WR (1986). Effect of variable versus fixed exposure levels on the toxicity of acetaldehyde in rats. J Appl Toxicol 6(5): 331-336.

Aranyi C, O'Shea WJ, Graham JA and Miller FJ (1986). The effects of inhalation of organic chemical air contaminants on murine lung host defenses. Fundam Appl Toxicol 6(4): 713-720.

Babiuk C, Steinhagen WH and Barrow CS (1985). Sensory irritation response to inhaled aldehydes after formaldehyde pretreatment. Toxicol Appl Pharm 79(1): 143-149.

Baselt RC and Cravey RH (1989). Disposition of Toxic Drugs and Chemicals in Man. 3rd. Edition. Chicago (IL): Year Book Medical Publishers Inc.

Beall JR and Ulsamer AG (1981). Toxicity of volatile organic compounds present indoors. Bull N Y Acad Med 57(10): 978-996.

Blakley PM and Scott Jr. WJ (1984a). Determination of the proximate teratogen of the mouse fetal alcohol syndrome. 1. Teratogenicity of ethanol and acetaldehyde. Toxicol Appl Pharm 72(2): 355-363.

Blakley PM and Scott Jr. WJ (1984b). Determination of the proximate teratogen of the mouse fetal alcohol syndrome. 2. Pharmacokinetics of the placental transfer of ethanol and acetaldehyde. Toxicol Appl Pharm 72(2): 364-371.

Bogdanffy MS, Plowchalk DR, Sarangapani R, Starr TB and Andersen ME (2001). Mode-of-action-based dosimeters for interspecies extrapolation of vinyl acetate inhalation risk. Inhal Toxicol 13(5): 377-96.

Bogdanffy MS, Sarangapani R, Kimbell JS, Frame SR and Plowchalk DR (1998). Analysis of vinyl acetate metabolism in rat and human nasal tissues by an in vitro gas uptake technique. Toxicol Sci 46(2): 235-246.

Bogdanffy MS, Sarangapani R, Plowchalk DR, Jarabek A and Andersen ME (1999). A biologically based risk assessment for vinyl acetate-induced cancer and noncancer inhalation toxicity. Toxicol Sci 51(1): 19-35.

Bos PMJ, Busschers M and Arts JHE (2002). Evaluation of the sensory irritation test (Alarie test) for the assessment of respiratory tract irritation. J Occup Environ Med 44(10): 968-976.

Brown VM, Crump DR, Gardiner D and Gavin M (1994). Assessment of a passive sampler for the determination of aldehydes and ketones in indoor air. Environ Technol 15: 679-685.

Burt J, Nelson NA and Kaufman JD (1996). Effects of ventilation flushout on indoor air quality in a newly constructed office building. Appl Occup Environ Hyg 11(6): 546-552.

CARB. (1993). *Acetaldehyde as a Toxic Air Contaminant. Part B, Health Assessment*. Sacramento (CA): California Air Resources Board, California Environmental Protection Agency.

Casanova-Schmitz M, David RM and Heck HD (1984). Oxidation of formaldehyde and acetaldehyde by NAD^+-dependent dehydrogenases in rat nasal mucosal homogenates. Biochem Pharmacol 33(7): 1137-1142.

Cassee FR, Arts JH, Groten JP and Feron VJ (1996a). Sensory irritation to mixtures of formaldehyde, acrolein, and acetaldehyde in rats. Arch Toxicol 70(6): 329-337.

Cassee FR, Groten JP and Feron VJ (1996b). Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde, and acrolein. Fundam Appl Toxicol 29(2): 208-218.

CCR (2007). California Code of Regulations Section 93001 Hazardous Air Pollutants Identified as Toxic Air Contaminants. Sacramento, CA: California Office of Administrative Law. 8-20-07. http://ccr.oal.ca.gov/linkedslice/default.asp?SP=CCR-1000&Action=Welcome.

Cicero TJ and Bell RD (1980). Effects of ethanol and acetaldehyde on the biosynthesis of testosterone in the rodent testes. Biochem Biophys Res Commun 94(3): 814-819.

Cicero TJ, Bell RD and Badger TM (1980a). Acetaldehyde directly inhibits the conversion of androstenedione to testosterone in the testes. Adv Exp Med Biol 132: 211-217.

Cicero TJ, Bell RD, Meyer ER and Badger TM (1980b). Ethanol and acetaldehyde directly inhibit testicular steroidogenesis. J Pharmacol Exp Ther 213(2): 228-233.

Cometto-Muniz JE and Cain WS (1995). Relative sensitivity of the ocular trigeminal, nasal trigeminal and olfactory systems to airborne chemicals. Chem Senses 20(2): 191-198.

Cometto-Muniz JE and Cain WS (1998). Trigeminal and olfactory sensitivity: Comparison of modalities and methods of measurement. Int Arch Occup Environ Health 71(2): 105-110.

Cometto-Muniz JE, Cain WS and Abraham MH (1998). Nasal pungency and odor of homologous aldehydes and carboxylic acids. Exp Brain Res 118(2): 180-188.

Cometto-Muniz JE, Cain WS, Abraham MH and Gola JM (1999). Chemosensory detectability of 1-butanol and 2-heptanone singly and in binary mixtures. Physiol Behav 67(2): 269-276.

Cometto-Muniz JE, Cain WS, Abraham MH and Gola JM (2001). Ocular and nasal trigeminal detection of butyl acetate and toluene presented singly and in mixtures. Toxicol Sci 63(2): 233-244.

Cometto-Muniz JE, Cain WS, Abraham MH and Gola JM (2002). Psychometric functions for the olfactory and trigeminal detectability of butyl acetate and toluene. J Appl Toxicol 22(1): 25-30.

Delfino RJ, Gong H, Jr., Linn WS, Pellizzari ED and Hu Y (2003). Asthma symptoms in hispanic children and daily ambient exposures to toxic and criteria air pollutants. Environ Health Perspect 111(4): 647-656.

Dellarco VL (1988). A mutagenicity assessment of acetaldehyde. Mutat Res 195(1): 1-20.

Donohue Jr. TM, Tuma DJ and Sorrell MF (1983). Acetaldehyde adducts with proteins: Binding of [^{14}C]acetaldehyde to serum albumin. Arch Biochem Biophys 220(1): 239-246.

Dorman DC, Struve MF, Wong BA, Gross EA, Parkinson C, Willson GA, Tan YM, Campbell JL, Teeguarden JG, Clewell HJ, 3rd and Andersen ME (2008). Derivation of an inhalation reference concentration based upon olfactory neuronal loss in male rats following subchronic acetaldehyde inhalation. Inhal Toxicol 20(3): 245-56.

Doty RL, Cometto-Muniz JE, Jalowayski AA, Dalton P, Kendal-Reed M and Hodgson M (2004). Assessment of upper respiratory tract and ocular irritative effects of volatile chemicals in humans. Crit Rev Toxicol 34(2): 85-142.

Egle Jr JL (1970). Retention of inhaled acetaldehyde in man. J Pharmacol Exp Ther 174(1): 14-9.

Egle Jr. JL (1972a). Effects of inhaled acetaldehyde and propionaldehyde on blood pressure and heart rate. Toxicol Appl Pharm 23(1): 131-135.

Egle Jr. JL (1972b). Retention of inhaled acetaldehyde in the dog. Arch Environ Health 24(5): 354-357.

Eriksson CJ (2001). The role of acetaldehyde in the actions of alcohol (update 2000). Alcohol Clin Exp Res 25(5 Suppl ISBRA): 15S-32S.

Eriksson CJ, Sippel HW and Forsander OA (1977). The occurrence of acetaldehyde binding in rat blood but not in human blood. FEBS Lett 75(1): 205-208.

Fenske JD and Paulson SE (1999). Human breath emissions of VOCs. J Air Waste Manag Assoc 49(5): 594-598.

Feron VJ, Arts JH, Kuper CF, Slootweg PJ and Woutersen RA (2001). Health risks associated with inhaled nasal toxicants. Crit Rev Toxicol 31(3): 313-347.

Feron VJ, Kruysse A and Woutersen RA (1982). Respiratory tract tumours in hamsters exposed to acetaldehyde vapour alone or simultaneously to benzo(a)pyrene or diethylnitrosamine. Eur J Cancer Clin Oncol 18(1): 13-31.

Frederick CB, Bush ML, Lomax LG, Black KA, Finch L, Kimbell JS, Morgan KT, Subramaniam RP, Morris JB and Ultman JS (1998). Application of a hybrid computational fluid dynamics and physiologically based inhalation model for interspecies dosimetry extrapolation of acidic vapors in the upper airways. Toxicol Appl Pharm 152(1): 211-231.

Frederick CB, Gentry PR, Bush ML, Lomax LG, Black KA, Finch L, Kimbell JS, Morgan KT, Subramaniam RP, Morris JB and Ultman JS (2001). A hybrid computational fluid dynamics and physiologically based pharmacokinetic model for comparison of predicted tissue concentrations of acrylic acid and other vapors in the rat and human nasal cavities following inhalation exposure. Inhal Toxicol 13(5): 359-376.

Fujimura M, Myou S, Kamio Y, Ishiura Y, Iwasa K, Hashimoto T and Matsuda T (1999). Increased airway responsiveness to acetaldehyde in asthmatic subjects with alcohol-induced bronchoconstriction. Eur Respir J 14(1): 19-22.

Gaines KC, Salhany JM, Tuma DJ and Sorrell MF (1977). Reaction of acetaldehyde with human erythrocyte membrane proteins. FEBS Lett 75(1): 115-119.

Hodgson AT, Beal D and McIlvaine JE (2002). Sources of formaldehyde, other aldehydes and terpenes in a new manufactured house. Indoor Air 12(4): 235-242.

Hodgson AT, Shendell DG, Fisk WJ and Apte MG (2004). Comparison of predicted and derived measures of volatile organic compounds inside four new relocatable classrooms. Indoor air 14 Suppl 8: 135-44.

Hoffmann T, Meyer RJ, Sorrell MF and Tuma DJ (1993). Reaction of acetaldehyde with proteins: Formation of stable fluorescent adducts. Alcohol Clin Exp Res 17(1): 69-74.

Hollie MC, Malone RA, Skufca RM and Nelson HS (1991). Extreme variability in aerosol output of the DeVilbiss 646 jet nebulizer. Chest 100(5): 1339-44.

HSDB (2004). Hazardous Substances Data Bank. Bethesda (MD): National Library of Medicine. htttp://toxnet.nlm.nih.gov.

Isse T, Oyama T, Matsuno K, Ogawa M, Narai-Suzuki R, Yamaguchi T, Murakami T, Kinaga T, Uchiyama I and Kawamoto T (2005). Paired acute inhalation test reveals that acetaldehyde toxicity is higher in aldehyde dehydrogenase 2 knockout mice than in wild-type mice. J Toxicol Sci 30(4): 329-337.

IWMB. (2003). *Building Material Emissions Study*. Integrated Waste Management Board, California Environmental Protection Agency. http://www.ciwmb.ca.gov/Publications.

Jones AW (1995). Measuring and reporting the concentration of acetaldehyde in human breath. Alcohol Alcohol 30(3): 271-285.

Kinney PL, Chillrud SN, Ramstrom S, Ross J and Spengler JD (2002). Exposures to multiple air toxics in New York City. Environ Health Perspect 110 (Suppl 4): 539-546.

Kruysse A, Feron VJ and Til HP (1975). Repeated exposure to acetaldehyde vapor. Studies in Syrian golden hamsters. Arch Environ Health 30(9): 449-452.

Lacroix G, Tissot S, Rogerieux F, Beaulieu R, Cornu L, Gillet C, Robidel F, Lefevre JP and Bois FY (2002). Decrease in ovalbumin-induced pulmonary allergic response by benzaldehyde but not acetaldehyde exposure in a guinea pig model. J Toxicol Env Heal A 65(14): 995-1012.

Lee RD, An SM, Kim SS, Rhee GS, Kwack SJ, Seok JH, Chae SY, Park CH, Choi YW, Kim HS, Cho HY, Lee BM and Park KL (2005). Neurotoxic effects of alcohol and acetaldehyde during embryonic development. J Toxicol Env Heal A 68(23-24): 2147-2162.

Lindstrom AB, Proffitt D and Fortune CR (1995). Effects of modified residential construction on indoor air quality. Indoor Air 5: 258-269.

Mannino DM, Homa DM, Pertowski CA, Ashizawa A, Nixon LL, Johnson CA, Ball LB, Jack E and Kang DS (1998). Surveillance for asthma--United States, 1960-1995. MMWR CDC Surveill Summ 47(1): 1-27.

Miller SL, Branoff S and Nazaroff WW (1998). Exposure to toxic air contaminants in environmental tobacco smoke: an assessment for California based on personal monitoring data. J Expo Anal Env Epid 8(3): 287-311.

Mohammad A, Olcott HS and Fraenkel-Conrat H (1949). The reaction of proteins with acetaldehyde. Arch Biochem 24(2): 270-280.

Morris JB (1997a). Dosimetry, toxicity and carcinogenicity of inspired acetaldehyde in the rat. Mutat Res 380(1-2): 113-124.

Morris JB (1997b). Uptake of acetaldehyde vapor and aldehyde dehydrogenase levels in the upper respiratory tracts of the mouse, rat, hamster and guinea pig. Fundam Appl Toxicol 35(1): 91-100.

Morris JB and Blanchard KT (1992). Upper respiratory tract deposition of inspired acetaldehyde. Toxicol Appl Pharm 114(1): 140-146.

Myou S, Fujimura M, Nishi K, Matsuda M, Ohka T and Matsuda T (1994). Potentiating effect of inhaled acetaldehyde on bronchial responsiveness to methacholine in asthmatic subjects. Thorax 49(7): 644-648.

Myou S, Fujimura M, Nishi K, Ohka T and Matsuda T (1993). Aerosolized acetaldehyde induces histamine-mediated bronchoconstriction in asthmatics. Am Rev Respir Dis 148(4 Pt 1): 940-943.

Myou S, Fujimura M, Kamio Y, Bando T, Nakatsumi Y and Matsuda T (1995). Repeated inhalation challenge with exogenous and endogenous histamine released by acetaldehyde inhalation in asthmatic patients. Am J Respir Crit Care Med 152(2): 456-460.

NAS. (2004). *Acute Exposure Guideline Levels (AEGLs) for Acetaldehyde* CAS Reg. No. 75-07-0. Washington D.C.: National Advisory Committee/AEGL, U.S. Environmental Protection Agency.

Nazaroff WW (2004). Inhalation of hazardous air pollutants from environmental tobacco smoke in US residences. J Expo Anal Env Epid 14: S71-S77.

OEHHA. (2001). *Prioritization of Toxic Air Contaminants - Children's Health Protection Act - Final Report*. Sacramento (CA): Air Toxics and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/air/toxic_contaminants/SB25finalreport.html.

OEHHA. (2006). *Safe Drinking Water and Toxic Enforcement Act of 1986. Chemicals Known to the State to Cause Cancer or Reproductive Toxicity. August 11, 2006*. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/prop65/prop65_list/files/P65single081106.pdf.

Plowchalk DR, Andersen ME and Bogdanffy MS (1997). Physiologically based modeling of vinyl acetate uptake, metabolism, and intracellular pH changes in the rat nasal cavity. Toxicol Appl Pharmacol 142(2): 386-400.

Pratt OE (1980). The fetal alcohol syndrome: Transport of nutrients and transfer of alcohol and acetaldehyde from mother to fetus. Psychopharmacol Alcohol: 229-256.

Prieto L. (2008) Personal communication with author regarding 95% confidence interval on data from Prieto et al., 2000. Communication via email received August 21, 2008 and November 20, 2008.

Prieto L, Gutierrez V, Cervera A and Linana J (2002a). Airway obstruction induced by inhaled acetaldehyde in asthma: Repeatability and relationship to adenosine 5'-monophosphate responsiveness. J Invest Allerg Clin Immuno 12(2): 91-98.

Prieto L, Sanchez-Toril F, Brotons B, Soriano S, Casan R and Belenguer JL (2000). Airway responsiveness to acetaldehyde in patients with asthma: Relationship to methacholine responsiveness and peak expiratory flow variation. Clin Exp Allergy 30(1): 71-78.

Prieto L, Sanchez-Toril F, Gutierrez V and Marin MJ (2002b). Airway responsiveness to inhaled acetaldehyde in subjects with allergic rhinitis: Relationship to methacholine responsiveness. Respiration 69(2): 129-135.

Saito Y, Sasaki F, Tanaka I, Sato M, Okazawa M, Sakakibara H and Suetsugu S (2001). Acute severe alcohol-induced bronchial asthma. Intern Med 40(7): 643-645.

Saldiva PH, do Rio Caldeira MP, Massad E, Calheiros DF, Cardoso LM, Bohm GM and Saldiva CD (1985). Effects of formaldehyde and acetaldehyde inhalation on rat pulmonary mechanics. J Appl Toxicol 5(5): 288-292.

Shaskan EG and Dolinsky ZS (1985). Elevated endogenous breath acetaldehyde levels among abusers of alcohol and cigarettes. Prog Neuro-Psychoph 9(3): 267-272.

Shiohara E, Tsukada M, Chiba S, Yamazaki H, Nishiguchi K, Miyamoto R and Nakanishi S (1984). Subcellular aldehyde dehydrogenase activity and acetaldehyde oxidation by isolated intact mitochondria of rat brain and liver after acetaldehyde treatment. Toxicology 30(1): 25-30.

Silverman L, Schultes HF and First MW (1946). Further studies on sensory response to certain industrial solvent vapors. J Ind Hyg Toxicol 28: 262-266.

Skog E (1950). A toxicological investigation of lower aliphatic aldehydes. I. Toxicity of formaldehyde, acetaldehyde, propionaldehyde, butyraldehyde, as well as acrolein and crotonaldehyde. Acta Pharmacol Toxicol 6: 299-318.

Sreenathan RN, Padmanabhan R and Singh S (1982). Teratogenic effects of acetaldehyde in the rat. Drug Alcohol Depend 9(4): 339-350.

Stanek JJ and Morris JB (1999). The effect of inhibition of aldehyde dehydrogenase on nasal uptake of inspired acetaldehyde. Toxicol Sci 49(2): 225-231.

Steinhagen WH and Barrow CS (1984). Sensory irritation structure-activity study of inhaled aldehydes in B6C3F1 and Swiss-Webster mice. Toxicol Appl Pharm 72(3): 495-503.

Tardif R, Liu L and Raizenne M (2004). Exhaled ethanol and acetaldehyde in human subjects exposed to low levels of ethanol. Inhal Toxicol 16(4): 203-207.

Teeguarden JG, Bogdanffy MS, Covington TR, Tan C and Jarabek AM (2008). A PBPK model for evaluating the impact of aldehyde dehydrogenase polymorphisms on comparative rat and human nasal tissue acetaldehyde dosimetry. Inhal Toxicol 20(4): 375-90.

Thompson PA and Folb PI (1982). An in vitro model of alcohol and acetaldehyde teratogenicity. J Appl Toxicol 2(4): 190-195.

Tuma DJ and Sorrell MF (1985). Covalent binding of acetaldehyde to hepatic proteins: Role in alcoholic liver injury. Prog Clin Biol Res 183: 3-17.

Tyulina OV, Prokopieva VD, Boldyrev AA and Johnson P (2006). Erythrocyte and plasma protein modification in alcoholism: A possible role of acetaldehyde. Biochim Biophys Acta-Molecular Basis of Disease 1762(5): 558-563.

U.S. EPA. (2003). Benchmark Dose Software. National Center for Environmental Assessment, United States Environmental Protection Agency.

Watanabe A, Hobara N and Nagashima H (1986). Blood and liver acetaldehyde concentration in rats following acetaldehyde inhalation and intravenous and intragastric ethanol administration. Bull Environ Contam Toxicol 37(4): 513-516.

West JR (1994). Recent findings on the mechanisms by which alcohol damages the developing nervous system. Alcohol Clin Exp Res 18(2): 9A.

Wickramasinghe SN, Thomas S and Hasan R (1994). Reaction of ^{14}C-acetaldehyde with whole blood in vitro: Further evidence for the formation of unstable complexes with plasma proteins and red cells. Alcohol Alcohol 29(1): 51-57.

Woutersen RA, Appelman LM, Feron VJ and Van der Heijden CA (1984). Inhalation toxicity of acetaldehyde in rats. II. Carcinogenicity study: Interim results after 15 months. Toxicology 31(2): 123-133.

Woutersen RA, Appelman LM, Van Garderen-Hoetmer A and Feron VJ (1986). Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. Toxicology 41(2): 213-231.

Woutersen RA and Feron VJ (1987). Inhalation toxicity of acetaldehyde in rats. IV. Progression and regression of nasal lesions after discontinuation of exposure. Toxicology 47(3): 295-305.

Yang X, Zhang YP, Chen D, Chen WG and Wang R (2001). Eye irritation caused by formaldehyde as an indoor air pollution--a controlled human exposure experiment. Biomed Environ Sci 14(3): 229-36.

Zorzano A and Herrera E (1989a). Decreased in vivo rate of ethanol metabolism in the suckling rat. Alcohol Clin Exp Res 13(4): 527-532.

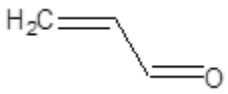
Zorzano A and Herrera E (1989b). Disposition of ethanol and acetaldehyde in late pregnant rats and their fetuses. Pediatr Res 25(1): 102-106.

Zweidinger R, Hoffman A and Gage L (1990). Comparison of Indoor and Outdoor Aldehyde Concentrations During the IACP Roanoke, Virginia USA Residential Study. Measurement of Toxic and Related Air Pollutants International Symposium.Atmospheric Research and Exposure Assessment Laboratory and Air and Waste Mangement Association, United States Environmental Protection Agency. pp 994-999. Raleigh (NC), May 1-4, 1990

Acrolein Reference Exposure Levels

(2-propenal, acrylic aldehyde, acryladehyde, acraldehyde)

CAS 107-02-8



1. Summary

Acrolein is a powerful irritant. Due to its highly reactive nature, the effects of acrolein are generally limited to the site of contact; skin, eyes and mucous membranes. Inhalation exposure to low levels (≤ 1 ppm) causes irritation of the eyes, nose and throat. Acute exposures to levels above 1 ppm result in mucous hypersecretion and exacerbation of allergic airway response in animal models. Moderately higher exposures may result in severe lacrimation, and irritation of the mucous membranes of the respiratory tract. Death due to respiratory failure has been associated with high level exposures. Long term exposure to acrolein may result in structural and functional changes in the respiratory tract, including lesions in the nasal mucosa, and pulmonary inflammation. The studies reviewed for this document include those published through Spring, 2008.

1.1 Acrolein Acute REL

Reference Exposure Level	**2.5 µg/m³ (1.1 ppb)**
Critical effect(s)	Subjective ocular irritation
Hazard Index target(s)	Eyes

1.2 Acrolein 8-Hour REL

Reference Exposure Level	**0.70 µg/m³ (0.30 ppb)**
Critical effect(s)	Lesions in respiratory epithelium
Hazard Index target(s)	Respiratory

1.3 Acrolein Chronic REL

Reference Exposure Level	**0.35 µg/m³ (0.15 ppb)**
Critical effect(s)	Lesions in respiratory epithelium
Hazard Index target(s)	Respiratory

2. Physical & Chemical Properties

Description	Colorless or yellow liquid with piercing disagreeable odor
Molecular formula	C_3H_4O
Molecular weight	56.1 g/mol
Density	0.843 g/cm^3 @ 20° C
Boiling point	53° C
Melting point	-87° C
Vapor pressure	220 mm Hg @ 20° C
Flashpoint	-26° C
Explosive limits	2.8% - 31% by volume
Solubility	soluble in ethanol, diethyl ether, and up to 20% w/v in water
Odor threshold	0.5 ppm
Metabolites	glycidaldehyde, acrylic acid
Conversion factor	1 ppm in air = 2.3 mg/m^3 @ 25° C

3. Occurrence and Major Uses

Acrolein is principally used as a chemical intermediate in the production of acrylic acid and its esters. Acrolein is used directly as an aquatic herbicide and algicide in irrigation canals, as a microbiocide in oil wells, liquid hydrocarbon fuels, cooling-water towers and water-treatment ponds, and as a slimicide in the manufacture of paper (IARC, 1995). Combustion of fossil fuels, tobacco smoke, and pyrolyzed animal and vegetable fats contribute to the environmental prevalence of acrolein. Acrolein is a byproduct of fires and is one of several acute toxicants to which firefighters are exposed. It is also formed from atmospheric reactions of 1,3-butadiene. The annual statewide emissions of acrolein from mobile, stationary and natural sources (not including atmospheric transformation) reported in the California Toxics Inventory for 2004 were estimated to be 2,242 tons contributing to a statewide ambient level of 0.53 ppb (CARB, 2005b).

4. Metabolism

The metabolism of acrolein comprises several pathways. It rapidly reacts with sulfhydryl groups, especially protein cysteine residues and glutathione. The glutathione conjugate may be oxidized or reduced to mercapturic acids (N-acetyl –S-2-carboxyethylcysteine and N-acetyl-S-3-hydroxypropylcysteine, respectively), with the reduction pathway predominating, followed by urinary elimination. Alternatively, acrolein may be epoxidized to glycidaldehyde, which is in turn attacked by glutathione and oxidatively processed to the mercapturic acid, N-acetyl-S-2-carboxy-2-hydroxyethylcysteine. In a third pathway, the Michael addition of water to acrolein is followed by oxidation to malonic and finally oxalic acids (Parent et al., 1998). The formation of homopolymers of acrolein is thought to occur but appears to be limited to the gut. Acrolein may also be oxidized to acrylic acid, mainly in the liver. Following

inhalation exposure, the predominant metabolites are the 3-hydroxypropyl and 2-carboxyethyl mercapturic acids mentioned above (Linhart et al., 1996).

5. Acute Toxicity of Acrolein

5.1 Acute Toxicity to Adult Humans

Sensory irritation is the primary adverse effect associated with acute, low level exposures to acrolein. The irritative effects of acrolein are noticeable at low levels of exposure (≤0.25 ppm) and rapidly become more pronounced with increasing concentration; brief exposure (1.5 min) to 0.3 ppm (0.7 mg/m³) causes irritation of the eyes and nose (Weber-Tschopp et al., 1977). The powerful irritant and lacrimator properties of acrolein led to its use in gas grenades and artillery shells by the French in 1916. At a concentration of 7 mg/m³, acrolein caused severe lacrimation and irritation of the mucous membranes of the respiratory tract (Prentiss, 1937). A case report of respiratory failure and death in individuals exposed to vapors from overheated frying pans containing fat and food items implicated acrolein as the principal toxicant (Gosselin et al., 1979).

Ocular irritation is one of the most sensitive responses to acrolein. In a study by Darley et al. (1960), 36 human volunteers were exposed to 0.06, 1.3-1.6, and 2.0-2.3 ppm for 5 minutes. Acrolein was dissolved in water and delivered to the eyes in a stream of oxygen through face masks. Carbon-filter respirators were worn during exposure so that only the eyes were exposed to the test material. The subjects, who were without a history of chronic upper respiratory or eye problems, rated the degree of eye irritation every 30 seconds during exposure as none (0), medium (1), or severe (2). The individuals' maximum values were used in the analysis that revealed a concentration-dependent incidence of eye irritation (Table 5.1.1). The lowest observed adverse effect level (LOAEL) for eye irritation in human volunteers was estimated by an unspecified method to be 0.06 ppm (0.14 mg/m³) acrolein during the five minute exposures. A NOAEL was not observed in this study.

Table 5.1.1 Ocular Irritation with Acrolein (from Darley et al., 1960)

Acrolein concentration	Irritation score
Filtered air	0.283
0.06 ppm	0.471
1.3-1.6 ppm	1.182
2.0-2.3 ppm	1.476

Ocular and upper respiratory tract irritation were also examined in a chamber study by Weber-Tschopp et al. (1977) involving healthy volunteers. Thirty one men and 22 women were exposed to increasing acrolein levels (0-0.60 ppm) for 40 min, while 21 men and 25 women were exposed to a constant 0.3 ppm for 60 min. Subjective reports of irritation and annoyance, and objective measures of eye-blink and respiratory rates were taken during the exposure periods. During exposure to increasing levels of acrolein, eye irritation, as measured by subjective report and blink frequency, was a

more sensitive measure of irritation than nasal irritation. By comparison, for less reactive volatile compounds in studies surveyed by Doty et al. (2004), the thresholds for ocular and intranasal irritation were of the same magnitude. In the Weber-Tschopp study of acrolein, significantly ($p<0.01$) higher eye irritation was first observed at 0.07 ppm, and nasal irritation at 0.26 ppm compared to controls. Significant depression of respiratory rates was observed at 0.60 ppm ($p<0.05$). With continuous exposure to 0.3 ppm acrolein, subjective eye and nasal irritation increased rapidly during the first 20 minutes and tended to plateau by 40 min. After 10 min of continuous exposure, a decrease in respiratory rate of 10% was evident in 47% of the subjects, while eye blink rate doubled in 66%. The authors suggest a threshold for adverse effects in the range of 0.09-0.30 ppm.

The effects of irritants such as acrolein may be accentuated in individuals with prior sensitization. Roux et al. (1999) investigated the interaction between passive sensitization of human isolated airways and exposure to pollutants (specifically, ozone and acrolein). Lung tissue from nonatopic, nonasthmatic patients was immunologically sensitized by incubation in sera from atopic asthmatic patients. Roux et al. reported that *in vitro* passive sensitization of the isolated tissues and exposure to acrolein act in a synergistic manner on human bronchial smooth muscle reactivity in response to both specific and nonspecific agonists. In tissues sensitized by incubation in sera from asthmatic patients, preexposure to 0.3 µM acrolein for 10 or 20 minutes significantly increased the maximal contractile response to a specific antigen of the dust mite, *Dermatophagoides pteronyssinus*, by 20.5 ± 6.5 % and 34.9 ± 7.4%, respectively. In addition, in sensitized tissue pre-exposed to 0.3 µM acrolein for 10 minutes, contractile response was increased by 33.5 ± 6.2% and 32.5 ± 5.1% for carbachol and histamine, respectively. Thus acrolein exposure potentially exacerbates asthma.

Mucus hypersecretion is one of the hallmarks of inflammatory airway disorders, including asthma. Borchers et al. (1999b) examined the effect of 0.01-100 nM acrolein on mucus glycoprotein (mucin) gene expression in cultured human airway epithelial cells. After a 4 hour exposure to acrolein in vitro, epithelial cells were found to have elevated mucin mRNA levels. It is not clear whether acrolein acts directly on epithelial cells or indirectly through inflammatory mediators released after acrolein exposure, however, asthma exacerbation is a likely result of acrolein exposure in susceptible individuals.

Persons with pre-existing eye, skin, respiratory, allergic, asthmatic or heart conditions might be at increased risk due to acrolein exposure. As a respiratory irritant, there is evidence that acrolein exacerbates asthma via the induction of bronchial hyper-responsiveness (Leikauf et al., 1989a; Leikauf et al., 1989b; Borchers et al., 1998; Borchers et al., 1999a; Borchers et al., 1999b). Acrolein has been listed as a TAC that may disproportionately impact children due to concerns related to asthma exacerbation.

Cancer patients treated with cyclophosphamide could be at increased risk because acrolein is a metabolite of cyclophosphamide (NTIS, 1981).

The effects of acrolein as an ocular irritant may be enhanced among those who wear contact lenses. Although no data specific to acrolein in this context were located, observations of ocular irritation following exposure to formaldehyde in an anatomy dissecting laboratory may be germane. Tanaka et al. (2003) reported that formaldehyde levels in an anatomy lab peaked at 0.62 ppm shortly after the exposure of cadavers for dissection, with a gradual decrease to 0.11 ppm. Formaldehyde-related irritation of the eyes, nose, throat, airways and skin was reported by 59% of the students. Ocular irritation was significantly ($p < 0.001$) higher among wearers of contact lenses compared with students without contacts. The ability of contact lenses to trap and concentrate volatile compounds, and to extend the exposure time by limiting the eye's normal self-cleansing, may make contact lens wearers more susceptible to ocular exposure and irritation by acrolein.

5.2 Acute Toxicity to Infants and Children

The literature specifically examining the effects of acrolein inhalation in infants and children is limited and comprises case studies of accidental exposure, and exposures to multiple substances. The most frequent sources of acrolein in childhood exposures are environmental tobacco smoke (ETS) and acrolein formed from overheated cooking oils. Mahut et al. (1993) describe the case of a 27 month-old boy hospitalized for acute respiratory failure following exposure for about an hour to acrid smoke from vegetable oil burning on an electric hot plate. The child was reportedly cyanotic with labored, crackling breathing, and was experiencing severe respiratory acidosis. Eighteen months following exposure, X-ray and CT scans showed bronchial thickening, massive over-inflation, patchy emphysema and diffuse bronchiectasis. In this case, and in cases of exposure to ETS, infants may be more susceptible to the adverse effects of acrolein in part due to an inability to escape exposure. Children also may be more susceptible to the effects of respiratory irritants due to the immature state of their airways.

As noted in OEHHA (2001): "*OEHHA considers asthma to impact children more than adults. Children have higher prevalence rates of asthma than do adults (Mannino et al., 1998). In addition, asthma episodes can be more severe due to the smaller airways of children, and result in more hospitalizations in children, particularly from the ages of 0 to 4 years, than in adults (Mannino et al., 1998; CDHS, 2000)." "Thus, on a population-wide basis, children are more impacted by asthma than adults, and since acrolein exacerbates asthma, children may be more impacted by acrolein toxicity than adults."* Data strongly suggesting that acrolein exacerbates asthma derive from studies using human tissue *in vitro* (Roux et al., 1999; Borchers et al., 1999a) and in animals *in vivo* (Leikauf et al., 1989a; 1989b; Borchers et al., 1998; Borchers et al., 1999b).

5.3 Acute Toxicity to Experimental Animals

Experimental exposures of rodents to acrolein at and above levels that are irritating to the eyes and respiratory tract in humans provide evidence for several adverse effects and their possible mechanisms. Acrolein prompts a proliferative response in nasal epithelium as shown by increased DNA synthesis (Roemer et al., 1993) and expression of mucin genes (Borchers et al., 1998). The latter effect in turn is associated with the

hyper-secretion of mucus that may contribute to chronic obstructive pulmonary disease and asthma (Borchers et al., 1998). Bronchial hyper-responsiveness, a hallmark of asthma, increases with acrolein exposure (Leikauf et al., 1989a) supporting a connection between acrolein exposure and exacerbation of asthma in humans. The dose-dependent decreases in protective epithelial enzyme activities (Cassee et al., 1996b) and levels of sulfhydryls (Lam et al., 1985; McNulty et al., 1984) are likely to be involved in the observed formation of lesions in the nasal epithelium (Cassee et al., 1996b).

Table 5.3.1 Acrolein Effects in Experimental Animals

Study	Model	Exposure	Outcome
Roemer et al. 1993	Proliferation of rat nasal and tracheal epithelium	0, 0.2, 0.6 ppm 6 h/d, 1 or 3 d	Increased DNA synthesis at 0.2 ppm (LOAEL)
Borchers et al. 1998	Mucus hyper-secretion, mucin gene expression in rat trachea and lungs	0.3, 0.75, 1.5, 3.0 ppm 6 h/d, 5 d/w	Hyper-secretion and gene expression at 0.75 ppm. (NOAEL = 0.3 ppm)
Leikauf et al. 1989a	Bronchial hyper-responsiveness and airway resistance in guinea pigs	1.3 ppm, 2 h	Resistance increased from 0.86 to 1.29 ml·cm H_2O/ml. Acetylcholine to double airway resistance dropped from 114 to 44.7 µg/kg/min
Buckley et al. 1984	Nasal histopathology at (RD_{50}) in mice;	1.7 ppm, 6 h/d, 5d	Exfoliation and squamous metaplasia of epithelium
Morris et al. 2003	Decrease in respiratory rate (RD_{50}) in mice	0.3, 1.6, 3.9 ppm, 10 min	Control RD_{50} at 1.50 ppm vs 0.82 ppm in allergic mice
Kane et al. 1979	Decrease in respiratory rate (RD_{50}) in mice	15 min	RD_{50} 1.7 ppm
Cassee et al. 1996b	Histopathology of rat nasal epithelium	0, 0.25, 0.67, 1.4 ppm, 6 h/d, 1-3 d	Dose-dependent lesions and decreased enzyme activities in nasal epithelium
Lam et al. 1985	Sulfhydryl depletion in rat respiratory mucosa	0, 0.1, 0.5, 1.0, 2.5 ppm 3 h	Dose-dependent depletion of non-protein sulfhydryls
McNulty et al. 1984	Sulfhydryl depletion in rat respiratory mucosa and liver	0.1, 0.3, 1, 2.5, 5 ppm 3 h	Dose-dependent depletion of non-protein sulfhydryls in nasal mucosa but not liver

Roemer et al. (1993) exposed Male Sprague Dawley rats by inhalation to 0, 0.2 or 0.6 ppm acrolein for 6 hours per day on one or three successive days. Nasal and tracheal epithelial and free lung cells were analyzed for proliferative responses using 5-bromodeoxyuridine (BrdU) labeling to identify DNA synthesizing cells. A single exposure to acrolein increased the DNA synthesizing cells 3-fold. After three exposures the increase was distinctly lower. All sites analyzed showed approximately the same concentration/response pattern. Since significant changes in cell proliferation were detected at 0.2 ppm (0.46 mg/m^3) acrolein, it is a LOAEL for this experiment.

Enhanced mucus secretion is a normal airway response to inhaled irritants. However, mucus hypersecretion is involved in the development of chronic obstructive pulmonary diseases; as such, it is considered an adverse effect. Borchers et al. (1998) exposed male rats to 3.0 ppm acrolein for 6 hours/day, 5 days/week for up to 12 days and examined the lungs and trachea for mucin cell metaplasia and expression of the mucin genes MUC2 and MUC5ac. The effects of acrolein concentration on mucin mRNA levels were further examined in rats exposed daily to 0.3, 0.75, 1.5, 3.0 ppm. Acrolein exposure resulted in a time-dependent increase in mucous cell differentiation and mucus hypersecretion in rat lungs. These changes were accompanied by increases in lung MUC5ac mRNA to levels 3-fold higher than in controls, and readily immunohistochemically detectable levels of MUC5ac. MUC5ac mRNA was elevated by concentrations as low as 0.75 ppm while MUC2 mRNA was not affected by any of the levels tested. Thus 0.3 ppm (0.69 mg/m^3) is a NOEL for this effect. The trachea of treated animals showed sloughing of the epithelium accompanied by excessive mucus and inflammatory cells in the lumen.

Bronchial hyper-responsiveness is a hallmark of reactive airway diseases such as asthma, and may be induced by inhaled irritants. Leikauf et al. (1989a) exposed guinea pigs to 1.3 ppm acrolein for 2 hours and measured the induction of bronchial hyperresponsiveness by the amount of infused acetylcholine necessary to double specific airway resistance 1, 2, 6, and 24 hours after exposure compared to baseline. The dose of acetylcholine required to double airway resistance decreased from 114.0 ± 6.6 to 44.7 ± 4.2 µg/kg/min ($p < 0.001$) at 2 hours following acrolein exposure and remained low for at least 24 hours. Acrolein exposure was found to increase levels of the bronchoconstrictor leukotriene C_4 (LTC_4) in bronchoalveolar lavage fluids prior to the observation of bronchial hyperresponsiveness. This hyperresponsiveness was prevented by treatment with an inhibitor of LTC_4 synthesis or an LTC_4 receptor antagonist. Acrolein was thus shown to induce bronchial hyperresponsiveness, an effect apparently mediated by LTC_4.

Buckley et al. (1984) investigated whether lesions occur in the respiratory tract of Swiss-Webster mice after exposure to the RD_{50} concentrations of ten sensory irritants including acrolein. After exposure of mice for 6 hr/day for 5 days to 1.7 ppm acrolein, the respiratory tract was examined for histopathologic changes. Acrolein (and all other irritants) produced lesions in the nasal cavity with a distinct anterior-posterior severity gradient. Acrolein specifically caused severe exfoliation and squamous metaplasia of

the respiratory epithelium and moderate ulceration of the olfactory epithelium. Acrolein did not induce lesions in the lower respiratory tract.

Morris et al. (2003) compared the respiratory responses to acrolein in healthy mice with those in mice previously sensitized to ovalbumin. Inhalation exposure to ovalbumin prior to acrolein exposure elicited an allergic response in the sensitized mice that was characteristic of allergic airway disease. Upon subsequent acrolein exposure, the RD_{50}, a measure of the dose required to reduce the respiratory rate by 50%, was 1.50 ppm in naïve mice and 0.82 ppm in the mouse model of allergic airway disease. Thus in sensitized animals, a lower concentration of acrolein is required to elicit the same changes in breathing rate observed in non-allergic animals. In both intact mice and in isolated mouse upper respiratory tracts, acrolein exposure caused a significant ($P < 0.05$) increase in flow resistance, an effect that was immediate and not exposure time dependent. Pretreatment with capsaicin to defunctionalize sensory neurons significantly attenuated the breathing rate and obstructive responses supporting the role of sensory neuron stimulation in the response to acrolein. For comparison, Kane et al. (1979) also used the RD_{50} as a measure of sensory irritation and estimated an RD_{50} of 1.7 ppm in mice during 15 minutes of acrolein exposure.

Cassee et al. (1996b) exposed male Wistar rats to 0, 0.25, 0.67, or 1.4 ppm acrolein for 6 hours per day on one or three successive days. Immediately following the last exposure, the rats were killed. Mucosae from the respiratory or olfactory parts of the nose were collected from 3 rats per group for biochemical analyses. The skulls of the other rats in each group were prepared for histopathology and cell proliferation measurements. Nasal epithelium, examined microscopically, showed dose-dependent evidence of disarrangement, necrosis, thickening, and desquamation of the respiratory/transitional epithelium (Table 5.3.2). Significant basal cell hyperplasia, observed at the lowest dose (0.25 ppm), increased with exposure. The activity of glutathione reductase (GR) was reduced after one-day exposure to acrolein, while the activities of GR, glutathione-S-transferase and aldehyde dehydrogenase were reduced following the three-day exposures. These results and those mentioned below suggest that acrolein interferes with enzyme systems involved in its detoxification.

Table 5.3.2 Nasal Lesions in Rats with Acrolein Exposure

(from Cassee et al., 1996b)

Site and type of lesion	Extent	Incidence	
		Low	Medium
Noses examined		5	6
Disarrangement, necrosis, desquamation of respiratory, transitional epithelium	Slight (mainly disarrangement)	4	3
	Moderate	1	3
	Severe and extensive	0	0
Basal cell hyperplasia and/or increased mitotic figures	Slight (focal)	3	2
	Moderate	0	4
	Severe (extensive)	0	0

Pronounced and possibly irreversible biochemical changes occur with acrolein levels that are extremely irritating. Acrolein depletes glutathione (GSH) and other free thiol groups both in vitro and in vivo (McNulty et al., 1984; Lam et al., 1985; Grafstrom et al., 1987; U.S.EPA, 2003; Yang et al., 2004). Inhalation exposure of rats to a concentration of 5 ppm (11.4 mg/m³) for 3 hours caused irreversible depletion of non-protein sulfhydryls in the nasal mucosa (Lam et al., 1985). Under similar exposure conditions, 5 ppm (11.5 mg/m³) for 3 hours, McNulty et al. (1984) reported a 63% decrease in glutathione in nasal mucosal but not in liver. In addition, ^{14}C-labeled acrolein has been shown to bind irreversibly to sulfhydryl groups on cytochrome P450 in rats (Gurtoo et al., 1981). The binding of acrolein to sulfhydryl groups is localized to the area of contact (e.g., nasal membranes or lung epithelium), and is not a systemic effect (Lam et al., 1985).

The pulmonary immunological defense against a bacterial challenge using *Staphylococcus aureus* in mice was impaired in a dose-dependent manner following a single exposure to acrolein at concentrations of 3 and 6 ppm (6.9 and 13.8 mg/m^3) for 8 hours (Astry and Jakab, 1983). In this study, the control exposure was not described.

The efficiency with which acrolein enters cells of the respiratory tract in large part determines the inspired levels at which toxic effects are observed. Struve et al. (2008) measured the uptake efficiency of 0.6, 1.8 or 3.6 ppm acrolein in isolated upper respiratory tracts of anesthetized, naïve rats under constant velocity, unidirectional flow rates of 100 or 300 ml/min for up to 80 min. Similar studies were also performed on rats with previous exposure to 0.6 or 1.8 ppm acrolein for 6 hours per day, 5 days per week for 14 exposure days prior to nasal uptake studies with 1.8 or 3.6 ppm at 100 ml/min flow rate. Acrolein levels entering and exiting the isolated respiratory tract were measured to determine uptake efficiency. At the end of the exposure period, the animals were killed and the nasal respiratory and olfactory mucosa isolated for measurements of protein, and total and oxidized glutathione. The efficiency of acrolein uptake by the rat nose was dependent on the concentration, flow rate, and duration of acrolein exposure. Uptake efficiency was significantly higher at the lowest exposure than at the higher levels ($0.6 > 1.8 \approx 3.6$; $p < 0.0001$), and at the lower flow rate ($100 > 300$; $p < 0.0061$). At both flow rates, the efficiency of uptake significantly declined over the 80 min exposure period ($p < 0.001$), with a significant interaction between concentration and time ($p = 0.01$). In naïve rats, glutathione levels dropped in respiratory epithelium but remained largely the same in olfactory epithelium. By comparison, in pre-exposed rats, the acrolein uptake efficiency was higher than in naïve rats. However, the GSH levels at the end of exposure were also higher, perhaps suggesting an adaptive response.

6. Chronic Toxicity of Acrolein

6.1 Chronic Toxicity to Adult Humans

Information regarding the chronic toxicity of acrolein in humans is limited. There is inadequate direct evidence for carcinogenicity of acrolein in humans or experimental animals. However, a metabolite of acrolein, the reactive epoxide glycidaldehyde, has been shown to be mutagenic and carcinogenic in mice and rats (IARC, 1985). Therefore, acrolein has been designated a Group C substance, with possible human carcinogenic potential by the U.S.EPA (1987). In addition, acrolein-DNA adducts have been found in aortic tissue following 6 hour inhalation exposure to 1 and 10 ppm acrolein (Penn et al., 2001).

A source of chronic acrolein exposure for some individuals is tobacco smoking. Much of the pulmonary irritancy associated with tobacco smoke has been attributed to acrolein and research in this area suggests mechanisms for some of acrolein's pulmonary effects. As part of a defense response, pulmonary neutrophils release oxidants, proteases and cytokines such as IL-8, all of which may promote inflammation and potentiate tissue damage. To limit tissue damage and resolve the inflammation, neutrophils normally undergo constitutive apoptosis. Experiments with isolated human neutrophils exposed to acrolein at levels achievable during active smoking (1-50 µM) found that acrolein inhibited neutrophil apoptosis, increased IL-8 production, and activated mitogen-activated protein kinases (MAPK) (Finkelstein et al., 2001). At acrolein concentrations up to 10 µM, inhibition of apoptosis was accompanied by increased cell viability. At higher acrolein levels, cell viability decreased as necrotic cell death increased. While the mechanisms behind acrolein's concentration-dependent effects on neutrophils are not clear, the effects observed at the lower exposure levels suggest that acrolein may contribute to pulmonary inflammation and exacerbate allergic responses by prolonging the survival of neutrophils, and stimulating the production of inflammation-related cytokines and enzymes. At higher levels, frank cellular toxicity becomes more prominent.

6.2 Chronic Toxicity to Infants and Children

No data addressing the effects of chronic acrolein exposure among infants and children were located. Inasmuch as acrolein is one of the major irritants in environmental tobacco smoke (Takabe et al., 2001) at relatively high concentrations in smokers' homes (1.6-3.6 µg/m^3; 0.70-1.57 ppb (Nazaroff and Singer, 2004)), children living with smokers may be disproportionately exposed to acrolein as they are less able to avoid exposure than are adult nonsmokers. To the extent that respiratory irritants such as acrolein elicit bronchoconstriction and excessive mucus secretion characteristic of asthma, children, with their smaller airways and greater prevalence of asthma, may experience more diminution of pulmonary function and more episodes of asthma with chronic exposure.

6.3 Chronic Toxicity to Experimental Animals

Long-term exposure to acrolein causes structural and functional changes in the respiratory tract. Nasal and pulmonary effects following acrolein exposure for 13 weeks (6 hours/day, 5 days/week) were described by Dorman et al. (2008) in 360 male F344 rats. The whole-body exposures were to air concentrations of 0, 0.02, 0.06, 0.2, 0.6, and 1.8 ppm acrolein, with evaluation of respiratory tract histopathology after 4, 14, 30 and 65 days of exposure, and at 60 days following the end of the 13 week exposure. Body weights of all acrolein exposed rats were depressed but there were reportedly no other significant increases in clinical signs. Formalin-fixed noses were sectioned transversely providing six sections of the nasal cavity at standard levels. Larynx, trachea and lungs were fixed, stained with hematoxylin and eosin, and examined histologically. The study examined both respiratory and olfactory epithelia with the former being the more sensitive as evidenced by inflammation, hyperplasia and squamous metaplasia. Mild hyperplasia of the respiratory epithelia was first observed after 4 days of exposure to ≥ 0.6 ppm. The NOAEL for pathology of nasal respiratory epithelia was 0.2 ppm in the lateral walls of level II, and for olfactory epithelia, 0.6 ppm. At the highest concentration, 1.8 ppm, mild squamous metaplasia was also observed in the larynx and trachea, but no treatment related effects were seen in the lungs. Two months following cessation of exposure, only partial recovery of the olfactory epithelium was observed; primarily in caudal areas where lesions developed more slowly and were less severe.

Schroeter et al. (2008) used data from the above study by Dorman et al. (2008) for the development of a physiological computational fluid dynamics (CFD) model of acrolein nasal dosimetry. The CFD models of Kimbell et al. (1997) and Subramaniam et al. (1998) were modified to estimate kinetic parameters of acrolein flux in rat nasal passages, and allow a cross-species prediction of acrolein flux in humans associated with histopathology. Based on a NOAEL of 0.6 ppm and a LOAEL of 1.8 ppm for olfactory neuronal loss from Dorman et al. (2008), the CFD model predicted a threshold acrolein flux of 72 pg/cm^2-s at region 11, comprising portions of the third ethmoturbinate. Assuming equal tissue doses of acrolein elicit similar responses in the olfactory epithelium of rats and human, an exposure level that may be expected to represent the threshold for olfactory neuronal loss in humans may be estimated. The 99th percentile olfactory flux value that is equal to the threshold of 72 pg/cm^2-s was estimated to be 45 ppb. The authors use this concentration to estimate a human equivalent NOAEL of 8 ppb, and a reference concentration (RfC) of 0.27 ppb. However, the threshold acrolein flux associated with the lower NOAEL of 0.2 ppm, reported by Dorman et al. (2008) for respiratory epithelium, was not estimated, and an equivalent human threshold and NOAEL is not available. The rationale for this, presented in Dorman et al. (2008), is *"Our CFD modeling efforts have revealed that although the observed NOAEL for the respiratory epithelium is lower than that seen for the olfactory epithelium (i.e., 0.2 vs. 0.6 ppm), in actuality the olfactory epithelial lesion arises at an appreciably lower delivered tissue dose suggesting that the olfactory epithelium is more sensitive to the effects of inhaled acrolein than is the respiratory epithelium (Schroeter et al. (2008)."* The RfC of 0.27 ppb estimated by the authors is thus based on lesion formation at the lowest modeled tissue dose rather than on the

more relevant value of the lowest applied acrolein concentration associated with an adverse effect.

Structural and functional changes in the respiratory tract were also examined in male Fischer-344 rats exposed for 6 hours/day, 5 days/week for 62 days to acrolein at concentrations of 0, 0.4, 1.4, and 4.0 ppm (0, 0.92, 3.2, and 9.2 mg/m^3)(Kutzman, 1981; Kutzman et al., 1985). Each group of 24 animals was assessed for pulmonary function immediately prior to the end of the experiment. Pulmonary function tests included lung volumes, forced respiratory capacity, pulmonary resistance, dynamic compliance, diffusing capacity of carbon monoxide, and multi-breath nitrogen washout. At the end of the experiment, animals were killed and histopathological changes in the lungs were recorded. Eight additional rats were designated for histopathology and 8 rats were used for reproductive testing only. All analyses were performed at 6 days post-exposure to minimize the acute effects of acrolein. Mortality was high (56%) in rats exposed to 4.0 ppm (9.2 mg/m^3). The observed mortality was due to acute bronchopneumonia in these cases. The animals from this group that survived had reduced body weight. No histological changes were observed in extra-respiratory tissues in any group. There was a concentration-dependent increase in histological changes to the nasal turbinates (increased submucosal lymphoid aggregates), beginning at 0.4 ppm. Concentration-dependent damage to the peribronchiolar and bronchiolar regions included epithelial necrosis and sloughed cells lying free in the lumen. No lung lesions were observed in the 0.4 ppm group. The LOAEL for nasal lesions (squamous epithelial metaplasia and neutrophil infiltration) in this study was 0.4 ppm.

Feron et al. (1978) exposed groups of 20 Syrian golden hamsters, 12 SPF Wistar rats and 4 Dutch rabbits (of both sexes) to acrolein vapor at 0, 0.4, 1.4 and 4.9 ppm (0, 0.92, 3.2, and 11.3 mg/m^3) 6 hours/day, 5 days/week for 13 weeks. The most prominent effects at the highest level included mortality in rats (3 of each sex), and ocular and nasal irritation, growth depression, and histopathological changes of the respiratory tract in each species. The changes in the airways induced by acrolein consisted of destruction, and hyperplasia and metaplasia of the lining epithelium accompanied by inflammatory alterations. Rats were the most susceptible species examined and showed treatment-related histopathological abnormalities in the nasal cavity down to 0.4 ppm (LOAEL), whereas this level was a NOAEL in hamsters and rabbits. The results for individual rats at 0.4 ppm were not given.

Bouley et al. (1975; 1976) exposed male SPF OFA rats continuously to 0.55 ppm (1.3 mg/m^3) of acrolein for up to 63 days. This level of acrolein led to a greater susceptibility to airborne *Salmonella enteritidis* infection during the first three weeks compared to control rats but it disappeared spontaneously when exposure was continued beyond three weeks. The general toxic effect of diminished weight gain (due to reduced feeding) compared to the control group lasted as long as exposure and disappeared only after acrolein was discontinued. Sneezing, a sign of nasal irritation, was consistently observed in the exposed animals on days 7 through 21 but ceased thereafter. No histopathology of the nasal cavity of or any other tissue was reported.

In one of the few chronic studies reported, Feron and Kruysse (1977) exposed hamsters (18/gender) to 4 ppm (9.2 mg/m^3) acrolein for 7 hours/day, 5 days/week, for 52 weeks. Mild to moderate histological changes were observed in the upper and lower respiratory tract. No evidence of toxicity to other organs was apparent at necropsy, although body weight was decreased. Hematology, urinalysis, and serum enzymes were not affected by exposure. Thus 4 ppm is a chronic LOAEL for hamsters. As noted above, hamsters appear to be a less sensitive species than rats (Feron et al., 1978).

Exposures of rodents have generally formed the basis for the determination of acrolein's chronic effects. However, an interspecies comparison was conducted by Lyon and associates (Lyon et al., 1970) who investigated the effects of repeated or continuous exposures of acrolein on Sprague-Dawley rats (n = 15/exposure group), guinea pigs (n = 15), beagle dogs (n = 2), and male squirrel monkeys (n = 9). Animals were exposed to 0.7 or 3.7 ppm (1.6 or 8.5 mg/m^3) acrolein for 8 hours/day, 5 days/week, for 6 weeks, or continuously to 0.22, 1.0, or 1.8 ppm (0.5, 2.3, or 4.1 mg/m^3) for 90 days. The results below suggest that dogs and monkeys were more susceptible to acrolein's effects than were the rodents.

Two monkeys in the 3.7 ppm intermittent exposure group died within 9 days. Monkeys and dogs salivated excessively during the first week. Squamous metaplasia and basal cell hyperplasia of the trachea were observed in monkeys and dogs; 7 of the 9 monkeys repeatedly exposed to 3.7 ppm also exhibited bronchiolitis obliterans with squamous metaplasia in the lungs. Bronchopneumonia was noted in the dogs. Inflammation in the lung interstitiae was more prominent in the dogs than in the monkeys. Rats and guinea pigs did not exhibit signs of toxicity when exposed intermittently to 3.7 ppm. Continuous exposure to 1.0 and 1.8 ppm, but not 0.22 ppm acrolein, resulted in salivation and ocular discharge in the monkeys and dogs. Rats and guinea pigs appeared normal at all concentrations. Rats exhibited significant weight loss in the 1.0 and 1.8 ppm continuous exposure groups. Nonspecific inflammatory changes were observed in sections of brain, heart, lung, liver and kidney from all species exposed to 1.8 ppm. The lungs from the dogs showed confluent bronchopneumonia. Focal histological changes in the bronchiolar region and the spleen were detected at 0.22 ppm in dogs. Nonspecific inflammatory changes at the 0.22 ppm level were apparent in liver, lung, kidney and heart from monkeys, guinea pigs and dogs. Unfortunately the nasal cavity was not examined in this study. While there were no unexposed control animals for any species, the cross-species comparison shows substantial interspecies variability in susceptibility.

7. Developmental and Reproductive Toxicity

There are no reports of reproductive or developmental toxicity following inhalation exposure to acrolein in humans. Kutzman (1981) studied reproductive fitness in male and female rats following acrolein inhalation for 6 hours/day, 5 days/week for 62 days. Treated males were mated with untreated females, and treated females with untreated males. No treatment-related differences were found in the parameters assessed including pregnancy rate, number of corpora lutea, embryo viability, early and late deaths, and preimplantation losses. Similarly, the morphology of sperm collected from

the epididymides of treated males was examined and reportedly not affected. Bouley et al. (1975; 1976) exposed three male and 21 female SPF-OFA rats continuously to 0.55 ppm (1.26 mg/m^3) acrolein vapor for 25 days. The rats were allowed to mate on day 4 of the exposure. The number of acrolein-exposed pregnant rats and the number and mean body weight of their fetuses were similar to controls.

In rats, acrolein can induce teratogenic and embryotoxic effects when administered directly into the amniotic fluid, or when added to cultured rat embryos (Slott and Hales, 1986). Additionally, acrolein injected into chicken embryos resulted in embryotoxicity and some teratogenic effects at moderate to high doses (0.001-0.1 mg/egg) (Chhibber and Gilani, 1986). However, intravenous injection of acrolein in pregnant rabbits showed no developmental effects in the offspring (Claussen et al., 1980). Based on this latter study, the World Health Organization (1992) concluded that human exposure to acrolein was unlikely to affect the developing embryo.

8. Derivation of Reference Exposure Levels

8.1 Acrolein Acute Reference Exposure Level

Study	Darley et al., 1960
Study population	36 healthy human volunteers
Exposure method	5 min exposure: carbon-filter respirators worn
Exposure continuity	Single exposure
Exposure duration	5 min
Critical effects	subjective ocular irritation
LOAEL	0.06 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	n/a
LOAEL uncertainty factor (UF_L)	6 (default: mild effect, no NOAEL)
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children to asthma exacerbation)
Cumulative uncertainty factor	60
Reference Exposure Level	2.3 µg/m³ (1.0 ppb)

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document).

The study by Darley et al. (1960) was selected as the best available acute exposure study employing human subjects. In addition, the ocular mucosa and the nasal mucosa are both innervated by cranial nerve V (trigeminal nerve). As noted by Doty et al. (2004), numerous studies employing n-alcohols, ketones, alkylbenzenes, terpenes, butyl acetate and toluene, report thresholds for ocular and intranasal irritation to be of the same magnitude suggesting that for most volatiles, tests of ocular and nasal irritancy are of equivalent sensitivity. Thus the endpoint of ocular irritancy used in this study is expected to also reflect irritancy of the upper respiratory tract. Confidence in this REL calculation is moderate as the LOAEL used is based on an estimated LOAEL of 0.06 ppm rather than a measured level. A default uncertainty factor of 6 is associated with the use of a LOAEL for mild effects in the absence of a NOAEL in acute REL derivations (see Section 4.4.5 of the TSD). Due to its high reactivity, the effects of exposure to acrolein in the air are largely confined to the site of contact, in this case the eyes, with negligible or no systemic effects. This localization of effects to the site of contact is supported by the confinement of acrolein's effects to the upper respiratory tract in the animal studies of acute inhalation exposure. Based on modeling of adults and 3-month old children that takes into account age-related ventilation rates and respiratory tract surface area, the deposition kinetics of reactive gases are generally thought not to be greatly different between adults and children (Ginsberg et al., 2005). Because of this, a value of 1 is used for the kinetic component of the intraspecies uncertainty factor (UF_{H-k}), rather than a more extended value of $\sqrt{10}$ or 10 which are used where metabolic processes also contribute to inter-individual variability. While ocular irritation is not expected to be substantially different between children and adults, the respiratory irritant effect, with documented potential to exacerbate asthma, is clearly an effect with the potential to differentially impact infants and children. The toxicodynamic component of the intraspecies uncertainty factor UF_{H-d} is therefore assigned an increased value of 10 to account for potential asthma exacerbation. These considerations are applied equally to the acute, 8-hour and chronic REL. Based on this study, an acute REL for acrolein exposure is calculated to be 2.3 $\mu g/m^3$ (1.0 ppb).

As noted in Section 5.1, contact lens wearers may be at greater risk for ocular irritation with acrolein exposure. However, since contact lens users, and infants and children are generally mutually exclusive groups, it is expected that with the ten-fold toxicodynamic UF_{H-d} described above, the acute REL should be adequately protective of these individuals as well.

The acute REL above is supported by a study in humans by Weber-Tschopp et al. (1977). During a 40 minute exposure to increasing concentrations of acrolein, significant ocular irritation was first reported at 0.07 ppm. This represents the LOAEL for this effect and is similar to the LOAEL of 0.06 ppm in Darley et al. (1960). The same uncertainty and adjustment factors, and rationale apply as in Darley, giving an acute REL of 2.7 $\mu g/m^3$ (1.2 ppb).

Study	Weber-Tschopp et al. (1977)
Study population	54 healthy human volunteers
Exposure method	Exposure chamber
Exposure continuity	Increasing concentration (0-0.6 ppm)
Exposure duration	40 min
Critical effects	subjective ocular irritation
LOAEL	0.07 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	n/a
LOAEL uncertainty factor (UF_L)	6 (no NOAEL)
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	60
Reference Exposure Level	2.7 µg/m³ (1.2 ppb)

Sensory irritancy is the critical response to acute acrolein exposure. For this effect both the Darley and Weber-Tschopp studies found similar effect levels resulting in similar estimates for the acute REL. In consideration of this, we took the geometric mean of the REL values from these studies to derive the acute REL of **2.5 µg/m³ (1.1 ppb).**

A similar acute REL was calculated as shown below based on lesions in nasal epithelium in rats exposed to acrolein for 6 hours/day for 3 days (Cassee et al., 1996b). There were sufficient data in this study to permit the application of the BMD method in preference to the NOAEL/LOAEL approach. A $BMCL_{05}$ of 56 µg/m³ was derived based on the incidence of moderate to severe lesions at each exposure level. Irritancy was not the endpoint in this study so a time adjustment was applied using $C^n * T = K$ (n = 3) to adjust the 18 hours of exposure to 1 hour that gave 147 µg/m³ (see Section 5.6.1 of the TSD). Interspecies uncertainty factors of 2 for toxicokinetic differences with use of a dosimetric adjustment factor (DAF) of 0.85 (dosimetric adjustment factor – described below and in Section 4.4.7.2.2 of the TSD), and √10 for toxicodynamic variability were combined with a combined intraspecies UF of 10 (1 for kinetic and 10 for dynamic variability, reflecting the expectation of greater toxicodynamic variability) for a cumulative UF of 60 and an acute REL of 0.91 ppb.

Study	Cassee et al., 1996b
Study population	11 rats
Exposure method	Nose-only inhalation
Exposure continuity	6 hr/day
Exposure duration	3 days
Critical effects	lesions of the respiratory epithelium
LOAEL	0.25 ppm (0.58 mg/m^3)
NOAEL	not observed
Benchmark concentration ($BMCL_{05}$)	56 µg/m^3
Time-adjusted exposure	C^n*T n = 3
Extrapolated concentration	147 µg/m^3 $(56^3*6/1*3/1)^{1/3}$
Human concentration adjustment	125 µg/m^3 = 147*0.85 (DAF)
LOAEL uncertainty factor (UF_L)	not applied
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (DAF adjustment with analogue chemical)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	60
Reference Exposure Level	2.1 µg/m^3 (0.91 ppb)

The DAF is a factor derived by OEHHA based on the modeled comparative flux of formaldehyde in the upper respiratory tracts of rats, rhesus monkeys and humans by Kimbell et al. (2001) (see Section 4.4.7.2.2 of the TSD). Kimbell et al. used three-dimensional, anatomically realistic, computational flow dynamic models to estimate mass flux across 20 consecutive bins representing the nasal passages. The mean flux at each bin was weighted by the percent of non-squamous epithelium in that bin to derive a weighted average flux for each bin. Averaging across all 20 bins provides an overall estimate of the flux for comparison between species (rat, 13.63 pmol/mm^2; human, 30.80 pmol/mm^2). Peak flux values were also estimated for the rat (2620 pmol/mm^2) and human (2082 pmol/mm^2), and averaged with the mean flux values to estimate the DAF (0.85). The DAF is the ratio of this value for the rat to that for humans. Although acrolein is more reactive than formaldehyde, both compounds appear to have their effects primarily on the respiratory (vs. olfactory) epithelium (Cassee et al., 1996a). This supports the assumption that in applying the DAF to acrolein, acrolein and formaldehyde deposit similarly in the nasal passages. In the absence of acrolein-specific modeling data, any residual uncertainty associated with this assumption is reflected in the use of an interspecies UF_{A-k} of 2.

8.2 Acrolein 8-Hour Reference Exposure Level

Study	Dorman et al., 2008
Study population	360 adult Fischer-344 rats
Exposure method	Discontinuous whole body 0.02 – 1.8 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	65 days
Critical effects	Lesions in the respiratory epithelium
LOAEL	0.6 ppm
NOAEL	0.2 ppm
Benchmark concentration	not derived
Time-adjusted exposure	C * T = K
Extrapolated 8 hour concentration	71 ppb = (0.2*6 /24*5/7*20/10)
Human concentration adjustment	60 ppb = 71*0.85 (DAF)
LOAEL uncertainty factor (UF_L)	1 (NOAEL observed)
Subchronic uncertainty factor (UFs)	√10
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (DAF adjustment with analogue chemical)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	200
Reference Exposure Level	**0.70 µg/m³ 0.30 ppb)**

The 8-hour Reference Exposure Level is a concentration at or below which adverse non-cancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the TSD).

The 8-hour and chronic RELs are based on the observation of lesions in rat respiratory epithelium by Dorman et al. (2008). In this study, a LOAEL of 0.6 ppm and a NOAEL of 0.2 ppm were observed for respiratory lesions in rats. The observation of a NOAEL eliminates the need for a UF for the LOAEL to NOAEL conversion. The critical effect of lesion formation is not a sensory irritancy effect so a time (T) adjustment was applied using C*T = K to extrapolate from the 6 to 24 hours and from 5 to 7 days. This chronic exposure was converted to an 8 hour exposure with the 8-hr breathing rate conversion of 20/10 and yields an extrapolated 8 hour concentration of 71 ppb. A human concentration of 60 ppb was estimated using a DAF of 0.85. Although the use of the DAF is expected to correct for pharmacokinetic differences between species, an interspecies kinetic UF of 2 was used because the DAF is based on an analogue (formaldehyde). The default interspecies UF_{A-d} of √10 was applied to compensate for the absence of data on pharmacodynamic differences between species. An intraspecies UF_{H-k} of 1 was used since, although the data are only for adult animals, the pharmacokinetic differences between adult and young animals are not expected to be great based on the similar inhalation dosimetry associated with reactive gases in adults and infants (Ginsberg et al., 2005). The potential pharmacodynamic differences among

individuals (especially those with and without asthma) and between adults and infants (due to the immaturity of the infants respiratory tract) are expected to be greater. For example, irritant gases more readily stimulate the hyper-reactive airways of asthmatics while enhanced mucus production in response to irritant gases may more easily block the infant's narrower airways. As described in Section 5.2, exacerbation of asthma by acrolein is expected to disproportionately affect children. For these reasons, an intraspecies UF_{H-d} of 10 was employed. The UF_{H-d} of 10 is the default in the absence of human kinetic data. This resulted in a cumulative UF of 200 and an 8-hour REL of 0.70 µg/m³ (0.30 ppb).

These results are supported by studies of Kutzman et al. (1985) and Feron et al. (1978) following exposure of rats to acrolein for 6 hours/day, 5 days/week for 62 days. In these studies, a LOAEL of 0.4 ppm was observed for nasal lesion formation.

Study	Kutzman et al., 1985; Feron et al. (1978)
Study population	96 adult Fischer-344 rats
Exposure method	Discontinuous whole body 0.4 – 4.0 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	62 days
Critical effects	Lesions in the respiratory epithelium
LOAEL	0.4 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	$C^n * T = K$, where n =1.2
Extrapolated 8 hour concentration	143 ppb = (0.4*6 /24*5/7*20/10)
Human concentration adjustment	122 ppb = 143*0.85 (DAF)
LOAEL uncertainty factor (UF_L)	3 (mild effect; no NOAEL)
Subchronic uncertainty factor (UFs)	√10
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (DAF adjustment with analogue chemical)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	600
Reference Exposure Level	0.46 µg/m³ (0.20 ppb**)**

The experimental designs and results from these two studies were essentially identical. As above, the critical effect of lesion formation is not a sensory irritancy effect so a time (T) adjustment was applied using C*T = K to extrapolate to an 8 hour concentration of 143 ppb. A UF of 3 for the use of a LOAEL reflects the expectation that the NOAEL, while not reported in either of these studies, will not be far from the LOAEL. This is based on the steepness of the dose-response in a plot of the nasal histopathology scoring vs acrolein concentration in the Feron paper, and the observation of a NOAEL

three-fold lower than the LOAEL in the Dorman study (0.2 vs 0.6 ppm). An adjusted human concentration of 122 ppb was estimated using a DAF of 0.85. The rest of the uncertainty factors were the same as for the crtitical Dorman study This resulted in a cumulative UF of 600 and an 8-hour REL of 0.46 µg/m³ (0.20 ppb). Thus, this derivation is supportive of the REL derived from Dorman et al. (2008).

8.3 Acrolein Chronic Reference Exposure Level

Study	Dorman et al., 2008
Study population	360 adult Fischer-344 rats
Exposure method	Discontinuous whole body 0.02 – 1.8 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	65 days
Critical effects	Lesions in the respiratory epithelium
LOAEL	0.6 ppm
NOAEL	0.2 ppm
Benchmark concentration	not derived
Time-adjusted exposure	36 ppb = (0.2*6/24*5/7)
Human concentration adjustment	30 ppb = 36*0.85 (DAF)
LOAEL uncertainty factor (UF_L)	1 (NOAEL observed)
Subchronic uncertainty factor (UFs)	√10 (exposure 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (DAF adjustment based on analogue chemical)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	200
Reference Exposure Level	**0.35 µg/m³ (0.15 ppb)**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from continuous chronic exposures (see Section 7 in the Technical Support Document).

The chronic REL was developed using the same study as the 8-hr REL but with a time extrapolation to continuous exposure since the endpoint was not trigeminal irritancy (see Section 1.2.3 in the TSD). It is based on the observed NOAEL of 0.2 ppm for respiratory lesions in rats. The observation of a NOAEL eliminates the need for a UF for the LOAEL to NOAEL conversion. Time adjustment from the experimental to continuous exposure gave 36 ppb (0.2*6 hr/24 hr*5 days/7 days). A DAF of 0.85 gave an equivalent human exposure of 30 ppb. Use of the DAF for an analogue chemical entails an uncertainty factor of 2 as described previously. The same UFs and rationale as used in the derivation of the 8-hour REL are applied to the chronic REL. The resulting cumulative UF of 200 gave an estimated reference exposure level of 0.35 µg/m³ (0.15 ppb).

These results were supported by those of Kutzman et al. (1985) and Feron et al. (1978)

Study	Kutzman et al., 1985; Feron et al. (1978)
Study population	96 adult Fischer-344 rats
Exposure method	Discontinuous whole body to 0 – 4.0 ppm
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	62 days
Critical effects	Lesions in the respiratory epithelium
LOAEL	0.4 ppm
NOAEL	not observed
Benchmark concentration	not derived
Time adjusted exposure	0.071 ppm = 0.4*6/24*5/7
Human concentration adjustment	60 ppb = 0.071 * 0.85 (DAF)
LOAEL uncertainty factor (UF_L)	3 (no NOAEL)
Subchronic uncertainty factor (UFs)	√10 (exposure 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2 (with DAF adjustment)
Toxicodynamic (UF_{A-d})	√10 (default: no interspecies toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	600
Reference Exposure Level	**0.10 µg/m³ (0.04 ppb)**

The LOAEL of 0.4 ppm was adjusted to a continuous exposure of 0.071 ppm (0.4*6 hr/24 hr*5 days/7 days). Application of a DAF of 0.85 gave a human equivalent concentration of 60 ppb (138 µg/m^3). The UFs applied here are the same as those for the Dorman study with the inclusion of a LOAEL to NOAEL UF of 3. The cumulative UF of 600 gives a chronic REL of 0.10 µg/m³ (0.04 ppb). The study by Dorman et al. was selected in preference to these studies because it identified a NOAEL for the critical effect.

The U.S. EPA (2003) based its RfC of 0.02 µg/m^3 on the study by Feron et al. (1978) from which a HEC of 0.02 mg/m^3 was derived based on a regional gas dosimetric ratio (RGDR) of 0.14 and an adjusted LOAEL of 0.16 mg/m^3 (0.14 * 0.16 = 0.02). U.S. EPA applied a total uncertainty factor of 1,000 (3 for interspecies extrapolation from a dosimetrically adjusted dose; 10 for intra-human variability; 3 for the use of a LOAEL; 10 for subchronic to chronic extrapolation). In contrast to the RGDR of 0.14, to better account for differences in rat and human exposures to reactive gases, OEHHA used a DAF of 0.85 based on comparative modeling of gas flux in human and rat nasal passages described above. This, combined with UFs of 6 for interspecies uncertainty (2 for use of the DAF, √10 for toxicodynamic differences), √10 for the use of a subchronic study, and 3 for the use of a LOAEL (vs US EPA's 3, 3, and 10, respectively) account for the difference between the REL and the U.S. EPA RfC.

For comparison, the state of Minnesota Department of Health reports a subchronic Health Risk Value (HRV) for acrolein of 0.2 μg/m^3, a level thought to be without significant risk following inhalation exposure for 13 weeks (MDH, 2002).

8.4 Acrolein as a Toxic Air Contaminant

Acrolein was identified by the ARB as a toxic air contaminant (TAC) in accordance with section 39657(b) of the California Health and Safety Code on April 8, 1993 (Title 17, California Code of Regulations, Section 93001)(CCR, 2007). In view of the differential impacts on infants and children identified in Section 6.2 (more severe effects associated with bronchoconstriction and asthma exacerbation, less ability to escape or avoid exposure), OEHHA listed acrolein as a TAC which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c) .

9. References

Astry CL and Jakab GJ (1983). The effects of acrolein exposure on pulmonary antibacterial defenses. Toxicol Appl Pharmacol 67(1): 49-54.

Borchers MT, Carty MP and Leikauf GD (1999a). Regulation of human airway mucins by acrolein and inflammatory mediators. Am J Physiol 276(4 Pt 1): L549-55.

Borchers MT, Wert SE and Leikauf GD (1998). Acrolein-induced MUC5ac expression in rat airways. Am J Physiol 274(4 Pt 1): L573-81.

Borchers MT, Wesselkamper S, Wert SE, Shapiro SD and Leikauf GD (1999b). Monocyte inflammation augments acrolein-induced Muc5ac expression in mouse lung. Am J Physiol 277(3 Pt 1): L489-97.

Bouley G, Dubreuil A, Godin J, Boisset M and Boudene C (1976). Phenomena of adaptation in rats continuously exposed to low concentrations of acrolein. Ann Occup Hyg 19(1): 27-32.

Bouley G, Dubreuil A, Godin J and Boudene C (1975). [Effects in the rat of a weak dose of acrolein inhaled continuously]. Eur J Toxicol Environ Hyg 8(5): 291-7.

Buckley LA, Jiang XZ, James RA, Morgan KT and Barrow CS (1984). Respiratory tract lesions induced by sensory irritants at the RD50 concentration. Toxicol Appl Pharmacol 74(3): 417-29.

CARB (2005b). Annual Statewide Toxics Summary - acrolein Sacramento, CA. http://www.arb.ca.gov/adam/toxics/statepages/acrostate.html.

Cassee FR, Arts JH, Groten JP and Feron VJ (1996a). Sensory irritation to mixtures of formaldehyde, acrolein, and acetaldehyde in rats. Arch Toxicol 70(6): 329-37.

Cassee FR, Groten JP and Feron VJ (1996b). Changes in the nasal epithelium of rats exposed by inhalation to mixtures of formaldehyde, acetaldehyde, and acrolein. Fundam Appl Toxicol 29(2): 208-18.

CCR (2007). California Code of Regulations Section 93001 Hazardous Air Pollutants Identified as Toxic Air Contaminants. Sacramento, CA: California Office of Administrative Law. 8-20-07. http://ccr.oal.ca.gov/linkedslice/default.asp?SP=CCR-1000&Action=Welcome.

Chhibber G and Gilani SH (1986). Acrolein and embryogenesis: an experimental study. Environ Res 39(1): 44-9.

Claussen U, Hellmann W and Pache G (1980). The embryotoxicity of the cyclophosphamide metabolite acrolein in rabbits, tested in vivo by i.v. injection and by the yolk-sac method. Arzneimittelforschung 30(12): 2080-3.

Darley E, Middleton J and Garber M (1960). Plant damage and eye irritation from ozone-hydrocarbon reactions. Agricul Food Chem 8(6): 483-484.

Dorman DC, Struve MF, Wong BA, Marshall MW, Gross EA and Willson GA (2008). Respiratory tract responses in male rats following subchronic acrolein inhalation. Inhal Toxicol 20(3): 205-16.

Doty RL, Cometto-Muniz JE, Jalowayski AA, Dalton P, Kendal-Reed M and Hodgson M (2004). Assessment of upper respiratory tract and ocular irritative effects of volatile chemicals in humans. Crit Rev Toxicol 34(2): 85-142.

Feron VJ and Kruysse A (1977). Effects of exposure to acrolein vapor in hamsters simultaneously treated with benzo[a]pyrene or diethylnitrosamine. J Toxicol Environ Health 3(3): 379-94.

Feron VJ, Kruysse A, Til HP and Immel HR (1978). Repeated exposure to acrolein vapour: subacute studies in hamsters, rats and rabbits. Toxicology 9(1-2): 47-57.

Finkelstein EI, Nardini M and van der Vliet A (2001). Inhibition of neutrophil apoptosis by acrolein: a mechanism of tobacco-related lung disease? Am J Physiol Lung Cell Mol Physiol 281(3): L732-9.

Ginsberg GL, Foos BP and Firestone MP (2005). Review and analysis of inhalation dosimetry methods for application to children's risk assessment. J Toxicol Environ Health A 68(8): 573-615.

Gosselin B, Wattel F, Chopin C, Degand P, Fruchart JC, Van der Loo D and Crasquin O (1979). [A case of acute acrolein poisoning]. Nouv Presse Med 8(30): 2469-72.

Grafstrom RC, Sundqvist K, Dypbukt JM and Harris CC (1987). Pathobiological effects of aldehydes in cultured human bronchial cells. IARC Sci Publ(84): 443-5.

Gurtoo HL, Marinello AJ, Struck RF, Paul B and Dahms RP (1981). Studies on the mechanism of denaturation of cytochrome P-450 by cyclophosphamide and its metabolites. J Biol Chem 256(22): 11691-701.

IARC (1995). Dry Cleaning, Some Chlorinated Solvents and Other Industrial Chemicals. IARC monographs 63: 337-371.

Kane LE, Barrow CS and Alarie Y (1979). A short-term test to predict acceptable levels of exposure to airborne sensory irritants. Am Ind Hyg Assoc J 40(3): 207-29.

Kimbell JS, Godo MN, Gross EA, Joyner DR, Richardson RB and Morgan KT (1997). Computer simulation of inspiratory airflow in all regions of the F344 rat nasal passages. Toxicol Appl Pharmacol 145(2): 388-98.

Kimbell JS, Overton JH, Subramaniam RP, Schlosser PM, Morgan KT, Conolly RB and Miller FJ (2001). Dosimetry modeling of inhaled formaldehyde: Binning nasal flux predictions for quantitative risk assessment. Toxicological Sciences 64(1): 111-121.

Kutzman R. (1981). A subchronic inhalation study of Fischer 344 rats exposed to 0, 0.4, 1.4, or 4.0 ppm acrolein. National Toxicology Program: Interagency Agreeement No. 222-Y01-ES-9-0043. Brookhaven National Laboratory. Upton, NY

Kutzman RS, Popenoe EA, Schmaeler M and Drew RT (1985). Changes in rat lung structure and composition as a result of subchronic exposure to acrolein. Toxicology 34(2): 139-51.

Lam CW, Casanova M and Heck HD (1985). Depletion of nasal mucosal glutathione by acrolein and enhancement of formaldehyde-induced DNA-protein cross-linking by simultaneous exposure to acrolein. Arch Toxicol 58(2): 67-71.

Leikauf GD, Doupnik CA, Leming LM and Wey HE (1989a). Sulfidopeptide leukotrienes mediate acrolein-induced bronchial hyperresponsiveness. J Appl Physiol 66(4): 1838-45.

Leikauf GD, Leming LM, O'Donnell JR and Doupnik CA (1989b). Bronchial responsiveness and inflammation in guinea pigs exposed to acrolein. J Appl Physiol 66(1): 171-8.

Linhart I, Frantik E, Vodickova L, Vosmanska M, Smejkal J and Mitera J (1996). Biotransformation of acrolein in rat: excretion of mercapturic acids after inhalation and intraperitoneal injection. Toxicol Appl Pharmacol 136(1): 155-60.

Lyon JP, Jenkins LJ, Jr., Jones RA, Coon RA and Siegel J (1970). Repeated and continuous exposure of laboratory animals to acrolein. Toxicol Appl Pharmacol 17(3): 726-32.

Mahut B, Delacourt C, de Blic J, Mani TM and Scheinmann P (1993). Bronchiectasis in a child after acrolein inhalation. Chest 104(4): 1286-7.

Mannino DM, Homa DM, Pertowski CA, Ashizawa A, Nixon LL, Johnson CA, Ball LB, Jack E and Kang DS (1998). Surveillance for asthma--United States, 1960-1995. MMWR CDC Surveill Summ 47(1): 1-27.

McNulty M, Heck HD and Csasnova-Schmitz M (1984). Depletion of glutathione in rat respiratory mucosa by inhaled acrolein. Fed Proc 43: 575, abstract 1695.

MDH (2002). Health Risk Values for Air - Subchronic HRVs March 2002. Minnesota Department of Health,. http://www.health.state.mn.us/divs/eh/air/hrvtable.htm#sub.

Morris JB, Symanowicz PT, Olsen JE, Thrall RS, Cloutier MM and Hubbard AK (2003). Immediate sensory nerve-mediated respiratory responses to irritants in healthy and allergic airway-diseased mice. J Appl Physiol 94(4): 1563-71.

Nazaroff WW and Singer BC (2004). Inhalation of hazardous air pollutants from environmental tobacco smoke in US residences. J Expo Anal Environ Epidemiol 14 Suppl 1: S71-7.

NTIS. (1981). Acrolein health effects. Final task 6 report. Contract # 68-03-2928. National Technical Information Service. Arlington (VA)

OEHHA. (2001). *Prioritization of toxic air contaminants - children's health protection act - final report*. Available on-line at: <http://www.oehha.ca.gov/air/toxic_contaminants/SB25finalreport.html>.

Parent RA, Paust DE, Schrimpf MK, Talaat RE, Doane RA, Caravello HE, Lee SJ and Sharp DE (1998). Metabolism and distribution of [2,3-14C]acrolein in Sprague-Dawley rats. II. Identification of urinary and fecal metabolites. Toxicol Sci 43(2): 110-20.

Penn A, Nath R, Pan J, Chen L, Widmer K, Henk W and Chung FL (2001). 1,N(2)-propanodeoxyguanosine adduct formation in aortic DNA following inhalation of acrolein. Environ Health Perspect 109(3): 219-24.

Prentiss A (1937). Chemicals in War. In. McGraw-Hill. New York: 139-140.

Roemer E, Anton HJ and Kindt R (1993). Cell proliferation in the respiratory tract of the rat after acute inhalation of formaldehyde or acrolein. J Appl Toxicol 13(2): 103-7.

Roux E, Hyvelin JM, Savineau JP and Marthan R (1999). Human isolated airway contraction: interaction between air pollutants and passive sensitization. Am J Respir Crit Care Med 160(2): 439-45.

Schroeter JD, Kimbell JS, Gross EA, Willson GA, Dorman DC, Tan YM and Clewell HJ, 3rd (2008). Application of physiological computational fluid dynamics models to predict interspecies nasal dosimetry of inhaled acrolein. Inhal Toxicol 20(3): 227-43.

Slott VL and Hales BF (1986). The embryolethality and teratogenicity of acrolein in cultured rat embryos. Teratology 34(2): 155-63.

Struve MF, Wong VA, Marshall MW, Kimbell JS, Schroeter JD and Dorman DC (2008). Nasal uptake of inhaled acrolein in rats. Inhal Toxicol 20(3): 217-25.

Takabe W, Niki E, Uchida K, Yamada S, Satoh K and Noguchi N (2001). Oxidative stress promotes the development of transformation: Involvement of a potent mutagenic lipid peroxidation product, acrolein. Carcinogenesis (Oxford) 22(6): 935-941.

Tanaka K, Nishiyama K, Yaginuma H, Sasaki A, Maeda T, Kaneko SY, Onami T and Tanaka M (2003). [Formaldehyde exposure levels and exposure control measures during an anatomy dissecting course]. Kaibogaku Zasshi 78(2): 43-51.

U.S.EPA. (2003). Toxicological review of acrolein. EPA/635/R-03/003. U.S. Environmental Protection Agency. Washington DC

Weber-Tschopp A, Fischer T, Gierer R and Grandjean E (1977). [Experimentally induced irritating effects of acrolein on men (author's transl)]. Int Arch Occup Environ Health 40(2): 117-30.

WHO. (1992). *Environmental health criteria 127. Acrolein*. World Health Organization. www.inchem.org/documents/ehc/ehc/ehc127.htm#SectionNumber:10.1.

Yang XM, Wu XL, Choi YE, Kern JC and Kehrer JP (2004). Effect of acrolein and glutathione depleting agents on thioredoxin. Toxicology 204(2-3): 209-218.

Inorganic Arsenic Reference Exposure Levels

1. Summary

Acute, 8-hour and chronic reference exposure levels (RELs) were derived for inorganic arsenic including arsine. Inorganic arsenic causes a wide variety of toxic effects in humans and experimental animals including effects on development, the vascular system, the nervous system, blood, lung, and skin. The most sensitive acute effects were seen in mice (fetal development) whereas the most sensitive 8-hour and chronic effects were decreased intellectual function in children. The relevant literature evaluated in this assessment was published before April 1, 2008. The key values are summarized below.

1.1 Inorganic Arsenic Acute REL

Reference Exposure Level	**0.2 µg As/m³**
Critical effect(s)	Decreased fetal weight in mice
Hazard Index target(s)	Development (teratogenicity); cardiovascular system; nervous system

1.2 Inorganic Arsenic 8-Hour REL

Reference Exposure Level	**0.015 µg/ As/m³**
Critical effect(s)	Decreased intellectual function in 10 year old children
Hazard Index target(s)	Development; cardiovascular system; nervous system; lung; skin

1.3 Inorganic Arsenic Chronic REL

Reference Exposure Level	**0.015 µg As/m³**
Oral Reference Exposure Level	0.0035 µg/kg bw-day
Critical effect(s)	Decreased intellectual function in 10 year old children
Hazard Index target(s)	Development; cardiovascular system; nervous system; lung; skin

2. Physical & Chemical Properties

Table 2.1 Arsenic and Arsenic Species*

Molecular formula	Molecular weight	Percent As by weight	Synonyms	CAS Registry Number
As	74.92	100%	Arsenic black, metallic arsenic	7440-38-2
As_2O_3 As_4O_6	197.82 395.68	75.7%	Arsenious oxide, arsenic (III) trioxide, arsenic oxide, arsenous acid, arsenous acid anhydride, Crude Arsenic, White Arsenic	1327-53-3
$AsCl_3$	181.28	41.3%	Arsenic butter, trichloroarsine, arsenious chloride	7784-34-1
As_2O_5	229.82	65.2%	Arsenic pentoxide, arsenic anhydride, arsenic oxide, arsenic acid anhydride	1303-28-2
$AsHNa_2O_4$	185.91	40.3%	Arsenic acid disodium salt, disodium arsenate, sodium arsenate dibasic	7778-43-0
$AsHNa_2O_3$	130.92	57.2%	Arsenous acid disodium salt, arsenious acid sodium salt	7784-46-5
AsH_3	77.94	96.12	Arsine, arsane, arsenic hydride, arsenous hydride, hydrogen arsenide, arsenic trihydride	7784-42-1
$As(OH)_3$	125.94	59.49	Arsenous acid	13464-58-9
$AsO(OH)_3$	141.93	52.78	Arsenic acid, orthoarsenic acid	7778-39-4
As_4S_4	427.92	70.03	Arsenic disulfide, realgar, red arsenic sulfide	
$CH_3AsO(OH)_2$	139.97	53.51	Monomethylarsonic acid	124-58-3
$CH_3As(OH)_2$	123.77	60.41	Monomethylarsonous acid	25400-23-1
$(CH_3)_2AsO(OH)$	137.99	54.28	Dimethylarsinic acid, cacodylic acid	75-60-5
$(CH_3)_2AsOH$	121.99	61.40	Dimethylarsinous acid	55094-22-9
$(CH_3)_3AsO$	136.02	55.06	Trimethylarsine oxide	4964-14-1

*Note: Methylated arsenic species occurring naturally and as metabolites (IARC, 2004)

2.1 Arsenic (Metallic) (ATSDR, 2000)

Description	Yellow, black or gray solid
Molecular formula	see Table 2.1
Molecular weight	see Table 2.1
Specific gravity (water = 1)	5.778 g/cm^3 @ 25°C
Boiling point	613°C (sublimes) at 760 mm Hg
Vapor pressure	7.5 x 10^{-3} mmHg at 280 °C
Flashpoint	not applicable
Explosive limits	not applicable
Solubility	soluble in nitric acid, insoluble in water
Odor threshold	not applicable
Odor description	odorless
Metabolites	dimethylarsinic acid, methylarsonic acid
Conversion factor	not applicable for As

2.2 Arsenic Trioxide (ATSDR, 2000)

Description	As_2O_3: White solid, glassy, amorphous lumps or crystal
Molecular formula	See Table 2.1
Molecular weight	197.84
Density	As_2O_3: 3.865 g/cm^3
Boiling point	As_2O_3: 460°C
Melting point	As_2O_3: 274°C
Solubility	Oxides: slightly soluble in water 17g/L, insoluble in alcohol, chloroform, ether.
Metabolites	Dimethylarsinic acid, methylarsonic acid

2.3 Arsine (U.S. EPA, 2006a)

Description	Colorless gas
Molecular formula	AsH_3
Molecular weight	77.93
Specific gravity (Water = 1)	1.689 @ 84.9°C
Boiling point	-62.55°C
Melting point	-117°C
Vapor pressure	Greater than 1 atm
Vapor density (Air = 1)	2.695
Solubility	soluble in chloroform and benzene, slightly soluble in water (20 mL/100 mL at 20 C), ethyl alcohol and in alkalis
Odor threshold	0.5 ppm
Odor description	garlic-like or fishy odor
Metabolites	oxidation to arsenite, arsenate, other unidentified (Landrigan *et al*., 1982; Carter *et al*., 2003)

Conversion factor 1 ppm = 3.19 mg/m³ @ 25°C

3. Occurrence and Major Uses

Arsenic is ubiquitous and is found in small amounts in soils and water throughout the world and also in foods, particularly seafood (NIOSH, 1975). Ore refining processes, including the smelting of copper and lead, are the major sources of release of arsenic dust and inorganic arsenic compounds. Arsenic trioxide is the form of inorganic arsenic most commonly produced. It is used as a raw material for the production of other inorganic arsenic compounds (Asi), alloys, and organic arsenic compounds (Grayson, 1978).

Pesticides have historically constituted the largest single use (50%) of arsenic compounds (HSDB, 1995). The major arsenic herbicides manufactured are monosodium methyl arsonate (MSMA), disodium methyl arsonate (DSMA), and dimethyl arsenic acid (cacodylic acid). Inorganic arsenic compounds are also used as herbicides (arsenite), insecticides (arsenic trioxide, calcium and other arsenates), or rodenticides (sulfides) (ACGIH, 1992). Arsenic trichloride, for example, is used mainly as a chemical intermediate in the production of insecticides, but has other applications in the ceramics and pharmaceutical industries (HSDB, 1995). Arsenic was used as a pesticide to treat tobacco; thus, cigarette smoke was another common source of exposure (U.S.EPA, 1984). The use of arsenic compounds in agriculture has reduced in recent years and U.S. EPA is considering ending their uses under the pesticide reregistration program (U.S. EPA, 2006b).

Arsenic-based wood preservatives have constituted the next largest use (40%) of arsenic compounds (HSDB, 1995). In December 2003 the U.S. EPA terminated all residential uses of wood preservatives containing arsenic limiting such products to restricted use by certified pesticide applicators (U.S. EPA, 2002).

The highly toxic trivalent arsenic compounds, such as arsenic trioxide, are typically introduced into the environment as a result of industrial processes including the smelting of metal ores. Pentavalent arsenic compounds are generally considered to be less toxic and are most frequently found naturally.

Processes such as smelting, galvanizing, soldering, and etching, that require the treatment of metal with strong acids, are possible sources of arsine gas. Acid treatment of metals contaminated with arsenic can result in the release of arsine gas. Arsine is used to provide arsenic as an ingredient in semiconductor manufacture. Combustion of fossil fuels may produce arsine gas.

4. Toxicokinetics

A knowledge of the metabolism of inorganic arsenic has long been thought to be essential to understanding the mode(s) of action of inorganic arsenic toxicity. Trivalent (+3, As^{III}) arsenic species (e.g., arsenite) have often exhibited greater acute toxicity than pentavalent (+5, As^{V}) species (e.g., arsenate). The terms arsenite and arsenate refer to

the ionized anions of arsenous acid and arsenic acid, respectively, as they exist in aqueous solution at physiological pH. Since the metabolism of inorganic arsenic in mammalian species generally proceeds via alternate reductive and oxidative methylation steps to mono- (MMA) and dimethyl (DMA) arsenic acids, it was believed that methylation represented detoxication of inorganic arsenic. However, recent evidence supports the idea that trivalent methylated species are in some cases more toxic than inorganic precursors and may play a key role in arsenic toxicity for selected endpoints. The metabolism of arsine (As^{-III}), while less studied, appears to progress similarly after its oxidation to arsenite (As^{V}) and is in part the basis for including arsine in the RELs for inorganic arsenic.

Several comprehensive reviews of the absorption, distribution, metabolism and elimination of arsenic have been published (Vahter, 1983; Thompson, 1993; ATSDR, 2000; NRC, 2001). Most information on the toxicokinetics of arsenic derives from oral exposure studies. The kinetics of arsenic varies depending on the chemical form of arsenic and on the animal species. The following discussion is limited to the oxidized forms found in water and air and forms that are ingested via the aquatic food chain. These include the inorganic, soluble forms of arsenite (As^{III}) and arsenate (As^{V}), as well as the organic monomethylarsonate (MMA), dimethylarsinic acid (DMA), trimethylarsine (TMA), and or arsenobetaine (in fish).

4.1 Inorganic Arsenic Oxides

Owen (1990) reported inhalation absorption of 32 percent (range 30 to 34 %) from arsenic containing aerosols, however it is uncertain if this figure included the gastrointestinal absorption of arsenic particles from the upper respiratory tract. The International Commission on Radiological Protection Human Respiratory Tract Model (ICRP, 1994) gives total deposition fractions for 10 yr old children inhaling 1 µm activity median thermodynamic diameter particles at 0.31 to 2.03 m^3/hr of 0.42 to 0.58. There are relatively few data on the kinetics of airborne arsenic excretion. Mann *et al*. (1996a) modeled inhalation exposures based on the occupational data of Vahter *et al*. (1986) and Offergelt *et al*. (1992). For simulated occupational exposures of 10 µg/m^3 of arsenic aerosol of MMAD of 5.0 µm, GSD of 2.1, 1.2 L tidal volume and a breathing rate of 16 /min, urinary excretion increased over the work week's exposure from 7 to 25 µg As/g creatinine.

The MMAD refers to the mass median aerodynamic diameter and GSD the geometric standard deviation. These values characterize a distribution of particles in an aerosol. The units refer to the first order rate constant for the absorption of arsenic into the blood plasma from the model lung compartments. The model has separate compartments for the nasopharynx, tracheobroncheal, and pulmonary regions of the lung. Deposition of particles in these lung compartments, in units of µg/hr, depends on breathing rate, tidal volume, concentration of particles in the air, and their aerodynamic diameters. Absorption of deposited particles into blood plasma is first order but depends upon the surface area of the region in question, hence the units of /cm^2-hr.

Model predictions of arsenic metabolites (Asi, MMA, DMA) in postshift urine generally fell within the range of observations for 18 workers in the exposure range of 10-1000 µg As/m^3. After daily inhalation exposure of 100 µg As (III)/m^3 for three weeks, the model predictions for urinary metabolite distribution closely matched observed values (predicted/observed means: Asi, 1.05; MMA, 1.0; DMA, 1.0). From the model, Mann *et al*. (1996b) derived a fitted lung absorption first order rate constant for arsenic trioxide dust of 0.01/cm^2-hr.

In general, investigations that have monitored arsenic excretion of experimental animals following parenteral administration have demonstrated that only a small fraction of the administered arsenic is excreted in the feces. Thus, to estimate the amount of inorganic arsenic absorbed following oral administration, most kinetic and metabolic studies have monitored the urine. Soluble compounds of inorganic arsenic, whether in the trivalent or pentavalent form, are readily absorbed (80-90 percent) in most animal species following oral administration (Charbonneau *et al*., 1978; Vahter, 1981; Hughes *et al*., 1994; Freeman *et al*., 1995). However, only about 40-50 percent absorption has been reported in hamsters (Yamauchi and Yamamura, 1985; Marafante and Vahter, 1987). Absorption of orally administered inorganic arsenic in humans has been shown to range between 54-80 percent (Tam *et al*., 1979; Buchet *et al*., 1981b; a; Kurttio *et al*., 1998).

Inorganic arsenic compounds are poorly absorbed through the skin (Ca.1-5%); the trivalent is more rapidly absorbed than the pentavalent (Wester *et al*., 1993; Wester *et al*., 2004).

Organic forms of arsenic are also extensively absorbed from the gastrointestinal tract. Experimental studies examining the absorption of MMA, DMA, TMA and arsenobetaine in humans have demonstrated 75-92 percent absorption. At low-level exposures, excretion of arsenic and its metabolites seems to balance absorption of inorganic arsenic. With increasing arsenic intake, there is suggestive evidence that methylation appears less complete. Studies, which examine the effect of dose on excretion patterns, have been conducted in mice and humans (Buchet *et al*., 1981b; a; Vahter, 1981). As the dose of inorganic arsenic increases, the percent of arsenic excreted as DMA decreases, accompanied by an increased excretion in the percent as inorganic arsenic. The percent excreted as MMA remains virtually unchanged. *In vitro* metabolism studies on the methylation of inorganic arsenic have demonstrated that the liver is the site of methylating activity and that S-adenosylmethionine and reduced glutathione are required as methyl donors (Buchet and Lauwerys, 1985; 1987).

While absorption from the gastrointestinal tract is the most important route of exposure for waterborne arsenic, some potential for dermal absorption has been reported. Rahman *et al*. (1994) conducted *in vitro* studies with sodium [^{74}As] arsenate and clipped full-thickness mouse skin in a flow-through system. Doses of 5, 50, 500, or 5000 ng were applied to 0.64 cm^2 of skin as a solid, in aqueous vehicle, or in soil. Absorption of sodium arsenate increased linearly with applied dose from all vehicles. The maximum absorption of 62 percent of applied dose was obtained with the aqueous vehicle and the least (0.3 percent) with soil. Wester *et al*. (1993) evaluated the percutaneous absorption of [^{73}As] arsenate from soil or water *in vivo* in Rhesus monkeys and *in vitro* in

human cadaver skin. Water solutions of [^{73}As] arsenate at low (0.024 ng/cm^2) or high (2.1 µg/cm^2) surface concentrations were compared. With topical administration for 24 hr, *in vivo* absorption in the Rhesus monkey was 6.4 ± 3.9 (SD) percent from the low dose and 2.0 ± 1.2 (SD) percent from the high dose. *In vitro* percutaneous absorption of the low dose from water in human skin was 0.93 ± 1.1 percent in receptor fluid and 0.98 ± 0.96 percent in the washed skin; the total was about 1.9 percent. Absorption from soil (0.4 ng/cm^2) was less, at 6.4 percent in the monkey *in vivo* and 0.8 percent in human skin *in vitro*.

The retention and distribution patterns of arsenic are in part determined by its chemical properties. Arsenite (As^{III}) reacts and binds to sulfhydryl groups while arsenate (As^{V}) has chemical properties similar to those of phosphate. As^{V} also has affinity for sulfhydryl groups; however, its affinity is approximately 10-fold less than As^{III} (Jacobson-Kram and Montalbano, 1985). The distribution and retention patterns of As^{III} and As^{V} are also affected by species, dose level, methylation capacity, valence form, and route of administration.

Vahter *et al*. (1984) studied tissue distribution and retention of ^{74}As-DMA in mice and rats. About 80 percent of an oral dose of 0.4 mg As/kg was absorbed from the gastrointestinal tract. In mice >99 percent of the dose was excreted within 3 d compared to only 50 percent in rats, due largely to accumulation in blood, which delayed excretion. Tissue distribution in mice showed the highest initial (0.5-6 hr) concentrations in kidneys, lungs, intestinal mucosa, stomach, and testes. Tissues with the longest retention times were lungs, thyroid, intestinal walls, and lens.

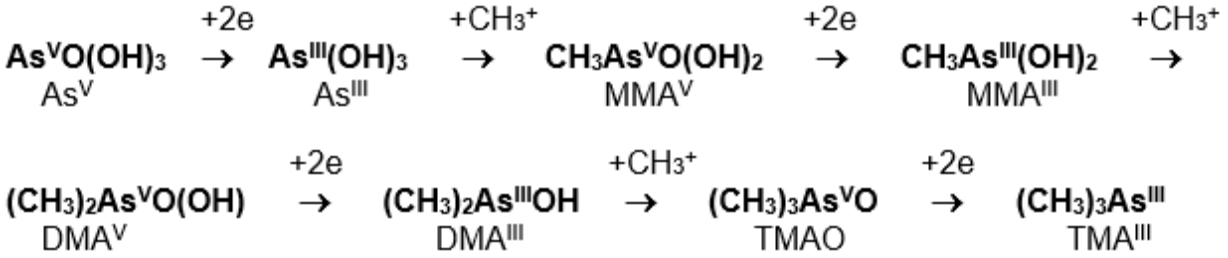
The effect of dose on arsenate disposition was evaluated in adult female B6C3F$_1$ mice dosed orally with 0.5 to 5000 µg/kg [^{73}As]-arsenate in water (Hughes *et al*., 1994). Urine was collected at several time points over a 48-hr period, and feces at 24 and 48 hr post-exposure. The recovery of As-derived radioactivity in excreta and tissues ranged from 83.1 to 89.3 percent of dose. As-derived radioactivity was detected in several tissues (urinary bladder, gall bladder, kidney, liver, lung) although the sum for each exposure level was very low (<0.5 percent of dose). The principal depot was the liver, followed by the kidneys. As the dose of arsenate increased there was a significant increase in the accumulation of radioactivity in the urinary bladder, kidney, liver, and lungs. The greatest concentration of As radioactivity was in the urinary bladder.

Most studies of arsenic metabolism have involved administration of inorganic arsenic (Asi) as arsenate (As^{V}) or arsenite (As^{III}) to an experimental animal or a human, and detection of Asi and the methylated metabolites methylarsonic acid (MMA^{V}) and dimethylarsinic acid (DMA^{V}) in urine, feces, and tissues.

Thompson (1993) conducted an extensive review and analysis of the mammalian metabolic data on arsenic. The metabolism of arsenate can be viewed as a cascade of reductive and oxidative methylation steps leading successively to As^{III}, MMA^{V}, MMA^{III}, DMA^{V}, DMA^{III}, $TMAO^{V}$, and TMA as outlined in Scheme 1. Recently Hayakawa *et al*. (2005) proposed a new metabolic pathway for arsenite, which does not involve oxidative methylation but rather is mediated by As-glutathione complexes, S-adenosylmethionine

(SAM) and human arsenic methyltransferase Cyt19. In this pathway arsenic triglutathione ($As(SG)_3$) is converted to monomethyl-(MADG) and dimethyl-(DMAG) conjugates which are hydrolyzed to MMA^{III} and DMA^{III}, respectively. Thus pentavalent methylated metabolites might arise via oxidation of their trivalent forms rather than the reverse as shown in Scheme 1.

Scheme 1. Biomethylation of Arsenic Involving Alternate Reduction of Pentavalent Arsenic to Trivalent Arsenic Followed by Oxidative Addition of a Methyl Group (after Jiang et al. (2003))



MMA^{III} and DMA^{III} have only recently been detected as stable urinary metabolites in human subjects (Aposhian *et al*., 2000a; Aposhian *et al*., 2000b; Le *et al*., 2000a; Le *et al*., 2000b), and trimethylarsine oxide (TMAO) and trimethylarsine (TMA) are rarely seen and are very minor metabolites in most mammals if found at all. Few data are available on the tissue concentrations of trivalent methylated As species (Kitchin, 2001). Gregus *et al*. (2000) found that in bile duct-cannulated rats, As^{III} and its metabolites were preferentially excreted into bile (22 percent) versus eight percent into urine in two hr. Arsenite appeared in bile rapidly and constituted the large majority in the first 20 min. Thereafter As^{III} declined and MMA^{III} output gradually increased. From 40 min after i.v. As^{III} administration, MMA^{III} was the dominant form of biliary arsenic. Within two hr 9.2 percent of the dose was excreted in the bile as MMA^{III}. Injection of arsenate produced a mixture of As^{V}, As^{III} and MMA^{III} in the bile. Curiously, rats injected with MMA^{V} did not excrete MMA^{III}.

The metabolism results of Styblo *et al*. (1995) in rat liver cytosol *in vitro* seem to support the overall metabolic scheme noted above; MMA^{III} and MMA^{III}-diglutathione complex are more rapidly methylated to the dimethyl forms than MMA^{V}. Thompson also suggests that the data support the presence of two inhibitory loops: (1) competitive inhibition by MMA^{III} of the $As^{III} \rightarrow MMA^{V}$ step catalyzed by monomethyltransferase (MMTase); and (2) possibly noncompetitive inhibition by As^{III} of the $MMA^{III} \rightarrow DMA^{V}$ step catalyzed by dimethyltransferase (DMTase).

Styblo *et al*. (1996) observed 50 µ*M* arsenite inhibition of DMA^{V} production in rat liver cytosol *in vitro*. Healy *et al*. (1998) studied the activity of MMTase in tissues of mice. The activity was determined with sodium arsenite and *S*-[methyl-^{3}H]-adenosyl-L-methionine by measuring the formation of [methyl-^{3}H] monomethylarsonate. The mean MMTase activities (units/mg ± SEM) measured in cytosol of mouse tissues were: liver, 0.40 ± 0.06; testis, 1.45 ± 0.08; kidney, 0.70 ± 0.06; and lung, 0.22 ± 0.01. When mice were given arsenate in drinking water for 32 or 92 days at 25 or 2500 µg As/L, the

MMTase activities were not significantly increased compared to controls. MMTases and DMTases have been partially purified from the livers of rabbits (Zakharyan *et al*., 1995), Rhesus monkeys (Zakharyan *et al*., 1996) and hamsters (Wildfang *et al*., 1998). All of the enzyme preparations exhibited Michaelis-Menten enzyme kinetics with Km values ranging from $8x10^{-4}$ *M* for hamster DMTase to $1.8x10^{-6}$ *M* for hamster MMTase. Vmax values ranged from 0.007 pmol/mg protein/hr for hamster DMTase to 39.6 pmol/mg protein/hr for rabbit MMTase. Comparative studies have shown several species to be deficient in methyltransferase activities, notably New World monkeys, marmosets, tamarin, squirrel, chimpanzee, and guinea pig (Vahter *et al*., 1995b; Aposhian, 1997). While comparisons with human arsenic methyl transferase are limited by lack of a purified human enzyme, based on excretion profiles of urinary metabolites the rabbit and hamster appear most pharmacokinetically similar to humans than the other species studied. Walton *et al*. (2003) compared the methylation of arsenite by rat and human primary hepatocytes in vitro (control values in their Tables 1 and 2). For the rat the methylation rate after a 3 hr incubation with 0.1 µM arsenite was 99.3 $\pm$ 1.87 pmol CH_3/hr/10^6 cells (mean $\pm$ SD, N =4). The human hepatocytes similarly exposed for 24 hr had a methylation rate of 1.68 $\pm$ 0.24 pmol CH_3/hr/10^6 cells, over a 50-fold difference in apparent methylation rate.

While the reduction of arsenate and MMA^V can be accomplished nonenzymatically *in vitro*, and arsenate reduction by glutathione occurs in mammalian blood *in vivo* (Vahter and Envall, 1983; Winski and Carter, 1995), these reductive steps are most likely enzymatically mediated *in vivo*. An arsenate reductase has been partially purified from human liver and described (Radabaugh and Aposhian, 2000). The approximate mass of the enzyme was 72,000. It was specific for arsenite (i.e., did not reduce [^{14}C] MMA^V) and exhibited substrate saturation at about 300 µM. The human arsenate reductase requires a thiol and a heat-stable cofactor and is apparently distinct from those isolated from bacteria (Ji and Silver, 1992; Gladysheva *et al*., 1994; Krafft and Macy, 1998).

Monomethyl arsonate (MMA^V) reductases have been isolated and described for rabbit (Zakharyan and Aposhian, 1999) and hamster (Sampayo-Reyes *et al*., 2000). In the latter study the distribution of MMA^V reductase activity was 91.4 nmol MMA^{III}/mg protein/hr in brain and 61.8 nmol MMA^{III}/mg protein/hr in bladder. Skin, kidney and testis all had less than 15 nmol/mg/hr. Spleen, liver, lung, and heart were all between 15 and 62 nmol/mg/hr. The high activity of MMA^V reductase in brain is curious and may help explain some of the neurotoxic effects of arsenic. Due to relatively low affinity of the MMA^V reductase (K_M = $2.2x10^{-3}$ M) compared to the methyl transferases (K_M = 5-$9x10^{-6}$ M), the MMA^V reduction is thought to be the rate-limiting step in arsenic metabolism (Zakharyan and Aposhian, 1999). The partially purified human liver MMA^V reductase has been shown to be identical with human glutathione S-transferase Omega class hGSTO 1-1 (Zakharyan *et al*., 2001).

DMA is the main metabolite found in the tissues and urine of most experimental animals administered inorganic arsenic. Humans are also somewhat unique in that MMA has been found to be an important metabolite of inorganic arsenic in addition to DMA. Studies conducted on human volunteers given a single oral dose of inorganic arsenic

demonstrated that within 4-7 days, 46-62 percent of the dose was excreted in the urine (Tam *et al*., 1979; Pomroy *et al*., 1980; Buchet *et al*., 1981b; a). Approximately 75 percent of the excreted arsenic is methylated, about one-third as MMA and two-thirds as DMA.

The possibility of genetic polymorphism in arsenic metabolism has been suggested by Vahter *et al*. (1995a), who studied native Andean women in northwestern Argentina who were exposed to a wide range of As concentrations in drinking water (2.5 to 200 µg As/L). The women exposed to the highest As concentration in water exhibited surprisingly low levels of MMA in their urine (2.3 percent of metabolites). The percentage of arsenic urinary metabolites as MMA in typical human urine ranges from 12 to 20. Chiou *et al*. (1997a) studied the relationships among arsenic methylation capacity, body retention, and genetic polymorphisms of glutathione-*S*-transferase (GST) M1 and T1 in 115 human subjects. Percentages of As species in urine (mean ± SE) were: Asi, 11.8 ± 1.0; MMA, 26.9 ± 1.2; and DMA, 61.3 ± 1.4. Genetic polymorphisms of GST M1 and T1 were significantly associated with As methylation. Subjects with the null genotype of GST M1 had an increased percentage of Asi in urine, while those with the null genotype GST T1 had elevated DMA in their urine samples.

Marnell *et al*. (2003) reported six polymorphisms in the MMA^V reductase hGSTO1 gene in DNA isolated from peripheral blood of 75 Mexican subjects. Two subjects with the same polymorphism showed 5 to 10 fold higher concentrations (µg/g creatinine) of Asi in their urine than other subjects.

Yu *et al*. (2003) screened DNA of 22 subjects of European ancestry (EA) and 24 of indigenous American ancestry (IA) for polymorphisms in arsenate reductase and MMA^V reductase genes. For the arsenate reductase gene (hPNP) 48 polymorphic sites were identified while 33 were found in the MMA^V reductase gene (hGSTO1-1). For the EA individuals the MMA^V reductase gene showed greater polymorphism than the arsenate reductase gene whereas the reverse was seen in the IA individuals. In the latter group only one polymorphism had a frequency of > 10%. Meza *et al*. (2005) screened 135 As-exposed subjects from Sonora, Mexico for polymorphisms in arsenic metabolism genes: arsenate reductase (hPNP); MMA^V reductase (hGSTO); and arsenic 3 methyltransferase (CYT19). The subjects were exposed to drinking water with 5.5 to 43.3 ppb arsenic. The screening was based on urinary DMA^V/MMA^V (D/M) ratios. The analysis revealed that all of the variation was due to a very strong association between CYT19 and D/M in children only (7-11 yr). With children removed no significant association was seen in adults (18-79 yr). This developmentally regulated association between CYT19 and arsenic metabolism raises questions about the adequacy of arsenic risk assessment for children.

Several authors have studied the kinetics of As excretion in humans. Tam *et al*. (1979) administered ^{74}As arsenic acid (0.01 µg, ca. 6 µCi) to six adult males (age: 28-60; body weight: 64-84 kg) following an overnight fast. The urine was analyzed at 24 hr intervals for five days following As administration. In the first 24 hr period Asi excretion exceeded that of the methylated metabolites but thereafter the usual DMA > MMA > Asi pattern persisted, with DMA increasing in percentage of cumulative excretion at the later time

points. A follow up study (Pomroy *et al*., 1980) followed ^{74}As excretion for periods up to 103 days using a whole body counter, with measurement of excreta for the first seven days. Their results indicate that the excretion data were best represented by a three-component exponential function. The coefficients for the pooled data accounted for 65.7 percent of excretion with a half-life of 2.09 days, 30.4 percent with a half-life of 9.5 days, and 3.7 percent with a half-life of 38.4 days. A four-exponent function showed a better fit to one of the six subjects (half-lives: 0.017, 1.42, 7.70 and 44.1 days).

Physiologically-based pharmacokinetic (PBPK) models employ data from various sources to mathematically simulate the uptake, distribution, metabolism and excretion of toxic chemicals in species of interest. Such models are used in risk assessment to estimate target tissue doses and to facilitate route-to-route and interspecies extrapolations. By contrast, pharmacodynamic (PD) models simulate biological responses to chemical exposures. A number of PBPK models for arsenic disposition and metabolism have been developed for experimental animals and humans (Mann *et al*., 1994; Menzel *et al*., 1994; Mann *et al*., 1996a; 1996b; Yu, 1999; Gentry *et al*., 2004). Although these models are based on somewhat different principles, they all seem to do a fair job in predicting the overall disposition of arsenic in animals and man. However, while the models often incorporate the latest ideas on the metabolism of inorganic arsenic with respect to oxidation state, methylated metabolites, and enzyme inhibition, due to limitations in our understanding of the modes of action of arsenic toxicity, they have yet to include representations of biological responses or pharmacodynamic (PD) capabilities, such as dosimetry linked alterations of DNA methylation, cell signaling pathways, DNA repair inhibition or generation of reactive oxygen species.

As an example of the complexity of arsenic action, Gentry *et al*. (2004) observed that pharmacodynamic changes occurred in mice without changes in PBPK predicted arsenic tissue dosimetry. These authors used the PBPK model of Mann *et al*. (1996a,b) extended to mice to evaluate possible dosimetry differences between mouse a strain susceptible to arsenic induced tumors (C57Bl/6J) and those that lacked susceptibility (e.g., Swiss CD-1, Swiss CD: NIH(S), C57Bl/6p53 (+/-)). The model was parameterized using published acute mouse data for arsenate, arsenite, MMA and DMA and validated with acute exposure data from the C57Black mouse strain. Model predictions for acute exposure were then compared with data from acute (24 hr) and chronic exposures (26 weeks). No differences were seen in the volume of distribution or tissue-plasma concentration ratios between acute and chronic exposures. Comparison of metabolite profiles in blood, liver and urine also showed little difference between acute and chronic exposures. Model predictions compared well with observed values. The authors concluded "… that pharmacokinetic factors do not provide an explanation for the difference in outcomes across the various mouse bioassays." This conclusion may be overly broad since all the metabolites of arsenic and its metabolic pathways were not included in the PBPK modeling.

Liao et al. (2008) employed PBPK models with age-specific parameters to estimate urinary excretion of methylated arsenic metabolites in children. The results were coupled with skin lesion data from West Bengal, Bangladesh and Taiwan to derive dose-response relationships based on MMA^{III} in urine and concentration and duration of

exposure to inorganic arsenic in drinking water using the Weibull (dose and time) model. While MMA^{III} was not specifically modeled a ratio of 7.4/2.8% MMA^{III}/MMA^{V} in total urinary MMA excretion was assumed. Age-specific risks at the $ED_{0.1}$ level (10^{-3} risk) were calculated for 0 -<1, 1-6, 7-12, and 13-18 yr age groups. Hyperpigmentation was a more sensitive endpoint than keratosis and males gave lower $ED_{0.1}$ values than females with values of 2.82, 1.51, 1.08, and 0.91 µg As/L for hyperpigmentation in males in the respective age groups. Age specific median daily drinking water consumption rates of 0.65, 1.29, 1.75, and 2.22 L/d, respectively, were used. Although the authors claim these concentrations as "Recommended Safe" levels, they are specific for 1/1000 risk and the skin lesion endpoint, which is not the most sensitive adverse effect for arsenic in exposed children.

4.2 Arsine

Although most studies of arsenic metabolism have centered on arsenate and arsenite, other forms of arsenic are also metabolized in humans. Apostoli *et al*. (1997) reported on the metabolism of arsine gas ($As^{-III}H_3$) in an occupationally exposed worker. Arsenic species were analyzed in urine over a five-day post-exposure period by liquid chromatography and inductively coupled plasma mass spectroscopy. The As species most excreted were MMA, DMA, As^{III}, arsenobetaine (AsB), and to a lesser extent As^{V}. The data indicate a capability to oxidize As^{-III} to As^{V} species probably via arsenite $As(OH)_3$. Arsenobetaine, an important form of arsenic in food, does not undergo subsequent biotransformation and is excreted via the urine. Curiously, arsenobetaine does not appear to be a metabolite of arsine in rats exposed for 1 hour to 4 to 80 mg/m^3 arsine (Buchet *et al*., 1998). The apparent similarity of the metabolism of arsine and arsenite is important and supports the use of the inorganic arsenic RELs for arsine.

Carter *et al*. (2003) have reviewed the metabolism of arsenic oxides, gallium arsenide and arsine. These authors describe three reactions that appear to occur in aqueous solutions of arsine (-III): (1) the formation of elemental As^0 and hydrogen; (2) reaction of AsH_3 with oxidized thiols to form diarsine AsH_2-AsH_2 (proposed) and reduced thiol RSH; and (3) possible reaction between arsine and oxygen species, producing arsine hydroperoxide H_2AsOOH (Hatlelid *et al*., 1995; 1996). Relatively few studies of arsine metabolism have been conducted in experimental animals. In vitro studies indicate that arsine was rapidly distributed to red blood cells. In plasma arsine appeared to decompose over a few hours. Arsine apparently undergoes rapid oxidative metabolism although the intermediary metabolites have not been identified and apparently are not identical with those shown above for arsenite metabolism (Scheme 1) (Carter *et al*., 2003). A hypothetical scheme based on the same alternate application of oxidative methylation and reduction steps might look as follows with double arrows indicating four electron oxidation steps and single arrows two electron reduction steps:

$$\mathbf{As^{-III}H_3 \Rightarrow H_2As^{I}(O)CH_3 \rightarrow H_2As^{-I}CH_3 \Rightarrow HAs^{III}(O)(CH_3)_2 \rightarrow HAs^{I}(CH_3)_2 \Rightarrow As^{V}(O)(CH_3)_3}$$

Arsine **TMAO**

According to this scheme the intermediary metabolites would include methylated arsine and arsine oxide species. Alternatively nonmethylative oxidation of arsine could lead to arsenite and arsenate via hydroxylated arsine species. Other metabolites possibly based on the oxidation of elemental As or arising via the postulated arsine hydroperoxide are also possible.

5. Acute Toxicity of Arsenic and Arsenic Compounds

5.1 Acute Toxicity to Adult Humans

The relative acute toxicity of arsenic compounds decreases as follows: arsine (As^{-III}) > organo-arsine derivatives > arsenites (As^{III}) > arsenoxides ($As^{II}I$) > arsenates (As^{V}) > pentavalent organic compounds (As^{V}) > arsonium metals (As^{I}) > metallic arsenic (As^{0}), where the Roman numeral indicates the oxidation state (HSDB, 1995).

Acute inhalation exposure may result in severe irritation of the mucous membranes of the upper and lower respiratory tract with symptoms of cough, dyspnea, and chest pain (Friberg *et al*., 1986). These may be followed by garlicky breath and gastrointestinal symptoms including vomiting and diarrhea (HSDB, 1995). Signs of acute poisoning are dermatitis, nasal mucosal irritation, laryngitis, mild bronchitis, and conjunctivitis (Friberg *et al*., 1986). The acute toxic symptoms of trivalent arsenic poisoning are due to severe inflammation of the mucous membranes and increased permeability of the capillaries (HSDB, 1995). Ingestion of 2 grams of As_2O_3 was fatal to an adult male (Levin-Scherz *et al*., 1987).

5.2 Acute Toxicity to Infants and Children

Relatively little data are available on acute toxicity of arsenic compounds to children. Childhood poisonings due to arsenic have been reported in the medical literature, often with little dosimetry. Campbell & Oates (1992) surveyed 200 child poisonings and found of the four deaths reported one was due to arsenic-containing weed killer (probably cacodylic acid). Alternatively, the use of arsenic trioxide in cancer chemotherapy seems well tolerated. George *et al*. (2004) reported the treatment of 11 children with acute promyelocytic leukemia with i.v. 0.15 mg As_2O_3/kg-d (8 treatment cycles over a period of 12 months). The toxic effects noted, including leukocytosis and skin hyperpigmentation, were considered minimal. Relapse-free survival was 81%.

5.3 Acute Toxicity to Experimental Animals

The lethal concentration low (LC_{Lo}) for $AsCl_3$ in the cat for a 20-minute inhalation exposure is 100 ppm (740 mg/m³) (Flury, 1921). In the mouse, the LC_{Lo} of $AsCl_3$ for a 10-minute exposure is 338 ppm (2500 mg/m³) (Flury, 1931).

A single intratracheal instillation of 17 mg As_2O_3/kg in rats resulted in multifocal interstitial pneumonia and focal proliferative bronchiolitis and alveolitis observed at necropsy 14 days post-exposure (Webb *et al*., 1986). The authors suggest that As_2O_3 induced an acute fibrogenic response.

Changes in host resistance from inhalation exposure to As_2O_3 aerosol were examined in female CD1 mice using a streptococcus infectivity model and an assay for pulmonary bactericidal activity (Aranyi *et al*., 1981; Aranyi *et al*., 1985). Mice (100-200/group) were exposed to As_2O_3 aerosol (or filtered air) for 3 hours/day, 5 days/week, for 1, 5 or 20 days. Aerosol exposed and control mice were then combined before challenge with *Streptococcus zoopidemicus* aerosol (4-8 replicate exposures). Statistically significant increases in mortality ($P < 0.05$) were observed in mice exposed: (1) once to 271, 496, and 940 µg As/m^3; (2) 5 times to 519 µg As/m^3; and (3) 20 times to 505 µg As/m^3. Multiple exposures at a given exposure level did not correlate with increased mortality, suggesting an adaptation mechanism. Single exposures did, however, show a dose-response for increased mortality with increasing level of arsenic exposure. Bactericidal activity was evaluated by measuring the ratio of viable bacteria count to radioactive count in the lung 3 hours after infection with ^{35}S-labeled *Klebsiella pneumoniae*. A single exposure to 271, 496, and 940 µg As/m^3, but not 123 µg As/m^3, resulted in significantly decreased bactericidal activity. Five exposures to 519 µg As/m^3 and twenty exposures to both 245 and 505 µg As/m^3 resulted in decreased bactericidal activity. The studies indicate a NOAEL for immunotoxicity of 123 µg As/m^3. This study provides a partial mode of action of arsenic-induced increase in mortality due to experimental lung infections with the mouse pathogen *S. zooepidemicus*. The second bactericidal assay with radiolabelled *K. pneumoniae* provides a plausible explanation, namely that arsenic exposure above 123 µg/m^3 inhibits normal immune bactericidal response in the lung.

Among the other adverse effects of inorganic arsenic noted in experimental animals, the most interesting and relevant to the 8-hour and chronic RELs are those on the brain and nervous system. These include changes in brain histology and conditioned reflexes, changes in locomotor activity, and decreased acetyl cholinesterase, GAD, and GABA levels in the hypothalamus, brain stem and cerebellum. Arsenic induced alterations of brain structure and function are consistent with the more subtle neuro-developmental effects seen in children exposed to inorganic arsenic at lower environmental levels.

5.4 Developmental and Reproductive Toxicity

Arsenic is listed under California Proposition 65 (Cal/EPA, Safe Drinking Water and Toxic Enforcement Act of 1986) as a developmental toxicant. The oxidation state of arsenic determines the teratogenic potential of its inorganic compounds; trivalent (III) arsenic compounds possess greater teratogenic potential than pentavalent (V) compounds. In hamsters, a single maternal intravenous injection of 20 mg/kg sodium arsenate (V) ($AsHNa_2O_4$) on gestation day 8 was lethal to 44% of all embryos (Willhite and Ferm, 1984). A smaller dose (10 mg/kg) of sodium arsenite (As^{III}) ($AsHNaO_2$) administered in the same manner resulted in 90% embryonic lethality.

Fetal malformations, including exencephaly, resulted from an intravenous injection of $AsH_3Na_2O_4$ (As^V) into pregnant hamsters on gestation day eight (Ferm and Carpenter, 1968). The reproductive NOAEL in this experiment was 5 mg/kg. A significant reduction in fetal body weight, but no malformations were observed following a maternal

dose of 5 mg/kg AsH_2NaO_3 (As^{III}) by the same route on gestation day eleven or twelve (Harrison and Hood, 1981).

A significant increase in pre-implantation mortality followed exposure of pregnant rats to aerosolized As_2O_3 at 1 mg/m³ for 5 months; no maternal toxicity was observed (Kamkin, 1982). At the LOAEL, 0.3 mg/m³, slightly elevated pre-implantation lethality was observed. The validity of this report cannot be evaluated, however, because key experimental details were not reported

A significant decrease in spermatozoa motility was observed in male rats following continuous exposure to As_2O_3 at a concentration of 40 mg/m³ for 48 hours (Kamil'dzhanov, 1982). Intravenous injection of radioactive arsenate (As^{V}) or arsenite (As^{III}) in several rodent species, including mice and hamsters, resulted in accumulation of arsenic in the lumen of the epididymal duct, which suggests that long term exposure of sperm may occur *in vivo* following acute exposure to As (Danielsson *et al*., 1984).

Nagymajtenyi *et al*., (1985) exposed pregnant CFLP mice (8-11 females/group) to As_2O_3 aerosol for 4 hours/day on gestational days 9-12 at concentrations of 0, 0.26, 2.9, or 28.5 mg As_2O_3/m^3 (~0.2, 2.2, and 21.6 mg As/m^3. The aerosol was generated by spraying an aqueous solution of As_2O_3. On the 18th day of gestation the mice were sacrificed and the fetuses removed. The numbers of live and dead fetuses were recorded, weighed, and examined microscopically. Fifty fetuses were stained with Alizarin red-S for skeletal examination. Chromosome preparations were made from livers of 10 fetuses per exposure group. Twenty mitoses in each fetus (200/group) were scored for chromosomal damage and 10 percent of these were karyotyped. The data were analyzed with either Fisher's exact test or in the case of fetal weights with the Dunnett multiple comparison t-test.

A statistically significant decrease in fetal weight was observed in all of the dose groups ($P < 0.05$), with a 3, 9, and 29% reduction in average fetal weight with increasing dose (Table 6.4.1). Significantly delayed bone maturation (ossification defects) was observed only in the highest dose group (sternum 14/50; limbs 32/50, both $p < 0.05$). However, an apparent positive dose-related trend in the number of fetuses with skeletal malformations was observed (2 [control], 3, 7, 31, respectively). A similar dose-related trend in chromosome aberrations in liver cells was also observed in the number of cells with damage (6[control], 10, 13, 24), chromatid gaps, chromatid breaks, chromosome fragments, and chromosome breaks (5[control], 10, 13, 27). Only the number of damaged cells and chromosome breaks at the high dose were significantly different from the control ($p < 0.05$).

Table 6.4.1 Data from Table 1 of Nagymajtényi et al. (1985).

As_2O_3 (mg/m^3)	Number of litters	Living fetuses per mother	Number of fetuses examined	% dead fetuses	Average fetal weight (grams)
28.5±0.3	11	9.6	100	29	0.981±0.04*
2.9±0.04	8	12.8.	100	13	1.146±0.03*
0.26±0.01	8	12.5	100	12	1.225±0.03*
0	8	12.5	100	8	1.272±0.02

* Significantly different from control ($p<0.05$)

This study demonstrates that inhalation exposure to inorganic arsenic is markedly fetotoxic. Arsenic concentrations of 28.5 mg/m^3 caused a reduction in the number of live fetuses, in fetal weight, and an increase in fetuses with delayed osteogenesis.

Rats exposed to 1 µg As_2O_3/m^3 (0.76 µg As/m^3) for 5 months showed increased preimplantation mortality and delayed ossification in fetuses (Kamkin, 1982). Experimental detail was not presented, thus limiting the usefulness of this study.

A significant decrease in spermatozoa motility was observed in male rats following continuous exposure to 32.4 mg As_2O_3/m^3 for 48 hours (Kamil'dzhanov, 1982). Similarly, motility was decreased after: (1) a 120-hour exposure to 7.95 mg/m^3; (2) a 252-hour exposure to 1.45 mg/m^3; and (3) an 800-hour exposure to 0.36 mg/m^3.

Holson *et al*. (1999) administered arsenic trioxide (As_2O_3) by whole body inhalation to groups of 25 Crl:CD (SD)BR female rats every day for six hours per day, beginning fourteen days prior to mating and continuing throughout mating. The target exposure levels were 0.3, 3.0, and 10.0 mg As_2O_3/m^3 (measured means: 0.24, 2.6, 8.3 mg As/m^3). Maternal toxicity evidenced by the occurrence of rales, a decrease in net body weight gain, and decreased food intake during pre-mating and gestation exposure, was observed only at the high dose. The NOAEL for maternal toxicity was 2.6 mg As/m^3 (3.4 mg As_2O_3/m^3). No treatment-related malformations or developmental variations were observed at any exposure level. The NOAEL for developmental toxicity was 8.3 mg As/m^3 (11 mg As_2O_3/m^3). The median mass aerodynamic diameter of particle sizes generated in the exposure chambers ranged from 1.9 to 2.2 µm for the three doses indicating that the dusts were respirable. However there were no blood or urine arsenic analytical data to assess delivered doses.

Nemec *et al*. (1998) evaluated the developmental toxicity of inorganic arsenic in mice and rabbits. CD-1 mice (25/dose group) and New Zealand White rabbits (20/dose group) were gavaged with aqueous arsenic acid (H_3AsO_4) doses of 0, 7.5, 24, or 48 mg/kg-d on gestation days (GD) six through 15 (mice) or 0, 0.19, 0.75, or 3.0 mg/kg-d on GD six through 18 (rabbits). The animals were examined at necropsy (GD 18, mice;

GD 29, rabbits). Treatment related maternal toxicity including mortality (2/25) was observed only in the highest dose administered to mice. Effects on maternal weight gain were noted only on GD 6-9 ($P < 0.01$) and GD 15-18 ($P < 0.05$) of the mid dose and on GD 6-9 ($p < 0.05$) of the low dose. While overall maternal weight gains were statistically significantly reduced only at the top dose there was an apparent negative trend in decreased GD18 body weights with increasing dose (56.2 g control, 54.9 g, 52.7g, 46.7g, respectively). While the authors identified a NOAEL for maternal toxicity of 7.5 mg/kg-d, the apparent negative trend noted above suggests that this may be a LOAEL (4.0 mg As/kg-d).

Statistically significant adverse effects on offspring growth or survival were seen only at the highest dose of 48 mg/kg-d. However, there was an apparent negative trend in the number of live fetuses per litter with increasing dose (12.3 control, 11.6, 11.0, 6.6, respectively). An increased incidence of resorptions per litter was seen in the 48 mg/kg-d dose group ($P \leq 0.01$), (mainly early resorptions). Early and total resorptions showed an apparent positive trend (6.4% total control, 6.1%, 9.6%, 41.9%, respectively). Mean fetal weight showed an apparent negative trend (1.3 g control, 1.32 g, 1.23 g, 0.99 g, respectively). There were no statistically significant dose-related increases in the overall incidence of fetal malformations; however, the mean percent of litter malformation was about three-fold higher in the 48 mg/kg-d dose group than in the lower doses and control. The NOAEL for developmental toxicity would appear to be 7.5 mg/kg-d (4.0 mg As/kg-d).

Maternal toxicity in rabbits, including mortality, slight body weight loss, and clinical signs (decreased urination and defecation, occasional prostration and ataxia), occurred only at the high arsenic acid dose of 3.0 mg/kg-d. The number of does with decreased urination and defecation appeared to be slightly higher in the mid- and low-dose groups, but these effects may not have been treatment related and no effects on body weight were seen. At sacrifice on GD 29 maternal body weight appeared to be reduced in the high dose group. A significant loss in mean maternal gravid body weight occurred during the first six days of high-dose treatment (GD 6-12) ($p \leq 0.01$). This effect persisted and was significantly different from controls for the entire treatment interval (GD 6-18). There were no statistically significant increases in the incidences of any developmental parameters, including malformations. Fetal survival, mean fetal weight, and sex ratio on GD 29 were not affected by the treatment. The number of live fetuses per litter was reduced and resorptions per litter increased in the high-dose group. The latter findings were mainly due to one doe with a totally resorbed litter. The overall values were the range from laboratory historical controls. The authors identified a NOAEL of 0.75 mg/kg-d (0.4 mg As/kg-d) for both maternal toxicity and developmental toxicity.

Stump *et al*. (1999) administered either sodium arsenate (As^{V}) i.p. or arsenic trioxide (As^{III}) i.p. or by gavage on GD 9 to 25 Crl:CD (SD) BR rats. The doses of sodium arsenate were 0, 5, 10, 20, and 35 mg/kg (0, 1.2, 2.4, 4.8, 8.4 mg As/kg). The doses of arsenic trioxide were: i.p. 0, 1, 5, 10, and 15 mg/kg (0, 0.8, 3.8, 7.6, and 11.4 mg As/kg); and by gavage (p.o.) 0, 5, 10, 20, 30 mg/kg (0, 3.8, 7.6, 15.2, 22.7 mg As/kg). Sodium arsenate (i.p.) caused decreased maternal food consumption (GD 9-20), decreased

body weights and body weight gains at the highest dose of 35 mg/kg. Decreased food consumption was also seen in the 20 mg/kg dose group at GD 9-10 and GD 9-20. Arsenic trioxide (i.p.) resulted in excessive mortality in the highest dose-group (19/25) and significant reductions in maternal food consumption, body weight at GD20, body weight change, and net body weight in the next highest dose-group (10 mg/kg). Arsenic trioxide (p.o.) resulted in less mortality in the highest dose-group (7/25). Clinical signs were noted in the 20 and 30 mg/kg dose-groups including changes in fecal consistency and decreased defecation. Food consumption (GD 9-10) was decreased in a dose-dependent manner across As treatment groups. The study identified single dose maternal effects NOAELs of 2.4 mg As/kg for sodium arsenate (i.p.) and 3.8 mg As/kg for arsenic trioxide i.p. A LOAEL of 3.8 mg As/kg was identified for arsenic trioxide p.o.

Intraperitoneal administration of sodium arsenate or arsenic trioxide caused neural tube and ocular defects (exencephaly, microphthalmia/anophthalmia, and other craniofacial defects) in the offspring of treated rats. These effects were statistically significant only at doses causing maternal toxicity or mortality (35 and 10 mg/kg, respectively). Oral administration of arsenic trioxide caused no treatment-related malformations. The study identified single dose developmental NOAELs of 2.4 mg As/kg for sodium arsenate i.p., 3.8 mg As/kg for arsenic trioxide i.p., and 15.2 mg As/kg for arsenic trioxide p.o.

DeSesso *et al*. (1998), in a comprehensive review of the developmental toxicity of inorganic arsenic, concluded that cranial neural tube defects (NTDs) were induced in rodents only when exposure occurred early in gestation, at high maternally toxic doses, and by parenteral routes of administration. They argued that such NTD effective doses are unlikely to be achieved by the oral, inhalation, or dermal routes in rodents, and that inorganic arsenic does not represent a realistic developmental risk in humans subjected to any environmentally relevant exposure scenarios.

Male and female Charles River CD mice (10/group) were treated with 0 or 5 ppm arsenite in drinking water continuously through three generations (Schroeder and Mitchener, 1971). Endpoints examined included the interval between litters, the age at first litter, the ratio of males to females, the number of runts, stillborn offspring, failures to breed, and congenital abnormalities. The study showed an alteration in the number of small litters in the arsenic exposed group.

Female CD-1 mice (8-15/group) were treated by oral gavage with 0, 20, 40, or 45 mg sodium arsenite/kg on a single day of gestation between days 8 and 15 (Baxley *et al*., 1981). Maternal mortality, fetal malformations, and increased prenatal death were observed among animals treated with 40 and 45 mg sodium arsenite/kg.

Pregnant golden hamsters (>10/group) were treated by oral gavage with a single administration of 0, 20, or 25 mg/kg sodium arsenite on one of gestational days 8-12 (Hood and Harrison, 1982). Prenatal mortality was increased among animals receiving 25 mg/kg on gestational days 8 and 12 and fetal weights were decreased among animals receiving 25 mg/kg on gestational day 12. One dam died following administration of 20 mg/kg.

Intravenous injection of radioactive arsenate (V) or arsenite (III) in several rodent species, including mice and hamsters, resulted in accumulation of arsenic in the lumen of the epididymal duct, which suggested that long term exposure of sperm to arsenic may occur *in vivo* following acute exposure (Danielsson *et al*., 1984).

6. Chronic Toxicity of Arsenic and Arsenic Compounds

6.1 Chronic Toxicity to Adult Humans

Arsenic in drinking water is carcinogenic to humans (Group 1, IARC, 2004). Arsenic compounds show limited to sufficient evidence of carcinogenicity in experimental animals (IARC, 2004). The U.S. Environmental Protection Agency has classified arsenic as Group A; a human carcinogen, based on sufficient evidence from human data including increased lung cancer mortality in multiple human populations exposed primarily through inhalation, increased mortality from multiple internal organ cancers (liver, kidney, lung, bladder), and increased skin cancers observed in populations exposed to arsenic in drinking water (IRIS online file www.epa.gov/iris/subst/0278.htm). Since this document deals with noncancer risks, the carcinogenicity of arsenic is not covered here in any detail (see OEHHA (1999)).

Smelter workers, exposed to concentrations of arsenic up to 7 mg As/m^3, showed an increased incidence in nasal septal perforation, rhinopharyngolaryngitis, tracheobronchitis, and pulmonary insufficiency (Lundgren, 1954).

In a case-control study, copper smelter workers (n = 47) exposed to arsenic for 8-40 years (plus 50 unexposed controls matched for age, medical history, and occupation) were examined by electromyography and for nerve conduction velocity in the arms and legs (Blom *et al*., 1985). The workers were found to have a statistically significant correlation between cumulative exposure to arsenic and reduced nerve conduction velocities in three peripheral nerves (upper and lower extremities). Slightly reduced nerve conduction velocity in 2 or more peripheral nerves was reported as “more common” among arsenic exposed workers. Minor neurological and electromyographic abnormalities were also found among exposed workers. Occupational exposure levels were estimated to be 0.05-0.5 mg As/m^3, with As_2O_3 the predominant chemical form. Except for three arsenic exposed workers who had long-term exposure to lead, exposure to other heavy metals was insignificant.

The smelter workers described by Blom *et al*. (1985) (number of controls reduced to 48) were further examined for prevalence of Raynaud’s phenomenon and for vasospastic tendency by measurement of finger systolic pressure at 10°C and/or 15°C relative to that at 30°C (FSP%) (Lagerkvist *et al*., 1986). The FSP% was found to covary with the duration of exposure to arsenic, and the prevalence of Raynaud’s phenomenon was significantly increased among exposed workers. Daily arsenic uptake was estimated at less than 300 µg/day and was confirmed with urinary excretion data.

Hyperpigmentation and hyperkeratinization were observed in workers exposed to 0.4 - 1 mg/m^3 inorganic arsenic for two or more years (Perry *et al*., 1948).

Most of the relevant epidemiological data on arsenic adverse effects comes from studies of arsenic exposure via drinking water. These studies are relevant because arsenic exerts similar toxic effects once it enters the body. For example, arsenic causes lung cancer in humans by both oral and inhalation routes. The adverse effects summarized below include skin lesions(keratosis and altered pigmentation), vascular effects on the heart, brain and peripheral vasculature, peripheral neuropathy, and lung disease.

6.1.2.1 Skin Effects

Mazumder *et al*. (1998) investigated arsenic-associated skin lesions of keratosis and hyperpigmentation in 7683 exposed subjects in West Bengal, India. While water arsenic concentrations ranged up to 3400 µg/L, over 80% of the subjects were consuming water with < 500 µg/L. The age-adjusted prevalence of keratosis was strongly related to water As concentration, rising from zero in the lowest exposure level (< 50 µg/L) to 8.3% for females drinking water containing >800 µg As/L, and from 0.2 to 10.7% in males, respectively. A similar dose-response was observed for hyperpigmentation: 0.3 to 11.5% for females; and 0.4 to 22.7% for males. Overall males had 2-3 times the prevalence of both keratosis and hyperpigmentation than females apparently ingesting the same doses of arsenic per body weight. Subjects that were more than 20% below standard body weight for their age and sex had a 1.6-fold increase in the prevalence of keratoses, suggesting that malnutrition may play a role in increasing susceptibility.

Rahman *et al*. (2006) evaluated arsenic exposure and age- and sex-specific risk for skin lesions in a population-based case-referent study in Bangladesh. The entire population over four years of age of Matlab, Bangladesh (N = 166,934) was screened for skin lesions. Skin lesions were classified as hyperpigmentation (melanosis), hypopigmentation (leukomelanosis), or keratosis. A total of 504 cases with skin lesions were identified. A randomly selected referent group of 1830 subjects was included in the study. Arsenic exposure was assessed by personal history of tube well use since 1970 or year of birth if later. Water samples from all functioning tube wells were measured for arsenic concentration by hydride-generation atomic absorption spectroscopy. A dose-response relationship was observed for increased skin lesions and arsenic exposure for both sexes (P < 0.001). For males using the metric of As µg/L the highest exposure quintile (≥ 300 µg/L) gave an adjusted odds ratio (OR) of 9.56 (95% CI = 4.20-21.8). Females gave a corresponding OR of 6.08 (3.06-15.5). The cumulative As exposure metric (µg/L x years) gave OR's of 10.4 and 9.19, respectively. In an analysis with males and females combined, adjusted for age and socioeconomic status, males had significantly higher risk of As-related skin lesions than females, when females' lowest average exposure quintile was used as the reference. For the highest quintile, the males OR was 10.9 (5.8-20.4) and the females OR was 5.78 (3.10-10.8), P = 0.005.

Dermatitis and irritation of the mucous membranes have been observed in arsenic-exposed workers (Vallee *et al*., 1960). Hepatic fatty infiltration, central necrosis, and

cirrhosis were observed in two patients who ingested As_2O_3 (1% in Fowler's solution) for three or more years (Morris *et al*., 1974). Daily consumption of 0.13 mg As/kg in contaminated well water resulted in the chronic poisoning and death of four children; at autopsy, myocardial infarction and arterial thickening were noted (Zaldivar and Guillier, 1977).

6.1.2.2 Vascular Disease

Vascular diseases have long been noted to be associated with chronic arsenic exposures among German vineyard workers (Grobe, 1976) and inhabitants of Antofagasta, Chile (Borgono *et al*., 1977). Peripheral vascular diseases have been reported to be associated with the occurrence of arsenic in well waters in Taiwan (Chen and Wu, 1962; Chi and Blackwell, 1968; Tseng, 1977; Chen *et al*., 1988). Concentrations in one study were characterized as 0.10 – 1.8 ppm (Yu *et al*., 1984). The term arseniasis or arsenosis connotes vascular disease associated with chronic exposure to arsenic, specifically blackfoot disease (BFD). BFD is characterized by progressive narrowing of the peripheral arteries, particularly those of the lower extremities. This can lead to ulceration, gangrene and amputation. The etiology of BFD is unclear but arsenic is thought to be the principal cause. The term arsenicosis refers to arsenic induced skin lesions ranging in severity over four stages, seven grades and 20 sub-grades from diffuse melanosis (skin pigmentation or depigmentation) to aggressive skin and internal malignancy (Saha, 2003).

Wu *et al*. (1989) found significant trends of mortality rates from peripheral vascular diseases and cardiovascular diseases with concentrations of arsenic in well water. However, no significant association was observed for cerebrovascular accidents. Engel and Smith (1994) evaluated arsenic in drinking water and mortality from vascular disease in 30 U.S. counties from 1968 to 1984. Mean As levels in drinking water ranged from 5.4 to 91.5 µg/L. Standardized mortality ratios (SMRs) for diseases of arteries, arterioles, and capillaries (DAAC) for counties exceeding 20 µg/L were 1.9 (90% C.I. = 1.7-2.1) for females and 1.6 (90% C.I. = 1.5-1.8) for males. SMRs for three subgroups of DAAC including arteriosclerosis and aortic aneurysm were also elevated as were congenital abnormalities of the heart and circulatory system.

Tseng *et al*. (1996) studied the dose relationship between peripheral vascular disease (PVD) and ingested inorganic arsenic in blackfoot disease endemic villages in Taiwan. A total of 582 adults (263 men and 319 women) underwent Doppler ultrasound measurement of systolic pressures on bilateral ankle and brachial arteries and estimation of long-term arsenic exposure. The diagnosis of PVD was based on an ankle-brachial index of < 0.9 on either side. Multiple logistic regression analysis was used to assess the association between PVD and As exposure. A dose-response relationship was observed between the prevalence of PVD and long-term As exposure. The odds ratios (95% confidence intervals) after adjustment for age, sex, body mass index, cigarette smoking, serum cholesterol and triglyceride levels, diabetes mellitus and hypertension were 2.77 (0.84-9.14), and 4.28 (1.26-14.54) for those who had cumulative As exposures of 0.1 to 19.9 and ≥ 20 (mg/L) x yr, respectively. A follow up study (Tseng *et al*., 1997) indicated that PVD was correlated with ingested As and not

with abnormal lipid profiles. The lipid profiles studied were total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c), apolipoprotein AI, and apolipoprotein B. Other lipids such as modified LDL, subclasses of LDL and HDL, and other lipoproteins such as lipoprotein (a), which may track as better indicators of atherosclerosis, were not included. Also, the roles of platelet aggregation and coagulation profiles were not studied.

Chen *et al*. (1996) evaluated the dose-response relationship between ischemic heart disease (ISHD) mortality and long-term arsenic exposure. Mortality rates from ISHD among residents in 60 villages in an area of Taiwan with endemic arseniasis from 1973 through 1986 were analyzed for association with As concentrations in drinking water. Based on 1,355,915 person-years and 217 ISHD deaths, the cumulative ISHD mortalities from birth to age 79 yr were 3.4%, 3.5%, 4.7%, and 6.6% for the median As concentrations of < 0.1, 0.1-0.34, 0.35-0.59, and ≥ 0.6 mg/L, respectively. Multivariate-adjusted relative risks (RRs (95% C.I.)) associated with cumulative arsenic exposure from well water were 2.46 (0.53-11.36), 3.97 (1.01-15.59), and 6.47 (1.88-22.24) for 0.1-9.9, 10.0-19.9, and 20+ (mg/L)-yr, respectively, compared with those without As exposure.

Chiou *et al*. (1997b) evaluated the dose-response relationship between prevalence of cerebrovascular disease and ingested arsenic among residents of the Lanyang Basin in northeast Taiwan. A total of 8102 adults from 3901 households were recruited for the study. Arsenic in well water of each household was determined by hydride generation and atomic absorption spectrometry. Logistic regression analysis was used to estimate multivariate-adjusted odds ratios and 95% confidence intervals for various risk factors of cerebrovascular disease. A significant dose-response relationship was observed between As concentration in well water and prevalence of cerebrovascular disease after adjustment for age, sex, hypertension, diabetes mellitus, cigarette smoking, and alcohol consumption. The dose-response relationship was even more prominent for cerebral infarction with multivariate-adjusted odds ratios (95% C.I.) of 1.0, 3.4 (1.6-7.3), 4.5 (2.0-9.9), and 6.9 (3.0-16), respectively, for those who consumed well water with As concentrations of 0, 0.1-50.0, 50.1-299.9, and > 300 µg/L. For cumulative arsenic exposures of <0.1, 0.1-4.9, and ≥ 5.0 (mg/L)-yr, the odds ratios were 1.00, 2.26, and 2.69 for cerebrovascular disease and 1.00, 2.66, and 3.39 for cerebral infarction, respectively. All of the values above for As exposed groups were significantly greater than unexposed at $P < 0.05$.

Chen *et al*. (1995) also investigated the association between long-term exposure to inorganic arsenic and the prevalence of hypertension. A total of 382 men and 516 women were studied in villages where arseniasis was endemic. Hypertension was defined as a systolic blood pressure of 160 mm Hg or greater, or a history of hypertension treated with antihypertensive drugs. The long-term arsenic exposure was calculated from the history of artesian well water consumption obtained through subject questionnaires and the measured arsenic concentration in well water. Residents in villages where long-term arseniasis was endemic had a 1.5-fold increase in age- and sex-adjusted prevalence of hypertension compared with residents in nonendemic areas. The duration of well water consumption, average As water concentration, and

cumulative As exposure were all significantly associated with hypertension. For the cumulative As exposure in (mg/L)-yr, the percent prevalence values were: 0, 5.0%; 0.1-6.3 (mg/L)-yr, 4.9%; 6.4-10.8 (mg/L)-yr, 12.8%; 10.9-14.7 (mg/L)-yr, 22.1%; 14.8-18.5 (mg/L)-yr, 26.5%; > 18.5 (mg/L)-yr, 29.2%.

As part of a study of arsenic exposure via drinking water and mortality outcome in Millard County, Utah, Lewis *et al*. (1999) found a statistically significant association with mortality from hypertensive heart disease. Median drinking water concentration of arsenic ranged from 14 to 166 µg/L for the 946 subjects in the study. The standard mortality ratios (SMR) without regard to specific exposure levels were SMR = 2.20 (95% C.I., 1.36-3.36) for males and SMR = 1.73 (95% C.I., 1.11-2.58) for females. When analyzed by cumulative exposure groups of low (< 1.0 (mg/L)-yr), medium (1.0-4.9 (mg/L)-yr), and high (≥ 5.0 (mg/L)-yr), there was no apparent dose response relationship. However the cumulative dose estimates in this study were lower than in the Chen *et al*. (1995) discussed above so the results of the two studies are not inconsistent.

Chen *et al*. (2006) conducted a cross-sectional analysis of the association of arsenic exposure from drinking water and blood pressure in 10,910 subjects. Time-weighted well arsenic concentrations (TWA) based on current and past well usage were derived. Odds ratios (OR's) for high pulse pressure (systolic – diastolic pressure ≥ 55 mmHg) by increasing TWA quintiles (≤ 8, 8.1-40.8, 40.9-91.0, 91.1-176.0, 176.1-864.0 µg/L) were: 1.00 (referent); 1.39 (95% C.I. 1.14, 1.71); 1.21 (0.9, 1.49); 1.19 (0.97, 1.45); 1.19 (0.97,1.46). OR's for systolic hypertension (≥ 140 mmHg) suggested a similar but weaker association. Participants with lower than average intake of B vitamins and folate showed somewhat higher OR's. No associations were apparent for TWA and diastolic hypertension.

In a study related to those above, Lai *et al*. (1994) studied inorganic arsenic ingestion and the prevalence of diabetes mellitus. A total of 891 adult residents of villages in southern Taiwan where arseniasis is endemic were included in the study. Diabetes status was determined by an oral glucose tolerance test and a history of diabetes regularly treated with sulfonylurea or insulin. Cumulative arsenic exposure in ppm-yr was determined from the detailed history of drinking artesian well water. There was a dose-response relation between cumulative arsenic exposure and prevalence of diabetes mellitus. The relation remained significant after adjustment for age, sex, body mass index, and activity level at work by a multiple logistic regression analysis giving multivariate-adjusted odds ratios of 6.61 and 10.05, respectively, for exposures of 0.1-15 ppm-yr and > 15.0 ppm-yr versus an unexposed group. In an effort to confirm this association between diabetes mellitus and arsenic observed for drinking water in Taiwan, Rahman and Axelson (1995) reviewed 1978 case-control data from a Swedish copper smelter. Twelve cases of diabetes mellitus (death certificate) were compared with 31 controls without cancer, cardiovascular and cerebrovascular disease. The odds ratios for diabetes mellitus with increasing arsenic exposure categories were 1.0 (reference level), 2.0, 4.2, and 7.0 with the 95% confidence level including unity. The trend was weakly significant, p = 0.03. Albeit with limited numbers, the study provides some support for a role of arsenic exposure in the development of diabetes mellitus.

6.1.2.3 Neurological Disease

Hafeman *et al*. (2005) evaluated the association between arsenic exposure and peripheral neuropathy in a cross-sectional study of 137 adults in Bangladesh. Exposure measures included individual arsenic water concentration, cumulative arsenic index (CAI), and urinary arsenic concentration. Experimental measures were primarily vibrotactile threshold testing of the index finger (IVT) and toe (TVT) and secondarily tapping speed, grip strength, ankle reflex, and proprioception. The cumulative arsenic index and urinary arsenic were both significantly associated with elevated TVT (P = 0.02 and P = 0.009, respectively) after adjustment for age and gender. While dose-response relations were difficult to define, a linear regression analysis of TVT (vibration units) versus the continuous measures of urinary arsenic and CAI gave slopes of 0.02 and 0.0025 TVT units/50 µg As/mg urinary creatinine, respectively. The association between IVT and arsenic exposure was not statistically significant. No association was found between any measure of arsenic exposure and grip strength, tapping speed, ankle reflex, or proprioception.

6.1.2.4 Lung Disease

Several studies have reported effects of arsenic exposure through drinking water on the lung. Mazumder et al. (2000) reported increasing respiratory symptoms, including cough, shortness of breath, and chest sounds, with increasing arsenic concentrations in the drinking water in people residing in West Bengal, India. The effects seen were marked in individuals who also had arsenic related skin lesions. In a later study also in West Bengal, these investigators also reported a large increase (OR = 10; 95% CI 2.7-37) in bronchiectasis in individuals with skin lesions compared to those without arsenic-related skin lesions (Mazumder et al., 2005).

Von Ehrenstein *et al*. (2005) studied the relation between lung function, respiratory symptoms, and arsenic in drinking water among 287 adults, including 132 with arsenic-induced skin lesions in West Bengal, India. Arsenic levels in drinking water and the number of male subjects with or without skin lesions were: 0-99 µg/L, 9, 36; 100-399 µg/L, 66, 34; ≥400 µg/L, 18, 15, respectively. For respiratory symptoms of "shortness of breath at night" and "morning cough", the odds ratios (ORs) for men with skin lesion versus those without was 2.8 with 95% confidence intervals (C.I.) of (1.1, 7.6) and (1.2, 6.6), respectively. For men with skin lesions, the average forced expiratory volume in one second (FEV_1) was reduced by 256.2 mL (95% C.I.; 113.9, 398.4) P < 0.001. Average forced vital capacity (FVC) was reduced by 287.8 mL (95% C.I.; 134.9, 440.8) P < 0.001. In men a 100 µg/L increase in arsenic level was associated with a 45.0 mL decrease (95% C.I.; 6.2, 83.9) in FEV_1 (P = 0.02) and a 41.4 mL decrease (95% C.I.; -0.7, 83.5) in FVC (P = 0.054). The findings were adjusted for age, height and smoking in both males and females. Women participating in the study (N = 109) had a lower risk of developing skin lesions than men and exhibited few respiratory symptoms.

6.2 Chronic Toxicity to Infants and Children

The adverse effects of inorganic arsenic exposure reported in children include skin lesions, neurodevelopmental effects (IQ and related effects), lung disease expressed in later years, and reproductive effects (decreased birth weight, spontaneous abortion, neonatal death).

As noted above Mazumder *et al*. (1998) observed a dose-response for arsenic-associated skin lesions in a cross-sectional survey of 7683 subjects in West Bengal, India. The study population was divided by age decades such that the effect on young children (≤ 9 yr) and adolescents (10-19 yr) could be analyzed separately. The prevalence of keratosis in females and males was 0.2 and 0.5 percent in young children and 1.0 and 1.7 percent in adolescents, respectively. The comparable values for hyperpigmentation were 1.7 and 2.0 percent and 2.2 and 3.5 percent, respectively. Overall 1149 young children and 1599 adolescents were surveyed. The low- to mid-dose quantal responses for combined skin lesions in young children using the mid points of the arsenic concentration ranges (µg/L) were: 25, 0/414; 75, 0/95; 125, 4/118; 175, 2/50; 275, 6/161; 425, 11/101. For the adolescents the comparable values were: 1/730; 2/147; 2/107; 7/110; 26/213; 9/58.

The adverse effects of inorganic arsenic on the developing intellectual function of exposed children have been reported in several studies summarized in this section. While some of the studies have deficiencies, as a group they indicate that arsenic exposure, like lead exposure, presents a risk to children. The neurodevelopmental endpoint has been selected by OEHHA as the critical effect for deriving 8-hour and chronic RELs for inorganic arsenic.

Calderon *et al*. (2001) conducted a cross-sectional study to examine the effects of chronic exposure to lead (Pb) and arsenic (As), and also nutrition, on the neuropsychological development of children. Two populations of children aged six to nine years (N = 41, 39) with differing As exposure levels (63 vs. 40 µg/g) but similar Pb exposures (8.9 vs. 9.7 µg Pb/dL blood, respectively) were compared using the Wechsler Intelligence Scale for Children (WISC) Revised Version for Mexico. After controlling for significant potential confounders verbal IQ was observed to decrease with increasing urinary arsenic ($P < 0.01$). Language, verbal comprehension and long-term memory also appeared to be adversely affected by increasing arsenic exposure (concepts and knowledge factors, $P < 0.05$ each). Blood lead was significantly associated with a decrease in attention (sequential factor, $P < 0.05$). However since blood lead is an imprecise measure of lead burden there could be some residual confounding in this study.

The relationship between arsenic exposure via drinking water and neurological development as indicated by intelligence (IQ) was assessed in Thailand (Siripitayakunkit *et al*., 1999) in 529 children aged six to nine years using a cross-sectional design. Arsenic levels in hair were used to assess exposure and the WISC test for children was used to assess IQ. The range of arsenic concentrations in hair was 0.48 to 26.94 µg/g (mean = 3.52, SD = 3.58). The mean IQ of the study was 90.44

(range 54 to 123). Most of the IQs were classified as average (45.7%) or dull normal (31.6%). Approximately 14% and 3% of the children were in the borderline and mental defective groups, respectively. The percentage of children in the average IQ group decreased significantly from 57 percent to 40 percent with increasing arsenic exposure. The percentage in the lower IQ group increased with increasing As (23% to 38%) and in the low IQ group (zero to six percent). In a comparison of IQ between children with As hair levels ≤ two ppm or > two ppm, arsenic was found to explain 14 percent of the variance in IQ after controlling for father's occupation, mother's intelligence score, and family income. Arsenic levels in hair above 2 ppm were associated with a 0.75-point decrease in IQ below the grand mean and As levels above 5 ppm with a two point decrease. Although the cross-sectional study design does not allow for establishment of the time precedence of exposure to arsenic, the investigators stated that the subjects of the study were born in a period of chronic arsenic poisoning and that this cohort has been continuously exposed since birth due to their non-mobility. The study suffers from small numbers of children exposed to low arsenic (hair arsenic ≤ 1 ppm) so this group could not be compared to the high arsenic children. Also the possible exposure to chemical confounders like lead was not discussed.

In a parallel cross-sectional study (Siripitayakunkit *et al*., 2001) the 529 children (above) were subjected to the Motor-Free Visual Perception Test (MVPT) and the Visual-Motor Integration Test (VMI). The visual perception score of each child was compared with the score of children in a control sub-district of the same age. The cutoff point for poor perception was the mean minus one standard deviation (SD) in each age level. Among arsenic-exposed children, 21 percent had poor visual perception and 17.6 percent had poor VMI. The comparable values in the control population were 16.5 percent and 15.8 percent, respectively. Potential confounders were controlled by multiple classification analysis. Only five percent of the variance in visual perception of children was significantly explained by arsenic (P = 0.01). The grand mean perception score was 20.57 and the adjusted values at low, medium and high hair As were 20.92, 20.51, and 20.03, respectively. Alternatively, these authors did not find an effect of arsenic on visual-motor integration.

Like the study of IQ decrements noted above, this study has the advantage of associating an adverse effect in children with a metric of chronic arsenic exposure, hair arsenic concentration. Disadvantages include a limited level of reporting and possible confounding with exposure to other metals.

Tsai *et al*. (2003) performed a cross-sectional study of the effect of arsenic exposure on the development of cognitive function among adolescents. Forty-nine 13-year old students were divided into low and high exposure groups and were compared with 60 13-year old unexposed children. Four neurobehavioral tests were conducted: continuous performance test (CPT); symbol digit (SD); pattern memory (PM); and switching attention (SA). Exposure in terms of As concentration in drinking water averaged 0 (<0.15), 131.2, and 185.0 ppb for control and exposure groups, respectively. Average cumulative arsenic exposures were 0, 252.1, and 768.2 mg (e.g., 184.99 ppb x 1008.6 cm^3/d x 11.28 yr x 365 d/yr x 10^{-3}). Neurobehavioral analysis revealed significant dose-response effects of arsenic exposure on CPT (P = 0.005), PM (P =

0.009) and SA (P = 0.0001), but not on SD (P = 0.23). A multiple linear regression analysis of the dose-response relationship between cumulative arsenic exposure and neurobehavioral endpoints showed a strong arsenic effects for CPT (low exposure group, P = 0.001), PM (high exposure group, P = 0.003) and SA (high and low exposures, P = 0.0001). This study is limited by low numbers but seems in line with other findings of As-induced CNS effects. The authors note that "the central nervous system of child and adolescents might be more vulnerable than adult to neurotoxicant". Although no dose-response relationship between As exposure and nerve conduction velocities was observed, the authors could not exclude the possibility of peripheral nerve dysfunction.

Wasserman *et al*. (2004) conducted a cross-sectional study of intellectual function in 201 As-exposed 10-year old children in Bangladesh. Children's intellectual function was assessed with tests drawn from the Wechsler Intelligence Scale for Children version III including Verbal, Performance, and Full-Scale raw scores. Children provided urine for arsenic and creatinine and blood samples for blood lead and hemoglobin measurements. After adjustment for sociodemographic covariates such as maternal education, height and head circumference, and waterborne levels of manganese (Mn), As in drinking water was associated with reduced intellectual function, in a dose-dependent manner. Children exposed to water arsenic of > 50 µg/L had significantly lower Performance and Full-Scale scores than did children with water As levels < 5.5 µg/L. Using the Full-Scale raw score, As water concentrations of 10 and 50 µg/L were associated with decrements of 3.8 and 6.4 points, respectively. The relationships between urinary arsenic concentration (µg As/g creatinine) and child intellectual function were not statistically significant but were in the expected (negative) direction (Full-Scale, P = 0.09; Performance, P = 0.14; Verbal, P = 0.11). Since there was no standard of intelligence for use in Bangladesh these decrements could not be directly equated with U.S. standard IQ points. However, "other simpler predictors of child intellectual function, such as maternal education and child height and head circumference, were significantly related to intellectual raw scores in the expected directions." In this study, as in others of this type exposure is inferred from water concentration.

Smith *et al*. (1998) studied lung and urinary bladder cancer mortality in a region of northern Chile (Region II, Antofagasta) where the residents were exposed to arsenic in their drinking water. Arsenic levels ranged from a population weighted average of 570 µg/L between 1955 and 1969 to 100 µg/L by 1980. Standardized mortality ratios (SMRs) were estimated for Region II as follows. Census data were used to calculate the person-years at risk during 1989-1993 by 10-year age groups, for men and women separately. National mortality data were obtained for 1991, the midpoint of the study period, and age- and sex-specific mortality rates were calculated for each cause of death of interest for the rest of Chile excluding Region II. The expected number of deaths was then calculated for Region II by multiplying the rest of the Chile 1991 age- and sex-specific mortality rates by the person-years at risk for residents in Region II for the period 1989-1993. Standardized mortality ratios were estimated by dividing observed deaths by expected deaths. Statistical tests of significance were based on the Poisson distribution, and 95 percent confidence intervals were calculated using exact methods.

The SMRs (observed/expected deaths) for bladder, kidney, liver, and skin cancers, and all other cancers combined, were not related to age in either sex. However, lung cancer mortality ratios were particularly high in younger men aged 30-39 yr (SMR = 11.7, 95 percent C.I. 6.4-19.6, P < 0.001). The estimated SMRs were not as elevated in all groups. The values for the subsequent 10-year age groups were: 5.9; 4.9; 2.9; 4.0; 2.8; and 3.8 for the total with a 95%CI of 3.5-4.1. Also observed was a decreasing trend in chronic obstructive pulmonary disease deaths (COPD), with higher rates among younger men, particularly those aged 30-39. Four COPD deaths were reported among men (0.8 expected), and six deaths among women (0.1 expected). These ten individuals who died of COPD would have been young children at the time of peak arsenic water levels in 1955-1970. Smoking was accounted for but not in men and women separately.

In a later study Smith *et al*. (2006) reported increased mortality from lung cancer and bronchiectasis in young adults following arsenic exposures *in utero* and in early childhood. For subjects born just before the high exposure period (1950-1957) and exposed in early childhood the SMR for bronchiectasis was 12.4 (95% C.I., 3.3-31.7; P < 0.001). For those born during the high exposure period (1958-1970) with likely *in utero* and early childhood exposure the SMR for bronchiectasis was 46.2 (C.I., 21.1-87.7; P < 0.001). The authors conclude that "exposure to arsenic in drinking water during early childhood or *in utero* has pronounced pulmonary effects, greatly increasing subsequent mortality in young adults form both malignant and nonmalignant lung disease."

Additional evidence supporting a link between childhood arsenic exposure and subsequent lung disease comes from autopsies of children in the affected area. The results of five autopsies of children, who died in 1968 and 1969 in Antofagasta and showed skin lesions and other evidence of arsenic poisoning, also showed lung abnormalities in four of the children. Two of these cases exhibited interstitial fibrosis (Rosenberg, 1974). Also, a survey of 144 children in Antofagasta with skin pigmentation due to arsenic exposure reported a history of bronchopulmonary disease 2.5-fold more frequent than children with normal skin (15.9 vs. 6.2 percent, respectively) (Borgono *et al*., 1977).

Chronic exposure to arsenic has been associated with decreased birth weight and an increased rate of spontaneous abortion in female smelter workers. However, this association is confounded by the presence of other toxicants in the smelting process, including lead (Nordstrom *et al*., 1979). Anemia and leukopenia have been reported in infants ingesting approximately 3.5 mg As/day in contaminated milk over a period of 33 days (Hammamoto, 1955).

Premature birth and subsequent neonatal death was reported in a single individual following ingestion of arsenic (Lugo *et al*., 1969).

Ihrig *et al*. (1998) conducted a hospital-based case-control study of stillbirths and environmental arsenic exposure using an atmospheric dispersion model linked to a geographical information system. They collected data on 119 cases and 267 controls in

a central Texas area including a facility with 60-year history of arsenic-based agricultural product manufacture. Four exposure groups were categorized (0; < 10 ng/m^3; 10-100 ng/m^3; and > 100 ng/m^3). For the period 1983-93 they fit a conditional logistic regression model including maternal age, race/ethnicity, parity, income group, exposure as a categorical variable, and exposure-race/ethnicity interaction. Effects were only seen in the Hispanic group with the medium exposure group having a prevalence odds ratio and 95% confidence interval of 1.9 (0.5-6.6) and the high exposure group 8.4 (1.4-50.1). The authors postulate a possible influence of a genetic polymorphism affecting folate metabolism in Hispanic populations possibly leading to increased neural tube defects and stillbirths. Small numbers limits this study; for example, there were only seven cases in the high exposure group and five of these were Hispanic.

Von Ehrenstein *et al*. (2006) studied pregnancy outcomes, infant mortality, and arsenic exposure via drinking water in West Bengal, India. The reproductive histories of 202 women were reviewed including measurements of 409 drinking water wells. The total number of pregnancies was 660 and the number of live births plus stillbirths was 558. Odds ratios for spontaneous abortion, stillbirth, neonatal mortality (death in the first month) and infant mortality (death in the first year) were estimated by logistic regression. Exposure to arsenic concentrations ≥ 200 µg/L during pregnancy was associated with a six-fold increased risk of stillbirth after adjustment for potential confounders (OR = 6.07; 95% C.I. 1.24-24.0, p = 0.01). The odds ratio for neonatal death was 2.81 (95% C.I. 0.73-10.8). No significant associations were found for arsenic exposure and spontaneous abortion (OR = 1.01; 95% C.I. 0.38-2.70) or overall infant mortality (OR = 1.33; 95% C.I. 0.43-4.04). Arsenic related skin lesions were observed in12 women who had increased risk of stillbirth (OR = 13.1; 95% C.I. 3.17-54.0).

6.3 Subchronic and Chronic Toxicity to Experimental Animals

Female albino rats (20 per group) were exposed to 0, 1.3, 4.9, or 60.7 µg As_2O_3/m^3 as aerosol continuously for 3 months (Rozenshtein, 1970). Decreased whole blood sulfhydryl group content, histological changes in the brain, bronchi, and liver, changes in conditioned reflexes, and changes in chronaxy ratio were observed in both the high- and mid-dose groups. Among animals in the high dose group, eosinophilia, decreased blood cholinesterase activity, decreased serum sulfhydryl content, and increased blood pyruvic acid were observed. No significant changes were observed in the low-dose group.

Male mice (8-10 per group) were exposed to 0, 0.5, 2.0, or 10.0 ppm sodium arsenite in drinking water for 3 weeks followed by a 28-day recovery period (Blakley *et al*., 1980). The primary immune response of the spleen (as indicated by changes in IgM-production assayed by plaque-formation) was suppressed at all dose levels. The secondary immune response was also suppressed at all dose levels as indicated by a decrease in the number of IgG producing cells.

Male Sprague-Dawley rats (7-28 per group) were exposed to 0, 40, 85, or 125 ppm sodium arsenate in drinking water for 6 weeks (Brown *et al*., 1976). Rats from all

arsenic exposed groups showed increased relative kidney weights, decreased renal mitochondrial respiration, and ultrastructural changes to the kidney.

Male ddY mice (number not stated) received 0, 3, or 10 mg As_2O_3/kg/day orally for 14 days and were examined for changes in concentrations of monoamine-related substances in various brain regions and for changes in locomotor activity (Itoh *et al*., 1990). Locomotor activity was increased in the low-dose group and decreased in the high-dose group. Several monoamine-related compounds were altered in both dose groups in the cerebral cortex, hippocampus, hypothalamus, and corpus striatum. The study indicates an effect of arsenite on brain chemistry but is inconclusive with respect to dose response.

Male and female Wistar rats (7-10 per group) were treated from age 2 to 60 days by oral gavage with daily administration of 0 or 5 mg As/kg body weight (as sodium arsenate) (Nagaraja and Desiraju, 1993; 1994). After 160 days, body weights, brain weights, and food consumption were decreased in the arsenic exposed group. Acetylcholinesterase (AChE) and GAD activity and GABA levels were decreased in the hypothalamus, brain stem, and cerebellum during the exposure period; all but AChE activity returned to normal during the post-exposure period. Changes in operant conditioning were also observed among the exposed animals.

Female Holtzman rats (>5 per group) were treated with 0, 100, 500, 1000, 2000, or 5000 ppm As_2O_3 in feed for 15 days (Wagstaff, 1978). Hexibarbitone sleeping time was altered in all arsenic exposed groups. Body weight and feed consumption were decreased among animals in the groups exposed to $\geq$ 500 ppm As_2O_3. Clinical signs of toxicity observed among arsenic exposed animals included roughened hair, diarrhea, and decreased physical activity.

Male Sprague-Dawley rats and C57 black mice (12 per group) were treated with 0, 20, 40, or 85 ppm sodium arsenate in drinking water for up to 6 weeks (Woods and Fowler, 1978). Among arsenic exposed rats, heme synthetase activity was decreased in all exposed groups. Among animals exposed to $\geq$ 40 ppm sodium arsenate, hepatic ALA synthetase activity was decreased and urinary uroporphyrin and coproporphyrin were increased. Among exposed mice, heme synthetase activity was decreased and uroporphyrinogen I synthetase activity was increased in all exposed groups. Among animals exposed to $\geq$ 40 ppm sodium arsenate, urinary uroporphyrin and coproporphyrin were increased.

Administration of 3.7 mg As_2O_3/kg/day to Rhesus monkeys for 12 months did not result in any neurologic change detectable by an EEG (Heywood and Sortwell, 1979). Two of the 7 animals exposed to this concentration died before the conclusion of the 52-week period. Of the surviving animals, two were retained for a 52-week recovery period after which they were sacrificed and necropsied. No significant changes in organ weights or gross appearance were noted.

7. Toxicity of Arsine

7.1 Toxicity to Adult Humans

Numerous case reports of accidental arsine poisoning exist in the literature, but reliable estimates of concentrations during acute human intoxication do not exist. This is due in large part to the insidious nature of arsine toxicity - arsine is a colorless gas, has a mild odor at low concentrations, produces no mucous membrane irritation, and usually results in delayed symptoms of toxicity (Klimecki and Carter, 1995). In mammalian systems, arsine primarily targets the erythrocyte and causes hemolysis and methemoglobinemia with acute exposure (NRC, 1984). Jaundice, hemoglobinuria, anuria, hepatic and renal damage, anoxia, and anemia are secondary effects resulting from hemolysis. Before the advent of dialysis, there were no reports of patients surviving if renal failure developed (Buchanan, 1962). Other acute symptoms reported include headache, weakness, dizziness, dyspnea, nausea, vomiting, diarrhea, and abdominal cramping (Klimecki and Carter, 1995). Central and peripheral nervous systems may be affected by acute arsine exposure, leading to agitation, disorientation, hallucinations, psychopathologic abnormalities, and peripheral nerve degeneration (Frank, 1976; Klimecki and Carter, 1995). The psychopathologic and peripheral abnormalities are thought to be secondary to the conversion of arsine to arsenate or arsenite. The first signs and symptoms of toxicity, hemoglobinuria and/or nausea, are usually delayed 2 to 24 hours following exposure (Kleinfeld, 1980).

A case report documents hemolytic anemia, hematuria, and renal failure following intermittent exposure to arsine gas over 2.5 hours (Parish *et al*., 1979). Symptoms of gastrointestinal distress, headache, and malaise were also reported following this exposure. The concentration of arsine gas sampled 3 days after exposure was 0.1 ppm (0.3 mg/m³), but the concentration at the time of poisoning was unknown. Another typical accidental poisoning resulted when 2 men were exposed to arsine gas in a metal smelting works (Coles *et al*., 1969). Symptoms included nausea, vomiting, red urine, generalized aching, shivering, epigastric pain, and jaundice. However, the more severely affected worker developed symptoms within 1 hour of exposure while the other did not develop symptoms for 24 hours. The more severely affected worker developed acute renal failure that required peritoneal dialysis.

In an occupational study, the highest average concentration of arsine recorded in a battery formation area of a battery manufacturing plant was 20.6 µg/m³ (0.006 ppm) (Landrigan *et al*., 1982). Elevated levels of urinary arsenic were observed in some workers but effects on the hematopoietic system were apparently not examined.

A study by Williams *et al*. (1981) collected personal and area air samples after 2 workers exhibited symptoms of arsine poisoning while restoring a large 19th century painting. Symptoms included headaches, nausea, weakness, vomiting, and red urine. The control-corrected air concentration of arsine ranged from 0.010 to 0.067 mg/m³. While these concentrations are below the OSHA PEL (permissible exposure level) 8-hour TWA (time weighted average) of 0.2 mg/m³, the results may indicate that these

workers are sensitive responders or that humans in general may be more sensitive to the effects of arsine than experimental animals. However, the air samples may not represent the actual concentration of arsine that caused the symptoms of poisoning in the workers since the workplace air was not analyzed for arsine until after symptoms were reported. The study also notes that 'appreciable concentrations' of lead and arsenic were found in the workplace air.

No studies were identified addressing the chronic toxicity of arsine in humans.

7.2 Toxicity to Infants and Children

No studies were identified allowing quantitative assessment of arsine toxicity in infants and children. Arsine's mode of toxic action is not completely understood but appears to involve binding to erythrocyte sulfhydryl groups followed by intracellular ion loss and hemolysis (Rael *et al*., 2000). Clinical treatment of arsine poisoning usually involves exchange transfusion. It seems plausible that infants and children would be more sensitive to the irreversible hematotoxicity of arsine than adults due to their greater breathing rate per unit body weight.

7.3 Toxicity to Experimental Animals

A number of studies were reviewed to understand the time-concentration relationship of arsine lethality. The most complete and relevant study was the IRDC (1985), which allowed determination of 1% and 5% lethality benchmark doses for exposure durations of 0.5 to 4 hours in rats. The most important acute non-lethal effects noted were hemolysis and reticulocytosis (Peterson and Bhattacharyya, 1985). Longer term effects of arsine also involved significant changes in hematological parameters (hemoglobin and mean corpuscular volume) (Blair, 1990).

LC_{50} values (estimate of concentration resulting in 50 percent mortality of exposed animals) reported by Gates (1946) are as follows: 120-210 ppm (380-670 mg/m³) for 10 minutes in rats, 110 ppm (350 mg/m³) for 30 minutes in dogs (equivalent to 190 ppm (610 mg/m³) for 10 minutes), and 200-300 ppm (640-960 mg/m³) for 10 minutes in rabbits. An LC_{50} in mice was reported as 31 ppm (99 mg/m³) for a 50-minute exposure (Levvy, 1947). The survival time of the fatalities (4 days) was reported to be more or less independent of exposure concentration (2500 mg/m³ to 25 mg/m³) and exposure duration.

The study by Levvy (1947) in mice varied exposure durations for each given concentration of arsine. Because the mortality data were not presented in conventional form by the standard LC_{50} method, the data were normalized to a 1-hour exposure using the modified form of Haber's equation (as described in Section 5.7.1 of the TSD):

$$C^n \times T = K,$$

where C = concentration, T = time, K = a constant determined at a given C, T and the exponent n is a constant determined experimentally. The exponent "n" of 1.8 was

determined by varying the term n in a log-normal probit analysis (Crump and Howe, 1983; Crump, 1984) until the lowest chi-square value was achieved. Fifty-four data points were used to estimate the exponent n because these points were of sufficient duration (≥ 5 minutes) and resulted in the best chi-square fit for the line and obvious heterogeneity (Table 7.3.1). This relationship indicates that the toxicity of arsine varies approximately with the product of the square of concentration times time rather that simply concentration times time.

Table 7.3.1 Arsine Mortality in Mice: Results from Levvy (1947) and 1-Hour Adjusted Concentrations Using Haber's Equation ($C^n \times T = K$, where n = 1.8).

Concentration (ppm)	Exposure Duration (min)	Mortality (no. died/total)	1-Hour Adjusted Concentration (ppm)
157*	10	30/30	58
	5	28/30	39
	2.5	17/30	27
	1.7	0/30	22
78.4*	15	21/30	36
	9	10/30	27
31.4	70	30/30	34
	50	15/30	28

** Shaded rows include data used for determination of the ED_{05} and BD_{05}*

Craig and Frye (1988) reported a 4-hour LC_{50} of 42.6 ppm in rats. However, when the rats were separated by sex for statistical purposes, there was slightly greater mortality among females than males (38.9 ppm LC_{50} for females vs. 46.8 ppm LC_{50} for males). No abnormalities were seen at necropsy except red discharge from nose, mouth, and genitalia at the higher concentrations. A concentration-related suppression of body weight gain was observed during the first week of the 14-day post-observation period.

The most comprehensive arsine lethality study was undertaken by IRDC (1985). LC_{50}s of 240, 178, and 45 ppm were determined in rats (10 rats/sex/group) for 30 minute, 1 hour, and 4-hour exposures, respectively. Deaths generally occurred within 3 days following 30-minute exposure to arsine. As in the previous study (Craig and Frye, 1988), there was slightly greater mortality in females than males. Adverse effects noted during exposure included dyspnea, while effects noted post-exposure included a concentration-related increase in hematuria, dark material around the head or the anogenital area, and pallor of ears, eyes, and feet. The higher concentrations resulted in weight loss immediately following exposure, suppressed weight gain during the first week and compensatory weight gains during the second week post-exposure. Necropsy on animals that died showed red, yellow or orange fluid in the bladder, stomach, or intestine, and discoloration of the kidneys, lungs, and liver.

Data in the IRDC (1985) report were used to determine the exponent "n" in the equation $C^n \times T = K$. This was done by varying the term n in a log-normal probit analysis (Crump, 1984; Crump and Howe, 1983) until the lowest chi-square value was achieved. The

value of "n" for extrapolation to 1-hour exposure was dependent on exposure duration. For extrapolation from 30 minutes to 1-hour exposure, n = 2.2; for extrapolation from 4-hours to 1-hour exposure, n = 1.0.

Table 7.3.2 contains the studies which provided adequate raw mortality data from which a maximum likelihood estimate corresponding to 5% lethality (ED_{05}) and benchmark dose at the 95% lower confidence interval of the ED_{05} and ED_{01} (BD_{05} and BD_{01}, respectively) could be determined.

Table 7.3.2 Animal Lethality Benchmark Dose Determinations in ppm for Arsine

Reference	Species	Exposure Time (min)	LC_{50} 60 min[1]	ED_{05} 60 min[1]	BD_{05} 60 min[1]	BD_{01} 60 min[1]
IRDC, 1985	rat	30	175	120	105	86
	rat	60	178	112	88	66
	rat	240	181	118	101	80
Craig and Frye, 1988	rat	240	170	125	102	84
Levvy, 1947	mice	varied[2]	29	20	16	13

[1] Exposure time was extrapolated to 60 minutes, if needed, using a modification of Haber's equation ($C^n * T = K$). For rats, n = 2.2 for extrapolation from 30 minutes to 1-hour, or n = 1.0 for extrapolation from 4 hours to 1-hour; for mice, n = 1.8.

[2] Lethality data for 5 exposure durations were pooled and normalized to a 1-hour exposure using the equation $C^n \times T = K$ (see Table 1).

In other experimental animal studies, a reduction in hematocrit as a function of arsine concentration was observed in mice following a 1-hour exposure (Peterson and Bhattacharyya, 1985). A LOAEL of 9 ppm (29 mg/m³) and a NOAEL of 5 ppm (16 mg/m³) were reported. The demarcation between the NOAEL and LOAEL for this non-lethal effect was well defined, not only among the exposure groups (5 ppm vs. 9 ppm), but also among individual mice in each exposure group (Peterson, 1990). Hematologic recovery of the surviving mice was gradual but nearly complete within 11 days after exposure (Peterson and Bhattacharyya, 1985). The study also reported a NOAEL of 15 ppm (100% survival) and LOAEL of 26 ppm (100% lethality) for lethality.

A continuous benchmark dose analysis of these data was performed. The full data set on hematocrit reduction 24 hours after exposure gave a BMD_{05} of 7.81 ppm and a $BMDL_{05}$ of 5.2 ppm (quadratic continuous model fit P= 0.16). The only other data sets that were adequately fit were the 24 hour increase in reticulocyte count (%) with the 11 and 26 ppm outliers removed (power continuous model, P = 0.50) and the 5 days values with the 9 ppm outlier removed (cubic continuous model, AIC = 61.8). Several response levels were evaluated including 25% relative, 1 and 2 % absolute increases and 1 and 2 standard deviations. The latter SD levels were closest to the minimal significant increase levels and exceeded the control plus one control SD values of 0.88 ppm (24 hr) and 2.0 (5 days). For a 1 SD response level at 24 hours the BMD_{1SD} = 3.29 ppm and the $BMDL_{1SD}$ = 2.17 ppm. The values for 2SD were BMD_{2SD} = 4.69 ppm and $BMDL_{2SD}$ = 3.50 ppm. For the 5 days data set the BMD_{2SD} = 4.32 ppm and the

$BMDL_{2SD}$ = 2.70 ppm. Reticulocytosis may be a more sensitive indicator of adverse hematologic effects of arsine exposure than hematocrit reduction.

A subchronic study in male and female rats and female mice (Fowler *et al*., 1989) supports the sharp increase in dose-response noted by Peterson and Bhattacharyya (1985). All treatment groups exposed to arsine (6 hr/day, 5 days/week) at concentrations of 10 ppm and above showed 100 percent mortality within 4 days while those exposed to 5 ppm or less showed no mortality or overt signs of toxicity. Other effects observed included a dose-related increase in spleen weight and a slight increase in liver weight. Blood samples taken at necropsy showed a slight dose-related decrease in hematocrit and a marked dose-related increase in the activity of red blood cell ALAD (δ-aminolevulinic acid dehydratase).

In a 90-day study, male and female mice were exposed to 0, 0.025, 0.5, and 2.5 ppm arsine gas for 6 hours/day, 5 days/week (Blair *et al*., 1990). After 5, 15, and 90 days, blood was collected for hematologic analysis. Exposure to 2.5 ppm had significant effects on all hematological parameters for nearly the entire exposure period, while 0.5 ppm caused only a few significant changes in hematological parameters at day 90 of exposure (decreased hemoglobin in males and increased MCV in females). Exposure to 0.025 ppm was without effect.

A continuous benchmark dose analysis was performed on the data sets of Blair *et al*. 1990. Adequate fits to the hematocrit data were obtained with the linear and quadratic models with $BMDL_{025}$ (relative risk) values ranging from 0.128 to 0.894 ppm (P values for model fits of 0.11 to 0.96). Absolute reticulocyte count increases gave continuous $BMDL_{10}$'s ranging from 0.22 to 0.68 ppm with linear and quadratic models (P values of 0.31 to 0.99). However, due to the poor dose spacing, essentially a missing dose level between 0.025 and 0.5 ppm, these results are considered inconclusive in determining an alternative NOAEL to 0.025 ppm.

7.4 Developmental and Reproductive Toxicity

In an unpublished study, workers in one semiconductor plant were reported to have a 39% rate of miscarriage, almost twice the national average (Sanger, 1987). Workers were exposed to unidentified levels of arsine gas, but other possible exposures were not identified.

A developmental toxicity study exposed pregnant rats and mice to 0.025, 0.5, or 2.5 ppm (0.079, 1.5, or 7.9 mg/m³) arsine for 6 hours per day on gestation days 6 through 15 (Morrissey *et al*., 1990). The rats exposed to 2.5 ppm exhibited a significant increase in fetal body weight, but no other endpoints of developmental toxicity were observed. The incidence of malformations observed in arsine exposed mice at 0.025 ppm (exencephaly) and at 2.5 ppm (unfused eyelids) was not significantly different from control mice.

8. Derivation of Reference Exposure Levels

8.1 Acute Reference Exposure Level for Inorganic Arsenic

Study	Nagymajtenyi *et al*., 1985
Study population	pregnant mice
Exposure method	maternal inhalation exposure
Exposure continuity	
Exposure duration	4 hours per day on gestation days 9, 10, 11, and 12
Critical effects	decreased fetal weight
LOAEL	0.26 mg/m³ As_2O_3 (0.197 mg As/m³)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	n/a
Human Equivalent Concentration	n/a
LOAEL uncertainty factor (UF_L)	10 (no NOAEL)
Subchronic uncertainty factor (UFs)	n/a
Interspecies Uncertainty Factor	
Toxicokinetic (UF_{A-k})	√10 (animal study)
Toxicodynamic (UF_{A-d})	√10 (animal study)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF_{H-k})	√10 (remaining interindividual variation: study considered effects on fetus or infant)
Toxicodynamic (UF_{H-d})	√10 (interindividual variation)
Cumulative uncertainty factor	1,000
Reference Exposure Level	**0.0002 mg As/m³ (0.20 µg As/m³,)**

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 in the Technical Support Document). The most appropriate study for the basis of an acute REL for arsenic is Nagymajtenyi *et al*. (1985). This study was selected since it measured a sensitive toxicological endpoint with a relevant route of exposure, and the experimental design and reporting were considered adequate (as specified in the Non-cancer Risk Assessment technical support document, Section 4.1.1). It involved a significant number of animals exposed by inhalation to three dose levels plus a control. Unfortunately, no NOAEL was obtained. However, a significant dose-related reduction in fetal weight and increased incidences of intrauterine growth retardation, skeletal malformations, and hepatocellular chromosomal aberrations were observed in mice following maternal inhalation exposure to 200 µg As/m^3 (260 µg As_2O_3/m^3) for 4 hours on gestation days 9, 10, 11, and 12 ($p<0.05$). The most sensitive effect, decreased fetal weight, was observed at 200 µg As/m^3, so 200 µg As/m^3 was taken as a LOAEL. Maternal toxicity data were not reported. This study is used as the basis of the acute REL:

0.2 mg/m^3/1000 = 0.0002 mg/m^3 = 0.2 µg As/m^3 (equivalent to 0.065 ppb arsine gas)

No temporal adjustment was made for the critical study since the critical period of exposure for a developmental effect may be very short relative to the study duration (OEHHA, 2007). The study concentration with appropriate uncertainty factors is a "not to exceed" value. An uncertainty factor of 10 (UF_L) was used to account for the lack of a no observed adverse effect level (NOAEL). A second uncertainty factor of 10 was used to account for interspecies differences between the test species and humans. This factor is the product of two components addressing pharmacokinetic (UF_{A-k}) and pharmacodynamic (UF_{A-d}) differences, each assumed to be the $\sqrt{10}$. A final uncertainty factor of 10 was applied to address human interindividual differences in pharmacokinetics (UF_{H-k}) and pharmacodynamics (UF_{H-d}) also assumed to be $\sqrt{10}$ each. The overall uncertainty of extrapolating from 4-hour exposures in mice (LOAEL) to no anticipated effects in humans is 1000 as noted in table above and the calculation of the acute REL. The rationale for the choice and value of uncertainty factors used by OEHHA is provided in the Non-cancer Risk Assessment technical support document (Section 4.4.3).

Inorganic arsenic (oxides) are listed as developmental toxicants under the California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). The studies reviewed in this document support the conclusion that exposure to inorganic arsenic may affect fetal weight, spontaneous abortion, neonatal death and postnatal neurological development.

In humans, the logarithm of infant mortality (death) increases linearly as birth weight decreases from 3500 to 1000 grams (Hogue *et al*., 1987; Rees and Hattis, 1994). This log-linear relationship exists on both sides of the weight (2500 g) conventionally used as a cutoff defining low birth weight. There is no evidence for a threshold. Thus any reduction in fetal weight is a cause for concern since it increases mortality. In the absence of certainty, OEHHA takes the health protective approach that the reduced weight effect in the animal fetuses may be biologically significant, particularly when viewed from a population perspective.

8.2 Inorganic Arsenic 8-Hour Reference Exposure Level

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures which might include daily occupational, in-home or in-school exposures. (see Section 6 in the Technical Support Document).

Due to the possibility of repeated exposure and the relatively slow clearance of arsenic compounds, the 8-hour REL is taken to be equivalent to the chronic REL. The half-life of the initial exponential phase of excretion of arsenic after a single dose is typically between one and two days, but there are also several much slower excretion processes. So a single exposure to arsenic would take several days to be cleared, mainly via urinary metabolites. Repeated exposures can significantly prolong the clearance of arsenic as the internal dose accumulates, so that in terms of internal

dosimetry it would be difficult to distinguish repeated periodic exposure from chronic exposure scenarios. An individual exposed daily via air and/or drinking water might show very similar urinary arsenic excretion to another individual exposed only periodically at work, school etc.

8.3 Inorganic Arsenic Chronic Reference Exposure Level

Study	Wasserman *et al*. (2004); Tsai *et al*. (2003)
Study population	201 children 10 years of age
Exposure method	drinking water
Exposure continuity	continuous
Exposure duration	9.5 to 10.5 years
Critical effects	Decrease in intellectual function, adverse effects on neurobehavioral development
LOAEL	0.23 µg As/m^3 based on est. LOAEL of 2.27 µg/L (Wasserman *et al*., 2004; see Section 8.3.1.1)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	none, exposure considered continuous
Human equivalent concentration	n/a
LOAEL uncertainty factor (UF_L)	3 (LOAEL estimated by quantitative analysis of study data)
Subchronic uncertainty factor (UFs)	1 (default: duration >8% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (remaining interindividual variation: study considered effects on 10 year-old but not infant)
Toxicodynamic (UF_{H-d})	√10 (default, interindividual variation)
Cumulative uncertainty factor	30
Inhalation Reference Exposure Level	**0.015 µg As/m^3**
Oral Reference Exposure Level	0.0035 µg/kg-d

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

8.3.1.1 Child Based Values

A number of studies have indicated potentially greater toxicity of arsenic exposure during childhood (see below). Although some PBPK modeling has been applied to inorganic arsenic and its methyl metabolites, the modes of toxic action and relevant internal dosimetry are not sufficiently understood at present to use this modeling directly in REL development. In this section we compare quantitative analyses of dose-

responses and LOAELs in key studies involving arsenic exposures in children. Health protective exposure levels derived from these analyses will be compared with similar analyses from studies in adults in the following section.

The study of Wasserman *et al*. (2004) indicated a dose-response of decreasing Full-Scale intellectual function raw scores with increasing drinking water arsenic exposure in 10-year olds. The values in their Fig.2 give an exact fit to a quadratic model ($Y = Y_0 + aX + bX^2$; Y_0 intercept = 0, a = -0.443, b = 0.0063, R^2 = 1.0) with a low dose slope of – 0.44 points/µg/L. Assuming an adverse effect level of one point loss, then the corresponding arsenic concentration can be calculated as:

-1point/-0.44 point/µg/L = 2.27 µg/L.

This level might be equivalent to a LOAEL. Further, assuming water intake of 1 Liter/day (L/d) and essentially complete intestinal absorption, this can be converted to an intake of 2.3 µg/d. If we assume a drinking water intake based on the 95% upper confidence level (UCL) for U.S. children aged 1 to 10 years of 1564 mL/day the intake would be somewhat higher at 3.6 µg/d (OEHHA, 2000; Table 8.3). Since 10-year old males would inhale about 9.9 m^3/d (OEHHA, 2000), if airborne arsenic were 100% absorbed, this oral effect level would be equivalent to an inhalation level of 2.3 µg/day/9.9 m^3/day = 0.23 µg/m^3. Assuming a more realistic inhalation absorption of 50 % would give a value of 0.46 µg/m^3. Applying a 3-fold UF for an estimated LOAEL based on a quantitative dose response analysis (a higher value would be used without a dose response analysis) and 10-fold for inter-individual variation since only 10-year olds were studied, a health protective air concentration of 0.015 µg/m^3 can be calculated. An oral value based on the average study body weight of 21.9 kg and 100% oral absorption would be 2.3 µg/d/21.9 kg = 0.105 µg/kg-day. Applying the same overall uncertainty factor of 30 the oral health protective value would be 0.105 µg/kg-day/30 = 0.0035 µg/kg-day.

The data of Tsai et al. (2003) for 13 year old children gave dose response relationships for arsenic exposure metrics of ppb As in drinking water and cumulative arsenic intake (mg) vs. the pattern memory (PM) and switching attention (SA) endpoints (ms). A continuous benchmark response analysis for ppb As vs. ms test duration was conducted for PM (BMD05 = 49.75; BMDL05 = 31.2 ppb) and SA (BMD05 = 28.81; BMDL05 = 19.73 ppb) both using a linear model. For cumulative As intake the PM endpoint data were similarly fit by a linear model (BMD05 = 194.1; BMDL05 = 122.7 mg) and the SA data by a polynomial (quadratic) model (BMD05 = 39.1; BMDL05 = 25.4 mg; see Fig. 1). The SA endpoint appears to be the most sensitive. Based on the SA BMDL05 of 19.7 ppb and 1 L/d drinking water intake a minimum effect level of 19.7 µg/d is estimated. If we assume a drinking water intake, based on the 95% UCL for U.S. children aged 11 to 19 years of 2.143 L/d the intake would be 2-fold higher at 42.2 µg/day (OEHHA, 2000; Table 8.3). Using uncertainty factors of 10 for interindividual variation and 3 for extrapolation from a minimum to a no effect level, a health protective intake of 19.7 µg/d/30 = 0.658 µg/d is calculated. Assuming inhalation of 10 m3/d and 50 % absorption (default) this value can be converted to an inhalation value of 0.658 µg/day/(0.50 x 10 m^3/day) = 1.32 µg/m^3. Using the SA cumulative $BMDL_{05}$ of 25.4 mg

As and 10 years exposure, an effect level of 25.4 mg/(10 yr x 365days/yr) = 6.96 µg/day is calculated. Using the same assumptions and UFs as above, an inhalation value of 0.044 µg/m^3 can be derived based on As concentration. The cumulative dose metric is a more accurate estimate of arsenic exposure than As water concentration, so the value of 0.046 µg/m^3 or 0.05 µg/m^3 (rounded) is preferred over the concentration based value. An oral value based on an average body weight for a 13-14 year old child (OEHHA, 2000) of 50 kg is 6.96 µg/day/50 kg = 0.139 µg/kg-d. Applying the same overall uncertainty factor of 30 would give 0.139 µg/kg-day/30 = 0.0046 µg/kg-day.

The quantal responses for skin lesions in young children (≤ 9 yr) and adolescents (10-19 yr) from Mazumder *et al*. (1998) were subjected to benchmark dose analysis. For young children, the quantal linear model adequately fit the data (X^2 = 6.1, P = 0.30) with a BMD_{01} = 54.4 µg/L and a $BMDL_{01}$ = 39.3 µg/L. For adolescents, the best fitting model was the log probit (X^2 = 0.77, P = 0.68) with a BMD_{01} = 77.3 µg/L and a $BMDL_{01}$ = 47.4 µg/L. These values are similar to the analysis of all age groups combined (above) and application of a 10-fold UF for intraspecies variation seems adequate for these data. Thus the health protective intake for children for skin effects would be in the range of 3.9 to 4.7 µg/d for one liter/day water intake. For conversion to inhalation equivalent, young children are assumed to inhale 9.9 m^3/day and drink 1 liter/day and adolescents to inhale 14 m^3/day and drink 1.5 liter/day (OEHHA, 2000). It is further assumed that 50 percent of inhaled arsenic is absorbed via the pulmonary and gastro-intestinal routes. The resulting health protective values would be 0.68 to 0.79 µg/m^3.

A study in Thailand (Siripitayakunkit *et al*., 1999) related drinking water arsenic exposure, indicated by hair arsenic, to IQ in 529 six to nine year old children. A continuous benchmark dose response analysis of this data set gave a BMD_{05} = 0.035 µg As/g hair and $BMDL_{05}$ = 0.0155 µg As/g (polynomial model). A slope of –3.2 IQ points/µg/g was derived from the $BMDL_{05}$. Using the conversion factor of 0.01µg As/g hair/µg As/Liter of water (Kurttio *et al*. 1998), a decrease of 1 IQ point would be equivalent to chronic consumption of 30 µg As/L water (OEHHA, 2004). At one liter/day water consumption the 30 µg/d value is over an order of magnitude higher than the analogous estimate indicated by the Wasserman *et al*. (2004) study above. An inhalation value was derived as above: 30 µg/day/(10 m^3/day x 0.50 x 30UF) = 0.20 µg/m^3.

The visual perception data from Siripitayakunkit *et al*. (2001) was subjected to continuous benchmark dose analysis. The $BMDL_{035}$ of 2.40 µg/g hair (polynomial model) was near the low level mean minus one SD score (20.5), presumably an adverse effect level on visual perception as defined by the authors. The linear model gave a higher value (3.69 µg/g) but did not fit the data as well in the low exposure range. Using the conversion factor above, one liter per day water consumption, and a 30-fold cumulative UF results in a presumptive health protective intake of 8 µg/d for this endpoint (2.40 µg/g ÷ 0.01 µg/g/µg/Liter x 1 Liter/day ÷ 30UF = 8.00 µg/d). An inhalation value was derived as above: 8.0 µg/day/(10 m^3/day x 0.50) = 1.6 µg/m^3.

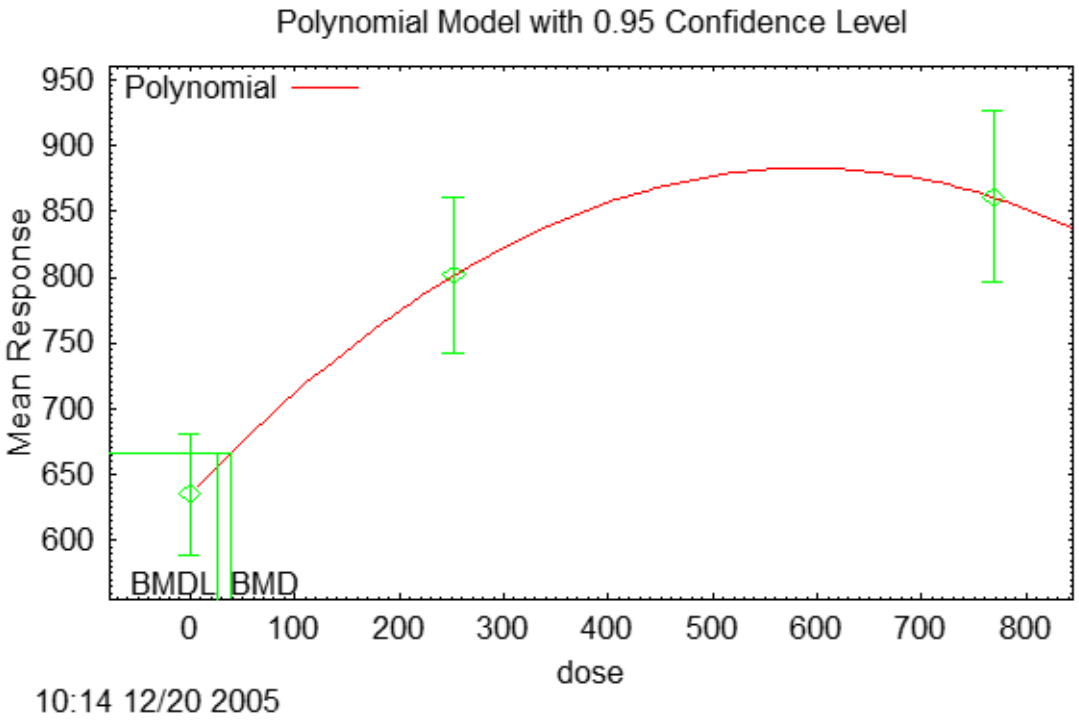
Chronic arsenic exposure appears to have adverse effects on intellectual development and visual perception in children. While the quantitation of these effects and the

toxicological significance of the criteria selected are somewhat uncertain, OEHHA thinks they are sufficient to support a chronic reference exposure level (cREL). It is uncertain whether neurological effects are the most sensitive caused by chronic arsenic exposure in children. Additional studies in exposed children are needed to adequately quantify adverse effects. The values above are summarized in Table 8.3.1. The child-based values range from 0.015 to1.6 μg/m^3. The geometric mean of the three cognitive endpoint values (0.015, 0.20, 0.05) is 0.053 μg/m^3.

Table 8.3.1. Inhalation Values Derived from Human Child Studies

Study	Toxic Endpoint	Criterion	Value	Derived cREL, μg/m^3
Wasserman *et al*. (2004)	Intellectual function	One point loss	2.27 μg/d	0.015
Siripitayakunkit *et al*. (1999)	IQ	One point loss	-3.2 IQ/μg/g hair As	0.20
Siripitayakunkit *et al*. (2001)	Visual perception loss	LOAEL	240 μg/d	1.6
Mazumder *et al*. (1998)	Skin Lesions	LED_{01}	39-47 μg/d	0.68-0.79
Tsai *et al*. (2003)	Neurobehavioral effects	LED_{05}	7 μg/d	0.05
Smith *et al*. (2006)	Bronchiectasis mortality	LED_{01}	213 μg/d	1.4

Figure 8.3.1 Switching attention (ms) in 13-year old children versus cumulative arsenic intake in mg (Tsai et al., 2003).



Inorganic arsenic is apparently more potent in its neurotoxic effects in humans than in experimental animals. The values of 2.27 µg/day in Wasserman *et al* (2004) and 7 µg/day in Tsai *et al* (2003) for cognitive effects in 10-13 year-old children are much lower than brain effects seen in animals e.g., 5 mg/kg-day in rats (Nagaraja and Desiraju, 1993; 1994) and 3.7 mg/kg-day in Rhesus monkeys (Heywood and Sortwell, 1979).

The bronchiectasis data from Smith et al. (2006) were subjected to benchmark dose analysis. A control value based on a background incidence rate of 0.04% (1/2500) and exposure of 40 µg As/L x 10 yr were used together with observed incidence values of 4/651 (90 µg As/L x 10 yr) and 9/488 (870 µg As/L x 13 yr). No statistically significant model fits were obtained. The best fitting model was the log probit (X^2 = 4.95, P = 0.026) which gave an LED_{01} ($BMDL_{01}$, 1% response) of 2.77 (mg/L) x yr. This value can be converted to an inhalation value of 1.42 µg/m^3 (2.77mg yr/L x 1000 µg/mg/(13 yr x 10 m^3/d x 30UF x 0.5) = 1.42 µg/m^3). This value has been added to Table 8.3.1 for comparison only due to the poor model fit.

8.3.1.2 Adult Based Values

In this section we review toxicological criteria from studies in adults that may serve as the basis for a chronic REL for inorganic arsenic or otherwise provide supporting information.

Studies in experimental animals show that inhalation exposure to arsenic compounds can produce immunological suppression, developmental defects, and histological or biochemical effects on the nervous system and lung, thus providing supportive evidence of the types of toxicity observed in humans. Among the inhalation studies, the lowest adverse effect level (LOAEL) was quite consistent:

245 µg As/m^3 for decreased bactericidal activity in mice (Aranyi *et al*., 1985);
200 µg As/m^3 for decreased fetal weight in mice (Nagymajtenyi *et al*., 1985); and
270 µg As/m^3 for decreased sperm motility in rats (Kamil'dzhanov, 1982).

Reports of human inhalation exposure to arsenic compounds, primarily epidemiological studies of smelter workers, indicate that adverse health effects occur as a result of chronic exposure. Among the targets of arsenic toxicity are the respiratory system (Lundgren, 1954), the circulatory system (Lagerkvist *et al*., 1986), the skin (Perry *et al*., 1948), the nervous system (Blom *et al*., 1985), and the reproductive system (Nordstrom *et al*., 1979). Occupational exposure levels associated with these effects ranged from 50 to 7000 µg As/m^3. These epidemiological studies suffer, however, from confounding as a result of potential exposure to other compounds, which limits their usefulness in the development of the chronic REL.

A single study showed effects occurring at 4.9 µg As_2O_3/m^3 (Rozenshtein, 1970). However, lack of detail with respect to endpoints and experimental design limits this study's usefulness for developing a Reference Exposure Level.

The cerebrovascular disease (CVD) and cerebrovascular infarct (CI) data of Chiou *et al*. (1997b) were subjected to benchmark dose analysis (BMD). The data were best fit using the quantal linear regression (QL) dose-response equation. Since the responses were of the order of 0.1 to 2 percent, the values calculated were for the 1 percent response (ED_{01}) and its 95% lower confidence limit (LED_{01}), rather than the usual 5 percent response values for the analysis of animal study data.

The values for CI were marginally better fit by the dose-response equation than those for CVD. Also the QL models gave better fits to the unadjusted data sets for both endpoints. The unadjusted ED_{01} and LED_{01} values with goodness of fit P value meeting the acceptable fit criterion of $P \geq 0.1$ were 359 and 189 µg/L for CVD and 268 and 166 µg/L for CI, respectively. Using the cumulative dose metric these values were 5.1, 3.0, 5.9, and 3.5 (mg/L)-yr, respectively. Due to the severity of these and other endpoints analyzed below, the uncertainty in the dose assignments (range mid-points instead of averages), and the fact that the chosen points of departure or LEDs were generally two-fold or more above concurrent control levels, the LED_{01} should be considered equivalent to a LOAEL for the purposes of risk assessment. Due to the severity of the CI endpoint, a 100 UF was used to derive a health protective water concentration of 0.1 to 0.3 µg/L based on the two dose metrics. For CVD with a 30 UF the corresponding values were 0.28 to 1.3 µg/L (for details of analysis see OEHHA, 2004). Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption, the corresponding inhalation values for these vascular effects would be for CI 0.10 to 0.33 µg/m^3 and for CVD 0.28 to 1.26 µg/m^3.

BMD analysis of the ISHD data from Chen *et al*. (1996) showed that these data were well fit by the QL dose-response equation (ED_{01} = 8.27 (mg/L)-yr, X^2 = 0.26, P = 0.88). The LED_{01} of 5.53 (mg/L)-yr should be considered an effect level for this endpoint. In this analysis the cumulative arsenic dose metric of (mg/L)-yr and resultant benchmark doses were divided by 70 yr to yield comparable lifetime drinking water concentrations of arsenic. Using a cumulative uncertainty factor of 100, a health protective concentration of 0.16 µg/L can be derived (OEHHA, 2004). Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption the corresponding health protective inhalation value for ISHD would be 0.16 µg/m^3.

The Chen *et al*. (1995) data on the association of hypertension (HT) and cumulative arsenic intake via drinking water were subjected to BMD analysis. The QL dose-response equation fit the unadjusted data well but was somewhat less than adequate for the adjusted prevalence values. The acceptable criterion for the X^2 goodness of fit test for the benchmark dose is $P \geq 0.10$. In the case of arsenic induced hypertension, the 10 percent effect level was chosen due to the higher background and greater dose response range compared to other human studies evaluated where 1% or 5% response levels were used. For HT the LED_{10} is considered an appropriate LOAEL for risk assessment. In the case of the adjusted data set, removal of the highest cumulative dose allows an acceptable fit of the QL equation with an LED_{10} of 7.4 (mg/L)-yr. The data of Rahman *et al*. (1999) were also analyzed. Both crude and adjusted data sets were well fit by the QL model with P values much greater than 0.1. The unadjusted LED_{10} value of 6.3 (mg/L)-yr from Bangladesh is quite similar to comparable value of 7.2 (mg/L)-yr from the Taiwan study (OEHHA, 2004). Health protective drinking water concentrations with a cumulative uncertainty factor of 30 ranged from 0.55 to 0.68 µg/Liter. Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption the corresponding health protective inhalation value for HT would be 0.55 to 0.70 µg/m^3.

The data of Chen *et al*. (2006) indicate a supralinear dose-response. The data were analyzed for benchmark response using metrics of time weighted average (TWA) and cumulative arsenic exposure of TWA times years of exposure or (mg/L)-yr. Systolic hypertension quantal responses of the first four quintiles of the overall population (N = 8726) were fit by the log-logistic model of BMDS (v 1.4.1). The $BMDL_1$ values (1% response) of 71.5 µg/L and 0.66 (mg/L)-yr were obtained (X^2 = 3.8, P = 0.15, d.f. = 2). The pulse hypertension data were similarly fit using the longer-term exposure subpopulation (N = 6319). In this case the 10% response level was used for $BMDL_{10}$'s of 0.49 µg/L and 0.004 (mg/L)-yr (X^2 = 4.45, P = 0.11, d.f = 2). TWA BMDLs for systolic and pulse hypertension in arsenic exposed subpopulations with lower intakes of B vitamins were also evaluated. The $BMDL_{10}$ values for populations with low dietary folate ranged from 62 to 405 µg/L TWA. The results indicate a higher sensitivity of the pulse hypertension effect to low level arsenic than the systolic hypertension effect. The supralinearity of dose-response makes comparison with earlier studies problematic. For example, projected 10^{-4} extra risk levels for pulse and systolic hypertension from this study are at least an order of magnitude less than values seen earlier with Chen *et al*. (1995) or Rahman *et al*. (1999) although cumulative arsenic exposures were 5-10 times

higher in the latter studies (Table 6). A cREL estimated from the 0.49 µg/L value above would be 0.0033 µg/m^3 (0.49 µg/L x 2L/d/(20m^3/d x 0.5 absorption x 30UF).

Similarly, the diabetes mellitus (DM) data of Lai *et al*. (1994) and Rahman *et al*.(1998) were analyzed. In this case, the QL dose-response model adequately fit both unadjusted and multivariate-adjusted prevalences. EDs and LEDs were determined for the 1 and 5 percent response levels. The LED_{05} for the adjusted values appear the best choice for a chronic criterion for arsenic-induced diabetes mellitus, i.e., 8.8 (mg/L)-yr from Lai *et al*. and 0.21 mg/L from Rahman *et al*. The health protective drinking water derived from these values with a cumulative UF of 30 were 0.84 and 1.4 µg/L, respectively (OEHHA, 2004). Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption, the corresponding health protective inhalation values for diabetes mellitus would be 0.85 to 1.4 µg/m^3.

In addition to the values noted above, an estimated LOAEL of 20 (mg/L)-yr for peripheral vascular disease from Tseng *et al*. (1996) was also included in this analysis. Using a cumulative UF of 30, a drinking water value of 1.9 µg/L was derived (OEHHA, 2004). Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption, the corresponding health protective inhalation value for peripheral vascular disease would be 1.9 µg/m^3. The study of Wang *et al*. (2002) on arsenic induced carotid atherosclerosis (subclinical) also gave an estimated LOAEL of 20 (mg/L)-yr and would yield the same health protective values.

The arsenic-induced skin keratosis and hyperpigmentation data of Mazumder *et al*. (1998) were analyzed as above (OEHHA, 2004). For both male and female skin keratosis data sets, adequate fits were obtained by the QL model with lower bound values (LED_{01}) of 49.6 µg/L for males and 124 µg/L for females. Adequate fits could not be obtained for both hyperpigmentation data sets with the models available in the benchmark dose program; however, the dose-response graphs appeared to be linear in the lower exposure groups with respective LED_{01}s of 18.9 and 34.7 µg/L. It appears that a single dose level outlier (125 µg/L) was largely responsible for the failure of the statistical test. Mazumder also included an assessment of skin keratosis and hyperpigmentation prevalence by dose per body weight. Using the dose metric of µg/kg-day, the skin hyperpigmentation data were still unable to be fit by the BMDS models. Therefore only the skin keratosis endpoint appears suitable for the development of a health protective value for arsenic-induced noncancer effects. Using a cumulative UF of 30, a drinking water value of 1.7 µg/L was derived. Assuming 20 m^3/day inhalation, 2 Liters/day water consumption and 50 percent inhalation absorption, the corresponding health protective inhalation value for skin keratosis would be 0.34 µg/m^3.

The skin lesion data of Rahman *et al*. (2006) was analyzed for benchmark response. The unadjusted data reported in Rahman's Table 3 was used with the mid points of the exposure concentration ranges (e.g., 5, 30, 100, 224, 450 µg/L) and the mean As exposures in Rahman's Table 4 (e.g., 9.8, 59.3, 127, 199, 344 µg/L). For the unadjusted male data, no adequate fit could be obtained. The female data was adequately fit by the quantal linear (P = 0.43) and log-logistic (P = 0.51) models. The

latter giving a $BMDL_{10}$ of 6.28 µg/L with mid-point based exposure estimates, and the former giving a $BMDL_{10}$ of 108.2 µg/L with mean As concentrations. Similarly, for the cumulative As dose metric of (mg/L)-yr no adequate fit was obtained with the male data, while the female data were best fit by the log-probit model (P = 0.86) for a $BMDL_{10}$ of 2.80 (mg/L)-yr. Using the age and asset adjusted data with the average As concentrations, an adequate fit to the male data could be obtained with the multistage model if the top dose group was removed, $BMDL_{10}$ = 96.0 µg/L (X^2 = 0.60, P = 0.74). The female adjusted data set gave a lower BMDL of 65.4 µg/L despite the authors' finding that the males were more sensitive. This may simply reflect the difficulty of fitting the male data. In almost all cases, the BMDL values are lower (indicating higher risk) than seen in the earlier study by Mazumder *et al*. (1998) analyzed above.

The Von Ehrenstein et al. (2005) study of decrements in lung function related to arsenic exposure via drinking water reported slopes of -45.0 mL forced expiratory volume in 1 second (FEV_1) and -41.1 mL forced vital capacity (FVC) per 100 µg/L increase in arsenic concentration for exposed men. Assuming low dose linearity these values can be converted to inhalation values of 0.044 µg/m^3 (FEV_1) and 0.048 µg/m^3 (FVC) corresponding to respective 1 mL losses in lung function (e.g., 45/100 = 2.22 µg/L/mL decrement; 2.22 µg/L/mL x 2L water/d /(20m^3/d x 10UF x 0.5) = 0.044 µg/m^3/mL).

The inhalation values derived from oral human exposure studies above are summarized in Table 8.3.2. With the exception of the very low value derived from the pulse hypertension endpoint, the derived health protective inhalation values range over approximately forty fold from 0.044 to 1.7 µg/m^3. These adult values exceed the child-based values (range 0.015 to 1.6 µg/m^3). Therefore the proposed chronic REL value of 0.015 µg/m^3 is derived from the child arsenic exposure studies evaluated above and the adult studies provide supporting information.

In addition to being inhaled, airborne arsenic can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for arsenic of 0.0035 µg/kg-day is also proposed. (From section 8.3.1.1, 2.3 µg/kg-d/(21.9 kg x 30UF) = 0.0035 µg/kg-d).

Table 8.3.2 Inhalation Values Derived from Adult Human Drinking Water Studies

Study	Toxic Endpoint	Criterion	Value	Derived chronic REL, ($\mu g/m^3$)
Chiou *et al*. (1997b)	Cerebrovascular disease	LED_{01}	378 µg/d	1.26
Chiou *et al*. (1997b)	Cerebrovascular infarct	LED_{01}	332 µg/d	0.33
Chen *et al*. (1996a)	Ischemic Heart Disease Mortality	LED_{01}	5.53 (mg/L)-yr	0.16
Chen *et al*. (1995)	Hypertension	LED_{10}	5.8 (mg/L)-yr	0.55
Chen *et al*. (2006)	Systolic hypertension	SHT LED_{01}	71.5 µg/L	1.43
	pulse hypertension	PHT LED_{10}	0.49 µg/L	0.0033
Lai *et al*. (1994)	Diabetes mellitus	LED_{05}	8.8 (mg/L)-yr	0.85
Rahman *et al*. (1998)	Diabetes mellitus	LED_{05}	0.21 mg/L	1.4
Mazumder *et al*. (1998)	Skin keratosis	LED_{01}	50 µg/L	0.33
Rahman *et al*. (2006)	Skin keratosis or altered pigmentation	LED_{10}	65.4 µg/L	0.44
Tseng *et al*. (1996)	Peripheral vascular disease	est. LOAEL	20 (mg/L)-yr	1.69
Wang *et al*. (2002)	Carotid atherosclerosis	est. LOAEL	20 (mg/L)-yr	1.69
Von Ehrenstein *et al*. (2005)	Lung Function decrements	-1 mL FEV_1	2.22 µg/L	0.044
		-1 mL FVC	2.42 µg/L	0.048

9. Arsine Based Calculations

The NAC/NRC (National Advisory Committee on Acute Exposure Guideline Levels for Hazardous Substances/National Research Council Subcommittee on Acute Exposure Guideline Levels) derived an Acute Exposure Guidance Level-2 (AEGL-2, disabling) of 0.17 ppm (500 μg/m^3) for one-hour exposure to arsine based on the hemolysis mouse data of Peterson and Bhattacharyya (1985) (Thomas and Young, 2001). Due to the steepness of the dose response the derivation of an AEGL-1 (Non-disabling) was considered inappropriate. Also the reliance on animal data was considered more "scientifically valid than AEGLs estimated from limited anecdotal human data". The panel used a total UF of 30 (10 for interspecies differences and 3 for intraspecies differences).

Based on the same study data, OEHHA calculated a continuous $BMDL_{1SD}$ of 2.17 ppm (6.9 mg/m^3) for reticulocytosis. When this value was adjusted with uncertainty factors of 10 for interspecies and 30 for intraspecies differences (including 10 for the intraspecies toxicokinetic sub-factor, as proposed in OEHHA, 2007 draft) the potential acute reference exposure level (aREL) for a one hour exposure was 2.17 ppm/300 = 0.0072 ppm (23 μg/m^3).

Despite the additional 10-fold margin of safety and more sensitive endpoint incorporated in the OEHHA derivation summarized above, there is still residual uncertainty in this comparison aREL value for arsine. There is particular concern with respect to the lack of adequate human data, given that rodents appear more resistant to the effects of acute exposure to various inorganic forms of arsenic than humans. The analogy between arsine and other inorganic forms of arsenic is supported by the observation that arsine exposure in humans and experimental animals results in similar metabolites excreted in urine as result from other inorganic arsenic exposure (Landrigan *et al*., 1982; Buchet *et al*., 1998). A further source of concern with a REL based on the Peterson and Bhattacharyya (1985) study is that while the margin of exposure for hemolysis is greater than 1000, the margin for total lethality is less than 4000. Although a steep dose-response slope for acute lethality is not unprecedented, it is a problematic feature when combined with the uncertainty in animal-to human extrapolation noted above. Thus, OEHHA staff have low confidence in using the Peterson and Bhattacharyya study as a basis of an aREL value for arsine and instead will rely on the aREL based on arsenic trioxide inhalation in mice (0.2 μg/m^3 arsenic, equivalent to 0.065 ppb arsine), which is sufficiently protective for all inorganic arsenic species.

A comparison of various possible values for an 8-hour REL for arsine is shown in Table 8.3.3. Adjustment of the one-hour NOAEL from Peterson and Bhattacharyya (1985) to eight hours using the modified Haber equation for mice gives a value of 1.6 ppm (4.98 mg/m^3)/300UF = 0.053 ppm (17 μg/m^3). This value is much higher than the values observed by Williams *et al*. (1981) in workers exposed to arsine concentrations estimated at 0.01 to 0.07 mg/m^3. The adverse effects noted included headache, nausea, weakness and vomiting. Although based on only a couple of subjects, the Williams *et al*. study would indicate an 8-hour value of about 0.04 mg/m^3/30 UF = 0.001 mg/m^3 or 1μg/m^3.

Alternatively, the 90-day study of Blair *et al*. (1990) gives a NOAEL for hematologic effects in mice of 0.025 ppm arsine at 6 hours/day, 5 days/week. Applying the same 300 UF as above gives 0.083 ppb or 0.26 µg/m^3. This latter figure seems more in line with the limited human observations and more suitable for potentially repeated 8-hour exposures to arsine. The intraspecies extrapolation includes additional uncertainty factors (PK + PD UF) for exposure of infants and children to arsine.

Table 8.3.3. Development of Health Protective Values for Arsine

Study	Toxic Endpoint	NOAEL/LOAEL/ BMDL	*Derived REL µg/m^3*
Peterson and Bhattacharyya, 1985	Reticulocytosis in mice 1 hour exposure	$BMDL_{1SD}$ 2.17 ppm 6.9 mg/m^3	*Acute 23*
Peterson and Bhattacharyya, 1985	As above with 8-hour adjustment	1.6 ppm 4.98 mg/m^3	*8-hour 17*
Williams *et al*., 1981	Headache, nausea, weakness, and vomiting in exposed workers	0.01 to 0.07 mg/m^3, average 0.04 mg/m^3 LOAEL.	*8-hour 1.0*
Blair et al*., 1990*	*Hematologic effects*	*NOAEL 0.025 ppm 6 hr/day*	*8-hour 0.26*

PBPK modeling of arsenic species in experimental animals and humans is presently considered inadequate to apply directly to the derivation of RELs for repeated arsine exposures.

Arsine exposure at atmospheric concentrations that caused adverse maternal effects did not adversely affect endpoints of developmental toxicity in mice or rats (Morrissey *et al*., 1990). In the absence of neurodevelopmental studies with arsine, it is assumed that such an effect would be comparable to those of other inorganic forms of arsenic. In view of the observed effect levels for hematological effects noted in the animal studies, both 8 hour and chronic effects of arsine are considered to be adequately covered by the respective cREL for inorganic arsenic based on neurodevelopmental effects observed in children (i.e., 0.015 µg/m^3 arsenic, equivalent to 0.005 ppb arsine)). In view of the concern over neurodevelopmental effects for all inorganic forms of arsenic, OEHHA concludes that it is appropriate to apply this value for 8-hour and chronic exposures to arsine.

10. Arsenic as a Toxic Air Contaminant that Disproportionately Impacts Children

In view of the neurodevelopmental toxicity studies discussed above, it is clear that infants and children are more susceptible to the toxicity of arsenic than adults. OEHHA recommends that inorganic arsenic and arsine be identified as a Toxic Air Contaminant the disproportionately impacts children under the California Health and Safety Code Section 39699.5.

11. References

ACGIH (1992). Documentation of the threshold limit values and biological exposure indices. Cincinnati (OH): American Conference of Government Industrial Hygienists, Inc.

Aposhian HV (1997). Enzymatic methylation of arsenic species and other new approaches to arsenic toxicity. Annu Rev Pharmacol Toxicol 37: 397-419.

Aposhian HV, Gurzau ES, Le XC, Gurzau A, Healy SM, Lu X, Ma M, Yip L, Zakharyan RA, Maiorino RM, Dart RC, Tircus MG, Gonzalez-Ramirez D, Morgan DL, Avram D and Aposhian MM (2000a). Occurrence of monomethylarsonous acid in urine of humans exposed to inorganic arsenic. Chem Res Toxicol 13(8): 693-7.

Aposhian HV, Zheng B, Aposhian MM, Le XC, Cebrian ME, Cullen W, Zakharyan RA, Ma M, Dart RC, Cheng Z, Andrewes P, Yip L, O'Malley GF, Maiorino RM, Van Voorhies W, Healy SM and Titcomb A (2000b). DMPS-arsenic challenge test. II. Modulation of arsenic species, including monomethylarsonous acid (MMA(III)), excreted in human urine. Toxicol Appl Pharmacol 165(1): 74-83.

Apostoli P, Alessio L, Romeo L, Buchet JP and Leone R (1997). Metabolism of arsenic after acute occupational arsine intoxication. J Toxicol Environ Health 52(4): 331-42.

Aranyi C, Bradof JN, Fenters JD, Graham JA and Miller FJ (1981). Effects of inhalation of arsenic trioxide aerosols in the pulmonary defenses of mice. In: International Conference. Heavy Metals in the Environment. Commission of the European Communities and the World Health Organization. 450-453.

Aranyi C, Bradof JN, O'Shea WJ, Graham JA and Miller FJ (1985). Effects of arsenic trioxide inhalation exposure on pulmonary antibacterial defenses in mice. J Toxicol Environ Health 15(1): 163-72.

ATSDR. (2000). *Arsenic. Toxicological Profile (Update)*. U.S. DHHS, Atlanta GA. http://www.atsdr.cdc.gov/toxprofiles/fp2-c4.pdf.

Baxley MN, Hood RD, Vedel GC, Harrison WP and Szczech GM (1981). Prenatal toxicity of orally administered sodium arsenite in mice. Bull Environ Contam Toxicol 26(6): 749-56.

Blair PC, Thompson MB, Bechtold M, Wilson RE, Moorman MP and Fowler BA (1990). Evidence for oxidative damage to red blood cells in mice induced by arsine gas. Toxicology 63(1): 25-34.

Blakley BR, Sisodia CS and Mukkur TK (1980). The effect of methylmercury, tetraethyl lead, and sodium arsenite on the humoral immune response in mice. Toxicol Appl Pharmacol 52(2): 245-54.

Blom S, Lagerkvist B and Linderholm H (1985). Arsenic exposure to smelter workers. Clinical and neurophysiological studies. Scand J Work Environ Health 11(4): 265-9.

Borgono JM, Vicent P, Venturino H and Infante A (1977). Arsenic in the drinking water of the city of Antofagasta: epidemiological and clinical study before and after the installation of a treatment plant. Environ Health Perspect 19: 103-5.

Brown MM, Rhyne BC and Goyer RA (1976). Intracellular effects of chronic arsenic administration on renal proximal tubule cells. J Toxicol Environ Health 1(3): 505-14.

Buchanan WD (1962). Arsine. In: Toxicity of Arsenic Compounds. Browning E. Elsevier. New York (NY): 67-75.

Buchet JP, Apostoli P and Lison D (1998). Arsenobetaine is not a major metabolite of arsine gas in the rat. Arch Toxicol 72(11): 706-10.

Buchet JP and Lauwerys R (1985). Study of inorganic arsenic methylation by rat liver in vitro: relevance for the interpretation of observations in man. Arch Toxicol 57(2): 125-9.

Buchet JP and Lauwerys R (1987). Study of factors influencing the in vivo methylation of inorganic arsenic in rats. Toxicol Appl Pharmacol 91(1): 65-74.

Buchet JP, Lauwerys R and Roels H (1981a). Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. Int Arch Occup Environ Health 48(1): 71-9.

Buchet JP, Lauwerys R and Roels H (1981b). Urinary excretion of inorganic arsenic and its metabolites after repeated ingestion of sodium metaarsenite by volunteers. Int Arch Occup Environ Health 48(2): 111-8.

Calderon J, Navarro ME, Jimenez-Capdeville ME, Santos-Diaz MA, Golden A, Rodriguez-Leyva I, Borja-Aburto V and Diaz-Barriga F (2001). Exposure to arsenic and lead and neuropsychological development in Mexican children. Environ Res 85(2): 69-76.

Campbell D and Oates RK (1992). Childhood poisoning--a changing profile with scope for prevention. Med J Aust 156(4): 238-40.

Carter DE, Aposhian HV and Gandolfi AJ (2003). The metabolism of inorganic arsenic oxides, gallium arsenide, and arsine: a toxicochemical review. Toxicol Appl Pharmacol 193(3): 309-34.

Charbonneau SM, Spencer K, Bryce F and Sandi E (1978). Arsenic excretion by monkeys dosed with arsenic-containing fish or with inorganic arsenic. Bull Environ Contam Toxicol 20(4): 470-7.

Chen CJ, Chiou HY, Chiang MH, Lin LJ and Tai TY (1996). Dose-response relationship between ischemic heart disease mortality and long-term arsenic exposure. Arterioscler Thromb Vasc Biol 16(4): 504-10.

Chen CJ, Hsueh YM, Lai MS, Shyu MP, Chen SY, Wu MM, Kuo TL and Tai TY (1995). Increased prevalence of hypertension and long-term arsenic exposure. Hypertension 25(1): 53-60.

Chen CJ, Wu MM, Lee SS, Wang JD, Cheng SH and Wu HY (1988). Atherogenicity and carcinogenicity of high-arsenic artesian well water. Multiple risk factors and related malignant neoplasms of blackfoot disease. Arteriosclerosis 8(5): 452-60.

Chen KP and Wu HY (1962). Epidemiologic studies on blackfoot disease: II. A study of source of drinking water in relation to the disease. J Formosan Med Assoc 61: 611-618.

Chen Y, Factor-Litvak P, Howe GR, Graziano JH, Brant-Rauf P, Parvez F, Van Geen A, Ahsan H. (2006). Arsenic exposure from drinking water, dietary intakes of B vitamins and folate, and risk of high blood pressure in Bangladesh: A population-based, cross-sectional study. Am J Epidemiol 165:541-552.

Chi IC and Blackwell RQ (1968). A controlled retrospective study of blackfoot disease, an endemic peripheral gangrene disease in Taiwan. Am J Epidemiol 88: 7-24.

Chiou HY, Hsueh YM, Hsieh LL, Hsu LI, Hsu YH, Hsieh FI, Wei ML, Chen HC, Yang HT, Leu LC, Chu TH, Chen-Wu C, Yang MH and Chen CJ (1997a). Arsenic methylation capacity, body retention, and null genotypes of glutathione S-transferase M1 and T1 among current arsenic-exposed residents in Taiwan. Mutat Res 386(3): 197-207.

Chiou HY, Huang WI, Su CL, Chang SF, Hsu YH and Chen CJ (1997b). Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 28(9): 1717-23.

Coles GA, Davies HJ, Daley D and Mallick NP (1969). Acute intravascular haemolysis and renal failure due to arsine poisoning. Postgrad Med J 45(521): 170-2.

Craig DK and Frye J. (1988). Acute LC50 nose only inhalation toxicity studies of arsine in rats (final report). N0512-4700. Batelle Columbus Labs. Columbus (OH)

Crump KS (1984). A new method for determining allowable daily intakes. Fundam Appl Toxicol 4(5): 854-71.

Crump KS and Howe R. (1983). Probit - A computer program to extrapolate quantile animal toxicological data to low doses. Crump K.S. & Company Inc.

Danielsson BR, Dencker L, Lindgren A and Tjalve H (1984). Accumulation of toxic metals in male reproduction organs. Arch Toxicol Suppl 7: 177-80.

DeSesso JM, Jacobson CF, Scialli AR, Farr CH and Holson JF (1998). An assessment of the developmental toxicity of inorganic arsenic. Reprod Toxicol 12(4): 385-433.

Engel RR and Smith AH (1994). Arsenic in drinking water and mortality from vascular disease: an ecologic analysis in 30 counties in the United States. Arch Environ Health 49(5): 418-27.

Ferm VH and Carpenter SJ (1968). Malformations induced by sodium arsenate. J Reprod Fertil 17(1): 199-201.

Flury F (1921). [Uber Kampfgasvergiftungen. IX. Lokal reizende Arsenverbindungen]. Zeichschrift fur die Gesamte Experimentelle Medizin 13: 527-528.

Flury F (1931). Schadliche Gase - dampfe, nebel, rauch - und staubarten. Berlin: Verlag von Julius Springer.

Fowler BA, Moorman MP, Adkins BJ, Bakewell WEJ, Blair PC and Thompson MB (1989). Arsine: toxicity data from acute and short-term inhalation exposures. In: ACGIH. Hazard assessment and control technology in semiconductor manufacturing. Lewis Publishers. Chelsea (MI): 85-89.

Frank G (1976). [Neurological and psychiatric disorders following acute arsine poisoning (author's transl)]. J Neurol 213(1): 59-70.

Freeman GB, Schoof RA, Ruby MV, Davis AO, Dill JA, Liao SC, Lapin CA and Bergstrom PD (1995). Bioavailability of arsenic in soil and house dust impacted by smelter activities following oral administration in cynomolgus monkeys. Fundam Appl Toxicol 28(2): 215-22.

Friberg L, Nordberg GF and Vouk VB, eds. (1986). Handbook on the Toxicology of Metals. Elsevier Amsterdam. p 59

Gates M, Williams J and Zapp JA. (1946). *Arsenicals. Summary technical report of Division 9, National Defense Research Committee. Vol. 1. Chemical warfare agents and related chemical problems.*: Office of Scientific Research and Development.

Gentry PR, Covington TR, Mann S, Shipp AM, Yager JW and Clewell HJ, 3rd (2004). Physiologically based pharmacokinetic modeling of arsenic in the mouse. J Toxicol Environ Health A 67(1): 43-71.

George B, Mathews V, Poonkuzhali B, Shaji RV, Srivastava A and Chandy M (2004). Treatment of children with newly diagnosed acute promyelocytic leukemia with arsenic trioxide: a single center experience. Leukemia 18(10): 1587-90.

Gladysheva TB, Oden KL and Rosen BP (1994). Properties of the arsenate reductase of plasmid R773. Biochemistry 33(23): 7288-93.

Grayson M, ed. (1978). Kirk-Othmer Encyclopedia of Chemical Technology. John Wiley and Sons New York. 247-251

Gregus Z, Gyurasics A and Csanaky I (2000). Biliary and urinary excretion of inorganic arsenic: monomethylarsonous acid as a major biliary metabolite in rats. Toxicol Sci 56(1): 18-25.

Grobe VJ (1976). [Peripheral circulatory disorders and acrocyanosis in arsenic exposed Moselle wine-growers]. Berufsdermatosen 24(3): 78-84.

Hafeman DM, Ahsan H, Louis ED, Siddique AB, Slavkovich V, Cheng Z, van Geen A and Graziano JH (2005). Association between arsenic exposure and a measure of subclinical sensory neuropathy in Bangladesh. J Occup Environ Med 47(8): 778-84.

Hammamoto E (1955). [Infant arsenic poisoning by powdered milk]. Japanese Medical Journal 1649: 2-12 [cited in ATSDR, 2006].

Harrison WP and Hood RD (1981). Prenatal effecrs following exposure of hamsters to sodium arsenite by oral or intraperitoneal routes [abstract]. Teratology 23: 40A [cited in Willhite and Ferm, 1984].

Hatlelid KM, Brailsford C and Carter DE (1995). An in vitro model for arsine toxicity using isolated red blood cells. Fundam Appl Toxicol 25(2): 302-6.

Hatlelid KM, Brailsford C and Carter DE (1996). Reactions of arsine with hemoglobin. J Toxicol Environ Health 47(2): 145-57.

Hayakawa T, Kobayashi Y, Cui X and Hirano S (2005). A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19. Arch Toxicol 79(4): 183-91.

Healy SM, Casarez EA, Ayala-Fierro F and Aposhian H (1998). Enzymatic methylation of arsenic compounds. V. Arsenite methyltransferase activity in tissues of mice. Toxicol Appl Pharmacol 148(1): 65-70.

Heywood R and Sortwell RJ (1979). Arsenic intoxication in the rhesus monkey. Toxicol Lett 3: 137-144.

Hogue CJ, Buehler JW, Strauss LT and Smith JC (1987). Overview of the National Infant Mortality Surveillance (NIMS) project--design, methods, results. Public Health Rep 102(2): 126-38.

Holson JF, Stump DG, Ulrich CE and Farr CH (1999). Absence of prenatal developmental toxicity from inhaled arsenic trioxide in rats. Toxicol Sci 51(1): 87-97.

Hood RD and Harrison WP (1982). Effects of prenatal arsenite exposure in the hamster. Bull Environ Contam Toxicol 29(6): 671-8.

HSDB (1995). Hazardous Substances Data Bank. National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.

Hughes MF, Menache M and Thompson DJ (1994). Dose-dependent disposition of sodium arsenate in mice following acute oral exposure. Fundam Appl Toxicol 22(1): 80-9.

IARC. (2004). *Some Drinking -water Disinfectants and Contaminants, including Arsenic*. 84. International Agency for Research on Cancer, World Health Organization, Lyon, France.

ICRP (1994). Human Respiratory Tract Model for Radiological Protection. International Commission on Radiological Protection. Publication 66 Annals of the ICRP. Tarrytown (NY): Elsevier Science Inc. 24(1-3).

Ihrig MM, Shalat SL and Baynes C (1998). A hospital-based case-control study of stillbirths and environmental exposure to arsenic using an atmospheric dispersion model linked to a geographical information system. Epidemiology 9(3): 290-4.

IRDC. (1985). Three acute inhalation toxicity studies of arsine on rats (final reports). 533-001, 533-002, 533-003. International Research and Development Corporation. Mattawan (MI)

Itoh T, Zhang YF, Murai S, Saito H, Nagahama H, Miyate H, Saito Y and Abe E (1990). The effect of arsenic trioxide on brain monoamine metabolism and locomotor activity of mice. Toxicol Lett 54(2-3): 345-53.

Jacobson-Kram D and Montalbano D (1985). The reproductive effects assessment group's report on the mutagenicity of inorganic arsenic. Environ Mutagen 7(5): 787-804.

Ji G and Silver S (1992). Reduction of arsenate to arsenite by the ArsC protein of the arsenic resistance operon of Staphylococcus aureus plasmid pI258. Proc Natl Acad Sci U S A 89(20): 9474-8.

Jiang G, Gong Z, Li XF, Cullen WR and Le XC (2003). Interaction of trivalent arsenicals with metallothionein. Chem Res Toxicol 16(7): 873-80.

Kamil'dzhanov AX (1982). Hygiene basis for the maximum permissible concentration of arsenic trioxide in the ambient air. Gig Sanit 2: 74-75.

Kamkin AB (1982). [Review of the maximum permissible concentration of arsenic trioxide in the atmosphere of populated places]. Gig Sanit(1): 6-9.

Kitchin KT (2001). Recent advances in arsenic carcinogenesis: modes of action, animal model systems, and methylated arsenic metabolites. Toxicol Appl Pharmacol 172(3): 249-61.

Kleinfeld MJ (1980). Arsine poisoning. J Occup Med 22(12): 820-1.

Klimecki WT and Carter DE (1995). Arsine toxicity: chemical and mechanistic implications. J Toxicol Environ Health 46(4): 399-409.

Krafft T and Macy JM (1998). Purification and characterization of the respiratory arsenate reductase of Chrysiogenes arsenatis. Eur J Biochem 255(3): 647-53.

Kurttio P, Komulainen H, Hakala E, Kahelin H and Pekkanen J (1998). Urinary excretion of arsenic species after exposure to arsenic present in drinking water. Arch Environ Contam Toxicol 34(3): 297-305.

Lagerkvist B, Linderholm H and Nordberg GF (1986). Vasospastic tendency and Raynaud's phenomenon in smelter workers exposed to arsenic. Environ Res 39(2): 465-74.

Lai MS, Hsueh YM, Chen CJ, Shyu MP, Chen SY, Kuo TL, Wu MM and Tai TY (1994). Ingested inorganic arsenic and prevalence of diabetes mellitus. Am J Epidemiol 139(5): 484-92.

Landrigan PJ, Costello RJ and Stringer WT (1982). Occupational exposure to arsine. An epidemiologic reappraisal of current standards. Scand J Work Environ Health 8(3): 169-77.

Le XC, Lu X, Ma M, Cullen WR, Aposhian HV and Zheng B (2000a). Speciation of key arsenic metabolic intermediates in human urine. Anal Chem 72(21): 5172-7.

Le XC, Ma M, Cullen WR, Aposhian HV, Lu X and Zheng B (2000b). Determination of monomethylarsonous acid, a key arsenic methylation intermediate, in human urine. Environ Health Perspect 108(11): 1015-8.

Levin-Scherz JK, Patrick JD, Weber FH and Garabedian C, Jr. (1987). Acute arsenic ingestion. Ann Emerg Med 16(6): 702-4.

Levvy GA (1947). A study of arsine poisoning. Quart J Exp Physiol 34: 47-67.

Lewis DR, Southwick JW, Ouellet-Hellstrom R, Rench J and Calderon RL (1999). Drinking water arsenic in Utah: A cohort mortality study. Environ Health Perspect 107(5): 359-65.

Lugo G, Cassady G and Palmisano P (1969). Acute maternal arsenic intoxication with neonatal death. Am J Dis Child 117(3): 328-30.

Lundgren KD (1954). [Damage to respiratory organs in workers in a smelting plant] cited in U.S. EPA, 1984. Nord Hyg Tidskr 3-4: 66-82.

Mann S, Droz PO and Vahter M (1994). A physiologically based pharmacokinetic model for four major arsenic species in mammals. In: Arsenic Exposure and Health Effects. Special Issue of Environmental Geochemistry and Health. Science and Technology Letters

Chappell W. R., Abernathy C. O. and Cothern C. R. Northwood. Middlesex, England: 16: 219-231.

Mann S, Droz PO and Vahter M (1996a). A physiologically based pharmacokinetic model for arsenic exposure. I. Development in hamsters and rabbits. Toxicol Appl Pharmacol 137(1): 8-22.

Mann S, Droz PO and Vahter M (1996b). A physiologically based pharmacokinetic model for arsenic exposure. II. Validation and application in humans. Toxicol Appl Pharmacol 140(2): 471-86.

Marafante E and Vahter M (1987). Solubility, retention, and metabolism of intratracheally and orally administered inorganic arsenic compounds in the hamster. Environ Res 42(1): 72-82.

Marnell LL, Garcia-Vargas GG, Chowdhury UK, Zakharyan RA, Walsh B, Avram MD, Kopplin MJ, Cebrian ME, Silbergeld EK and Aposhian HV (2003). Polymorphisms in the human monomethylarsonic acid (MMA V) reductase/hGSTO1 gene and changes in urinary arsenic profiles. Chem Res Toxicol 16(12): 1507-13.

Mazumder DNG, Haque R, Ghosh N, De BK, Santra A, Chakraborty D and Smith AH (1998). Arsenic levels in drinking water and the prevalence of skin lesions in West Bengal, India. Int J Epidemiol 27(5): 871-7.

Mazumder DN, Haque R, Ghosh N, De BK, Santra A, Chakraborti D, and Smith AH (2000). Arsenic in drinking water and the prevalence of respiratory effects in West Bengal, India. Int J Epidemiol 29(6):1047-1052.

Mazumder DN, Steinmaus C, Bhattacharya P, von Ehrenstein OS, Ghosh N, Gotway M, Sil A, Balmes JR, Haque R, Hira-Smith MM and Smith AH (2005). Bronchiectasis in persons with skin lesions resulting from arsenic in drinking water. Epidemiology 16(6): 760-765.

Menzel DB, Ross M, Oddo SV, Bergstrom PD, Greene H and Roth RN (1994). A physiologically based pharmacokinetic model for ingested arsenic. In: Arsenic Exposure and Health Effects. Special Issue of Environmental Geochemistry and Health. Science and Technology Letters. Chappell W. R., Abernathy C. O. and Cothern C. R. Northwood

Middlesex, England: 16: 209-218.

Meza MM, Yu L, Rodriguez YY, Guild M, Thompson D, Gandolfi AJ and Klimecki WT (2005). Developmentally restricted genetic determinants of human arsenic metabolism: association between urinary methylated arsenic and CYT19 polymorphisms in children. Environ Health Perspect 113(6): 775-81.

Morris JS, Schmid M, Newman S, Scheuer PJ and Sherlock S (1974). Arsenic and noncirrhotic portal hypertension. Gastroenterology 66(1): 86-94.

Morrissey RE, Fowler BA, Harris MW, Moorman MP, Jameson CW and Schwetz BA (1990). Arsine: absence of developmental toxicity in rats and mice. Fundam Appl Toxicol 15(2): 350-6.

Nagaraja TN and Desiraju T (1993). Regional alterations in the levels of brain biogenic amines, glutamate, GABA, and GAD activity due to chronic consumption of inorganic arsenic in developing and adult rats. Bull Environ Contam Toxicol 50(1): 100-7.

Nagaraja TN and Desiraju T (1994). Effects on operant learning and brain acetylcholine esterase activity in rats following chronic inorganic arsenic intake. Hum Exp Toxicol 13(5): 353-6.

Nagymajtenyi L, Selypes A and Berencsi G (1985). Chromosomal aberrations and fetotoxic effects of atmospheric arsenic exposure in mice. J Appl Toxicol 5(2): 61-3.

Nemec MD, Holson JF, Farr CH and Hood RD (1998). Developmental toxicity assessment of arsenic acid in mice and rabbits. Reprod Toxicol 12(6): 647-58.

Nordstrom S, Beckman L and Nordenson I (1979). Occupational and environmental risks in and around a smelter in northern Sweden. V. Spontaneous abortion among female employees and decreased birth weight in their offspring. Hereditas 90(2): 291-6.

NRC. (1984). *Emergency and Continuous Exposure Limits for Selected Airborne Contaminants.* . 1. National Academy Press, Washington, DC.

NRC. (2001). *Arsenic in Drinking Water 2001 Update*. National Academy Press, Washington, DC.

OEHHA. (1999). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part II: Technical Support Document for Describing Available Cancer Potency Factors*. Air Toxicology and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

OEHHA. (2000). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part IV: Technical Support Document for Exposure Assessment and Stochastic Analysis*. Air Toxicology and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/air/hot_spots/pdf/May2005Hotspots.pdf.

OEHHA. (2004). *Public Health Goals for Arsenic in Drinking Water*. Pesticide and Environmental Toxicology Branch, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. http://www.oehha.ca.gov/water/phg/allphgs.html.

OEHHA. (2007). *The Air Toxics Hot Spots Program Risk Assessment Guidelines. Part V. Technical Support Document for the Derivation of Noncancer Reference Exposure Levels (5-15-07, draft).* Air Toxicology and Epidemiology Branch. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

Offergelt JA, Roels H, Buchet JP, Boeckx M and Lauwerys R (1992). Relation between airborne arsenic trioxide and urinary excretion of inorganic arsenic and its methylated metabolites. Br J Ind Med 49(6): 387-93.

Owen BA (1990). Literature-derived absorption coefficients for 39 chemicals via oral and inhalation routes of exposure. Regul Toxicol Pharmacol 11(3): 237-52.

Parish GG, Glass R and Kimbrough R (1979). Acute arsine posioning in two workers cleaning a clogged drain. Arch Environ Health 34(4): 224-7.

Perry K, Bowler RG, Bucknell HM, Druett HA and Scilling RSF (1948). Studies in the incidence of cancer in a factory handling inorganic compounds of arsenic. II. Clinical and environmental investigations. Br J Ind Med 5: 6-15.

Peterson DP (1990). Personal communication.

Peterson DP and Bhattacharyya MH (1985). Hematological responses to arsine exposure: quantitation of exposure response in mice. Fundam Appl Toxicol 5(3): 499-505.

Pomroy C, Charbonneau SM, McCullough RS and Tam GK (1980). Human retention studies with 74As. Toxicol Appl Pharmacol 53(3): 550-6.

Radabaugh TR and Aposhian HV (2000). Enzymatic reduction of arsenic compounds in mammalian systems: reduction of arsenate to arsenite by human liver arsenate reductase. Chem Res Toxicol 13(1): 26-30.

Rael LT, Ayala-Fierro F and Carter DE (2000). The effects of sulfur, thiol, and thiol inhibitor compounds on arsine-induced toxicity in the human erythrocyte membrane. Toxicol Sci 55(2): 468-77.

Rahman M and Axelson O (1995). Diabetes mellitus and arsenic exposure: a second look at case-control data from a Swedish copper smelter. Occup Environ Med 52(11): 773-4.

Rahman M, Tondel M, Ahmad SA and Axelson O (1998). Diabetes mellitus associated with arsenic exposure in Bangladesh. Am J Epidemiol 148(2): 198-203.

Rahman M, Tondel M, Ahmad SA, Chowdhury IA, Faruquee MH and Axelson O (1999). Hypertension and arsenic exposure in Bangladesh. Hypertension 33(1): 74-8.

Rahman M, Vahter M, Sohel N, Yunus M, Wahed MA, Streatfield PK, Ekstrom EC, Persson LA. (2006). Arsenic exposure and age- and sex-specific risk for skin lesions: A population-based case-referent study in Bangladesh. Environ Health Perspect 114:1847-1852.

Rahman MS, Hall LL and Hughes MF (1994). In vitro percutaneous absorption of sodium arsenate in B6C3F1 mice. Toxicol in Vitro 8(3): 441-448.

Rees DC and Hattis D (1994). Developing quantitative strategies for animal to human exprapolation. In: Principles and Methods of Toxicology. Hayes A. W. Raven Press. New York.

Rosenberg HG (1974). Systemic arterial disease and chronic arsenicism in infants. Arch Pathol 97(6): 360-5.

Rozenshtein IS (1970). [Sanitary toxicological assessment of low concentrations pf arsenic trioxide in the atmosphere]. Gig Sanit 35: 16-21.

Saha KC (2003). Saha's grading of arsenicosis progression and treatment. In: Arsenic Exposure and Health Effects V. Chappell W. R., Abernathy C. O. and Thomas D. J. Elsevier. San Diego: 391.

Sampayo-Reyes A, Zakharyan RA, Healy SM and Aposhian HV (2000). Monomethylarsonic acid reductase and monomethylarsonous acid in hamster tissue. Chem Res Toxicol 13(11): 1181-6.

Sanger DE (1987). Unpublished study cited in Blair *et al*., 1990. Amherst, MA: University of MassachusettsSchool of Public Health.

Schroeder HA and Mitchener M (1971). Toxic effects of trace elements on the reproduction of mice and rats. Arch Environ Health 23(2): 102-6.

Siripitayakunkit U, Lue S and Choprapawon C (2001). Possible effects of arsenic on visual perception and visual-motor integration of children in Thailand. In: Arsenic Exposure and Health Effects. Chappell W. R., Abernathy C. O. and Calderon R. L. Elsevier Science.

Siripitayakunkit U, Visudhiphan P, Pradipasen M and Vorapongsathron T (1999). Association between chronic arsenic exposure and children's intelligence in Thailand. In: Arsenic Exposure and Health Effects. Chappell W. R., Abernathy C. O. and Calderon J. Elsevier Science.

Smith AH, Goycolea M, Haque R and Biggs ML (1998). Marked increase in bladder and lung cancer mortality in a region of Northern Chile due to arsenic in drinking water. Am J Epidemiol 147(7): 660-9.

Stump DG, Holson JF, Fleeman TL, Nemec MD and Farr CH (1999). Comparative effects of single intraperitoneal or oral doses of sodium arsenate or arsenic trioxide during in utero development. Teratology 60(5): 283-91.

Styblo M, Delnomdedieu M and Thomas DJ (1996). Mono- and dimethylation of arsenic in rat liver cytosol in vitro. Chem Biol Interact 99(1-3): 147-64.

Styblo M, Yamauchi H and Thomas DJ (1995). Comparative in vitro methylation of trivalent and pentavalent arsenicals. Toxicol Appl Pharmacol 135(2): 172-8.

Tam GK, Charbonneau SM, Bryce F, Pomroy C and Sandi E (1979). Metabolism of inorganic arsenic (74As) in humans following oral ingestion. Toxicol Appl Pharmacol 50(2): 319-22.

Thomas R and Young R (2001). Arsine. Acute exposure guideline levels. Inhal Toxicol 13 (Suppl): 43-77.

Thompson DJ (1993). A chemical hypothesis for arsenic methylation in mammals. Chem Biol Interact 88(2-3): 89-14.

Tsai SY, Chou HY, The HW, Chen CM and Chen CJ (2003). The effects of chronic arsenic exposure from drinking water on the neurobehavioral development in adolescence. Neurotoxicology 24(4-5): 747-53.

Tseng CH, Chong CK, Chen CJ and Tai TY (1996). Dose-response relationship between peripheral vascular disease and ingested inorganic arsenic among residents in blackfoot disease endemic villages in Taiwan. Atherosclerosis 120(1-2): 125-33.

Tseng WP (1977). Effects and dose--response relationships of skin cancer and blackfoot disease with arsenic. Environ Health Perspect 19: 109-19.

U.S. EPA. (2002). *Notice of Receipt of Requests to Cancel Certain Chromated Copper Arsenate (CCA) Wood Preservative Products and Amend to Terminate Certain Uses of CCA Products* OPP-66300; FRL-6826-8. U.S. Environmental Protection Agency.

U.S. EPA. (2006a). *EPA Chemical Profiles: Arsine*. U. S. Environmental Protection Agency. http://yosemite.epa.gov/oswer/ceppoehs.nsf/Profiles/1303-28-2?OpenDocument.

U.S. EPA. (2006b). *Organic herbicides (MSMA, DSMA, CAMA, and Cacodylic Acid), Registration Eligibility Decision: Notice of Availability*. Volume 71, No 153. Federal Register: August 9, 2006.

Vahter M (1981). Biotransformation of trivalent and pentavalent inorganic arsenic in mice and rats. Environ Res 25(2): 286-93.

Vahter M (1983). Metabolism of arsenic. In: Bilogical and Environmental Effects of Arsenic. Fowler B. A. Elsevier. Amsterdam: 171-198.

Vahter M, Concha G, Nermell B, Nilsson R, Dulout F and Natarajan AT (1995a). A unique metabolism of inorganic arsenic in native Andean women. Eur J Pharmacol 293(4): 455-62.

Vahter M, Couch R, Nermell B and Nilsson R (1995b). Lack of methylation of inorganic arsenic in the chimpanzee. Toxicol Appl Pharmacol 133(2): 262-8.

Vahter M and Envall J (1983). In vivo reduction of arsenate in mice and rabbits. Environ Res 32(1): 14-24.

Vahter M, Friberg L, Rahnster B, Nygren A and Nolinder P (1986). Airborne arsenic and urinary excretion of metabolites of inorganic arsenic among smelter workers. Int Arch Occup Environ Health 57(2): 79-91.

Vahter M, Marafante E and Dencker L (1984). Tissue distribution and retention of 74As-dimethylarsinic acid in mice and rats. Arch Environ Contam Toxicol 13(3): 259-64.

Vallee BL, Ulmer DD and Wacker WEC (1960). Arsenic toxicology and biochemistry. Arch Ind Health 21: 132-151.

von Ehrenstein OS, Guha Mazumder DN, Hira-Smith M, Ghosh N, Yuan Y, Windham G, Ghosh A, Haque R, Lahiri S, Kalman D, Das S and Smith AH (2006). Pregnancy outcomes, infant mortality, and arsenic in drinking water in West Bengal, India. Am J Epidemiol 163(7): 662-9.

von Ehrenstein OS, Mazumder DN, Yuan Y, Samanta S, Balmes J, Sil A, Ghosh N, Hira-Smith M, Haque R, Purushothamam R, Lahiri S, Das S and Smith AH (2005). Decrements in lung function related to arsenic in drinking water in West Bengal, India. Am J Epidemiol 162(6): 533-41.

Wagstaff DJ (1978). Alteration of hepatic detoxication enzyme activity by dietary arsenic trioxide. Food Cosmet Toxicol 16(5): 423-6.

Walton FS, Waters SB, Jolley SL, LeCluyse EL, Thomas DJ and Styblo M (2003). Selenium compounds modulate the activity of recombinant rat AsIII-methyltransferase and the methylation of arsenite by rat and human hepatocytes. Chem Res Toxicol 16(3): 261-5.

Wang CH, Jeng JS, Yip PK, Chen CL, Hsu LI, Hsueh YM, Chiou HY, Wu MM and Chen CJ (2002). Biological gradient between long-term arsenic exposure and carotid atherosclerosis. Circulation 105(15): 1804-9.

Wasserman GA, Liu X, Parvez F, Ahsan H, Factor-Litvak P, van Geen A, Slavkovich V, LoIacono NJ, Cheng Z, Hussain I, Momotaj H and Graziano JH (2004). Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect 112(13): 1329-33.

Webb DR, Wilson SE and Carter DE (1986). Comparative pulmonary toxicity of gallium arsenide, gallium(III) oxide, or arsenic(III) oxide intratracheally instilled into rats. Toxicol Appl Pharmacol 82(3): 405-16.

Wester RC, Hui X, Barbadillo S, Maibach HI, Lowney YW, Schoof RA, Holm SE and Ruby MV (2004). In vivo percutaneous absorption of arsenic from water and CCA-treated wood residue. Toxicol Sci 79(2): 287-95.

Wester RC, Maibach HI, Sedik L, Melendres J and Wade M (1993). In vivo and in vitro percutaneous absorption and skin decontamination of arsenic from water and soil. Fundam Appl Toxicol 20(3): 336-40.

Wildfang E, Zakharyan RA and Aposhian HV (1998). Enzymatic methylation of arsenic compounds. VI. Characterization of hamster liver arsenite and methylarsonic acid methyltransferase activities in vitro. Toxicol Appl Pharmacol 152(2): 366-75.

Willhite CC and Ferm VH (1984). Prenatal and developmental toxicology of arsenicals. In: Nutritional and Toxiclogical Aspects of Food Safety. Friedman M. Plenum Publishing Corp. New York (NY): 9: 205-228.

Williams PL, Spain WH and Rubenstein M (1981). Suspected arsine poisoning during the restoration of a large cyclorama painting. Am Ind Hyg Assoc J 42(12): 911-3.

Winski SL and Carter DE (1995). Interactions of rat red blood cell sulfhydryls with arsenate and arsenite. J Toxicol Environ Health 46(3): 379-97.

Woods JS and Fowler BA (1978). Altered regulation of mammalian hepatic heme biosynthesis and urinary porphyrin excretion during prolonged exposure to sodium arsenate. Toxicol Appl Pharmacol 43(2): 361-71.

Wu MM, Kuo TL, Hwang YH and Chen CJ (1989). Dose-response relation between arsenic concentration in well water and mortality from cancers and vascular diseases. Am J Epidemiol 130(6): 1123-32.

Yamauchi H and Yamamura Y (1985). Metabolism and excretion of orally administrated arsenic trioxide in the hamster. Toxicology 34(2): 113-21.

Yu D (1999). A physiologically based pharmacokinetic model of inorganic arsenic. Regul Toxicol Pharmacol 29(2 Pt 1): 128-41.

Yu HS, Sheu HM, Ko SS, Chiang LC, Chien CH, Lin SM, Tserng BR and Chen CS (1984). Studies on blackfoot disease and chronic arsenism in southern Taiwan: with special reference to skin lesions and fluorescent substances. J Dermatol 11(4): 361-70.

Yu L, Kalla K, Guthrie E, Vidrine A and Klimecki WT (2003). Genetic variation in genes associated with arsenic metabolism: glutathione S-transferase omega 1-1 and purine nucleoside phosphorylase polymorphisms in European and indigenous Americans. Environ Health Perspect 111(11): 1421-7.

Zakharyan R, Wu Y, Bogdan GM and Aposhian HV (1995). Enzymatic methylation of arsenic compounds: assay, partial purification, and properties of arsenite methyltransferase and monomethylarsonic acid methyltransferase of rabbit liver. Chem Res Toxicol 8(8): 1029-38.

Zakharyan RA and Aposhian HV (1999). Enzymatic reduction of arsenic compounds in mammalian systems: the rate-limiting enzyme of rabbit liver arsenic biotransformation is MMA(V) reductase. Chem Res Toxicol 12(12): 1278-83.

Zakharyan RA, Sampayo-Reyes A, Healy SM, Tsaprailis G, Board PG, Liebler DC and Aposhian HV (2001). Human monomethylarsonic acid (MMA(V)) reductase is a member of the glutathione-S-transferase superfamily. Chem Res Toxicol 14(8): 1051-7.

Zakharyan RA, Wildfang E and Aposhian HV (1996). Enzymatic methylation of arsenic compounds. III. The marmoset and tamarin, but not the rhesus, monkeys are deficient in methyltransferases that methylate inorganic arsenic. Toxicol Appl Pharmacol 140(1): 77-84.

Zaldivar R and Guillier A (1977). Environmental and clinical investigations on endemic chronic arsenic poisoning in infants and children. Zentralbl Bakteriol [Orig B] 165(2): 226-34.

Benzene Reference Exposure Levels

(benzol; benzole; cyclohexatriene)

CAS: 71-43-2

1 Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360(b)(2)). In response to this statutory requirement, OEHHA developed a Technical Support Document (TSD) that describes acute, 8-hour, and chronic Reference Exposure Levels (RELs). The TSD was adopted in December 2008 (OEHHA, 2008) and presents methodology reflecting the latest scientific knowledge and techniques, and in particular explicitly includes consideration of possible differential effects on the health of infants, children, and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Chapter 731, Statutes of 1999, Health and Safety Code Sections 39669.5 *et seq.*). These guidelines have been used to develop the RELs for benzene presented in this document; this document will be added to Appendix D of the TSD.

Benzene is a solvent and chemical intermediate. Acute, high inhalation exposure may lead to eye, nose, and throat irritation and central nervous system depression in humans. Prolonged or repeated exposures have been associated with both blood cell proliferation and reduction in blood cell numbers due to bone marrow suppression, including peripheral lymphocytopenia, pancytopenia, and aplastic anemia. The non-cancer adverse health effects of benzene result from the ability of its metabolites to adversely affect rapidly dividing cells, especially in the bone marrow where detoxifying enzymes for its toxic metabolites are present at lower levels than the liver. Children may be more sensitive to benzene because so many of their tissues are undergoing rapid cell division and differentiation for growth and development to stimulate and maintain growth. This review includes relevant material published through December 2013 and is a technical review of those studies specifically applicable to developing non-cancer acute, 8-hour, and chronic inhalation RELs for benzene.

Although benzene is a known human carcinogen (IARC Group 1), this document does not discuss issues related to the cancer potency factor. That was derived previously and is available at www.oehha.ca.gov/air/hot_spots/index.html.

1.1 Benzene Acute REL

Reference Exposure Level	**27 µg/m³ (0.008 ppm; 8 ppb)**
Critical effect(s)	Developmental hematotoxicity in fetal and neonatal mice
Hazard Index target(s)	Developmental; Immune System; Hematologic System

1.2 Benzene 8-Hour REL

Reference Exposure Level	**3 µg/m³ (0.001 ppm; 1 ppb**)
Critical effect(s)	Decreased peripheral blood cells in Chinese workers
Hazard Index target(s)	Hematologic System

1.3 Benzene Chronic REL

Reference Exposure Level	**3 µg/m³ (0.001 ppm; 1 ppb)**
Critical effect(s)	Decreased peripheral blood cells in Chinese workers
Hazard Index target(s)	Hematologic System

2 Physical and Chemical Properties **(HSDB, 2007)**

Description	clear, colorless liquid
Molecular formula	C_6H_6
Molecular weight	78.1 g/mol
Density/Specific gravity	0.8787 @ 15°C/4°C
Boiling point	80.1°C
Melting point	5.5°C
Vapor pressure	94.8 mm Hg @ 25°C (0.125 atm)
Flashpoint	-11°C
Explosive limits	upper = 8.0% by volume in air lower = 1.4% by volume in air
Solubility	miscible with ethanol, chloroform, ether, carbon disulfide, acetone, oils, and glacial acetic acid; slightly soluble in water (1,790 mg/L @ 25°C)
Octanol/water partition coefficient	log Kow = 2.13
Odor threshold	0.875 ppm (2.8 mg/m³) (Haley, 1977) 4.68 ppm (15.3 mg/m³) (HSDB, 2007)
Odor description	aromatic odor (sweet); gasoline-like odor
Metabolites	hydroquinone, benzoquinone, catechol, phenol
Conversion factor	1 ppm = 3.26 mg/m³

3 Occurrences and Major Uses

Benzene was widely used in the past as a multipurpose organic solvent. The tire industry and shoe factories used benzene extensively. This use was discouraged due to its high toxicity and, at least in the United States, its use as a solvent has decreased. Present uses include benzene as a raw material in the synthesis of styrene, phenol, cyclohexane, aniline, and alkyl benzenes and in the manufacture of various plastics, resins, and detergents. Synthesis of many pesticides and pharmaceuticals also involves benzene as a chemical intermediate. Benzene is a natural constituent of crude oil as well as a product of petroleum refining (e.g., cracking) and is emitted in large quantities from oil refineries and petroleum storage facilities, which are a major source of exposure for the general public nearby. Mobile sources together emit the most benzene into the ambient air. Annual production in the U.S. was estimated to be 12.32 billion pounds (6.16 million tons) in 1993 (HSDB, 2007). In 2010 estimated U.S. production was 1.8 billion gallons (13.3 billion pounds) (Balboa, 2011).

Benzene exposure also arises from cigarette smoking (including passive smoking), home use of solvents or gasoline, and leaking underground storage tanks (Goldstein and Witz, 2009).

Estimates for benzene emissions from the Statewide 2008 California Toxics Inventory (CTI) were 1,284 tons from stationary sources, 117 tons from area-wide sources, 5,024 tons from on-road mobile sources, 4,393 tons from other mobile sources, and 46 tons from natural sources (CARB, 2008). The top 25 benzene-emitting facilities in the Air Toxics Hot Spots program in 2008 emitted between 4,000 and 49,000 pounds per year.

A survey of indoor levels of volatile organic chemicals reported a geometric mean of 0.69 μg/m^3 (0.22 ppb) for benzene (range = 0.29 – 2.11 μg/m^3) in 29 small- and medium-sized commercial buildings in California (Wu et al., 2011).

Indoor benzene levels were measured prior to and after the Ireland Public Health Tobacco Act of 2002 ban on smoking in pubs (McNabola et al., 2006). The average ambient concentration of benzene measured inside two Dublin pubs in March 2004 prior to the ban was 4.83 μg/m^3 (1.5 ppb). The average ambient level outside the pubs was 0.84 μg/m^3 (0.26 ppb). In August 2004 after the ban the average indoor level was 0.54 μg/m^3 (0.2 ppb). The average ambient outside level was 0.13 μg/m^3 (0.04 ppb).

The TEACH (Toxic Exposure Assessment, Columbia/Harvard) study characterized personal exposures to urban air toxics among 41 high school students living in Los Angeles in 2000 (Sax et al., 2006). Exposure was analyzed using 48-hr personal monitoring, outdoor ambient monitoring, and in-home ambient monitoring. The students were mainly Hispanic (93%), and were required to be non-smokers from non-smoking families. The mean outdoor concentration of benzene was 3.32 μg/m^3 (1 ppb) (maximum = 5.56 μg/m^3 (1.7 ppb)), while the mean in-home concentration was 3.87 μg/m^3 (1.2 ppb) (maximum = 11.4 μg/m^3 (3.5 ppb)). The mean personal concentration

was 4.64 μg/m^3 (1.4 ppb) (maximum =11.27 μg/m^3 (3.5 ppb)).

Nazaroff and Singer studied hazardous air pollutants including benzene within US residences. Data analyses indicated that some 16 million US juveniles (2 months to 16 years old) were exposed to benzene from Environmental Tobacco Smoke (ETS) in the home (Nazaroff and Singer, 2004). Assuming that from 14 to 20 cigarettes are smoked per day in each residence, with an average of 430 μg benzene per cigarette, the resulting indoor air level was calculated to be 1.1-2.5 μg/m^3 (0.3-0.8 ppb) and the daily intake of benzene for juveniles was 14 – 31 μg.

In 2002 the estimated statewide ambient concentration of benzene was approximately 0.6 ppb (~2 μg/m^3) (CARB, 2004). Statewide the annual average benzene concentration has decreased from ~2.5 ppb in 1990 to ~0.5 ppb in 2007 (CARB, 2009). The Bay Area Air Quality Management District maintains a 32-station air monitoring network to determine whether the Bay Area is in compliance with California and National Ambient Air Quality Standards. Some of the monitoring sites include toxics sampling equipment. Table 3.1 shows benzene levels in ambient air at various sampling stations in the Bay Area Air Quality Management District in 2008. Benzene levels were determined by a modification of USEPA method TO-15, which uses gas chromatography/mass spectrometry. The minimal detectable level for benzene is 0.014 ppb (0.046 μg/m^3).

Table 3.1. Benzene Levels (ppb) at Monitoring Stations in the Bay Area in 2008

Station	Arithmetic Mean	Maximum	Minimum	Samples
Benicia – VIP	0.106	0.350	0.030	60
Berkeley	0.269	1.00	0.050	122
Bethel Island	0.135	0.510	0.040	62
Concord - Treat Blvd.	0.167	0.450	0.050	62
Crockett - Kendall Ave	0.116	0.250	0.040	62
Fremont-Chapel Way 1	0.195	0.630	0.070	40
Fremont-Chapel Way 2	0.230	0.590	0.100	31
Fort Cronkhite	0.0681	0.200	0	62
Livermore - Rincon Ave.	0.197	0.540	0.060	62
Martinez - Jones St	0.172	0.610	0.030	60
Napa - Jefferson St	0.321	1.05	0.080	62
Oakland	0.234	0.520	0.060	62
Oakland - Filbert St.	0.191	0.610	0.040	60
Redwood City	0.244	0.710	0.090	62
Richmond - 7th St	0.165	0.320	0.020	62
San Francisco - Arkansas 1	0.176	0.410	0	62
San Francisco - Arkansas 2	0.182	0.470	0.060	31
San Jose - Jackson St. 1	0.316	1.11	0.050	120
San Jose - Jackson St. 2	0.296	1.00	0.110	31
San Pablo - Rumrill	0.232	0.440	0.100	62
San Rafael	0.190	0.370	0.050	62
Santa Rosa - 5th St	0.210	0.800	0.030	62
Sunnyvale - Ticonderoga	0.153	0.430	0.050	56
Vallejo - Tuolumne St	0.196	0.660	0.060	62

Source: BAAQMD. Toxic Air Contaminant Air Monitoring Data for 2008. Available at: http://www.baaqmd.gov/Divisions/Engineering/Air-Toxics/Toxic-Air-Contaminant-Control-Program-Annual-Report.aspx. The values are 24-hour integrated samples.

Table 3.2 shows benzene levels in ambient air at ten fixed sampling locations in the South Coast Air Quality Management District between 2004 and 2006 taken as part of the Multiple Air Toxics Exposure Study (MATES) III study. The MATES III study included an air monitoring program, an updated emissions inventory of toxic air contaminants, and a modeling effort to characterize risk, especially the carcinogenic risk from exposure to air toxics. Year 1 was April 2004 through March 2005. Year 2 was April 2005 through March 2006. The analytical method used generally followed the US EPA Method TO-15: determination of volatile organic compounds collected in specially prepared canisters and analyzed by gas chromatography/mass spectrometry.

Table 3.2. Benzene Levels (ppb) at 10 Fixed Sites in South Coast in 2004 - 2006

	Year 1 (4/2004 - 3/2005)				Year 2 (4/2005 – 3/2006)			
Location	Mean	SD	N	Max	Mean	SD	N	Max
Anaheim	0.44	0.28	118	1.44	0.42	0.33	115	2.06
Burbank	0.73	0.42	118	2.16	0.69	0.44	122	1.85
Central LA	0.59	0.30	117	1.83	0.57	0.31	121	1.53
Compton	0.82	0.70	118	3.50	0.78	0.67	118	3.53
Inland Valley	0.49	0.24	115	1.26	0.49	0.24	116	1.24
Huntington Park	0.76	0.46	98	2.20	-	-	-	
North Long Beach	0.56	0.35	119	1.62	0.48	0.34	118	1.70
Pico Rivera	0.57	0.32	121	1.86	-	-	-	
Rubidoux	0.45	0.25	114	1.23	0.43	0.26	120	1.32
West Long Beach	0.57	0.44	114	1.95	0.50	0.38	120	1.77

Source: http://www.aqmd.gov/prdas/matesIII/Final/Appendices/f-MATESIIIAppendixVIFinal92008.pdf. Values are 24 hour integrated samples.

Note that all the levels in Table 3.2 are higher than the highest mean value (0.321 ppb) in the Bay Area (Table 3.1). Table 3.3 shows benzene levels from ten sites in a subproject of MATES III in which mobile monitoring stations (microscale) were intentionally put near a known (fixed) emission source and the results compared to a fixed site nearby over a period of three to ten months.

Table 3.3. Benzene Levels (ppb) at Monitoring Stations in South Coast 2004-2006

Site	Monitor	Mean (ppb)	SD	Max	Samples	Time interval
Commerce	Microscale*	0.69	0.33	1.72	62	11/2004 – 5/2005
Huntington Park	Fixed	0.93	0.52	2.20	46	"
Indio	**Microscale**	**0.21**	**0.1**	**0.37**	**26**	**3/2005 – 5/2005**
Rubidoux	**Fixed**	**0.39**	**0.23**	**0.94**	**26**	**"**
San Bernardino	Microscale	0.73	0.38	1.52	45	10/2004 – 2/2005
Inland Valley(SB)	Fixed	0.51	0.28	1.11	46	"
Sun Valley	**Microscale**	**0.52**	**0.23**	**1.11**	**91**	**6/2005 – 3/2006**
Burbank	**Fixed**	**0.75**	**0.46**	**1.85**	**101**	**"**
Santa Ana	Microscale	1.04	0.6	2.84	47	9/2005 – 1/2006
Anaheim	Fixed	0.61	0.4	2.06	46	"

*A mobile monitoring device was intentionally located near a known but not specifically identified emission source of air toxics.
Sources: http://www.aqmd.gov/prdas/matesIII/Final/Document/e-MATESIIIChapter5Final92008.pdf; Dr. Jean Ospital, South Coast AQMD

The South Coast Air Quality Management District has recently conducted MATES IV. Preliminary results show that benzene levels at the ten monitoring stations have decreased since MATES III.

Benzene exists mostly in the vapor phase. It reacts with photochemically produced hydroxyl radicals with a calculated half-life of 13.4 days. In atmospheres polluted with NOx or SO_2 the half-life can be as short as 4-6 hours (http://www.epa.gov/ogwdw/pdfs/factsheets/voc/tech/benzene.pdf).

4 Metabolism

Inhalation of benzene is the principal route of concern for the general public; approximately half the benzene inhaled by humans is absorbed (Nomiyama and Nomiyama, 1974; Pekari et al., 1992). In men and women exposed to 52-62 ppm benzene for 4 hours, 46.9 percent of the inhaled dose was absorbed. Of this, 30.1 percent was retained and 16.8 percent was excreted unchanged in the expired air (Nomiyama and Nomiyama, 1974).

After absorption, a fraction of benzene is metabolized in the liver, and benzene and its metabolites reach the kidney, the lung, the brain, and the bone marrow. Benzene itself is neurotoxic, but its metabolites have other toxic properties. Important aspects of benzene metabolism are depicted in Figure 4.1.

(1) Benzene is metabolized in the liver and bone marrow to benzene epoxide by the cytochrome P450 system, primarily CYP2E1 (but also to varying extents by CYP1A1, CYP2B1, CYP2F1, and CYP2F2). Benzene epoxide has a half-life of approximately 8 minutes in rat blood (Lindstrom et al., 1997) and thus could travel from the liver to other organs including bone marrow.

(2) Benzene epoxide rearranges nonenzymatically to phenol, initially the major metabolite of benzene. Phenol is oxidized, also by CYP2E1, to hydroquinone. Hydroquinone can be oxidized to the toxic metabolite 1,4-benzoquinone non-enzymatically by reacting with O_2 or enzymatically by myeloperoxidase (MPO) in bone marrow. 1,4-Benzoquinone can be converted back to hydroquinone by NAD(P)H:quinone oxidoreductase (NQO1), a detoxifying reaction (Ross, 2005).

(3) Benzene epoxide is enzymatically transformed by microsomal epoxide hydrolase to benzene dihydrodiol, which is then dehydrogenated to catechol by dihydrodiol dehydrogenase.

Most of the catechol and phenol metabolites are excreted within 24 hours in the urine, while hydroquinone requires 48 hours (Teisinger et al., 1952).

(4) Benzene epoxide is also metabolized to a ring-opened product, trans, trans-muconaldehyde. t,t-Muconaldehyde (or E,E-muconaldehyde using the EZ

nomenclature system for geometric isomers) can be metabolized to t,t-muconic acid (E,E-muconic acid) (oxidation) and to 6-hydroxy-t,t-2,4-hexadienal (reduction) (Short et al., 2006).

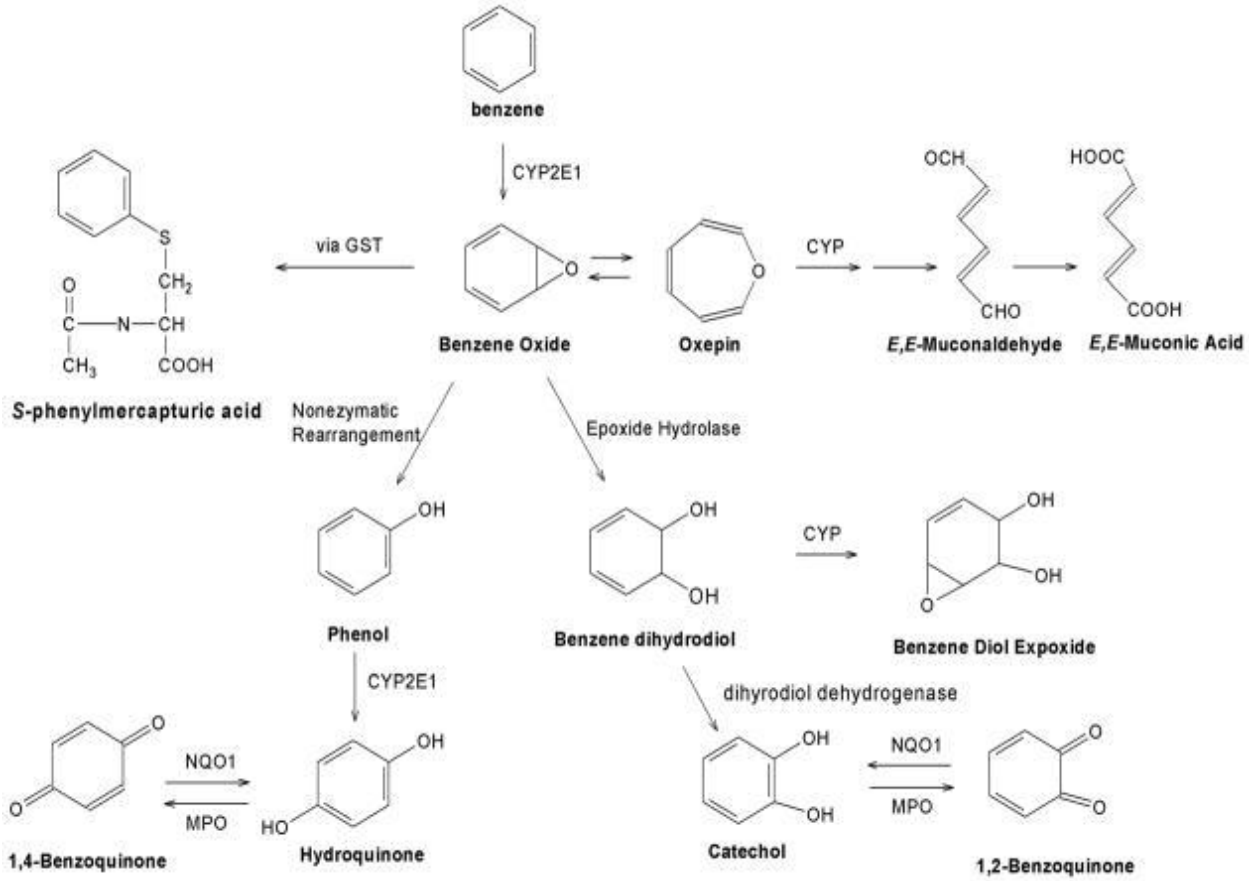
(5) Benzene epoxide can be conjugated with glutathione by glutathione-S-transferases to ultimately form S-phenylmercapturic acid (SPMA).

(6) Benzene epoxide also equilibrates with its oxepin, a seven-atom monocyclic structure, which may be an intermediate in one or more of the previous pathways (Figure 4.1).

(7) The benzene metabolites with hydroxyl groups (phenol, catechol, hydroquinone, and 1,2,4-benzenetriol) can form sulfates and glucuronidates (Nebert et al., 2002), and quinol thioethers after reaction with glutathione (Bratton et al., 1997) as part of phase II metabolism (not shown in Figure 4.1).

(8) Benzene metabolites can form adducts with DNA and proteins, especially albumin.

Figure 4.1. Intermediary metabolism of benzene



Source: (Rappaport et al., 2010)

The metabolites of benzene found in individual experiments depend on the species and the exposure conditions. As an example, Table 4 compares the percentage of water soluble metabolites in 24 hour urine samples from mice and rats exposed to either 5 ppm or 600 ppm benzene for the first 6 of the 24 hours (Sabourin et al., 1989).

Table 4.1. Percentage of water soluble metabolites in 24 hour urines (Sabourin et al., 1989)

Metabolite	5 ppm (3 mice)	600 ppm (3 mice)	5 ppm (2 rats)	600 ppm (3 rats)
Phenyl glucuronide	1	15	1.4	1
Catechol glucuronide	Non-detect	Non-detect	Non-detect	Non-detect
Phenyl sulfate	36	52	56	73
Hydroquinone monoglucuronide	26	9	8.5	0.5
Hydroquinone sulfate	6.6	2.3	2.9	1.5
Pre-phenylmercapturic acid	6	15	9.5	17
Phenylmercapturic acid	1	Non-detect	1.3	1
Muconic acid	23	5	18.5	4
Unknown	Non-detect	Non-detect	1.4	Non-detect
Total	99.6	98.3	99.5	98

4.1 Mechanistic Studies

Benzene causes hematotoxicity through its metabolites, which lead to DNA strand breaks, chromosomal damage, sister chromatid exchange (SCE), inhibition of topoisomerase II, and damage to the mitotic spindle. The hematotoxic (and carcinogenic) effects of benzene are associated with free radical formation either as benzene metabolites, particularly 1,4-benzoquinone, or as lipid peroxidation products (Smith et al., 1989; USEPA, 2002; Rana and Verma, 2005). The effects of intraperitoneal injection of benzene and various of its metabolites on erythropoiesis were studied in mice in vivo (Snyder et al., 1989). The most potent inhibitor of red blood cell (RBC) production was a mixture of hydroquinone (50 mg/kg) and t,t-muconaldehyde (1 mg/kg), which implicates at least two pathways of benzene metabolism in toxicity (Figure 4.1). Several other metabolites also inhibited red cell formation. Most of the studies which investigated the role of genetic polymorphisms in xenobiotic metabolizing enzymes focused on the CYP2E1 and various detoxifying pathways.

Transgenic mice, in which the gene for CYP2E1 has been knocked out ($cyp2e1^{-/-}$), do not show toxicity when exposed to 200 ppm (650 mg/m^3) benzene for 6 hr (Valentine et al., 1996). CYP2E1 protein and its associated enzymatic activity were not detected in early fetal human liver samples (Vieira et al., 1996).

Transgenic male mice, in which the gene for microsomal epoxide hydrolase was knocked out, did not show benzene toxicity (e.g., decreased white blood cell (WBC) counts) when exposed to 50 ppm (160 mg/m^3) benzene for two weeks, while control male mice did (Bauer et al., 2003). In humans, susceptibility to chronic benzene poisoning has been related to the epoxide hydrolase genotype (Sun et al., 2008). Specifically the risk of benzene poisoning increased in the subjects with microsomal epoxide hydrolase (EPHX1) GGAC/GAGT diplotype (P = 0.00057) or AGAC/GAGT diplotype (P = 0.00086). Surprisingly, neither diplotype altered the level of microsomal epoxide hydrolase enzyme activity. Thus the mechanism involved is not obvious. The authors also reported that less prevalent combinations of four single nucleotide polymorphisms (SNPs) could either increase or decrease the odds of chronic benzene poisoning relative to the more prevalent combinations (Table 8.5). This exemplifies the potentially broad distribution in metabolism and thus toxicity in humans.

Yoon and co-workers investigated the involvement of the aryl hydrocarbon receptor (AhR) in benzene hematotoxicity using wild-type (AhR$^{+/+}$), heterozygous (AhR$^{+/-}$), and homozygous null (AhR$^{-/-}$) male mice (Yoon et al., 2002). No hematotoxicity and no changes in peripheral blood and bone marrow cells were induced in AhR$^{-/-}$ mice by a 2-week inhalation exposure to 300 ppm (978 mg/m^3) benzene. The lack of hematotoxicity was associated with the lack of p21 over-expression, regularly seen in wild-type mice following benzene inhalation. (p21, also known as Cdkn1a in mice and CDKN1A in humans, **is a** cyclin-dependent kinase inhibitor important in the braking of the cell division cycle.) Combined treatment of AhR$^{-/-}$ mice with two benzene metabolites (phenol and hydroquinone) induced hematopoietic toxicity. The aryl hydrocarbon receptor may have a role in the regulation of hematopoiesis and in benzene-induced hematotoxicity (Gasiewicz et al., 2010).

In Wistar rats of both sexes with a large body fat content, benzene was eliminated more slowly and remained in the body for a longer time than in rats with a small body fat content. Accordingly, the decrease in WBC during chronic benzene exposure was seen only in rats with large volumes of fat tissue. In humans, the elimination of benzene was slower in women than in men (slope of the benzene concentration in blood curve starting 3 hrs post-exposure was -0.00198/minute in women vs. -0.0033/minute in men). The authors suggest that the slower elimination in women is due primarily to the higher percentage and distribution of body fat tissue. The authors concluded that women may be inherently more susceptible to benzene, which has a high affinity for fat tissue (Sato et al., 1975). Based on this observation, obese adults and children may also be more susceptible to benzene toxicity than non-obese adults and children.

4.2 Toxicokinetic studies:

The pharmacokinetics of benzene follows a two-compartment model in the rat. The rapid phase for benzene elimination in expired air has a half-life ($t_{1/2}$) of 0.7 hours, and the $t_{1/2}$ for the longer phase is 13.1 hours (Rickert et al., 1979). The benzene metabolites catechol, quinone, and hydroquinone were found to be preferentially

retained in the bone marrow (Greenlee et al., 1981). These reactive metabolites are not readily excreted, and are cytotoxic to the stem cells in the bone marrow.

Based partly on an early PBPK model for styrene (Ramsey and Andersen, 1984), Medinsky and coworkers developed a model to describe the uptake and metabolism of benzene in F344/N rats and B6C3F1 mice and to determine if the observed differences in toxic effects between mice and rats could be explained by differences in metabolic pathways or by differences in uptake (Medinsky et al., 1989a). For inhalation concentrations up to 1,000 ppm (3,260 mg/m^3) for six hours, mice metabolized at least two to three times as much total benzene (per kg body weight) as rats. In regard to metabolites, rats primarily formed phenyl sulfate, a detoxification product (see Table 4.1). In addition to phenyl sulfate, mice formed hydroquinone glucuronide and muconic acid which are part of toxicity pathways. The formation of hydroquinone showed the greatest difference between mice and rats. Metabolic rate parameters (Vmax and Km) were very different for hydroquinone conjugation and muconic acid formation compared to formation of phenyl conjugates and phenyl mercapturic acids. Assumed toxication pathways had high affinity, low capacity kinetics; detoxification pathways were low affinity, high capacity. Model simulations suggested that hydroquinone and muconic acid comprised a larger fraction of the total benzene metabolized at lower benzene levels for both rats and mice than at higher levels (where detoxification metabolites predominated). (See also Table 4.1).

The animal model of Medinsky et al. (1989a) was extended to predict benzene metabolism in people exposed near occupational exposure limits in effect at the time of the paper's publication (Medinsky et al., 1989b). For 8 hr inhalation exposures less than 10 ppm (32.6 mg/m^3), metabolites of hydroquinone, the precursor of the toxic 1,4-benzoquinone, were predicted to predominate in people. Lower levels of muconic acid, a metabolite of muconaldehyde, were predicted below 10 ppm. Above 10 ppm, detoxification metabolites, including phenyl conjugates, predominated (Medinsky et al., 1989a).

A PBPK model, based on that of Medinsky above, was developed to describe the disposition of benzene in 3 and 18 month-old C57BL/6N mice and to examine the key parameters affecting changes in benzene disposition with age (McMahon et al., 1994). The model included a rate constant for urinary elimination of metabolites as an age-related increase in Km for production of hydroquinone conjugates from benzene. The study indicated that age-related changes in physiology, including decreased elimination of hydroquinone conjugates at 18 months, were responsible for altered disposition of benzene in aged mice.

Travis and colleagues developed three PBPK models to describe the pharmacokinetics of benzene in mice, rats, and humans, respectively, using five anatomical compartments: liver, fat, bone marrow, muscle (poorly perfused), and other richly perfused organs (e.g., brain, heart, kidney, and viscera), all interconnected by the arterial and venous blood flow (Travis et al., 1990). Benzene metabolism showed Michaelis-Menten kinetics and occurred primarily in the liver (human Vmax = 29.04

mg/h) and secondarily in the bone marrow (human Vmax = 1.16 mg/h). Graphical comparisons of model results with empirical data in the three species from previously published reports were quite favorable for exposure by inhalation and gavage, and from intraperitoneal and subcutaneous injection.

Cole and coworkers developed a PBPK model in mice to relate inhaled benzene levels to tissue doses of benzene, benzene oxide, phenol, and hydroquinone (Cole et al., 2001). Parameter values in the literature were used. Additional parameters, estimated by fitting the model to published data, were first-order rate constants (k_i) for pathways lacking in vitro data and the concentrations of microsomal and cytosolic protein. The model was constrained by using the in vitro metabolic parameters (Vmax, first-order rate constants (k_i), and saturation parameters), rather than in vivo values. Even though data from multiple laboratories and experiments were used, model simulations matched the data reasonably well in most cases. No extrapolation to humans was attempted.

A three-compartment model was fit to human data on benzene disposition and bone-marrow metabolism (Watanabe et al., 1994). The general relationship between cumulative quantity of metabolites produced and inhalation concentration, was S-shaped, inflecting upward at low concentrations, and saturating at high concentrations.

Kim, Rappaport and associates studied the relationships between levels of five benzene metabolites (E,E-muconic acid, S-phenylmethylmercapturic acid, phenol, hydroquinone, and catechol) and benzene exposure among 250 exposed and 136 control Chinese workers (Kim et al., 2006a; Kim et al., 2006b). Benzene metabolism was nonlinear with increasing benzene levels above 0.03 ppm. They then statistically tested whether human metabolism of benzene is better fit by a kinetic model having two pathways rather than only one (i.e., CYP2E1) (Rappaport et al., 2009). Michaelis-Menten-like models were fit to individual urinary benzene metabolites and the corresponding air concentrations of benzene (range: < 0.001 ppm - 299 ppm (0 - 975 mg/m^3)) for 263 nonsmoking Chinese females. The different values of Akaike's information criterion (AIC) obtained with the two models gave strong statistical evidence favoring two metabolic pathways. The low-affinity pathway (likely due to CYP2E1) had an affinity ("Km") of 301 ppm (981 mg/m^3) for benzene in air; the value for the high-affinity pathway (unknown but possibly due to CYP2F1 (Sheets et al., 2004) or CYP2A13) was 0.594 ppm, a 500-fold difference. The exposure-specific metabolite level predicted by the two-pathway model at non-saturating benzene concentrations was 184 µM/ppm of benzene, which is close to an independent estimate of 194 µM/ppm for a nonsmoking Chinese female (Weisel et al., 2003). Rappaport estimated that a nonsmoking woman would metabolize about three times more benzene from the ambient environment under the two-pathway model (184 µM/ppm) than under the one-pathway model (68.6 µM/ppm). A follow-up study examined the individual urinary metabolites of benzene in each woman (Rappaport et al., 2010). The data indicated that the predicted high-affinity enzyme pathway is predominant at less than 1 ppm and metabolism favors the ring-opening pathway to t,t-muconaldehyde in this exposure scenario (see Figure 4.1).

The concept of increased dose specific benzene metabolism at levels below 3 ppm has important implications for low level environmental exposures. The degree of increased efficiency has been debated (Price et al., 2012; 2013) and defended by the original authors (Rappaport et al., 2013)

Kim and coworkers also studied the effect of various reference single nucleotide polymorphisms (SNPs) affecting metabolic enzymes on the metabolism of benzene and the relative amounts of different metabolites produced (Kim et al., 2007). They found that polymorphisms in GSTT1 (glutathione-S-transferase), NQO1, CYP2E1, and EPHX1 (rs1051740 or rs2234922) all affect the production of metabolites changing the relative formation of S-phenyl mercapturic acid, phenol, catechol, and hydroquinone by the liver. Interactions with smoking were also observed.
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Bois and colleagues modeled the distribution and metabolism of benzene in humans using population pharmacokinetics, Bayesian statistical inference, and PBPK modeling (Bois et al., 1996). Based on existing experimental data on three subjects from Pekari et al. (1992), they used Markov chain Monte Carlo methods to derive distributions of variability and uncertainty for model parameters. The model adequately fit both prior physiological information and the experimental data. For benzene exposures up to 10 ppm (32.6 mg/m^3), the median population fraction metabolized in the bone marrow was 52 percent (90% CI = 47-67%), and was linear in the three subjects studied. Inter-individual variation for metabolic parameters showed geometric standard deviations (GSD) of between 1.2 and 1.4. However, the posterior distribution of estimates of the quantity of benzene metabolized in the bone marrow was very broad. At 1 ppm (3.26 mg/m^3) continuous exposure (the occupational inhalation exposure threshold in the U.S), this estimate ranged from 2 to 40 mg/day. The authors pointed out that this large (20-fold) spread reflects a substantial element of uncertainty since the extent of metabolism in the bone marrow is not a measured parameter, but is inferred from the model inputs and assumptions which are themselves subject to uncertainty.

Yokley and associates developed a human PBPK model that quantified tissue levels of benzene, benzene oxide, phenol, and hydroquinone after inhalation and oral benzene exposures (Yokley et al., 2006). The model was integrated into a statistical framework that acknowledges sources of variation due to inherent intra- and inter-individual variation, measurement error, and other data collection issues. They estimated the population distributions of key PBPK model parameters. They hypothesized that observed interindividual variability in the dosimetry of benzene and its metabolites resulted primarily from known or estimated variability in key metabolic parameters and that a statistical PBPK model that explicitly included variability in only those metabolic parameters would sufficiently describe the observed variability. They identified parameter distributions for the PBPK model to characterize observed variability through the use of Markov chain Monte Carlo analysis applied to two data sets. The identified parameter distributions described most of the observed variability, but variability in physiological parameters such as organ weights may also be helpful to predict the observed human-population variability in benzene dosimetry.

Various benzene exposure scenarios were simulated for adult men and women using PBPK modeling (Brown et al., 1998). Women had a higher blood/air partition coefficient (8.20 vs. 7.80) and maximum velocity of metabolism (19.47 vs. 13.89 mg/hr) for benzene than men. Women generally had a higher body fat percentage than men (30% vs. 20%). Physicochemical gender differences resulted in women metabolizing 23-26 percent more benzene than men in the same scenario, although benzene blood levels are generally higher in men. These authors also stated that women may be at higher risk for certain effects of benzene exposure.

Knutsen and coworkers adapted a PBPK model of benzene inhalation based on a mouse model (Cole et al., 2001; Yokley et al., 2006) to include bone marrow and bladder compartments (Knutsen et al., 2013). They used data on human liver microsomal protein levels and linked CYP2E1 activities (Lipscomb et al., 2003a; Lipscomb et al., 2003b) and estimated metabolite-specific conversion rate parameters by fitting model equations to biomonitoring data (Waidyanatha et al., 2004) and adjusting for background levels of urinary metabolites (Qu et al., 2000). Human studies of benzene levels in blood and breath, and of phenol levels in urine (Pekari et al., 1992) were used to validate the rate of conversion of benzene to benzene oxide. Urinary benzene metabolites in Chinese workers (Kim et al., 2006a) provided model validation for rates of human conversion of benzene to muconic acid and phenylmercapturic acid, phenol, catechol, hydroquinone, and 1,2,4-benzenetriol. The model predicts that (1) liver microsomal protein and CYP2E1 activities are lower on average in humans than in mice, (2) mice show far lower rates of benzene conversion to muconic acid and phenylmercapturic acid, and (3) mice show far higher conversion of benzene to hydroquinone and 1,2,4-benzenetriol. Several metabolic rate parameters used in the model differ from other human models.

In summary benzene metabolism has been studied in animals and humans and has been the subject of several pharmacokinetic models, some of which adequately fit the observed data. The most important finding from the models for humans is that benzene is efficiently metabolized at low doses. The studies of the kinetics of benzene metabolism in humans have not only identified some quantitative differences from the results seen in animals, but have also allowed the exploration of dose level effects and the impact of various gene polymorphisms in the enzymes involved. This is of particular interest in the case of the studies by Kim, Rappaport and colleagues which were of a substantial number of individuals from the population in the epidemiological studies of benzene toxicity by Lan et al. (2004) (see below).

4.3 Metabolic Interaction with Other Chemicals

In the environment exposure to multiple chemicals occurs, both voluntarily and involuntarily. Benzene is only one of hundreds of chemicals emitted from refineries and vehicles and of thousands of chemicals in cigarette smoke. Humans vary widely in their intake of xenobiotics including alcohol. Some xenobiotics interact with benzene. For example, toluene is a competitive inhibitor of benzene oxidation by CYP2E1, and co-

exposure may decrease benzene toxicity. Ethanol exposure may also change susceptibility to benzene toxicity.

CYP2E1 (also known as Microsomal Ethanol Oxidizing System (MEOS)) has been reported to activate/metabolize more than 80 chemicals (Lieber, 1997) including benzene (Figure 4.1) and ethanol. Ethanol induces CYP2E1. For example, a 4-fold increase in CYP2E1 has been observed in human alcoholics (Lieber, 1997), while moderate drinking increased CYP2E1 25% (Snawder and Lipscomb, 2000).

Daiker and coworkers investigated the interactive effects of ethanol consumption and benzene inhalation in animals (Daiker et al., 2000). They used a liquid diet containing 4.1% ethanol to induce liver CYP2E1 activity 4-fold in female CD-1 mice. Groups of six ethanol-exposed or pair-fed control mice were exposed to benzene or air for 7 hours/day, 5 days/week for 6 or 11 weeks. One experiment using this protocol studied immunotoxicity endpoints. No statistically significant alterations were found in spleen lymphocyte cellularity, subtype (e.g., CD4+, CD8+) profile, mitogen-induced proliferation, cytokine production (IL2, IFNγ), or natural killer cell lytic activity after 6 weeks of ethanol diet, 0.44 ppm (1.4 mg/m^3) benzene exposure, or both. Subsequent experiments exposed animals to 4.4 ppm (14.3 mg/m^3) benzene. Bone marrow and spleen cells were evaluated for DNA-protein cross-links, and spleen lymphocytes were monitored for hypoxanthine guanine phosphoribosyl transferase (hprt)-mutant frequency. No changes in either endpoint were found in mice exposed to 4.4 ppm (14.3 mg/m^3) benzene for 11 weeks with or without co-exposure to 4.1% ethanol. Benzene and ethanol did not have interactive adverse effects under these specific conditions at 0.44 to 4.4 ppm benzene exposure. In an older study, 5% and 15% ethanol ingestion potentiated the effect of 300 ppm (980 mg/m^3) benzene on decreasing bone marrow and spleen cell levels in C57Bl/6J male mice (Baarson et al., 1982).

5 Acute Toxicity of Benzene

5.1 Acute Toxicity to Adult Humans

Deaths from acute exposure to benzene are often related to physical exertion and release of epinephrine with subsequent respiratory depression. Frequently, the person trying to rescue a collapsed victim will die during the effort of lifting the unconscious person (HSDB, 2007). Anesthesia may develop at concentrations above 3,000 ppm (9,600 mg/m³). At exposures greater than 1,000 ppm (3,200 mg/m³) (duration unspecified), CNS symptoms include giddiness, euphoria, nausea, and headaches; heightened cardiac sensitivity to epinephrine-induced arrhythmias may develop (Snyder, 1987). These effects may be accompanied by symptoms of mild irritation to the eyes and mucous membranes. Acute hemorrhagic pneumonitis is highly likely if benzene is aspirated into the lung (HSDB, 2007). Respiratory tract inflammation, pulmonary hemorrhages, renal congestion, and cerebral edema have been observed at autopsy in persons with acute benzene poisoning by inhalation. In these cases, blood levels of 2 mg percent (2 mg/100 ml) benzene were not associated with hematological changes (Winek and Collom, 1971).

A case report described three deaths due to acute benzene poisoning from a shipboard accident (Avis and Hutton, 1993). Exposure levels were not estimated. Autopsies showed skin, respiratory, and cerebral injury. Benzene levels in body fluids and tissues were consistent with the lipophilicity of benzene. In a single fatal acute case of benzene intoxication aboard a chemical cargo ship (Barbera et al., 1998), the authors found blood clots inside the heart and main vessels, multi-organ congestion, and pulmonary edema. They also measured benzene (rounded to whole numbers) in several organs: liver (379 µg/g tissue), heart (183 µg/g tissue), brain (179 µg/g tissue), kidneys (75 µg/g tissue), lungs (22 µg/g tissue), blood (32 µg/mL), and urine (2 µg/mL).

Systemic poisoning by benzene can occasionally result in neuroretinal edema and in retinal and conjunctival hemorrhage (Grant, 1986). Additionally, petechial hemorrhages of the brain, pleura, pericardium, urinary tract, mucous membranes, and skin may occur in cases of fatal, acute benzene poisoning (Haley, 1977).

Major concerns of systemic benzene toxicity include pancytopenia and acute myelogenous leukemia (IARC, 1982). Both are typically seen in chronic and subchronic exposures. Cells of the myeloid pathway, erythroid in particular, are specific targets of benzene toxicity.

Fifteen degassers were acutely exposed over several days to > 60 ppm benzene during removal of residual fuel from fuel tanks on ships (Midzenski et al., 1992). The maximal level was approximately 653 ppm (2,129 mg/m^3). Volatilization of benzene from the residual fuel was the suspected source of benzene. Twelve workers reported mucous membrane irritation. Eleven reported neurotoxic symptoms. Workers with more than 2 days (8 hours/day) of acute exposure were significantly more likely to report dizziness and nausea than those with 2 or fewer days. Blood cell analyses over a 4-month period

after exposure found at least one hematologic abnormality consistent with benzene exposure in 9 degassers. For example, white blood cell counts were below normal in 4 workers. At one year, 6 workers had persistent abnormalities; an additional worker, with normal hematologic parameters initially, later developed an abnormality consistent with benzene exposure. Many large granular lymphocytes were found in the peripheral blood smears of six of the workers. There were no significant associations between the presence of hematologic abnormalities and either the number of hours of acute benzene exposure or the duration of employment as a degasser in this study.

Two studies may indicate an acute NOAEL for adult humans. Japanese students between the age of 18 and 25 breathed one of seven organic solvents for 2.7 to 4 hours in a 60 m^3 chamber. No adverse effects were reported in three males and three females exposed to 52-62 ppm benzene for the full 4 hours (Nomiyama and Nomiyama, 1974). In a study of the absorption and elimination of inhaled benzene (Srbova et al., 1950), the authors mentioned that no adverse effects were seen in 23 adult volunteers exposed to 47 to 110 ppm benzene for 2 to 3 hours. Thus 110 ppm (359 mg/m^3) is a possible 3-hour NOAEL for acute effects of benzene in humans (National Academy of Sciences, 2009) .

5.2 Acute Toxicity to Infants and Children

Children were among those exposed to benzene and other chemicals following an extended (40 day) flaring incident at a petroleum refinery in Texas City, Texas in 2010 (D'Andrea and Reddy, 2013). The total release of chemicals was more than 500,000 pounds of which more than 17,000 pounds (3.4%) were benzene. Some of the other chemicals released were toluene, hydrogen sulfide, nitrogen oxides, and carbon monoxide. A total of 157 subjects < 17 years (mean age = 15.4 years) were exposed and compared to 155 (mean age = 11.8 years) unexposed children. Somatic symptoms included neurological problems (e.g., unsteady gait, memory loss, headaches) in 80% of the exposed and upper respiratory symptoms in 48%. Blood samples were taken 143 days (median time; range = 117 to 290 days) after the incident. WBC counts (x $10^3/\mu L$) were statistically significantly decreased in exposed children compared with unexposed (6.8 ± 2.1 vs. 7.3 ± 1.7, p = 0.022), and platelet counts (x $10^3/\mu L$) were significantly increased in the exposed group compared with unexposed (278.4 ± 59.9 vs. 261.6 ± 51.7, p = 0.005). Exposed children also had significantly higher levels of alkaline phosphatase (183.7 ± 95.6 vs. 165 ± 70.3 IU/L, p =0 .04), aspartate aminotransferase (23.6 ± 15.3 vs. 20.5 ± 5.5 IU/L, p = 0.015), and alanine amino-transferase (19.2 ± 7.8 vs. 16.9 ± 6.9 IU/L, p = 0.005) compared with the unexposed indicating an adverse effect on the liver. Unfortunately no reports of benzene levels in the air during the incident are publically available.

5.3 Acute Toxicity to Experimental Animals

The oral LD_{50} in rats was calculated to be 3.4 g/kg in young rats and 4.9 g/kg in older rats (Kimura et al., 1971). Death was observed in 2 out of 10 rats exposed to 33,000 mg/m³ (10,300 ppm) for 12.5-30 minutes daily for either 1 or 12 days ((IARC, 1982)). A 4-hour LC_{50} of 13,700 ppm (43,800 mg/m³) was reported in female rats (Drew and Fouts, 1974; IARC, 1982). An LC_{Lo} of 45,000 ppm (144,000 mg/m³) is reported in rabbits (NIOSH, 1994). In mice, an LC_{50} of 9,800 ppm (31,400 mg/m³) is reported (NIOSH, 1994). Leukopenia has been demonstrated to occur in rabbits exposed to 240 ppm (767 mg/m³) for 10 hours/day for 2 weeks (IARC, 1982).

Brief inhalation of air saturated with benzene vapor (concentration unknown) resulted in ventricular extrasystole in cats and primates, with periods of ventricular tachycardia that occasionally terminated in ventricular fibrillation (Sandmeyer, 1981b).

The RD_{50} is a chemical concentration that depresses the respiratory rate in mice by 50 percent due to sensory irritation of the upper respiratory tract. An attempt to determine the inhalation RD_{50} for benzene was not successful (Nielsen and Alarie, 1982). The investigators showed that inhalation of 5,800 ppm (18,800 mg/m³) benzene in mice caused an increase in respiratory rate beginning at 5 minutes following onset of exposure. They speculated that the stimulation of respiratory rate resulted from the action of benzene on the central nervous system. In this study, the authors reported that benzene was not irritating to the upper airways of the animals up to 8,500 ppm (27,710 mg/m^3).

Repeated subcutaneous dosing of mice with benzene for 6 to 20 days resulted in a dose-related decrease in red blood cell production as measured by the incorporation of ^{59}Fe into developing erythrocytes. The DBA mouse strain was more sensitive than the CD-1 and C57BL6 strains. Research using multiple species indicated that mice are more sensitive to adverse effects on erythropoiesis from benzene than are rabbits which are more sensitive than rats (Longacre et al., 1980; Longacre et al., 1981a; IARC, 1982).

Acute exposure to benzene may disrupt erythropoiesis and result in genotoxicity. Subcutaneous injection of 5, 13, 33, and 80 mmol/kg (390, 915, 2,577, and 6,248 mg/kg) benzene in 8- to 10-week-old, male, Swiss-Webster mice inhibited erythropoiesis in a dose-dependent manner, as measured by uptake of radiolabeled iron in the bone-marrow 48 hours after benzene injection (Bolcsak and Nerland, 1983). Three metabolites of benzene (phenol, hydroquinone, and catechol) also inhibited iron uptake.

Results from subacute exposures further illustrate the hematotoxic effects of benzene and the potential for immunotoxicity. Inhalation of 103 ppm (334 mg/m³) benzene for 6 hours/day for 7 days by mice caused decreased spleen and marrow cellularity and decreased spleen weights (Green et al., 1981). Benzene inhalation at 0, 10, 30, 100, and 300 ppm (0, 32.6, 97.3, 326, and 973 mg/m³) for 6 hours/day for 5 days resulted in

a decreased host-resistance to bacterial infection by *Listeria monocytogenes* (Rosenthal and Snyder, 1985). The numbers of *L. monocytogenes* isolated from the spleen were increased in a dose-dependent manner on day 4 of infection. The total numbers of T- and B-lymphocytes in the spleen and the proliferative ability of the splenic lymphocytes were decreased in a dose-dependent manner by benzene exposures of 30 ppm (97.3 mg/m³) or greater. No decrement in host resistance or immune response was observed at 10 ppm (32.6 mg/m³) benzene. Later studies in mice have also shown that exposure to 10 ppm for a subacute duration does not significantly alter hematological parameters in blood, spleen, thymus, or bone marrow.

Inhalation of 0, 10, 31, 100, or 301 ppm (0, 32.6, 100.4, 326, or 978 mg/m^3) benzene for 6 hours/day for 6 days resulted in a dose-dependent reduction in peripheral lymphocytes, and a reduced proliferative response of B- and T-lymphocytes to mitogenic agents in mice (Rozen et al., 1984). Total peripheral lymphocyte numbers and B-lymphocyte proliferation in response to lipopolysaccharide (LPS) were significantly reduced at 10 ppm (32.6 mg/m^3). The proliferation of T-lymphocytes was significantly reduced at 31 ppm (100.4 mg/m^3).

Farris et al. (1997) reported the hematological consequences of benzene inhalation in 12-week-old male B6C3F1/CrlBR mice exposed to 1, 5, 10, 100, and 200 ppm (3.26, 16.3, 32.6, 326, and 652 mg/m^3) benzene for 6 hr/day, 5 days/week for 1, 2, 4, or 8 weeks (Farris et al., 1997). The study also evaluated hematology in small recovery subset groups at each concentration (4 weeks exposure to benzene, then up to 25 days in air). There were no significant effects on hematopoietic parameters from exposure to 10 ppm benzene or less. Thus 10 ppm (32.6 mg/m^3) was a NOAEL for up to 8 weeks of exposure in this study. Exposure to 100 and 200 ppm benzene reduced the number of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, and most blood parameters. Replication of primitive progenitor cells in the bone marrow was increased during the exposure period as a compensation for the cytotoxicity. At 200 ppm, the primitive progenitor cells maintained an increased percentage of cells in S-phase through 25 days of recovery compared with controls.

Evans and coworkers used CD1 and C57BL/6J mice in a time-sampling protocol to quantify seven categories of behavior (stereotypic, sleeping, resting, eating, grooming, locomotion, and fighting). Animals were exposed 6 hours per day for 5 days to 0, 300, or 900 ppm (0, 960 or 2,930 mg/m³) benzene followed by two weeks of no benzene exposure. The authors designed the inhalation exposures to “reflect” occupational exposure at that time. An increase in active behavior in the form of eating and grooming was observed in both strains of mice following exposure to 300 ppm and 900 ppm benzene for 6 hours/day for 5 days (Evans et al., 1981).

Exposure of BALB/c male mice to 50 ppm (162 mg/m^3) benzene on 14 consecutive days resulted in a significantly reduced blood leukocyte count (Aoyama, 1986).

6 Chronic Toxicity of Benzene

6.1 Chronic Toxicity to Adult Humans

The primary toxicological effects of chronic benzene exposure are on the hematopoietic system. Neurological effects are also of concern at slightly higher concentrations. Impairment of immune function and/or various types of anemia may result from the hematotoxicity. Developmental and Reproductive toxicity are covered in Section 7.0 below.

Tissues often react to injury by dysplasia, including hypoplasia or hyperplasia, which may progress to more serious conditions such as aplastic anemia, fibrosis, or neoplasia. Myeloproliferative disorders are a group of diseases in which the bone marrow produces excess red blood cells, white blood cells, or platelets. In myelodysplastic syndrome (MDS), the bone marrow produces low numbers of blood cells or defective blood cells. In 10-30% of cases MDS may progress to acute myeloid leukemia (Schnatter et al., 2012). In 1999 the World Health Organization classified both myeloproliferative disorders and MDS as myeloid neoplasms (Harris et al., 1999). An update of three nested case control studies of petroleum distribution workers in Australia, Canada, and the United Kingdom indicated a monotonic dose-response relationship of MDS with cumulative benzene exposure (Schnatter et al., 2012). OEHHA currently considers both myeloproliferative disorders and MDS to be cancer endpoints.

The hematologic lesions in the bone marrow can lead to peripheral lymphocytopenia or pancytopenia following chronic exposure. Repeated benzene exposures can also lead to life-threatening aplastic anemia. These lesions may lead to the development of leukemia years later, after apparent recovery from the hematologic damage (Degowin, 1963).

Investigators observed 28 cases of pancytopenia among 32 patients in Turkey, who were chronically exposed to benzene vapors from adhesives ranging from 150 to 650 ppm (489 to 2,119 mg/m^3) for 4 months to 15 years (Aksoy et al., 1972). Bone marrow samples revealed variable hematopoietic lesions, ranging from acellularity to hypercellularity. Central nervous system (CNS) abnormalities were reported in four of six individuals with pancytopenia following chronic occupational exposure to unknown concentrations of benzene for an average of 6 years (Baslo and Aksoy, 1982). The abnormalities included reduced sensory and motor nerve conduction velocities.

A retrospective longitudinal study correlated average benzene exposure with total white blood cell counts in a cohort of 459 Pliofilm rubber workers in Ohio (Kipen et al., 1988). The authors found a significant ($p < 0.016$) negative correlation between average workplace benzene concentrations and white blood cell counts for the years 1940-1948. A reanalysis of these data (Cody et al., 1993) showed significant decreases in red (RBC) and white (WBC) blood cell counts among a group of 161 workers during the 1946-1949 period compared with their pre-exposure blood cell counts. The decline in

blood counts was measured over the course of 12 months following start of exposure. During the course of employment, workers with low monthly blood cell counts were transferred to other areas with lower benzene exposures; this potentially created a bias towards non-significance by removing sensitive subjects from the study population. Since there was a reported 75 percent rate of job change within the first year of employment, this bias could be very strong. In addition, there was some indication that blood transfusions were used to treat some "anemic" workers, which would cause serious problems in interpreting the RBC data, since RBCs have a long lifespan (120 days) in the bloodstream. Two of Cody's co-authors performed the exposure analysis and determined a range of monthly median exposures of 30-54 ppm throughout the 12-month segment examined (Crump and Allen, 1984). Despite the above-mentioned potential biases, workers exposed above the median concentrations displayed significantly decreased WBC and RBC counts compared with workers exposed to the lower concentrations using a repeated measures analysis of variance (ANOVA).

In a separate analysis of the workers studied by Kipen et al., staff at NIOSH studied the relationship of benzene exposure to WBC and RBC counts collected from 1940 through 1975 for 657 employees (Ward et al., 1996). The study estimated benzene exposures with a job exposure matrix developed earlier (Rinsky et al., 1987). The maximum daily benzene exposure estimate was 34 ppm (111 mg/m^3). The authors used conditional logistic regression to analyze the effects of (1) benzene exposure in the 30, 90, and 180 days before blood testing and (2) cumulative exposure up until the blood test date. For WBCs all exposure metrics showed a statistically significant relationship ($p < 0.05$) with low cell counts. For RBCs there was a weak positive exposure-response, which was significant ($p = 0.03$) for one of the exposure metrics (in total cumulative dose in ppm-yr prior to testing). The authors found no evidence for a threshold for the hematologic effects of benzene.

The mortality from all cancers and leukemia, in addition to hematologic parameters, was investigated in male workers exposed to benzene for 1-21 years between 1952 and 1978 at a Gulf Oil refinery in Texas (Tsai et al., 1983). The cohort of 454 included maintenance workers and utility men and laborers assigned to benzene units on a "regular basis". Exposures to benzene were determined using personal monitors. The median air concentration was 0.53 ppm (1.7 mg/m^3) in the work areas of greatest exposure to benzene and the average length of employment was 7.4 years. The analysis of overall mortality revealed no significant excesses. Mortality from all causes (SMR = 0.58, $p \leq 0.01$) and from diseases of the circulatory system (SMR = 0.54, $p \leq 0.05$) was significantly below expected values based on comparable groups of U.S. males. The authors concluded that a healthy worker effect was present. An internal comparison group of 823 people, including 10 percent of the workers who were employed in the same plant in operations not related to benzene, showed relative risks (RR) of 0.90 and 1.31 for all causes and cancer at all sites, respectively ($p < 0.28$ and 0.23). A subset of 303 workers was followed for medical surveillance. Up to four hematological tests per year were conducted. Total and differential WBC counts, hemoglobin, hematocrit, RBC, platelets, and clotting times were found to be within normal (between 5th and 95th percentile) limits in this group. (OEHHA considered 0.53

ppm (1.7 mg/m^3) to be a NOAEL. This study was the basis of OEHHA's previous chronic REL of 60 µg/m^3.)

Complete blood count (CBC) data from employees who had ever participated in the Shell Benzene Medical Surveillance Program were compared to employees who had not participated (Tsai et al., 2004). The study included 1,200 employees in the surveillance program (mean eight hour TWA benzene exposure of 0.60 ± 5.60 ppm (median = 0.1 ppm) (2 ± 18 mg/m^3) from 1977 to 1988 and of 0.14 ± 0.82 ppm (median = 0.1 ppm) (0.5 ± 2.7 mg/m^3) since 1988) and 3,227 comparison employees. The study evaluated abnormality of six blood count parameters (WBC, lymphocytes, RBC, hemoglobin, mean corpuscular volume (MCV), and platelets) and the adjusted mean values of these parameters in the exposed group. No increased abnormality of the six parameters was found among exposed employees, however a significant decrease ($p = 0.02$) in the MCV was seen in the exposed workers. The mean values of the exposed employees, adjusted for age, gender, race, amount of time between first and last exam, and current smoking, were similar to those in the comparison group. No adverse hematological effects were found. However, the "exposed" group had a very wide range of benzene exposure levels as evidenced by the reported mean, standard deviation, and median values. The coefficient of variation (SD/mean) was greater than 100 percent. The median exposure value of 0.1 ppm (0.326 mg/m^3) means that half the workers were exposed below that level.

Routine data collected from 1980 to 1993 for Monsanto's medical/industrial hygiene system were used to study 387 workers with daily 8-hour time-weighted average exposures (TWA) of 0.55 ppm benzene (1.8 mg/m^3) (range = 0.01 – 87.69 ppm; based on 4213 personal monitoring samples; less than 5 percent exceeded 2 ppm) (Collins et al., 1997). Controls were 553 unexposed workers. There was no increase in the prevalence of lymphopenia, an early, sensitive indicator of benzene toxicity, among exposed workers (odds ratio = 0.6; 95% confidence interval (CI) = 0.2 to 1.8), taking into account smoking, age, and sex. There also was no increase in risk among 266 workers exposed for 5 or more years (odds ratio = 0.6; 95% CI = 0.2 to 1.9). There were no differences between exposed and unexposed workers for other measures of hematotoxicity, including mean corpuscular volume and counts of total white blood cells, red blood cells, hemoglobin, and platelets.

Between 1967 and 1994 a cohort of 105 workers exposed to low levels of benzene (as measured by personal monitors) was studied at a small petroleum company in Texas (Khuder et al., 1999). The exposure ranged from 0.14 ppm to 2.08 ppm (0.46 to 6.78 mg/m^3) (8-hour TWA) (mean ± 1 SD = 0.81 ± 0.72 ppm). The mean complete blood counts (CBC) were within the normal range, as were the WBC. Other CBC values (RBC, hemoglobin level, MCV, and platelet count) were significantly reduced during the follow-up period. Duration of employment was significantly related to the changes in MCV and platelet counts. The reductions in MCV were statistically significant only among workers employed for more than 10 years. The study suggests that low levels of benzene may affect some CBC values.

A collaboration among the National Cancer Institute, the Shanghai Hygiene and Anti-Epidemic Center, the University of California Berkeley, and other institutions has produced an impressive amount of data on levels of benzene exposure and their effects on nearly 75,000 Chinese workers in 672 factories in 12 cities (Dosemeci et al., 1994; Yin et al., 1994; Hayes et al., 1996; Rothman et al., 1996b; Qu et al., 2002; Lan et al., 2004). The initial studies were on exposure between 1949 and 1987 (Dosemeci et al., 1994), but subsequent reports include later years. Chronic benzene poisoning, defined by a WBC level less than 4000 cells per microliter over several months duration, is a compensable adverse health effect for workers in China and a precursor of chronic disease.

In a cross-sectional study, hematologic outcomes were assessed in 44 (23 male and 21 female) workers heavily exposed to benzene (median = 31 ppm (101 mg/m^3) as an 8-hr TWA) for six months to 16 years (mean = 6.3 years) at three workplaces, each manufacturing a different product in Shanghai (Rothman et al., 1996a). Controls were 44 age and gender-matched unexposed workers at two other workplaces. Hematologic parameters (total WBC, absolute lymphocyte count, platelets, RBC, and hematocrit) were decreased among exposed workers compared to controls (Table 6.1); an exception was the red blood cell mean corpuscular volume (MCV), which was higher among exposed subjects. In a subgroup of 11 workers with a median 8 hr TWA of 7.6 ppm (24.8 mg/m^3) (range = 1-20 ppm) and not exposed to more than 31 ppm (101 mg/m^3) on any of 5 sampling days, only the absolute lymphocyte count was significantly different between exposed workers and controls ($p = 0.03$). Among exposed subjects, a dose response relationship with various measures of current benzene exposure (i.e., personal air monitoring, benzene metabolites in urine) was present only for the total WBC count, the absolute lymphocyte count, and the MCV. The results support the use of the absolute lymphocyte count as the most sensitive indicator of benzene-induced hematotoxicity.

Table 6.1. Selected peripheral blood cell count data from Rothman *et al*. (1996)

	Controls (N=44)	≤ 31 ppm benzene Median (8 h TWA) = 13.6 ppm (N=22)	> 31 ppm benzene Median (8 h TWA) = 91.9 ppm (N=22)
WBC (mean (SD)/µL)	6800 (1700)	6400 (1800)	5600 (1900)[a]
Absolute lymphocyte count (mean (SD)/µL)	1900 (400)	1600 (300)[a]	1300 (300)[c]
RBC(mean x10^3 (SD)/µL)	4700 (600)	4600 (460)[b]	4200 (600)[c]
Platelets (mean (SD)/µL)	166 (59)	132 (45)[b]	121 (43)[a]
MCV (µm^3)(mean(SD))	88.9 (4.9)	89.8 (3.9)	92.9 (3.4)[c]

[a] $p < 0.01$; [b] $p < 0.05$; [c] $p < 0.001$

Subsequently, the research group examined genetic influences related to metabolism of benzene on chronic benzene poisoning. They reported that, in a case-control study (50

cases, 50 controls) within the Shanghai worker cohort, benzene poisoning was two to three times more likely if a person had either rapid chlorzoxazone metabolism (ascribed by the authors to CYP2E1, although chlorzoxazone is metabolized by both CYP2E1 and CYP1A2 (Neafsey et al., 2009)), or no NAD(P)H:quinone oxidoreductase (NQO1) activity (NQO1*2/*2 null genotype), and seven to eight times more likely with both rapid chlorzoxazone metabolism and no NQO1 activity (Table 6.2) (Rothman et al., 1997). Although several polymorphisms of CYP2E1 have been identified (Neafsey et al., 2009), Rothman and colleagues examined only one (the c2 allele) and found no effect on the risk of chronic benzene poisoning.

Table 6.2. Joint effects of chlorzoxazone metabolism activity and NQOI genotype on benzene poisoning from Rothman et al. (1997)

Chlorzoxazone metabolism	NQO1 genotype	Odds ratio	95% CI	No. cases
Slow	+/+ and +/-	1	-	8
Slow	-/-	2.4	0.6-9.7	6
Rapid	+/+ and +/-	2.9	1.0-8.2	21
Rapid	-/-	7.6	1.8-31.2	13

The incidence of the NQO1*2/*2 null activity genotype varies approximately 8-fold among tested ethnic groups (Table 6.3). The percentage of Chinese with the null activity NQO1*2/*2 genotype is five times that of non-Hispanic whites (22.4 % vs 4.4 %) (Kelsey et al., 1997; Ross, 2005) (Table 6.3). Investigators found an even higher incidence of 34% of the null phenotype among 198 Hmong refugees from Cambodia now living in Minnesota (Kiffmeyer et al., 2004). All the ethnic groups in Table 6.3 are found in California. The data on increased susceptibility to benzene due to null activity of NQO support the role of benzoquinone as a key metabolite in benzene-induced toxicity.

Table 6.3 Percent of NQO1*2/*2 individuals in different populations (Ross, 2005)

Population	NQO1*2/*2 (%)	# of Persons genotyped
Hmong	34.0	198
Chinese	22.4	49
Korean	18.8	69
Native American	17.9	56
Mexican Hispanic	15.5	61
Japanese	12.2	156
African American	5.2	136
Non-hispanic white	4.4	114

GST metabolic enzymes are involved in the detoxification of benzene by conjugating benzene oxide with glutathione to S-phenylmercapturic acid, and by conjugating other hydroxyl –containing metabolites with glutathione. GST null activity variants are very common in the human population (Table 6.4). People who are GST null (no activity of

the particular transferase) are less able to detoxify benzene. The GSTM1 null activity is slightly more prevalent in the Chinese population than among Caucasians (58% vs 53%), while the GSTT1 null activity is nearly three times higher in Chinese than Causasians (57% vs 20%). [Conjugation with glutathione is usually a detoxification step, although in some instances the glutathione conjugate can show toxicity (Ginsberg et al., 2009).]

Table 6.4 GSTM1 and GSTT1 genotypes in populations (Ginsberg et al., 2009)

	% GSTM1 genotype			% GSTT1 genotype		
Population	+/+	+/-	-/- (null)	+/+	+/-	-/- (null)
Chinese	6	36	58	6	37	57
Caucasian	7.3	39	53	34	46	20
Japanese	11	44	45	11	45	44
Mexican-American	13	46	41	45	44	11
African-American	29	50	21	28	50	22
Korean	-	-	-	7	40	53

In a study that partially confirmed and extended the study of Rothman and colleagues, Chen and coworkers studied single nucleotide polymorphisms (SNPs) in CYP2E1, NQO1, MPO, GSTM1 and GSTT1 in 100 benzene-exposed workers diagnosed with chronic benzene poisoning and 90 benzene-exposed matched controls (Chen et al., 2007). The SNPs can lend different degrees of functionality to the protein product, in this case metabolic enzymes. Benzene poisoning was defined according to the criteria from the Ministry of Health in China. The criteria include: (1) total white cell count < 4000 per µl or white cell count between 4000 and 4500 per µl and platelet count < 80,000 per µl, and (2) evidence of chronic benzene exposure. There was a 2.82-fold (95% CI = 1.42-5.58) increased risk of benzene poisoning in the workers with the NQO1 609C > T mutation genotype (T/T) compared with the heterozygote and the wild-type (C/C) (Table 6.5)(Chen et al., 2007). Workers with the GSTT1 null genotype had a 1.91-fold (95% CI = 1.05-3.45) increased risk of poisoning compared with those with GSTT1 non-null genotype. A three genes' interaction revealed a 20.41-fold (95% CI = 3.79-111.11) increased risk of poisoning in subjects with the NQO1 609C > T T/T genotype and with the GSTT1 null genotype and the GSTM1 null genotype compared with those carrying the NQO1 609C > T C/T and C/C genotype, GSTT1 non-null genotype, and GSTM1 non-null genotype (Table 6.5). Multiplying the null genotype frequencies in Tables 6.3 and 6.4 results in an estimation that 7.4% of the Chinese population has this combination. The confidence intervals are large both for the adjusted OR value of 20.41 and the unadjusted OR of 16.13 due to only 2 controls in the denominator. These authors found no association of CYP2E1 (using wild type and two SNPs) and MPO (wild type and one SNP) genotype with chronic benzene poisoning in these 190 benzene-exposed Chinese workers.

Table 6.5 Interaction of three genes in benzene poisoning

NQO1	GSTT1	GSTM1	Cases	Controls	OR (95% CI)	ORadj (95% CI)
T/T	null	null	17	2	16.13 (3.2–83)**	20.41 (3.9–111)**
T/T	+	null	9	3	5.7 (1.3–25.6)*	5.3 (1.26–23.8)*
T/T	null	+	3	6	0.95 (0.20–4.57)	0.96 (0.19–4.78)
T/T	+	+	9	5	3.4 (0.92–12.8)	3.8 (0.99–14.3)
C/C&C/T	null	+	14	11	2.43 (0.83–7.14)	2.59 (0.87–7.75)
C/C&C/T	null	null	21	18	2.23 (0.85–5.85)	2.69 (0.99–7.25)
C/C&C/T	+	null	16	24	1.27 (0.48–3.34)	1.37 (0.51–3.73)
C/C&C/T	+	+	11	21	1.0	1.0

**p<0.01, *p<0.05.

Personal benzene exposure and blood cell counts were measured in 130 exposed workers (62 men and 68 women) in three factories in Tianjin, China (near Bejing) and in 51 age- and gender-matched unexposed subjects (Qu et al., 2002). Benzene air levels on the day of blood sampling ranged from 0.06 to 122 ppm (0.2 to 398 mg/m^3). The 4-week average exposure levels were 0.08 to 54.5 ppm (0.26 to 178 mg/m^3). Significant decreases of RBC, WBC, and neutrophils were observed (Table 6.6). The decreases correlated with both personal benzene exposures and levels of biomarkers: the urinary metabolites S-phenylmercapturic acid and trans,trans-muconic acid, and the albumin adducts of benzene oxide and 1,4-benzoquinone. The depressions in RBC, WBC, and neutrophils were exposure dependent and were also significantly different in the lowest exposed group (≤ 0.25 ppm) compared with unexposed subjects. The results suggested to the authors that lymphocytes may not be more sensitive than neutrophils to chronic benzene exposure. Some of the workers were likely co-exposed to toluene. Since toluene protects against the adverse effects of benzene (Snyder et al., 1989), the presence of this co-exposure in this study could have masked some of the possible effects that benzene may have in other benzene-exposed workers. The statement that some cell counts were significantly depressed at ≤ 0.25 ppm benzene is potentially important, but it was difficult to ascertain from the presentation of the data how many workers were exposed at that level.

Table 6.6. Selected peripheral blood cell count data from Qu *et al*. (2002)

Workers (N)	51	54	36	29	11
Mean (SD) cumulative exposure (ppm-yr)	0	32 (21)	74 (51)	123 (65)	237 (188)
Current exposure (ppm) (last four weeks)	0.004 (0.003)	3.07 (2.9)	5.89 (4.8)	17.4 (15.5)	50.6 (55.4)
RBC ($x10^4$/µL)*	463 (52)	403 (62) #	396 (57)	404 (51)	391 (39)
WBC (per µL)*	6671 (1502)	6383 (1330)	6089 (1455)	6103 (1560)	4727 (548)
Neutrophils (per µL)*	4006 (1108)	3377 (868) #	3491 (1121)	3501 (1314)	2480 (451)

*$p < 0.001$, test for exposure-response trend
#$p < 0.01$ vs. control by t test for difference between the means (only lowest benzene tested)

A cross-sectional survey studied 250 (86 male and 164 female) Chinese workers exposed in two shoe manufacturing facilities near Tianjin to glues containing 0.6 to 34 percent benzene for 6.1 ± 2.1 years (Lan et al., 2004). For each worker, individual benzene (and toluene) exposure was monitored repeatedly (up to 16 months) before blood samples were drawn. WBC and platelet counts were significantly lower than in the 140 control garment workers, even for exposure below 1 ppm benzene in air (mean = 0.57 ± 0.24 ppm) (1.86 ± 0.78 mg/m^3) (Table 6.7). Total lymphocytes and the specific subtypes B cells and $CD4^+$-T cells (the last also a target of the Human Immunodeficiency Virus (HIV) (Wigzell, 1988)) were also lower than controls. Progenitor cell colony formation declined significantly with increasing benzene exposure and was more sensitive to the effects of benzene than was the number of mature blood cells. Genetic variants in myeloperoxidase (MPO) and NQO1 influenced susceptibility to benzene hematotoxicity. Increased myeloperoxidase activity and decreased NQO1 activity were associated with increased hematotoxicity. The authors concluded that hematotoxicity from benzene exposure may be evident among genetically susceptible subpopulations. A confounder is the co-exposure of the workers to toluene, a competitive inhibitor of benzene metabolism.

Table 6.7. Selected peripheral blood cell count data from Table 1 of Lan *et al*. (2004)

	Controls (< 0.04 ppm) (n = 140)	Low exposure 0.57 ppm (n = 109)	Medium 2.85 ppm (n = 110)	High 28.73 ppm (n = 31)	*p* for 0.57 ppm vs. controls
WBC	6480 (1710)*	5540 (1220)	5660 (1500)	4770 (892)	< 0.0001
Granulocytes	4110 (1410)	3360 (948)	3480 (1170)	2790 (750)	< 0.0001
Lymphocytes	2130 (577)	1960 (541)	1960 (533)	1800 (392)	0.018
$CD4^+$ T cells	742 (262)	635 (187)	623 (177)	576 (188)	0.003
B cells	**218 (94)**	**186 (95)**	**170 (75)**	**140 (101)**	0.003
Monocytes	241 (92)	217 (97)	224 (93)	179 (74)	0.018
Platelets	230 (60) x 10^3	214 (49) x 10^3	200 (53) x 10^3	172 (45) x 10^3	0.023

* Unadjusted mean cell number (± 1 SD) per microliter of blood

When trend analysis was used to examine only the 219 workers exposed to <10 ppm benzene, inverse associations of cell count decrease with benzene increase were each statistically significant ($p < 0.05$) for total WBCs, granulocytes, lymphocytes, B cells, and platelets. In 60 workers exposed to mean benzene <1 ppm over the most recent year, and in a subset of 50 who also had <40-ppm-years lifetime cumulative benzene exposure, all five cell types were decreased compared to controls ($p < 0.05$). A group of 30 workers exposed to <1 ppm benzene, with negligible exposure to other solvents including toluene, had decreased levels of WBCs, granulocytes, lymphocytes, and B cells compared to controls ($p < 0.05$) but not of platelets (Lan et al., 2004). These findings confirm hematotoxicity in Chinese workers exposed to ≤ 1 ppm benzene.

In response to criticism by (Lamm and Grunwald, 2006) of the adequacy of their dose-response data, the authors (Lan et al., 2006) confirmed the monotonicity of their data by spline regression analysis of benzene exposure and WBC counts. They found no apparent threshold in their exposure range of 0.2 to 75 ppm (0.65 to 245 mg/m^3) benzene.

To assess possible toxicity in progenitor cells, peripheral blood from 29 benzene-exposed workers and 24 controls were examined by Lan et al. (2004) for effects of benzene on progenitor cell colony formation including colony forming units-granulocyte-macrophage (CFU-GM), blast forming units-erythroid (BFU-E), and colony forming units-granulocyte, erythroid, macrophage, megakaryocyte (CFU-GEMM). Exposed workers were split into < 10 ppm and > 10 ppm benzene groups. Statistically significant ($p < 0.01$), dose-dependent decreases were observed in colony formation in all three classes of progenitor cells.

In contrast, investigators in Israel compared growth of BFU-E and CFU-GM colonies in 17 male petroleum workers exposed to 0.28–0.41 ppm benzene with 20 healthy control subjects (Quitt et al., 2004). Benzene-exposed workers had significantly increased growth of autonomous BFU-E and unstimulated CFU-GM when compared with controls.

Surprisingly, unexposed smokers had increased colony growth without the addition of cytokines (erythropoietin and granulocyte colony stimulating factor) compared to unexposed nonsmokers. Colony growth was not significantly different between the groups after the addition of cytokines.

In order to identify specific genes involved in the cell count changes above, the authors used a commercial assay (Golden Gate assay by Illumina) to analyze 1,395 single nucleotide polymorphisms (SNPs) in 411 genes in the 250 benzene-exposed workers and the 140 unexposed controls (Lan et al., 2009). Statistically significant findings clustered in five genes (BLM, TP53, RAD51, WDR79, and WRN) that have important roles in DNA repair and genomic maintenance. TP53 (tumor protein p53), the "guardian of the genome," is a tumor suppressor gene and is mutated in up to 50 percent of human cancers. WDR79 is located next to TP53 on chromosome 17 and is also known as TCAB1 and WRAP43 (for WD40-encoding RNA antisense to p53). BLM (for Bloom syndrome) codes for a member of the RecQ helicase family which is involved in DNA replication fork repair processes. WRN (Werner's syndrome) codes for an enzyme(s) with helicase, exonuclease, and ATPase properties. RAD is a homologue of RecA involved in homologous recombination and repair. One or more SNPs in each gene were associated with statistically significant reductions of 10-20 percent in the WBC count among benzene-exposed workers ($p = 0.0011$ to 0.0002) but not among controls.

A further study of this cohort involved 1,023 tag SNPs in 121 gene regions important for benzene effects (Hosgood et al., 2009). Linear regression was used to investigate possible relationships between genetic polymorphisms and total white blood cell (WBC) count and its subtypes. The minp (minimal p value) test assessed the association on the gene region level. The false discovery rate (FDR) method was used to control for multiple comparisons.[1] Vascular endothelial growth factor (VEGF) (minp = 0.0030) and ERCC3 (a gene involved in nucleotide excision repair of DNA) (minp = 0.0042) were the gene regions most significantly associated with altered WBC counts among benzene-exposed workers, after accounting for multiple comparisons. Statistically significant changes were also found for WBC subtype counts, including granulocytes, CD4+ T cells, and lymphocytes for VEGF, and granulocytes and NK cells for ERCC3. Further, in workers exposed to <1 ppm benzene, a SNP in VEGF was associated with changes in WBC and granulocyte counts, and SNPs in ERCC3 were associated with changes in WBC, NK cell, and granulocyte counts.
A cross-sectional study of the same workers evaluated the association between SNPs in genes involved in innate immunity and benzene hematotoxicity. A total of 1,236 tag SNPs in 149 gene regions of six pathways were analyzed (Shen et al., 2011). Six regions were significant for their association with WBC counts based on gene-region ($p < 0.05$) and SNP analyses (False Discovery Rate < 0.05): MBP (myelin basic protein), VCAM1 (vascular cell adhesion molecule 1), ALOX5 (arachidonate 5-lipoxygenase),

[1] The False Discovery Rate (FDR) of a set of predictions is the expected percent of false predictions in the set. If the algorithm returns 100 genes with an FDR of 0.3, expect 30 of them to be false and 70 to be correct.

MPO (myeloperoxidase), RAC2 (a member of a group of small GTPases), and CRP (C reactive protein). Specific SNPs for VCAM1, ALOX5, and MPO were the three most significant SNPs and showed similar effects on WBC subtypes: granulocytes, lymphytes, and monocytes. A 3-SNP block in ALOXE3 showed a global association (omnibus P = 0.0008) with WBCs, but the SNPs were not individually significant.

In a different population of Chinese workers, investigators from ExxonMobil, Fudan University, and the University of Colorado studied peripheral blood counts in 855 workers using benzene glues in five factories (including one shoe factory "B") in and around Shanghai, China (Schnatter et al., 2010). A group of 73 controls was used. The workers were exposed to weekly benzene levels of 0.07 to 872 mg/m^3 (median = 7.4 mg/m^3 or 2.3 ppm). Lifestyle and demographic information was obtained by questionnaire. Possible genetic influences were assessed with five single nucleotide polymorphisms (SNPs) in the NQO1(2 SNPs), MPO, CYP2E1, and GSTT1 genes. Effects on peripheral blood were seen for red cell indices, including anemia and macrocytosis, for benzene exposures above 10 ppm (32.6 mg/m^3). The most sensitive parameters to benzene exposure based on change point regression analysis in this study were decreases in neutrophil counts and an increase in the mean platelet volume (MPV) at and above 7.77 and 8.24 ppm (25.1 and 26.9 mg/m^3), respectively. These "change points" appear to be LOAELs for benzene effects in this study. In addition, there was a statistically significant decrease in red cells in workers exposed to less than 1 ppm benzene and in those exposed to greater than 10 ppm benzene (based on the confidence intervals of the odds ratios, which did not include 1), but not in those workers between 1 and 10 ppm (Table 6.8). Note, however, that the confidence intervals were very wide. The report tells when the factories opened but does not indicate the years of employment of the workers. However, since factories D and E opened in 1999 and 1998, respectively, and the sampling was done in 2003 and 2007, those workers would have been exposed for 4 to 9 years.

Table 6.8. Odds ratios for blood parameters at different benzene exposure levels

Parameter	< 1 ppm (95% CI)	1-10 ppm (95% CI)	> 10 ppm (95% CI)
WBC	2.49 (0.31 - 20.0)	1.92 (0.23 - 15.7)	4.07 (0.51 – 32.4)
RBC	10.8 (1.41 – 82.5)	5.13 (0.66 – 39.9)	16.0 (2.11 – 121)
MCV*	5.65 (0.63 – 51.1)	5.91 (0.75 – 46.5)	17.7 (2.35 – 134.1)
Platelets	2.18 (0.24 – 19.8)	1.76 (0.20 – 15.2)	4.54 (0.56 – 36.7)

Data are from Table 5 of (Schnatter et al., 2010). Benzene exposure was based on the average weekly readings of individual workers. Workers were sampled 1-14 times (average = 4) between 2003 and 2007.
*MCV = mean corpuscular volume of RBCs

Other recent studies have not found effects of benzene on blood cells at low levels. Swaen and colleagues studied hematological effects at low benzene air levels among Dow Chemical Company employees in the Netherlands (Swaen et al., 2010). They compared 8,532 blood samples from 701 male workers with low benzene exposure to 12,173 samples from 1,059 male workers with no occupational benzene exposure for the years 1981-2007. A Job Exposure Matrix was constructed using 21,584 benzene

air measurements for the exposed employees. The Matrix was used to estimate benzene exposure in the year in which each blood sample was collected. The average lymphocyte counts for the unexposed and exposed group were similar: 2,090.29 and 2,097.02 cells per microliter, respectively. The lymphocyte levels are similar to controls in other populations. Adjustments for smoking, age, and month of blood sample did not change the results. No adverse effect on any blood parameters was seen. Stratification into three exposure subgroups (<0.5 ppm (1.63 mg/m^3), 0.5-1 ppm, and >1 ppm (3.26 mg/m^3)) showed no significant differences for any of the blood parameters among the exposure categories including the non-exposed.

In 1981, South Korea banned use of glues or solvents containing more than 1 percent benzene from the workplace, except where benzene is used in a completely sealed process. After the ban (in 2000), the number of workers possibly exposed to benzene was estimated to be 196,182 workers in 6,219 factories. Benzene exposure in different industries was assessed by reviewing the claimed cases for workmen's compensation due to hematopoietic diseases related to benzene which were investigated by Korea OSHA between 1992 and 2000 (Kang et al., 2005). Six factories were evaluated for benzene exposure. Personal air monitoring was performed in 61 workers; urine samples were collected from 57 to measure trans,trans-muconic acid (t,t-MA); and hematologic examination was performed. The geometric mean of benzene in air was 0.094 ppm (range = 0.005-5.311 ppm) (0.3 mg/m^3; range = 0.02-17 mg/m^3). Seven samples were higher than 1 ppm but less than 10 ppm, the occupational exposure limit in Korea. The geometric mean of t,t-MA in urine was 0.966 mg/g creatinine (range = 0.24-2.74). The benzene exposure level was low except in a factory where benzene was used to polymerize other chemicals. Ambient benzene from 0.1 to 1 ppm (0.326 to 3.26 mg/m^3) was significantly correlated with urinary t,t-MA concentration ($r=0.733$, $p<0.01$). Hematologic parameters did not show any significant differences among groups based on the level of exposure. Korean workers were not highly exposed to benzene and the level of exposure was mostly less than 1 ppm.

In summary, some recent studies have reported reduced peripheral blood cell counts at workplace benzene levels at and below 1 ppm, especially among Chinese workers. Lymphocytes and lymphocyte subtypes are the most sensitive to benzene, but some studies find other cell types, such as red blood cells, most sensitive to benzene. Many genes involved in benzene metabolism, in DNA repair, in genome maintenance, and in other cellular functions influence the peripheral blood cell count in exposed workers. (Genes, and the enzymes they code for, involved in the formation of muconaldehyde and muconic acid (Figure 4.1), have not been reported to affect cell count, perhaps because of the large number of enzymes catalyzing alcohol dehydrogenation (Edenberg, 2000) and aldehyde dehydrogenation, oxidation, and reduction (Marchitti et al., 2008)).

6.2 Chronic Toxicity to Infants and Children

Several epidemiological and ecological studies have been published, which examine the association between benzene exposure and health outcomes in children, notably leukemia (Brosselin et al., 2009). However, most of these studies use proximity to gasoline stations or roads with high traffic volumes as proxies for benzene exposure. Since the resulting exposures are to complex mixtures of VOCs and/or vehicular exhaust of which benzene is one component among many, the role of benzene in the reported health effects is not clear. Nonetheless, we briefly describe some below.

In France, Brosselin and colleagues studied the association between acute childhood leukemia and residing next to gas stations and automotive repair shops for 2003-2004 (the ESCALE study (SFCE))(Brosselin et al., 2009). A total of 765 cases of acute leukemia and 1,681 controls under 15 years of age was studied. Acute leukemia was significantly associated with residence next to either gas stations or automotive-repair garages (odds ratio (OR) = 1.6 [95% CI = 1.2-2.2]) and next to a gas station only (OR = 1.9 [95% CI = 1.2-3.0]). The OR did not show a tendency to increase with exposure duration. The results were not modified by adjustment for potential confounders including urban/rural status and type of housing (Brosselin et al., 2009). In a further study of this population, acute leukemia was significantly associated with higher estimates of traffic NO_2 levels at the home (> 27.7 µg/m^3) compared with lower NO_2 levels (< 21.9 µg/m^3) [OR = 1.2; 95% CI = 1.0-1.5] and with the presence of a heavily-trafficked road within 500 meters (m) of the home compared with the absence of such a road in the same area (OR=2.0; 95% CI = 1.0-3.6). There was a significant association between acute leukemia and a high density of heavy-traffic roads within 500 m compared with the reference category with no heavy-traffic road within 500 m (OR = 2.2; 95% CI= 1.1-4.2), and a significant positive linear trend of the association of acute leukemia with the total length of heavy-traffic road within 500 m (Amigou et al., 2011).

An earlier case-control study in France for the years 1995-1999 involved 280 leukemia cases and 285 controls. There was no association between the mothers' work exposure to hydrocarbons during pregnancy and leukemia, or between residential traffic density and leukemia. There was a statistically significant association between residences near a gas station or an automotive repair shop during childhood and the risk of childhood leukemia (OR = 4.0, 95% CI = 1.5-10.3), with a positive duration trend. The association was strong for acute non-lymphocytic leukemia (OR = 7.7, 95% CI = 1.7-34.3) and was not altered by adjustment for potential confounders (Steffen et al., 2004).

An individual analysis with an ecologic measure of exposure studied 977 cases of childhood lymphohematopoietic cancer diagnosed from 1995-2004 in Texas (Whitworth et al., 2008). Exposure values were the U.S. Environmental Protection Agency's 1999 modeled estimates of benzene and 1,3-butadiene for 886 census tracts surrounding Houston. Census tracts with the highest benzene levels had elevated rates of all leukemia [rate ratio (RR) = 1.37; 95% CI = 1.05-1.78]. The association was higher for acute myeloid leukemia (RR = 2.02; 95% CI, 1.03-3.96) than for acute lymphocytic

leukemia (RR = 1.24; 95% CI, 0.92-1.66). Among census tracts with the highest 1,3-butadiene levels, the authors observed RRs of 1.40 (95% CI, 1.07-1.81), 1.68 (95% CI, 0.84-3.35), and 1.32 (95% CI, 0.98-1.77) for all leukemia, acute myeloid leukemia, and acute lymphocytic leukemia, respectively.

In a case–control study of California children under the age of six, 69 cases of acute lymphoblastic leukemia (ALL) and 46 cases of acute myeloid leukemia (AML) were identified in the California Cancer Registry, and 19,209 controls were culled from California birth records for 1990 through 2007 (Heck et al., 2013). The children resided within 2 km (for ALL) or 6 km (for AML) of an air toxics monitoring station. The authors used logistic regression to estimate the risk of leukemia associated with one interquartile range increase in air toxics exposure. Risk of ALL was elevated with 3rd trimester exposure to benzene (OR = 1.50, 95% CI = 1.08-2.09), and to five other air toxics related to fuel combustion. Risk of AML was increased with 3rd trimester exposure to benzene (OR = 1.75, 95% CI = 1.04-2.93), as well as to chloroform and two other traffic-related toxics. During the child's first year, exposure to benzene did not increase the risk to either leukemia, while exposure to butadiene, ortho-xylene, and toluene increased risk for AML and exposure to selenium increased risk for ALL.

6.3 Chronic Toxicity to Experimental Animals

A number of animal studies have demonstrated that benzene exposure can induce bone marrow damage, changes in circulating blood cells, developmental and reproductive effects, alterations of the immune response, and cancer. With respect to chronic toxicity, hematological changes appear to be the most sensitive indicator (Table 6.9).

Wolf and coworkers conducted repeat benzene exposures (7-8 h/day, 5 days/week) in several species. Rabbits were exposed to 80 ppm (261 mg/m^3) for 175 total exposures; rats were exposed to 88 ppm (287 mg/m^3) for 136 total exposures; and guinea pigs were exposed to 88 ppm (287 mg/m^3) for 193 total exposures (Wolf et al., 1956). The observed effects included leukopenia, increased spleen weight, and histological changes to the bone marrow.

Hematologic effects, including leukopenia, were observed in rats exposed to mean concentrations of 44 ppm (143 mg/m^3) or greater for 5 to 8 weeks. Exposure to 31 ppm (100 mg/m^3) benzene or less did not result in leukopenia after 3 to 4 months of exposure (Deichmann et al., 1963).

Among Sprague-Dawley rats and AKR/J mice exposed to 300 ppm (972 mg/m^3) benzene, 6 hours/day, 5 days/week for life, Snyder and coworkers found lymphocytopenia, anemia, and decreased survival time (Snyder et al., 1978).

Male mice exposed to 400 ppm (1,304 mg/m^3) benzene, 6 hours/day, 5 days/week for 9.5 weeks showed depressed bone marrow cellularity, decreased stem cell count, and altered morphology in spleen colony-forming cells (Cronkite et al., 1982)

Mice are more sensitive than rats or rabbits to the hematologic and leukemic effects of benzene (IARC, 1982; Sabourin et al., 1989). Metabolism of benzene to hydroquinone, muconic acid, and hydroquinone glucuronide is much greater in mice than rats, whereas the detoxification pathways are approximately equivalent between the two species (Sabourin et al., 1988).

A study on the chronic hematological effects of benzene exposure in C57 Bl/6 male mice (5 or 6 per group) showed that peripheral lymphocytes, red blood cells and colony-forming units (CFUs) in the bone marrow and spleen were significantly decreased in number after treatment with 10 ppm (32.4 mg/m^3) benzene for 6 hours/day, 5 days/week for 178 days compared to unexposed controls (Baarson et al., 1984). Ten ppm, the only concentration studied, was the workplace exposure standard at the time.

Male and female mice (9 or 10 per group) were exposed to 10, 25, 100, 300 and 400 ppm benzene for 6 hours/day, 5 days/week for 2 to 16 weeks (Cronkite et al., 1985). After 2 weeks at 100 ppm (326 mg/m^3) benzene and higher, mice showed both decreased bone marrow cellularity and a reduction of pluripotent stem cells in the bone marrow. The decrease in marrow cellularity continued for up to 25 weeks following a 16-week exposure to 300 ppm (972 mg/m^3) benzene. Peripheral blood lymphocytes (PBLs) were dose-dependently decreased with benzene exposures of greater than 25 ppm (81 mg/m^3) for 16 weeks, but recovered to normal levels following a 16-week recovery period.

Fifty Sprague-Dawley rats and 150 CD-1 mice of both sexes were exposed to 0, 1, 10, 30, or 300 ppm (0, 3.26, 32.6, 97.2, or 972 mg/m^3) benzene, 6 hours/day, 5 days/week for 13 weeks (Ward et al., 1985). Serial necropsies were conducted at 7, 14, 28, 56, and 91 days (20 percent of each group of rodents at each time point). No hematological changes were found for mice and rats at 1, 10, or 30 ppm. In male and female mice significant increases in mean cell volume and mean cell hemoglobin values and decreases in hematocrit, hemoglobin, lymphocyte percentages, and decreases in red cell, leukocyte and platelet counts were observed at 300 ppm (978 mg/m^3) . The changes were first observed after 14 days of exposure. Histological changes in mice included myeloid hypoplasia of the bone marrow, lymphoid depletion in the mesenteric lymph node, increased extramedullary hematopoiesis in the spleen, and periarteriolar lymphoid sheath depletion. Effects were less severe in the rats. In this subchronic study 30 ppm (97.2 mg/m^3) was a NOAEL.

The National Toxicology Program (NTP, 1986) conducted a chronic (2 year) toxicity “bioassay” in F344 rats and B6C3F1 mice of benzene by gavage in corn oil. Doses were 0, 25, 50, and 100 mg/kg-day for females and 0, 50, 100, and 200 mg/kg-day for males. Dose-related lymphocytopenia and leukocytopenia were observed in both species in all dosed groups. Mice exhibited lymphoid depletion of the thymus and spleen and hyperplasia of the bone marrow.

Investigators at Brookhaven National Laboratory exposed CBA/Ca mice to 10, 25, 100, 300, 400 and 3,000 ppm (32.6, 82, 326, 972, 1,304 and 9,720 mg/m^3) benzene 6 hours/day, 5 days/week for up to 16 weeks (Cronkite et al., 1989). No effects were observed at 10 ppm. Lymphopenia was observed in the 25 ppm (82 mg/m^3) exposure group. Higher concentrations of benzene produced dose-dependent decreases in blood lymphocytes, bone marrow cellularity, spleen colony-forming units (CFU-S), and an increased percentage of CFU-S in S-phase synthesis.

Farris et al. exposed B6C3F$_1$ mice to 1, 5, 10, 100, and 200 ppm (3.26, 16.3, 32.6, 326, and 652 mg/m^3) benzene for 6 hr/day, 5 days/week, for 1, 2, 4, or 8 weeks (Farris et al., 1997). In addition some animals were allowed to recover from the exposure for up to 25 days. There were no significant effects on hematopoietic parameters from exposure to 10 ppm benzene or less. Exposure to higher levels reduced the number of total bone marrow cells, progenitor cells, differentiating hematopoietic cells, and most blood parameters. The replication of primitive progenitor cells was increased. The authors suggested that this last effect, in concert with the genotoxicity of benzene, could play a role in the carcinogenicity of benzene.

Table 6.9. Important non-acute animal inhalation studies of benzene

Study	Animal	Exposure	Duration	Effect	NOAEL	LOAEL
(Farris et al., 1997)	Mice	6 h/d, 5 d/wk	1, 2, 4, or 8 wk	hemato-poiesis	10 ppm	100 ppm
(Cronkite et al., 1989)	Mice	6 h/d, 5 d/wk	Up to 16 wk	hemato-poiesis	10 ppm	25 ppm
(Aoyama, 1986)	Mice	6 h/d	14 days	hemato-poiesis	not found	50 ppm
(Ward et al., 1985)	Mice & rats	6 h/d, 5 d/wk	13 weeks	hemato-poiesis	30 ppm	300 ppm
(Cronkite et al., 1985)	Mice	6 h/d, 5 d/wk	2-16 wk	hemato-poiesis	25 ppm	100 pm
(Baarson et al., 1984)	Mice	6 h/d, 5 d/wk	178 d	hemato-poiesis	not found	10 ppm
(Deichmann et al., 1963)	Rats	5 h/d, 4 d/wk	5 wk-4 mo	hemato-poiesis	31 ppm	44 ppm
(Wolf et al., 1956)	Rabbits, rats, guinea pigs	7 h/d	136-193 exposures	hemato-poiesis	not found	80-88 ppm

7 Developmental and Reproductive Toxicity

7.1 Human Studies

Based on blood samples taken at birth from mother and infant, benzene can cross the human placenta and be in the umbilical cord at a level equal to or greater than in maternal blood (Dowty et al., 1976). The database of benzene effects on human

reproductive and developmental toxicity is limited to a few reports which usually have small samples, unmeasured exposure to benzene, and exposure to other chemicals. CYP2E1, which is a key enzyme in the pathway from benzene to its toxic metabolites, was not detected in human livers in the early fetal period (Vieira et al., 1996) but was detectable at low levels in some fetuses beginning in the second trimester (Johnsrud et al., 2003). In the third trimester CYP2E1 is present in most fetuses at 10-20 percent of adult levels (Table 7.1). However, many phase II enzymes which detoxify benzene metabolites are also low in the fetal period (McCarver and Hines, 2002). For example, for seven of eight substrates of UDP-glucuronyltransferase in human liver, the level during the fetal period ranged from less than 1 to 30 percent of adult levels and the level at the end of the fetal period ranged from 6 to 31 percent of adult levels. For only one substrate (5-hydroxytryptamine) were adult levels of UDP-glucuronyltransferase present in the fetal period (Leakey et al., 1987).

Table 7.1. Changes of CYP2E1 content in human liver with age

Age	N	pmol CYP2E1/mg protein
1st trimester fetus: 8-13.4 weeks	14	- (not detectable)
2nd trimester fetus: 13.6-25 weeks	45	0.3 ± 0.6 (mean ± SD)
3rd trimester fetus: 27-40 weeks	14	5.8 ± 4.6
Neonate: 0-29 days	42	13.4 ± 16.0
Infant: 1.1-11.3 months	64	36.2 ± 20.3
Prepubertal: 1.1-10.0 years	41	43.1 ± 20.6
Adolescent: 11.0-17.7 years	20	~68 (median)
Adult (Snawder and Lipscomb, 2000)	40[a]	52.2 ± 24.2
Adult (Shimada et al., 1994)	60[b]	22 ± 20

Values are from (Hines, 2007) except as noted
[a] black, caucasian, and hispanic subjects
[b] of the 60 subjects, 30 were Japanese and 30 caucasian

The EDEN Mother-Child Cohort Study Group assessed the relation between personal exposure to airborne benzene in non-smoking pregnant French women and fetal growth (Slama et al., 2009). A group of 271 mothers recruited from the University Hospitals of Nancy and Poitiers from September 2003 through June 2006 carried a diffusive air sampler during week 27 of gestation to assess benzene exposure. The authors estimated head circumference of the offspring by ultrasound measurements during the second and third trimesters of pregnancy and at birth. Median benzene exposure was 1.8 μg/m^3 (0.5 ppb) (5 and 95th percentiles, 0.5 and 7.5 μg/m^3). An increase of 1 in log-transformed benzene exposure was associated with a gestational age-adjusted decrease of 68 g in mean birth weight (95% CI, -135 to -1 g; p = 0.05) and of 1.9 mm in mean head circumference at birth (95% CI, -3.8 to 0.0 mm; p = 0.06). Similarly, this differential in exposure was also associated with an adjusted decrease of 1.9 mm in head circumference during the third trimester (95% CI, -4.0 to 0.3 mm; p = 0.09) and of 1.5 mm in head circumference at the end of the second trimester (95% CI, -3.1 to 0 mm; p = 0.05). The association cannot necessarily be attributed solely to benzene, particularly since the benzene exposure may reflect exposure to a mixture of associated

traffic-related air pollutants. Traffic-related air pollutants have been associated in a number of studies with adverse birth outcomes including low birth weight.

Lindbohm and colleagues reported a statistically significant increase in spontaneous abortions in women whose husbands worked at petroleum refineries or with petroleum derived solvents including benzene (16 spontaneous abortions among 93 pregnancies; odds ratio = 2.2; 95% CI = 1.3-3.8) (Lindbohm et al., 1991).

The Texas Birth Defects Registry contained data on neural tube defects (533 cases of spina bifida and 303 cases of anencephaly) in babies delivered between 1999 and 2004 (Lupo et al., 2011). Census tract-level estimates of annual benzene, toluene, ethylbenzene, and xylene (BTEX) levels were obtained from the U.S. EPA's 1999 Assessment System for Population Exposure Nationwide. Mothers living in census tracts with the highest benzene levels were more than twice as likely to have offspring with spina bifida than were women living in census tracts with the lowest levels (odds ratio = 2.30; 95% CI = 1.22-4.33). No other significant associations were observed for benzene and no associations were found for toluene, ethlybenzene, and xylene. A variety of confounders such as race/ethnicity, maternal age, and socioeconomic status were taken into account.

Ghosh and colleagues investigated the effect of ambient air pollution in Los Angeles County on birth weight among 8,181 term (≥ 37 wk gestation), low birth weight (LBW; <2,500 g) children and 370,922 term normal birth weight children born from 1995 through 2006; all mothers lived within 5 miles of at least one of four stationary toxic air contaminant monitors (Ghosh et al., 2012). The influence of local variation in traffic pollution was assessed by land-use-based, regression-modeled estimates of oxides of nitrogen. Adjustments were made for maternal age, race/ethnicity, education, and parity, and for infant gestational age (and gestational age squared). Logistic regression indicated that the odds of term LBW increased 2–5 percent (range of 95% CI = 0%-9%) per interquartile-range increase in modeled traffic pollution estimates and in monitoring-based air toxics exposure estimates for (1) the entire pregnancy, (2) the third trimester, and (3) the last month of pregnancy. Models stratified by monitoring station (to investigate air toxics associations based solely on temporal variations) resulted in 2-5 percent increased odds per interquartile-range increase in third-trimester exposures to benzene, toluene, ethyl benzene, and xylene (BTEX); some confidence intervals indicated statistically significant effects. However, benzene was not a better predictor of LBW than toluene, ethyl benzene, xylene, or PAHs (Polycyclic Aromatic Hydrocarbons excluding naphthalene).

In the latter 1990s, the level of benzene in gasoline sold in the United States decreased as the result of regulation. Zahran and coworkers investigated the relationship between maternal exposure to benzene and birth weight outcomes among US residents in 1996 and 1999. A total of 3.1 million singleton births registered with the U.S. National Center for Health Statistics were included (Zahran et al., 2012). Maternal benzene concentrations were estimated at the county level using data from the US EPA's National Air Toxics Assessment. Regression analysis estimated that a 1 $\mu g/m^3$ (0.3 ppb) increase in maternal exposure to benzene (1) reduced birth weight by 16.5 g (95%

CI, 17.6-15.4), (2) increased the odds of a low birth weight (LBW) child by 7 percent, and (3) increased the odds of a very LBW child by a multiplicative factor of 1.23 (95% CI, 1.19-1.28). In counties where benzene levels decreased 25 percent from 1996 to 1999, birth weight increased by 13.7 g (95% CI, 10.7-16.8) and the risk of low birth weight (LBW) decreased by a factor of 0.95 (95% CI, 0.93-0.98). The authors admit that concentrating on benzene is a limitation since PM also affects birth weight.

As noted above, in a California case-control study evaluating risk of childhood leukemia from ambient exposure to benzene and other air toxics, Heck et al (2013) observed elevated risk of ALL with 3rd trimester exposure to benzene (OR = 1.50, 95% CI = 1.08-2.09), and to five other air toxics related to fuel combustion. Risk of AML was increased with 3rd trimester exposure to benzene (OR = 1.75, 95% CI = 1.04-2.93), as well as to chloroform and two other traffic-related toxics.

Xing and coworkers used multicolor fluorescence in situ hybridization (FISH) to measure the incidence of sperm with numerical abnormalities of chromosomes X, Y, and 21 among 33 benzene-exposed men and 33 unexposed men from Chinese factories (Xing et al., 2010). Passive air monitors were used to measure benzene as well as toluene and xylene. Benzene levels for the exposed ranged from zero (i.e., limit of detection) to 24 ppm (78 mg/m^3); nine had levels ≤ 1 ppm (≤ 3.26 mg/m^3). Exposed men were grouped into low and high exposure based on levels of urinary t,t-muconic acid. Compared to controls, sperm aneuploidy increased across low- and high-exposed groups for disomy X [incidence rate ratio (IRR) for low = 2.0; 95% CI = 1.1-3.4; and IRR for high = 2.8; 95% CI = 1.5-4.9], and for overall hyperhaploidy for X, Y and 21 chromosomes (IRR for low = 1.6; 95% CI, 1.0-2.4; and IRR for high = 2.3; 95% CI, 1.5-3.6, respectively). Even for the nine exposed to ≤ 1 ppm, the authors found statistically significantly elevated disomy X (IRR = 1.8; 95% CI = 1.1-3.00) and hyperhaploidy (IRR = 2.0; 95% CI= 1.1-3.9) compared with the 33 unexposed men. In this study benzene increased the frequencies of aneuploid sperm for chromosomes associated with chromosomal abnormality syndromes in human offspring at surprisingly low levels. Further studies with this cohort using chromosome 1 and low, moderate and high exposure groups yielded IRRs and 95% CIs for all structural aberrations combined of 1.42 (95% CI= 1.10-1.83), 1.44 (CI = 1.12-1.85), and 1.75 (CI = 1.36-2.24) and for deletion of 1p36.3 alone of 4.31 (CI = 1.18-15.78), 6.02 (CI = 1.69-21.39), and 7.88 (CI = 2.21-28.05) for men with low, moderate, and high exposure, respectively, compared with unexposed men (Marchetti et al., 2012).

7.2 Animal Studies

Inhalation of ^{14}C-benzene by pregnant mice resulted in labeled material in the fetuses (Ghantous and Danielsson, 1986).

Groups of 40 female Sprague-Dawley rats were exposed to 0, 1, 10, 40, and 100 ppm (0, 3.26, 32.6, 129.6, or 326 mg/m³) benzene for 6 hours/day during days 6-15 of gestation (Coate et al., 1984). At least 80 percent of the 40 females in each group littered (mean litter size = 13 fetuses). The viscera and skeletons of the fetuses were evaluated for variants and the fetal body weight and length were measured. No increase in variants was noted. A significant decrease was noted in the body weights of fetuses from dams exposed to 100 ppm (326 mg/m³) benzene (Table 7.2). No effects were observed at 40 ppm (130 mg/m³), the NOAEL for this experiment.

Table 7.2. Fetal body weights from Table 5 of Coate *et al*. (1984)

Group	Litters	benzene ppm	Fetal male bw (g, mean ± SD)	Fetal female bw (g)	Live† fetuses per litter (mean±SD)
1	32/40	0	4.02 ± 0.349	3.78 ± 0.303	13.0 ± 3.10
2	33/40	0	4.06 ± 0.430	3.85 ± 0.477	12.5 ± 2.98
3	37/40	1	3.86 ± 0.381	3.69 ± 0.350	13.8 ± 2.47
4	37/40	10	3.88 ± 0.303	3.70 ± 0.385	13.3 ± 2.56
5	37/40	40	3.91 ± 0.492	3.64 ± 0.382	12.9 ± 2.90
6	35/40	100	3.77 ± 0.226*	3.56 ± 0.274*	13.8 ± 2.43

* significantly lower than 0 ppm groups; $p < 0.05$
† There were no dead fetuses.

Exposure of pregnant Swiss Webster mice to concentrations as low as 5 ppm (16 mg/m³) benzene on days 6-15 of gestation (6 hr/day) resulted in bone-marrow hematopoietic changes in the offspring that persisted into adulthood (Keller and Snyder, 1986). However, the hematopoietic effects (e.g., bimodal changes in erythroid colony-forming cells (CFU-E)) were of uncertain clinical significance.
In a subsequent, similar study, the authors (Keller and Snyder, 1988) found that exposure of mice in utero for 6 h/day to 5, 10 and 20 ppm (16.3, 32.6, and 65.2 mg/m^3) benzene on days 6-15 of gestation resulted in suppression of erythropoietic precursor cells and persistent, enhanced granulopoiesis in peripheral blood cells of 2-day neonates (Table 7.3) and increased granulocytes in the livers of 2-day neonates and the spleens of adults at 6 weeks (data not shown). There was a dose-dependent decrease in early nucleated red cells (basophilic normoblasts) (Table 7.3). The authors considered these effects to be significant bone-marrow toxicity. OEHHA staff previously used this study to develop a Maximum Allowable Daily Level (MADL) for Proposition 65 (OEHHA, 2001). The benzene MADL is 49 µg/day for an inhalation exposure and 24 µg/day for an oral exposure.

Table 7.3. Differential peripheral blood cell counts in fetuses of benzene-exposed pregnant mice.[#]

Exposure	Blasts	Dividing Granulo-cytes	Nondividing granulocytes	Early[&] nucleated red cells	Late[&] nucleated red cells	Primitive nucleated red cells	Lympho-cytes
16-day fetuses							
Air	0.00±0.00	0.50±0.16	1.60±0.50	5.10±1.34	0.40±0.22	92.4±1.95	
5 ppm	0.00±0.00	2.10±0.67	3.60±1.57	5.80±1.88	0.80±0.25	87.4±4.11	
10 ppm	0.10±0.00	0.90±0.28	1.30±0.33	4.00±0.60	1.20±0.39	92.4±1.20	
20 ppm	0.10±0.00	1.50±0.50	2.20±0.63	3.90±0.79	1.50±0.34	90.7±1.48	
2-day neonates							
Air	0.00±0.00	3.80±0.66	67.60±2.44	**7.30±1.36**	6.20±1.79		14.0±3.1
5 ppm	0.20±0.14	3.10±0.57	72.30±3.09	**1.70±0.62***	3.60±0.88		17.9±2.4
10 ppm	0.10±0.10	5.90±1.04	67.90±2.88	**0.50±0.22***	7.30±0.83		16.9±2.0
20 ppm	0.10±0.10	2.10±0.62	80.40±2.67*	**0.00±0.00***	1.60±0.45*		14.2±2.5
6-week adults							
Air	0.00±0.00	2.20±0.47	19.3±2.28	0.00±0.00	0.20±0.14		75.0±3.0
5 ppm	0.00±0.00	1.20±0.47	22.0±2.47	0.10±0.10	0.20±0.13		72.3±3.1
10 ppm	0.00±0.00	0.60±0.22	24.2±2.59	0.00±0.00	0.10±0.10		75.1±2.9
20 ppm	0.10±0.10	2.20±0.63	16.7±2.27	0.10±0.10	0.20±0.13		77.6±2.4

[#] 100 cells were counted from 1 male and 1 female from each of 5 litters per treatment (n=10)

* $p < 0.05$ vs corresponding control by Dunnett's test. Values are mean±SE.

[&] Late nucleated red cells contain hemoglobin; early nucleated red cells do not.

An exposure of 500 ppm (1,600 mg/m³) benzene for 7 hours per day through days 6-15 of gestation was teratogenic in the fetal brain of Sprague-Dawley rats, while 50 ppm (160 mg/m³) and 500 ppm resulted in reduced fetal weights on day 20 of gestation (Table 7.4) (Kuna and Kapp, 1981). The higher exposure levels also had significantly more fetuses with skeletal variants. No fetal effects were noted at an exposure of 10 ppm (32.6 mg/m^3), which is the NOAEL for this study.

Table 7.4. Fetal body weight and length (Kuna and Kapp, 1981)

Benzene	0 ppm	10 ppm	50 ppm	500 ppm
Inseminated rat dams (n)	17	18	20	19
Live fetuses/implants (n)	107/119	188/197	127/131	151/165
Mean body weight of live fetuses (g)	4.4 ± 0.6	4.4 ± 0.5	3.8 ± 0.7*	3.6 ± 0.8*
Mean crown-rump length (cm) in live fetuses	4.1 ± 0.2	4.1 ± 0.2	3.9 ± 0.3	3.8 ± 0.4*
Fetuses (litters) with skeletal or visceral variants (n)	3 (3)	2 (1)	23** (6)	30** (6)
Fetuses with brain anomalies or variants	0/35	0/56	5/35	7/44***

* statistically significant difference from control ($p < 0.05$); values are mean ± 1 SD.
** significantly different by chi-square test
*** statistically different from control ($p<0.05$) by Fisher Exact Test (2-tailed)

Inhalation of 500 ppm benzene (the only concentration tested) for 7 hours/day on gestational days 6 to 15 in CF-1 mice and days 6 to 18 in white New Zealand rabbits induced minor skeletal variations that the authors did not consider to be teratogenic (Murray et al., 1979).

Exposure of CFY rats to continuous benzene inhalation (24 h/day) at 150, 450, 1500, or 3000 mg/m^3 (50, 150, 500, or 1000 ppm) from days 7-14 of gestation led to decreased fetal body weights, elevated liver weights, and signs of skeletal retardation at 150 mg/m³ (50 ppm) benzene, the lowest concentration tested (Tatrai et al., 1980).

Female CFLP mice and NZ rabbits were exposed by inhalation to 0, 500, or 1,000 mg/m^3 (0, 153, or 307 ppm) benzene for 24 h/day from day 6 to day 15 of pregnancy (Ungvary and Tatrai, 1985). Maternal toxic effects were moderate and dose dependent. Benzene induced skeletal variations and weight retardation in fetuses of rabbits at 1,000 mg/m^3 and in fetuses of mice at 500 and 1,000 mg/m^3. Benzene increased the post-implantation loss (percent fetuses dead or resorbed) in rabbits at 1,000 mg/m^3. Benzene induced spontaneous abortion in rabbits at 1,000 mg/m^3.

In order to determine if prenatal exposure to benzene induces neurobehavioral changes in offspring, 0.1 mg/kg benzene was injected subcutaneously on gestation day 15 into four pregnant female Sprague-Dawley rats (Lo Pumo et al., 2006). There were no changes in total number of neonates, body weight, and eye opening time between progeny of benzene-exposed dams and controls, and no malformations. At birth, neonatal reflexes (cliff aversion, forelimb, placing, bar holding, forelimb grasping, startle) were scored in benzene-exposed pups. More benzene-exposed pups exhibited reflexes each day compared to controls. Also, the completion (maximum appearance, i.e. 100 percent of the litter exhibited each reflex) of neonatal reflexes in benzene-exposed animals preceded that of controls. Beginning at 2 months after birth, cognitive and motor performance was assessed in males of the prenatally benzene-exposed

progeny. Motor activity in the open-field test showed reduced ambulation in benzene-exposed rats compared to controls. Acquisition of active avoidance responses in the shuttle-box test was impaired in benzene-exposed rats vs. controls. Prenatal benzene exposure was associated with reduced retention latency in a step-through passive avoidance task. The authors concluded that acute exposure to benzene during gestational organogenesis may cause long-lasting changes in motor behavior and cognitive processes. It is problematic to extrapolate this acute subcutaneously administered dose to an equivalent inhalation exposure.

Exposure of rabbits to 80 ppm (261 mg/m^3) and of guinea pigs to 88 ppm (277 mg/m^3) benzene 7 hours/day, 5 days a week for 8 months caused testicular degeneration (Wolf et al., 1956).

In a one-generation reproduction study, groups of 26 female Sprague-Dawley rats were exposed for 6 hours per day by inhalation to 1, 10, 30, and 300 ppm benzene during a 10-week premating period and during mating (to proven fertile males), gestation, and lactation (Kuna et al., 1992). There was no effect on female reproductive performance at any benzene level. Performance measures included number of litters (range = 19-24), mean gestation length (21.6-21.9 days), mean pup number per litter (11.7-12.6), and viability index (96.9-99.5%). At 30 and 300 ppm there was a trend for 21-day-old pups toward reduced body and organ weight but differences were statistically significant ($p < 0.05$) only for female pups at 300 ppm (978 mg/m^3) (32.59 ± 5.05 g vs. 36.3 ± 5.20 g in controls).

Studies *in vitro* indicate that immature hematopoetic cells are more sensitive to hydroquinone than adult cells. Zhu and coworkers compared the effects of hydroquinone on mouse embryonic yolk sac hematopoietic stem cells (YS-HSCs) and adult mouse bone marrow hematopoietic stem cells (BM-HSCs) (Zhu et al., 2013). HSCs were isolated, enriched ~4-fold, and exposed to 1.25, 2.5, 5, or 10 µM hydroquinone. Hydroquinone decreased proliferation, differentiation and colony formation, but increased the apoptosis of both types of HSCs. The cytotoxic and apoptotic effects of hydroquinone were more apparent and the reduction in colony formation was more severe in YS-HSCs than in BM-HSCs; most differences were less than 3-fold.

7.3 Genotoxicity

A review of the data from more than 1,400 genotoxicity tests for benzene and its metabolites (Whysner et al., 2004) led to the conclusion that benzene and its metabolites do not produce reverse mutations in *Salmonella typhimurium* but are clastogenic and aneugenic, producing micronuclei (MN), chromosomal aberrations (CA), sister chromatid exchanges (SCE), and DNA strand breaks.

The International Agency for Research on Cancer (IARC) recently summarized the genotoxicity of benzene (IARC, 2012): “There is strong evidence that benzene metabolites, acting alone or in concert, produce multiple genotoxic effects at the level of

the pluripotent haematopoietic stem cell resulting in chromosomal changes in humans consistent with those seen in haematopoietic cancer. In multiple studies in different occupational populations in many countries over more than three decades a variety of genotoxic changes, including chromosomal abnormalities, have been found in the lymphocytes of workers exposed to benzene."

In the most sensitive inhalation study of genotoxicity in animals, inhalation of 3, 10, and 30 ppm (9.7, 32.6, and 97.8 mg/m^3) benzene for 6 hours by adult male Sprague-Dawley rats resulted in a significant increase over controls in the frequency of sister chromatid exchanges (SCE) in peripheral blood lymphocytes (Erexson et al., 1986). One ppm (3.26 mg/m^3) was a tentative NOAEL for the effect. Male DBA/2 mouse peripheral blood lymphocytes showed a significant concentration-related increase in SCE frequency over controls at 10, 100, and 1,000 ppm (32.6, 326, and 3,260 mg/m^3) benzene, the three concentrations tested. Mouse femoral bone marrow also showed a significant concentration-dependent increase in micronuclei at 10, 100, and 1,000 ppm over controls (Erexson et al., 1986).

7.4 Toxicogenomics

In order to study hematotoxicity at the level of altered multigene expression, cDNA microarray analyses were performed on mouse bone marrow tissue extracts during and after a 2-week exposure to 300 ppm (978 mg/m^3) benzene (Yoon et al., 2003). Expression of fifteen genes was at least doubled by benzene exposure compared to controls. Two of these were increased nearly five-fold (a polycomb binding protein and Metallothionein 1). CYP2E1 expression was increased 2.13 fold. Conversely this high-level benzene exposure decreased expression of a G-protein coupled receptor to 1 percent of its normal output.

One of the cohorts of Chinese workers described above (Lan et al., 2004) was analyzed by microarray analysis for global gene expression in the peripheral blood mononuclear cells (WBC) of 83 workers exposed to benzene levels ranging from < 1 ppm (3.26 mg/m^3) to > 10 ppm (32.6 mg/m^3). The workers were divided into 4 exposure groups and compared to a group of 42 controls (Table 7.5) (McHale et al., 2011). Changes in many metabolic pathways and extensive increases (and probably decreases, which are not discussed) of the expression of specific genes were found at all benzene exposure levels (McHale et al., 2011). The AML (acute myeloid leukemia) pathway was among the pathways most significantly associated with benzene exposure. Alterations in immune response pathways (e.g., toll-like receptor signaling pathway, T-cell receptor signaling pathway) were associated with most exposure levels. A 16-gene increased expression signature (relative to no exposure) was associated with all levels of benzene exposure. The three genes with the highest increased expression were a serpin peptidase inhibitor, a tumor necrosis factor, and interleukin 1 alpha.

The above summaries of acute and chronic toxicity of benzene are intended to give an overview of the data and to analyze reports most relevant to developing Reference Exposure Levels (RELs) for benzene, i.e., inhalation studies. More comprehensive

reviews of benzene toxicity are available (Sandmeyer, 1981a; World Health Organization, 1993; USEPA, 2002; ATSDR, 2007; Wilbur et al., 2008; Goldstein and Witz, 2009; IARC, 2012; Wang et al., 2012).

8 Derivation of Reference Exposure Levels

8.1 Acute Reference Exposure Level for Benzene

Key study	Keller and Snyder, 1988
Study population	pregnant female mice
Exposure method	inhalation of 0, 5, 10, or 20 ppm benzene
Exposure continuity	6 hours per day
Exposure duration	10 days (days 6-15 of gestation)
Critical effects	decreased early nucleated red cell counts (Table 7.3)
LOAEL	5 ppm (16 mg/m^3)
NOAEL	not found
BMCL$_{0.5SD}$	not used due to poor fit (Table 8.1)
Human equivalent concentration	*5* ppm (RGDR* = 1)(systemic effect)
Time adjustment factor	Not done (see below)
LOAEL uncertainty factor (UF$_L$)	√10 (see below)
Interspecies uncertainty factor	
Toxicokinetic (UF$_{A-k}$)	2 (default) (OEHHA, 2008)
Toxicodynamic (UF$_{A-d}$)	√10 (default)
Intraspecies uncertainty factor	
Toxicokinetic (UF$_{H-k}$)	10 (default)
Toxicodynamic (UF$_{H-d}$)	√10 (default)
Database uncertainty factor	1 (developmental studies are available)
Cumulative uncertainty factor	600
Acute Reference Exposure Level	**8 ppb (27 µg/m³)**

*The Regional Gas Dose Ratio (RGDR) is the ratio of the regional gas dose calculated for the respiratory region affected by the inhaled toxicant in the animal species to the regional gas dose in the corresponding region in humans. For a toxicant with a systemic effect, the default value is 1.

Reference Exposure Levels are based on the most sensitive, relevant health effect reported in the medical and toxicological literature. Acute Reference Exposure Levels are levels at which infrequent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document (OEHHA, 2008)). Studies of developmental toxicity usually use repeat exposures in utero, either throughout gestation or during organogenesis. The acute REL for benzene is based on a developmental study (Keller and Snyder, 1988) in which pregnant mice were exposed 6 hours per day during days 6 through 15 of gestation. However, developmental toxicity may occur in response to just one exposure during a specific window of susceptibility. A literature search found 133 single-day exposure developmental toxicity

studies involving 58 chemicals (Davis et al., 2009). The same endpoints observed in repeat dose studies are often observed with single exposures, an acute effect. The acute REL derived above is a level not to be exceeded in any one hour period, which is the default application for acute RELs based on developmental studies (OEHHA, 2008)

In the key study, which OEHHA earlier used to develop a Proposition 65 MADL for benzene (OEHHA, 2001), a monotonic dose response was seen for early nucleated red cells in 2 day neonates. The LOAEL was 5 ppm. A NOAEL was not detected. The several continuous models in BMDS version 2.2 were fit to the data. The Hill Model calculated a $BMCL_{0.5SD}$ of 0.0112 ppm, which was much smaller than the model's $BMC_{0.5SD}$ of 0.92 ppm, while other models had poor fits ($p < 0.1$) (data not shown). The poor results were in part due to the high adverse response (> 75 percent decrease in differential cell count) at 5 ppm, the lowest dose, and hitting a bound of 0 at 20 ppm, the highest dose. The data from the highest dose were omitted and the BMDS linear model was fit to the data. The value for fit was also below 0.1 (Table 8.1). The BMDS results were not used as the point of departure for the REL. However, despite the relatively poor fit to the data, the proximity of the $BMCL_{0.5SD}$ of 1.51 ppm to the LOAEL of 5 ppm provides some support for the use of √10 for the LOAEL to NOAEL UF.

Table 8.1. Benzene 2d neonate data (drop 20 ppm) in BMDS 2.2 Linear Model

Variance	Deviation	BMC(ppm)	BMCL(ppm)	p for fit	AIC* (fitted)
Constant	1 SD	4.14	3.01	0.0364	98.0831
Constant	**0.5 SD**	**2.07**	**1.51**	**0.0364**	**98.0831**
Constant	0.05 Relative	0.48	0.40	0.0364	98.0831
Not	1 SD	8.11	5.18	0.015	76.4167
Not	0.5 SD	4.06	2.59	0.015	76.4167
Not	0.05 Relative	0.548	0.512	0.015	76.4167

*Akaike Information Criterion

The default interspecies $UF_{A\text{-}k}$ of 2 for residual pharmacokinetic differences was used. As indicated above PBPK models for benzene are available in mice, rats, and humans. The hematological effects in the key study have a plausible mechanism involving the toxic metabolites of benzene.

A discussion of evidence for the extent of inter-individual variability appears in Section 8.3 describing derivation of the chronic REL. A number of toxicokinetic studies (described in Section 4) and studies of the association of genetic polymorphisms in metabolizing enzymes and chronic benzene poisoning suggest that the toxicokinetic variation in adults can be accommodated by the default factor of 10. However, one study (Chen et al., 2007) of variability in susceptibility to chronic benzene poisoning suggests a larger than 10 fold variability in response to benzene based on genetic polymorphisms affecting metabolism. These results are based on evaluation of chronically exposed workers. Thus, their application to the acute REL, meant for assessing the hazard of infrequent one hour exposures, does not appear warranted. Further, in the case of the acute REL, the critical effect is based on developmental toxicity following pre-natal exposure, and it is therefore reasonable to assume that

systematic differences associated with early lifestage (including effects *in utero*) are accommodated in the toxicity data. The default intraspecies UF_{H-K} of 10 coupled with a UF_{H-d} of $\sqrt{10}$ for a total UF for intraspecies variability of 30, was therefore considered to be sufficient for the acute benzene REL derivation.

CYP2E1, a principal enzyme in the pathway of benzene metabolism which produces toxic metabolites, has not been detected early in human fetal liver (Vieira et al., 1996) and rises to only 10-20 percent of the adult level by the third trimester (Johnsrud et al., 2003)(Table 7.1). However, since many detoxifying enzymes are also low during this period (McCarver and Hines, 2002), bone marrow toxicity from benzene metabolites might occur in the fetus. The variation in CYP2E1 levels between the third trimester fetus and the adult (Table 7.1) is also compatible with the default value of 10 for toxicokinetic variability among humans.

Additional inter-individual variability issues are addressed by the UF_{H-d} as discussed below.

The default intraspecies UF_{H-d} (toxicodynamics) of $\sqrt{10}$ was used to account for pharmacodynamic variability among pregnant women and their fetuses, the most sensitive group for the acute REL, and among infants, children, and adults (OEHHA, 2008). During embryonic and fetal development, hematopoiesis occurs first (mesoblastic period) in the extraembryonic yolk sac beginning in the 2nd week and ceasing by the eighth week of gestation, then in the liver (hepatic period) and to a lesser extent in thymus and spleen beginning at the 5th-6th week of gestation, and finally in the bone marrow (myeloid period) beginning at the 16th-18th week of gestation or even earlier (Charbord et al., 1996; Peault, 1996; Brugnara and Platt, 2003). The bone marrow volume increases linearly with body weight between 29-33 weeks of gestation and term (Hudson, 1965).

Blood cell type and rates of formation change with age (e.g., Table 7.3) and hematopoiesis would be expected to be more dynamic during developmental (growth) stages than in the adult. The erythroblast count, a marker of the contribution of the liver to erythropoiesis, decreases exponentially from 83 erythroblasts/100 leukocytes at 17 weeks gestation to 4/100 at term (Nicolaides et al., 1989). The third trimester fetus is said to produce red cells at three to five times the rate in adults at steady-state (Palis and Segel, 1998). The estimate is based on (1) the linear decline in reticulocytes per 100 red blood cells from ~10 percent at 17-24 weeks of gestation to ~4 percent at term (Matoth et al., 1971; Zaizov and Matoth, 1976; Nicolaides et al., 1989), (2) a comparison of reticulocytes/1000 red cells in newborns (mean = 51.9) versus adults (mean = 15.7) (Seip, 1955), and (3) a computerized simulation analysis of total red cell volume and life span at different life stages (Bratteby et al., 1968).

In a study of infants born prematurely, the body weight steadily increased (tripled) with gestational age, the percent reticulocytes declined overall, and the red blood cells (RBC) per volume was fairly constant (Table 8.2) (Zaizov and Matoth, 1976). Since both the bone marrow volume (Hudson, 1965) and the red blood cell volume (Bratteby,

1968) increase with gestational age and body weight, the net formation of RBCs must be substantial.

At birth a large drop in red cell production results in a transient physiological anemia of clinical concern (Palis and Segel, 1998). which reaches a low point in total red cells and hematocrit at 6-9 weeks of life after which red cell counts increase again (Matoth et al., 1971).

Table 8.2. Red cell values on first postnatal day in infants born prematurely (Zaizov and Matoth, 1976)

Gestation (wk)	N (infants)	Body wt (g)[a]	% retics.	RBC x 10^4
24-25	7	725 ± 185	6.0 ± 0.5	4.65 ± 0.43
26-27	11	993 ± 194	9.6 ± 3.2	4.73 ± 0.45
28-29	7	1174 ± 128	7.5 ± 2.5	4.62 ± 0.75
30-31	25	1450 ± 232	5.8 ± 2.0	4.79 ± 0.74
32-33	23	1816 ± 192	5.0 ± 1.9	5.00 ± 0.76
34-35	23	1957 ± 291	3.9 ± 1.6	5.09 ± 0.50
36-37	20	2245 ± 213	4.2 ± 1.8	5.27 ± 0.68
term[b]	19		3.2 ± 1.4	5.14 ± 0.70

[a] mean ± standard deviation

[b] term data based on (Matoth et al., 1971)

Although hematopoiesis is dynamic during development, we were unable to find pertinent quantitative data justifying a $UF_{H\text{-}d}$ factor greater than the default factor of $\sqrt{10}$.

In the study (Coate et al., 1984) that was the basis of OEHHA's previous acute REL for benzene (OEHHA, 1999), statistically significant decreased fetal body weight was seen only at 100 ppm, the highest dose tested (Table 7.2). A mechanism for the fetal effect of decreased body weight in the study is not obvious. The effects on fetal body weight may be due to the parent compound, which can cross the placental wall, and/or to one of more benzene metabolites. The Keller and Snyder (1988) study is a much more sensitive study than the Coate et al. study showing effects on the hematopoietic system in neonates at much lower gestational exposure levels than Coate et al. found affecting fetal body weight.

8.2 8 hour Reference Exposure Level for Benzene

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 of the Technical Support Document (OEHHA, 2008)).

For a health protective approach, OEHHA determined that the 8 hour REL should be the same as the chronic REL: 1 ppb (0.001 ppm; 3 μg/m^3). It is unclear whether the adverse effects of repeated benzene exposure are reversed by overnight or over-the-weekend periods of non-exposure and they are likely to continue to worsen with additional exposure.

8.3 Chronic Reference Exposure Level for Benzene

Study	Lan et al. (2004)
Study population	250 male and female Chinese shoe workers aged 29.9 ± 8.4 years (vs. 140 controls)
Exposure method	Discontinuous occupational exposure
Exposure continuity	8 hr/day (10 m^3 per 20 m^3 day), 6 days/week
Exposure duration	6.1 ± 2.1 years
Critical effects	Decreased peripheral blood cell counts (7 categories; see Table 6.4)
LOAEL	0.57 ± 0.24 ppm (1.86 ± 0.78 mg/m^3)
NOAEL	Not found
$BMCL_{0.5SD}$	0.476 ppm (Hill Model version 2.15)(Table 8.3)
Average continuous exposure	0.204 ppm (0.476 ppm x 10/20 x 6/7)
Human equivalent concentration	0.204 ppm (0.665 mg/m^3)
LOAEL uncertainty factor (UF_L)	Not applicable with BMC
Subchronic uncertainty factor (UF_S)	√10 (8-≤12% expected lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	60 (see explanation below)
Toxicokinetic (UF_{H-k})	
Toxicodynamic(UF_{H-d})	
Database uncertainty factor	1 (developmental studies are available)
Cumulative uncertainty factor	200
Chronic Reference Exposure Level	**1 ppb (0.001 ppm: 3 μg/m^3)**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from continuous chronic exposures (see Section 7 in the Technical Support Document (OEHHA, 2008)).

In the 219 workers exposed to <10 ppm benzene, inverse associations of cell count with benzene exposure were statistically significant ($p < 0.05$) for total WBCs, granulocytes, lymphocytes, B cells, and platelets. This allowed exploration of which cell might be the most sensitive indicator. The point of departure for the REL was derived using the changes in B cell levels (Lan et al., 2004), which were considered the most sensitive endpoint, as a function of benzene concentration (Table 6.1.4) and the continuous models in the BMDS software. The Hill model (version 2.15) in the BMDS software gave acceptable values for fit and the lowest AIC. We specified a risk of 0.5 estimated standard deviation from the control mean as the benchmark and obtained a BMC of 1.62 ppm and a BMCL of 0.476 ppm (p value for fit = 0.303) (Table 8.4). The U.S. Environmental Protection Agency (USEPA) has suggested use of 1 standard deviation from the control mean as a benchmark but a $BMCL_{1SD}$ could not be obtained with the B cell data using the Hill model (Table 8.4).

Table 8.4. Benzene B cell data in BMDS 2.2 (Hill Model Version: 2.12; 02/20/2007)

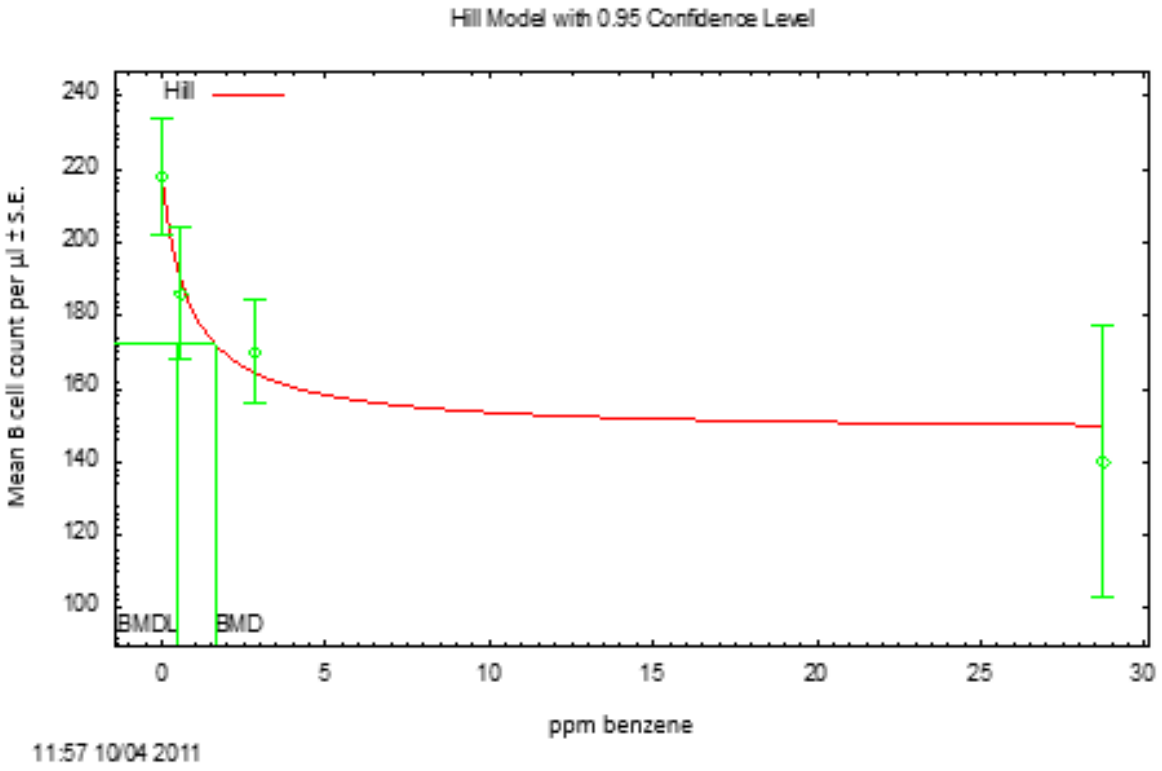
Model	BMC (ppm)	BMCL (ppm)	BMC/BMCL	p (test 4)	AIC (fitted)
Constant variance (rho = 0)					
Polynomial 0.5 SD	3.04	2.05	1.5	0.04099	3907.44
Exponential 0.5 SD (Models 4 and 5)	1.22	0.44	2.8	0.1105	3905.811
Power 0.5 SD	20.35	14.03	1.7	0.0006241	3916.02
Linear 0.5 SD	20.35	14.03	1.7	0.0006241	3916.02
Hill 0.1 SD	0.131	0.0299	4.4	0.303	3904.325
Hill 0.25 SD	0.422	0.1039	4.1	0.303	3904.325
Hill 0.5 SD	**1.624**	**0.4764**	**3.4**	**0.303**	**3904.325**
Hill 0.05 Relative Dev	0.164	0.038	4.3	0.303	3904.325
Hill 1.0 SD	Failed (BMR not in range of mean function)				
Variance not constant (rho ≠ 0)					
Hill 0.1 SD	0.129	Failed	-	0.663	3904.575
Hill 0.25 SD	0.427	Failed	-	0.663	3904.575
Hill 0.5 SD	1.813	Failed	-	0.663	3904.575
Hill 1.0 SD	Failed (BMR not in range of mean function)				

The Lan *et al*. (2004) study is more sensitive than both the Tsai *et al*. (1983) study, used previously by OEHHA for its chronic REL (OEHHA, 2000), and the Rothman *et al*. (1996) study used by USEPA for its Reference Concentration (RfC). Effects on the hematologic system are seen at a level where Tsai *et al*. did not find significant changes. However, as indicated above, at least half (≥ 50%) of the exposures in Tsai *et al*. were less than 0.1 ppm, the median concentration. In Lan *et al*., 0.09 ppm is two standard deviations below the mean of 0.57 ppm indicating that in Lan *et al*. only 2.5 percent of the exposures were below 0.09 ppm. The findings of significant depression in some blood cell counts at ≤ 0.25 ppm by Qu *et al*. (2002) and at 0.79 ppm by Khudar *et al*. (1999) are compatible with the results of Lan *et al*. (2004). Schnatter et al. (2010)

found decreases in total WBC, but the study did not differentiate cell types using cell markers. Differences in benzene effects on WBC sub-types could not be seen by the methods used in Scnhatter et al (2010). Thus, it was not as detailed or specific about which cells were affected by benzene exposure as the Lan et al (2004) study. Using the available information from the study on total WBC counts, OEHHA derived a comparison REL from Schnatter et al (2010) using a NOAEL approach (see below) that was higher than that derived from Lan et al (2004)

Figure 8.3 shows the graph of the Hill model fit to the data of benzene level in ppm on the X axis and mean B cell counts on the Y axis.

Figure 8.3. The continuous Hill model fit to the B cell data of Lan et al. (2004).



Human Inter-individual Variability and the Intraspecies Uncertainty Factor for Chronic Exposure (UF_H)

A combined intraspecies uncertainty factor (UF_H) of 60 was used instead of separate toxicokinetic and toxicodynamic factors since there were reasons for and against higher and lower values for each subfactor.

As described in Section 4 above, PBPK models for benzene are available in mice, rats, and humans. The hematological effects in the key study (Lan et al., 2004) have a plausible mode of action involving toxic metabolites of benzene, although the details of this mechanism are not completely characterized. A number of the human studies described in Sections 4 through 6 examined the extent of inter-individual variability in

the human population on benzene metabolism and toxicity, including the impacts of various genetic polymorphisms in enzymes believed to be involved in benzene metabolism. These have included enzymes believed to have a role in either activation or detoxification of reactive metabolites.

By default, OEHHA uses an intraspecies toxicokinetic uncertainty factor (UF_{H-k}) of 10 (OEHHA, 2008), which is generally thought to make allowance for the expected variability in kinetic factors including differences between infants and children and adults. However, for benzene there are sufficient compound-specific data in humans to address the question of whether this default is adequate in this case. Most benzene-specific reports of toxicokinetic influences on chronic benzene poisoning in adult workers are consistent with the default value of 10 (Table 8.5).

Table 8.5 Inter-individual Variability in Benzene Metabolism and Toxicity

Reference	Ratio	Comment
Variability in observed odds of chronic benzene poisoning due to occupational exposure		
(Rothman et al., 1997)	OR*=7.6 (95%CI 1.8-31.2)	Increased chlorzoxazone metabolism and null NQO1 (see Table 6.2)
(Chen et al., 2007)	OR=20.41 (95%CI 3.9-111)	3 genes interaction – NQO1,GSTT,GSTM; 95%CI very large due to only 2 controls in top denominator (see Table 6.4)
(Sun et al., 2008)	OR (see comment)	Epoxide hydrolase alleles very variable; GAGT haplotype increases (OR=7.8; 95%CI 2.73-18.38) and GGGT haplotype decreases (OR=0.19; 95%CI 0.06-0.57) odds of benzene poisoning
Variability in measured enzyme activity or content		
(Ross, 2005)	Ratio = 5	**NQO1** null activity is 5 times greater in Chinese population than white (see Table 6.3)
(Hines, 2007)	Up to 10 fold	Liver content of **CYP2E1** 3rd trimester fetus vs adults (see Table 7.1) pmol CYP2E1/mg protein about 10 fold less than adult liver
	2 fold	Ratio of pmol CYP2E1/mg protein in liver (Mean + 2SD)/Mean ~2 among pre-adolescents; similar in adults
	2.4 fold	Ratio of pmol CYP2E1/mg protein in liver (Mean + 3SD)/Mean ~2.4 among pre-adolescents; similar in adults
(Hines, 2008)	See comment	**GSTM** increases steadily during gestation; readily detectable at 30 weeks. No difference in GSTM content of infant (>2 weeks to 67 weeks) and adult liver GSTM content
(Hines, 2008)	See comment	**EPHX1** present in gestational liver samples; activity ranged from 41 to 306 pmol/min/mg protein in 8 to 22 week gestation samples; adult samples 424 ± 236 pmol/min/mg protein.
Population variability in benzene metabolism		
(Rappaport et al., 2013)	3.5	Ratio (90th percentile /median) for hydroquinone metabolite of benzene in Chinese workers (see Table 8.6 below)

*Odds ratio of benzene poisoning in adults

Chinese workers with high rates of chlorzoxazone metabolism (due to CYP2E1 and CYP1A2 (Neafsey et al., 2009)) and no NQO1 had an odds ratio (OR) of 7.6 for benzene poisoning compared to those with low chlorzoxazone metabolism and normal NQO1 activity (Table 6.2) (Rothman et al., 1997). The prevalence of no NQO1 activity is five times greater (22.4% vs. 4.4%) in the Chinese population compared to non-

Hispanic whites (Ross, 2005) (Table 6.3). A three genes' interaction revealed a 20.41-fold increased risk of poisoning in subjects with the NQO1 T/T genotype and with the GSTT1 null genotype and the GSTM1 null genotype compared with those carrying the NQO1 C/T and C/C genotype, GSTT1 non-null genotype, and GSTM1 non-null genotype (Chen et al., 2007). However, the 95% confidence interval for the OR of 20.41 was quite large (3.9-111) (Table 6.5), which indicates substantial uncertainty in the estimate of the OR.

GSTM1 null activity is slightly more prevalent in the adult Chinese population than among Caucasians (58% vs 53%), while GSTT1 null activity is nearly three times higher among Chinese than Causasians (57% vs 20%)(Table 6.4).

Some portion of these variations in genotype and enzyme levels, which make a person more sensitive to benzene, is present in the population of Chinese workers studied in Lan et al. (2004), which was used as the basis of the 8-hour and chronic RELs. Although the production of toxic metabolites and rates of detoxification are both involved in toxicity, the observation that CYP2E1 levels are up to 10-fold lower in the third trimester fetus relative to the adult liver (Table 7.1) provides some support for a 10-fold variability in toxicokinetic uncertainty among humans.

These variations in genotype and enzyme levels, which make a person more or less sensitive to benzene, were further studied by Kim, Rappaport and others. Rappaport presented supplemental material (Rappaport et al., 2013) in which they showed results of a reanalysis of the original studies (Kim et al., 2006b) correcting for sampling variability in the calibration model. Variation in the amounts of different metabolites of benzene was calculated at various exposure levels; those in the low dose range are the most relevant for consideration of REL development. Percentiles of these modeled values for a 30 ppb (98 $\mu g/m^3$) benzene exposure are shown in Table 8.6. The results of this model indicate about a 5 fold variation in production of total metabolites at a 30 ppb exposure in humans from the 10^{th} to the 90^{th} percentile. The ratio of the 90^{th} percentile of the toxic hydroquinone metabolite to the median is around 3.5. These results are consistent with a toxicokinetic variability factor of roughly 10.

Table 8.6 Percentiles of the predicted metabolite levels at 30 ppb benzene using a bootstrap procedure that accounts for sampling variability in the calibration model (Rappaport et al., 2013).

Benzene (ppb)	Metabolite	p10	p25	p50	p75	p90
30	Muconic acid	10.9**	16.2	22.4	28.6	34.7
30	SPMA*	0.0	0.0	0.1	0.1	0.1
30	Phenol	110.1	264.7	412.4	574	724.2
30	Catechol	0.0	0.0	50.2	98.3	132.8
30	Hydroquinone	0.0	0.0	13.3	29.0	47.1
30	Sum*	172.7	337.9	510.1	705.1	848.7

* SPMA, S-phenylmercapturic acid; sum, sum of predictions from the 5 models of individual metabolites

** µmol/L in urine

In a modeling study (Bois et al., 1996) based on three research workers in Finland Pekari et al., 1992), interindividual variation in toxicokinetic parameters was decribed by population-based distributions with geometric standard deviations between 1.2 and 1.4. On the other hand, the PBPK model-estimated quantity of benzene metabolized in human bone marrow at 1 ppm benzene continuous exposure ranged from 2 to 40 mg/day, a 20-fold variation (based on ± three standard deviations from the mean). The authors pointed out that this range is mainly reflective of the large model uncertainty because of the limited information on metabolism in human bone marrow, rather than indicating such considerable inter-individual variation in the actual values. The confidence in this result is limited by the small number of subjects and large model uncertainty.

These studies provide some indications of the range of variation in metabolism of benzene, and the relationship between some polymorphisms and odds of chronic benzene poisoning. The overall objective of applying an intraspecies uncertainty factor in risk assessment is to protect subpopulations which are recognized as present to a significant degree in the general population. It has been argued that a suitable value for a variability factor, such as the human toxicokinetic subfactor for intraindividual variability (UF_{H-k}), is the ratio of the (median + 3 SD), approximately the 99th percentile, to the median (Meek et al., 2002). This is distinct from the overall range (from some stated upper to lower confidence interval). On the other hand these distribution-based approaches to defining the uncertainty factor (UF) are hard to apply to situations where there are many factors contributing to human variability in toxicokinetics and toxicity, as is likely for benzene toxicity. Further, due to the complex network of interrelated metabolic pathways, a percentage variation in a particular enzyme level does not automatically translate into a similar difference in the overall level of toxic metabolites.

The studies for which these ranges of variation are reported are in healthy adults (workers or volunteers), so they do not necessarily reflect the variation expected for infants and children. We noted earlier that CYP2E1 levels are up to 10-fold lower in the third trimester fetus relative to an adult (Table 7.1, based on Hines, 2007). Since the levels early in fetal development are essentially zero, the range of variation is very large if the whole gestational period is included, although maternal metabolism is probably the dominant factor in the early part of this period. The detoxifying enzymes also vary; GSTM for example is low early in gestation increasing steadily through the second and third trimesters and postnatally. Such variation in expression of CYP2E1, GSTM and other enzymes involved in benzene metabolism between young and adult lifestages is in addition to that observed as interindividual variability among adults.

Obesity changes the kinetics of benzene metabolism since benzene is fat soluble (Sato et al., 1975). As noted in Section 4.1, there is evidence that clearance of benzene is slower in women than men likely due to a higher body fat content and thus larger volume of distribution. Interestingly, obesity also increases the level of CYP2E1. In overfed, obese male Sprague-Dawley rats (978 ± 233 g), the total P450 content of liver was elevated 88% over non-obese controls (583 ± 83.7 g). Microsomal ethanol oxidation, which is a function of CYP2E1, was 19% per gram of liver and 87% per total

liver higher in obese rats relative to controls (Salazar et al., 1988). Investigators in Tennessee compared nine obese women (119 ± 16 kg) with nine age-matched control women (72 ± 11 kg) and reported a significantly increased clearance of orally-administered chlorzoxazone in the obese women (6.23 ± 1.72 vs. 4.15 ± 0.81 ml/min-kg, normalized by body weight). The absolute mean difference between control and obese was 2.47 fold (O'Shea et al., 1994). Since increased chlorzoxazone metabolism is due in part to CYP2E1 and is associated with increased risk of benzene poisoning (Rothman et al., 1997), obesity may be another risk factor for adverse effects of benzene, and another consideration in the value of the intraspecies uncertainty factor.

The above discussion reflects only consideration of the toxicokinetic variation in the human population. Toxicodynamic variability is addressed below. However, that source of variability is partially included in the overall extent of variation noted in studies (e.g., Chen et al. 2007, shown in Table 8.5) where benzene poisoning was measured as a function of metabolic enzyme genotype.

Most of the available studies suggest that the default value of 10 for $UF_{H\text{-}k}$ covers the expected variability in metabolic capabilities. Although compared to the California population there is incomplete representation of ethnic groups, it is important to note that the population examined in the critical study (Lan et al., 2004) does include representation of the genetic polymorphisms most clearly recognized at this point as affecting metabolic capabilities, and was in fact the basis for one of the studies of diversity in metabolic capability. Therefore the response data reflect at least some, although not all, of the variability in toxicokinetics in the target population for REL derivation. There are still concerns for lack of information about toxicokinetic effects in young humans. Further, the study by Chen et al (2007) suggests a larger than 10 fold variability based on genetic polymorphisms for metabolic enzymes involved in the activation and detoxification of benzene.

The intraspecies $UF_{H\text{-}d}$ (toxicodynamics) is used to account for pharmacodynamic variability among pregnant women and their fetuses and among infants, children, and adults (OEHHA, 2008). During embryonic and fetal development hematopoiesis first occurs in the extraembryonic yolk sac, then in the liver and thymus, and finally in the bone marrow during the second and third trimesters (Peault, 1996). Blood cell type and rates of formation change with age (e.g., Table 7.3) and would be expected to be more dynamic during pre- and post-natal developmental stages than in adults. Further discussion of this subfactor can be found in the acute REL derivation above. However, unlike the acute REL where a sensitive developmental stage was the critical effect, the key study for the chronic REL involved only exposures and effects in adult workers.

In view of the remaining uncertainties with regard to toxicokinetics in infants and children, and the larger variation in response observed by Chen et al. (2007) based on specified metabolic enzyme gene polymorphism interactions, we consider it prudent to assign an overall uncertainty factor for human inter-individual variability of 60, which is twice the default. This UF_H is not further subdivided into $UF_{H\text{-}k}$ and $UF_{H\text{-}d}$ since this assignment is uncertain.

As a comparison chronic REL, OEHHA used the study by Schnatter and colleagues of a different group of Chinese workers exposed to benzene (Schnatter et al., 2010).

Study	Schnatter et al. (2010)
Study population	855 male and female exposed Chinese workers (vs. 73 controls)
Exposure method	Discontinuous occupational exposure
Exposure continuity	8 hr/day (10 m^3 per 20 m^3/day), 6 days/week
Exposure duration	Not specifically stated in text but only 4-9 years for factories D and E
Critical effects	Decreased neutrophils and decreased platelet volume
LOAEL	7.77 ppm (25 mg/m^3)("change point")
NOAEL	Not identifiable
BMCL	Data not available to calculate
Average continuous exposure	3.3 ppm (7.77 ppm x 10 m^3/20 m^3 x 6d/7d)
Human equivalent concentration	*3.3 ppm (10.8 mg/m^3)*
LOAEL uncertainty factor (UF_L)	√10 (change point near threshold)
Subchronic uncertainty factor (UF_S)	√10 (8-≤12% expected lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	60 (see above for key study)
Toxicokinetic (UF_{H-k})	
Toxicodynamic(UF_{H-d})	
Database uncertainty factor	1 (developmental studies are available)
Cumulative uncertainty factor	600
Chronic Reference Exposure Level	**6 ppb (18 µg/m^3)**

The comparison REL is higher than that derived using the key study. OEHHA staff did not consider the study superior to the key study (Lan et al., 2004). Among its specific limitations (1) it used a relatively small number of controls (1 control per ~11 exposed) which decreased its power, (2) length of employment of the workers was not given, and (3) the study did not examine the lymphocyte subtypes, whereas the Lan study did.

The federal Agency for Toxic Substances and Disease Registry (ATSDR) developed a draft Minimal Risk Level (MRL) for chronic (365 days or more) exposure to benzene of 0.003 ppm (10 µg/m^3) based on the Lan *et al*. (2004) study (ATSDR, 2005; Wohlers et al., 2006; Wilbur et al., 2008). ATSDR also used the data on B cells (see Table 3 above) in the continuous Hill model. However, they defined the BMR as 0.25 standard deviation from the mean, and got a BMC of 0.42 ppm and a BMCL of 0.10 ppm.

In 2003, USEPA derived a Reference Concentration (RfC) for benzene of 10 ppb (30 μg/m^3) using the decreased lymphocyte counts in the Rothman *et al*. (1996) study (see Table 6.1), a benchmark dose approach, and their judgment of appropriate uncertainty factors (a factor of 10 for use of a LOAEL due to lack of an appropriate NOAEL, a factor of 10 for intraspecies variability, a factor of 3 for subchronic-to-chronic extrapolation, and a factor of 3 for database deficiencies, especially the lack of two-generation reproductive and developmental toxicity studies for benzene) (IRIS, 2007). This RfC was derived prior to the publication of Lan et al. (2004).

8.4 Data Strengths and Limitations for Development of the RELs

Both the animal and human databases for benzene are extensive compared to many other chemicals in commerce. However, a major data gap is the absence of health effects data in infants and children and in young animals. The hematopoietic system of a growing child must not only keep up with blood cell turnover but must also supply extra cells for a growing body. Rapidly dividing cells are more prone to mutation and other cellular changes resulting from chemical exposure. Thus infants and children may be more sensitive to benzene and its metabolites.

A data gap of a specific test is that of a two-generation reproductive study (Chapin and Sloane, 1997). USEPA considered that this gap warranted an additional “database” uncertainty factor in its benzene RfC derivation. Due to the otherwise extensive animal and human data available for benzene, including developmental studies in animals, we did not apply such a factor. OEHHA’s acute REL is based on a developmental study in mice.

8.5 Benzene as a Toxic Air Contaminant Especially Affecting Infants and Children

Under Health and Safety code Section 39669.5, OEHHA establishes and maintains a list of Toxic Air Contaminants (TACs) that may disproportionately impact infants and children. OEHHA evaluates TACs for addition to this list as we develop Reference Exposure Levels for TACs. In view of the wide-spread exposure to benzene, the documented toxicokinetic variability in benzene metabolism, the transplacental genotoxicity and developmental toxicity of benzene, the documented increased sensitivity of early in life exposure to benzene carcinogenicity in animals, as well as the dynamic hematopoiesis that occurs during human development, there is valid concern that benzene exposure may disproportionately impact infants and children.

In addition to the concern regarding increased early-in-life exposure resulting in noncancer effects described in this document, increased sensitivity of the young to the carcinogenic effects of benzene is also a major concern and is described in this section as well. Benzene causes leukemia in exposed workers, including acute myeloid leukemia(AML) and acute non-lymphocytic leukemia. A positive association has also

been found between benzene exposure in workers and acute lymphocytic leukemia, chronic lymphocytic leukemia, multiple myeloma, and non-Hodgkin lymphoma. A recent report on petroleum workers found that cumulative benzene exposure showed a monotonic dose-response relationship with myelodysplastic syndrome, considered a type of cancer by IARC (Schnatter et al., 2012).

Acute lymphoblastic leukemia is the most common childhood cancer (~80%); other types of leukemia including AML also occur in children (IARC, 2012). Some epidemiological studies have reported statistically significant associations of increases in childhood leukemia, especially acute non-lymphocytic leukemia, with maternal exposures during pregnancy or paternal exposures prior to conception to benzene or benzene-containing mixtures (Shu et al., 1988; Buckley et al., 1989; McKinney et al., 1991). These findings are consistent with evidence in animals that exposure to benzene (1) induced transplacental genotoxicity (Ning et al., 1991), (2) altered hematopoiesis transplacentally (Keller and Snyder, 1988), (3) increased the frequency of micronuclei and DNA recombination events in haematopoietic tissue of fetal and post-natal mice (Lau et al., 2009), and (4) induced transplacental carcinogenicity (Badham et al., 2010), and with evidence that (5) Chinese workers incur chromosomal damage in their sperm at exposures < 1 ppm benzene (Xing et al., 2010; Marchetti et al., 2012). However, other epidemiological studies did not find an association between occupational paternal exposure to benzene and childhood leukemias.

In animals, exposures to benzene early in life and through adulthood resulted in a 2-fold higher increase in the incidences of cancer compared to exposures only as adults (Maltoni et al., 1989).

In an in vitro cell-based assay, the cytotoxic and apoptotic effects of hydroquinone were more apparent and the reduction in colony formation was more severe in embryonic hematopoietic stem cells (HSC) than in adult HSCs (Zhu et al., 2013).

Several studies, summarized in Section 7, suggest an association of exposure to benzene and adverse developmental outcomes in humans (Slama et al., 2009; Lupo et al., 2011; Zahran et al., 2012). Benzene is listed under California's Proposition 65 as a chemical known to the state to cause male developmental toxicity.

OEHHA recommends that benzene be identified as a toxic air contaminant which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

9 References

Aksoy M, Dincol K, Erdem S, Akgun T and Dincol G (1972). Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. Br J Ind Med 29(1): 56-64.

Amigou A, Sermage-Faure C, Orsi L, Leverger G, Baruchel A, Bertrand Y, Nelken B, Robert A, Michel G, Margueritte G, Perel Y, Mechinaud F, Bordigoni P, Hemon D and Clavel J (2011). Road traffic and childhood leukemia: the ESCALE study (SFCE). Environ Health Perspect 119(4): 566-72.

Aoyama K (1986). Effects of benzene inhalation on lymphocyte subpopulations and immune response in mice. Toxicol Appl Pharmacol 85(1): 92-101.

ATSDR. (2005). Toxicological Profile for Benzene (update). Draft for Public Comment. Agency for Toxic Substances and Disease Registry, U.S. Public Health Service.

ATSDR. (2007). *Toxicological Profile for Benzene*. Agency for Toxic Substances and Disease Registry.

Avis SP and Hutton CJ (1993). Acute benzene poisoning: a report of three fatalities. J Forensic Sci 38(3): 599-602.

Baarson KA, Snyder CA and Albert RE (1984). Repeated exposure of C57Bl mice to inhaled benzene at 10 ppm markedly depressed erythropoietic colony formation. Toxicol Lett 20(3): 337-42.

Baarson KA, Snyder CA, Green JD, Sellakumar A, Goldstein BD and Albert RE (1982). The hematotoxic effects of inhaled benzene on peripheral blood, bone marrow, and spleen cells are increased by ingested ethanol. Toxicol Appl Pharmacol 64(3): 393-404.

Badham HJ, LeBrun DP, Rutter A and Winn LM (2010). Transplacental benzene exposure increases tumor incidence in mouse offspring: possible role of fetal benzene metabolism. Carcinogenesis 31(6): 1142-8.

Balboa B. (2011). US benzene production rises 19% in 2010 - NPRA. ICIS, from http://www.icis.com/Articles/2011/02/25/9439086/us-benzene-production-rises-19-in-2010-npra.html.

Barbera N, Bulla G and Romano G (1998). A fatal case of benzene poisoning. J Forensic Sci 43(6): 1250-1.

Baslo A and Aksoy M (1982). Neurological abnormalities in chronic benzene poisoning. A study of six patients with aplastic anemia and two with preleukemia. Environ Res 27(2): 457-65.

Bauer AK, Faiola B, Abernethy DJ, Marchan R, Pluta LJ, Wong VA, Gonzalez FJ, Butterworth BE, Borghoff SJ, Everitt JI and Recio L (2003). Male mice deficient in microsomal epoxide hydrolase are not susceptible to benzene-induced toxicity. Toxicol Sci 72(2): 201-9.

Bois FY, Jackson ET, Pekari K and Smith MT (1996). Population toxicokinetics of benzene. Environ Health Perspect 104 Suppl 6: 1405-11.

Bolcsak LE and Nerland DE (1983). Inhibition of erythropoeisis by benzene and benzene metabolites. Toxicol Appl Pharmacol 69: 363-8.

Bratteby LE (1968). Studies on erythro-kinetics in infancy. X. Red cell volume of newborn infants in relation to gestational age. Acta Paediatr Scand 57: 132-6.

Bratteby LE, Garby L, Groth T, Schneider W and Wadman B (1968). Studies on erythro-kinetics in infancy. XIII. The mean life span and the life span frequency function of red blood cells formed during fetal life. Acta Paediatr Scand 57: 311-20.

Bratton SB, Lau SS and Monks TJ (1997). Identification of quinol thioethers in bone marrow of hydroquinone/phenol-treated rats and mice and their potential role in benzene-mediated hematotoxicity. Chem Res Toxicol 10(8): 859-65.

Brosselin P, Rudant J, Orsi L, Leverger G, Baruchel A, Bertrand Y, Nelken B, Robert A, Michel G, Margueritte G, Perel Y, Mechinaud F, Bordigoni P, Hemon D and Clavel J (2009). Childhood acute leukemia and residence next to gas stations and automotive repair garages: the ESCALE study (SFCE*). Occup Environ Med 66(9): 598-606.

Brown EA, Shelley ML and Fisher JW (1998). A pharmacokinetic study of occupational and environmental benzene exposure with regard to gender. Risk Anal 18(2): 205-13.

Brugnara C and Platt OS (2003). Chapter 2. The neonatal erythrocyte and its disorders. In: Nathan and Oski's Hematology of Infancy and Childhood. Nathan D. G., Orkin S. H., Ginsburg D. and Look A. T. Saunders. Philadelphia: 1: 19-55.

Buckley JD, Robison LL, Swotinsky R, Garabrant DH, LeBeau M, Manchester P, Nesbit ME, Odom L, Peters JM, Woods WG and et al. (1989). Occupational exposures of parents of children with acute nonlymphocytic leukemia: a report from the Childrens Cancer Study Group. Cancer Res 49(14): 4030-7.

CARB. (2004). ARB Almanac 2004. p. 217. Retrieved Jan. 13, 2006, from http://www.arb.ca.gov/aqd/almanac/almanac04/pdf/chap504.pdf

CARB. (2008). *California 2008 Toxics Inventory.* . http://www.arb.ca.gov/toxics/cti/cti.htm.

CARB. (2009). *ARB Almanac 2009*. http://www.arb.ca.gov/aqd/almanac/almanac09/almanac09.htm.

Chapin RE and Sloane RA (1997). Reproductive assessment by continuous breeding: evolving study design and summaries of ninety studies. Environ Health Perspect 105 Suppl 1: 199-205.

Charbord P, Tavian M, Humeau L and Peault B (1996). Early ontogeny of the human marrow from long bones: an immunohistochemical study of hematopoiesis and its microenvironment. Blood 87(10): 4109-19.

Chen Y, Li G, Yin S, Xu J, Ji Z, Xiu X, Liu L and Ma D (2007). Genetic polymorphisms involved in toxicant-metabolizing enzymes and the risk of chronic benzene poisoning in Chinese occupationally exposed populations. Xenobiotica; the fate of foreign compounds in biological systems 37(1): 103-12.

Coate W, Hoberman A and Durloo R (1984). Inhalation teratology study of benzene in rats. In: Advances in Modern Environmental Toxicology, Vol. VI. Applied Toxicology of Petroleum Hydrocarbons. . MacFarland H. Princeton Scientific Publishers, Inc. Princeton: 187-198.

Cody RP, Strawderman WW and Kipen HM (1993). Hematologic effects of benzene. Job-specific trends during the first year of employment among a cohort of benzene-exposed rubber workers. J Occup Med 35(8): 776-82.

Cole CE, Tran HT and Schlosser PM (2001). Physiologically based pharmacokinetic modeling of benzene metabolism in mice through extrapolation from in vitro to in vivo. J Toxicol Environ Health A 62(6): 439-65.

Collins JJ, Ireland BK, Easterday PA, Nair RS and Braun J (1997). Evaluation of lymphopenia among workers with low-level benzene exposure and the utility of routine data collection. J Occup Environ Med 39(3): 232-7.

Cronkite EP, Drew RT, Inoue T and Bullis JE (1985). Benzene hematotoxicity and leukemogenesis. Am J Ind Med 7(5-6): 447-56.

Cronkite EP, Drew RT, Inoue T, Hirabayashi Y and Bullis JE (1989). Hematotoxicity and carcinogenicity of inhaled benzene. Environ Health Perspect 82: 97-108.

Cronkite EP, Inoue T, Carsten AL, Miller ME, Bullis JE and Drew RT (1982). Effects of benzene inhalation on murine pluripotent stem cells. J Toxicol Environ Health 9(3): 411-21.

Crump K and Allen B. (1984). Quantitative estimates of risk of leukemia from occupational exposure to benzene. Occupational Safety and Health Administration.

D'Andrea MA and Reddy GK (2013). Health Effects of Benzene Exposure among Children Following a Flaring Incident at the British Petroleum Refinery in Texas City. Pediatr Hematol Oncol.

Daiker DH, Shipp BK, Schoenfeld HA, Klimpel GR, Witz G, Moslen MT and Ward JB, Jr. (2000). Effect of CYP2E1 induction by ethanol on the immunotoxicity and genotoxicity of extended low-level benzene exposure. J Toxicol Environ Health A 59(3): 181-96.

Davis A, Gift JS, Woodall GM, Narotsky MG and Foureman GL (2009). The role of developmental toxicity studies in acute exposure assessments: analysis of single-day vs. multiple-day exposure regimens. Regul Toxicol Pharmacol 54(2): 134-42.

Degowin RL (1963). Benzene exposure and aplastic anemia followed by leukemia 15 years later. JAMA 185: 748-51.

Deichmann WB, Macdonald WE and Bernal E (1963). The hemopoietic tissue toxicity of benzene vapors. Toxicol Appl Pharmacol 5: 201-24.

Dosemeci M, Li GL, Hayes RB, Yin SN, Linet M, Chow WH, Wang YZ, Jiang ZL, Dai TR, Zhang WU and et al. (1994). Cohort study among workers exposed to benzene in China: II. Exposure assessment. Am J Ind Med 26(3): 401-11.

Dowty BJ, Laseter JL and Storer J (1976). The transplacental migration and accumulation in blood of volatile organic constituents. Pediatr Res 10(7): 696-701.

Drew RT and Fouts JR (1974). The lack of effects of pretreatment with phenobarbital and chlorpromazine on the acute toxicity of benzene in rats. Toxicol Appl Pharmacol 27(1): 183-93.

Edenberg HJ (2000). Regulation of the mammalian alcohol dehydrogenase genes. Prog Nucleic Acid Res Mol Biol 64: 295-341.

Erexson GL, Wilmer JL, Steinhagen WH and Kligerman AD (1986). Induction of cytogenetic damage in rodents after short-term inhalation of benzene. Environ Mutagen 8(1): 29-40.

Evans HL, Dempster AM and Snyder CA (1981). Behavioral changes in mice following benzene inhalation. Neurobehav Toxicol Teratol 3(4): 481-5.

Farris GM, Robinson SN, Gaido KW, Wong BA, Wong VA, Hahn WP and Shah RS (1997). Benzene-induced hematotoxicity and bone marrow compensation in B6C3F1 mice. Fundam Appl Toxicol 36(2): 119-29.

Gasiewicz TA, Singh KP and Casado FL (2010). The aryl hydrocarbon receptor has an important role in the regulation of hematopoiesis: implications for benzene-induced hematopoietic toxicity. Chem Biol Interact 184(1-2): 246-51.

Ghantous H and Danielsson BR (1986). Placental transfer and distribution of toluene, xylene and benzene, and their metabolites during gestation in mice. Biol Res Pregnancy Perinatol 7(3): 98-105.

Ghosh JKC, Wilhelm M, Su j, Goldberg D, Cockburn M, Jerrett M and Ritz M (2012). Assessing the influence of traffic-related air pollution on risk of term low birth weight on the basis of land-use-based regression models and measures of air toxics. Am J Epidemiol 175(12): 1262-74.

Ginsberg G, Smolenski S, Hattis D, Guyton KZ, Johns DO and Sonawane B (2009). Genetic Polymorphism in Glutathione Transferases (GST): Population distribution of GSTM1, T1, and P1 conjugating activity. J Toxicol Environ Health B Crit Rev 12(5-6): 389-439.

Goldstein BD and Witz G (2009). Benzene. In: Environmental Toxicants: Human Exposures and Their Health Effects. 3rd ed. Lippman M. Wiley. New York.

Grant W (1986). Toxicology of the Eye. Springfield (IL) CC Thomas.

Green JD, Snyder CA, LoBue J, Goldstein BD and Albert RE (1981). Acute and chronic dose/response effect of benzene inhalation on the peripheral blood, bone marrow, and spleen cells of CD-1 male mice. Toxicol Appl Pharmacol 59(2): 204-14.

Greenlee WF, Sun JD and Bus JS (1981). A proposed mechanism of benzene toxicity: formation of reactive intermediates from polyphenol metabolites. Toxicol Appl Pharmacol 59(2): 187-95.

Haley TJ (1977). Evaluation of the health effects of benzene inhalation. Clin Toxicol 11(5): 531-48.

Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, Lister TA and Bloomfield CD (1999). World Health Organization classification of neoplastic diseases of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee meeting-Airlie House, Virginia, November 1997. J Clin Oncol 17(12): 3835-49.

Hayes RB, Yin SN, Dosemeci M, Li GL, Wacholder S, Chow WH, Rothman N, Wang YZ, Dai TR, Chao XJ, Jiang ZL, Ye PZ, Zhao HB, Kou QR, Zhang WY, Meng JF, Zho JS, Lin XF, Ding CY, Li CY, Zhang ZN, Li DG, Travis LB, Blot WJ and Linet MS (1996). Mortality among benzene-exposed workers in China. Environ Health Perspect 104 Suppl 6: 1349-52.

Heck JE, Park AS, Qiu J, Cockburn M and Ritz B (2013). Risk of leukemia in relation to exposure to ambient air toxics in pregnancy and early childhood. Int J Hyg Environ Health.

Hines RN (2007). Ontogeny of human hepatic cytochromes P450. J Biochem Mol Toxicol 21(4): 169-75.

Hines RN (2008). The ontogeny of drug metabolism enzymes and implications for adverse drug events. Pharmacol Ther 118(2): 250-67.

Hosgood HD, 3rd, Zhang L, Shen M, Berndt SI, Vermeulen R, Li G, Yin S, Yeager M, Yuenger J, Rothman N, Chanock S, Smith M and Lan Q (2009). Association between genetic variants in VEGF, ERCC3 and occupational benzene haematotoxicity. Occup Environ Med 66(12): 848-53.

HSDB (2007). Benzene. Available on-line at http://toxnet.nlm.nih.gov.

Hudson G (1965). Bone-Marrow Volume in the Human Foetus and Newborn. Br J Haematol 11: 446-52.

IARC (1982). IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Some Industrial Chemicals and Dyestuffs. Vol. 29. Lyon: International Agency for Research on Cancer.

IARC (2012). IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Chemical Agents and Related Occupations. Vol. 100F. Lyon: International Agency for Research on Cancer.

IRIS. (2007). Benzene. Retrieved Aug. 14, 2007, from http://www.epa.gov/iris/subst/0276.htm.

Johnsrud EK, Koukouritaki SB, Divakaran K, Brunengraber LL, Hines RN and McCarver DG (2003). Human hepatic CYP2E1 expression during development. J Pharmacol Exp Ther 307(1): 402-7.

Kang SK, Lee MY, Kim TK, Lee JO and Ahn YS (2005). Occupational exposure to benzene in South Korea. Chem Biol Interact 153-154: 65-74.

Keller KA and Snyder CA (1986). Mice exposed in utero to low concentrations of benzene exhibit enduring changes in their colony forming hematopoietic cells. Toxicology 42(2-3): 171-81.

Keller KA and Snyder CA (1988). Mice exposed in utero to 20 ppm benzene exhibit altered numbers of recognizable hematopoietic cells up to seven weeks after exposure. Fundam Appl Toxicol 10(2): 224-32.

Kelsey KT, Ross D, Traver RD, Christiani DC, Zuo ZF, Spitz MR, Wang M, Xu X, Lee BK, Schwartz BS and Wiencke JK (1997). Ethnic variation in the prevalence of a common NAD(P)H quinone oxidoreductase polymorphism and its implications for anti-cancer chemotherapy. Br J Cancer 76(7): 852-4.

Khuder SA, Youngdale MC, Bisesi MS and Schaub EA (1999). Assessment of complete blood count variations among workers exposed to low levels of benzene. J Occup Environ Med 41(9): 821-6.

Kiffmeyer WR, Langer E, Davies SM, Envall J, Robison LL and Ross JA (2004). Genetic polymorphisms in the Hmong population: implications for cancer etiology and survival. Cancer 100(2): 411-7.

Kim S, Lan Q, Waidyanatha S, Chanock S, Johnson BA, Vermeulen R, Smith MT, Zhang L, Li G, Shen M, Yin S, Rothman N and Rappaport SM (2007). Genetic polymorphisms and benzene metabolism in humans exposed to a wide range of air concentrations. Pharmacogenet Genomics 17(10): 789-801.

Kim S, Vermeulen R, Waidyanatha S, Johnson BA, Lan Q, Rothman N, Smith MT, Zhang L, Li G, Shen M, Yin S and Rappaport SM (2006a). Using urinary biomarkers to elucidate dose-related patterns of human benzene metabolism. Carcinogenesis 27(4): 772-81.

Kim S, Vermeulen R, Waidyanatha S, Johnson BA, Lan Q, Smith MT, Zhang L, Li G, Shen M, Yin S, Rothman N and Rappaport SM (2006b). Modeling human metabolism of benzene following occupational and environmental exposures. Cancer Epidemiol Biomarkers Prev 15(11): 2246-52.

Kimura ET, Ebert DM and Dodge PW (1971). Acute toxicity and limits of solvent residue for sixteen organic solvents. Toxicol Appl Pharmacol 19(4): 699-704.

Kipen HM, Cody RP, Crump KS, Allen BC and Goldstein BD (1988). Hematologic effects of benzene: a thirty-five year longitudinal study of rubber workers. Toxicol Ind Health 4(4): 411-30.

Knutsen JS, Kerger BD, Finley B and Paustenbach DJ (2013). A calibrated human PBPK model for benzene inhalation with urinary bladder and bone marrow compartments. Risk Anal 33(7): 1237-51.

Kuna R, Nicolich M, Schroeder R and Rusch G (1992). A female rat fertility study with inhaled benzene. J Am Coll Toxicol 11: 275-282.

Kuna RA and Kapp RW, Jr. (1981). The embryotoxic/teratogenic potential of benzene vapor in rats. Toxicol Appl Pharmacol 57(1): 1-7.

Lamm SH and Grunwald HW (2006). Benzene exposure and toxicity. Science 312: 998.

Lan Q, Vermeulen R, Zhang L, Li G, Rosenberg PS, Alter BP, Shen M, Rappaport SM, Weinberg RS, Chanock S, Waidyanatha S, Rabkin C, Hayes RB, Linet M, Kim S, Yin S, Rothman N and Smith MT (2006). Response (to Benzene exposure and toxicity). Science 312: 998-9.

Lan Q, Zhang L, Li G, Vermeulen R, Weinberg RS, Dosemeci M, Rappaport SM, Shen M, Alter BP, Wu Y, Kopp W, Waidyanatha S, Rabkin C, Guo W, Chanock S, Hayes RB, Linet M, Kim S, Yin S, Rothman N and Smith MT (2004). Hematotoxicity in workers exposed to low levels of benzene. Science 306(5702): 1774-6.

Lan Q, Zhang L, Shen M, Jo WJ, Vermeulen R, Li G, Vulpe C, Lim S, Ren X, Rappaport SM, Berndt SI, Yeager M, Yuenger J, Hayes RB, Linet M, Yin S, Chanock S, Smith MT and Rothman N (2009). Large-scale evaluation of candidate genes identifies associations between DNA repair and genomic maintenance and development of benzene hematotoxicity. Carcinogenesis 30(1): 50-8.

Lau A, Belanger CL and Winn LM (2009). In utero and acute exposure to benzene: investigation of DNA double-strand breaks and DNA recombination in mice. Mutat Res 676(1-2): 74-82.

Leakey JEA, Hume R and Burchell B (1987). Development of multiple activities of UDP-glucuronyltransferase in human liver. Biochem J 243(859-61.).

Lieber CS (1997). Cytochrome P-4502E1: its physiological and pathological role. Physiol Rev 77(2): 517-44.

Lindbohm ML, Hemminki K, Bonhomme MG, Anttila A, Rantala K, Heikkila P and Rosenberg MJ (1991). Effects of paternal occupational exposure on spontaneous abortions. Am J Public Health 81(8): 1029-33.

Lindstrom AB, Yeowell-O'Connell K, Waidyanatha S, Golding BT, Tornero-Velez R and Rappaport SM (1997). Measurement of benzene oxide in the blood of rats following administration of benzene. Carcinogenesis 18(8): 1637-41.

Lipscomb JC, Teuschler LK, Swartout J, Popken D, Cox T and Kedderis GL (2003a). The impact of cytochrome P450 2E1-dependent metabolic variance on a risk-relevant pharmacokinetic outcome in humans. Risk Anal 23(6): 1221-38.

Lipscomb JC, Teuschler LK, Swartout JC, Striley CA and Snawder JE (2003b). Variance of microsomal protein and cytochrome P450 2E1 and 3A forms in adult human liver. Toxicol Mech Methods 13(1): 45-51.

Lo Pumo R, Bellia M, Nicosia A, Micale V and Drago F (2006). Long-lasting neurotoxicity of prenatal benzene acute exposure in rats. Toxicology 223(3): 227-34.

Longacre S, Kocsis J and Snyder R (1980). Benzene metabolism and toxicity in CD-1, C57/B6, and DBA/2N mice. In: Microsomes, Drug Oxidations, and Chemical Carcinogenesis. Coon M. Academic. New York: 897-902.

Longacre SL, Kocsis JJ and Snyder R (1981a). Influence of strain differences in mice on the metabolism and toxicity of benzene. Toxicol Appl Pharmacol 60(3): 398-409.

Lupo PJ, Symanski E, Waller DK, Chan W, Langlois PH, Canfield MA and Mitchell LE (2011). Maternal exposure to ambient levels of benzene and neural tube defects among offspring: Texas, 1999-2004. Environ Health Perspect 119(3): 397-402.

Maltoni C, Ciliberti A, Cotti G, Conti B and Belpoggi F (1989). Benzene, an experimental multipotential carcinogen: results of the long-term bioassays performed at the Bologna Institute of Oncology. Environ Health Perspect 82: 109-24.

Marchetti F, Eskenazi B, Weldon RH, Li G, Zhang L, Rappaport SM, Schmid TE, Xing C, Kurtovich E and Wyrobek AJ (2012). Occupational exposure to benzene and chromosomal structural aberrations in the sperm of Chinese men. Environ Health Perspect 120(2): 229-34.

Marchitti SA, Brocker C, Stagos D and Vasiliou V (2008). Non-P450 aldehyde oxidizing enzymes: the aldehyde dehydrogenase superfamily. Expert Opin Drug Metab Toxicol 4(6): 697-720.

Matoth Y, Zaizov R and Varsano I (1971). Postnatal changes in some red cell parameters. Acta Paediatr Scand 60(3): 317-23.

McCarver DG and Hines RN (2002). The ontogeny of human drug metabolizing enzymes: Phase II conjugation enzymes and regulatory mechanisms. J Pharmacol Exp Ther 300: 361-366.

McHale CM, Zhang L, Lan Q, Vermeulen R, Li G, Hubbard AE, Porter KE, Thomas R, Portier CJ, Shen M, Rappaport SM, Yin S, Smith MT and Rothman N (2011). Global gene expression profiling of a population exposed to a range of benzene levels. Environ Health Perspect 119(5): 628-34.

McKinney PA, Alexander FE, Cartwright RA and Parker L (1991). Parental occupations of children with leukaemia in west Cumbria, north Humberside, and Gateshead. BMJ 302(6778): 681-7.

McMahon TF, Medinsky MA and Birnbaum LS (1994). Age-related changes in benzene disposition in male C57BL/6N mice described by a physiologically based pharmacokinetic model. Toxicol Lett 74(3): 241-53.

McNabola A, Broderick B, Johnston P and Gill L (2006). Effects of the smoking ban on benzene and 1,3-butadiene levels in pubs in Dublin. J Environ Sci Health A 41: 799-810.

Medinsky MA, Sabourin PJ, Lucier G, Birnbaum LS and Henderson RF (1989a). A physiological model for simulation of benzene metabolism by rats and mice. Toxicol Appl Pharmacol 99(2): 193-206.

Medinsky MA, Sabourin PJ, Lucier G, Birnbaum LS and Henderson RF (1989b). A toxikinetic model for simulation of benzene metabolism. Exp Pathol 37(1-4): 150-4.

Meek ME, Renwick A, Ohanian E, Dourson M, Lake B, Naumann BD, Vu V and International Programme on Chemical S (2002). Guidelines for application of chemical-specific adjustment factors in dose/concentration-response assessment. Toxicology 181-182: 115-20.

Midzenski MA, McDiarmid MA, Rothman N and Kolodner K (1992). Acute high dose exposure to benzene in shipyard workers. Am J Ind Med 22(4): 553-65.

Murray FJ, John JA, Rampy LW, Kuna RA and Schwetz BA (1979). Embryotoxicity of inhaled benzene in mice and rabbits. Am Ind Hyg Assoc J 40(11): 993-8.

National Academy of Sciences. (2009). *Benzene (CAS Reg. No. 71-43-2): Interim Acute Exposure Guideline Levels (AEGLs)* http://www.epa.gov/oppt/aegl/pubs/benzene_interim_dec_2008_v1.pdf.

Nazaroff WW and Singer BC (2004). Isolation of hazardous air pollutants from environmental tobacco smoke in US residences J Expo Anal Environ Epidemiol 14: S71-7.

Neafsey P, Ginsberg G, Hattis D, Johns DO, Guyton KZ and Sonawane B (2009). Genetic polymorphism in CYP2E1: Population distribution of CYP2E1 activity. J Toxicol Environ Health B Crit Rev 12(5-6): 362-88.

Nebert DW, Roe AL, Vandale SE, Bingham E and Oakley GG (2002). NAD(P)H:quinone oxidoreductase (NQO1) polymorphism, exposure to benzene, and predisposition to disease: a HuGE review. Genet Med 4(2): 62-70.

Nicolaides KH, Thilaganathan B and Mibashan RS (1989). Cordocentesis in the investigation of fetal erythropoiesis. Am J Obstet Gynecol 161(5): 1197-200.

Nielsen GD and Alarie Y (1982). Sensory irritation, pulmonary irritation, and respiratory stimulation by airborne benzene and alkylbenzenes: prediction of safe industrial exposure levels and correlation with their thermodynamic properties. Toxicol Appl Pharmacol 65(3): 459-77.

Ning H, Kado NY, Kuzmicky PA and Hsieh DP (1991). Benzene-induced micronuclei formation in mouse fetal liver blood, peripheral blood, and maternal bone marrow cells. Environ Mol Mutagen 18(1): 1-5.

NIOSH. (1994). *Registry of Toxic Effects of Chemical Substances (RTECS)*.

Nomiyama K and Nomiyama H (1974). Respiratory elimination of organic solvents in man. Benzene, toluene, n-hexane, trichloroethylene, acetone, ethyl acetate and ethyl alcohol. Int Arch Arbeitsmed 32(1): 85-91.

NTP. (1986). *NTP Technical Report on the Toxicology and Carcinogenesis Studies of Benzene (CAS No. 71-43-2) in F344/N Rats and B6C3F1 Mice (Gavage Studies)*.

O'Shea D, Davis SN, Kim RB and Wilkinson GR (1994). Effect of fasting and obesity in humans on the 6-hydroxylation of chlorzoxazone: a putative probe of CYP2E1 activity. Clin Pharmacol Ther 56(4): 359-67.

OEHHA. (1999). *Air Toxics Hot Spots Program Risk Assessment Guidelines. Part I. The Determination of Acute Reference Exposure Levels for Airborne Toxicants*. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

OEHHA. (2000). Air Toxics Hot Spots Program Risk Assessment Guidelines Part III Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

OEHHA (2001) Proposition 65 Maximum Allowable Daily Level (MADL) for Reproductive Toxicity for Benzene. DOI:

OEHHA. (2008). *Air Toxics Hot Spots Risk Assessment Guidelines. Technical Support Document for the Derivation of Noncancer Reference Exposure Levels*. Air Toxicology and Epidemiology Branch, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

Palis J and Segel GB (1998). Developmental biology of erythropoiesis. Blood Rev 12(2): 106-14.

Peault B (1996). Hematopoietic stem cell emergence in embryonic life: developmental hematology revisited. J Hematother 5(4): 369-78.

Pekari K, Vainiotalo S, Heikkila P, Palotie A, Luotamo M and Riihimaki V (1992). Biological monitoring of occupational exposure to low levels of benzene. Scand J Work Environ Health 18(5): 317-22.

Price PS, Rey TD, Fontaine DD and Arnold SM (2012). A reanalysis of the evidence for increased efficiency in benzene metabolism at airborne exposure levels below 3 p.p.m. Carcinogenesis 33(11): 2094-9.

Price PS, Rey TD, Fontaine DD and Arnold SM (2013). Letter to the editor in response to "Low-dose Metabolism of Benzene in Humans: Science and Obfuscation" Rappaport et al. (2013. Carcinogenesis.

Qu Q, Melikian AA, Li G, Shore R, Chen L, Cohen B, Yin S, Kagan MR, Li H, Meng M, Jin X, Winnik W, Li Y, Mu R and Li K (2000). Validation of biomarkers in humans exposed to benzene: urine metabolites. Am J Ind Med 37(5): 522-31.

Qu Q, Shore R, Li G, Jin X, Chen LC, Cohen B, Melikian AA, Eastmond D, Rappaport SM, Yin S, Li H, Waidyanatha S, Li Y, Mu R, Zhang X and Li K (2002). Hematological changes among Chinese workers with a broad range of benzene exposures. Am J Ind Med 42(4): 275-85.

Quitt M, Cassel A, Yoffe A, Anatol AM and Froom P (2004). Autonomous growth of committed hematopoietic progenitors from peripheral blood of workers exposed to low levels of benzene. J Occup Environ Med 46(1): 27-9.

Ramsey JC and Andersen ME (1984). A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. Toxicol Appl Pharmacol 73(1): 159-75.

Rana S and Verma Y (2005). Biochemical toxicity of benzene. J Environ Biol 26(2): 157-168.

Rappaport SM, Kim S, Lan Q, Li G, Vermeulen R, Waidyanatha S, Zhang L, Yin S, Smith MT and Rothman N (2010). Human benzene metabolism following occupational and environmental exposures. Chem Biol Interact 184(1-2): 189-95.

Rappaport SM, Kim S, Lan Q, Vermeulen R, Waidyanatha S, Zhang L, Li G, Yin S, Hayes RB, Rothman N and Smith MT (2009). Evidence that humans metabolize benzene via two pathways. Environ Health Perspect 117(6): 946-52.

Rappaport SM, Kim S, Thomas R, Johnson BA, Bois FY and Kupper LL (2013). Low-dose metabolism of benzene in humans: science and obfuscation. Carcinogenesis 34(1): 2-9.

Rickert DE, Baker TS, Bus JS, Barrow CS and Irons RD (1979). Benzene disposition in the rat after exposure by inhalation. Toxicol Appl Pharmacol 49(3): 417-23.

Rinsky RA, Smith AB, Hornung R, Filloon TG, Young RJ, Okun AH and Landrigan PJ (1987). Benzene and leukemia. An epidemiologic risk assessment. N Engl J Med 316(17): 1044-50.

Rosenthal GJ and Snyder CA (1985). Modulation of the immune response to Listeria monocytogenes by benzene inhalation. Toxicol Appl Pharmacol 80(3): 502-10.

Ross D (2005). Functions and distribution of NQO1 in human bone marrow: potential clues to benzene toxicity. Chem Biol Interact 153-154: 137-46.

Rothman N, Li GL, Dosemeci M, Bechtold WE, Marti GE, Wang YZ, Linet M, Xi LQ, Lu W, Smith MT, Titenko-Holland N, Zhang LP, Blot W, Yin SN and Hayes RB (1996a). Hematotoxicity among Chinese workers heavily exposed to benzene. Am J Ind Med 29(3): 236-46.

Rothman N, Li GL, Dosemeci M, Bechtold WE, Marti GE, Wang YZ, Linet M, Xi LQ, Lu W, Smith MT, Titenko-Holland N, Zhang LP, Blot W, Yin SN and Hayes RB (1996b). Hematotoxocity among Chinese workers heavily exposed to benzene. Am J Ind Med 29(3): 236-46.

Rothman N, Smith MT, Hayes RB, Traver RD, Hoener B, Campleman S, Li GL, Dosemeci M, Linet M, Zhang L, Xi L, Wacholder S, Lu W, Meyer KB, Titenko-Holland N, Stewart JT, Yin S and Ross D (1997). Benzene poisoning, a risk factor for hematological malignancy, is associated with the NQO1 609C-->T mutation and rapid fractional excretion of chlorzoxazone. Cancer Res 57(14): 2839-42.

Rozen MG, Snyder CA and Albert RE (1984). Depressions in B- and T-lymphocyte mitogen-induced blastogenesis in mice exposed to low concentrations of benzene. Toxicol Lett 20(3): 343-9.

Sabourin PJ, Bechtold WE, Birnbaum LS, Lucier G and Henderson RF (1988). Differences in the metabolism and disposition of inhaled [3H]benzene by F344/N rats and B6C3F1 mice. Toxicol Appl Pharmacol 94(1): 128-40.

Sabourin PJ, Bechtold WE, Griffith WC, Birnbaum LS, Lucier G and Henderson RF (1989). Effect of exposure concentration, exposure rate, and route of administration on metabolism of benzene by F344 rats and B6C3F1 mice. Toxicol Appl Pharmacol 99(3): 421-44.

Salazar DE, Sorge CL and Corcoran GB (1988). Obesity as a risk factor for drug-induced organ injury. VI. Increased hepatic P450 concentration and microsomal ethanol

oxidizing activity in the obese overfed rat. Biochem Biophys Res Commun 157(1): 315-20.

Sandmeyer EE (1981a). Benzene. In: Industrial Hygiene and Toxicology. 3rd ed. revised. Clayton G. D. and Clayton F. E. John Wiley and Sons. New York (NY): IIB: 3260-3283.

Sandmeyer EE (1981b). Benzene. In: Industrial Hygiene and Toxicology. 3rd ed. revised. Clayton G. D. and Clayton F. E. John Wiley and Sons. New York (NY): IIB: 3260-3283.

Sato A, Nakajima T, Fujiwara Y and Murayama N (1975). Kinetic studies on sex difference in susceptibility to chronic benzene intoxication--with special reference to body fat content. Br J Ind Med 32(4): 321-8.

Sax SN, Bennett DH, Chillrud SN, Ross J, Kinney PL and Spengler JD (2006). A cancer risk assessment of inner-city teenagers living in New York City and Los Angeles. Environ Health Perspect 114(10): 1558-66.

Schnatter AR, Glass DC, Tang G, Irons RD and Rushton L (2012). Myelodysplastic syndrome and benzene exposure among petroleum workers: an international pooled analysis. J Natl Cancer Inst 104(22): 1724-37.

Schnatter AR, Kerzic PJ, Zhou Y, Chen M, Nicolich MJ, Lavelle K, Armstrong TW, Bird MG, Lin L, Fu H and Irons RD (2010). Peripheral blood effects in benzene-exposed workers. Chem Biol Interact 184(1-2): 174-81.

Seip M (1955). The reticulocyte level, and the erythrocyte production judged from reticulocyte studies, in newborn infants during the first week of life. Acta Paediatr Scand 44(4): 355-69.

Sheets PL, Yost GS and Carlson GP (2004). Benzene metabolism in human lung cell lines BEAS-2B and A549 and cells overexpressing CYP2F1. J Biochem Mol Toxicol 18(2): 92-9.

Shen M, Zhang L, Lee KM, Vermeulen R, Hosgood HD, Li G, Yin S, Rothman N, Chanock S, Smith MT and Lan Q (2011). Polymorphisms in genes involved in innate immunity and susceptibility to benzene-induced hematotoxicity. Exp Mol Med 43(6): 374-8.

Shimada T, Yamazaki H, Mimura M, Inui Y and Guengerich FP (1994). Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs,

carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. J Pharmacol Exp Ther 270(1): 414-23.

Short DM, Lyon R, Watson DG, Barski OA, McGarvie G and Ellis EM (2006). Metabolism of trans, trans-muconaldehyde, a cytotoxic metabolite of benzene, in mouse liver by alcohol dehydrogenase Adh1 and aldehyde reductase AKR1A4. Toxicol Appl Pharmacol 210(1-2): 163-70.

Shu XO, Gao YT, Brinton LA, Linet MS, Tu JT, Zheng W and Fraumeni JF, Jr. (1988). A population-based case-control study of childhood leukemia in Shanghai. Cancer 62(3): 635-44.

Slama R, Thiebaugeorges O, Goua V, Aussel L, Sacco P, Bohet A, Forhan A, Ducot B, Annesi-Maesano I, Heinrich J, Magnin G, Schweitzer M, Kaminski M and Charles MA (2009). Maternal personal exposure to airborne benzene and intrauterine growth. Environ Health Perspect 117(8): 1313-21.

Smith MT, Yager JW, Steinmetz KL and Eastmond DA (1989). Peroxidase-dependent metabolism of benzene's phenolic metabolites and its potential role in benzene toxicity and carcinogenicity. Environ Health Perspect 82: 23-9.

Snawder JE and Lipscomb JC (2000). Interindividual variance of cytochrome P450 forms in human hepatic microsomes: correlation of individual forms with xenobiotic metabolism and implications in risk assessment. Regul Toxicol Pharmacol 32(2): 200-9.

Snyder CA (1987). Benzene. In: Ethyl Browning’s Toxicity and Metabolism of Industrial Solvents. Snyder R. Elsevier. Amsterdam: 3-37.

Snyder CA, Goldstein BD, Sellakumar A, Wolman SR, Bromberg I, Erlichman MN and Laskin S (1978). Hematotoxicity of inhaled benzene to Sprague-Dawley rats and AKR mice at 300 ppm. J Toxicol Environ Health 4(4): 605-18.

Snyder R, Dimitriadis E, Guy R, Hu P, Cooper K, Bauer H, Witz G and Goldstein BD (1989). Studies on the mechanism of benzene toxicity. Environ Health Perspect 82: 31-5.

Srbova J, Teisinger J and Skramovsky S (1950). Absorption and elimination of inhaled benzene in man. Arch Ind Hyg Occup Med 2(1): 1-8.

Steffen C, Auclerc MF, Auvrignon A, Baruchel A, Kebaili K, Lambilliotte A, Leverger G, Sommelet D, Vilmer E, Hemon D and Clavel J (2004). Acute childhood leukaemia and

environmental exposure to potential sources of benzene and other hydrocarbons; a case-control study. Occup Environ Med 61(9): 773-8.

Sun P, Qian J, Zhang ZB, Wan JX, Wu F, Jin XP, Fan WW, Lu DR, Zhao NQ, Christiani DC and Xia ZL (2008). Polymorphisms in phase I and phase II metabolism genes and risk of chronic benzene poisoning in a Chinese occupational population. Carcinogenesis 29(12): 2325-9.

Swaen GM, van Amelsvoort L, Twisk JJ, Verstraeten E, Slootweg R, Collins JJ and Burns CJ (2010). Low level occupational benzene exposure and hematological parameters. Chem Biol Interact 184(1-2): 94-100.

Tatrai E, Ungvary G, Hudak A, Rodics K, Lorincz M and Barcza G (1980). Concentration dependence of the embryotoxic effects of benzene inhalation in CFY rats. J Hyg Epidemiol Microbiol Immunol 24(3): 363-71.

Teisinger J, Bergerova-Fiserova V and Kudrna J (1952). The metabolism of benzene in man. Pracov Lek 4: 175-178.

Travis CC, Quillen JL and Arms AD (1990). Pharmacokinetics of benzene. Toxicol Appl Pharmacol 102(3): 400-20.

Tsai SP, Fox EE, Ransdell JD, Wendt JK, Waddell LC and Donnelly RP (2004). A hematology surveillance study of petrochemical workers exposed to benzene. Regul Toxicol Pharmacol 40(1): 67-73.

Tsai SP, Wen CP, Weiss NS, Wong O, McClellan WA and Gibson RL (1983). Retrospective mortality and medical surveillance studies of workers in benzene areas of refineries. J Occup Med 25(9): 685-92.

Ungvary G and Tatrai E (1985). On the embryotoxic effects of benzene and its alkyl derivatives in mice, rats and rabbits. Arch Toxicol Suppl 8: 425-30.

USEPA. (2002). *Toxicological review of benzene (noncancer effects). EPA/635/R-02/001F* United States Environmental Protection Agency.

Valentine JL, Lee SS, Seaton MJ, Asgharian B, Farris G, Corton JC, Gonzalez FJ and Medinsky MA (1996). Reduction of benzene metabolism and toxicity in mice that lack CYP2E1 expression. Toxicol Appl Pharmacol 141(1): 205-13.

Vieira I, Sonnier M and Cresteil T (1996). Developmental expression of CYP2E1 in the human liver. Hypermethylation control of gene expression during the neonatal period. Eur J Biochem 238(2): 476-83.

Waidyanatha S, Rothman N, Li G, Smith MT, Yin S and Rappaport SM (2004). Rapid determination of six urinary benzene metabolites in occupationally exposed and unexposed subjects. Anal Biochem 327(2): 184-99.

Wang L, He X, Bi Y and Ma Q (2012). Stem cell and benzene-induced malignancy and hematotoxicity. Chem Res Toxicol 25(7): 1303-15.

Ward CO, Kuna RA, Snyder NK, Alsaker RD, Coate WB and Craig PH (1985). Subchronic inhalation toxicity of benzene in rats and mice. Am J Ind Med 7(5-6): 457-73.

Ward E, Hornung R, Morris J, Rinsky R, Wild D, Halperin W and Guthrie W (1996). Risk of low red or white blood cell count related to estimated benzene exposure in a rubberworker cohort (1940-1975). Am J Ind Med 29(3): 247-57.

Watanabe KH, Bois FY, Daisey JM, Auslander DM and Spear RC (1994). Benzene toxicokinetics in humans: exposure of bone marrow to metabolites. Occup Environ Med 51(6): 414-20.

Weisel CP, Park S, Pyo H, Mohan K and Witz G (2003). Use of stable isotopically labeled benzene to evaluate environmental exposures. J Expo Anal Environ Epidemiol 13(5): 393-402.

Whitworth KW, Symanski E and Coker AL (2008). Childhood lymphohematopoietic cancer incidence and hazardous air pollutants in southeast Texas, 1995-2004. Environ Health Perspect 116(11): 1576-80.

Whysner J, Reddy MV, Ross PM, Mohan M and Lax EA (2004). Genotoxicity of benzene and its metabolites. Mutat Res 566(2): 99-130.

Wigzell H (1988). Immunopathogenesis of HIV infection. J Acquir Immune Defic Syndr 1(6): 559-65.

Wilbur S, Wohlers D, Paikoff S, Keith LS and Faroon O (2008). ATSDR evaluation of health effects of benzene and relevance to public health. Toxicol Ind Health 24(5-6): 263-398.

Winek CL and Collom WD (1971). Benzene and toluene fatalities. J Occup Med 13(5): 259-61.

Wohlers D, Stickney J and Wilbur S (2006). New epidemiological data support the derivation of a chronic-duration inhalation Minimal Risk Level (MRL) for benzene based on Benchmark Dose (BMD) analysis [abstract # 2306]. The Toxicologist 90(1): 471.

Wolf MA, Rowe VK, McCollister DD, Hollingsworth RL and Oyen F (1956). Toxicological studies of certain alkylated benzenes and benzene; experiments on laboratory animals. AMA Arch Ind Health 14(4): 387-98.

World Health Organization (1993). Benzene. Environmental Health Criteria 150.

Wu XM, Apte MG, Maddalena R and Bennett DH (2011). Volatile organic compounds in small- and medium-sized commercial buildings in California. Environ Sci Technol 45(20): 9075-83.

Xing C, Marchetti F, Li G, Weldon RH, Kurtovich E, Young S, Schmid TE, Zhang L, Rappaport S, Waidyanatha S, Wyrobek AJ and Eskenazi B (2010). Benzene exposure near the U.S. permissible limit is associated with sperm aneuploidy. Environ Health Perspect 118(6): 833-9.

Yin SN, Linet MS, Hayes RB, Li GL, Dosemeci M, Wang YZ, Chow WH, Jiang ZL, Wacholder S, Zhang WU and et al. (1994). Cohort study among workers exposed to benzene in China: I. General methods and resources. Am J Ind Med 26(3): 383-400.

Yokley K, Tran HT, Pekari K, Rappaport S, Riihimaki V, Rothman N, Waidyanatha S and Schlosser PM (2006). Physiologically-based pharmacokinetic modeling of benzene in humans: a Bayesian approach. Risk Anal 26(4): 925-43.

Yoon BI, Hirabayashi Y, Kawasaki Y, Kodama Y, Kaneko T, Kanno J, Kim DY, Fujii-Kuriyama Y and Inoue T (2002). Aryl hydrocarbon receptor mediates benzene-induced hematotoxicity. Toxicol Sci 70(1): 150-6.

Yoon BI, Li GX, Kitada K, Kawasaki Y, Igarashi K, Kodama Y, Inoue T, Kobayashi K, Kanno J, Kim DY and Hirabayashi Y (2003). Mechanisms of benzene-induced hematotoxicity and leukemogenicity: cDNA microarray analyses using mouse bone marrow tissue. Environ Health Perspect 111(11): 1411-20.

Zahran S, Weiler S, Mielke HW and Pena AA (2012). Maternal benzene exposure and low birth weight risk in the United States: A natural experiment in gasoline reformulation. Environ Res 112: 139-146.

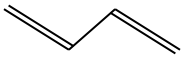
Zaizov R and Matoth Y (1976). Red cell values on the first postnatal day during the last 16 weeks of gestation. Am J Hematol 1(2): 275-8.

Zhu J, Wang H, Yang S, Guo L, Li Z, Wang W, Wang S, Huang W, Wang L, Yang T, Ma Q and Bi Y (2013). Comparison of toxicity of benzene metabolite hydroquinone in hematopoietic stem cells derived from murine embryonic yolk sac and adult bone marrow. PLoS One 8(8): e71153.

1,3-Butadiene Reference Exposure Levels

(butadiene; buta-1,3-diene; biethylene; bivinyl; divinyl; vinylethylene)

CAS 106-99-0



1 Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). In response to this statutory requirement OEHHA developed a Technical Support Document (TSD), adopted in December 2008,that describes acute, 8-hour and chronic RELs. The TSD presents methodology that explicitly includes consideration of possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, chapter 731, statutes of 1999, Health and Safety Code Sections 39669.5 et seq.). These guidelines have been used to develop the following RELs for butadiene; this document will be added to Appendix D of the TSD.

Butadiene is a major commodity product of the petrochemical industry. Workers acutely exposed to butadiene during rubber manufacturing experienced irritation of the eyes, nasal passages, throat, and lung. Some workers also experienced coughing, fatigue, and drowsiness. Inhalation of butadiene is mildly narcotic at low concentrations. Exposure to very high concentrations can result in narcosis, respiratory paralysis, and even death. Repeated exposure can damage human sperm cells and increase ovarian atrophy in mice. The studies reviewed for this document include those published through August 2012.

Although butadiene is a carcinogen (IARC Group 1, IARC, 2012), this document does not discuss issues related to the cancer potency factor. That has been previously derived and is available at www.oehha.ca.gov/air/hot_spots/index.html.

1.1 Butadiene Acute REL

Inhalation reference exposure level	**660 µg/m^3 (297 ppb)**
Critical effect(s)	Lowered male fetal weight
Hazard Index target(s)	Development

1.2 Butadiene 8-Hour REL

Inhalation reference exposure level	**9 µg/m^3 (4 ppb)**
Critical effect(s)	Increased incidence of ovarian atrophy in mice
Hazard Index target	Female reproductive system

1.3 Butadiene Chronic REL

Inhalation reference exposure level	**2 µg/m^3 (1 ppb)**
Critical effect	Increased incidence of ovarian atrophy in mice
Hazard Index target	Female reproductive system

2 Physical & Chemical Properties

Description	Colorless gas
Molecular formula	C_4H_6
Molecular weight	54.09 g/mol
Density	0.6211 g/ml at 20 ºC
Boiling point	-4.4 ºC
Melting point	-108.9 ºC
Vapor pressure	2100 mm Hg at 25 ºC
Flashpoint	- 76 ºC
Flammability limits	Extremely flammable
Solubility	Very slightly soluble in water ; soluble in ethanol, ether, acetone, benzene and organic solvents
Odor threshold	0.0014 mg/l; mildly aromatic with slight petroleum odor
Octanol-water partition coefficient	Log Kow = 1.99
Henry's Law constant	0.074 atm m^3/mole (HSDB, 2011)
Conversion factor	1 ppm = 2.21 mg/m^3 at 20 ºC, 1 bar 1 mg/m^3 = 0.445 ppm at 20 ºC, 1 bar

3 Occurrence and Exposure

1,3-Butadiene (butadiene, BD) is a major commodity product of the petrochemical industry. The largest quantities are used in the production of styrene-butadiene rubber copolymers for car and truck tire manufacturing. Butadiene polymers are also used in a variety of other industrial applications, including the production and synthesis of certain latex products, resins, and nitrile rubbers. Most environmental releases of butadiene (78.8%) are associated with non-point or mobile sources such as motor vehicles. About 1.6% of butadiene releases are fugitive or accidental emissions during manufacture,

use, transport, storage, or disposal. The remainder comes from other sources including direct volatilization from gasoline, stack emissions from incinerators, cigarette smoke, and the burning of plastics (U.S. EPA, 2002; HSDB, 2004). Butadiene is a component of gasoline (Kane and Newton, 2010) as well as a product of the combustion of minor gasoline components, olefins and cyclohexane (Zhang et al., 2008). Approximately 26 percent of butadiene emissions in California can be attributed to on-road motor vehicles, with an additional 27 percent attributed to other mobile sources, such as recreational boats, off-road recreational vehicles, and aircraft. Area-wide combustion sources contribute approximately 21 percent and include agricultural waste burning, open burning associated with forest management, and woodstoves and fireplaces. Stationary sources contribute less than one percent of the statewide 1,3-butadiene emissions. The primary stationary sources with reported 1,3-butadiene emissions are petroleum refining, manufacturing of man-made materials, and oil and gas extraction. The primary natural sources of 1,3-butadiene emissions are wildfires. The total statewide emission inventory for butadiene was estimated at 3,754 tons for 2008 (CARB, 2009).

Butadiene is subject to rapid destruction by reactions in the atmosphere in the presence of O_3, OH radical, and NO_x from which a number of reactive electrophiles are formed, including acrolein, formaldehyde, epoxybutene, and other aldehydes (Tuazon et al., 1999; Baker et al., 2005; Doyle et al., 2007). Baker et al. (2005) investigated the formation of butadiene and hydroxyl radical-initiated reaction products using atmospheric pressure ionization mass spectrometry (API-MS) and solid phase extraction followed by gas chromatography. The products from butadiene were identified as 4-hydroxy-2-butenal ($HOCH_2CH=CHCHO$) and its nitro derivative ($HOCH_2CH=CHCH_2ONO_2$) and isomers. The rate constant for the reaction of OH radicals (OH) with 1,3-butadiene was 6.66×10^{-11} $cm^3molecule^{-1}s^{-1}$ and with 4-hydroxy-2-butenal was $(5.7\pm1.4) \times 10^{-11}$ $cm^3molecule^{-1}s^{-1}$ both at 298±2K. The formation yields of acrolein and 4-hydroxy-2-butenal were 58±10% and 25+15-10%, respectively. Hydroxynitrates from the reaction of hydroxyperoxy radicals with NO account for an additional 7-10% of products from butadiene. Acrolein also reacts with OH radicals to form glycolaldehyde ($HOCH_2CHO$) with yields of 25 to 32 percent. For 1,3-butadiene the reaction with OH radicals is the dominant chemical loss process during daytime, with butadiene lifetimes of 1-2 hr at OH concentrations of 2×10^6 molecules/cm^3.

Sexton et al. (2007) investigated the photochemistry of 1,3-butadiene in an outdoor smog chamber. The butadiene atmospheric lifetime and transformation rates were dependent on concentrations of NO, NOx, other organic compounds, sunlight, temperature, and humidity. The results were compared with computer-based simulations of butadiene transformations (Morphecule Mechanism model). The major observed products with daytime BD/NOx were acrolein, formaldehyde, furan, 1,3-butadiene monoxide and an organic nitrate probably peroxyacylnitrate . Other carbonyls found were glyoxal, glycolaldehyde, glycidaldehyde, 3-hydroxy propanaldehyde, hydroxyl acetone, butenedial, and malonaldehyde. The model simulations were representative of the observed results.

Doyle et al. (2004) studied the photochemical degradation of 200 ppb 1,3-butadiene in presence of 50 ppb NO. The initial and major photochemical products were acrolein, acetaldehyde and formaldehyde. They observed that the photoproducts were more

toxic to A549 human lung cells in vitro than butadiene. In a similar study Doyle et al. (2007) showed that BD + NOx + 5 hours light was more toxic to A549 cells than a synthetic mixture of acrolein, formaldehyde and ozone photoproducts or ozone alone. The photoproducts of butadiene were primarily acrolein, acetaldehyde, formaldehyde, furan and ozone. Toxicity was assessed by increased lactate dehydrogenase (LDH) release and increased interleukin-8 (IL-8) and interleukin-6 (IL-6) protein release.

3.1 Ambient Exposures

Despite its rapid removal, butadiene is almost always present at very low concentrations in US cities and large suburban areas.. Elevated concentrations have been measured in the vicinity of heavily trafficked areas, refineries, chemical manufacturing plants, and plastic and rubber factories (ATSDR 1992). Statewide levels throughout California have consistently dropped from an annual average high of 0.41 ppb in 1990 to approximately 0.1 ppb in 2007. Sampling of 18 communities in the San Francisco Bay area showed a maximum butadiene concentration of 0.26 ppb (San Jose). The majority of all samples were below the minimum detection limit (MDL) of 0.05 parts per billion by volume (ppbv) (BAAQMD, 2008). Similar sampling of 10 communities in the Los Angeles air basin showed a maximum butadiene concentration of 0.78 ppb (Compton). The majority of all samples were below the MDL of 0.2 ppbv (SCAQMD, 2008). In general, mean values in southern California are about twice those in the San Francisco Bay area e.g., San Jose, 0.069 ppb vs. Compton, 0.14 ppb.

One recent study measured butadiene concentrations prior to and after the Ireland Public Health Tobacco Act of 2002 ban on smoking in pubs (McNabola et al., 2006). The average concentration of butadiene measured inside pubs prior to the ban was 4.15 $\mu g/m^3$ (± 1.61 $\mu g/m^3$). This is compared to average ambient outside measurement of 0.12 $\mu g/m^3$ (0.05 ppm) and an average indoor measurement of 0.22 $\mu g/m^3$ (0.1 ppm) following the smoking ban. Similar results were obtained in a survey of 10 Finnish restaurants with 4.3 ± 3.2 SD $\mu g/m^3$ (1.9 ppm) butadiene (geometric mean = 2.7, range = 0.26-10.1, N = 20 measurements) in smoking areas compared with 1.1 ± 1.3 SD (0.5 ppm) butadiene (geometric mean = 0.52, range = 0.11-3.9, N =20) in non-smoking areas (Vainiotalo et al., 2008).

The TEACH (Toxic Exposure Assessment, Columbia/Harvard) study characterized personal exposures to urban air toxics among high school students living in inner city neighborhoods of New York City (Kinney et al., 2002). Exposure to air toxics was analyzed in 46 high school students using 48-hr personal monitoring, outdoor ambient monitoring, and in-home ambient monitoring. The students were mainly African American and Hispanic, and were required to be non-smokers from non-smoking families. The subjects ranged in age from 12.5 to 19.6 years old, with 42% male and 58% female. Investigators measured levels of particulates and concentrations of 17 volatile organic compounds (VOCs). Butadiene levels tended to be higher in the home and from personal exposures than what was measured in ambient outdoor air. The mean outdoor concentrations of butadiene were 0.13 $\mu g/m^3$ (winter) and 0.14 $\mu g/m^3$ (summer), versus in-home ambient concentrations of 1.18 $\mu g/m^3$ in winter and 1.01

μg/m^3 in summer. Mean concentrations obtained from personal air monitors were 0.87 μg/m^3 (0.39 ppm) in winter and 1.16 μg/m^3 (0.52 ppm) in summer (Kinney et al., 2002).

Marshall et al. (2006) estimated the intake of butadiene from ambient air pollution in California's South Coast Air Basin. Butadiene concentrations were based on air dispersion modeling for the period April, 1998 to March, 1999. Surveys from 25,064 respondents were used in the study. The 28,746 person-days in the data set are from 11,749 households. Travel survey data were collected for 15-24 week periods in 2000-2002. The method used four main inputs: temporal information about people's position (latitude and longitude); microenvironment, and activity level; temporally and spatially explicit model estimates of ambient butadiene concentrations; and stochastically determined microenvironmental adjustment factors relating the exposure concentration to the ambient concentration, age-, gender-, and activity-specific breathing rates. The calculated average breathing rate (m^3/day-person) for the study was 13.1. The mean intake rate for 1,3-butadiene was 7.3 μg/day ±7.6 SD. This corresponds to an average concentration of 0.56 μg/m^3 (0.24 ppb)(7.3/13.1) or approximately 8% of the chronic REL for butadiene derived in this document.

A qualitatively similar difference between personal/indoor air and outdoor air concentrations was observed in metropolitan Mexico City (Serrano-Trespalacios et al., 2004). Personal exposures to 34 VOCs were measured for adolescents and their families living close to one of five central monitoring stations over the period of one year. Simultaneous 24-hr indoor, outdoor and central site measurements were also taken. Indoor butadiene concentrations (mean = 2.5 μg/m^3, 1.1 ppb) were 2 to 6 times higher than outdoors measurements (mean 0.9 μg/m^3, 0.40 ppb). Personal exposures for all participants including adolescents (mean = 2.9 μg/m^3, 1.3 ppb) were also significantly higher than ambient outdoor concentrations (Serrano-Trespalacios et al., 2004). Logue et al. (2011) identified 1,3-butadiene as one of nine indoor air pollutants categorized as priority hazards based on available acute and chronic health criteria. Two hundred and sixty-seven chemicals were evaluated with health criteria for 97. Butadiene was categorized based on its cancer risk, however the upper bound estimate of exposure (their Fig.2) is close to the U.S. EPA RfC of 0.9 ppb and the chronic REL derived in this document (1.0 ppb).

Gustafson et al. (2007) measured personal exposures and indoor air concentrations of butadiene, benzene, formaldehyde, and acetaldehyde due to wood burning in a small Swedish town. Ambient air concentrations of butadiene for 1 day sampling (N = 9) had a mean of 0.12μg/m^3(0.05 ppb) (95%CI, 0.04-0.16). Indoor personal monitoring in the wood burning group (N = 14) gave mean butadiene concentrations of 0.31 to 0.38 μg/m^3 (0.14 to 0.17 ppb) for 1 to 7 days sampling. The referent group (N=10) had mean values of 0.11 to 0.14 μg/m^3 (0.049 to 0.06 ppb) ($P < 0.015$-0.03). The authors concluded that domestic wood burning increased personal exposure to ambient butadiene as well as indoor exposures to butadiene.

Additional exposure analyses were conducted by Nazaroff and Singer (2004), who studied hazardous air pollutants including butadiene within US residences. The authors found that the population inhalation intake of butadiene was dominated by residential

environmental tobacco smoke (ETS). Mean individual intake of butadiene was also dominated by residential ETS. Data analyses indicated that some 16 million U.S. juveniles (2 months to 16 years old) are exposed to ETS in the home. Assuming between 14-20 cigarettes smoked per day in each residence, with an average of 515 µg butadiene/cigarette, the resulting daily intake of butadiene for juveniles was 16 – 37 µg. The total mean individual inhalation intake for juveniles was estimated to be approximately 10 mg butadiene/year from residential ETS, far exceeding the exposure to butadiene from ambient sources, estimated at approximately 0.3 mg/year (Nazaroff and Singer 2004).

3.2 Worker Exposures

Investigators in the US, Europe, and China report similar average exposures to butadiene in the workplace. However, approaches to exposure assessment vary. Because butadiene monomer production and extraction are generally operated currently as closed processes, exposures tend to be low. With certain tasks, or with accidents and engineering failures, there can be transiently high exposures that are brief and intense. Air measurement may not accurately reflect these peaks. Historical levels of butadiene exposure in the workplace are thought to have been much higher than present values (Lynch 2001). Few data are available for butadiene concentrations prior to the 1970s. Exposure modeling suggests that levels in the 1940s – 1950s were considerably higher, and estimated to be approximately 20 ppm (Lynch et al., 2001). There has been an annual trend of an approximate 6% drop in butadiene concentrations over time, with large decreases in the late 1950s and the early 1980s (Lynch 2001). More current measurements of butadiene in the workplace were provided by Chan et al. (2006), who measured worker exposure to air toxics in an 11-plant petrochemical complex in Taiwan. For the years 1997-1999, only 15.2% of the measurements were above the limit of detection (LOD), with a mean low concentration of 7.7 ppb, a mean high concentration of 10.5 ppb, and a maximum concentration of 3080 ppb butadiene. Specific LODs were not given for the 39 chemicals studied but the mean low concentrations were calculated using 0 for values below the LOD and the mean high concentrations were estimated using LOD/$\sqrt{2}$ for values below the LOD.

Sapkota and colleagues evaluated butadiene exposure of tollbooth workers in the Baltimore Harbor Tunnel during the summer of 2001. Mean ambient butadiene concentrations outside the tollbooths varied by shift, with the morning levels (19.8 µg/m^3) exceeding afternoon levels (14.9 µg/m^3). The lowest concentrations were measured at night, and averaged 4.9 µg/m^3. Considerable protection was offered by the tollbooth itself, within which ambient concentrations even in the height of traffic only measured 6.7 µg/m^3 (3 ppb) butadiene (Sapkota et al., 2005).

Ammenheuser et al. (2002) evaluated BD exposure in 49 workers in a styrene-butadiene rubber plant. The concentration of the butadiene metabolite 1,2-dihydroxy-4-(N-acetylcysteinyl-S)-butane (M1) in urine was used as a biomarker of butadiene exposure and the frequency of hypoxanthine-guanine phosphoribosyl transferase (*HPRT)* mutations in peripheral blood lymphocytes was used as a biomarker of effect. The workers were divided into high and low exposure groups according to job

description and work area. The average butadiene exposure concentrations (by personal organic vapor badge dosimeters) for the two groups were high, 1.48 ppm and low, 0.15 ppm ($P < 0.002$). Both mean M1 and *HPRT* mutant frequencies were more than three-fold higher in the high group than in the low group ($P < 0.0005$). For the high exposure group (N = 19-22) urine M1 mean was 2,046 ± 348 SE ng/mg creatinine and the *HPRT* frequency was 6.8±1.2 SE x 10^{-6}. The values for the low exposure group (N = 20-24) were 585±98 ng/mg creatinine and 1.8±0.2 x 10^{-6}, respectively. The study demonstrated correlation between the three measures of butadiene exposure. All workers, even those not exposed to butadiene, excreted some M1 suggesting other exogenous and/or endogenous sources. It is uncertain whether the observed increase in mutant frequency represents an adverse effect.

4 Metabolism

Common routes of exposure to butadiene include inhalation and dermal exposure.The inhalation absorption of butadiene is dependent on its partition coefficient (log Kow = 1.99) and Henry's Law constant (0.074 atm m^3/mole). Butadiene is absorbed very quickly into the lungs and blood. Physiological modeling uses blood/air partition coefficients of about 1 for butadiene (e.g., lung/air = 0.65 in humans, Pery & Bois, 2009; blood/air = 1.22 in humans, Brochot et al., 2007). The dosimetry of butadiene in rodents has been assessed by measuring steady-state concentrations of butadiene epoxides in blood and deposition in tissues following butadiene exposure. The steady-state in vivo concentrations of epoxybutene in the blood of mice exposed to 62.5, 625, and 1250 ppm butadiene for 4 to 6 hours was 4-8 times higher than those measured in rats at these BD concentrations and exposure periods, i.e. 0.4 to 8.6 µM in mice vs. 0.01 to 2.5µM in rats (Bond and Medinsky, 2001). Average tissue epoxides in mice for exposures of 625 ppm or 1250 ppm butadiene for 6 hours ranged from 2 to 3.5 mmol/g for lung epoxybutene, 0.5 to 0.75 mmol/g for liver epoxybutene, and 0.6 to 1.5 mmol/g for lung diepoxybutane, respectively. In rats at exposures of 625, 1250, or 8000 ppm butadiene, lung epoxybutene ranged from 0.2 to 1.1 mmol/g and liver epoxybutene ranged from 0.1 to 1.2 mmol/g (Bond et al., 1996).

Butadiene metabolites play a role in the toxicity of butadiene. It is not always clear which metabolites are responsible for various observed toxicities (e.g., cardiovascular). In some cases, strong evidence implicates specific toxicological endpoints are associated with particular metabolites.

In general, studies using human tissues showed that humans metabolized butadiene more like rats than mice, with lower rates of diepoxybutane formation (Bond et al., 1996). Perez et al. (1997) report the formation of N-(2,3,4-trihydroxybutyl)valine hemoglobin adducts (THBV) from the butadiene metabolite epoxybutanediol in rats and occupationally exposed humans. The adduct levels in rats exposed to 50, 200, or 500 ppm butadiene were 13.4, 5.9, and 1.15 pmol THBV/g globin/ppm butadiene, respectively. In humans exposed to 1 ppm butadiene, hemoglobin adducts were 8.2 and 10.7 pmol THBV/g globin/ppm. The results indicate that butadiene is absorbed into human blood at relatively low concentrations and forms hemoglobin adducts at levels comparable to those seen in rats.

Once in the body, butadiene is activated, creating the toxic metabolite(s) associated with adverse effects. Activation of butadiene typically occurs in liver microsomes via P450-mediated oxidative metabolism in mammals (Bond and Medinsky, 2001). Epoxybutene is the major oxidative metabolite, which is further oxidized to diepoxybutane and hydrolyzed to epoxybutane diol by microsomal epoxide hydrolase (Bond and Medinsky, 2001; Elfarra et al., 2001) (Figure 1).

Available data indicate that metabolism is qualitatively similar among all species studied, although the rates of metabolite formation differ. Mice form the initial oxidative metabolite, epoxybutene, approximately 6 to 8 times faster than rats or humans (Csanady et al., 1992) and produce a greater proportion of active epoxide metabolites than rats (Bond et al., 1986; Himmelstein et al., 1994; Thornton-Manning et al., 1995; Csanady et al., 2011).

This greater formation of epoxy metabolites may be related to differences in the amino acid composition of the binding pocket of CYP2E1 in rodents that facilitate hydrogen bonding and increase the probability of a second epoxidation. Compared to the rat, mouse CYP2E1 has an additional methylene group in the active site that binds epoxybutene more tightly, thus further enhancing formation of the second epoxide (Lewis et al., 1997). This observed difference in the quantity of epoxides formed correlates with the observed sensitivity and higher overall toxic response in mice versus rats. In vitro data from adult liver samples indicate that humans form epoxides of butadiene at rates (Vmax/Km, where Vmax = maximum reaction rate e.g. µmoles/hr and Km = reactant or substrate concentration (S) in µmol/L giving half the Vmax, where the overall reaction rate is more precisely defined by the Michaelis-Menten relation v = Vmax x S/(Km + S)) similar to mice but less than rats (Duescher and Elfarra 1994; Krause and Elfarra 1997), although it is unclear how this relates to in vivo toxicity in humans. Other authors, notably Csanady et al. (1992), reported that the Vmax/Km ratio obtained with mouse liver microsomes was 5.6- and 8.2-fold higher than the ratios obtained with human or rat liver microsomes, respectively. The observed differences in human butadiene oxidation rates between the studies may be attributed to variability in the expression of P450 2E1, 2A6 or other P450 enzymes in the human liver samples (Duescher and Elfarra, 1994). Detoxification of butadiene epoxides also varies across species, with human tissues preferentially detoxifying the derived epoxides through microsomal epoxide hydrolase to form butane diol followed by conjugation with GSH, whereas detoxification in mice occurs through glutathione-*S*-transferase (Boogaard and Bond 1996; Kemper et al., 2001). As a result, mice predominantly excrete 1-hydroxy-2-(N-acetylcysteinyl)-3-butene (MII), while adult humans excrete mainly 1,2-dihydroxy-4-(N-acetylcysteinyl-S)-butane (MI) (Figure 1). Boogard et al. (2001) reported urinary metabolites resulting from 8 hours occupational exposure to 1 ppm butadiene: MI, 2213 µg/L and MII, 39 µg/L.

The reactive metabolites of butadiene, including 1,2-epoxy-3-butene (EB), 3,4-epoxy-1,2-butane diol (EB diol), diepoxybutane (DEB), and hydroxymethylvinyl ketone (HMVK) have been shown to covalently bind to biological macromolecules, including DNA in the testes, lung, and liver, and certain proteins, including hemoglobin (Koivisto et al., 1998; Hayes et al., 2000; Begemann et al., 2001; Boogaard et al., 2004). In vivo experiments

have demonstrated species differences in adduct formation. For example, following a nose-only 6 hr inhalation exposure of 200 ppm ^{14}C-1,3-butadiene, measured uptake in mice was almost twice that for rats; the formation of specific DNA adducts was nearly 10 times higher in mice than rats (Boogaard et al., 2004). Quantitatively similar profiles of adducts were observed in an inhalation study using 20 ppm, although the overall level of DNA adducts formed in rats and mice was very low (Booth et al., 2004).

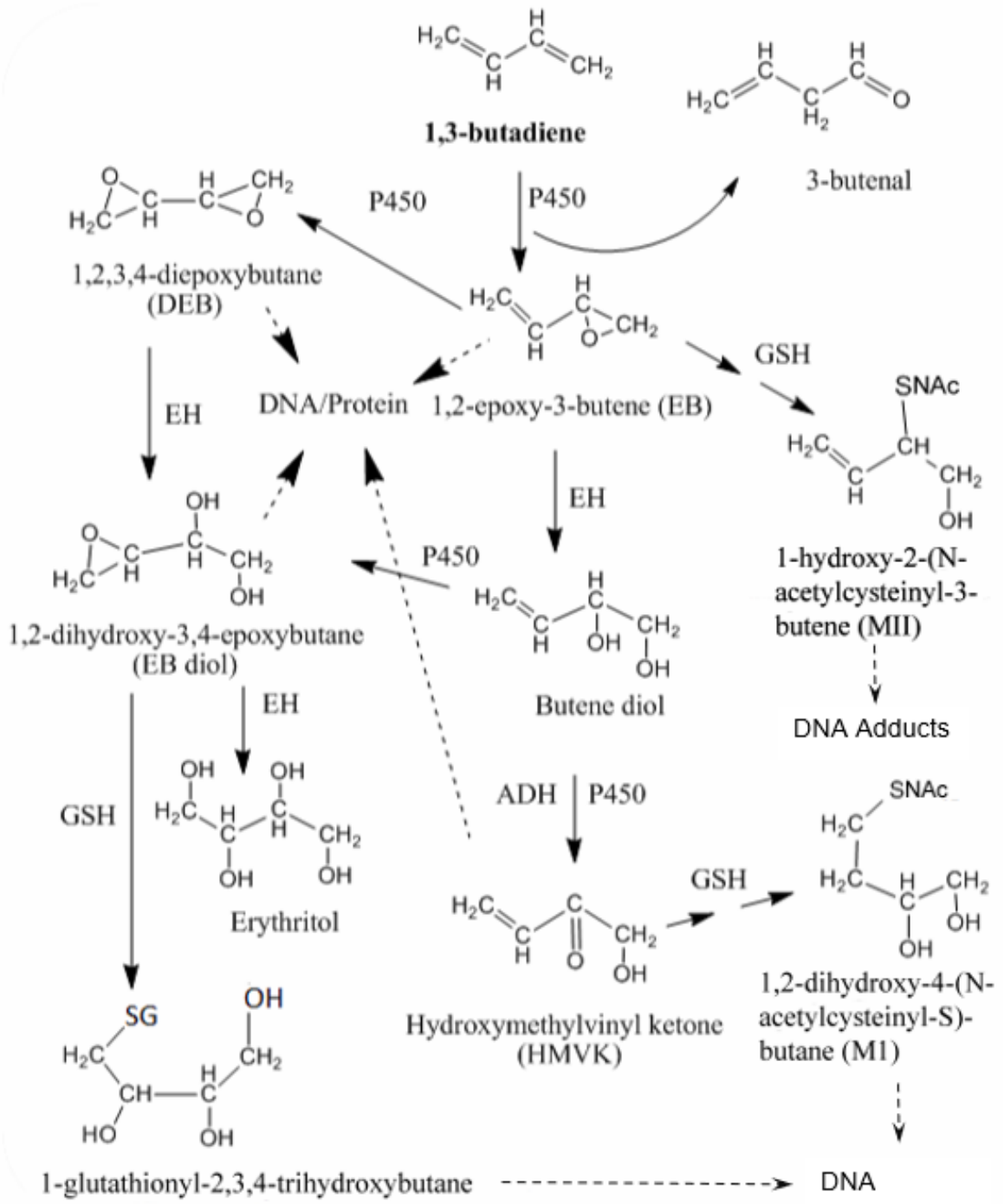


Figure 3. Metabolic scheme for 1,3-butadiene

This scheme presents the major identified reactive metabolites. Dashed arrows emanate from butadiene metabolites reported to form adducts with DNA or proteins. Abbreviations: EH, epoxy hydrolase; GSH, glutathione; ADH, alcohol dehydrogenase; P450, Cytochrome P450; NAc, N acetylcysteinyl; G, glutathionyl.

Cho and Guengerich (2012b) reported the mutagenicity in the *Escherichia* coli *rpoB* gene of the DEB-GSH conjugate *S*-(2-hydroxy-3,4-epoxybutyl)glutathione. Three DNA adducts were identified in the *E.coli* DEB/GST/GSH system: *S*-[4-(N(7)-guanyl)-2,3-dihydroxybutyl]GSH; *S*-[4-(N(3)-adenyl)-2,3-dihydroxybutyl]GSH; and *S*-[4-(N(6)-deoxyadenosinyl)-2,3-dihydroxybutyl]GSH.

Butadiene reactive metabolites also appear to form stable hemoglobin adducts in humans and experimental animals. In one study, Begemann et al. (2001) found that workers in a butadiene monomer production plant exposed to a median concentration of 440 µg/m^3 (0.2 ppm) had significantly increased levels of epoxybutene hemoglobin adducts versus unexposed controls. Results from another study indicated that levels of hemoglobin adducts of epoxybutane diol were substantially higher than those of epoxybutene in both inhalation-exposed rats and occupationally exposed humans (Perez et al., 1997). Forty-one butadiene polymer production workers in China were exposed to median butadiene concentrations of 2 ppm (6 hr time-weighted average) and had greater levels of hemoglobin adducts when compared with unexposed controls ($p < 0.0001$) (Hayes et al., 2000). These adduct levels were significantly correlated with butadiene concentrations ($p < 0.03$). The exposed workers did not differ from unexposed workers with respect to frequency of diepoxybutane-induced sister chromatid exchanges or aneuploidy, leading the authors to suggest that there were no correlations of biomarkers of butadiene exposure with these specific genotoxic effects (Hayes et al., 2000).

Sangaraju et al. (2012) used a more sensitive liquid chromatography-electrospray ionization tandem mass spectrometry method of measuring DEB-specific adducts in mouse liver DNA (bis-N7G-BD) following 2 weeks exposure (6hr/d, 5d/week) to various concentrations of 1,3-butadiene from 0.5 to 625 ppm. At low concentrations of 0.5, 1.0 and 1.5 ppm butadiene they found bis-N7G-BD (1,4-bis-(guan-7-yl)-2,3-butanediol) adducts at 0.17, 0.14, and 0.14/10^7 nucleotides/ppm BD, respectively. At higher concentrations of 6.25, 62.5, 200 and 625 ppm BD the adduct levels were significantly lower at 0.051, 0.013, 0.009, and 0.006/10^7 nucleotides/ppm BD, respectively. A similar pattern of higher efficiency of adduct formation at lower exposures was also seen with DEB-specific hemoglobin adducts by Georgieva et al. (2010). At the same low concentration butadiene exposures (0.5, 1.0 and 1.5 ppm), pyr-Val (N,N-(2,3-dihydroxy-1,4-butadiyl)valine) Hb adducts were formed at 21.0, 20.0 and 26.0 pmol pyr-Val-Hb/g globin/ppm BD. At 6.25, 62.5, 200 and 625 ppm butadiene concentrations, adduct formation was 12.0, 6.7, 3.7, and 2.5 pmol pyr-Val Hb/g globin/ppm BD, respectively.

Overall the results indicate that the efficiency of DEB reaction with cellular macromolecules is higher at lower exposure levels in mice. It is unknown whether a similar increased efficiency at low exposure levels occurs in humans. Boysen et al. (2012) have observed low but clearly detectable levels of pyr-Val-Hb in workers with 8-hr time weighted average butadiene exposures of 0.01 ppm (N = 23, controls), 0.24 ppm (N = 24, monomer workers), or 0.81 ppm (N = 34, polymerization workers). The levels of pyr-Val-Hb adducts found were 0.13, 0.16, and 0.29 ($P<0.05$ vs controls and monomer workers) pmol/g globin, respectively. The authors concluded that humans were less efficient in the formation of DEB than mice or rats at similar butadiene

exposures. HB-Val, indicative of epoxybutene concentrations, was found at 0.22, 0.47, and 2.23 pmol/g globin and THB-Val, indicative of epoxybutane diol concentrations, was found at 95, 179, and 716 pmol/g globin, respectively. The formation of DEB relative to other epoxides was significantly different in the highest exposed workers compared to controls and lower exposures. The pyr-Val concentrations in individual workers were significantly correlated with their HB-Val concentrations when analyzed combined or separately ($P < 0.01$, $P < 0.05$, respectively). In contrast the pyr-Val concentrations were significantly correlated with the THB-Val concentrations when analyzed combined or separately only for the high exposure group ($P < 0.01$).

5 Acute Toxicity of 1,3-Butadiene

5.1 Acute Toxicity to Adult Humans

There is a paucity of data on acute human exposure to butadiene. For eyes and skin, direct contact with liquid butadiene can cause burns similar to frostbite, while vapors or fumes may be irritating to the eyes. Some early occupational health studies indicated that workers exposed to butadiene during rubber manufacturing experienced irritation of the eyes, nasal passages, throat, and lung (Wilson 1944). Some workers also experienced coughing, fatigue and drowsiness, although the symptoms diminished after the exposure ceased. Inhalation of butadiene is mildly narcotic at low concentrations, and may result in a feeling of lethargy and drowsiness (Sandmeyer, 1981). Exposure to very high concentrations of butadiene can result in narcosis, respiratory paralysis, and even death. The first signs of acute exposure to high concentrations are blurred vision, nausea, paresthesia, and dryness of the mouth, throat, and nose. This may be followed by fatigue, headache, vertigo, hypotension, slowed pulse rate, and unconsciousness (ATSDR, 1992).

In more recent occupational health studies, while there were significant differences in short-term inhaled concentrations of butadiene between exposed workers (mean 1.5 $\mu g/m^3$, range 0.2 – 69.0 $\mu g/m^3$) and unexposed workers (mean 0.4 $\mu g/m^3$, range < 0.1 – 3.8 $\mu g/m^3$), biomarkers of effect, such as sister chromatid exchanges, were not different between groups of workers (Lovreglio et al., 2005). A study of 437 full-shift and 14 short-term samples indicated that exposure to butadiene in the polymer industry ranged from < 0.005 – 43.2 ppm for 8 hr samples, and 0.088 – 210 ppm for acute (15 – 120 min) exposures (Fajen et al., 1990). Data on potential adverse effects from acute exposure were not reported. Since the workers in many of these studies were exposed to mixtures of chemicals, the relationship of acute butadiene exposure and adverse effects remains unclear.

In an acute study of human physiological factors, 133 adult subjects (both male and female) were exposed to 2 ppm (4.42 mg/m^3) butadiene for 20 minutes (Lin et al., 2001) followed by purified air for 40 minutes. Five exhaled breath samples collected during exposure were used to measure the respiratory uptake of butadiene. The absorbed dose was defined as µg butadiene uptake/kg body weight. Although the subjects were given identical administered doses, there was a wide range of uptakes, 0.6-4.9 µg/kg.. The total butadiene concentration inhaled was significantly higher in males than in

females; however there was no significant difference in the respiratory uptake fraction of the total concentration inhaled, which ranged from 18-74% for all participants. Females had larger body burdens per kilogram of body weight than males. Additionally, both age and smoking were negatively associated with butadiene uptake. The authors suggested that the wide range of butadiene uptake values indicates that administered dose is a poor estimator of absorbed dose or body burden (Lin et al., 2001).

Khalil et al. (2007) evaluated neurological parameters for a random cohort of 310 subjects who had been exposed to 1,3-butadiene due to accidental leakage and release of BD in an industrial area and 50 matched unexposed controls. A set of clinical neurological tests was utilized including electroencephalograms (EEGs), somatosensory evoked potentials (SSEPs), brainstem auditory evoked potentials (BAEPs), visual evoked potentials (VEPs), and electromyography/nerve conduction tests (EMG/NCT). Exposed individuals were divided into mild (N = 212) and severe (N = 98) subgroups based on preliminary testing. Among the mild subgroup the most common finding was encephalopathy characterized by headaches and light headedness that resolved within hours to six months. The electroneurodiagnostic tests were normal except for abnormal BAEPs in 21/212 (9.91%) subjects. In the severe subgroup encephalopathy was characterized by confusion, irritability, impaired judgment, altered levels of consciousness, disequilibrium, numbness, paresthesia, ataxia, weakness, nausea, vomiting, and memory deficits. The EEGs were mildly abnormal (12/98, 12%) and BAEPs and VEPs strongly so (63/98, 49/98, respectively). Within the 50 controls, one had an abnormal EEG on heavy medication and 2 (4%) had polyneuropathy with diabetes and alcohol abuse, and two had abnormal BAEPs due to deafness or hearing abnormalities. All controls had normal VEPs. The authors concluded that butadiene met the definition of a neurotoxicant. The butadiene-induced neurotoxic effects of most of the subjects were reversible except for 18 (6%) subjects that demonstrated irreversible neurotoxic effects.

5.2 Acute Toxicity to Infants and Children

The exposures most infants and children have to butadiene tend to be chronic and low level. However, by correlating changes in health effects with short-term changes in ongoing measurements of butadiene and other VOC's, it may be possible to discern the potential toxicity associated with acute exposures. This is the approach taken below to determine the effects of air pollutants on asthma exacerbation in children.

Delfino and colleagues studied the relationship between asthma symptoms in children and concentrations of ambient VOCs and criteria air pollutants, including butadiene (Delfino et al., 2003). To be eligible to participate, children were required to have a minimum 1-year history of physician diagnosed asthma and at least two symptomatic days per week that required use of a β-agonist inhaler. Twenty-two Hispanic children, aged 10 – 16, who were living within the Los Angeles air basin and who were nonsmokers from nonsmoking households, were enrolled in the study. The children kept daily symptom diaries from November 1999 through January 2000 and the severity of their symptoms was analyzed for association with ambient concentrations of particulates, ozone, and VOCs. Regression analysis examined air pollution

concentrations on the same day or up to 4 days prior to the reported asthma symptoms, thereby allowing the analysis of the lag between the measured concentrations and the onset of health effects.

Adjusted odds ratios (OR's) for more severe asthma symptoms were as follows: for 1.4 ppb NO_2, 1.27 (95% CI, 1.05-1.54); 1.00 ppb benzene, 1.23 (95% CI, 1.02-1.48); 3.16 ppb formaldehyde, 1.37 (95% CI, 1.04-1.80); 37 $\mu g/m^3$ PM_{10}, 1.45 (95% CI, 1.11-1.90); 2.91 $\mu g/m^3$ elemental carbon, 1.85 (95% CI, 1.11-3.08); and 4.64 $\mu g/m^3$ organic carbon, 1.88 (95% CI, 1.12-3.17).

Butadiene was detected in 74 daily air pollution measurements with a mean concentration of 0.51 ppb (range 0.05 – 1.50 ppb). This study presented an OR of 1.32 (95% CI = 0.97-1.80) for the association between asthma symptoms and ambient butadiene concentrations on the previous day, and an OR of 1.16 (95% CI = 0.90-1.49) for the association between asthma symptoms and same-day butadiene levels. While these estimates are elevated, the confidence intervals included no effect. Thus, the authors interpreted these results as suggestive of an increased risk of asthma symptoms in children with butadiene exposure. However, the study was limited by small numbers, as only seven subjects reported symptom scores >2 and only 16 with scores >1. Also, associations for scores >2 were strongly influenced by one subject with moderate persistent asthma (Delfino et al., 2003). Since many of these pollutant chemicals occur together it will always be difficult to tease out effects due to specific pollutants. However, such studies may be of use in comparison with studies of accidental releases of butadiene where the problem of concurrent exposures is much less.

5.3 Acute Toxicity to Experimental Animals and In Vitro Toxicity

Few studies have reported the acute or short-term effects of butadiene. The lethal concentration resulting in 50% mortality in a population (LC_{50}) was measured in an acute inhalation study using 2 hr exposures for mice and 4 hr exposures for rats (Shugaev 1969). The mouse LC_{50} was determined to be 122,000 ppm, and the rat LC_{50} was 129,000 ppm; no other effects were reported. In a National Toxicology Program study, male and female B6C3F1 mice were exposed to 0, 625, 1,250, 2,500, 5,000, or 8000 ppm butadiene in air for 15 days (NTP, 1984). Survival rates were unaffected by dosing, and no respiratory effects, cardiovascular lesions, or hepatic/gastrointestinal histopathology were noted even at the highest concentration. There was, however, a non-significant decrease in mean body weight at 8000 ppm butadiene when compared with controls (NTP 1984). No severe immunological changes were noted in humoral or cell-mediated immunity in mice exposed to 1250 ppm butadiene for 6 weeks (Thurmond et al., 1986).

The effects of butadiene inhalation (12.5 and 1250 ppm) on birth outcomes following a single acute 6-hr exposure of male mice were reported by Anderson et al. (1996). The mean number of implants per female was reduced at both concentrations compared to controls, but statistically significant only at the higher level ($p < 0.05$). A dominant lethal

effect (see Section 7.2) was not evident with the acute exposure, and there was no significant increase in post-implantation losses or fetal abnormalities.

In contrast to the results of the studies of the parent compound cited above, short-term studies of butadiene metabolites have shown acute effects, some of which were severe. Henderson and colleagues investigated the acute and sub-acute toxicity of the oxidative metabolite butadiene diepoxide in female B6C3F1 mice and in Sprague Dawley rats. Examination of the organs, including the liver, kidney, marrow, heart, and ovaries revealed that a single 6 hr inhalation exposure to 18 ppm butadiene diepoxide (3 to 4/group) induced no clinical signs of toxicity. However, the mice showed a slight increase in the number of alveolar macrophages and focal necrosis of the olfactory epithelium that started to reverse 7 days after exposure (Henderson et al., 1999). In the sub-acute portion of the study, mice (15/group) were exposed to either 2.5 or 5 ppm butadiene diepoxide for 6 hr/day for 5 days. The animals showed no body weight changes and no histopathology of the nose, larynx, or lung. However, in an earlier part of the study there was significant toxicity when mice and rats (56/group) were exposed to either 10 or 20 ppm butadiene diepoxide for 6 hr/day for 7 days. Both mice and rats experienced significant weight loss, corneal opacity, labored breathing, ruffled coats and hunched posture, as well as nasal mucosa degeneration, necrosis, inflammation, and ulceration. Additionally there were individual instances of focal hepatic necrosis and bone marrow atrophy in the mice. All but one of 56 mice from the high dose group were dead by the end of 12 days (98.2 %); by comparison, mortality in the low dose group was only 29% at 10 days of the 21 day study duration.

In a study of the butadiene metabolite 3-butene-1,2-diol, male B6C3F1 mice (4 to 5/group) were given a one-time intraperitoneal (i.p.) dose of 250 mg/kg and male Sprague Dawley rats (4 to 5/group) were given either 125, 200, or 250 mg/kg 3-butene-1,2-diol i.p. (Sprague et al., 2004). Two of the four rats dosed with 250 mg/kg died within 24 hours. Prior to death, the rats were lethargic and experienced seizures. At necropsy, it was evident that rats dosed at the highest level experienced severe hepatic necrosis and hemorrhage. The rats dosed with 200 mg/kg also showed indications of hepatic necrosis, inflammation, and hemorrhage. No other rat organ examined showed consistent dose-related lesions, although dosed rats exhibited significant hematological differences and significantly lower hepatic levels of glutathione when compared with controls (Sprague et al., 2004). Mice necropsied 4 hrs after treatment with 250 mg/kg 3-butene-1,2-diol did not exhibit any lesions, or any significant hematological changes. However, the mice did have significantly lower levels of hepatic glutathione 1 hr after treatment when compared to controls (Sprague et al., 2004). Half-lives for 3-butene-1,2-diol were calculated to be 2.12 hr in the rat and 0.44 hr in the mouse, which may partially account for the observed differences in acute toxicity between the two species (Sprague et al., 2004).

Results from a human embryonic lung fibroblast study suggest that in vitro exposure to the butadiene metabolite 1,2:3,4-diepoxybutane (100 µM for 1 hr) is associated with substantial inhibition of cell proliferation and cell cycle arrest at G_1 (Schmiederer et al., 2005). The authors suggested that cell proliferation was inhibited by a diepoxybutane-mediated alteration to cell division (Schmiederer et al., 2005). Another in vitro study

found that products from the incomplete combustion of butadiene were differentially taken up and retained by human bronchial epithelial cells (Penn et al., 2005). Investigators found that the combustion-generated "ultrafine" particles (defined by the authors as <2.5 µm aerodynamic diameter) migrated from culture medium to the cell membrane, but not into the cell interior. The organic chemicals bound to the particles, however, were found to migrate from the particle surface, through the cell membrane into the cytosol, and into cellular vesicles. The authors suggested that toxicants, including butadiene, may transfer into cells directly without the cellular uptake of the carrier particles i.e., direct uptake from the vapor phase (Penn et al., 2005).

6 Developmental and Reproductive Toxicity

6.1 Developmental Effects

Many investigators have studied the potential for butadiene and its metabolites to cause reproductive or developmental toxicity. Earlier studies focused on overall reproductive fitness following short-term exposures, while more recent studies have focused on specific effects and mechanisms of toxicity.

Some of the first studies on the reproductive and developmental toxicity of butadiene were nested within chronic studies (Owen et al., 1987; Melnick et al., 1990). The National Toxicology Program developed the first series of short-term studies to evaluate developmental and reproductive toxicity of inhaled butadiene in mice and rats (Morrissey et al. 1990). In a three-part study, Sprague Dawley rats (24-28/group), and B6C3F1 and Swiss (CD-1) mice (18-22/group) were exposed to 0, 40, 200, or 1000 ppm butadiene on gestation days 6-15 for 6 hr/day and examined for teratological effects. A separate set of male mice was exposed to 0, 200, 1000, or 5000 ppm for 6 hr/day for 5 days for a sperm morphology study. Pregnant rats exhibited toxicity at 1000 ppm in the form of reduced extragestational weight gain and, during the first week of treatment, decreased body weight gain; there were no other differences in fertility or developmental parameters between exposed and control groups. In mice, however, there were increased numbers of fetal variations (supernumerary ribs, reduced ossification) in litters from dams exposed to 200 and 1000 ppm butadiene. These teratogenic effects occurred in the presence of maternal toxicity and fetal weight gain reductions. Male mice treated with 1000 or 5000 ppm butadiene showed significant increases in abnormal sperm 5 wks after exposure, consistent with an effect of butadiene on mature spermatozoa and spermatids (Morrissey et al., 1990).

Teratological effects at maternal butadiene exposures of 200 ppm and higher were also observed by Hackett et al. (1987a), along with signs of maternal toxicity. However, in their study of reproductive performance, and maternal and fetal toxicity, Hackett et al. included a lower dose of 40 ppm. For this study, 78 pregnant female CD-1 mice received whole-body exposure to 0, 40, 200, or 1000 ppm butadiene for 6 hr/day on gestation days (gd) 6-15, with necropsy on gd 18. Mice were weighed prior to mating and repeatedly during gestation. They were observed for signs of toxicity during exposure and examined for gross tissue abnormalities at necropsy. Reproductive parameters included numbers of implantations sites, resorptions, and live and dead

fetuses. Live fetuses were examined for signs of morphological anomalies and growth retardation. The incidences of fetal variations (supernumerary ribs and reduced ossification of the sternebrae) were significantly elevated in litters from mice exposed to 200 and 1000 ppm ($P < 0.05$). At these exposure levels, there was also evidence of maternal toxicity as shown by significantly lower maternal weight gain ($P \leq 0.05$). There was a significant dose-dependent reduction of fetal body and placental weights at the two higher doses for female fetuses, and at all doses in males ($P \leq 0.05$) (Table 1). The observation that males fetuses appeared to be susceptible to butadiene at levels that were not maternally toxic is the basis of the acute REL.

Green (2003) reanalyzed the data of Hackett et al. (1987) and found inconsistencies associated with the presentation and calculation of mean values for maternal and fetal body weights, sex ratio, and reproductive data. When the data were analyzed in the context of analysis of covariance (ANCOVA) with sex ratio and litter size as covariates, no statistically significant difference was found between fetal weights at the 40 ppm exposure level and the controls. The results of the Green (2003) re-analysis are shown in Table 2B. In addition to the covariance adjusted pairwise tests of means in Table 2, Green also evaluated the ratios of treatment means to control means and calculated a 95% confidence interval (CI) for this ratio using the variance-covariance matrix from the ANCOVA. For male fetal weights the ratios (and 95% CI) were: control, 100 (referent); 40 ppm, 95.7(100.1); 200 ppm, 82.6(87.2); and 1000 ppm, 77.0(81.5). Since the upper 95% CI for the 40 ppm exposure level includes 100 there is no statistically significant difference from the control at this level. The other data sets for combined sexes and female fetal weights gave similar results.

In a companion study, Hackett et al. (1987b) exposed female Sprague-Dawley rats to whole body inhalation of 1,3-butadiene for 6 hr/day at 0, 40, 200, or 1000 ppm. The female rats (24 to 28/dose group) that had been mated to unexposed males were exposed on gestation days 6 thru 15 and sacrificed on gd 20. There were no significant differences among treatment groups in maternal body weights or extragestational weights at 40 or 200 ppm, but at 1000 ppm depressed body weight gains were observed during the first 5 days of exposure and extragestational weight gains were lower than control values. Placental weights, fetal body weights and sex ratios were unaffected by butadiene treatment. There were no significant differences among groups for incidences of fetal malformations.

The dose-response analyses of the Hackett et al. (1987) and the Green (2003) re-analysis data sets are described and discussed in Section 8.1. Those analyses employ the benchmark dose software (BMDS v. 2.3.1) and do not depend on a specific no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL). Rather, the benchmark model uses the entire dose-response data set. The key value for a non-cancer dose response is usually the benchmark concentration level $BMCL_{05}$, or the 95% lower confidence limit on the concentration causing a 5% response. Considerable experience has shown that the $BMCL_{05}$ is a better choice for a point of departure than a single dose NOAEL.

Table 1. Body Weight and Fetal and Placental Measures after 1,3-Butadiene Exposure (Hackett et al., 1987a)

Observation	1,3-Butadiene Concentration (ppm)			
	0	40	200	1000
Number examined				
Mothers	18	19	21	20
Litters	18	19	21	20
Fetuses	211	237	259	244
Maternal weight gain (g)[a] (11-16 gd)	13.3 ± 0.6[b]	12.7 ± 0.4[b,c]	11.4 ± 0.5[c,d]	10.6 ± 0.4[d]
Pup body weight (g)[a]				
Females	1.30 ± 0.02[b]	1.25 ± 0.01[b]	1.10 ± 0.02[c]	1.02 ± 0.03[d]
Males	1.38 ± 0.03[b]	1.31 ± 0.02[c]	1.13 ± 0.02[d]	1.06 ± 0.02[e]
Sex ratio (% male)	51.6 ± 3.91	49.8 ± 3.06	51.5 ± 3.68	51.8 ± 3.29
Placental weight (mg)[a]				
Females	83.1 ± 3.03[b]	80.9 ± 2.46[b]	74.7 ± 3.52[b,c]	70.1 ± 2.33[c]
Males	89.3 ± 3.05[b]	89.5 ± 2.27[b]	80.1 ± 2.35[c]	74.5 ± 1.81[c]

[a]Mean ± standard error; [b-e] Values that do not share a common superscript letter are significantly different ($p \leq 0.05$) from one another.

Table 2. Body Weight and Fetal and Placental Measures after 1,3-Butadiene Exposure (Green, 2003 Reanalysis of Hackett et al., 1987a data)

Observation	1,3-Butadiene Concentration (ppm)			
	0	40	200	1000
Number examined				
Mothers	18	19	21	20
Litters	18	19	21	20
Fetuses	211	237	259	244
Pup body weight (g)	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Combined	1.350 ± 0.028	1.283 ± 0.013	1.126 ± 0.021*	1.038 ± 0.025*
Females	1.309 ± 0.021	1.253 ± 0.012	1.100 ± 0.022*	1.015 ± 0.026*
Males	1.382 ± 0.033	1.307 ± 0.016	1.132 ± 0.016*	1.060 ± 0.024*
Sex ratio (% male)	51.55 ± 3.866	49.66 ± 2.947	51.44 ± 3.677	51.80 ± 3.310
Placental weight (mg)				
Combined	86.99 ± 2.960	85.30 ± 2.283	78.65 ± 3.243	73.06 ± 1.826*
Females	83.15 ± 3.023	80.89 ± 2.474	74.33 ± 3.540	70.84 ± 2.284*
Males	89.58 ± 2.995	89.71 ± 2.263	80.27 ± 2.324	74.64 ± 1.785*

Note: * significantly different from controls ($P < 0.05$).

6.2 Dominant Lethal Effects

The primary purpose of the dominant lethal assay is to identify toxicants that have the ability to enter the gonad and cause a genetic lesion in the gamete, which results in cytogenetic damage in the fertilized zygote without recognized changes in fertilizability. Generally, male animals are either given 1 – 5 doses of the test agent, or are dosed over the entire cycle of spermatogenesis, then mated at weekly intervals with untreated females. The females are necropsied prior to giving birth and examined for uterine contents. The total number of implanted embryos is compared over dose groups and with controls. Dominant lethal assays use conceptus failure as an endpoint for potential male-mediated developmental toxicity.

Nested within the larger National Toxicology Program study discussed above, dominant lethality was studied in litters sired from male mice exposed to 70, 200, 1000, or 5000 ppm butadiene for 6 hr/day for 5 days (Morrissey et al., 1990). There was no mortality noted in the male mice even at the highest butadiene concentrations, and body weights were unaffected. One week following exposure to 1000 ppm, investigators observed a statistically significant increase in dead implants as a percentage of total implants ($p \leq 0.05$). There were smaller non-statistically significant increases in dead implants at 200 and 5000 ppm (p values not given). During the second week following exposure, there were significant increases in dead implants in both the 200 and 1000 ppm groups ($p \leq 0.05$). The investigators noted that the dose-dependency of the effects weakens at higher dose levels, but the effect in week 1 and 2 is consistent with an effect of butadiene on mature spermatids and spermatozoa. The authors suggested that because of the effects noted in sperm morphology and dominant lethality, butadiene might act as a germ cell mutagen in mice (Morrisey et al., 1990).

In 1993, Anderson and colleagues considered dominant lethality in a more focused study of male-mediated reproductive toxicity following acute and short-term butadiene exposure. In the acute study, male CD-1 mice were exposed to 0 (n = 25), 1250 (n = 25), or 6250 (n = 50) ppm butadiene one time for 6 hr, then mated with untreated females. In the short-term study, male CD-1 mice were also exposed to 0, 12.5, or 1250 ppm butadiene for 6 hr/day 5 days/wk for 10 weeks prior to mating, then mated with untreated females. The necropsied dams were examined for the number of live fetuses, the number of early and late post-implantation deaths, and the number and type of fetal gross malformations. A single 6 hr exposure did not elicit a dominant lethal effect, although there was a non-significant reduction in the mean number of implants in the 1250 and 6250 ppm dose groups. Such findings may indicate germ cell toxicity or pre-implantation losses, which are generally not associated with heritable changes (Anderson et al., 1993). Subchronic exposures to 12.5 ppm butadiene were associated with increased congenital malformations and late fetal deaths. In addition, there was a significant incidence of dominant lethality at 1250 ppm butadiene. The authors concluded that butadiene is mutagenic and teratogenic following subchronic exposures (Anderson et al., 1993).

To further elucidate the dominant lethality of butadiene, Adler and Anderson (1994) exposed CD-1 male mice to 1250 ppm for 6 hr/day, 5 day/week for 10 weeks prior to

mating, and then mated the exposed males with unexposed females for 4 successive weeks. There was a total (summed) incidence of 23.1% dominant lethality over 3 weeks of mating. The highest weekly incidence of dominant lethality was 12.4% after week 2 of mating. No other fertility differences were noted between exposed and control animals. From the timing of the dominant lethal effect, the authors suggested that spermatozoa and late spermatids were the most sensitive germ cell types to butadiene inhalation (Adler and Anderson, 1994). Most of these results were duplicated in another report by Anderson and colleagues (Anderson et al., 1996) and are consistent with dominant lethal findings by Morrisey et al. (1990).

In 1994, Adler and colleagues designed a cross-bred (102/E1 x C3H/E1) mouse study to consider not only dominant lethality but teratogenicity in pregnancies allowed to come to term. Male mice (20 per group) were exposed to 0 or 1300 ppm butadiene for 6 hr/day for 5 days, and then mated with unexposed females for 4 consecutive weeks. Groups of pregnant dams were sacrificed at gestation day 14 following successive weeks of mating. The percentage of dead implants and dominant lethals was elevated in litters that were sired during weeks 1, 2, and 3 following butadiene exposure in the males. The effect, however, was highly significant ($p \leq 0.01$) in week 2. All other fertility parameters were similar to controls (Adler et al., 1994). From the timing of effects, the authors concluded that dominant lethal mutations were induced in spermatozoa and late spermatids, suggesting that butadiene may induce heritable translocations in these germ cell stages (Adler et al., 1994), consistent with their previous work in CD-1 mice (Adler and Anderson 1994).

Two other studies have demonstrated a positive association between inhalation of low concentrations of butadiene and dominant lethality, with mice being more susceptible than rats (Anderson et al., 1998; Brinkworth et al., 1998). Brinkworth and colleagues exposed male CD-1 mice to 0, 12.5 or 125 ppm butadiene for 6 hrs/day for 5 days a week for 10 weeks followed by mating with untreated females. A significant increase in dominant lethality was noted, but only in the 125 ppm group. Anderson and colleagues exposed male CD-1 mice to 0, 12.5, 65, or 130 ppm butadiene for 6 hr/day, 5 days/week for four weeks and groups of male Sprague Dawley rats to 0, 65, 400, or 1250 ppm butadiene for 6 hr/day, 5 days/week for 10 weeks. There was a significant increase in dominant lethality in mice in the 65 and 130 ppm dose groups, although the effect was not dose-related. Male-specific effects in the rats were not observed at any exposure level (Anderson et al., 1998). Additionally, one study showed that the butadiene metabolite diepoxybutane is also associated with dominant lethality. Male mice were given either 0 or 54 mg/kg diepoxybutane (DEB), and mated for up to 16 days after treatment with untreated females. A single dose of 54 mg/kg DEB was toxic to mature spermatozoa; therefore, there was a low number of total implants in the first 8 days after treatment. However, there was an increase in dominant lethals throughout all mating intervals. The authors suggested that diepoxybutane is likely a late spermatid mutagen (Adler et al., 1995).

Accumulated data appear to suggest that inhalation exposure of butadiene is associated with an increase in dominant lethal effects even at concentrations below the threshold for acute toxicity. There is evidence of species and strain differences in

susceptibility, with mice being more susceptible than rats, and outbred CD-1 mice appearing to show dominant lethality at lower butadiene concentrations than other strains of mice. Regardless of the length of pre-mating dosing (i.e., a single 6 hr, 5 day, or 4-10 week exposures), dominant lethal effects were associated with butadiene effects in the more mature male germ cells, specifically mature sperm and late spermatids.

6.3 Germ Cell Mutagenicity

Results from dominant lethal experiments suggest that butadiene exposure may be associated with germ cell mutagenicity. As such, several researchers investigated the potential for butadiene to induce heritable mutations. In one study, C3H/E1 male mice were exposed to 1300 ppm butadiene for 6 hr/day for 5 days and mated with untreated females. Half the offspring were examined for dominant lethality while the other half were allowed to mature in order to study heritable effects in subsequent generations (Adler et al., 1995). There was a non-significant increase in dominant lethal mutations in F_1 litters sired from butadiene-exposed males. However, when the F_1 males were allowed to mature and mate, the resulting F_2 litters were approximately half as large as litters sired by control F_1 mice. Additionally, there was a 2.7% translocation frequency in F_2 pups following F_0 paternal butadiene exposure compared with a 0.05% translocation frequency from historical controls (Adler et al., 1995).

In a follow-up study, mice exposed to 0, 130, or 500 ppm butadiene for 6 hr/day for 5 days were mated for 4 consecutive weeks with untreated females. A positive dominant lethal effect was observed for 130 and 500 ppm during week one of mating (a sperm effect) and for 500 ppm during week two of mating (a late spermatid effect). Among 434 F_1 offspring from butadiene-exposed F_0 sires, there was a 1.15% incidence of translocation when compared with 0.05% translocation in historical controls ($p < 0.001$) (Adler et al., 1998). The heritable mutation was identified as a reciprocal translocation with insertion of the central portion of chromosome 9 into chromosome 19 (Adler et al., 1998). The genetic risk from butadiene exposure was calculated using data from these two studies with a linear dose-response model [$Y = 0.05 + 6.9 \times 10^{-5} X$] where Y is the percentage of offspring with heritable translocations and X is butadiene exposure in ppm hr. Results from the calculations suggest that butadiene has the potential to cause heritable mutations in mouse sperm that can be transmitted to subsequent generations with a doubling dose of 725 ppm-h butadiene (Adler et al., 1998).

Pacchierotti et al. (1998) investigated the paternal transmission of butadiene-induced chromosomal aberrations to one cell embryos. Male mice (102/E1 x C3H/E1) were exposed to 0, 130, 500, or 1300 ppm butadiene for 6 hr/day for 5 days then mated 1:2 with untreated B6C3F1 females. The resulting zygotes were arrested at first cleavage and analyzed for chromosomal aberrations in each of the first three weeks of mating following paternal exposure. Zygotes conceived in weeks one and two of mating were produced from oocytes from untreated females fertilized with either mature sperm or late spermatids. Zygotes conceived in week three of mating were the product of oocyte-early spermatid or oocyte-late prophase spermatocyte unions. Investigators found a dose-dependent increase in structural chromosomal aberrations in first-cleavage

embryos conceived in week one of mating, with significance ($p < 0.005$) achieved at 500 ppm butadiene. Embryos conceived during week two also showed dose-dependent increases in chromosomal aberrations, with significance achieved at 1300 ppm butadiene ($p < 0.025$). The authors concluded that the male germ cells associated with the observed embryo mutations were likely the same types as those associated with male-mediated dominant lethality in other studies, and that sperm in the final stages of maturation were most susceptible to the mutagenic effects of butadiene.

Anderson et al. (1997) evaluated the effects of 1,2-epoxybutene (BMO) and three other chemicals (β-estradiol, daidzein, dibromochloropropane) on human sperm using the alkaline Comet assay for DNA damage in vitro. Fresh and frozen samples from two fertile donors each and frozen samples from two infertile donors were analyzed. All the chemicals induced significant effects in all exposed samples ($P < 0.01$). For BMO exposures of 0, 80, 160, and 320 µM, significant dose-response model fits were obtained only with frozen samples. Using the continuous Hill and polynomial models, $BMCL_{05}$ values ranged from 0.6 to 24.2 µM with an arithmetic mean of 12.3 µM and a geometric mean of 4.64 µM. The effects seen with BMO were comparable to those seen with dibromochloropropane, a known male reproductive toxicant, at 20 to 200 µM. Internal exposure estimates obtained with human PBPK models based on Kohn and Melnick (2001) indicate that BMO blood concentrations in the range of $BMCL_{05}$'s might be achieved by acute occupational exposures to butadiene (e.g., 200 ppm butadiene x 2 hr = 6.8 to 15.6 µM BMO Cmax in venous blood). Alternatively, the human PBPK model of Brochot et al. (2007) predicts lower blood and tissue concentrations of BMO for the same exposure scenario (i.e., 0.1 to 0.7 µM).

The majority of acute reproductive toxicity studies of butadiene have focused on male animals. No similar studies were found for female animals. However, one study by Tiveron and colleagues (1997) considered the acute female reproductive toxicity of the butadiene metabolite diepoxybutane. Female B6C3F1 mice received a single intraperitoneal injection of 26, 34, 43, or 52 mg/kg diepoxybutane, and then mated with unexposed males. The embryos were arrested at first-cleavage metaphase and examined for chromosomal aberrations. There were no consistent diepoxybutane-associated effects on mating, fertilization, or cell-cycle progression of the fertilized oocytes, although there was a decrease in the average number of zygotes harvested from each female. There was, however, a significant increase in frequency and number of chromosomal aberrations in exposed zygotes when compared with controls in all dose groups ($p < 0.05$-0.001).

When comparing the sensitivity of male and female gametes to pre-conception exposures, it appears that zygotes produced from exposed sperm-nonexposed oocytes are many times more likely to contain chromosomal aberrations (Adler et al., 1995) than zygotes conceived with exposed oocytes (Tiveron et al., 1997). While chromosomal damage may be transmitted via female germ cells (Adler et al., 1995), sperm appear to be at much higher risk of the mutagenic effects of butadiene and butadiene metabolites.

6.4 Ovarian Atrophy

Reproductive organs appear to be critical targets of chronic butadiene exposure, and ovotoxicity is the basis of the chronic and 8-hour RELs. Effects in the female reproductive tract were identified in an NTP chronic study, where B6C3F1 mice were exposed to 0, 625, or 1250 ppm butadiene for 6 hr/day, 5 days/wk for a planned period up to 103 weeks (NTP, 1984). Significant ovarian atrophy was observed in female mice in both dose groups (40 of 45 females at 625 ppm; 40 of 48 females at 1250 ppm). NTP investigators identified a chronic LOAEL of 625 ppm, based on the observed gonadal effects (NTP, 1984). In an effort to further elucidate the reproductive toxicity, NTP investigators conducted a study in B6C3F1 mice with lower butadiene concentrations (Melnick et al., 1990; NTP 1993). The animals were exposed to 0, 6.25, 20, 62.5, 200, or 625 ppm for 6 hr/day, 5 days/wk for up to 65 weeks. While there was significant mortality, a concentration-related increase in ovarian atrophy was also observed. At 40 weeks, ovarian atrophy was present in females exposed to 200 and 625 ppm butadiene. At 65 weeks, ovarian atrophy was present in all groups exposed to ≥ 20 ppm butadiene, and female mice exposed to the lowest concentrations of butadiene (6.25 ppm) exhibited atrophy at the end of the study at 105 weeks (Melnick et al., 1990; NTP, 1993). Based on these results, NTP investigators identified a chronic LOAEL of 6.25 ppm for reproductive toxicity (NTP, 1993). The 9-, 15- and 24-month ovarian atrophy data from NTP (1993) were subjected to benchmark dose analysis as summarized in Table 3. Only the 9-month interim sacrifice data were adequately fit by the multistage model (X^2 = 2.47, P = 0.78, $BMCL_{05}$ = 19.2 ppm). The 24-month data were adequately fit by the log probit model if the top dose was excluded. If the dose response used the average mixed venous concentration of diepoxybutane (DEB) from the mouse PBPK model an excellent fit to all the 24 months data was obtained (X^2 = 0.1, P = 0.99, $BMCL_{05}$ = 0.58μM DEB equivalent to 0.71 ppm BD). If the data were included in a time-adjusted model, all of the data could be fit (N = 435). Using the log probit model, a $BMCL_{05}$ of 1.01 ppm butadiene was obtained with a X^2 of 1.7, P = 0.64 (BMDS v 2.2). This study forms the basis of the derivation of 8-hour and chronic RELs described in sections 8.2 and 8.3, respectively.

While atrophy may be associated with normal reproductive senescence in rodents, it is noteworthy that butadiene-associated ovarian atrophy was observed in female mice as early as 9 months of age in association with butadiene exposure (NTP 1984). Depletion of ovarian follicles and ovotoxicity were also observed following intraperitoneal administration of butadiene monoepoxide or diepoxide (Doerr et al., 1996). Doerr et al. (1996) administered butadiene monoepoxide (BMO; 0.005-1.43 mmol/kg b.w.) or the diepoxide (DEB; 0.002-0.29 mmol/kg b.w.) to female $B6C3F_1$ mice (n = 10/dose) intraperitoneally daily for 30 days. The investigators analyzed the data on ovarian and uterine weight depression in mice (% body weight ± SD) using a PBPK model to simulate i.p. administrations and extrapolate internal dose metrics to external butadiene inhalation equivalents.

Table 3a. Ovarian Atrophy in Female Mice in 2-Year Inhalation Study of 1, 3-Butadiene (NTP, 1993).

Exposure Period	Model	X^2	P	BMC_{05} ppm	$BMCL_{05}$ ppm	$BMCL_{05}$ continuous, ppm	Comments
9 months	Multistage	2.47	0.78*	35.0	19.25	3.44	Full data set, N = 58
15 months	Log probit	10.74	0.030	11.2	3.66	0.654	Full data set, N = 52
	Log probit	10.64	0.014	11.1	3.45	0.616	Without top dose, N = 50
24 months	Log probit	6.47	0.091	0.056	0.0034	0.00054	Full data set, N = 325
	Log probit	2.80	0.42*	0.254	0.031	0.0055	Without top dose, N = 246
24 months#	Log logistic PBPK mean blood DEB concn	0.1	0.99	1.21 µM	0.56 µM, 0.71 ppm BD equivalent	0.51 ppm BD equivalent	Full data set, N = 325
9-24 mo time adjusted**	Log probit	1.7	0.64*	2.04	1.01	0.18	Full data, N = 435
9-24 mo time adjusted**	Log logistic	1.13	0.89*	2.03	1.58	0.28	Full data, N = 435

Note: * indicates adequate model fit to data, P ≥ 0.1; ** time adjustment by fitting % atrophy data to the hyperbola $y\ (\%) = a - b/(1 + c \times dose)^d$ where a, b, c, and d are parameters, the dose was ppm butadiene (6hr/day, 5days/week), and the % atrophy response was weighted for time (PSI-Plot Hyperbola c, www.polysoftware.com). The fitted values were reconverted to quantal equivalents and analyzed by BMDS 2.2 with log probit, log logistic and multistage models (see Tables A8-A11 in appendix for additional details). # PBPK model simulation was for 24 hr and the 24 hr DEB AUC was divided by 24 to give average daily concentration so continuous adjustment is only 5/7 for 5 days/week exposure.

The values from Figure 2 of Doerr et al. (1996) were estimated as shown in Table 4. Internal dose metrics were then analyzed by benchmark dose methods using BMDS (versions 1.4.1c and 2.1.2). The benchmark dose BMD_{05} was the 0.05 relative deviation of a given dose metric typically using the Hill or Polynomial continuous dose response model and the $BMDL_{05}$ was the 95% lower confidence limit on that dose. The 5% response level is typically chosen by OEHHA for noncancer toxicity endpoints. The PBPK model was based on Kohn and Melnick (2001). The formation of hemoglobin adducts in the model was based in part on Csanady et al. (2003) with additional ACSL model code obtained from Prof. J.G. Filser (personal communication).

Table 4. Mean Ovarian and Uterine Weights (% Body Weight) in Mice Estimated from Figure 2 of Doerr et al. (1996).

Compound	Dose i.p., mmol/kg-d	Ovary	SD*	Uterus	SD*
BMO	0.005	0.0425	0.007	0.27	0.126
	0.02	0.0425	0.007	0.30	0.126
	0.09	0.038	0.007	0.28	0.126
	0.36	0.034	0.007	0.27	0.126
	1.43	0.020	0.007	0.10	0.126
DEB	0.002	0.0375	0.007	0.34	0.126
	0.009	0.034	0.007	0.33	0.126
	0.036	0.035	0.007	0.22	0.126
	0.14	0.020	0.007	0.11	0.126
	0.29	0.015	0.007	0.03	0.126

*SD values assumed equal for BMD analysis since they appear similar for low doses and decrease for the high doses.

Following the 30 days of exposure, ovaries and uteri were weighed, fixed, stained, and the follicular populations were assessed microscopically. In mice receiving 1.43 mmol BMO /kg, body weights were slightly decreased (10%) while ovarian and uterine weights were significantly decreased ($p < 0.05$). Administration of DEB caused significant decreases in both ovarian and uterine weights at 0.14 and 0.29 mmol/kg b.w. ($p < 0.05$), along with a 15% decrease in body weights at the higher dose. A dose-dependent decrease in ovarian follicle numbers was observed with both epoxides. To facilitate comparison, the dose required to reduce follicle numbers by 50% relative to controls (ED_{50}) was estimated. For small and growing follicles, the ED_{50} values for BMO were 0.29 and 0.40 mmol/kg, respectively. DEB showed greater potency with ED_{50} values for these same follicle populations of 0.10 and 0.14 mmol/kg, respectively. These authors suggested that butadiene ovotoxicity may be associated with metabolism of the parent compound to reactive diepoxides in situ.

Perez et al. (1997) measured N-(2,3,4-trihydroxybutyl)valine (THBV) adducts of hemoglobin in rats exposed to 1,3-butadiene or butadiene epoxides. Sprague-Dawley rats (2 to 3/group) were treated with single i.p. doses of either epoxybutene (78.3 mg/kg), epoxybutanediol (30 mg/kg), or diepoxybutane (16,7, 33.4, 60 mg/kg). The

animals were sacrificed and blood was collected after 48 hr. Rats (3/group) were exposed to 1,3-butadiene by inhalation for 6 hr/d for 5 days at concentrations of 0, 50, 200 or 500 ppm. Two humans occupationally exposed to 1 ppm butadiene were also analyzed. The HBI (hemoglobin binding index, pmol THBV adduct/g/µmol/kg for epoxides, or /ppm-hr for butadiene exposures) for rats was 0.07 for epoxybutene, 3.4 for epoxybutanediol, and 4.0 to 9.3 for diepoxybutane. For butadiene exposures in rats, THBV adducts ranged from 0.04 to 0.5 pmol/g/ppm-hr. For the exposed human subjects, the THBV adduct levels were 0.15 and 0.20 pmol/g. The results indicate that epoxybutanediol is comparable to DEB in it adduct forming ability and considerably more active than BMO.

In the Hazelton Laboratories study, Sprague Dawley rats were exposed to 0, 1000, or 8000 ppm butadiene for 6 hr/day, 5 day/week, for 105 weeks. Although investigators reported tumors in certain reproductive organs, there was no clear statement on the presence or absence of ovarian atrophy, or any other reproductive effect in females (Owen et al., 1987).

Male reproductive effects have also been observed in relation to chronic butadiene exposure, although generally at concentrations greater than those that elicited effects in female animals. In the NTP study, investigators observed a significant concentration-related decrease in testis weight at weeks 40 and 65 for males exposed to ≥ 62.5 ppm butadiene (Melnick et al., 1990). In addition, testicular atrophy (measured at weeks 40 and 60) was observed in males exposed to 625 ppm butadiene (Melnick et al., 1990). In the later NTP study (1993), investigators once again observed testicular atrophy at 625 ppm, and reduced testicular weights in the 200 ppm and 625 ppm exposure groups at the end of the study. However, no effect was seen at the lower butadiene concentrations, as noted in female mice (NTP 1993). In the Hazelton Laboratories study, there was no clear statement of the presence or absence of testicular atrophy in male rats exposed to up to 8000 ppm butadiene. However Leydig cell tumors were observed in 3 males in the 1000 ppm group and 8 males in the 8000 ppm group (Owen et al., 1987).

Kirman and Grant (2012) conducted a meta-analysis with dose-response data from rats and mice that were normalized using an internal dose estimate of diepoxybutane (DEB) in blood that is causally related to ovarian toxicity. A time-to-response (multistage-Weibull) model was used to fit the pooled data sets with exposures from 13 to 105 weeks. Reference concentration (RfC) values were derived for scenarios with average, low and high follicle counts at birth with differing durations of susceptibility to toxic action of DEB. The $BMCL_{01}$ points of departure for these scenarios in ppm continuous butadiene exposure were 17, 1.5, and 240 ppm, respectively. The authors applied a 10-fold uncertainty factor to these values to derive a range of presumably health protective values for human exposures.

7 Chronic Toxicity of 1,3-Butadiene

7.1 Chronic Toxicity to Adult Humans

Studies on the chronic effects of butadiene have been centered in the styrene-butadiene rubber manufacturing industry and the butadiene monomer industry. The Occupational Health Studies Group at the University of North Carolina, Chapel Hill, organized one of the earliest retrospective epidemiological studies conducted in the rubber industry. Investigators observed rubber workers during the period of 1964 – 1972 and reported an increase in overall mortality, emphysema, and cardiovascular diseases (chronic rheumatic and atherosclerotic heart disease) among the subjects (McMichael et al., 1976).

Penn and Snyder (2007) reviewed the evidence supporting an association between butadiene exposure and cardiovascular disease. Two independent longitudinal epidemiological studies have indicated an association. Matanoski et al. (1990) found that butadiene exposure in a styrene-butadiene manufacturing facility was associated with a significant increase in the standardized mortality ratio (SMR: the ratio for mortality in the workers compared to the general population) for arteriosclerotic heart disease in exposed black workers (SMR = 1.48 (95%CI, 1.23-1.76)). The SMRs were adjusted for age, calendar time, and race. Circulatory system disease was also significantly elevated in 214 of 1195 exposed black workers (SMR = 1.18 (95%CI, 1.03-1.35)). Significant differences in these diseases were not seen in 10,915 exposed white workers. In both cases the referent population was U.S. males. Devine (1990) and Devine and Hartman (1996) studied a cohort of nearly 2800 male workers employed at least 6 months at a 1,3-butadiene manufacturing facility. They found significantly elevated SMRs for all causes of death including arteriosclerotic heart disease in nonwhite workers (SMR = 1.28 (95%CI, 1.06-1.53)). For arteriosclerotic heart disease the SMR was 1.42 (95%CI, 0.95-2.04). The referent population was the U.S. nonwhite male mortality rates. Butadiene exposure was also associated with atherogenesis in an avian model (see 7.3 below).

Additional arguments have been made in regard to polymorphisms for xenobiotic metabolizing genes including GSTM1, GSTT1, CYP2E1, and microsomal epoxide hydrolase (EH) and susceptibility to atherosclerosis. Salama et al.(2002) studied 120 atherosclerotic subjects and 90 matched controls for associations between polymorphisms in metabolizing genes and atherosclerotic susceptibility. They found increased atherosclerotic susceptibility associated with the GSTT1 null allele and the fast allele of EH. Also individuals with one of three combined phenotypes (GSTM1 null/CYP2E1 5B, GSTM1 null/mEH YY and GSTT1 null/mEH) exhibited significantly increased atherosclerotic susceptibility (ORs = 3.4-15.42). The importance of EH as a potential cardiovascular disease susceptibility gene in population subgroups and as a key player in butadiene metabolism may influence the AR potential of butadiene in individuals exposed to ETS.

Johns Hopkins University and the National Institute for Occupational Safety and Health initiated two large cohort studies of North American synthetic rubber industry workers

(Meinhardt et al., 1982; Matanoski et al., 1990). Investigators studied male workers at eight synthetic rubber plants and two plant complexes in Texas, Louisiana, Kentucky, and Ontario, and included men who began their employment as far back as 1943. Results from these studies were the first to report increased risk of hematologic neoplasms from workplace exposure to butadiene. The University of Alabama designed a series of follow-up epidemiological studies, and has since reported an excess of leukemia among hourly workers with long duration of employment and high butadiene exposure (Delzell et al., 1996), as well as a positive association between cumulative exposure to butadiene and leukemia risk (Macaluso et al., 1996). More recently, University of Alabama investigators evaluated all-cause mortality for the same synthetic rubber industry workers from 1944 – 1998 (Sathiakumar et al., 2005). The subjects had an overall mortality rate 14% lower than expected, including lower rates of cardiovascular, digestive, and genitourinary deaths than the general population. However, the total group of 17,924 subjects had 16% more leukemia deaths than expected. While this increase was not limited to a single form of leukemia, it was concentrated in long-term hourly workers particularly those in polymerization, coagulation, and maintenance positions (Sathiakumar et al., 2005).

Results from studies of the genotoxic outcomes in workers exposed to butadiene have not been consistent. Begemann et al. (2001) analyzed blood samples from 17 workers in butadiene monomer production and 19 controls from a heat plant in the Czech Republic. Butadiene exposure was assessed by personal monitoring, with a median exposure concentration of 440 $\mu g/m^3$ for exposed workers and < 6 $\mu g/m^3$ for control subjects. The hemoglobin adduct HBVal, which is formed by a reaction of the *N*-terminal valine with the carbon-1 of epoxybutene, was measured with a limit of detection of 0.2 pmol/g globin. Results showed that the median butadiene-hemoglobin adduct level in monomer production workers was significantly higher than that of controls, with no consistent correlation with any other parameter except smoking (Begemann et al., 2001).

Hayes et al. (2000) also measured a spectrum of genotoxic outcomes, including aneuploidy and sister chromatid exchange, in 41 butadiene polymer production workers and 38 non-exposed controls in China. Full-shift personal exposure varied widely, and short-term breathing zone grab samples showed even greater extremes (median 6.5 ppm, range 0 – 1078 ppm). Hemoglobin adducts were significantly more common in butadiene exposed workers than controls ($p < 0.0001$). In addition, butadiene exposed workers had greater lymphocyte and platelet counts than controls. However, the frequency of total aneuploidy (chromosomes 1, 7, 8, 12) did not differ significantly between exposed and non-exposed workers, nor did structural or numerical chromosomal abnormalities. The authors found no correlation between hemoglobin adducts and any genotoxic markers, nor did they find a positive correlation between butadiene concentrations in air and sister chromatid exchanges (Hayes et al., 2000).

Lovreglio and colleagues (2005) conducted personal monitoring throughout an 8 hr work shift at a petrochemical plant where butadiene was produced and polymerized. Personal monitoring was conducted 3 to 4 times over a 6-week period, with the last session followed by blood sampling of the subjects. Twenty-seven healthy petroleum

plant workers were matched with 26 controls not occupationally exposed. Results indicated significantly higher airborne butadiene levels for exposed workers when compared with controls, ranging from 0.2 to 69 μg/m^3. Blood samples were analyzed for sister chromatid exchange and percent chromosomal aberrations in peripheral blood lymphocytes. After correcting for cigarette smoking, the investigators found no genetic marker differences between butadiene exposed and control workers. In their conclusion, the authors stated that there was a lack of genotoxicity in workers exposed to very low concentrations of butadiene similar to ambient levels currently characterized in the general population (Lovreglio et al., 2005).

The Health Effects Institute reported on a genetic epidemiology study of Czech workers exposed to butadiene (Albertini et al., 2003). The study was designed to determine whether biomarkers of exposure could be correlated with in situ levels of butadiene and personal workplace exposure. Blood and urine samples were collected from males, working in either a butadiene monomer production plant or in a butadiene-styrene polymerization facility. Samples were also collected from male administrative workers who had very limited occupational exposure to butadiene, and served as controls. In addition, full-shift personal monitoring was conducted on each of 10 days over a 60-day period for both groups of workers, including the days on which biological samples were collected. The mean concentrations ranged from 0.026 mg/m^3 for controls to 1.76 mg/m^3 for polymer workers, with maximum concentrations of 0.125 mg/m^3 and 39 mg/m^3, respectively (Albertini et al., 2003). Cytogenetic assays included chromosomal aberrations, breaks, translocations, and sister chromatid exchanges. No significant relations could be identified between group mean chromosomal aberrations, determined by the conventional method or by fluorescent in situ hybridization, and butadiene exposure levels after adjusting for age and smoking. Since tobacco smoke contains butadiene it is possible that this latter adjustment affected the results. In addition, there was no relationship found between butadiene exposure and aberrations associated with glutathione-S-transferase genotypes. Regression analyses showed no significant effects of butadiene exposure on sister chromatid exchanges, although the effect of smoking on sister chromatid exchanges was significant after adjusting for age and occupational butadiene exposure. Hemoglobin adducts of butadiene (to the valine residue) were analyzed as biomarkers for butadiene exposure. There was a significant correlation between the individual subject's exposure levels and two types of hemoglobin adducts. However, the authors cautioned that one adduct, trihydroxybutyl valine (THBVal), also appeared to be formed by an endogenous material other than butadiene and its metabolites, indicating relatively high natural background levels. Overall conclusions from the authors indicate that the cytogenetic endpoints did not prove to be sensitive indicators of butadiene exposure at the levels encountered in these facilities (Albertini et al., 2003).

Since butadiene exposures rarely occur in the absence of other air toxic contaminants it is usually difficult to isolate the effects due to butadiene from those due to other chemicals or combinations. It is OEHHA policy to use human data in preference to animal data when they are of suitable quality. However, in the case of butadiene there are no human dose-response data that can be used to derive a chronic REL with confidence.

7.2 Chronic Toxicity to Infants and Children

Infants and children are rarely exposed to butadiene alone but rather experience long-term exposure to butadiene as one of the combustion products in vehicle exhaust, environmental tobacco smoke (ETS), and other combustion processes. As can be expected with many toxic air contaminants, it is difficult to elucidate the effects of one particular pollutant when it exists in the presence of multiple co-pollutants. Studies of the chronic effects of butadiene in children primarily focus on carcinogenesis as the endpoint. Butadiene has been associated with asthma exacerbation and increased respiratory tract infections, but only in the presence of other pollutants. While some recent studies have aimed to quantify children's exposure to butadiene, ascribing chronic respiratory effects to butadiene alone remains problematic (see discussion of TEACH study in section 3 above).

7.3 Chronic Toxicity to Experimental Animals

Butadiene is considered a multi-site carcinogen in all identified long-term studies in mice and rats, although there appear to be strain and species differences in sensitivity and severity of effect (NTP 1984; Owen et al., 1987; Melnick et al., 1990; NTP 1993). Instances of tumors include malignant lymphomas, histiocytic sarcomas, cardiac hemangiosarcomas, as well as neoplasms of the liver, lung, mammary gland, ovary, and forestomach (NTP, 1993).

Far fewer data are available for the non-cancerous effects of long-term exposure to butadiene. However, based on these limited data, sensitivity to non-neoplastic events appears to be consistent with species variations in metabolism of butadiene and quantitative differences in the formation of butadiene metabolites (Hughes et al., 2001). For mice, National Toxicology Program (NTP) investigators exposed B6C3F1 males and females to 0, 625, or 1250 ppm butadiene for 6 hrs/day, 5 days/week, for up to 61 weeks (NTP 1984). Liver necrosis was observed in male mice at both doses and in female mice at 1250 ppm. At the highest dose, adverse changes were observed in the nasal cavity, including non-neoplastic lesions, chronic inflammation, fibrosis, cartilaginous metaplasia, osseous metaplasia, and atrophy of the sensory epithelium. In a later study, investigators studied the effects of lower concentrations of butadiene in mice exposed for 6 hrs/day, 5 days/week, for up to 2 years (Melnick et al., 1990; NTP 1993). Two-year survival was significantly decreased in mice exposed to ≥ 20 ppm, which the investigators attributed to butadiene-induced malignant neoplasms. Beyond the neoplastic changes, there were increased incidences of angiectasis, alveolar epithelial hyperplasia, forestomach epithelial hyperplasia, and cardiac endothelial hyperplasia.

Chronic effects in rats were studied by Hazelton Laboratories and reported later by Owen and colleagues (1987; 1990). Groups of 110 male and female Sprague Dawley rats were exposed to 0, 1000, or 8000 ppm butadiene for 6 hrs/day, 5 days/week, for up to 2 years. After the first 52 weeks, investigators euthanized multiple female rats in both dose groups because of the high incidence of large subcutaneous masses. Increased mortality in males was observed in the highest dose group and associated with

increased nephropathy. Body weight decreases were observed in the first twelve weeks in the high dose males and females, along with liver and kidney organ weight changes. Although there were minor clinical effects, including slight ataxia, no major changes in neuromuscular function could be definitively associated with butadiene exposure (Owen et al., 1987). Further reporting on this 2-year chronic investigation indicated that there were decreased survival rates at 105 weeks for females and 111 weeks for males (Owen and Glaister 1990). There were no effects on hematology, blood chemistry, and urinalysis, although changes in body and organs weights continued until study termination. Although somewhat equivocal, the rats displayed both common and uncommon tumor types, leading the investigators to suggest that butadiene might be a rat oncogen (Owen and Glaister 1990).

Other studies have identified hematological effects following chronic exposure to butadiene at concentrations at or below those that were associated with systemic toxicity (Irons et al., 1986a; Irons et al., 1986b; NTP 1993). Such hematotoxicity may be associated with the observed changes in organ weights (NTP, 1993). Butadiene exposure has also been associated with bone marrow changes, including bone marrow atrophy, bone marrow toxicity (regenerative anemia), and decreased cellularity as observed in mice exposed to ≥ 62.5 ppm (Melnick et al., 1990; NTP, 1993). Owen and colleagues (1987), however, found no hematological effects in rats exposed to as much as 8000 ppm butadiene in a 2-yr bioassay.

Penn and Snyder (2007) have identified vapor phase butadiene as the principal component in environmental tobacco smoke (ETS) promoting plaque development in an avian model of accelerated atherosclerosis (20 ppm butadiene x 16 weeks). The total butadiene doses that produced atherogenic effects were only $1/80^{th}$ and $1/1000^{th}$ the minimal doses causing cancer in mice and rats, respectively. The authors note that side-stream smoke, which represents about 90 % of ETS, contains butadiene at 205 to 400 µg/cigarette versus 16 to 77 µg/cigarette in main-stream tobacco smoke. Furthermore, Penn and Snyder (1996, 2007) found that the atherogenic effect of butadiene exposure was apparently not associated with either of the two principal epoxy metabolites of BD; 1,3-epoxybutene and 1,2:3,4-diepoxybutane. Neither of these metabolites was increased by butadiene exposure in avian liver in contrast to mouse liver.

7.4 Mode of Action

There is currently no accepted mode of action for the acute or chronic effects of butadiene exposure noted in this document. The metabolism of butadiene in all species studied produces epoxide, diepoxide and epoxydiol metabolites in addition to glutathione conjugates and 1,2-butene-3,4-diol. The epoxides all exhibit mutagenic activity in various test systems and react with DNA and proteins to form adducts. Butene diol has exhibited the ability to cause single strand breaks in DNA (Zhang et al., 2012). Adduct forming activity has also been observed for the glutathione conjugate of the butadiene metabolite DEB (Cho and Guengerich, 2012a). One of the DEB-conjugates (*S*-(2-Hydroxy-3,4-epoxybutyl)glutathione) was observed to be more mutagenic than DEB or other BD-epoxides in *Salmonella typhimurium* TA1535 (Cho and Guengerich, 2012b). The ability of DEB-GSH conjugates to form DNA adducts and cause mutations indicate a level of reactivity that may extend to protein binding. While mutagenicity of epoxide metabolites and other genetic toxicity is usually associated with oncogenic effects of butadiene and related chemicals, these genotoxic effects may also be associated with non-cancer effects such as cardiovascular disease, neurodegenerative disease, diabetes mellitus, rheumatoid arthritis, and aging (Burnet, 1974, Cooke et al., 2006). Recent reports indicate a specific mutation (*TREM2*) associated with increased risk of Alzheimer's disease in humans (Guerreiro et al., Jonsson et al., 2012).

In addition to the formation of pyr-Val-Hb and related hemoglobin adducts and their use as biomarkers of internal exposure to specific butadiene epoxy metabolites in butadiene exposed human subjects, such adducts are also indicative of more general reaction of these metabolites with cellular proteins. The generalized organ and body adverse effects induced by butadiene in mice: fetal body weight decreases, relative organ weight decreases (lung, liver and kidney), and tissue atrophy (ovary, testes, nasal olfactory epithelium) are indicative of, or at least could be plausibly associated with, epoxy adduct formation of key cellular proteins and/or nucleic acids. Such adducts may lead to impaired cellular function and observed organ/tissue effects. The observed neurotoxic effects of butadiene exposure may also be due to reactive epoxide metabolites binding to key proteins and nucleic acids of the central nervous system.

Oxidative stress induced by BD metabolism may also play an important role in the observed tissue/organ toxicity and possible chronic effects in humans exposed to butadiene (Primavera et al., 2008; Yadavilli et al., 2007). Products of butadiene combustion have been observed to inhibit catalase activity and induce oxidative DNA damage repair enzymes in human bronchial epithelial cells in vitro (Kennedy et al., 2009). While the atherogenic effect of butadiene seen in an avian model does not have a clear link to the principal epoxide metabolites of butadiene, it may be related to ETS-induced oxidative stress as seen in mouse heart tissue (Yang et al., 2004). Alternatively, previously unidentified metabolites like the GSH-conjugates of Cho and Guengerich (2012a,b) may be involved in atherogenesis.

8 Derivation of Reference Exposure Levels

8.1 1,3-Butadiene Acute Reference Exposure Level (aREL)

Study	Hackett et al. (1987)
Study population	78 Pregnant CD-1 mice and offspring
Exposure method	Whole body inhalation of 0, 40, 200 or 1000 ppm on GD 6 through 15
Critical effects	Lowered male fetal weight at GD 18
LOAEL	200 ppm
NOAEL	40 ppm (based on Green re-analysis)
$BMCL_{05}$	17.7 ppm (Hill Model)
Exposure duration	6 hr
Time-adjusted exposure	n/a
Human Equivalent Concentration	29.7 ppm (17.7 ppm * 1.68 DAF)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	√10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	√10
Cumulative uncertainty factor	100
Inhalation reference exposure level	297 ppb (660 µg/m^3)

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in noncancer adverse health effects (see Section 5 of the Technical Support Document).

The study by Hackett et al. (1987) was selected as it addressed the most sensitive noncancer endpoint associated with butadiene inhalation: developmental effects. A continuous benchmark dose analysis of the male mouse fetal weight data from Table 2 was conducted and the results are presented in Table 5. Both applied and PBPK doses were used but the latter showed little improvement in model fit or values derived. The best value based on the applied dose and the Hill model using the values from the Green (2003) re-analysis of the Hackett data was 17.7 ppm for the mouse and 29.7 ppm for the human equivalent concentration. This analysis in BMDS (v 2.3.1) was based on the male pup data and the homogenous variance assumption, due to the exact model fit and little evidence of differing variance in the exposed groups. We analyzed all the data (male and female) as well as the males only and the males consistently gave better model fits and lower $BMCL_{05}$ values (Table 12A). The nonhomogeneous variance assumption based BMC/BMCL values were about 50% lower than the homogeneous variance values and the model fit, while acceptable, was not exact (see Table A12). In the end we chose the lowest BMCL value giving the best model fit (male pups, Green, 17.7 ppm).

A second mouse PBPK model based on rat and human pregnancy (Gentry et al., 2002; Poet et al. 2010) allowed estimates of fetal exposure to BMO (mean fetal AUC GD 9-18). This metric also gave an exact fit to the fetal body weight data (Table 5). In the summary acute REL calculation above, we employed the standard intraspecies toxicokinetic uncertainty subfactor (UF_{H-k}) of 10: this is specifically justified to account for observed human variability due to polymorphisms in the microsomal epoxide hydrolase (mEH) gene reported by Abdel-Rahman, Wickliffe, Ward and co-workers (Abdel-Rahman et al., 2003, 2005; Wickliffe et al. 2003). When the interaction between BD exposure and inheritance of a variant ATT allele for mEH was evaluated in 49 exposed workers, the increase in HPRT variant mutant frequency was increased to $10.89 \pm 2.16 \times 10^{-6}$ (95% C.I. = 6.56-15.20 ($\times 10^{-6}$), p = 0.0027) over the background rate of $4.02 \pm 1.32 \times 10^{-6}$ (Abdel-Rahman et al. 2005). In this case, the background is not an unexposed general population but rather that portion of the worker study population (N= 33) with exposures < 150 (18.4 ± 5.5) ppb BD. Since mEH is a key enzyme in the detoxification of butadiene epoxide metabolites, low activities in this gene/enzyme translate to increased internal concentrations of these metabolites and risk of related toxic effects. The dosimetric adjustment factor (DAF) is related to the ratio of human and animal blood/air partition coefficients for butadiene and was estimated using mouse and human PBPK models. For example, the butadiene blood/air partition coefficient for rodents used by Kohn and Melnick (2001) was 1.95 and the butadiene blood/air partition coefficient for humans used by Brochot et al. (2007) was 1.22 ± 0.30. This would indicate a DAF based on these values of 1.60. OEHHA's estimates using rodent and human PBPK models was 1.68. That is:

DAF = PBPK predicted human blood concentration/PBPK predicted animal blood concentration and;

Human equivalent concentration (HEC) = Animal experimental concentration x DAF.

Table 5. Benchmark Dose Analysis of Male Mouse Fetal Weight Data of Hackett et al. (1987).

Dose Metric	N, male fetuses	Model	BMC_{05}	$BMCL_{05}$	$BMCL_{05}$ ppm BD equivalent	Human ppm equivalent 1.68 DAF
Applied BD ppm 6 hr/d						
0	109	Hill (Green reanalysis)	37.2**	17.7**	17.7**	29.7
40	118	Hill (Hackett et al. original values)	28.5**	13.4**	13.4**	22.5
200	133	Polynomial	48.8	41.1	41.1	69.0
1000	126	Power	261 n.s.	225 n.s	225 n.s.	378 n.s.
PBPK AUC Maternal BMO µMhr/d						
0	109	Hill	134**	70.1**	16.5**	27.8
166.4	118	Polynomial	100	69.2	14.9	25.0
371.9	133	Power	106	90.7	19.8	33.2
493.8	126					
PBPK AUC Fetal BMO µMhr/d[a]						
0	109	Hill	10.9**	5.1**	13.4**	22.5
15.2	118					
74.3	133					
356.7	126					

Note ** indicates exact model fit by graph and tabular output, P values were not applicable for exact fits of the Hill model to the continuous data sets or given as P <0.0001 for the other models despite obvious high degrees of fit visually and by tabular output of observed and predicted values; n.s. indicates insignificant model fit (P < 0.1)

[a] based on average fetal BMO AUC during gestation days 9-18.

8.2 1,3-Butadiene 8-Hour Reference Exposure Level

Study	NTP (1993) supported by Doerr et al. (1996)
Study population	B6C3F1 mice
Exposure method	Daily inhalation
Exposure continuity	6 hr/d, 5d/wk
Exposure duration	9-24 months
Critical effects	Increased incidence of ovarian atrophy
$BMCL_{05}$	1.01 ppm
Time-adjusted exposure	758 ppb (1.01 ppm x 6/8 hr/d)
Human Equivalent Concentration	1.27 ppm (0.758 ppm x1.68 DAF)
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	$\sqrt{10}$
Cumulative uncertainty factor	300
Reference Exposure Level	4.2 ppb (9 $\mu g/m^3$)

The 8-hour Reference Exposure Level is a concentration at or below which adverse non-cancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the TSD). The study of NTP (1993) and the endpoint of ovarian atrophy was selected as the basis of both the 8 hr and chronic RELs. The benchmark concentration analysis of this study is summarized in Table 3 above. We selected the value of 1.01 ppm for the $BMCL_{05}$ based on the full data sets of 9, 15 and 24 months exposures as the best basis for the derivations. This study is supported by that of Doerr et al. (1996). The Doerr study involves PBPK modeling (Table 6) to estimate internal dose metrics for ovarian effects resulting from i.p. doses of mono- or diepoxide metabolites of BD. Dose-response modeling (BMDS v.2.1.2) was used to estimate $BMDL_{05}$'s for the various dose metrics evaluated.

The selected best point of departure, 1.27 ppm, is the $BMCL_{05}$ adjusted for time and human equivalent exposure. The interspecies uncertainty subfactor for toxicokinetics (UF_{A-k}) was reduced from $\sqrt{10}$ to 1 because we think that the mouse is more active in metabolizing butadiene to reactive epoxide metabolites than either the rat or human, thus reducing the uncertainty in the interspecies toxicokinetic extrapolation.

The other departure from defaults which we have made in this derivation is that we have increased the toxicodynamic subfactor (UF_{A-d}) from the default value of $\sqrt{10}$ to 10, based on the uncertainty arising from potentially greater human response to the ovotoxic effects of butadiene epoxide metabolites, particularly diepoxybutane (DEB), as compared to the mouse model from which the primary data for risk calculations were derived. Humans differ substantially from mice in lifespan and in the time available for

chronic exposure to effect ovotoxicity which is far longer in humans, and the generally greater robustness of the mouse reproductive system relative to the human (e.g., see He et al., 2007). Chemicals structurally similar to butadiene, specifically the butadiene dimer 4-vinylcyclohexene (VCH) and its diepoxide (VCD) are established rodent ovotoxicants. These related chemical species produce selective destruction of primordial and primary follicles, premature ovarian failure and increased risk of ovarian tumors (Smith et al., 1990; Ito et al., 2009; Mark-Kappeler et al., 2011). Mechanistic studies of the ovotoxic effects of VCD indicate that activation of proapoptotic signaling events in the Bcl-2 and mitogen-activated protein kinases play a role (Hu et al., 2001a, b, 2002). VCD causes a physiological form of ovotoxicity in which follicle loss is "silent" and mimics normal follicular atresia. Thus the damage caused by VCD, were this to be paralleled in humans, could go unnoticed in exposed and affected individuals leading one investigator to surmise that "As a result, because VCD only targets ovarian preantral follicles, chronic exposure in women to low levels of this chemical may represent a risk for early menopause without prior evidence of disrupted menstrual cycles" (Mark-Kappeler et al., 2011) (also see Hoyer and Sipes, 2007).

Table 6. Modeled Butadiene Inhalation Resulting in Ovarian and Uterine Atrophy Following I.P. Butadiene Mono- and Diepoxide Exposures

Data Set	Metric	Model	P for fit	BMD_{05}	$BMDL_{05}$	6 hr ppm mouse equivalent
Ovary						
BMO i.p.	AUC BMO blood µMhr/d	Power	0.76	2688	1765	N.A.
	AUC DEB blood µMhr/d	Poly	0.92	57.4	20.5	1.80
BMO i.p.	Hb adducts nmol/g/d	Poly	0.92	34.8	15.6	N.A.
DEB i.p.	Hb adducts nmol/g/d	Hill	0.43	183.3	85.1	N.A.
Uterus						
BMO i.p.	AUC BMO blood µMhr/d	Power	0.36	2688	1765	N.A.
	AUC DEB blood µMhr/d	Poly	0.84	103.1	37.9	3.47
BMO i.p.	Hb adducts nmol/g/d	Poly	0.85	799	117	N.A.
DEB i.p.	Hb adducts nmol/g/d	Poly	0.85	799	116.8	N.A.

Notes: BMO = butadiene mono epoxide; DEB = diepoxybutane; EBD = epoxybutenediol; AUC = area under the blood concentration x time curve; Hb = hemoglobin,
table BMD/L values are the average of 30 consecutive daily i.p. administrations,
ppm equivalent is inhalation exposure of butadiene (BD) giving the same internal BMDL dose,
N.A. indicates values unachievable by BD inhalation,
Poly is the polynomial model for continuous dose response in BMDS software,
BMD_{05} indicates 5% relative risk and BMDL the 95% lower confidence limit on the BMD value.

VCH is an industrial chemical released during the manufacture of rubber tires, plasticisers and pesticides, and human exposures to that chemical are likely to be limited to occupational settings. The similarity of the ovotoxcity of VCD, as a model system, however, indicates that of DEB may pose risk derived from mechanistic similarities. Should such mechanistic similarities exist, human females exposed chronically to DEB via butadiene may be at risk of silent ovotoxic effects that would not be manifest for many years. Because of these reproductive uncertainties, OEHHA believes it is adviseable to reject the lower and less conservative default value in favor of a larger UFA-d until further relevant information is available.

The PBPK modeling used two related models. PBPK Model 1 simulates butadiene plus three metabolites BMO, DEB, and butadiene diol (BDD) and estimates EBD as a metabolized dose (AMET). PBPK Model 2 added EBD as a circulating metabolite and erythritol as a metabolized dose (AMET). Both models use parameters and model structure from Kohn & Melnick, 2001. The ovary and uterus data sets (% of body weight vs. i.p. dose) are from Doerr et al. (1996) Fig 2. The general metabolic scheme is: BD →BMO→DEB→EBD→Erythritol and BMO→BDD→EBD→Erythritol. All metabolic steps have Michaelis-Menten kinetics for P450 oxidation, epoxide hydrolases (EH) or the ordered bi bi rate law for glutathione sulfotransferases (GST). Further details of the GST kinetics can be found in Kohn and Melnick (2000). Essentially, the GST reaction rate (moles/hr) is the product of the Vmax, substrate (Ci) and glutathione concentrations (CGSH) divided by the sum of the cross products of the Km's (Kmi, Kmgsh) and tissue concentrations of the substrate and glutathione:

$$V = Vmax*Ci*CGSH/(Kmi*Kmgsh + Kmi*CGSH + Kmgsh*Ci + Ci*CGSH)$$

All model simulations were conducted with Berkeley Madonna software. Additional details on the PBPK modeling including parameters, graphical and tabular model output, benchmark dose analysis, and sample model code are given in the Appendix.

8.3 1,3-Butadiene Chronic Reference Exposure Level (cREL)

Study	NTP (1993) supported by Doerr et al. (1996),
Study population	B6C3F1 mice
Exposure method	Daily inhalation
Exposure continuity	6 hr/d, 5d/wk
Exposure duration	9-24 months
Critical effects	Increased incidence of ovarian atrophy
$BMCL_{05}$	1.01 ppm
Time-adjusted exposure	180 ppb (1.01 ppm x 6/24h x 5/7d)
Human Equivalent Concentration	302 ppb (180 ppb x 1.68 DAF)
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	√10
Cumulative uncertainty factor	300
Reference Exposure Level	1.0 ppb (2.2 μg/m^3)

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from continuous chronic exposures (see Section 8 in the Technical Support Document). In the derivation of the chronic REL above, the $BMCL_{05}$ is the same as the 8-hour REL but the time adjustment is for potentially continuous exposure rather than 8 hours/day. The uncertainty factors applied are the same in both cases (i.e., 8-hour and chronic) and the proposed RELs are similar to those derived from the supporting study of Doerr et al. (1996), namely 23 ppb and 8 ppb for 8-hour and chronic RELs , respectively. This latter study uses the same toxicity endpoint but with parenteral administration of the butadiene epoxide metabolites BMO and DEB.

Butadiene causes non-cancer effects following chronic exposure, the most important of which appears to be reproductive toxicity. Inhalation exposure to relatively low concentrations of butadiene over a lifetime is associated with adverse effects in the reproductive organs of female and male mice (NTP, 1984; Melnick et al., 1990; NTP, 1993; Doerr et al., 1996). A PBPK model was developed to model the disposition of butadiene and its metabolites based on a rodent model described by Kohn and Melnick (2001), and data on the ovarian toxicity of 1,3-butadiene epoxide in rats and mice (Doerr et al.,1996).

As reported earlier, ovarian atrophy was observed at all concentrations in the NTP chronic bioassays (NTP, 1984; Melnick et al., 1990; NTP, 1993), and showed a dose-dependent effect associated with increased inhalation exposure. In all exposure groups, ovarian atrophy was associated with what might be considered premature reproductive senescence. It is noteworthy that such senescence was observed as early

as nine months in female mice exposed to 200 or 625 ppm, and after 15 months in female mice exposed to ≥20 ppm butadiene (NTP, 1984; NTP, 1993). It is currently unknown how ovarian atrophy in rodents relates to human reproductive senescence. Reproductive senescence is a general term referring to the loss of reproductive function with age. In the female, this involves a loss of normal ovarian, and hypothalamic or pituitary function (Valdez and Petroff, 2004). The correlation between the loss of oocytes and declining fertility with age is well characterized, and it is generally accepted that depletion of follicles results in the final loss of fertility. The actual end of ovarian activity is preceded by a period of irregular reproductive cycles and subfertility in both women and rodents (Valdez and Petroff, 2004). Age-related changes in the rodent hypothalamus and pituitary appear to bring about the final cessation of ovarian cyclicity before follicular depletion occurs (Wise et al., 2002). However, exhaustion of ovarian follicular reserves appears to drive menopause in women (vom Saal et al., 1994). Despite these apparent differences in final reproductive senescence, the biological mechanisms leading to prolonged cycles and aberrant endocrinology appear similar in primates and rodents (Wise et al., 2002; Bellino and Wise 2003).

Currently, there are few data detailing the potential role of butadiene on the etiology of ovotoxicity and late-stage infertility (Hughes et al., 2001). Potential mechanisms might involve the in situ formation of butadiene reactive intermediates, which may have a direct effect on ovarian function (Doerr et al., 1995; Hughes et al., 2001), or a more general role on the hypothalamic-pituitary-gonadal axis. Available data seem to suggest that humans potentially form qualitatively similar metabolites to experimental rodent species, although the rates of formation and the quantities of specific metabolites differ (Bond et al., 1986;Csanady, 1992). Mice appear to produce a greater proportion of active epoxide metabolites than rats (Bond et al., 1986; Himmelstein et al., 1994; Thornton-Manning et al., 1995). However there are conflicting in vitro data that suggest humans may form epoxides of butadiene at comparable rates to mice or at rates several fold less than mice (Csanady et al., 1992; Duescher and Elfarra 1994; Krause and Elfarra 1997). Dahl et al. (1991) reported that following equivalent inhalation exposures, the concentrations of total butadiene metabolites in blood were 5-50 times lower in monkeys than in mice, and 4-14 times lower than in rats. Humans may more closely resemble the monkey than the mouse or the rat in their formation of epoxides from butadiene, and several models adjust for species differences in butadiene pharmacokinetics (Sweeney et al., 1997). In view of the fact that we have used a pharmacokinetic model to assess relevant dosimetry and the weight of published evidence that mice are more active butadiene metabolizers than other species studied, we used a UF of 1 to account for interspecies kinetic differences. However, an interspecies toxicodynamic adjustment for ovarian atrophy endpoint with butadiene is still needed to reflect the expected substantial toxicodynamic differences between mice and women (see the discussion in the derivation of the 8-hour REL). Therefore OEHHA used an interspecies toxicodynamic uncertainty factor of 10. We applied an intraspecies uncertainty factor of 30, 10 for toxicokinetics and $\sqrt{10}$ for toxicodynamics. The overall uncertainty factor was 300, and the cREL is 1.0 ppb.

For comparison, the U.S. EPA's RfC is 0.9 ppb. This value is based on a benchmark concentration (BMC) and a Weibull time-to response model for the ovarian atrophy

endpoint in mice in the NTP 2-year inhalation study (NTP, 1993; U.S.EPA, 2002). U.S.EPA (2002) calculated a BMC_{10} of 1.05 ppm and a $BMCL_{10}$ of 0.88 ppm (continuous) without the top dose. They applied an overall uncertainty factor (UF) of 1000 to derive a final value of 0.88 ppb or 0.9 ppb rounded. The composite UFs included √10 for interspecies, 10 for effect level, 10 for intraspecies, and √10 for data deficiencies.

The Texas Commission on Environmental Quality (TCEQ) used a similar analysis employing the top dose in the same data set to calculate a $BMCL_{05}$ of 462 ppb. Their chronic reference value of 15 ppb employs an overall UF of 30, which includes 10 for intraspecies differences and √10 for data deficiencies (Grant et al., 2010).

Kirman and Grant (2012) conducted a meta-analysis of pooled rodent data on ovarian atrophy resulting from butadiene exposure. They used data in mice from NTP (1993), NTP (1984) and Bevan et al. (1996) together with negative data in rats from Owen et al. (1987) and Bevan et al. (1996). Instead of using a PBPK model to estimate internal dosimetry they used an empirical equation relating average blood concentration of diepoxybutane (DEB) to DEB-specific pyr-Val hemoglobin adducts with separate rate constants and erythrocyte lifespans for mice and rats (4.7×10^{-5}, 40.3; 5.5×10^{-5}, 64.4, L/g hr., days, respectively). This resulted in estimated DEB blood concentrations in mice of 11 to 823 nM for butadiene exposures of 6.25 to 1250 ppm. For rats, estimated blood concentrations of 2.9 to 8.1 nM DEB were obtained for exposure concentrations of 1000 and 8000 ppm butadiene, respectively. Dose response analysis was conducted using the Multistage-Weibull (MSW) time and dose model for incidental effects (ovarian atrophy, extra risk, non-fatal analysis). Analyses were conducted for the mouse data at 823, 686, 471, 186, 73, 29, and 11 nM DEB, for rat data at 2.9 nM DEB, and for pooled mouse and rat data at 0 nM DEB (controls).

The advantage of this approach was the prediction of effects at specific exposure durations that could be compared with human ovarian reserves at times prior to menopause. Three human scenarios for duration of susceptibility to ovarian effects were defined by human menopause at 60 yr (low susceptibility), 49.6 yr. (average susceptibility) and 38.7 yr. (high susceptibility). For these scenarios points of departure (PODs) were determined at the 10% and 1% effect levels with 95% confidence limits i.e., $BMCL_{10}$, $BMCL_{01}$. The $BMCL_{01}$ PODs for high, average and low susceptibility were 1.3, 0.88, and 0.67 nM DEB in blood, respectively. The human equivalent concentrations of butadiene (continuous exposure) $BMCL_{01}$s were 10, 17, and 39 ppm butadiene, respectively. Comparable rodent durations for the human scenario durations were 53.7, 68.8, and 83.2 weeks, respectively. The MSW predicted dose response curve for low follicle count at birth (high susceptibility scenario) was shifted by factors of 3 and 8.5 (25.5 total) to the left to reflect a sensitive individual due to variation in toxicokinetics and toxicodynamics. Similarly, the curve for high follicle count at birth (low susceptibility) was shifted to the right by the same factors. The dose-response curve was not shifted for the average scenario. For the POD $BMCL_{01}$s of 17 ppm BD (average), 1.5 ppm BD (high, 25.5 TK+TD shift UF) , and 240 ppm BD (low,1/25.5 TK+TD shift UF) the derived reference values including an additional net uncertainty factor of 10 were 2, 0.2, and 20 ppm butadiene rounded, respectively. The lowest value

of 0.2 ppm or 200 ppb butadiene is over an order of magnitude higher than that proposed by Grant et al. (2010) for the Texas chronic reference value of 15 ppb butadiene and 67 times higher than the cREL proposed in this document.

This is a novel approach to butadiene non-cancer risk assessment. It rests on a number of assumptions which may not be justified. First of all, it assumes that the effects of butadiene in humans will be exactly as seen in mice, i.e. ovarian atrophy. Ovarian atrophy was the most sensitive non-neoplastic effect noted among several organ effects in mice (decreased lung, liver, and kidney weights) and uterine, testicular and nasal olfactory epithelial atrophies. Next it assumes that all the ovarian effects are due to DEB and that DEB acts the same in rats as in mice. An examination of their Table 3 shows some problems with this latter assumption since at a comparable estimated blood DEB concentration of 11 nM in mice 19/49 showed atrophy, whereas at 8.1 nM in rats no effect was seen (0/110). It appears that mice are much more sensitive to blood DEB than rats. There is also a concern that the effect of including the negative rat data (350 animals) may have reduced the overall response. The choice of a 1% response rate may also be problematic. Usually for non-cancer data the lower bound on the 5% response rate is chosen as the POD i.e., $BMCL_{05}$. While the authors note that several hundred animals were included in the analysis it is questionable if the analysis had the power to detect a 1% increase ($BMCL_{01}$) since the largest denominator for any specific dose level causing an effect was 79 mice. The $BMCL_{01}$ is an extrapolated response well beyond the bulk of observed values. There appears to be a discrepancy in Table 8 which lists a POD of 240 ppm and a reference level of 20 ppm (rounded) where the text gives a value of 255 ppm (10 x 25.5) which would give 26 ppm rounded.

In order to compare their method with ours we used the same TWA DEB internal dose estimates of Kirman & Grant (2012) and conducted a Multistage Weibull non-fatal time to effect analysis (MSW) using U.S.EPA software (U.S.EPA, 2010a) to estimate the benchmark concentration level time adjusted $BMCLt_{05}$ values for the largest and most important data set in their meta-analysis and our cREL derivation, namely the NTP (1993) 24 months data (N= 325). Using individual animal data (dose, time, incident, censored, number) we obtained a value of $BMCLt_{05}$ = 0.502 ppm butadiene equivalent (Table 7). This value is half the 1.01 ppm value based on our time-weighted analysis of 9, 15 and 24 months quantal data (dose, time, number with ovarian atrophy/total number exposed) and the $BMCL_{05}$ for a log probit model (N = 435). While there is no chi-square goodness of fit statistic for the $BMCLt_{05}$, graphical comparisons of the MSW parametric and nonparametric models in probability-time (pr), dose-response (dr), quantile-quantile (qq), and probability-probability (pp) plots indicate excellent fit of the MSW model to the 24 months ovarian atrophy data (Appendix B, U.S.EPA, 2010b). Since the $BMCLt_{05}$ is similar to the $BMCL_{05}$, but is based on fewer animals and conducted with new software with which we have limited experience, the MSW analysis is considered a supporting analysis for the cREL.

Table 7. Multistage Weibull Analysis of Ovarian Atrophy in Female Mice in 2-Year Inhalation Study of 1, 3-Butadiene (NTP, 1993).

Exposure Period	Model	AIC	$BMCt_{05}$ nM DEB	$BMCLt_{05}$ nM DEB	$BMCLt_{05}$ ppm BD equiv.	$BMCLt_{05}$ continuous ppm BD HEC	Comment
24 months	Multi-stage Weibull[1]	300.781[2]	1.56	1.28[3]	0.502[4]	0.151[5]	N = 325; good fit by pr, dr, qq, & pp plots[6]

Note: (1) P(response) = 1-exp{-(t-t0)^c * (beta_0 + beta_1*Dose^1 + beta_2*Dose^2 + beta_3*Dose^3)} run as nonfatal analysis, t0 = 0; (2) Akaike's Information Criterion; (3) $BMCLt_{05}$ 95% lower bound on the 5% response level by MSW with internal DEB dose metric of Kirman and Grant (2012); (4) external equivalent of 0.50 nM DEB in 6hr/d BD; (5) continuous human equivalent concentration of BD (6/24h x 5/7d x 1.68 dosimetric adjustment factor; (6) goodness of fit graphical tests: probability v. time (pr), probability v. dose (dr), time v time (qq), probability v. probability (pp) for parametric and nonparametric msw models (U.S.EPA, 2010b).

It is important to note that in deriving the cREL for butadiene, OEHHA makes no assumption concerning target site concordance in experimental animals and humans. We assume only that the most sensitive site and species with appropriate uncertainty factors will protect against any adverse effect in exposed humans including children.

9 References

Abdel-Rahman, S.Z., R.A. El-Zein, M.M. Ammenheuser, Z. Yang., T.H. Stock, M. Morandi and J.B.Ward Jr. (2003). Variability in human sensitivity to1,3-butadiene:Influence of the allelic variants of the microsomal epoxide hydrolase gene**.** Environ Mol Mutagen 41(2): 140-6**.**

Abdel-Rahman, S.Z., M.M. Ammerheuser, C.J. Omiecinski, J.K. Wickliffe J.L. Rosenblatt and J.B.Ward Jr. (2005). Variability in human sensitivity to 1,3-butadiene:influence of polymorphisms in the 5'-flanking region of the microsomal epoxide hydrolase gene (EPHX1). Toxicol Sci 85(1): 624-31.

Adler, I. D. and D. Anderson (1994). Dominant lethal effects after inhalation exposure to 1,3-butadiene. Mutat Res 309(2): 295-7.

Adler, I. D., J. Cao, J. G. Filser, P. Gassner, W. Kessler, U. Kliesch, A. Neuhauser-Klaus and M. Nusse (1994). Mutagenicity of 1,3-butadiene inhalation in somatic and germinal cells of mice. Mutat Res 309(2): 307-14.

Adler, I. D., J. Filser, H. Gonda and G. Schriever-Schwemmer (1998). Dose response study for 1,3-butadiene-induced dominant lethal mutations and heritable translocations in germs cells of male mice. Mutat Res 397(1): 85-92.

Adler, I. D., J. G. Filser, P. Gassner, W. Kessler, J. Schoeneich and G. Schriever-Schwemmer (1995). Heritable translocations induced by inhalation exposure of male mice to 1,3-butadiene. Mutation Research 347(3,4): 121-7.

Adler, I. D., U. Kliesch, C. Tiveron and F. Pacchierotti (1995). Clastogenicity of diepoxybutane in bone marrow cells and male germ cells of mice. Mutagenesis. Vol. 10(6): 535-541.

Albertini, R.J. (1999). 1,3-Butadiene: Evidence for Genotoxicity. In. A Partnership to Examine Emerging Health Effects:EC/HEI Workshop on 1,3-Butadiene. Brussels 29-30 June, 1998. HEI Communications Number 6, pp 53-84. Health Effects Institute, Cambridge, MA.

Albertini, R. J., R. J. Sram, P. M. Vacek, J. Lynch, J. A. Nicklas, N. J. van Sittert, P. J. Boogaard, R. F. Henderson, J. A. Swenberg, A. Tates, J. B. J. Ward. and M. Wright (2003). Biomarkers in Czech Workers Exposed to 1,3-Butadiene: A Transitional Epidemiologic Study. H. E. Institute.

Ammenheuser, M.M., W.E. Bechtold, S.Z. Abdel-Rahman, J. I. Rosenblatt, D.A. Hastings-Smith and J.B. Ward (2002). Assessment of 1,3-butadiene exposure in polymer production workers using *HPRT* mutations in lymphocytes as a biomarker. Environ Health Perspect 110:1249-1255.

Anderson, D., A. J. Edwards and M. H. Brinkworth (1993). Male-mediated F1 effects in mice exposed to 1,3-butadiene. IARC Sci Publ(127): 171-81.

Anderson, D., A. J. Edwards, M. H. Brinkworth and J. A. Hughes (1996). Male-mediated F1 effects in mice exposed to 1,3-butadiene. Toxicology 113(1-3): 120-7.

Anderson, D., M.M. Dobrzynska, T.W. Yu, L. Gandini, E. Cordelli and M. Spano (1997). DNA integrity in human sperm. Teratog Carcinog Mutagen 17: 97-102.

Anderson, D., J. A. Hughes, A. J. Edwards and M. H. Brinkworth (1998). A comparison of male-mediated effects in rats and mice exposed to 1,3-butadiene. Mutat Res 397(1): 77-84.

ATSDR (1992). Toxicological Profile for 1,3-butadiene, Clement International Corp.,Ruston,LA,USA.**:** 136 pp.

BAAQMD (2008). Toxic Air Contaminant Air Monitoring Data for 2008. Bay Area Air Quality Management District. http://www.baaqmd.gov/Divisions/Engineering/Air-Toxics/Toxic-Air-Contaminant-Control-Program-Annual-Report.aspx

Baker, J., J. Arey and R. Atkinson (2005). Formation and reaction of hydroxycarbonyls from the reaction of OH radicals with 1,3-butadiene and isoprene. Environ Sci Technol 39(11): 4091-9.

Begemann, P., R. J. Sram and H. G. Neumann (2001). Hemoglobin adducts of epoxybutene in workers occupationally exposed to 1,3-butadiene. Arch Toxicol 74(11): 680-7.

Bellino, F. L. and P. M. Wise (2003). Nonhuman primate models of menopause workshop. Biol Reprod 68(1): 10-8.

Bond, J. A., A. R. Dahl, R. F. Henderson, J. S. Dutcher, J. L. Mauderly and L. S. Birnbaum (1986). Species differences in the disposition of inhaled butadiene. Toxicology and Applied Pharmacology 84(3): 617-27.

Bond, J.A., M.W. Himmelstein, M. Seaton, P. Boogaard and M.A. Medinsky (1996). Metabolism of butadiene by mice, rats, and humans: a comparison of physiologically based toxicokinetic model predictions and experimental data. Toxicology 113:48-54.

Bond, J. A. and M. A. Medinsky (2001). Insights into the toxicokinetics and toxicodynamics of 1,3-butadiene. Chem Biol Interact 135-136: 599-614.

Boogaard, P. J. and J. A. Bond (1996). The role of hydrolysis in the detoxification of 1,2:3,4-diepoxybutane by human, rat, and mouse liver and lung in vitro. Toxicol Appl Pharmacol 141(2): 617-27.

Boogaard, P.J., N.J. van Sittert and H.J.J.J. Megans (2001). Urinary metabolites and haemoglobin adducts as biomarkers of exposure to 1,3-butadiene: a basis for 1,3-butadiene cancer risk assessment. Chem-Biol Interact 135-136:695-701.

Boogaard, P. J., K. P. de Kloe, E. D. Booth and W. P. Watson (2004). DNA adducts in rats and mice following exposure to [4-14C]-1,2-epoxy-3-butene and to [2,3-14C]-1,3-butadiene. Chem Biol Interact 148(1-2): 69-92.

Booth, E. D., J. D. Kilgour, S. A. Robinson and W. P. Watson (2004). Dose responses for DNA adduct formation in tissues of rats and mice exposed by inhalation to low concentrations of 1,3-[2,3-[(14)C]-butadiene. Chem Biol Interact 147(2): 195-211.

Boysen, G., N.I. Georgieva, N.K. Bordeerat, R.J. Sram, P. Vacek, R.J. Albertini and J.A. Swenberg (2012). Formation of 1,2:3,4-Diepoxybutane-specific hemoglobin adducts in 1,3-butadiene exposed workers. Toxicol Sci 125(1):30-40.

Brinkworth, M. H., D. Anderson, J. A. Hughes, L. I. Jackson, T. W. Yu and E. Nieschlag (1998). Genetic effects of 1,3-butadiene on the mouse testis. Mutat Res 397(1): 67-75.

Brochot, C., T.J.Smith and F.Y. Bois (2007). Development of a physiologically based toxicokinetic model for butadiene and four major metabolites in humans: Global sensitivity analysis for experimental design issues. Chem-Biol Interact 167:168-183.

Burnet, F.M. (1974). *Intrinsic Mutagenesis, a Genetic Approach to Aging*. J Wiley, New York.

CARB (2009). The California Almanac of Emissions and Air Quality - 2009 Edition, California Air Resources Board.

Carmical, J.R,, L.V. Nechev, C.M. Harris, T.M. Harris and R.S. Lloyd (2000). Mutagenic potential of adenine N6 adducts of monoepoxide and diolepoxide derivatives of butadiene. Environ Mol Mutagen 35: 48-56.

Chan, C. C., R. H. Shie, T. Y. Chang and D. H. Tsai (2006). Workers' exposures and potential health risks to air toxics in a petrochemical complex assessed by improved methodology. Int Arch Occup Environ Health 79(2): 135-42.

Cho, S.H. and F.P. Guengerich (2012a). Conjugation of butadiene diepoxide with glutathione yields DNA adducts in vitro and in vivo. Chem Res Toxicol 25:706-712.

Cho, S.H. and F.P. Guengerich (2012b). Mutation spectra of S-(2-hydroxy-3,4-epoxybutyl)glutathione:comparison with 1,3-butadiene and its metabolites in the Escherichia coli rpoB gene. Chem Res Toxicol 25(7):1522-30.

Cooke, M.S., R. Olinski and M.D. Evans (2006). Does measurement of oxidative damage to DNA have clinical significance? Clin Chim Acta 365(1-2): 30-49.

Csanady, G. A., F. P. Guengerich and J. A. Bond (1992). Comparison of the biotransformation of 1,3-butadiene and its metabolite, butadiene monoepoxide, by hepatic and pulmonary tissues from humans, rats and mice. Carcinogenesis 13(7): 1143-53.

Csanady, G.A., W. Kessler, H.D.Hoffmann and J.G.Filser (2003). A toxicokinetic model for styrene and its metabolite styrene 7,8-oxide in mouse, rat and human with special emphaisis on the lung. Toxicol Lett 138:75-102.

Csanady, G.A, R. Steinhoff, M.B.Riester, B.Semder,C.Putz,Q.Li, N.Richter,W.Kessler,D.Klein,and J.G.Filser. (2011). 1,2:3,4-Diepoxybutane in blood of male B6C3F1 mice and male Sprague-Dawley rats exposed to butadiene. Toxicol Lett 207:286-290.

Dahl, A. R., J. D. Sun, L. S. Birnbaum, J. A. Bond, W. C. Griffith, Jr., J. L. Mauderly, B. A. Muggenburg, P. J. Sabourin and R. F. Henderson (1991). Toxicokinetics of inhaled 1,3-butadiene in monkeys: comparison to toxicokinetics in rats and mice. Toxicol Appl Pharmacol 110(1): 9-19.

Delfino, R. J., H. Gong, Jr., W. S. Linn, E. D. Pellizzari and Y. Hu (2003). Asthma symptoms in Hispanic children and daily ambient exposures to toxic and criteria air pollutants. Environ Health Perspect 111(4): 647-56.

Delzell, E., N. Sathiakumar, M. Hovinga, M. Macaluso, J. Julian, R. Larson, P. Cole and D. C. Muir (1996). A follow-up study of synthetic rubber workers. Toxicology 113(1-3): 182-9.

Devine, B.J. (1990). An update on mortality among workers at a 1,3-butadiene facility - preliminary results.Environ Health Perspect 86:119-128.

Devine, B.J. and C.M.Hartman (1996). Mortality update of butadiene production workers. Toxicology 113:169-181.

Doerr, J. K., E. A. Hollis and I. G. Sipes (1996). Species difference in the ovarian toxicity of 1,3-butadiene epoxides in B5C3F1 mice and Sprague-Dawley rats. Toxicology 113(1-3): 128-136.

Doerr, J. K., S. B. Hooser, B. J. Smith and I. G. Sipes (1995). Ovarian toxicity of 4-vinylcyclohexene and related olefins in B6C3F1 mice: role of diepoxides. Chem Res Toxicol 8(7): 963-9.

Doyle, M., K.G. Sexton, H. Jeffries, K. Bridge and I. Jaspers (2004). Effects of 1,3-butadiene, isoprene, and their photochemical degradation products on human lung cells. Environ Health Perspect 112(15):1488-1495.

Doyle, M., K. G. Sexton, H. Jeffries and I. Jaspers (2007). Atmospheric photochemical transformations enhance 1,3-butadiene-induced inflammatory responses in human epithelial cells: The role of ozone and other photochemical degradation products. Chem Biol Interact 166(1-3): 163-9.

Duescher, R. J. and A. A. Elfarra (1994). Human liver microsomes are efficient catalysts of 1,3-butadiene oxidation: evidence for major roles by cytochromes P450 2A6 and 2E1. Arch Biochem Biophys 311(2): 342-9.

Elfarra, A. A., T. S. Moll, R. J. Krause, R. A. Kemper and R. R. Selzer (2001). Reactive metabolites of 1,3-butadiene: DNA and hemoglobin adduct formation and potential roles in carcinogenicity. Biological Reactive Intermediates Vi 500: 93-103.

Evans, M.D. and M.S. Cooke (2006). Oxidative damage to DNA in non-malignant disease: biomarker or biohazard? Genome Dyn 1: 53-66.

Fajen, J. M., D. R. Roberts, L. J. Ungers and E. R. Krishnan (1990). Occupational exposure of workers to 1,3-butadiene. Environ Health Perspect 86: 11-8.

Gentry,P.R.,T.R. Covington, M.E.Andersen and H.J.Clewell III (2002).Application of a physiologically based pharmacokinetic model for isopropanol in the derivation of a reference dose and reference concentration. Regul Toxicol Pharmacol 36: 51-68.

Georgieva, N.I., G. Boysen, N. Bordeerat , V.E.Walker and J.A. Swenberg (2010). Exposure-response of 1,2:3,4-diepoxybutane specific N-terminal valine adducts in mice and rats after inhalation exposure to 1,3-butadiene. Toxicol Sci 15:322-329.

Grant R. L. , J. Hanley, A. L. Curry and M. Honeycutt. (2010). A chronic reference value for 1,3-butadiene based on an updated noncancer toxicity assessment. J Toxicol Environ Health B 13:460-475.

Green, J.W. (2003). Statistical Analysis of Butadiene Mouse Data from Hackett et al. (1987). Haskell Laboratory for Health and Environmental Sciences, E.I. Dupont de Nemours & Co., Newark, DE, USA: 151 pp.

Guerreiro, R., A. Wojtas, J. Bras, M. Carrasquillo, E. Rogaeva, E. Majounie, C. Cruchaga, C. Sassi, J.S.K. Kauwe, S. Younkin, L. Hazrati, J. Collinge, J. Pocock, T. Lashley, J. Williams, J.C. Lambert, P. Amouyel, A. Goate, R. Rademakers, K. Morgan, J. Powell, P. St. george-Hyslop, A. Singleton and J. Hardy (2012). *TREM2* variants in Alzheimer's disease. NE J Med Nov14: 11pp.

Gustafson, P., L. Barregard, B. Strandberg and G. Sallsten (2007). The impact of domestic wood burning on personal, indoor and outdoor levels of 1,3-butadiene, benzene, formaldehyde and acetaldehyde. J Environ Monit 9:23-32.

Hackett, P. L., M. R. Sikov, T. J. Mast, M. G. Brown, R. L. Buschbom, M. L. Clark, J. R. Decker, J. J. Evanoff, R. L. Rommereim and S. E. Rowe (1987a). Inhalation developmental toxicology studies: teratology study of 1,3-butadiene in mice: final report, Pac. Northwest Lab.,Richland,WA,USA.**:** 92 pp.

Hackett, P. L., M. R. Sikov, T. J. Mast, M. G. Brown, R. L. Buschbom, M. L. Clark, J. R. Decker, J. J. Evanoff, R. L. Rommereim, S. E. Rowe and R.B. Westerberg (1987b). Inhalation developmental toxicology studies of 1,3-butadiene in the rat: final report, Pac. Northwest Lab.,Richland,WA,USA.**:** 22 pp.

Hayes, R. B., L. Zhang, S. Yin, J. A. Swenberg, L. Xi, J. Wiencke, W. E. Bechtold, M. Yao, N. Rothman, R. Haas, J. P. O'Neill, D. Zhang, J. Wiemels, M. Dosemeci, G. Li and M. T. Smith (2000). Genotoxic markers among butadiene polymer workers in China. Carcinogenesis 21(1): 55-62.

He, W., R.J. Greenwell, D.M. Brooks, L. Calderon-Garciduenas, H.D. Beall and J.D. Coffin (2007). Arsenic exposure in pregnant mice disrupts placental vasculogenesis and causes spontaneous abortion. Toxicol Sci 99(1):244-253.

Henderson, R. F., F. F. Hahn, J. M. Benson, E. B. Barr, W. E. Bechtold, D. G. Burt and A. R. Dahl (1999). Dosimetry and acute toxicity of inhaled butadiene diepoxide in rats and mice. Toxicol Sci 51(1): 146-52.

Himmelstein, M. W., M. J. Turner, B. Asgharian and J. A. Bond (1994). Comparison of blood concentrations of 1,3-butadiene and butadiene epoxides in mice and rats exposed to 1,3-butadiene by inhalation. Carcinogenesis 15(8): 1479-86.

HSDB. (2004). "Hazardous Substances Data Bank." 2009, from http://toxnet.nlm.nih.gov.

HSDB. (2011). "Hazardous Substances Data Bank." 2011, from http://toxnet.nlm.nih.gov.

Hoyer P.B. and I.G. Sipes (2007). Development of an animal model for ovotoxicity using 4-vinylcyclohexene: A case study. Birth Defects Res (B) 80:113-125.

Hu X., P.J. Christian, K.E. Thompson, I.G. Sipes and P.B. Hoyer (2001a). Apoptosis induced in rats by 4-vinylcycolhexene diepoxide is associated with activation of the caspase cascades. Biol Reprod 65:87-93.

Hu X., P. Christian, I.G. Sipes and P.B. Hoyer (2001b). Expression and redistrbution of cellular Bad, Bax, and Bcl-x_L protein is associated with VCD-induced ovotoxicity in rats. Biol Reprod 65:1489-1495.

Hu X., J.A. Flaws, I.G. Sipes and P.B. Hoyer (2002). Activation of mitogen-activated protein kinases and AP-1 transcription factor in ovotoxicity induced by 4-vinylcyclohexene diepoxide in rats. Biol Reprod 67:718-724.

Hughes, K., M. E. Meek and M. Walker (2001). Health risk assessment of 1,3-butadiene as a Priority Substance in Canada. Chem Biol Interact 135-136: 109-35.

IARC. (2012). 1,3-Butadiene. http://monographs.iarc.fr/ENG/Monographs/vol100F/

Irons, R. D., C. N. Smith, W. S. Stillman, R. S. Shah, W. H. Steinhagen and L. J. Leiderman (1986a). Macrocytic-megaloblastic anemia in male B6C3F1 mice following chronic exposure to 1,3-butadiene. Toxicol Appl Pharmacol 83(1): 95-100.

Irons, R. D., C. N. Smith, W. S. Stillman, R. S. Shah, W. H. Steinhagen and L. J. Leiderman (1986b). Macrocytic-megaloblastic anemia in male NIH Swiss mice following repeated exposure to 1,3-butadiene. Toxicol Appl Pharmacol 85(3): 450-5.

Ito, A., N. Mafune and T. Kimura (2009). Collborative work on evaluation of ovarian toxicity 4) Two- or Four-week repeated dose study of 4-vinylcyclohexene diepoxide in female rats. J Toxicol Sci 34:(Special Issue I):SP53-SP58.

Jonsson, T., H. Stefansson, S. Steinberg, I. Jonsdottir, P.V. Jonsson, J. Snaedal , S. Bjornsson, J. Huttenlocher, A.I. Levey, J.J. Lah, D. Rujescu, H. Hampel, I. Giegling, O.A. Andreassen, K. Engedal, I. Ulstein, S. Djurovic, C. Ibrahim-Verbaas, A. Hofman, M. A.Ikram, C.M. van Duijn, U. Thorsteinsdottir, A. Kong and K. Stefansson (2012). Variant of *TREM2* associated with the risk of Alzheimer's disease. NE J Med November 14:10 pp.

Kane, E.V. and R. Newton (2010). Occupational exposure to gasoline and the risk of non-Hodgkin lymphoma: a review and meta-analysis of the literature. Cancer Epidemiol 34(5):516-522.

Kemper, R. A., R. J. Krause and A. A. Elfarra (2001). Metabolism of butadiene monoxide by freshly isolated hepatocytes from mice and rats: different partitioning between oxidative, hydrolytic, and conjugation pathways. Drug Metab Dispos 29(6): 830-6.

Kennedy, C.H., W. J. Catallo, V.L. Wilson and J.B. Mitchell (2009). Combustion products of 1,3-butadiene inhibit catalase activity and induce expression of oxidative DNA damage repair enzymes in human bronchial epithelial cells . Cell Biol Toxicol 25:457-470.

Khalil , M., M. Abudiab and A.E. Ahmed (2007). Clinical evaluation of 1,3-butadiene neurotoxicity in humans. Toxicol Ind Health 23:141-146.

Kinney, P. L., S. N. Chillrud, S. Ramstrom, J. Ross and J. D. Spengler (2002). Exposures to multiple air toxics in New York City. Environ Health Perspect 110 Suppl 4: 539-46.

Kirman, C.R. and R.L. Grant (2012). Quantitative human health risk assessment for 1,3-butadiene based upon ovarian effects in rodents. Regul Toxicol Pharmacol 62:371-384.

Kohn, M.C. and R.L.Melnick (2000). The privileged access model of 1,3-butadiene disposition. Environ Health Perspect 108(Suppl 5):911-917.

Kohn, M. C. and R. L. Melnick (2001). Physiological modeling of butadiene disposition in mice and rats. Chem Biol Interact 135-136: 285-301.

Koivisto, P., I. D. Adler, F. Pacchierotti and K. Peltonen (1998). DNA adducts in mouse testis and lung after inhalation exposure to 1,3-butadiene. Mutat Res 397(1): 3-10.

Krause, R. J. and A. A. Elfarra (1997). Oxidation of butadiene monoxide to meso- and (+/-)-diepoxybutane by cDNA-expressed human cytochrome P450s and by mouse, rat, and human liver microsomes: evidence for preferential hydration of meso-diepoxybutane in rat and human liver microsomes. Arch Biochem Biophys 337(2): 176-84.

Lewis, D.F.V, M.G.Bird and D.V.Parke (1997). Molecular modelling of CYP2E1 enzymes from rat, mouse and man: An explanation for species differences in butadiene metabolism and potential carcinogenicity, and rationalization of CYP2E1 substrate specificity. Toxicology 118(2-3):93-113.

Lin, Y. S., T. J. Smith, K. T. Kelsey and D. Wypij (2001). Human physiologic factors in respiratory uptake of 1,3-butadiene. Environ Health Perspect 109(9): 921-6.

Logue, J.M., T.E. McKone, M.H. Sherman and B.C. Singer (2011). Hazard assessment of chemical air contaminants measured in residences. Indoor Air 21:92-109.

Lovreglio, P., N. Bukvic, S. Fustinoni, A. Ballini, I. Drago, V. Foa, G. Guanti and L. Soleo (2005). Lack of genotoxic effect in workers exposed to very low doses of 1,3-butadiene. Arch Toxicol: 1-4.

Lynch, J. (2001). Occupational exposure to butadiene, isoprene and chloroprene. Chem Biol Interact 135-136: 207-14.

Macaluso, M., R. Larson, E. Delzell, N. Sathiakumar, M. Hovinga, J. Julian, D. Muir and P. Cole (1996). Leukemia and cumulative exposure to butadiene, styrene and benzene among workers in the synthetic rubber industry. Toxicology 113(1-3): 190-202.

Mark-Kappeler, C.J., P.B. Hoyer and P.J. Devine (2011). Xenobiotic effects on ovarian preantral follicles. Biol Reprod 85:871-883.

Marshall, J.D., P.W. Granvold, A.S. Hoats, T.E. McKone, E. Deakins and W.W. Nazaroff (2006). Inhalation intake of ambient air pollution in California's South Coast Air basin. Atmos Environ 40:4381-4392.

Matanoski, G. M., C. Santos-Burgoa and L. Schwartz (1990). Mortality of a cohort of workers in the styrene-butadiene polymer manufacturing industry (1943-1982). Environ Health Perspect 86: 107-17.

McMichael, A., R. Spirtas, J. Gamble and P. Tousey (1976). Mortality among rubber workers: Relationship to specific jobs. Journal of Occupational Medicine 18(3) 178-85..

McNabola, A., B. Broderick, P. Johnston and L. Gill (2006). Effects of the smoking ban on benzene and 1,3-butadiene levels in pubs in Dublin. J Environ Sci Health A Tox Hazard Subst Environ Eng 41(5): 799-810.

Meinhardt, T. J., R. A. Lemen, M. S. Crandall and R. J. Young (1982). Environmental epidemiologic investigation of the styrene-butadiene rubber industry. Mortality patterns with discussion of the hematopoietic and lymphatic malignancies. Scand J Work Environ Health 8(4): 250-9.

Melnick, R. L., J. Huff, B. J. Chou and R. A. Miller (1990). Carcinogenicity of 1,3-butadiene in C57BL/6 x C3H F1 mice at low exposure concentrations. Cancer Res 50(20): 6592-9.

Melnick, R. L., J. E. Huff, J. H. Roycroft, B. J. Chou and R. A. Miller (1990). Inhalation toxicology and carcinogenicity of 1,3-butadiene in B6C3F1 mice following 65 weeks of exposure. Environ Health Perspect 86: 27-36.

Meng, Q., D. L. Redetzke, L. C. Hackfeld, R. P.,Hodge, D. M. Walker and V. E. Walker (2007). Mutagenicity of stereochemical configurations of 1,2-epoxybutene and 1,2:3,4-diepoxybutane in human lymphoblastoid cells. Chem Biol Interact 166(1-3): 207-18.

Morrissey, R. E., B. A. Schwetz, P. L. Hackett, M. R. Sikov, B. D. Hardin, B. J. McClanahan, J. R. Decker and T. J. Mast (1990). Overview of reproductive and developmental toxicity studies of 1,3-butadiene in rodents. Environ Health Perspect 86: 79-84.

Nazaroff, W. W. and B. C. Singer (2004). Inhalation of hazardous air pollutants from environmental tobacco smoke in US residences. J Expo Anal Environ Epidemiol 14 Suppl 1: S71-7.

NTP, N. T. P. (1984). Toxicology and carcinogenesis studies of 1,3-butadiene (CAS No. 106-99-0) in B6C3F1 mice (inhalation studies). N. T. Program, U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health.

NTP, N. T. P. (1993). NTP Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No. 106-99-0) in B6C3F1 Mice (Inhalation Studies). Natl Toxicol Program Tech Rep Ser 434: 1-389.

Owen, P. E. and J. R. Glaister (1990). Inhalation toxicity and carcinogenicity of 1,3-butadiene in Sprague-Dawley rats. Environ Health Perspect 86: 19-25.

Owen, P. E., J. R. Glaister, I. F. Gaunt and D. H. Pullinger (1987). Inhalation toxicity studies with 1,3-butadiene. 3. Two year toxicity/carcinogenicity study in rats. Am Ind Hyg Assoc J 48(5): 407-13.

Pacchierotti, F., I. D. Adler, D. Anderson, M. Brinkworth, N. A. Demopoulos, J. Lahdetie, S. Osterman-Golkar, K. Peltonen, A. Russo, A. Tates and R. Waters (1998). Genetic effects of 1,3-butadiene and associated risk for heritable damage. Mutat Res 397(1): 93-115.

Penn A. and C.A. Snyder (1996). 1,3-Butadiene, a vapor phase component of environmental tobacco smoke, accelerates arteriosclerotic plaque development. Circulation 93(3):552-557.

Penn, A. and C.A. Snyder (2007). 1,3-Butadiene exposure and cardiovascular disease. Mutat Res 621:42-49.

Penn, A., G. Murphy, S. Barker, W. Henk and L. Penn (2005). Combustion-derived ultrafine particles transport organic toxicants to target respiratory cells. Environ Health Perspect 113(8): 956-63.

Perez, H. L., J. Lahdetie, H. Landin, I. Kilpelainen, P. Koivisto, K. Peltonen and S. Osterman-Golkar (1997). Haemoglobin adducts of epoxybutanediol from exposure to 1,3-butadiene or butadiene epoxides. Chem Biol Interact 105(3): 181-98.

Pery, A. R. R. and F. Y. Bois (2009). Stochasticity in physiological based kinetics models:implications for cancer risk assessment. Risk Anal 29(8):1182-1191.

Poet, T.S., C.R. Kirman, M. Bader, C.van Thriel, M.L.Gargas and P.M.Hinderliter (2010).Quantitative risk analysis for N-methyl pyrrolidone using physiologically based pharmacokinetic and benchmark dose modeling.Toxicol Sci 113(2):468-482.

Primavera, A., S. Fustinoni, A. Biroccio, S. Ballerini, A. Urbani, S. Bernardini, G. Federici, E. Capucci, M. Manno and M. Lo Bello (2008). Glutathione transferases and glutathionylated hemoglobin in workers exposed to low doses of 1,3-butadiene. Cancer Epidemiol Biomarkers Prev 17(11):3001-3012.

Salama, S.A., W.W.Wu, G.C. Hunter, R.G. Sheahan, O.A. Badary, A.B. Abdel-Naim and F.M.A. Hamada (2002). Polymorphic metabolizing genes and susceptibility to atherosclerosis among cigarette smokers. Emviron Mol Mutagen 40:153-160.

Sandmeyer, E.E. (1981). Apiphatic Hydrocarbons: Butadiene. In: Clayton, G.D. and F.E. Clayton eds. *Patty's Industrial Hygiene and Toxicology*. Vol 2B, 3rd Revised Edition, J. Wiley and Sons, New York, N.Y.

Sangaraju, D., M. Goggin, V. Walker, J. Swenberg and N. Tretyakova (2012). NanoHPLC-nanoESI$^+$-MS/MS quantitation of *Bis*-N7-Guanine DNA-DNA cross-links in tissues of B6C3F1 mice exposed to subppm levels of 1,3-butadiene. Anal Chem 84:1732-1739.

Sapkota, A., D. Williams and T. J. Buckley (2005). Tollbooth workers and mobile source-related hazardous air pollutants: how protective is the indoor environment? Environ Sci Technol 39(9): 2936-43.

Sathiakumar, N., J. Graff, M. Macaluso, G. Maldonado, R. Matthews and E. Delzell (2005). An updated study of mortality among North American synthetic rubber industry workers. Occup Environ Med 62(12): 822-9.

Schmiederer, M., E. Knutson, P. Muganda and T. Albrecht (2005). Acute exposure of human lung cells to 1,3-butadiene diepoxide results in G1 and G2 cell cycle arrest. Environ Mol Mutagen 45(4): 354-64.

Serrano-Trespalacios, P. I., L. Ryan and J. D. Spengler (2004). Ambient, indoor and personal exposure relationships of volatile organic compounds in Mexico City Metropolitan Area. J Expo Anal Environ Epidemiol 14 Suppl 1: S118-32.

Sexton, K.G., M.L. Doyle, H.E. Jeffries and S. Ebersviller (2007). Development and testing of a chemical mechanism for atmospheric photochemical transformations of 1,3-butadiene. Chem-Biol Interact 166:156-162.

Shugaev, B. B. (1969). Concentrations of hydrocarbons in tissues as a measure of toxicity. Arch Environ Health 18(6): 878-82.

Smith B.J., D.R. Mattison and I.G. Sipes (1990). The role of epoxidation in 4-vinylcyclohexene-induced ovarian toxicity. Toxicol Appl Pharmacol 105:372-381.

SCAQMD. (2008). Multiple Air Toxics Exposure Study in the South Coast Air Basin (MATES III). Final Report. Table VI-2. South Coast Air Quality Management District, September, 2008.

Sprague, C. L., L. A. Phillips, K. M. Young and A. A. Elfarra (2004). Species and tissue differences in the toxicity of 3-butene-1,2-diol in male Sprague-Dawley rats and B6C3F1 mice. Toxicol Sci 80(1): 3-13.

Sweeney, L. M., P. M. Schlosser, M. A. Medinsky and J. A. Bond (1997). Physiologically based pharmacokinetic modeling of 1,3-butadiene, 1,2-epoxy-3-butene, and 1,2:3,4-diepoxybutane toxicokinetics in mice and rats. Carcinogenesis 18(4): 611-25.

Thornton-Manning, J. R., A. R. Dahl, W. E. Bechtold, W. C. Griffith, Jr. and R. F. Henderson (1995). Disposition of butadiene monoepoxide and butadiene diepoxide in various tissues of rats and mice following a low-level inhalation exposure to 1,3-butadiene. Carcinogenesis 16(8): 1723-31.

Thurmond, L. M., L. D. Lauer, R. V. House, W. S. Stillman, R. D. Irons, W. H. Steinhagen and J. H. Dean (1986). Effect of short-term inhalation exposure to 1,3-butadiene on murine immune functions. Toxicol Appl Pharmacol 86(2): 170-9.

Tiveron, C., R. Ranaldi, B. Bassani and F. Pacchierotti (1997). Induction and transmission of chromosome aberrations in mouse oocytes after treatment with butadiene diepoxide. Environ Mol Mutagen 30(4): 403-9.

Tuazon, E. C., A. Alvarado, S. M. Aschmann, R. Atkinson and J. Arey (1999). Products of the gas-phase reactions of 1,3-butadiene with OH and NO3 radicals. Environmental Science & Technology 33(20): 3586-3595.

U.S. EPA. (2002). Health assessment of 1,3-butadiene. EPA/600/P-98/001F. National Center for Environmental Assessment , Office of Research and Development, U.S. Environmental Protection Agency, Washington, DC.

U.S.EPA. (2010a). User Manual for "MSW" Multistage Weibull Time-To-Tumor Model. Quantitative Risk Management Group, NCEA, ORD, U.S. Environmental Protection Agency, Washington, DC. 21 pp. (www.epa.gov/ncea/bmds/msw.html)

U.S. EPA.(2010b). User Manual for gofplot_msw( ): Producing Plots to Access Goodness-of-Fit for the Multistage Weibull (MSW) Time-To-Tumor Model. Quantitative Risk Management Group, NCEA, ORD, U.S. Environmental Protection Agency, Washington, DC. 12 pp. (www.epa.gov/ncea/bmds/msw.html)

Vainiotalo, S., V. Vaananen and R. Vaaranrinta (2008). Measurement of 16 volatile organic compounds in restaurant air contaminated with environmental tobacco smoke. Environ Res 108:280-288.

Valdez, K. E. and B. K. Petroff (2004). Potential roles of the aryl hydrocarbon receptor in female reproductive senescence. Reprod Biol 4(3): 243-58.

vom Saal, F. S., C. E. Finch and J. F. Nelson (1994). Natural history and mechanisms of reproductive aging in humans, laboratory rodents and other selected vertebrates. The Physiology of Reproduction pp 1213-1314. Eds Knobil E Neill JD. Raven Press, New York. K. E. and N. J.D. New York, Raven Press**:** 1213-1314.

Wickliffe, J. K., M. M. Ammenheuser, J. J. Salazar, S. Z. Abdel-Rahman, D. A. Hastings-Smith, E. M. Postlethwait, R. S. Lloyd and J. B. Ward Jr. (2003). A model of sensitivity: 1,3-butadiene increases mutant frequencies and genomic damage in mice lacking a functional microsomal epoxide hydrolase gene. Environ Mol Mutagen 42(2):106-10.

Wickliffe, J. K., M. M. Ammenheuser, P. J.Adler, S. Z. Abdel-Rahman and J. B.Ward Jr. (2009). Evaluation of frequencies of *HPRT* mutant lymphocytes in butadiene polymer workers in a Southeast Texas facility. Environ Mol Mutagen 50(2):82-7.

Wilson, R. (1944). Health hazards encountered in the manufacture of synthetic rubber. JAMA 124(11): 701-703.

Wise, P. M., M. J. Smith, D. B. Dubal, M. E. Wilson, S. W. Rau, A. B. Cashion, M. Bottner and K. L. Rosewell (2002). Neuroendocrine modulation and repercussions of female reproductive aging. Recent Prog Horm Res 57: 235-56.

Yadavilli, S., E. martinez-Ceballos, J. Snowden-Aikens, A. Hurst, T. Joseph, T. Albrecht and P.M. Muganda (2007). Diepoxybutane activates the mitochondrial apoptotic pathway and mediates apoptosis in human lymphoblasts through oxidative stress. Toxicol In Vitro 21:1429-1441.

Yang, Z., C.A. Knight, M.M. Mamerow, K. Vickers, A. Penn, E.M. Postlethwait and S.W. Ballinger (2004). Prenatal environmental tobacco smoke exposure promotes adult atherogenesis and mitochondrial damage in apolipoprotein $E^{-/-}$ mice fed a chow diet. Circulation 110:3715-3720.

Zhang, H.R., E.G. Eddings and A.F. Serofim (2008). Pollutant emissions from gasoline combustion 1. Dependence on fuel structural functionalities. Environ Sci Technol 42:5615-5621.

Zhang, P.P., Y. Wen, J. An, Y.X. Yu, M.H. Wu and X.Y. Zhang (2012). DNA damage induced by three major metabolites of 1,3-butadiene in human hepatocyte L02 cells. Mutat Res 747:240-245.

Appendix A

This appendix includes information on genotoxicity of butadiene, except discussion of dominant lethal mutations and germ cell mutagenicity, which are covered in the developmental and reproductive toxicity section of the main document. An overview of the genotoxicity of butadiene and its metabolites is presented in Table A1. Also included are data on PBPK modeling of butadiene employed in the derivation of the $BMCL_{05}$ and the 8-hr and chronic RELs. Table A2 gives a historical overview of the PBPK and related models that were more or less available for use.

While genetic toxicology generally provides key supporting documentation for cancer risk assessment rather than the present noncancer assessment, we believe that mutagenicity and other genetox effects, particularly oxidative DNA damage, may contribute to chronic diseases such as heart disease, neurodegenerative diseases, diabetes mellitus, rheumatoid arthritis, and aging, irrespective of their role in initiation and promotion of tumors (F.M.Burnet,1974; Cooke et al., 2006).

A.1 Genetic Toxicity: Introduction

This section includes information on genetic toxicity. Albertini et al. (2010) have provided a comprehensive review of 1, 3-butadiene genotoxicity. As noted above, the metabolism of butadiene produces the major electrophilic metabolites EB, EBD, and DEB. These epoxide metabolites are responsible both for the mutagenicity and carcinogenicity of 1, 3-butadiene. They are DNA reactive forming a variety of adducts, and all are genotoxic in vitro and in vivo. Also butadiene epoxide metabolites form adducts with proteins notably hemoglobin and may exert or influence noncancer endpoints. In general genotoxicity, specifically oxidative DNA damage, may influence a number of chronic diseases and aging in addition to cancer (Cooke et al., 2006; Evans & Cooke, 2006).

A.2 Microbial test systems

Butadiene has induced mutagenic responses in all microbial systems that were provided with exogenous microsomal activation (S-9) that allowed the formation of oxidative metabolites. The purified metabolites epoxybutene (EB) and diepoxybutane (DEB) have also been shown to be mutagenic in all microbial systems without exogenous activation (Albertini, 1999). Carmical et al. (2000) found evidence of stereospecific mutagenicity of diolepoxide derived adducts in an *E.coli* system. The BDE R,R isomer induced a mutantion frequency of 0.14% (A→G mutations exclusively), whereas the S,S isomer induced 0.25% (A→C mutations exclusively). The mutation frequencies induced by BMO isomers were not significantly different from controls (<0.05%). Curiously, a recent report by Cho et al. (2010) demonstrated the mutagenicity in *S. typhimurium* TA1535 of the glutathione conjugate of DEB, *S*-(2-hydroxy-3,4-epoxybutyl)GSH. In general GSH conjugates are regarded as detoxification products but in this case the conjugate was reported to have higher mutagenic potency than DEB.

A.3 Higher organism test systems

Butadiene and its diepoxide have been tested in the fruit fly *Drosophila melanogaster*. BD was negative in sex-linked recessive lethal mutations and the spot wing test whereas DEB was positive in these tests. Studies in mammalian cell systems parallel those in microbial systems with BD, EB, DEB and epoxybutane diol (EBD) showing positive results in systems provided with exogenous activation. In these systems the rank order of mutagenic potency was generally DEB> EB (BMO)>EBD>BD. DEB was generally 40 to 100-fold more potent than EDB. DEB is more likely to cause deletions, whereas all the epoxides induce base substitutions with a preference for A:T base pairs (Albertini, 1999). Both EB and DEB occur in different stereochemical configurations, which have been evaluated in the TK6 human lymphoblastoid cell line for increases in hypoxanthine-guanine phosphoribosyltransferase (HPRT) and thymidine kinase (TK) mutant frequencies. Meng et al. (2007) found no differences in cytotoxicity or mutagenicity among three isomers of DEB or the two isomers of EB. Thus, stereochemistry seems likely to play little role in the mutagenicity of BD. These findings were consistent with in vivo results, which showed little difference in the mutagenic efficiencies of racemic-DEB or meso-DEB in rodents. However, the results seem somewhat at odds with those of Carmical et al. (2000) above, where *S*,*S*-EBD derived adducts preferentially induced mutagenicity in *E.coli*.

Mutagenic studies in whole mammals have generally given positive responses when the animals (mice or rats) were exposed to butadiene by inhalation (Albertini et al., 2010). The tests included the mouse spot test, which involves in utero exposure of a developing embryo, which results in mutations in hair coloring in the offspring. Most of the assays were for mutations in the *hprt* locus in lymphocytes of exposed rodents.

A.4 Genotoxicity in humans

Genotoxicity in humans is usually assessed by the molecular epidemiology of exposed populations via the monitoring of genetic endpoints, including mutations. Several specific biomarkers of BD exposure are available which allow measurement of internal dosimetry of different BD metabolites. Two BD urinary metabolites: M1 (1, 2-dihydroxy-4-[*N*-acetylcysteinyl]-butane) and M2 (an isomeric mixture of 1-hydroxy-2-[*N*-acetylcisteinyl]-3-butene and 2-hydroxy-1-[*N*-acetylcisteinyl]-3-butene have frequently been used as measures of BD exposure (Albertini et al., 2010). N-terminal valine adducts of hemoglobin have also been used as in vivo BD dosimeters (Perez et al., 1997; Boogaard et al., 2001).

There have been several small studies involving one or more biomarkers of exposure and nine populations that have been subjected to comprehensive studies with biomarkers of BD exposure and specific genotoxicity endpoints including somatic gene mutations and chromosome aberrations (Albertini et.al., 2010). Overall the results of these investigations have been mixed. With respect to mutational endpoints, only studies of BD-exposed workers in Texas have reported significantly increased frequencies of *hprt* gene mutations in peripheral blood lymphocytes determined by autoradiography (Albertini et al., 2010; Wickliffe et al., 2009).

Albertini et al. (2007) conducted a molecular epidemiological study of 53 BD-exposed Czech workers and 51 controls. The mean 8-hr time weighted average (TWA) concentrations of BD were 0.008 mg/m^3 (3.5 ppb) and 0.397 mg/m^3 (180 ppb) for female controls and exposed, respectively, with individual 8-hr TWA values up to 9.8 mg/m^3 (4.45 ppm). For male workers, mean TWA values for control and exposed workers were 3.2 and 370 ppb, respectively with single individual values up to 5.72 ppm. For both sexes, the urinary BD metabolites M1 and M2 were elevated in exposed vs. control subjects, reaching statistical significance in males. *HPRT* mutations, via cloning assays, and multiple measures of chromosome level changes (SCEs) were not associated with BD exposures.

Wickliffe et al. (2009) evaluated the frequencies of HPRT mutant lymphocytes in BD polymer workers in a Southeast Texas facility. Thirty workers were exposed to BD concentrations of 93.5 ppb (mean and 2.5 ppb (median) with only one individual's exposure estimate (1684 ppb) exceeding the OSHA PEL of 1.0 ppm. The minimum detection level was 2.5 ppb and 50% of exposures were below this level. *HPRT* mutant frequencies by autoradiographic lymphocyte assay were not significantly associated with current exposures or age (N = 29). However *HPRT* mutant frequencies were significantly associated with occupational longevity (≥ 30 yr, N = 29, R^2 = 0.107, $P < 0.046$). The authors speculate that chronic or past high-level BD exposures may have produced retention of mutant long-term memory T-cells.

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
Microbial, Procaryotic			
BD	*Salmonella typhimurium* TA 1530, TA1535	+	Rat and human S-9, multiple tests
BD	*Salmonella typhimurium* TA 1530, TA1535	-	Without S-9, usually negative, positive results attributed to volatile metabolites
EB (BMO)	*Salmonella typhimurium* TA 1530, TA1535,TA100	+	Without S-9, LEC = 2431 µM
EB (BMO)	*Escherichia coli*	+	Without S-9
EB N^6 adenine adducts	*E.coli* transfected with adducted oligonucleotides	-	Neither isomer adduct was significantly mutagenic, Carmical et a. 2000
EB (BMO)	*Klebsiella pneumoniae*	+	Without S-9
DEB	*Salmonella typhimurium*	+	Without S-9, more potent than EB, LEC = 302µM
DEB	*E.coli*	+	Without S-9
DEB	*Bacillus megaterium*	+	Without S-9
DEB	*Pseudomonas pyocyanea*	+	Without S-9
DEB	*K. pneumonia*	+	Without S-9
DEB-GSH	*Salmonella typhimurium* TA1535	+	*S*-(2-hydroxy-3,4-epoxybutyl)GSH reported more mutagenic than DEB (Cho et al., 2010)
EBD	*Salmonella typhimurium*	+	With or without S-9, LEC = 980 µM
EBD N^6 adenine adducts	*E.coli* transfected with adducted oligonucleotides	+	S,S- stereoisomer of EBD gave higher mutagic frequency than the R,R isomer, Carmical et al., 2000

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
Microbial, Eucaryotic			
DEB	*Neurospora crassa*	+	Without S-9
DEB	*Schizosaccharomyces pombe*	+	Without S-9
DEB	*Saccharomyces cerevisiae*	+	Without S-9
DEB	*Aspergillus nidulans mitotic crossive over and recombinant selection, gene mutations*	+	20μM, 1 min; LEC = 50 mM, 13 min
DEB	*Penicillium multicolor*	+	Both stereoisomers
Insect			
BD	*Drosophila melanogaster*	-	Sex-linked recessive lethal mutations, spot wing test somatic mutations
ED (BMO)	*Drosophila melanogaster* adult males exposed to EB by inhalation for 24 hr. Gene mutations scored	+	Sex-linked recessive lethal mutations induced highest exposure level on 3800 ppm EB (91,200 ppm-hr) w/ DNA repair or at 2000 ppm (24,000 ppm-hr) w/o DNA repair.
DEB	*Drosophila melanogaster,* gene mutations, deletions, CAs	+	Sex-linked recessive lethal mutations, semi lethal and visible mutations, LEDs 100-800 μg/kg by injection.
DEB	*Drosophila melanogaster,* heritable gene deletions, point mutations	+	500 μM (single dose), high frequency of deletions vs. point mutations. Males fed DEB progeny phenotypes scored, or adults from treated larvae.

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
Mammalian cells in vitro			
BD	Mouse Lymphoma	+	S-9
DEB	Human TK6 (*hprt* and *tk* loci)	+	Mutations induced preferentially at A:T base pairs, 40-100x more potent than BDE, LECs = 3.5, 2.3 µM, respectively.
EB (BMO)	Human TK6 (*hprt* and *tk* loci)	+	Mutations induced preferentially at A:T base pairs, 3.5x more potent than BDE, LECs = 122, 243 µM, respectively.
BDE	Human TK6 (*hprt* and *tk* loci)	+	Mutations induced preferentially at A:T base pairs, LECs = 200, 300 µM, respectively.
DEB	Human TK6 (*hprt* locus)	+	Mutations induced preferentially at A:T base pairs
EB (BMO)	Human TK6 (*hprt* locus)	+	Mutations induced preferentially at A:T base pairs
Mammalian, whole animal			
BD inhalation	Mouse melanocyte mutations, mouse spot test	+	500 ppm, 6hr/d on gestation days 8-12, offspring inspected for color spots at 2-3 weeks of age.
BD inhalation	Mouse B6C3F1 *hprt* splenic lymphocytes	+	Mutagenic potency mouse/rat = 5
BD inhalation	Mouse B6C3F1 *hprt* thymic lymphocytes	+	
BD inhalation	Rat Fischer 344 *hprt* splenic lymphocytes	+	
BD inhalation	Rat Fischer 344 *hprt* thymic lymphocytes	+	
BD inhalation	Mouse 102XC3h *hprt* splenic lymphocytes	+	
BD inhalation	Mouse CD1 *hprt* splenic lymphocytes	-	
BD inhalation	Mouse MM *lac I*	+, -	Lung positive, bone marrow and liver negative

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
BD inhalation	Mouse BB *lac I*	+	
BD inhalation	Mouse 2-year Bioassay K-ras mutation	+	Lung and liver tumors, lymphoma
BD inhalation	Mouse 2-year Bioassay H-ras mutation	+	Liver tumors
BD inhalation	Mouse 2-year Bioassay p53, Rb, Chr.4 gene, allele loss	+	Lung and mammary tumors
EB (BMO)	Mouse B6C3F1 *hprt* splenic lymphocytes	+	
EB (BMO)	Mouse 102XC3h *hprt* splenic lymphocytes	±	
EB (BMO)	Rat Fischer 344 *hprt* splenic lymphocytes	±	
EB (BMO)	Rat Lewis *hprt* splenic lymphocytes	-	
DEB	Mouse B6C3F1 *hprt* splenic lymphocytes	+	
DEB	Mouse 102XC3h *hprt* splenic lymphocytes	-	
DEB	Mouse C57B1 *hprt* splenic lymphocytes	-	
DEB	Rat Fischer 344 *hprt* splenic lymphocytes	+	
DEB	Rat Lewis *hprt* splenic lymphocytes	-	
Mammalian cytogenetics in vitro			
BD	Chinese hamster ovary cells, sister chromatid exchanges (SCEs)	+	S-9
BD	Human lymphocytes, SCEs	±	± S-9, S-9 not necessary

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
EB (BMO)	Mouse, rat and human lymphocytes, Cas	-	EB negative for all species tested
EB (BMO)	Mouse and rat splenocytes, Cas	-	EB negative in both species, HIC = 931 µM EB
EB (BMO)	Human peripheral blood lymphocytes, MN induction	+	LEC = 1.0 µM
EB (BMO)	Rat spermatids, MN. Treatment of late pachytene-diakinetic spermatocytes for 4 days, score MN in meiosis.	-	Negative at 1000 µM
DEB	Rat spermatids, MN. Treatment of late pachytene-diakinetic spermatocytes for 4 days, score MN in meiosis.	+	LEC = 5 µM
DEB	Human peripheral blood lymphocytes, MN induction	+	LEC = 0.5 µM
DEB	Mouse and rat splenocytes, Cas	+	DEB positive in both species, 0.47 and 0.36 aberrant metaphases/100 metaphases/µM, respectively. LEC mouse = 40µM, LEC rat = 80µM.
DEB	Mouse, rat and human lymphocytes, Cas	+	DEB positive for all species tested, aberrant metaphases = 0.36/100/µM DEB for mice and rats.
DEB	Rat embryo fibroblasts, chromosome aberrations	+	Induction of Cas and high degree of tetraploidy, LEC = 93 µM
DEB	Rat liver cells, cytological scoring	+	Concentration not stated.

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
DEB	Human skin fibroblasts	+	Cas positive in Fanconi's anemia heterozygotes, LEC = 0.1 µM.
EBD	Rat spermatid, MN induction. Treatment of late pachytene-diakinetic spermatocytes for 4 days, score MN in meiosis.	-	HIC = 100 µM
Mammalian cytogenetics in vivo			
BD inhalation	Mouse Chromosome aberrations (Cas)	+	Lymphocytes and bone marrow multiple tests
BD inhalation	Mouse induced micronuclei (MN)	+	Lymphocytes and bone marrow multiple tests
BD inhalation	Mouse, induced MN in lung fibroblasts, 500 ppm (males) and 1300 ppm, 6hr/d x 5d	+	Significant increase in kinetochore (+) MN in males at 500 ppm, and kinetochore (+) and (-) MN in females at 1300 ppm.
BD inhalation	Mouse SCEs	+	Lymphocytes and bone marrow multiple tests
BD inhalation	Rat Cas	-	
BD inhalation	Rat MN	-	
BD inhalation	Rat SCEs	±	
EB (BMO), i.p. injection	Mouse, Cas in bone marrow cells	+	Positive dose response 25-150 mg/kg
EB (BMO), i.p. injection	Mouse and Rat, MN in splenic lymphocytes	+	Positive in both species, LED mice = 40 mg/kg, LED rats = 80 mg/kg
DEB, s.c. injection	Rat, peripheral blood lymphocytes Cas	+	Stickiness and clumping of chromosomes at 1 mg/kg, chromosome fragmentation at 3 mg/kg

Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
DEB, i.p. injection	Mouse and Rat, MN in splenic lymphocytes by cytokinesis-block method; characterization of induced MN by FISH.	+	Positive in both species, LED mice = 15 mg/kg, LED rats = 30 mg/kg
DEB, i.p. injection	Mouse, Cas in spermatogenic cells	+	Significant increase in chromosome breaks.
EBD, i.p. injection	Rat, MN in bone marrow cells at 30 and 60 mg.kg	±	Weak positive at 30 mg/kg only.
EBD, i.p. injection	Rat, MN in spermatids at 30 amd 60 mg/kg	+	Significant induction of MN on days 2 and 3 post-exposure (diploteme and late pachyteme)
Mammalian germ cell genotoxicity in vivo			
BD inhalation	Mouse dominant lethals	+	Multiple tests
BD inhalation	Mouse heritable translocation	+	2 studies
BD inhalation	Mouse sperm head abnormalities	+	Increases at 200 ppm, statistically significant at 1,000 and 5,000 ppm, 6hr/d x 5 d
BD inhalation	Mouse spermatid micronuclei	+	
BD inhalation	Mouse testicular cells Comet Assay	+	DNA fragmentation
BD inhalation	Rat dominant lethal	-	
EB (BMO), i.p. injection	Mouse splenic lymphocytes, SCEs	+	Statistically significant increases at 48.8 and 73.2 mg/kg. HID = 24.4 mg/kg
DEB, i.p., i.v. injection	Mouse SCEs in bone marrow cells, alveolar macrophages and liver cells	+	Positive SCE induction in all cell types.
DEB, 1 hr aerosol inhalation, i.p. injection.	Mouse and Chinese hamsters, SCEs in bone marrow cells	+	Positive in mice, LED = 20-30 mg/kg (based on blood concentration). Hamster less sensitive.

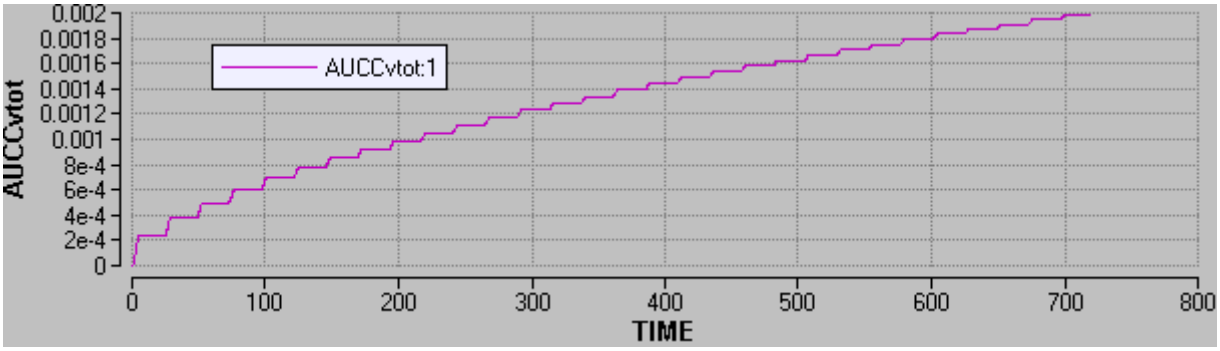
Table A1. Genotoxicity of 1, 3-Butadiene and Metabolites
(Adapted from Albertini 1999 and Albertini et al., 2010).

Compound	Test System	Results	Comments
EBD, i.p. injection	Mouse, dominant lethal mutations	-	Single EBD dose of 120 mg/kg
Human cytogenetics in vivo			
BD occupational exposure	Cas -	±	+ for $GST^{-/-}$ genotype
BD occupational exposure	SCEs	±	
BD occupational exposure	MN	-	
BD occupational exposure	Comet Assay	-	
BD occupational exposure	*hprt* T-cell (Autoradiography)	+	Variant frequencies significantly associated with occupational longevity (N = 29, R^2 = 0.107, P < 0.046), Wickliffe et al., 2009.
BD occupational exposure	*hprt* T-cell (Cloning Assay)	-	
BD occupational exposure	*ras* oncoprotein	-	

A.5 PBPK Modeling Data

A general summary of the PBPK models and related reports in the literature is given in Table A2. Of the available models, the one that seemed the most developed in view of relevant metabolites covered in mice and rats was that of Kohn and Melnick (2001). Unfortunately, due to the untimely death of the senior author we were unable to secure the actual model code for this model and were limited to the published report. The values given for the physiological parameters for mice and rats are reproduced in Table A3. Average reported values for alveolar ventilation rates of 20.2 and 20.0 L/hr/kg$^{0.7}$ for mice and rats, respectively were used in the model. For cardiac output the values used were 15.3 and 14.7 L/hr/kg$^{0.7}$, for mice and rats respectively. Typical body weights were 0.025 to 0.030 kg for mice and 0.215 to 0.360 kg for rats. Model partition coefficients are shown in Table A4. The biochemical parameters are listed in Table A5. The activities of P450 and epoxide hydrolase (EH) in mice and rats were multiplied by 30, 9, and 9 mg of microsomal protein per gram of tissue for liver, lung, and kidney, respectively. The glutathione *S*-transferase activities were multiplied by 82.8 or 108 mg of cytosolic protein per gram of tissue for mice and rats, respectively. Michaelis-Menten kinetics were used for P450 and EH and the ordered bi-bi rate law was used for GST.

The results of PBPK simulations of Doerr et al (1996) are shown in Table A6. Daily i.p. doses of BMO are administered to a young mouse over 30 days (720 hr). The cumulative AUC for DEB in the mixed venous blood (Cvtot) is divided by 30 to give the dose metric of μMhr/d, which is then analyzed by the benchmark dose method (Table A7) to obtain a $BMDL_{05}$. A Berkeley Madonna graph of the 30 days simulation of the 0.09 mmol/kg mid dose is shown in the Figure below. The units are AUC_{DEB} in mixed venous blood Mhr versus time in hours. The terminal value (0.002Mhr) corresponds to the 1985 μMhr value in Table A5 obtained from the tabular output of the same simulation.



An example of code for the OEHHA implementation of the Kohn and Melnick (2001) butadiene model is shown after the tables.

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Hattis & Wasson, 1987	Mice, Rats	BD	Pb = 0.35 Pf = 118.2 Plvrg = 5.4 Pm = 5.26 P_{450} Vmax = 400µmol/kg-hr (mice), 200µmol/kg-hr (rats) Km = 5.0 µM	3 compartment model liver combined with vessel rich group. Model runs in moles, minutes, liters and Vmax = 1.46 x 10^{-6} mol/min (rat)
Csanady et al., 1992	Mice, Rats, Humans	BD, BMO	P_{450} Vmax/Km = 157-1295; EH Vmax/Km = 3.6-35; GST Vmax/Km = 4.3-17 nmol/mg protein/min/mM, all values for liver	2-compartment kinetic model for BD and BMO metabolism in vitro: BD gas and liquid, BMO gas and liquid.
Johanson & Filser, 1993	Mice, Rats	BD, BMO, GSH (liver)	BD Pb = 3.03; Pf = 7.2; Pmusc = 0.24; Pl = 0.31; Pkid = 0.30; Pbrain = 0.14; BMO Pb = 83.4; Pf = 1.85; Pmusc = 0.72; Pl = 0.65; Pkid = 0.84; Pbrain = 0.62.	Closed chamber experiments simulated.
Evelo et al., 1993	Mice, Rats	BD	Pb = 1.184, 0.603 Pf = 32.36; Pl = 2.675; Pmusc = 1.871; Pkidney = 1.69; Plung = 1.272; Pbrain = 2.355; Pvrg = 2.02	2-compartment lung with gas exchange in alveoli, metabolism in bronchi and alveoli; Mice Vmax liver = 318, pulmonary = 70, bronchi = 77 µmol/hr-kg, Km = 8 µM

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Kohn & Melnick, 1993	Mice, Rats, Humans	BD, BMO	BD Pb = 1.5; BD Pf = 118.2; BD Pl = 5.49; BD Pmusc = 5.26; BD Pviscera = 5.34; BMO Pb = 60; BMO Pf = 1.81; BMO Pl = 0.6545; BMO Pmusc = 0.6533; BMO Pviscera = 0.6348	Sensitivity Analysis; Vmax BD P_{450} = 155.4 (mouse), 35.4 (rat), 70.8 (human) nmol/hr-mg prot., liver microsomal protein = 11.6, 16.8, 14.5 g/L, Km = 2, 3.75, 5.14 µM, respectively.
Medinsky et al., 1994	Mice, Rats	BD, BMO	BD Pb = 1.49, 1.34; BD Pf = 14.9, 14.3; BD Pl = 0.799, 1.01 BD Pm = 0.99, 2.99 BMO Pb = 50.4, 36.6; BMO Pf = 2.74, 2.49, BMO Pl = 1.43, 1.15 BMO Pm = 0.39, 0.64 (rats and mice, respectively.)	BD liver and lung oxidation; BMO liver oxidation, hydrolysis, and conjugation. Liver oxidation Vmax/Km = 16.5, 169 µmol/hr/kg/µM, rats and mice, respectively.
Johanson & Filser,1996	Mice, Rats, Humans	BD,BMO,GSH (liver)	BD Km = 83 µM GST Km(BMO) = 100 mM	Intrahepatic oxidation of BD and hydrolysis of BMO
Csanady et al., 1996	Mice, Rats, Humans	BD,BMO,DEB	Rats BD Vmax = 220 µmol/kg-hr, Km = 36 µM; Mice BD Vmax = 400 µmol/kg-hr, Km = 26 µM; BMO k_{P450} = 0.00126 L/hr; DEB k_{elim} = 0.0046 L/hr	Hemoglobin adducts from BMO reaction with N-terminal valine:2.9E-5 L/hr/g Hb

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Sweeney et al., 1997	Mice, Rats	BD, BMO, DEB in Liver and Lung;	DEB P_{liver} = 1.53, P_{kidney} = 1.41, P_{muscle} = 1.82, P_{fat} = 2.20; Mice BD Liver Vmax = 338 µmol/kg-hr, Km = 2.0µM, Lung Vmax = 21.6 µmol/kg-hr, Km = 5.01 µM; Liver DEB EH Vmax = 4193µmol/kg-hr, Km = 8100 µM; GST Vmax = 5.03 x 10^4 µmol/kg-d, Km = 6400 µM	Mice BMO Liver P_{450} Vmax = 176.6 µmol/kg-hr, Km = 145 µM, EH Vmax= 754 µmol/kg-hr, Km = 1590 µM, GST = 1.54 x 10^5 µmol/kg-hr, Km = 3.53 x 10^4 µM
Kohn & Melnick, 2000	Mice, Rats	BD, BMO, DEB	BD Pb = 1.95 BD Pf = 10.8 BMO Pb = 56.8 BMO Pf = 2.25 DEB Pf = 2.19 BD liver Vmax/Km = 1292, 578 nmol/min/mg protein/mM for mice and rats, respectively	Diffusion-limited model, liver, lung and kidney metabolism P_{450}, EH, GST; P_{450}-EH complex ER bound.
Kohn & Melnick, 2001	Mice, Rats	BD, BMO, DEB, BDD, EBD	BD Pb = 1.95 BD Pf = 10.8 BMO Pb = 56.8 BMO Pf = 2.25 DEB Pf = 2.19 BDD Pf = 0.573 EDB Pf = 0.496	Diffusion-limited model, liver, lung and kidney metabolism P_{450}, EH, GST; P_{450}-EH complex ER bound.

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Sweeney et al., 2001	Human	BD,BMO, DEB,BDD,EBD	Vmax/Km: BD→BMO, 0.0132/0.7; BD→DEB, 0.031/880; BMO→BDD, 1.4/540; BMO→$GS_{conj,}$ 2.7/10,400; DEB→GS_{conj}, 0.4/3390; BDD→EBD, 0.031/880; DEB→EBD, 9.2/4605; EBD→Erythritol, 4.6/4605; EBD→GS_{conj}, 0.2/339, µmol/mg prot.-hr/µM.	Tissue/blood partition coefficients for BDD and EBD assumed to be 1.0. Sensitivity analysis (SA) for exhaled BD (2hr human exposure to 5 ppm BD): BD blood/air = 0.36; alveolar ventilation = -0.36; cardiac output = 0.28; Qliver = -0.40; Qfat = -0.23. SA for DEB AUC: Vmax EB→DEB = -0.98;Vliver = -0.96; Km DEB→EBD = 0.94; Vmax DEB→EBD = -0.93; Vmax BMO→BDD = -0.87, microsomal protein liver = -0.80; BD blood/air = 0.78.
Smith et al. 2001	Humans	BD 3 compartment model fit to data from human subjects, N = 133	BD uptake µg/kg; kmet /min, CYP2E1 genotype, phenotype	BD uptake and rate of metabolism not related in this analysis. No significant differences in uptake or kmet with genetic makeup.

Table A2. Summary of Butadiene PBPK and Related Modeling Literature.

Study	Species	Chemicals modeled	Key parameters	Comments or other factors
Mezzetti et al., 2003	Humans	BD 3-compartment model fit to data from human subjects, N= 130 8 parameters analyzed	1120 parameters sampled, the most sensitive: W_{wp_i} the weight fraction of the well perfused tissues; P_{fat}, the fat/blood partition coeff; P_{wp} = wp/blood partition coeff.	Bayesian Analysis Model, MCMC analysis. Subgroups analyzed: males, females; age<30yr; age ≥30 yr; African-American; Hispanic; Asian, Caucasian
Brochot et al., 2007	Humans	BD, BMO, DEB, BDD, EBD	BD Pb = 1.22 BD Pf = 18.4 BD P_{450} Kmet = 0.19 ± 0.06 /min	Global sensitivity analysis
Pery & Bois, 2009	Human	BD	Pb = 0.653 Pf = 22.0 Kmet = 0.3 L/min	23 tissue compartments PBPK model coupled to PD model of carcinogenicity
Beaudouin et al., 2010	Humans, N = 133	BD	BD P_{450} Vmax = 2.28 x 10^{-5} mmol/min/mg microsomal protein, Km = 0.39 mM	Acute and chronic (lifetime) exposures modeled. Human inter-individual variability in exhaled BD well described, within 95% CI: median x 0.3 to 1.6.

Table A3. Physiological Parameter Values for Kohn & Melnick (2001) PBPK Model for Butadiene and Metabolites

Tissue compartment volumes, % body weight	Mouse	Rat
Liver	5.5	3.7
Lung (bronchi)	0.6	0.52
Alveolar	0.5	0.515
Kidney	1.67	1.48
GI Tract	7.5	7.5
Viscera	3.93	14.3
Fat	6.0	5.4
Muscle and skin	64.5	54.2
Blood	6.0	5.4
Capillary blood volume, % tissue volume		
Liver	11.0	13.8
Lung	11.0	18.0
Kidney	10.2	16.0
GI tract	2.9	2.65
Viscera	7.1	7.1
Fat	3.0	2.0
Muscle and skin	1.3	2.0
Blood flow rate, % cardiac output		
Liver (hepatic artery only)	4.4	3.9
Kidney	16.3	13.3
GI tract	18.1	18.1
Viscera	22.4	24.8
Fat	5.0	6.5
Muscle and skin	33.8	33.4

Table A4. Partition Coefficients for Kohn & Melnick (2001) PBPK Model for Butadiene and Metabolites

Partition Coefficient	Butadiene	Epoxybutene	Butenediol	Epoxybutanediol	Diepoxybutane
Blood:air	1.95	56.8	-	-	-
Liver:blood	0.595	0.984	1.04	0.903	1.41
Lung:blood	0.615	0.977	1.107	0.958	1.41
Kidney:blood	0.472	0.842	0.962	0.833	1.54
GI tract:blood	0.446	0.908	1.22	1.06	1.41
Viscera:blood	0.446	0.908	1.22	1.06	1.41
Fat	10.8	2.25	0.573	0.496	2.19
Muscle and Skin:blood	0.564	0.736	1.139	0.986	1.82

Table A5. Biochemical Parameters for Kohn & Melnick (2001) PBPK Model for Butadiene and Metabolites

	Mouse Liver	Rat Liver	Mouse Lung	Rat Lung	Mouse Kidney	Rat Kidney
Butadiene						
P450 Vmax	155	130	139	9.6	1430	30
P450 Km	0.002	0.00375	0.00501	0.00775	0.00501	0.00216
Epoxybutene						
P450 Vmax	45.1	24.3	10.2	9.84	48.6	12.6
P450 Km	0.0156	0.145	0.0156	0.145	0.0156	0.145
EH Vmax	347	584	34.8	42.8	113	14.7
EH Km	1.59	0.26	1.59	0.7	1.59	0.7
GST Vmax	6420	4260	720	196	960	494
GST Kmx, adjusted	3.59	2.59	3.59	4.94	3.59	4.39
Butenediol						
P450 Vmax	16.3	67.1	1.0	31.5	1.0	85.0
P450 Km	0.0156	0.145	0.0156	0.145	0.0156	0.145
GST Vmax	3280	1230	491	276	1070	658
GST Kmx	34	34	34	34	34	34
Epoxybutanediol						
EH Vmax	363	1150	69.5	169	10.0	152
EH Km	8.1	2.76	7.5	7.1	7.5	7.1
GST Vmax	2260	271	50.0	100	50.0	138
GST Kmx	6.40	4.17	6.40	4.17	6.40	4.17
Diepoxybutane						
EH Vmax	1920	3170	10.0	1160	35.2	1000
EH Km	8.1	2.76	7.5	7.1	7.5	7.1
GST Vmax	9720	1940	100	100	100	100
GST Kmx, adjusted	6.40	4.17	6.40	4.17	6.40	4.17
Cysteine						
Tissue cysteine	0.193	0.195	0.171	0.127	0.280	0.326
γ-GCS Vmax	420	396	54	50	7920	6080

Table Vmax values in nmol/hr/mg protein, Km values in mM.

Table A6. PBPK Modeling ot the Doerr et al. (1996) Data on Ovarian Atrophy in Mice (Model based on Kohn and Melnick, 2001)

BMO i.p. dose mmol/kg-d x 30 d	AUC DEB in blood µM hr (30 d)	AUC DEB in blood µM hr/day	Hb adducts nmol THBV/g globin-day
0.005	449	15.0	2.43
0.02	1046	34.9	21.8
0.09	1985	66.2	41.4
0.36	2717	90.6	56.7
1.43	3971	132.4	82.9

Table A7. Benchmark Dose Analysis of AUC DEB and Hb Adduct Dose Metrics with the Doerr et al. (1996) Data Set on Ovarian and Uterine Atrophy in Mice Induced by Parenteral Administration of Epoxybutene (BMO).

Model, continuous, site	Fit Statistic, P	BMD_{05} µMhr/d	$BMDL_{05}$ µMhr/d
Polynomial, ovary	0.919	57.4	20.5
Power, ovary	0.0284 n.s.	2688	1765
Polynomial, uterus	0.84	103.1	37.9
		BMD_{05} nmol Hb/g-d	$BMDL_{05}$ nmol Hb/g-d
Hill, ovary	0.661	31.75	18.3
Polynomial, ovary	0.9225	34.81	15.60
Polynomial, uterus	0.80	58.2	23.9

Table A8 Ovarian Quantal Toxicity Data (NTP, 1993)

Dose group	9 months	15 months	24 months	N
0	0/10	0/10	4/49	69
6.25	0/10	0/10	19/49	69
20.0	0/10	0/10	32/48	68
62.5	0/10	9/10	42/50	70
200.0	9/10	7/10	43/50	70
625.0	8/8	2/2	69/79	89
$BMCL_{05}$	3.44	0.65	0.00054	435

Table A9 Ovarian Toxicity Continuous Data for Weighted Analysis (Time Adjustment)

Dose group, Xi, ppm	Yi, %	Wi, weight time in months*
0	0	9
0	0	15
0	8.46	24
6.25	0	9
6.25	0	15
6.25	38.78	24
20	0	9
20	0	15
20	66.67	24
62.5	0	9
62.5	90.0	15
62.5	84.0	24
200	90.0	9
200	70.0	15
200	86.0	24
625	100.0	9
625	100.0	15
625	87.3	24

Note:* weighting by $X^2 = \sum_i W_{i(Yi - Y(Xi, P1 \ldots Pm))}^2$, $Y = a - b/(1 + c(X))^d$, fitted parameters $a = 93.52 \pm 0.236$, $b = 89.23 \pm 0.2933$, $c = 0.01558 \pm 0.000632$, $d = 1.7097 \pm 0.05887$ (SD).

Table A10 Ovarian Continuous to Quantal Conversion

Dose group	Y predicted ,% with parameters a, b, c, d	Adjusted quantal response for BMC analysis	Observed at 24 months, %	Observed average 9-24 months, %
0	4.29	3/69	8.16	2.8
6.25	17.4	12/69	38.78	12.5
20.0	37.4	25/68	66.67	22.2
62.5	65.6	46/70	84.0	58.0
200.0	85.6	60/70	86.0	82.0
625.0	91.98	82/89	87.3	95.8

Table A11. Continuous Benchmark Analysis of Adjusted Quantal Response from Time Weighted Regression*

Model	X^2, P	BMC_{05} ppm	$BMCL_{05}$ ppm	$BMLC_{05}$ ppm continuous
Log Logistic	1.13, 0.8896	2.05	1.58	0.28
Log Probit	**1.70, 0.6364**	**2.04**	**1.009**	**0.18**
Multistage	58.35, -	7.54	6.16	n.s.
Multistage	10.19, 0.017	4.10	3.37	Without top dose, n.s.

Note:* BMDS version 2.2

Table A 12. Benchmark Dose Analysis of Fetal Weight Data from Hackett et al. (1987) and Green (2003) Re-Analysis.

Data Set	BMC_{05}	$BMCL_{05}$	Variance assumption	Fit*	Model	Comment
Hackett et al. (1987), total males and females	44.4 183.9	20.0 111.2	Equal Unequal	Exact No	Hill Hill	Best fit
	57.6 89.6	45.9 62.2	Equal Unequal	Exact No	Polynomial Polynomial	Nonmonotonic
Male fetuses	39.5 28.5	19.3 13.4	Equal Unequal	Exact Yes	Hill Hill	Best fit
Green (2003), total males and females	40.3 172.5	19.2 48.0	Equal Unequal	Exact No	Hill Hill	Best fit
	58.2 54.4	46.7 44.4	Equal Unequal	Exact No	Polynomial Polynomial	Nonmonotonic
Male fetuses	37.2 22.6	**17.7** 9.6	Equal Unequal	Exact Yes	Hill Hill	**Best fit overall**

Note * Exact = predicted values equal observed by table and plot, all absolute value residuals <3E-7, Yes = test 4 for fit, A3 vs. fitted, all absolute value residuals <2 . All values obtained with BMDS version 2.3.1.

The following model code is an example of OEHHA implementation of the Kohn and Melnick (2001) butadiene model in Berkeley Madonna v. 8.3.9. In Berkeley Madonna lines preceded by semi colons or enclosed in curved brackets are not executed.

```
METHOD Stiff

STARTTIME = 0
STOPTIME = 720
DT = 0.0005
DTOUT = 0.25
;Butadiene multimetabolite mouse model based on Kohn & Melnick (Chemico-Biol. Interact. 135-136:285-301(2001)). Symbols: A = mass of BD in moles, AB = BMO, AC = DEB, AD = BDD; AE = BDE; f = fat, l = liver, m = muscle, vrg = vessel rich group, br = bronchi, pu = alveoli, uo = uterus-ovary, kid, k = kidney, cap = tissue capillary bed; exh = exhaled, perit = peritoneum,C = concentration, Cv = concentration leaving tissue, Cvtot = mixed venous concentration, AUC = area under the time x concentration curve, V = volume, Q = flow, P = partition coeff.,Vmax = metabolic rate, Km = affinity constant, P450 = oxidase; EH = epoxide hydrolase, GST = glutathione sulfotransferase, BW = body weight for adult or young mouse for 30 day simulations, Amet = amount metabolized
{butadiene, moles}
init Af = 0
Limit Af >= 0
init Al = 0
Limit Al >= 0
init Am = 0
Limit Am >= 0
init Avrg = 0
Limit Avrg >= 0
init Abr = 0
Limit Abr >= 0
init Apu = 0
Limit Apu >= 0
init Auo = 0
Limit Auo >= 0
init AUCvtot = 0
Limit AUCvtot >= 0
init Aexh = 0
Limit Aexh >= 0
init Akid = 0
Limit Akid >= 0

init Afcap = 0
Limit Afcap >= 0
init Alcap = 0
Limit Alcap >= 0
init Amcap = 0
Limit Amcap >= 0
init Avrgcap = 0
Limit Avrgcap >= 0
;init Abrcap = 0
;init Apucap = 0
init Auocap = 0
Limit Auocap >= 0
```

```
init Akidcap = 0
Limit Akidcap >= 0

{butadienemonoxide moles}
init ABperit = 0
init ABf = 0
Limit ABf >= 0
init ABl = 0
Limit ABl >= 0
init ABlfree = 0
init ABm = 0
Limit ABm >= 0
init ABvrg = 0
Limit ABvrg >= 0
init ABbr = 0
Limit ABbr >= 0
init ABbrfree = 0
init ABpu = 0
Limit ABpu >= 0
init ABpufree = 0
init ABuo = 0
Limit ABuo >= 0
init AUCBuo = 0
Limit AUCBuo >= 0
init AUCBvtot = 0
Limit AUCBvtot >= 0
init ABkid = 0
Limit ABkid >= 0
init ABkidfree = 0

init ABfcap = 0
Limit ABfcap >= 0
init ABlcap = 0
Limit ABlcap >= 0
init ABmcap = 0
Limit ABmcap >= 0
init ABvrgcap = 0
Limit ABvrgcap >= 0
;init ABbrcap = 0
;init ABpucap = 0
init ABuocap = 0
Limit ABuocap >= 0
init ABkidcap = 0
Limit ABkidcap >= 0

{diepoxybutane moles}
init ACperit = 0
init ACf = 0
Limit ACf >= 0
init ACl = 0
Limit ACl >= 0
init AClfree = 0
init ACm = 0
Limit ACm >= 0
init ACvrg = 0
Limit ACvrg >= 0
```

```
init ACbr = 0
Limit ACbr >= 0
init ACbrfree = 0
init ACpu = 0
Limit ACpu >= 0
init ACpufree = 0
init ACuo = 0
Limit ACuo >= 0
init AUCCuo = 0
Limit AUCCuo >= 0
init AUCCvtot = 0
Limit AUCCvtot >= 0
init ACkid = 0
Limit ACkid >= 0
init ACkidfree = 0

init ACfcap = 0
init AClcap = 0
init ACmcap = 0
init ACvrgcap = 0
;init ACbrcap = 0
;init ACpucap = 0
init ACuocap = 0
init ACkidcap = 0

{dihydroxybutene}
init ADf = 0
Limit ADf >= 0
init ADl = 0
Limit ADl >= 0
init ADm = 0
Limit ADm >= 0
init ADvrg = 0
Limit ADvrg >= 0
init ADbr = 0
Limit ADvrg >= 0
init ADpu = 0
Limit ADpu >= 0
init ADuo = 0
Limit ADuo >= 0
init AUCDvtot = 0
init ADkid = 0
Limit ADkid >= 0

init ADfcap = 0
init ADlcap = 0
init ADmcap = 0
init ADvrgcap = 0
;init ADbrcap = 0
;init ADpucap = 0
init ADuocap = 0
init ADkidcap = 0

{epoxybutanediol}
init AEf = 0
Limit AEf >= 0
```

```
init AEl = 0
Limit AEl >= 0
init AElfree = 0
init AEm = 0
Limit AEm >= 0
init AEvrg = 0
Limit AEvrg >= 0
init AEbr = 0
init AEbrfree = 0
Limit AEvrg >= 0
init AEpu = 0
Limit AEpu >= 0
init AEpufree = 0
init AEuo = 0
Limit AEuo >= 0
init AUCEvtot = 0
init AEkid = 0
Limit AEkid >= 0
init AEkidfree = 0

init AEfcap = 0
init AElcap = 0
init AEmcap = 0
init AEvrgcap = 0
;init AEbrcap = 0
;init AEpucap = 0
init AEuocap = 0
init AEkidcap = 0

{moles of butadiene metabolized}
init Ametl = 0
init Ametpu = 0
init Ametbr = 0
init Ametk = 0

{moles of butadienemonoxide metabolized}
init ABmetl1 = 0
Limit ABmetl1 >= 0
init ABmetl2 = 0
Limit ABmetl2 >= 0
init ABmetpu1 = 0
Limit ABmetpu1 >= 0
init ABmetpu2 = 0
Limit ABmetpu2 >= 0
init ABmetbr1 = 0
Limit ABmetbr1 >= 0
init ABmetbr2 = 0
Limit ABmetbr2 >= 0
init ABmetl3 = 0
Limit ABmetl3 >= 0
init ABmetpu3 = 0
Limit ABmetpu3 >= 0
init ABmetbr3 = 0
Limit ABmetbr3 >= 0

{moles of dihydroxybutene metabolized}
```

```
init ADmetpu6 = 0
init ADmetbr6 = 0
init ADmetl6 = 0

{moles of diepoxybutane metabolized}
init ACmetpu4 = 0
init ACmetbr4 = 0
init ACmetl4 = 0

{Cysteine metabolism, moles, mol/L}
init AFl = Ccysl*Vl
init AFlu = Ccyslu*Vlu
init AFkid = Ccyskid*Vkid
Ccysl = 1.93E-4
Ccyslu = 1.71E-4
Ccyskid = 2.8E-4
Vmax10lC = 420E-9
Vmax10l = Vmax10lC*CPL*Vl
Vmax10luC = 54E-9
Vmax10lu = Vmax10luC*CPLu*Vlu
Vmax10kidC = 7920E-9
Vmax10kid = Vmax10kidC*CPLu*Vkid
Kmcys = 3.5E-4
Kl = 2.3E-3
Kl0 = 1.5*Vl
Klu0 = 1.5*Vlu
Kkid0 = 1.5*Vkid

{Glutathione metabolism, moles, mol/L}
init AGl = CGSHl*Vl
Limit AGl >= 0
init AGlu = CGSHlu*Vlu
Limit AGlu >= 0
init AGkid = CGSHkid*Vkid
Limit AGkid >= 0
CGSHl = 5E-3
CGSHlu = 1E-3
CGSHkid = 2E-3
CGl = AGl/Vl
Limit CGl >= 0
CGlu = AGlu/Vlu
Limit CGlu >= 0
CGkid = AGkid/Vkid
Limit CGkid >= 0
Ka = 3E-3

{Adduct formation}
init Adduct = 0

{tissue flows L/hr}
Qtot = 15.3*BW^0.7
Qalv = 20.2*BW^0.7
Qf = 0.05*Qtot
Ql = 0.044*Qtot
Qkid = 0.163*Qtot
Qm = Qtot - (Qf + Ql + Qvrg + Quo + Qkid)
```

```
Qvrg = 0.389*Qtot
Qpu = 0.928*Qtot
Qbr = 0.072*Qtot
Quo = 0.016*Qtot

{tissue volumes, L}
Vf = 0.06*BW
Vl = 0.055*BW
Vkid = 0.0167*BW
Vm = BW - (Vf + Vl + Vvrg + Vlu + Vuo + Vkid)
Vvrg = 0.1143*BW
Vlu = 0.011*BW
Vpu = 0.454*Vlu
Vbr = 0.545*Vlu
Vuo = 0.001*BW
;BW = 0.030
BW = 0.01263 + 3.69E-4*(T/24) - 5.59E-6*(T/24)^2

{capillary blood volumes, L}
Vfcap = 0.03*Vf
Vlcap = 0.11*Vl
;Vbrcap = 0.11*Vbr
;Vpucap = 0.11*Vpu
Vkidcap = 0.102*Vkid
Vvrgcap = 0.071*Vvrg
Vuocap = 0.071*Vuo
Vmcap = 0.013*Vm

{blood/air and tissue/blood partition coefficients, butadiene}
Pb = 1.95
Pl = 0.595
Pf = 10.8
Pkid = 0.472
Pm = 0.564
Pvrg = 0.472
Ppu = 0.615
Pbr = 0.615
Puo = 0.446
Pibd = 1.183

{blood/air and tissue/blood partition coefficients, butadienemonoxide}
PBb = 56.8
PBl = 0.984
PBkid = 0.842
PBf = 2.25
PBm = 0.736
PBvrg = 0.842
PBpu = 0.977
PBbr = 0.977
PBuo = 0.908
Pibmo = 2.125

{blood/air and tissue/blood partition coefficients, diepoxybutane}
PCl = 4.41
PCkid = 1.54
PCf = 2.19
```

PCm = 1.82
PCvrg = 1.54
PCpu = 1.41
PCbr = 1.41
PCuo = 1.41
Pideb = 1.174

{blood/air and tissue/blood partition coefficients, dihydroxybutene}
PDl = 1.04
PDkid = 0.833
PDf = 0.573
PDm = 1.139
PDvrg = 0.962
PDpu = 1.107
PDbr = 1.107
PDuo = 1.22
Pidhb = 1.198

{blood/air and tissue/blood partition coefficients, epoxybutanediol}
PEl = 0.903
PEkid = 1.06
PEf = 0.496
PEm = 0.986
PEvrg = 1.06
PEpu = 0.833
PEbr = 0.833
PEuo = 1.06
Piebd = 1.237

{butadiene oxidation metabolic parameters, mol/hr, mol/L}
Vmaxlu = 139E-9*9.0E3*Vlu
Vmaxbr = 0.50*Vmaxlu
Vmaxpu = 0.50*Vmaxlu
Kmlu = 5.01E-6
Vmaxl = 155.0E-9*30.0E3*Vl
Km = 2.0E-6
VmaxkC = 1430E-9
Vmaxk = VmaxkC*Vkid*30.0E3
Kmk = Kmlu
MPL = 3.0E4
MPLu = 9.0E3

{butadienemonoxide metabolic parameters, mol/hr, mol/L, /hr; 1 = hydrolysis EH, 2 = conjugation GST, 3 = oxidation P450}
;EH
Vmaxl1 = 347.0E-9*30.0E3*Vl
Km1 = 1.59E-3
Vmaxlu1C = 34.8E-9
Vmaxbr1 = Vmaxlu1C*Vbr*9.0E3
Vmaxpu1 = Vmaxlu1C*Vpu*9.0E3
Kmlu1 = 1.59E-3
Vmaxk1C = 113E-9
Vmaxk1 = Vmaxk1C*Vkid*9.0E3
Kmk1 = Kmlu1
krel = 3.76
R = 0.052

```
;GST
Vmaxl2C = 6420E-9
Vmaxl2 = Vmaxl2C*Vl*8.28E4
Km2 =3.59E-3
Vmaxlu2C = 720.0E-9
Vmaxbr2 = Vmaxlu2C*Vbr*8.28E4
Vmaxpu2 = Vmaxlu2C*Vpu*8.28E4
Kmlu2 = 3.59E-3
Vmaxk2C = 960.0E-9
Vmaxk2 = Vmaxk2C*Vkid*8.28E4
Kmk2 = 3.59E-3
CPL = 8.28E4
CPLu = 8.28E4
Kmgsh = 1.0E-3

;P450
Vmaxl3 = 45.1E-9*30.0E3*Vl
Vmaxlu3C = 10.2E-9
Vmaxpu3 = Vmaxlu3C*Vpu*9.0E3
Vmaxbr3 = Vmaxlu3C*Vbr*9.0E3
Km3 = 1.56E-5
Kmlu3 = Km3
Kmk3 = Km3
Vmaxk3C = 48.6E-9
Vmaxk3 = Vmaxk3C*Vkid*9.0E3

{Diepoxybutane (DEB) metabolic parameters, 4 = EH, 5 = GST, mol/hr, mol/L}
Vmaxl4C = 1920E-9
Vmaxl4 = Vmaxl4C*Vl*30E3
Km4 = 8.1E-3
Vmaxlu4C = 10E-9
Vmaxbr4 = Vmaxlu4C*Vbr*9.0E3
Vmaxpu4 = Vmaxlu4C*Vpu*9.0E3
Kmlu4 = 7.5E-3
Vmaxk4C = 35.2E-9
Vmaxk4 = Vmaxk4C*Vkid*9.0E3
Kmk4 = Kmlu4
k4rel = 9.75

Vmaxl5C = 9720E-9
Vmaxl5 = Vmaxl5C*Vl*CPL
Km5 = 6.4E-3
Vmaxlu5C = 100E-9
Vmaxbr5 = Vmaxlu5C*Vbr*CPlu
Vmaxpu5 = Vmaxlu5C*Vpu*CPlu
Vmaxk5C = 100E-9
Vmaxk5 = Vmaxk5C*Vkid*CPlu

{Dihydroxybutene (DHB) metabolic parameters, 6 = P450, 7 = GST, mnol/hr, mol/L}
Vmaxl6C = 16.3E-9
Vmaxl6 = Vmaxl6C*Vl*30E3
Km6 = 1.56E-5
Vmaxlu6C = 1.0E-9
Vmaxbr6 = Vmaxlu6C*Vbr*9.0E3
Vmaxpu6 = Vmaxlu6C*Vpu*9.0E3
```

```
Vmaxk6C = 1.0E-9
Vmaxk6 = Vmaxk6C*Vkid*9.0E3

Vmaxl7C = 3480E-9
Vmaxl7 = Vmaxl7C*Vl*CPL
Km7 = 34E-3
Vmaxlu7C = 491E-9
Vmaxbr7 = Vmaxlu7C*Vbr*CPlu
Vmaxpu7 = Vmaxlu7C*Vpu*CPlu
Vmaxk7C = 1070E-9
Vmaxk7 = Vmaxk7C*Vkid*CPlu

{Epoxybutanediol metabolic parameters 8 = EH, 9 = GST}
Vmaxl8C = 363E-9
Vmaxl8 = Vmaxl8C*Vl*30E3
Km8 = 8.1E-3
Vmaxlu8C = 69.5E-9
Vmaxbr8 = Vmaxlu8C*Vbr*9.0E3
Vmaxpu8 = Vmaxlu8C*Vpu*9.0E3
Kmlu8 = 7.5E-3
Vmaxk8C = 10E-9
Vmaxk8 = Vmaxk8C*Vkid*9.0E3
Kmk8 = Kmlu8
k8rel = 50.6

Vmaxl9C = 2260E-9
Vmaxl9 = Vmaxl9C*Vl*CPL
Km9 = 6.4E-3
Vmaxlu9C = 50E-9
Vmaxbr9 = Vmaxlu9C*Vbr*CPlu
Vmaxpu9 = Vmaxlu9C*Vpu*CPlu
Vmaxk9C = 50E-9
Vmaxk9 = Vmaxk9C*Vkid*CPlu

Kadduct = 1E-4

Kip = 1.25
T = TIME
{exposure in ppm converted to moles}
Cair = IF TIME <= 6 THEN 0*(1E-6/25.45) ELSE 0

{calculated concentrations of butadiene}
Cart = (Qpu*Cvpu + Qbr*Cvbr)/Qtot
Cvf = Af/(Vf*Pf)
Cvl = Al/(Vl*Pl)
Cvkid = Akid/(Vkid*Pkid)
Cvm = Am/(Vm*Pm)
Cvvrg = Avrg/(Vvrg*Pvrg)
Cvpu = Apu/(Vpu*Ppu)
Cvbr = Abr/(Vbr*Pbr)
Cvuo = Auo/(Vuo*Puo)
Cvtot = (Ql*Cvl + Qf*Cvf + Qm*Cvm + Qvrg*Cvvrg + Quo*Cvuo + Qkid*Cvkid)/Qpu
Cvtotg = Cvtot*MWBD*1000
Cvipu = (Qalv*Cair + Qpu*Cvtot)/((Qalv/Pb) + Qpu)
Cexh = Cvipu/Pb
MWBD = 54.1
```

{calculated concentrations of butadienemonoxide}
CBart = (Qpu*CBvpu + Qbr*CBvbr)/Qtot
CBvf = ABf/(Vf*PBf)
CBvl = ABl/(Vl*PBl)
CBvkid = ABkid/(Vkid*Pkid)
CBvm = ABm/(Vm*PBm)
CBvvrg = ABvrg/(Vvrg*PBvrg)
CBvpu = ABpu/(Vpu*PBpu)
CBvbr = ABbr/(Vbr*PBbr)
CBvuo = ABuo/(Vuo*PBuo)
CBvtot = (Ql*CBvl + Qf*CBvf + Qm*CBvm + Qvrg*CBvvrg + Quo*CBvuo + Qkid*CBvkid)/Qtot
CBvtotg = CBvtot*MWEB*1000
CBair = CBvtot/PBb
CBvipu = (Qalv*CBair + Qpu*CBvtot)/((Qalv/PBb) + Qpu)
CBexh = CBvipu/PBb
MWEB = 70.1

{calculated concentrations of diepoxybutane}
CCart = (Qpu*CCvpu + Qbr*CCvbr)/Qtot
CCvf = ACf/(Vf*PCf)
CCvl = ACl/(Vl*PCl)
CCvkid = ACkid/(Vkid*PCkid)
CCvm = ACm/(Vm*PCm)
CCvvrg = ACvrg/(Vvrg*PCvrg)
CCvpu = ACpu/(Vpu*PCpu)
CCvbr = ACbr/(Vbr*PCbr)
CCvuo = ACuo/(Vuo*PCuo)
CCvtot = (Ql*CCvl +Qf*CCvf + Qm*CCvm + Qvrg*CCvvrg + Quo*CCvuo + Qkid*CCvkid)/Qtot
CCvtotg = CCvtot*MWDEB*1000
CCvipu = (Qalv + Qpu*CCvtot)/(Qalv + Qpu)
MWDEB = 86.0

{calculated concentrations of dihydroxybutene}
CDart = (Qpu*CDvpu + Qbr*CDvbr)/Qtot
CDvf = ADf/(Vf*PDf)
CDvl = ADl/(Vl*PDl)
CDvkid = ADkid/(Vkid*PDkid)
CDvm = ADm/(Vm*PDm)
CDvvrg = ADvrg/(Vvrg*PDvrg)
CDvpu = ADpu/(Vpu*PDpu)
CDvbr = ADbr/(Vbr*PDbr)
CDvuo = ADuo/(Vuo*PDuo)
CDvtot = (Ql*CDvl + Qf*CDvf + Qm*CDvm + Qvrg*CDvvrg + Quo*CDvuo + Qkid*CDvkid)/Qtot
CDvtotg = CDvtot*MWDHB*1000
CDvipu = (Qalv + Qpu*CDvtot)/(Qalv +Qpu)
MWDHB = 72.0

{calculated concentrations of epoxybutanediol}
CEart = (Qpu*CEvpu + Qbr*CEvbr)/Qtot
CEvf = AEf/(Vf*PEf)
CEvl = AEl/(Vl*PEl)
CEvkid = AEkid/(Vkid*PEkid)
CEvm = AEm/(Vm*PEm)
CEvvrg = AEvrg/(Vvrg*PEvrg)

```
CEvpu = AEpu/(Vpu*PEpu)
CEvbr = AEbr/(Vbr*PEbr)
CEvuo = AEuo/(Vuo*PEuo)
CEvtot = (Ql*CEvl + Qf*CEvf + Qm*CEvm + Qvrg*CEvvrg + Quo*CEvuo + Qkid*CEvkid)/Qtot
CEvtotg = CEvtot*MWEBD*1000
CEvipu = (Qalv + Qpu*CEvtot)/(Qalv +Qpu)
MWEBD = 102.1

{differential equations for butadiene uptake and metabolism}
d/dt(Apu) = Qpu*(Cvipu - Cvpu) - Vmaxpu*Cvpu/(Kmlu + Cvpu)
d/dt(Abr) = Qbr*(Cart - Cvbr) - Vmaxbr * Cvbr/(Kmlu + Cvbr)
d/dt(Alcap) = Ql*(Cart - Alcap/Vlcap) - Ql*Pibd*(Cart - Cvl)
d/dt(Al) = Ql*Pibd*(Alcap/Vlcap - Cvl) - Vmaxl*Cvl/(Km + Cvl)
d/dt(Akidcap) = Qkid*(Cart - Akidcap/Vkidcap) - Qkid*Pibd*(Cart - Cvkid)
d/dt(Akid) = Qkid*Pibd*(Cart - Cvkid) - Vmaxk*Cvkid/(Kmk + Cvkid)
d/dt(Afcap) = Qf*(Cart - Afcap/Vfcap) - Qf*Pibd*(Cart - Cvf)
d/dt(Af) = Qf*Pibd*(Cart - Cvf)
d/dt(Amcap) = Qm*(Cart - Amcap/Vmcap) - Qm*Pibd*(Cart - Cvm)
d/dt(Am) = Qm*Pibd*(Cart - Cvm)
d/dt(Avrgcap) = Qvrg*(Cart - Avrgcap/Vvrgcap) - Qvrg*Pibd*(Cart - Cvvrg)
d/dt(Avrg) = Qvrg*Pibd*(Cart - Cvvrg)
d/dt(Auocap) = Quo*(Cart - Auocap/Vuocap) - Quo*Pibd*(Cart - Cvuo)
d/dt(Auo) = Quo*Pibd*(Cart - Cvuo)
d/dt(AUCvtot) = Cvtot
d/dt(Aexh) = Cexh*0.15

{amount of butadiene metabolized in liver and lung}
d/dt(Ametl)  = Vmaxl*Cvl/(Km + Cvl)
d/dt(Ametpu) = Vmaxpu*Cvpu/(Kmlu + Cvpu)
d/dt(Ametbr) = Vmaxbr*Cvbr/(Kmlu + Cvbr)
d/dt(Ametk) = Vmaxk*Cvkid/(Kmk + Cvkid)

{differential equations for BMO metabolism}
d/dt(ABperit) = PULSE(1.31*9.0E-5*BW,0,24) -ABperit*Kip
init AUCABperit = 0
d/dt(AUCABperit) = ABperit

d/dt(ABpufree) = ABpu*krel

d/dt(ABpu) = Qpu*(CBart - CBvpu) + Vmaxpu*Cvpu/(Kmlu + Cvpu) - Vmaxpu1*ABpufree/(Km1*Vpu +
ABpufree) - Vmaxpu1*ABpu/(R*Km1*Vpu + ABpu) - Vmaxpu2*CBvpu*CGSHlu/(Kmlu2*Kmgsh +
CBvpu*Kmgsh + CGSHlu*Kmlu2 + CBvpu*CGSHlu) - Vmaxpu3*CBvpu/(Kmlu3 + CBvpu)

d/dt(ABbrfree) = ABbr*krel

d/dt(ABbr) = Qbr*(CBart - CBvbr) + Vmaxbr * Cvbr/(Kmlu + Cvbr) - Vmaxbr1*ABbrfree/(Km1*Vbr +
ABbrfree) - Vmaxbr1*ABbr/(R*Km1*Vbr + ABbr) -Vmaxbr2*CBvbr*CGSHlu/(Kmlu2*Kmgsh +
CBvbr*Kmgsh + CGSHlu*Kmlu2 + CGSHlu*CBvbr) - Vmaxbr3*CBvbr/(Kmlu3 + CBvbr) + ABperit*Kip

d/dt(ABlcap) = Ql*(CBart - ABlcap/Vlcap) - Ql*Pibmo*(CBart - CBvl)

d/dt(ABlfree) = ABl*krel

d/dt(ABl) = Ql*Pibmo*(CBart - CBvl) + Vmaxl*Cvl/(Km + Cvl) - Vmaxl1*ABlfree/(Km1*Vl + ABlfree) -
Vmaxl1*ABl/(R*Km1*Vl + ABl) - Vmaxl2*CBvl*CGSHl/(Km2*Kmgsh + CBvl*Kmgsh + CGSHl*Km2 +
CGSHl*CBvl) - Vmaxl3*Cbvl/(Km3 + CBvl)
```

```
d/dt(ABkidcap) = Qkid*(CBart - ABkidcap/Vkidcap) - Qkid*Pibmo*(CBart - CBvkid)

d/dt(ABkidfree) = ABkid*krel

d/dt(ABkid) = Qkid*Pibmo*(CBart - CBvkid) + Vmaxk*Cvkid/(Kmk + Cvkid) -
Vmaxk1*ABkidfree/(Km1*Vkid + ABkidfree) - Vmaxk1*ABkid/(R*Km1*Vkid + ABkid) -
Vmaxk2*CBvkid*CGSHkid/(Kmk2*Kmgsh + CBvkid*Kmgsh + CGSHkid*Kmk2 + CGSHkid*CBvkid) -
Vmaxk3*CBvkid/(Kmk3 + CBvkid)

d/dt(ABfcap) = Qf*(CBart - ABfcap/Vfcap) - Qf*Pibmo*(CBart - CBvf)
d/dt(ABf) = Qf*Pibmo*(CBart - CBvf)

d/dt(ABmcap) = Qm*(CBart - ABmcap/Vmcap) - Qm*Pibmo*(CBart - CBvm)
d/dt(ABm) = Qm*Pibmo*(CBart - CBvm)

d/dt(ABvrgcap) = Qvrg*(CBart - ABvrgcap/Vvrgcap) - Qvrg*Pibmo*(CBart - CBvvrg)
d/dt(ABvrg) = Qvrg*pibmo*(CBart - CBvvrg)

d/dt(ABuocap) = Quo*(CBart - ABuocap/Vuocap) - Quo*Pibmo*(CBart - CBvuo)
d/dt(ABuo) = Quo*Pibmo*(CBart - CBvuo)

{Hb and DNA adducts based on styrene oxide induced formation rates}
init HB1 = 0
d/dt(HB1) = CCart*kher ; mole /hr/g Hb
kher = 3.2E-5 ; L/hr/g Hb
Ter = 960 ; lifetime of RBC in hr
HB = (1E9)*HB1*(1 - (T/(2*Ter))) ; nmol/g Hb
init DNA1 = 0
d/dt(DNA1) = CCart*kfdna - DNA*keldna/1E9
DNA = DNA1*1E9 ; nmol/gDNA
kfdna = 9.6E-5 ; N7 DNA adducts/gDNA/hr
keldna = 0.0077 ; elimination of DNA adducts /hr

{AUCs for butadienemonoxide}
d/dt(AUCBuo) = CBvuo
d/dt(AUCBvtot) = CBvtot

{amounts of butadienemonoxide metabolized}
d/dt(ABmetl1) = Vmaxl1*ABlfree/(Km1*Vl + ABlfree) + Vmaxl1*ABl/(R*Km1*Vl + ABl)
d/dt(ABmetl2) = Vmaxl2*CBvl*CGSHl/(Km2*Kmgsh + CBvl*Kmgsh + CGSHl*Km2 + CGSHl*CBvl)

d/dt(ABmetpu1) = Vmaxpu1*ABpufree/(Km1*Vpu + ABpufree) + Vmaxpu1*ABpu/(R*Km1*Vpu + ABpu)
d/dt(ABmetpu2) = Vmaxpu2*CBvpu*CGSHlu/(Km2*Kmgsh + CBvpu*Kmgsh + CGSHlu*Km2 +
CGSHlu*CBvpu)

d/dt(ABmetbr1) = Vmaxbr1*ABbrfree/(Km1*Vbr + ABbrfree) + Vmaxbr1*ABbr/(R*Km1*Vbr + ABbr)
d/dt(ABmetbr2) = Vmaxbr2*CBvbr*CGSHlu/(Km2*Kmgsh + CBvbr*Kmgsh + CGSHlu*Km2 +
CGSHlu*CBvbr)

d/dt(ABmetl3) = Vmaxl3*CBvl/(Km3 + CBvl)
d/dt(ABmetpu3) = Vmaxpu3*CBvpu/(Km3 + CBVpu)
d/dt(ABmetbr3) = Vmaxbr3*CBvbr/(Km3 + CBvbr)

init ABmetk1 = 0
init ABmetk2 = 0
```

```
init ABmetk3 = 0
d/dt(ABmetk1) = Vmaxk1*ABkidfree/(Km1*Vkid + ABkidfree) + Vmaxk1*ABkid/(R*Km1*Vkid + ABkid)
d/dt(ABmetk2) = Vmaxk2*CBvkid*CGSHkid/(Km2*Kmgsh + CBvkid*Kmgsh + CGSHkid*Km2 +
CGSHkid*CBvkid)
d/dt(ABmetk3) = Vmaxk3*CBvkid/(Km3 + CBvkid)


{differential equations for diepoxybutane}
d/dt(ACperit) = PULSE(0*BW,0,24) -ACperit*Kip

d/dt(ACpufree) = ACpu*k4rel

d/dt(ACpu) = Qpu*(CCart - CCvpu) + Vmaxpu3*CBvpu/(Km3 + CBvpu) - Vmaxpu4*ACpufree/(Kmlu4*Vpu
+ ACpufree) - Vmaxpu4*ACpu/(R*Kmlu4*Vpu + ACpu) - Vmaxpu5*CCvpu*CGSHlu/(Km5*Kmgsh +
CCvpu*Kmgsh + CGSHlu*Km5 + CGSHlu*CCvpu)

d/dt(ACbrfree) = ACbr*k4rel

d/dt(ACbr) = Qbr*(CCart - CCvbr) + Vmaxbr3*CBvbr/(Km3 + CBvbr)  - Vmaxbr4*ACbrfree/(Kmlu4*Vbr +
ACbrfree) - Vmaxbr4*ACbr/(R*Kmlu4*Vbr + ACbr) - Vmaxbr5*CCvbr*CGSHlu/(Km5*Kmgsh +
CCvbr*Kmgsh + CGSHlu*Km5 + CGSHlu*CCvbr) + ACperit*Kip

d/dt(AClfree) = ACl*k4rel

d/dt(AClcap) = Ql*(CCart - AClcap/Vlcap) - Ql*Pideb*(CCart - CCvl)
d/dt(ACl) = Ql*Pideb*(CCart - CCvl) + Vmaxl3*CBvl/(Km3 + CBvl) - Vmaxl4*AClfree/(Km4*Vl + AClfree) -
Vmaxl4*ACl/(R*Km4*Vl + ACl) - Vmaxl5*CCvl*CGSHl/(Km5*Kmgsh + CCvl*Kmgsh + CGSHl*Km5 +
CGSHl*CCvl)

d/dt(ACkidfree) = ACkid*k4rel

d/dt(ACkidcap) = Qkid*(CCart - ACkidcap/Vlcap) - Ql*Pideb*(CCart - CCvkid)
d/dt(ACkid) = Qkid*Pideb*(CCart - CCvkid) + Vmaxk3*CBvkid/(Km3 + CBvkid) -
Vmaxk4*ACkidfree/(Kmlu4*Vkid + ACkidfree) - Vmaxk4*ACkid/(R*Kmlu4*Vkid + ACkid) -
Vmaxk5*CCvkid*CGSHkid/(Km5*Kmgsh + CCvkid*Kmgsh + CGSHkid*Km5 + CGSHkid*CCvkid)

d/dt(ACfcap) = Qf*(CCart - ACfcap/Vfcap) - Qf*Pideb*(CCart - CCvf)
d/dt(ACf) = Qf*Pideb*(CCart - CCvf)

d/dt(ACmcap) = Qm*(CCart - ACmcap/Vmcap) - Qm*Pideb*(CCart - CCvm)
d/dt(ACm) = Qm*Pideb*(CCart - CCvm)

d/dt(ACvrgcap) = Qvrg*(CCart - ACvrgcap/Vvrgcap) - Qvrg*Pideb*(CCart - CCvvrg)
d/dt(ACvrg) = Qvrg*Pideb*(CCart - CCvvrg)

d/dt(ACuocap) = Quo*(CCart - ACuocap/Vuocap) - Quo*Pideb*(CCart - CCvuo)
d/dt(ACuo) = Quo*Pideb*(CCart - CCvuo)

{metabolism of diepoxybutane to epoxybutanediol}
d/dt(ACmetpu4) = Vmaxpu4*ACpufree/(Kmlu4*Vpu + ACpufree) + Vmaxpu4*ACpu/(R*Kmlu4*Vpu +
ACpu)

d/dt(ACmetbr4) = Vmaxbr4*ACbrfree/(Kmlu4*Vbr + ACbrfree) + Vmaxbr4*ACbr/(R*Kmlu4*Vbr + ACbr)

d/dt(ACmetl4) =  Vmaxl4*AClfree/(Km4*Vl + AClfree) + Vmaxl4*ACl/(R*Km4*Vl + ACl)
init ACmetk4 = 0
```

d/dt(ACmetk4) = Vmaxk4*ACkidfree/(Kmlu4*Vkid + ACkidfree) + Vmaxk4*ACkid/(R*Kmlu4*Vkid + ACkid)

{AUCs for diepoxybutane}
d/dt(AUCCuo) = CCvuo
d/dt(AUCCvtot) = CCvtot

{metabolism of diepoxybutane to DEB-SG}
init ACmetpu5 = 0
init ACmetbr5 = 0
init ACmetl5 = 0
Limit ACmetl5 >= 0
init ACmetk5 = 0

d/dt(ACmetpu5) = Vmaxpu5*CCvpu*CGSHlu/(Km5*Kmgsh + CCvpu*Kmgsh + CGSHlu*Km5 + CGSHlu*CCvpu)
d/dt(ACmetbr5) = Vmaxbr5*CCvbr*CGSHlu/(Km5*Kmgsh + CCvbr*Kmgsh + CGSHlu*Km5 + CGSHlu*CCvbr)
d/dt(ACmetl5) = Vmaxl5*CCvl*CGSHl/(Km5*Kmgsh + CCvl*Kmgsh + CGSHl*Km5 + CGSHl*CCvl)
d/dt(ACmetk5) = Vmaxk5*CCvkid*CGSHkid/(Km5*Kmgsh + CCvkid*Kmgsh + CGSHkid*Km5 + CGSHkid*CCvkid)

{differential equations for dihydroxybutene}
d/dt(ADpu) = Qpu*(CDart - CDvpu) + Vmaxpu1*ABpufree/(Km1*Vpu + ABpufree) + Vmaxpu1*ABpu/(R*Km1*Vpu + ABpu) - Vmaxpu6*CDvpu/(Km6 + CDvpu) - Vmaxpu7*CDvpu*CGSHlu/(Km7*Kmgsh + CDvpu*Kmgsh + CGSHlu*Km7 + CGSHlu*CDvpu)

d/dt(ADbr) = Qbr*(CDart - CDvbr) + Vmaxbr1*ABbrfree/(Km1*Vbr + ABbrfree) + Vmaxbr1*ABbr/(R*Km1*Vbr + ABbr) - Vmaxbr6*CDvbr/(Km6 + CDvbr) - Vmaxbr7*CDvbr*CGSHlu/(Km7*Kmgsh + CDvbr*Kmgsh + CGSHlu*Km7 + CGSHlu*CDvbr)

d/dt(ADlcap) = Ql*(CDart - ADlcap/Vlcap) - Ql*Pidhb*(CDart - CDvl)
d/dt(ADl) = Ql*Pidhb*(CDart - CDvl) + Vmaxl1*ABlfree/(Km1*Vl + ABlfree) + Vmaxl1*ABl/(R*Km1*Vl + ABl) - Vmaxl6*CDvl/(Km6 + CDvl) - Vmaxl7*CDvl*CGSHl/(Km7*Kmgsh + CDvl*Kmgsh + CGSHl*Km7 + CGSHl*CDvl)

d/dt(ADkidcap) = Qkid*(CDart - ADkidcap/Vkidcap) - Qkid*Pidhb*(CDart - CDvkid)
d/dt(ADkid) = Qkid*Pidhb*(CDart - CDvkid) + Vmaxk1*ABkidfree/(Km1*Vkid + ABkidfree) + Vmaxk1*ABkid/(R*Km1*Vkid + ABkid) - Vmaxl6*CDvkid/(Km6 + CDvkid) - Vmaxl7*CDvkid*CGSHkid/(Km7*Kmgsh + CDvkid*Kmgsh + CGSHkid*Km7 + CGSHkid*CDvkid)

d/dt(ADfcap) = Qf*(CDart - ADfcap/Vfcap) - Qf*Pidhb*(CDart - CDvf)
d/dt(ADf) = Qf*Pidhb*(CDart-CDvf)

d/dt(ADmcap) = Qm*(CDart - ADmcap/Vmcap) - Qm*Pidhb*(CDart - CDvm)
d/dt(ADm) = Qm*Pidhb*(CDart - CDvm)

d/dt(ADvrgcap) = Qvrg*(CDart - ADvrgcap/Vvrgcap) - Qvrg*Pidhb*(CDart - CDvvrg)
d/dt(ADvrg) = Qvrg*Pidhb*(CDart - CDvvrg)

d/dt(ADuocap) = Quo*(CDart - ADuocap/Vuocap) - Quo*Pidhb*(CDart - CDvuo)
d/dt(ADuo) = Quo*Pidhb*(CDart - CDvuo)

d/dt(AUCDvtot) = CDvtot
init (AUCDvuo) = 0
d/dt(AUCDvuo) = CDvuo

{metabolism of DHB to EBD }
d/dt(ADmetpu6) = Vmaxpu6*CDvpu/(Km6 + CDvpu)
d/dt(ADmetbr6) = Vmaxbr6*CDvbr/(Km6 + CDvbr)
d/dt(ADmetl6) = Vmaxl6*CDvl/(Km6 + CDvl)
init ADmetk6 = 0
d/dt(ADmetk6) = Vmaxk6*CDvkid/(Km6 + CDvkid)

{metabolism of DHB to DHB-SG conjugate}
init ADmetpu7 = 0
init ADmetbr7 = 0
init ADmetl7 = 0
init ADmetk7 = 0

d/dt(ADmetpu7) = Vmaxpu7*CDvpu*CGSHlu/(Km7*Kmgsh + CDvpu*Kmgsh + CGSHlu*Km7 + CGSHlu*CDvpu)
d/dt(ADmetbr7) = Vmaxbr7*CDvbr*CGSHlu/(Km7*Kmgsh + CDvbr*Kmgsh + CGSHlu*Km7 + CGSHlu*CDvbr)
d/dt(ADmetl7) = Vmaxl7*CDvl*CGSHl/(Km7*Kmgsh + CDvl*Kmgsh + CGSHl*Km7 + CGSHl*CDvl)
d/dt(ADmetk7) = Vmaxl7*CDvkid*CGSHkid/(Km7*Kmgsh + CDvkid*Kmgsh + CGSHkid*Km7 + CGSHkid*CDvkid)

{differential equations for epoxybutanediol}
d/dt(AEpufree) = AEpu*k8rel

d/dt(AEpu) = Qpu*(CEart - CEvpu) + Vmaxpu4*ACpufree/(Kmlu4*Vpu + ACpufree) + Vmaxpu4*ACpu/(R*Kmlu4*Vpu + ACpu) + Vmaxpu6*CDvpu/(Km6 + CDvpu) - Vmaxpu8*AEpufree/(Km8*Vpu + AEpufree) - Vmaxpu8*AEpu/(R*Km8*Vpu + AEpu) - Vmaxpu9*CEvpu*CGSHlu/(Km9*Kmgsh + CEvpu*Kmgsh + CGSHlu*Km9 + CGSHlu*CEvpu)

d/dt(AEbrfree) = AEbr*k8rel

d/dt(AEbr) = Qbr*(CEart - CEvbr) + Vmaxbr4*ACbrfree/(Kmlu4*Vbr + ACbrfree) + Vmaxbr4*ACbr/(R*Kmlu4*Vbr + ACbr) + Vmaxbr6*CDvbr/(Km6 + CDvbr) - Vmaxbr8*AEbrfree/(Km8*Vbr + AEbrfree) - Vmaxbr8*AEbr/(R*Km8*Vbr + AEbr) - Vmaxbr9*CEvbr*CGSHlu/(Km9*Kmgsh + CEvbr*Kmgsh + CGSHlu*Km9 + CGSHlu*CEvbr)

d/dt(AElfree) = AEl*k8rel

d/dt(AElcap) = Ql*(CEart - AElcap/Vlcap) - Ql*Piebd*(CEart - CEvl)
d/dt(AEl) = Ql*Piebd*(CEart - CEvl) + Vmaxl4*AClfree/(Km4*Vl + AClfree) + Vmaxl4*ACl/(R*Km4*Vl + ACl) + Vmaxl6*CDvl/(Km6 + CDvl) - Vmaxl8*AElfree/(Km8*Vl + AElfree) - Vmaxl8*AEl/(R*Km8*Vl + AEl) - Vmaxl9*CEvl*CGSHl/(Km9*Kmgsh + CEvl*Kmgsh + CGSHl*Km9 + CGSHl*CEvl)

d/dt(AEkidfree) = AEkid*k8rel

d/dt(AEkidcap) = Qkid*(CEart - AEkidcap/Vkidcap) - Qkid*Piebd*(CEart - CEvkid)
d/dt(AEkid) = Qkid*Pidhb*(CEart - CEvkid) + Vmaxk4*ACkidfree/(Kmlu4*Vkid + ACkidfree) + Vmaxk4*ACkid/(R*Kmlu4*Vkid + ACkid) + Vmaxl6*CDvkid/(Km6 + CDvkid) - Vmaxk8*AEkidfree/(Km8*Vkid + AEkidfree) - Vmaxk8*AEkid/(R*Km8*Vkid + AEkid) - Vmaxl9*CEvkid*CGSHkid/(Km9*Kmgsh + CEvkid*Kmgsh + CGSHkid*Km9 + CGSHkid*CEvkid)

d/dt(AEfcap) = Qf*(CEart - AEfcap/Vfcap) - Qf*Piebd*(CEart - CEvf)
d/dt(AEf) = Qf*Piebd*(CEart-CEvf)

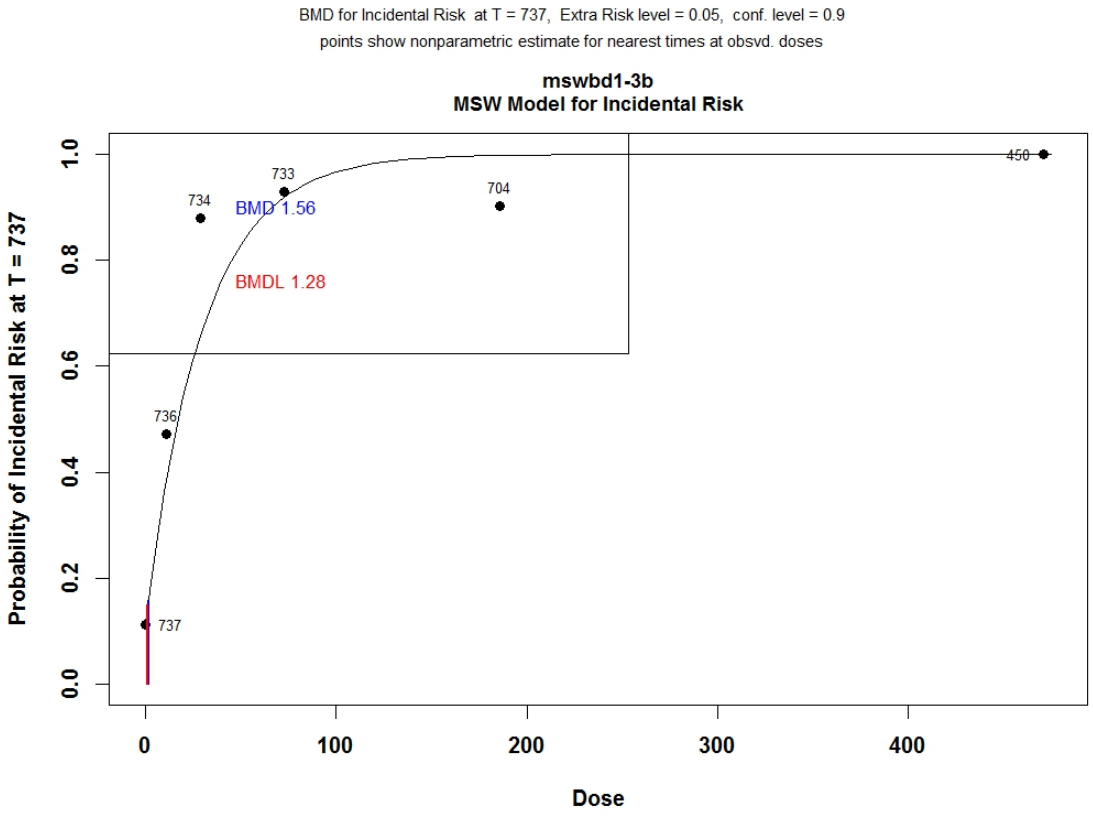
```
d/dt(AEmcap) = Qm*(CEart - AEmcap/Vmcap) - Qm*Piebd*(CEart - CEvm)
d/dt(AEm) = Qm*Piebd*(CEart - CEvm)

d/dt(AEvrgcap) = Qvrg*(CEart - AEvrgcap/Vvrgcap) - Qvrg*Piebd*(CEart - CEvvrg)
d/dt(AEvrg) = Qvrg*Piebd*(CEart - CEvvrg)

d/dt(AEuocap) = Quo*(CEart - AEuocap/Vuocap) - Quo*Piebd*(CEart - CEvuo)
d/dt(AEuo) = Quo*Piebd*(CEart - CEvuo)

d/dt(AUCEvtot) = CEvtot
init (AUCEvuo) = 0
d/dt(AUCEvuo) = CEvuo

{metabolism of  epoxybutanediol to erythitol}
init AEmetpu8 = 0
init AEmetbr8 = 0
init AEmetl8 = 0
init AEmetk8 = 0
d/dt(AEmetpu8) =  Vmaxpu8*AEpufree/(Km8*Vpu + AEpufree) + Vmaxpu8*AEpu/(R*Km8*Vpu + AEpu)
d/dt(AEmetbr8) = Vmaxbr8*AEbrfree/(Km8*Vbr + AEbrfree) + Vmaxbr8*AEbr/(R*Km8*Vbr + AEbr)
d/dt(AEmetl8) = Vmaxl8*AElfree/(Km8*Vl + AElfree) + Vmaxl8*AEl/(R*Km8*Vl + AEl)
init AEmetk8 = 0
d/dt(AEmetk8) = Vmaxk8*AEkidfree/(Km8*Vkid + AEkidfree) + Vmaxk8*AEkid/(R*Km8*Vkid + AEkid)

{metabolism of EBD to EBD-SG}
init AEmetpu9 = 0
init AEmetbr9 = 0
init AEmetl9 = 0
init AEmetk9 = 0

d/dt(AEmetpu9) = Vmaxpu9*CEvpu*CGSHlu/(Km9*Kmgsh + CEvpu*Kmgsh + CGSHlu*Km9 +
CGSHlu*CEvpu)
d/dt(AEmetbr9) = Vmaxbr9*CEvbr*CGSHlu/(Km9*Kmgsh + CEvbr*Kmgsh + CGSHlu*Km9 +
CGSHlu*CEvbr)
d/dt(AEmetl9) = Vmaxl9*CEvl*CGSHl/(Km9*Kmgsh + CEvl*Kmgsh + CGSHl*Km9 + CGSHl*CEvl)
d/dt(AEmetk9) = Vmaxl9*CEvkid*CGSHkid/(Km9*Kmgsh + CEvkid*Kmgsh + CGSHkid*Km9 +
CGSHkid*CEvkid)

{differential equations for cysteine metabolism}
d/dt(AFl) = Kl0 - Vmax10l*AFl/((Kmcys*Vl + AFl)*(1 + AGl/(KI*Vl)))
d/dt(AFlu) = Klu0 - Vmax10lu*AFlu/((Kmcys*Vlu + AFlu)*(1 + AGlu/(KI*Vlu)))
d/dt(AFkid) = Kkid0 - Vmax10kid*AFkid/((Kmcys*Vkid + AFkid)*(1 + AGkid/(KI*Vkid)))

{differential equations for glutathione metabolism}
d/dt(AGl) = Vmax10l*AFl/((Kmcys*Vl + AFl)*(1 + AGl/(KI*Vl))) - ABmetl2 - ACmetl5 - ADmetl7- AEmetl9
d/dt(AGlu) = Vmax10lu*AFlu/((Kmcys*Vlu + AFlu)*(1 + AGlu/(KI*Vlu))) - ABmetpu2 - ABmetbr2 -
ACmetpu5 - ACmetbr5 - ADmetpu7 - ADmetbr7 - AEmetpu9 - AEmetbr9
d/dt(AGkid) = Vmax10kid*AFkid/((Kmcys*Vkid + AFkid)*(1 + AGkid/(KI*Vkid))) - ABmetk2 - ACmetk5 -
ADmetk7 - AEmetk9

{differential equation for  Hb adduct formation, pmol/g Hb-ppm-hr}
d/dt(Adduct) = CEvtot*Kadduct*1E12/(476.7*8000)
; Mass balance
MassA = Af + Al + Am + Avrg + Abr + Apu + Auo + Aexh + Akid
MassB = ABf + ABl + ABm + ABvrg + ABbr + ABpu + ABuo + ABkid
MassBfree = ABlfree + ABpufree + ABbrfree + ABkidfree
```

```
MassC = ACf + ACl + ACm + ACvrg + ACbr + ACpu + ACuo + ACkid
MassCfree = AClfree + ACpufree + ACbrfree + ACkidfree
MassD = ADf + ADl + ADm + ADvrg + ADbr + ADpu + ADuo + ADkid
MassE = AEf + AEl + AEm + AEvrg + AEbr + AEpu + AEuo + AEkid
MassEfree = AElfree + AEpufree + AEbrfree + AEkidfree
Massfree = MassBfree + MassCfree + MassEfree
MassG2 = ABmetl2 + ABmetpu2 + ABmetbr2 + ABmetk2
MassG5 = ACmetl5 + ACmetpu5 + ACmetbr5 + ACmetk5
MassG7 = ADmetl7 + ADmetpu7 + ADmetbr7 + ADmetk7
MassG9 = AEmetl9 + AEmetpu9 + AEmetbr9 + AEmetk9
MassG = MassG2 + MassG5 + MassG7 + MassG9
MassE8 = AEmetl8 + AEmetpu8 + AEmetbr8 + AEmetk8
TotMass = MassA + MassB + MassC + MassD + MassE + MassG + MassE8 + Massfree
init Inh = 0
d/dt(Inh) = Cair*Qalv
```

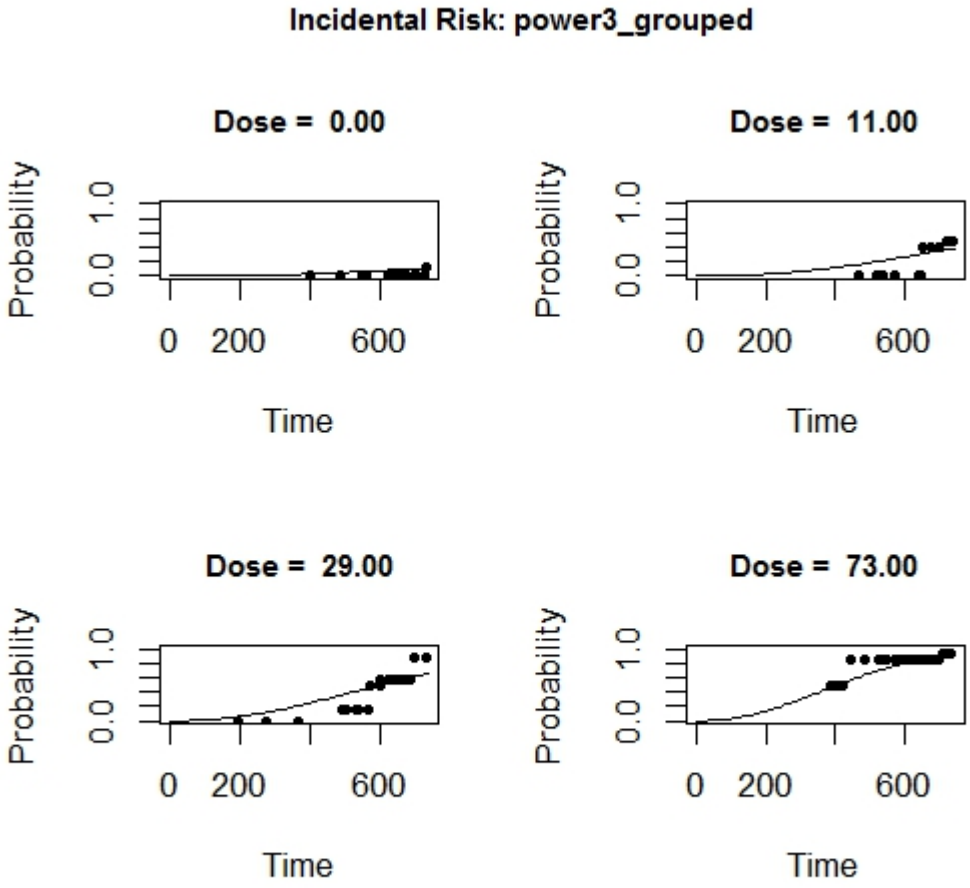
APPENDIX B

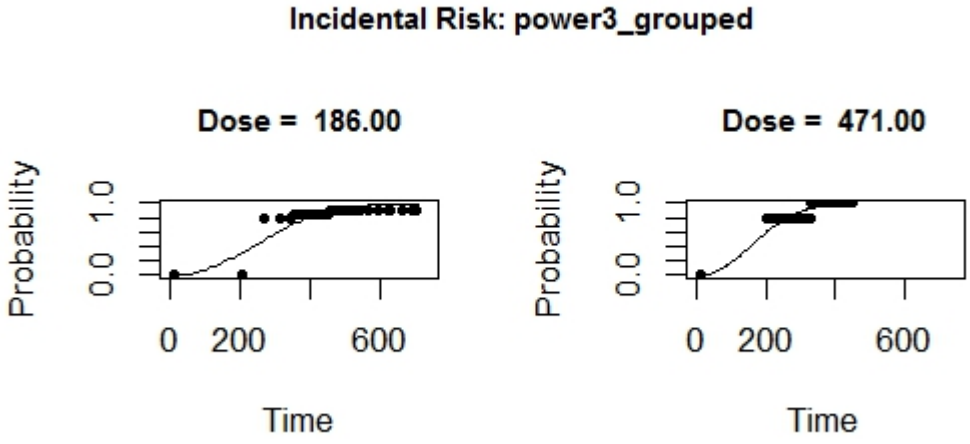
This appendix contains the goodness of fit (gof) plots for the Multistage Weibull model (MSW) fits to the 24 month mouse ovarian atrophy data in NTP (1993). The plots are generated in the statistical program R using slightly modified input files that were used in the main MSW analysis.

Appendix B1. GOFPLOT: DR Plot for Incidental Risk of Ovarian Atrophy in Mice Versus Butadiene Exposure ppm for 24 months. Solid line is Multistage Weibull Model Prediction, Point are Nonparametric Model Estimates. BMD and BMDL are for 95% Lower Bounds on 5% Response Level. Points Closer to Line Indicate Better Model Fit.

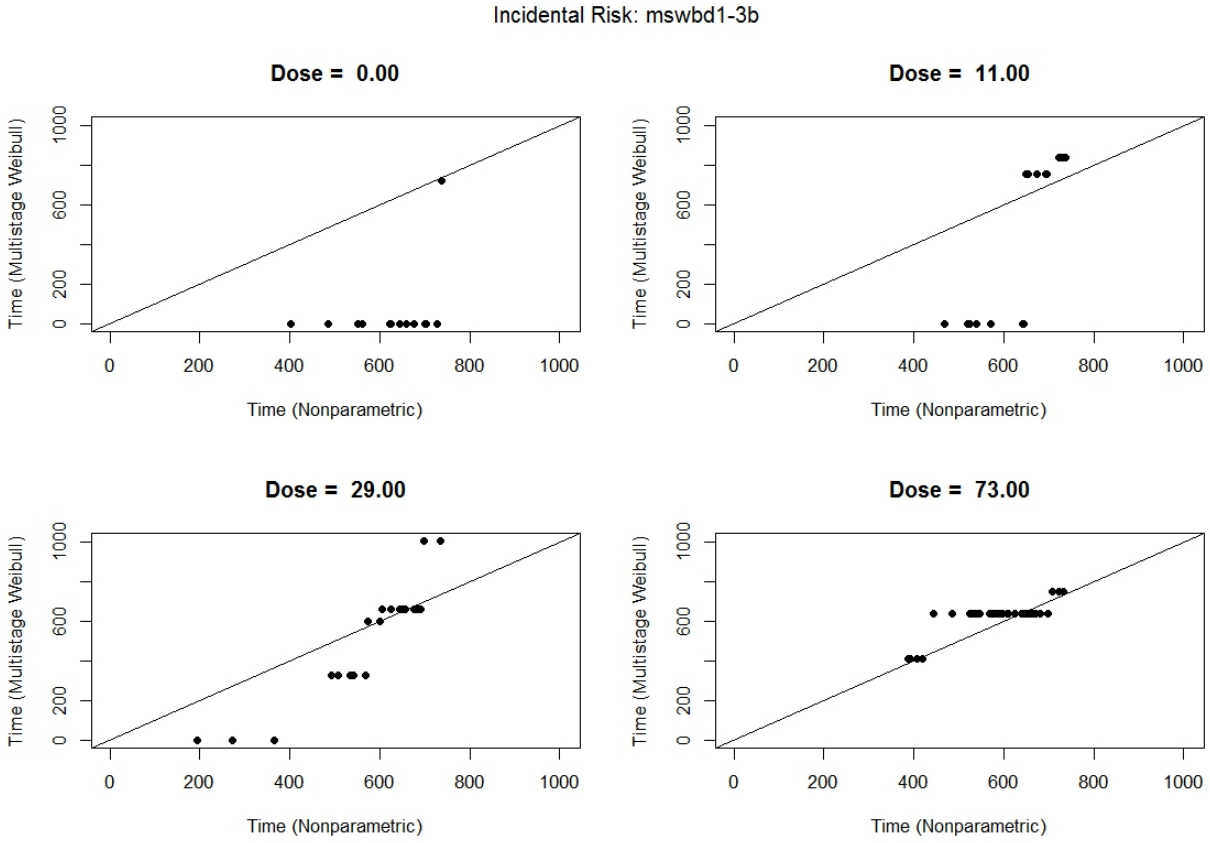

BMD for Incidental Risk at T = 737, Extra Risk level = 0.05, conf. level = 0.9
points show nonparametric estimate for nearest times at obsvd. doses
mswbd1-3b
MSW Model for Incidental Risk
Probability of Incidental Risk at T = 737
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
Dose
737
736
734
733
704
450
BMD 1.56
BMDL 1.28

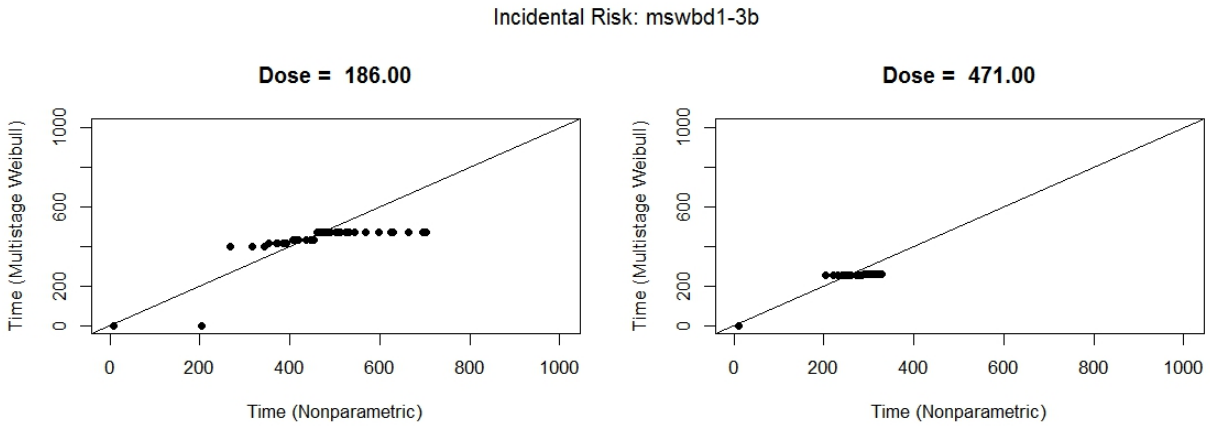
Appendix B2. GOFPLOT: PR Plot. Probability of Ovarian Atrophy in Butadiene Exposed Mice by Multistage Weibull Model (Solid Line) versus Time for Butadiene Internal Doses nM Diepoxybutane in Blood. Points Represent Nonparametric Model Estimates. More Points Closer to the Line Indicates Better Model Fit.



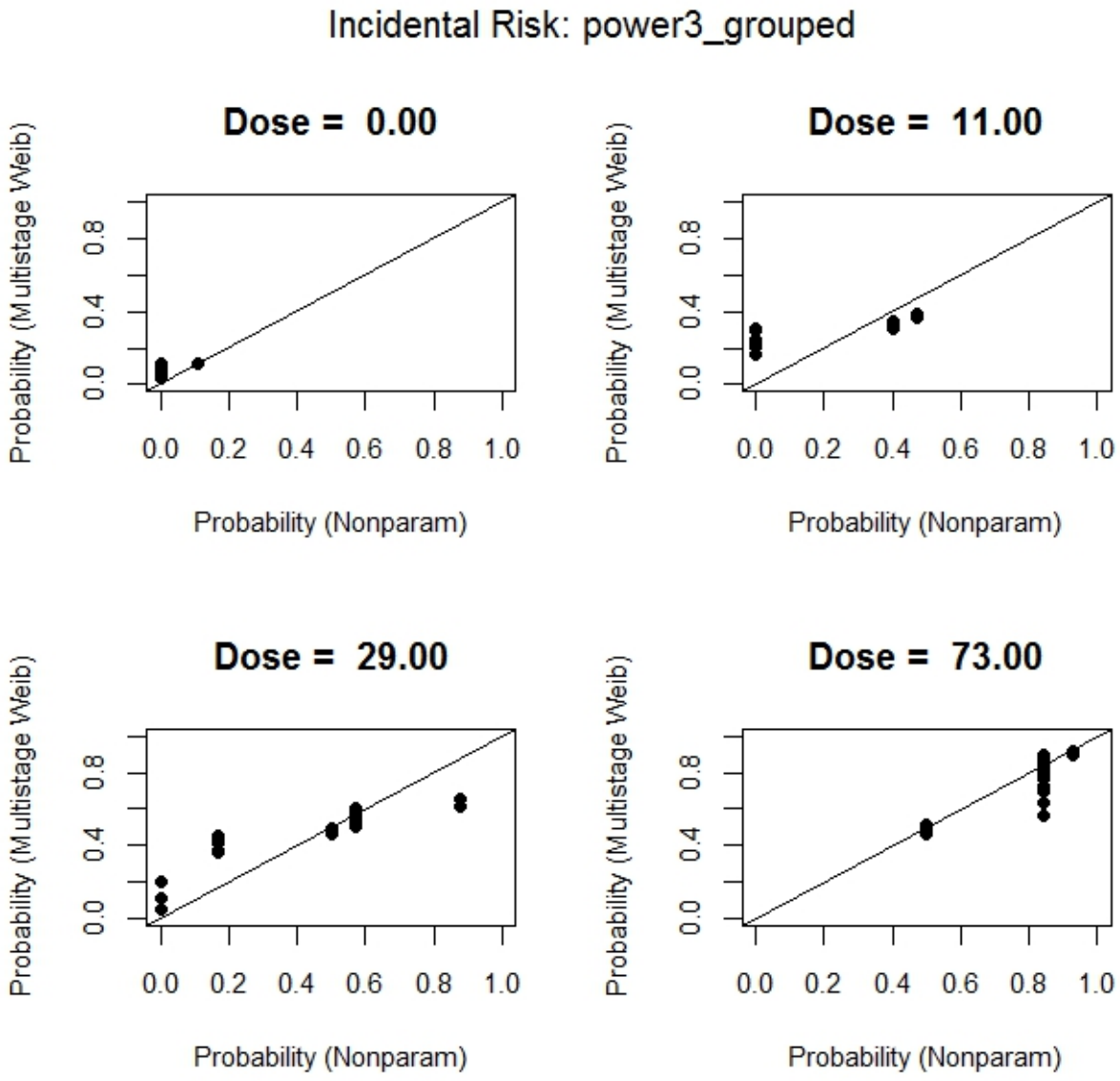

Incidental Risk: power3_grouped
Dose = 186.00
Probability
0.0
1.0
0
200
600
Time
Dose = 471.00
Probability
0.0
1.0
0
200
600
Time

Appendix B3. GOFPLOT: QQ Plot. Incidental Risk of Ovarian Atrophy in Butadiene Exposed Mice. Time-Multistage Weibull Model versus Time-Nonparametric Model Estimates for Internal Blood Diepoxybutane nM. Solid line is for Equivalence of Model Predictions. More Points Closer to Line Indicates Better Model Fit.




Incidental Risk: mswbd1-3b
Dose = 186.00
Dose = 471.00
Time (Multistage Weibull)
Time (Nonparametric)
0
200
400
600
800
1000

Appendix B4. GOFPLOT: PP Plot. . Incidental Risk of Ovarian Atrophy in Butadiene Exposed Mice. Probability-Multistage Weibull Model versus Probability-Nonparametric Model Estimates for Internal Blood Diepoxybutane nM. Solid line is for Equivalence of Model Predictions. More Points Closer to Line Indicates Better Model Fit.



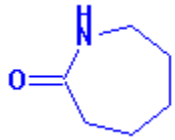
Caprolactam Reference Exposure Levels

(Aerosol, Vapor & Particulate)

(Aminocaproic lactam; epsilon-Caprolactam; Hexahydro-2H-azepin-2-one;

2-Oxohexamethylenimine; 2-Ketohexamethylenimine)

CAS 105-60-2



1 Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). OEHHA developed a Technical Support Document (TSD) in response to this statutory requirement that describes acute, 8-hour and chronic RELs and was adopted in December 2008. The TSD presents methodology reflecting the latest scientific knowledge and techniques, and in particular explicitly includes consideration of possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, chapter 731, statutes of 1999, Health and Safety Code Sections 39669.5 *et seq.*). These guidelines have been used to develop the following RELs for caprolactam: this document will be added to Appendix D of the TSD.

Exposure to caprolactam has been found to cause upper respiratory and eye irritation in both animals and humans. Exposure causes inflammation of the nasal and laryngeal epithelium in exposed rodents. The site-of-contact nature of the lesion to the most sensitive cells lining the upper respiratory tract indicates that caprolactam is primarily a direct-acting irritant, rather than a chemical requiring metabolic activation in nasal mucosa to cause tissue injury. Although there is no evidence for reproductive or developmental effects at levels that produce sensory irritation, considerably higher doses administered orally to pregnant rats have resulted in reduced weight of offspring. Literature summarized and referenced in this document covers the relevant published literature for caprolactam through Fall 2011.

1.1 Acute REL Summary

Acute 1-Hour inhalation reference exposure level	**50 µg/m^3 (11 ppb)**
Critical effect(s)	Increased eye blink frequency in humans
Hazard index target(s)	Eyes

1.2 8-Hour REL Summary

8-Hour inhalation reference exposure level	**7 µg/m^3 (1.4 ppb)**
Critical effect(s)	Inflammatory changes of nasal and laryngeal epithelium in rodents
Hazard index target(s)	Upper respiratory system

1.3 Chronic REL Summary

Chronic inhalation reference exposure level	**2.2 µg/m^3 (0.5 ppb)**
Critical effect(s)	Inflammatory changes of nasal and laryngeal epithelium in rodents
Hazard index target(s)	Upper respiratory system

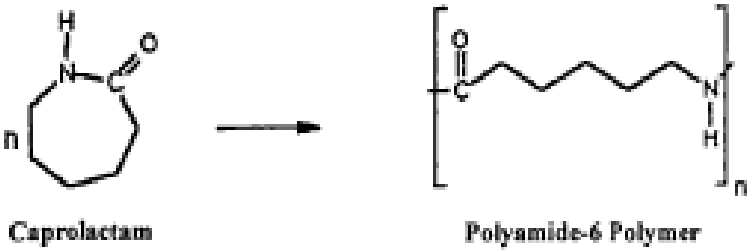
2 Physical and Chemical Properties [from HSDB (2006), unless noted otherwise]

Description	A semi-volatile white, hygroscopic, crystalline solid or flakes with unpleasant odor
Molecular formula	$C_6H_{11}NO$
Molecular weight	133.16 g/mol
Density	1.05 g/cm^3 @ 25 °C
Boiling point	270 °C
Melting point	69.3 °C
Vapor pressure	0.0011 mm Hg @ 20 ºC (68ºF) 0.0021 mm Hg @ 25 °C (77ºF), saturated vapor concentration = 13 mg/m^3 0.153 Pa @ 20 ºC, and 0.275 Pa @ 25 °C (Zaitsau et al., 2006)
Odor threshold	≤0.15 mg/m^3 (Ziegler et al., 2008)
Solubility	Very soluble in water, benzene, diethyl ether, and ethanol. Soluble in chlorinated solvents, petroleum distillates, and cyclohexene
Conversion factor	1 ppm = 4.63 mg/m^3 (as vapor) @ 25 °C

3 Occurrence and Major Uses

Caprolactam is the monomer used in the polymerization process to manufacture synthetic fibers and resins, known as Nylon-6 (Cooper et al., 1993; NPG, 2007). Nylon-6 was first developed for commercial use in the 1930's. This type of nylon has also been called polyamide-6, which refers to the type of polymer produced by the interaction of an amino group of one caprolactam molecule and a carboxylic group of another caprolactam molecule to give a protein-like structure. The reaction, shown in Figure 1, entails a ring-opening polymerization:

Figure 1. Ring opening polymerization of caprolactam (Lander, 2002)



Nylon-6 is the most widely used type of nylon, representing more than 60% in volume of all nylon types used worldwide (NPG, 2007). U.S. EPA (2006) notes in their IUR (Inventory Update Reporting) that the aggregated national production volume of

caprolactam was 1 billion lbs and greater in 2006. Nylon-6 is found in many products, such as in:

- Carpets, rugs and home textiles
- Engineering plastics (automotive, E&E, equipment housing)
- Tire cord (for reinforcement of heavy-duty tires)
- Textiles (apparel, hosiery, lingerie, sportswear, swimwear, casual wear, fashion wear, socks, umbrellas, luggage, tents, parachutes, sleeping bags, etc.)
- Film (food packaging, industrial packaging, medical applications)

Bongard (2000) reported that about 75% of manufactured Nylon-6 is used in fibers (textile, industrial, carpet) and 25% in engineering plastics and food packaging film. It was expected that this ratio would gradually change more in favor of plastics and film over time. Minor uses of caprolactam include use as a solvent in paint, and for the synthesis of the amino acid lysine (IARC, 1999; Bradley et al., 2004). Another common type of nylon known as Nylon-6,6 is produced from hexamethylene diamine and adipic acid and does not contain caprolactam (Hegde et al., 2004).

In industrial processes, exposure occurs during the production of the caprolactam monomer when the monomer is polymerized, extruded, chilled, or cut into pellets, or when the polymer pellets are re-melted, re-extruded, and drawn into fiber or film (ACGIH, 2003). Exposure may also occur during recycling of waste Nylon-6 carpets, which can be reprocessed in full back to its raw material (NPG, 2007). The monomer vaporizes from the heated processes and condenses as fume (i.e., a cloud of small particles suspended in air) (ACGIH, 2003). In early industrial studies, in which high concentrations of caprolactam were recorded in workplace air, contact of the fume with cooler surfaces resulted in formation of light feathery flakes (Hohensee, 1951; Kelman, 1986). Exposure is primarily as an aerosol, although the vapor form would also be expected to be present at concentrations relevant to the RELs (ACGIH, 2003). In the ambient air, caprolactam has been observed as a fine aerosol collected on $PM_{2.5}$ filters as a result of the probable release from a facility that used caprolactam as a raw material (Cheng et al., 2006). Wilkins et al. (1993) found caprolactam in floor dust following a thermal desorption process to analyze VOC emission profiles. Thus, caprolactam also appears to adsorb onto dust particles.

Measurable levels of caprolactam have been found primarily in indoor air as a result of release of the vapor or particulate from carpeting containing Nylon-6 (IWMB, 2003). Caprolactam may also migrate into foods packaged in Nylon-6 film (Bradley et al., 2004). Caprolactam was detected in foodstuffs packaged in Nylon-6 in the range of 2.8 to 13 mg/kg.

The polymerization process of caprolactam to nylon polymer is not 100 percent efficient, thus allowing some of the un-polymerized caprolactam into the final product. Goldblatt et al. (1954) noted that the polymerized fiber contains approximately one percent of the unreacted caprolactam monomer. A more recent study suggests that total caprolactam contaminants, plus lesser amounts of its various oligomers, are present at 1% or less in some Nylon-6 products (Venema et al., 1993). These oligomers found following polymerization include cyclic oligomers (i.e., the cyclic dimer, trimer and tetramer, etc.),

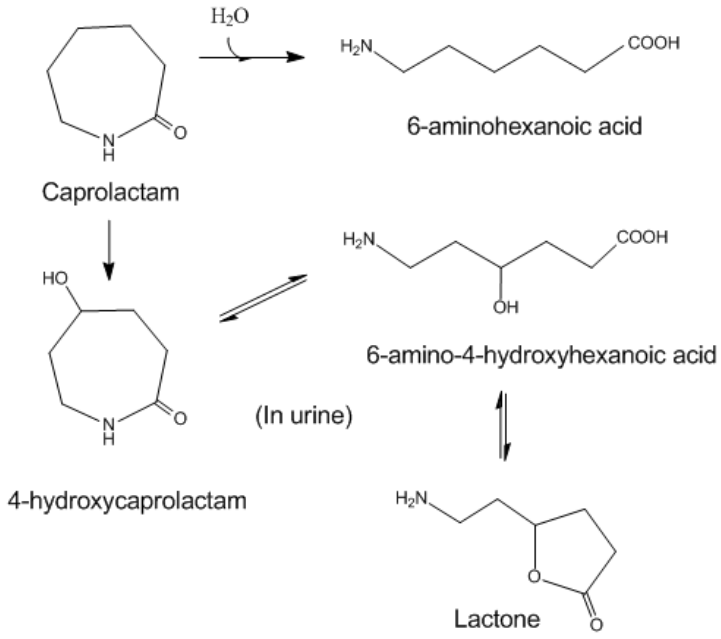
as well as some linear oligomers (Krajnik et al., 1982; Ballistreri et al., 1987; Bonifaci et al., 1991; Venema et al., 1993). No investigations regarding the health effects of caprolactam oligomers could be located in the literature. *In vitro* cytotoxicity testing with polymers and their corresponding monomers, one of which was the monomer vinylcaprolactam, showed that the monomers can have much greater cytotoxicity with respect to the corresponding polymers (Vihola et al., 2005). The authors stated that the polymers tested in this study were 156,000 to 1,500,000 g/mol. Whether caprolactam and its oligomers would show a similar pattern of cytotoxicity was unclear from this study.

Based on the measured emission rate of caprolactam from carpet samples, modeled air concentrations for office and classroom scenarios ranged from 39 to 450 µg/m^3 (IWMB, 2003). A chamber study found caprolactam emissions from some polyamide carpets resulted in chamber concentrations ranging from 6 to 97 µg/m^3 on the 28th day of chamber testing (Wilke et al., 2004). In indoor monitoring studies, caprolactam was detected in all floor dust samples collected during an indoor air study in nine public buildings (Wilkins et al., 1993). In another study, the average caprolactam concentration in a new California portable classroom during school hours over 8 weeks following installation of a Nylon-6 broadloom carpet was 22.2 µg/m^3 (range: 10.6 - 30.1 µg/m^3) (Hodgson et al., 2004). The emission rate of caprolactam following installation of the carpet was about 5 mg/h prior to occupancy, and dropped to 3 mg/h 27 weeks after first occupancy. Similar portable classrooms that installed upgraded carpets containing Nylon-6,6 emitted low to non-detectable concentrations of caprolactam (maximum: 1.4 µg/m^3).

4 Metabolism

In rats, Kirk et al. (1987) observed that approximately 16% of ingested caprolactam in diet was excreted in urine as 4-hydroxycaprolactam and a small amount as the non-hydroxylated acid, 6-aminohexanoic acid (Figure 2). Following a single oral dose of [^{14}C]caprolactam in male rats, 77.6% of the radioactivity was excreted in urine, 3.5% in the feces, and 1.5% in the expired air in 24 hrs (Unger et al., 1981). The half-life of disappearance of radioactivity from the blood was 3.0 hr. Similar to the findings by Kirk et al., Unger et al. (1981) observed two metabolites following oral administration, which comprised 79.3 and 17.7% of the total urinary radioactivity. However, Unger et al. made no attempt to identify these urinary metabolites. Unchanged caprolactam represented only 2.3% of the total urinary radioactivity. The radiolabeled caprolactam was widely distributed among the tissues of the rat, including the brain, with concentrations mostly similar to that in the blood. The radioactivity was consistently lower in fat relative to the blood in the first 24 hrs, indicating a low affinity of caprolactam and its metabolites for adipose tissue.

Figure 2. Metabolism of caprolactam



Oral delivery of [^{14}C]caprolactam in male and female mice also showed that the chemical is rapidly absorbed from the stomach and freely distributed into all tissues (Waddell et al., 1984). Almost all radioactivity was eliminated in 24 hours, although some retention of radioactivity during this time was noted in the brain, nasal epithelium, olfactory lobe of the brain, liver, optic lens and Harderian gland. In pregnant mice, sites of localization of the radiolabel were similar to non-pregnant mice, with the exception that some residual radioactivity was also noted in the umbilical cord, amnion, and yolk sac. No radioactivity was retained in any other fetal tissues. It was speculated that metabolism of caprolactam in the nasal tissue may produce a metabolite that was slow to clear. The apparent retention of radioactivity in the olfactory bulb was thought to represent an artifact caused by the washing of radioactivity from the nasal epithelium by hexane during the freezing procedure.

5 Acute Toxicity of Caprolactam

5.1 Acute Toxicity to Adult Humans

Occupational exposure to caprolactam emitted during Nylon-6 processes is known to cause acute eye and upper respiratory tract irritation (Hohensee, 1951; Ferguson and Wheeler, 1973; Kelman, 1986). Dermal irritation and dermatitis also occur in occupational settings with short-term and repeated exposure to the solid form of caprolactam, or caprolactam particulate that has “condensed” (sic) onto surfaces from the airborne vapor or aerosol phase (Hohensee, 1951; Ferguson and Wheeler, 1973; Kelman, 1986; NIOSH, 1995a).

Two published studies, an industrial observational exposure study by Ferguson and Wheeler (1973), and a chamber controlled human exposure study by Ziegler et al. (2008) examined the acute sensory irritant effects resulting from directly measured concentrations of caprolactam. These two studies are summarized and assessed for REL development below. No peer-reviewed studies have been conducted specifically examining toxicological effects of caprolactam released from finished products that contain the chemical (e.g., finished Nylon-6 carpets).

Additionally, several case reports of workers heavily exposed to caprolactam developing seizures are summarized in this section. This is a particular concern given that, in general, children are more vulnerable than adults to the neurologic effects of chemicals. Because caprolactam is a respiratory irritant, the available data for the potential of caprolactam also acting as a sensitizing agent for allergic responses is presented. Lastly, the basis for caprolactam occupational exposure limits (e.g., ACGIH and NIOSH) is summarized at the end of this section.

Ferguson and Wheeler (1973)

Ferguson and Wheeler (1973) exposed 5 healthy unacclimated male worker "volunteers" at a caprolactam polymerization plant. Exposures were to caprolactam vapor at concentrations of 10, 14, 25, and 104 ppm (46, 65, 116, and 482 mg/m^3, respectively) while subjects were standing or conversing for several minutes downwind from a known emission source. The smoking status of the workers was not reported. 'Unacclimated' was defined as workers who were experienced employees of the work environment, but reportedly were not continuously exposed in their ordinary duties. Although the authors report exposure was to the "vapor" form of caprolactam, the concentrations were above the saturated vapor concentration of 13 mg/m^3, indicating that exposure included caprolactam aerosol.

Air sampling for the exposures was area level rather than personal monitoring and was set at a height of about 60 inches from the floor and was averaged over a minimum of 30 minutes (Ferguson and Wheeler, 1973). The instrumentation for these exposures was presumably the same used for estimating 8-hour time weighted average concentrations in the second part of the study: a liquid absorption train that consisted of three flasks in series set up to collect the caprolactam in measured volumes of air. The flasks were half-filled with water and connected with one another by fritted glass delivery tubes. The train was connected to a fixed vacuum supply through a wet test meter. A similar arrangement was also employed that used gas traps rather than flasks, and a portable vacuum pump was used as a vacuum source. Two-mm glass beads were used in the first trap as a sparger. Both sampling techniques were considered effective, as no caprolactam was found in the last trap in either train. Analysis was by gas chromatography (GC) with a flame ionization detector.

The sampling and analytics methods above are presented in detail because of the implications for error in exposure assessment. This air sampling technique was a commonly used method in the 1970's, but some particulates could have been missed or lost due to the use of fritted glass (Gill, 2011). Further, using GC for analysis of

caprolactam is less sensitive than using high pressure liquid chromatography (HPLC) because the solvent peak tends to interfere with detection of caprolactam (Nau et al., 1984; Gill, 2011). Current methods for caprolactam air sampling recommended by OSHA include XAD resins or other absorbents, and analysis using HPLC and mass spectrometry (OSHA, 1988).

Most or all of the subjects in the Ferguson and Wheeler study reported transient nasal and throat irritation at all concentrations, including 4 out of 5 individuals exposed to 10 ppm (46 mg/m^3). Eye irritation was noted only in one volunteer at the highest concentration. The authors did not indicate whether throat irritation was a result of mouth-breathing (due to the unpleasant odor and/or nasal irritation) by those exposed. The degree of discomfort felt by the workers was considered dose-responsive, but was not quantified, reportedly due to wide differences in the degree of discomfort among the individual subjects. Thus, scalar functions that had been used in an attempt to evaluate degrees of discomfort were not presented. Some of the study participants also were exposed to similar concentrations for up to 30 min, but the sensory effects were not clearly stated or quantified. Brief exposure to 400-1200 ppm caprolactam was described as “extremely irritating”, resulting in a “choking” response.

A similar acute exposure study was arranged at a caprolactam monomer plant, although specific conditions of the exposure were not presented by the authors (Ferguson and Wheeler, 1973). In that part of the study, 14 ppm (65 mg/m^3) did not result in distress or discomfort. The authors speculated that the conditions of 100% humidity at the monomer plant (the polymer plant was described as having near normal humidity) may have been a factor in reducing sensory irritation. The authors also noted that caprolactam concentrations at the monomer plant appeared to be more uniform, suggesting that the greater variability in the concentration at the polymer plant might have resulted in brief high exposures leading to sensory irritation. The authors, however, did not present quantitative data documenting the variation in caprolactam concentration during the acute exposures.

Ferguson and Wheeler concluded that the irritant response threshold for the workers was at or near 10 ppm (46 mg/m^3) caprolactam, and that 5 ppm (23 mg/m^3) would be 50% of the discomfort threshold and somewhat below the no-effect level. The authors further state that additional support for their worker threshold value of 5 ppm is based on their findings of no reported distress among the employees in active and semi-active areas at concentrations up to about 7 ppm, although this did not appear to reflect responses to a systematic survey of the workforce (discussed in Section 6.1).

Ziegler et al. (2008)

Ziegler et al. (2008) conducted chamber exposures of 20 adult subjects (10 men and 10 women) to 0, 0.15, 0.5, and 5 mg/m^3 caprolactam vapor for 6 hours on 4 successive days. The stated study goal was to address possible chemosensory effects of caprolactam at low concentrations reflecting the indoor environment. Chemosensory subjective effects were assessed by a standardized questionnaire. Objective measures of exposure were assessed by eye blink frequency using a standard manual counting

procedure and a new semi-automated method; conjunctival hyperemia (eye redness) based on digital slit lamp photographs taken during exposure; and by nasal resistance using active anterior rhinometry before and after exposures. Except for nasal resistance, the questionnaire and objective tests were given at time 0 (i.e., just after entering the chamber), 1 hr, 3 hrs and 6 hrs during exposure. Nasal resistance was measured only at baseline and once more at the end of the exposure period at 6 hrs.

Two techniques were used to determine eye blink frequency at each time point during caprolactam exposures, a manual count method followed immediately by a semi-automated approach. For the manual method, Ziegler et al. digitally recorded the eyes of the participants for a specified duration under dim lighting conditions. The recording was later reviewed in a double blind fashion and the number of eye blinks manually recorded. The second method involves a neon light shining on the eye surface of the participants and a sensor records the change in beam length that reflects back when the participant blinks. The authors noted in their report that the semi-automated method is still in the testing phase and its applicability and reproducibility needs to be verified in further studies. The manual method is a traditional procedure used in many studies to record eye blinks. OEHHA concurred with Ziegler et al. that for eye blink frequency, greater confidence should be placed on the findings using the standard manual method.

The questionnaire consisted of 29 items related to sensory irritant symptoms and perceptions (e.g., odor). This multi-item battery was used to generate a total daily score. Six adjectives were presented for rating the intensity of symptoms on a Likert-type scale from zero (not at all) to 5 (very severe). The 29 items were also categorized into seven subscores for assessment. The subscores include: 1) non-specific symptoms: *feeling of weakness, headache, dizziness, felling of being unwell*; 2) not classified: *blurred vision, irritation to the throat, skin irritation*; 3) sensations of bad taste: *very unpleasant taste in the mouth, unpleasant taste, foul taste*; 4) respiratory symptoms: *pressure on the chest, coughing, dyspnea*; 5) olfactory symptoms: *perception of bad air, foul smell, unpleasant smell, stink*; 6) nasal irritation: *nasal irritation, itching nose, dry nose, runny nose, burning nose*; 7) irritation to the eyes: *tiredness of the eyes, itchy eyes, burning eyes, eye irritation, dry eyes, watery eyes, redness of the eyes*. A second section evaluating well-being (i.e., tension, tiredness, annoyance and general well-being) was rated on a visual analog scale from one (no symptoms) to seven (severe symptoms).

Ziegler et al. (1998) found that caprolactam exposure was associated with a statistically significant increase in the subjects' detection of an unpleasant odor beginning at 0.15 mg/m^3. However, at 5 mg/m^3 the average intensity score of 1.2 was only slightly pronounced (i.e., between "barely" (1) and "somewhat" (2) for an odor nuisance). Subscores for eye and nasal irritation and eye/nasal irritation combined showed no statistically significant concentration-response relationships using the analysis of variance (ANOVA) test. Discounting odor nuisance, there were no statistically significant differences among the other individual symptoms and subscores associated with caprolactam exposure. At 5 mg/m^3 the total symptom score based on all 29 acute symptom items was significantly elevated ($p \leq 0.05$). No statistically significant increase or decrease in the total symptom score was observed in the course of the day. Thus,

these results do not indicate any adaptation or habituation processes in the course of the 6 hour exposure.

For the three objective measures of sensory irritation, the authors found no statistically significant concentration-response relationships by the ANOVA test, but noted there was a “non-significant trend” towards higher blink frequency and nasal resistance associated with increasing caprolactam concentrations. As noted below, there appear to be statistical issues with these analyses that could have obscured effects of caprolactam.

Because there was little variation over time among most of the subjective and objective measures during the six hours of exposure, the authors had combined the data collected at time 0 (i.e., just after entering the chamber), 1 hr, 3 hrs and 6 hrs for their statistical analyses. OEHHA obtained the individual raw data, kindly provided by Dr. Ziegler, with the primary goal of running several statistical analyses on the data collected at 1 hour of exposure, the exposure duration that forms the basis of an acute REL, as well as the other time points. The combined data collected up to 6 hrs of exposure were also analyzed by OEHHA.

For statistical evaluation of the raw data, we used the non-parametric Page’s trend test to verify any associations between caprolactam exposure among both the objective and subjective endpoints. In the Page’s trend test, the response for each ordered category (caprolactam concentration in this case) is ranked for each participant. Ranking is based on an ordinal scale (i.e., 1, 2, 3 and 4 since there are four exposure concentrations). For example, over ordered doses, if a person's blink response is 24, 15, 70 and 90 blinks/90 sec for the 0, 0.15, 0.5 and 5 mg/m^3 caprolactam concentrations, respectively, then the participants’ ranks are 2-1-3-4. The ranks for each dose are then summed over all the individuals and those sums are used to calculate an L-statistic and corresponding p-value to determine if an association between caprolactam concentration and eye blink frequency exists.

Ziegler et al. originally used a parametric repeated measures ANOVA for their statistical analysis of normally distributed data and a non-parametric Kruskal-Wallis ANOVA for non-normally distributed data, although they did not specify which outcomes each was applied to in every situation. To avoid any assumptions of normality in this data, we chose non-parametric methods. For this set of data, Page’s trend test is a more appropriate non-parametric method for assessing the possibility of a dose-response relationship. This is because Page’s test is able to use the order of the dose categories to assess whether a general trend in response is present over increasing dose, which is essentially the question of interest. Moreover, Page’s trend test accounts for multiple measurements of the same subjects at different exposure times (i.e., repeated measures) and the within-subject correlation in values that such a design creates, whereas the Kruskal-Wallis test does not. In the instances where a statistically significant trend ($p \leq 0.05$) was found, we utilized the Wilcoxon signed-rank test to identify any statistically significant differences at $p \leq 0.05$ between the control exposure and each dose group.

Table 1 presents the statistical results for eye redness and nasal resistance based on Page's trend test. No statistically significant dose-response relationship was found for the eye redness score at 1 hour of exposure. In addition, no dose-response relationship was found at the other time points of 0, 3 and 6 hours (data not shown). Nasal resistance was also not statistically significantly affected by the 6 hour exposure to caprolactam. Nasal resistance values in Table 1 represent the difference between resistance just before entering the exposure chamber and resistance at the end of the 6 hr exposures. A negative mean for nasal resistance (0 and 0.15 mg/m^3 caprolactam exposures) indicates resistance was less at the end of 6 hr exposure than before entering the chamber. In addition to mean, standard deviation, and interquartile range (IQR), mean rankings for each dose group are shown because the rankings are what Page's test ultimately utilizes. Though the L-statistic is calculated using the sum of ranks for each dose group, the mean is provided here to keep ranks on the original ordinal 1 to 4 scale for ease of comprehension.

Table 1. Page's trend test results (mean ± standard deviation, median, interquartile range and mean rank) for eye redness at 1 hour of caprolactam exposure, and for nasal resistance at 6 hours of caprolactam exposure, performed by OEHHA

Outcome & Statistic	Caprolactam concentration (mg/m^3)				Page's trend test result
	0	0.15	0.5	5.0	
Eye redness score[a]					
Mean±SD	2.00±0.00[b]	2.00±0.00[b]	2.00±0.00[b]	2.05±0.15	p=0.64
Median	2	2	2	2	
IQR	0	0	0	0	
Mean rank	2.45	2.45	2.45	2.65	
Nasal resistance[c]					
Mean±SD	-0.049±0.21	-0.029±0.22	0.013±0.12	0.020±0.12	p>0.99
Median	0.00	0.04	0.03	0.01	
IQR	0.14	0.09	0.09	0.09	
Mean rank	2.45	2.45	2.75	2.35	

[a] Score ratings for eye redness were: (1) very slight, (2) slight, (3) moderate, (4) severe
[b] No standard deviation or IQR because all subjects rated a (2) for slight eye redness
[c] Nasal resistance presented in units of kPa/L/sec. Nasal resistance values in Table 1 are different from those presented in the original paper by Ziegler et al. (2008). The correct formula for calculating nasal resistance used in Table 1: LRxRR/(LR+RR) was inadvertently calculated as LRxRR/LR+RR in the original paper, where LR is left nostril resistance and RR is right nostril resistance (A. Ziegler, personal communication). Nevertheless, use of either formula resulted in the same outcome: no effect of caprolactam on nasal resistance.

Based on the manual method for recording eye blinks, the Page's trend test confirmed a statistically significant association for increasing caprolactam exposure and eye blink frequency at 1 hour of exposure (Table 2). The Wilcoxon sign-rank test showed that exposure to 5 mg/m^3 caprolactam resulted in a statistically significant increase (p=0.01) in eye blink frequency compared to the control group. A concentration-response association was not observed at 0 hour (defined by Ziegler et al. as within 5 minutes after entering the chamber). For reasons not specified in the Ziegler study, only 4 to 8 individuals were assessed by the manual eye blink count method at each caprolactam concentration during the 3 and 6 hour exposure effects assessment. OEHHA did not consider this to be a sufficient number of individuals for statistical analysis for these exposure durations.

Applying Page's trend test to the novel semi-automated approach for estimating eye blink frequency, a concentration-response association was not observed at 0 or 1 hour of exposure (Table 2). A statistically significant association for increasing caprolactam exposure and eye blink frequency was observed at 3 and 6 hours of exposure, and for the 0, 1, 3, and 6 hour eye blink data when they were combined. In all cases when an association was found at p<0.05 using the Page's trend test, the Wilcoxon sign rank test observed a statistically significant difference (p<0.05) between the control exposure and the 5 mg/m^3 exposure. No other statistically significant comparisons between exposure concentrations were found.

Table 2. Page's trend test results (mean ± standard deviation (SD), median, interquartile range (IQR) and mean rank) by OEHHA for eye blink frequency

Exposure time & Statistic	Caprolactam concentration (mg/m^3)				Page's trend test result
	0	0.15	**0.5**	**5**	
Manual method					
0 hr[a]					
Mean±SD	25.6±19.9	27.3±23.2	24.0±18.1	30.4±23.1	*p*=0.88
Median	18.5	18.0	19.0	26.0	
IQR	15.5	22.5	15.8	28.0	
Mean rank	2.53	2.48	2.38	2.63	
1 hr					
Mean±SD	18.7±11.8	25.2±24.6	23.5±14.7	34.4±29.2	*p*=0.002
Median	17.0	18.0	21.5	26.0	
IQR	12.8	22.0	19.0	29.8	
Mean rank	1.90	2.38	2.58	3.15[b]	
Semi-automated method					
0 hr[a]					
Mean±SD	21.9±14.4	25.2±24.2	23.8±20.4	28.4±20.6	*p*=0.15
Median	20.5	17.5	19.5	27.0	
IQR	10.5	18.5	13.8	24.5	
Mean rank	2.38	2.28	2.40	2.95	
1 hr					
Mean±SD	18.0±10.5	25.5±25.3	22.8±14.6	29.7±25.8	*p*=0.23
Median	14.0	20.5	19.0	21.5	
IQR	13.5	29.0	14.0	27.3	
Mean rank	2.15	2.63	2.53	2.70	
3 hr					
Mean±SD	21.5±21.0	19.4±14.9	18.2±13.0	29.1±25.0	*p*=0.01
Median	15.5	17.5	16.5	22.5	
IQR	16.5	14.5	11.3	20.5	
Mean rank	2.18	2.28	2.28	3.28[c]	
6 hr					
Mean±SD	16.5±11.4	21.2±19.7	18.9±18.1	25.9±21.8	*p*<0.001
Median	14.5	16.0	13.0	15.5	
IQR	15.5	22.0	14.8	22.3	
Mean rank	1.88	2.30	2.28	3.55[d]	
Combined					
Mean±SD	19.5±10.8	22.8±20.0	20.9±15.2	28.2±21.0	*p*=0.02
Median	17.3	18.4	15.8	23.3	
IQR	9.10	17.3	10.0	22.5	
Mean rank	2.28	2.30	2.10	3.33[e]	

[a] "0 hour" exposure means eye blink data collection started within 5 minutes after entering the chamber

[b] Sign rank test: control (0 mg/m^3) different from 5 mg/m^3 group (*p*=0.01)

[c] Sign rank test: control (0 mg/m^3) different from 5 mg/m^3 group (*p*=0.02)

[d] Sign rank test: control (0 mg/m^3) different from 5 mg/m^3 group (*p*=0.01)

[e] Sign rank test: control (0 mg/m^3) different from 5 mg/m^3 group (*p*=0.01)

For the subjective questionnaire subscores at one hour of exposure, we found a statistically significant concentration-response relationship (p=0.02) for perceived eye irritation (Table 3) by the Page's trend test, consistent with the objective eye blink measure. The 5.0 mg/m^3 exposure differed from the control group (p<0.05) by the sign-rank test. A statistically significant trend was also verified at 6 hours of exposure, but the ensuing sign-rank test comparing control vs. 5.0 mg/m^3 groups was not statistically significant (data not shown). There was no concentration-response relationship at 0 or 3 hours (data not shown).

Combining the 0, 1, 3 and 6 hr eye irritation subscore measurements for statistical analysis, as Ziegler et al. (2008) had done in the original report, also yielded a statistically significant association by the Page's trend test (p=0.002) (data not shown in Table 3). Using the sign-rank test, the control group was different from both the 5 mg/m^3 group (p=0.01) and the 0.5 mg/m^3 group (p=0.03).

The subjective nasal irritation score at one hour of exposure did not demonstrate a statistically significant concentration-response relationship at 1 hour of exposure (Table 3). No statistically significant concentration-response was observed at any of the other time points (0, 3 and 6 hours), or when all the time point data was combined (data not shown in Table 3).

Table 3. Page's trend test results (mean ± standard deviation (SD), median, interquartile range (IQR), and mean rank) performed by OEHHA for selected subjective questionnaire results at 1 hour of exposure: eye and nose irritation subscores, odor nuisance subscore, and total symptom and complaint score of 29 questions with and without odor subscore [a].

Outcome & Statistic	Caprolactam concentration (mg/m^3)				Page's trend test result
	0	0.15	0.5	5	
Eye irritation					
Mean±SD	0.18±0.24	0.26±0.50	0.34±0.48	0.36±0.44	p=0.02
Median	0.14	0	0.21	0.21	
IQR	0.29	0.18	0.32	0.46	
Mean rank	2.13	2.18	2.83	2.88[b]	
Nose irritation					
Mean±SD	0.14±0.23	0.18±0.28	0.18±0.31	0.24±0.33	p=0.42
Median	0	0	0	0	
IQR	0.25	0.2	0.2	0.45	
Mean rank	2.3	2.53	2.53	2.65	
Odor nuisance					
Mean±SD	0.10±0.22	0.20±0.30	0.24±0.30	1.09±0.90	p<0.001
Median	0	0	0	1	
IQR	0	0.31	0.5	0.94	
Mean rank	1.83	2.15	2.40[c]	3.63[c]	
Total score					
Mean±SD	0.13±0.16	0.19±0.33	0.21±0.26	0.37±0.32	p<0.001
Median	0.07	0.07	0.12	0.22	
IQR	0.16	0.13	0.16	0.44	
Mean rank	1.83	2.15	2.68	3.35[d]	
Total score w/o odor					
Mean±SD	0.13±0.17	0.18±0.36	0.20±0.28	0.26±0.31	p=0.003
Median	0.08	0.06	0.1	0.12	
IQR	0.19	0.07	0.09	0.28	
Mean rank	2	2.18	2.73	3.10[e]	

[a] Score ratings were: (0) not at all, (1) barely, (2) somewhat, (3) quite pronounced, (4) severe, and (5) very severe
[b] Control (0 mg/m^3) group different from 5 mg/m^3 group by the sign-rank test (p=0.02)
[c] *Control* (0 mg/m^3) group different from the 5 mg/m^3 (p<0.001) and 0.5 mg/m^3 group (p=0.04) by the sign-rank test
[d] Control (0 mg/m^3) group different from 5 mg/m^3 group by the sign-rank test (p<0.001)
[e] Control (0 mg/m^3) group different from 5 mg/m^3 group by the sign-rank test (p=0.01)

For the odor subscore, a significant concentration-response relationship was observed (Table 3). Ziegler et al. observed a difference between no exposure and all three exposure groups when odor response data collected at 0 (just after entering the chamber), 1, 3, and 6 hrs of exposure were combined. Utilizing just the one hour

questionnaire data, however, we observed a statistically significant difference only for the 0.5 and 5.0 mg/m^3 exposures compared to the control exposure.

The total symptom and complaint score was evaluated both with and without the odor responses. The latter analysis was performed in order to evaluate how strong a driver odor was for the total subjective complaint score. Ziegler et al. had observed a statistically significant difference at $p<0.05$ between the non-exposed and the 5 mg/m^3 caprolactam exposure groups when the questionnaire data (including the odor questions) was combined from all four time points. Using Page's trend test, OEHHA found a statistically significant concentration-response relationship at 1 hour of exposure, when the odor subscore was included (Table 3). The sign-rank test showed a difference in response between the control and the 5 mg/m^3 exposure ($p<0.001$). When the odor questions were excluded, there was still a significant concentration-response relationship at one hour of exposure ($p=0.003$). The sign-rank test for odor-excluded scores indicated a statistically significant difference between the control and 5 mg/m^3 exposures ($p=0.009$).

A benchmark concentration analysis was carried out by OEHHA using U.S. EPA (2009c) continuous modeling methodology on the eye blink frequency data (Table 2) and eye irritation data from Table 3. For the eye blink and eye irritation datasets, continuous modeling demonstrated a significant dose-related trend ($p<0.05$, test for difference among dose groups), but was unable to provide a fit to the data (chi-square test for goodness of fit, where $p\geq0.10$ for acceptability) using the available models. Specifically, the models could not be fit to the data because one or more of the observed means was not positioned reasonably close enough to the estimated means.

OEHHA applied the Spearman rho test to look for correlations in response to caprolactam concentration between the eye blink frequency and subjective eye irritation score at 1 hour of exposure. First, individual caprolactam responses were characterized by comparing response at the 5 mg/m^3 dose to response at the baseline 0 mg/m^3 dose. For subjective eye irritation, an absolute measure of response was calculated by simply subtracting an individual's control dose response from their high dose response. For eye blink, however, wide intra-individual and inter-individual variation in blink frequencies made simple differences difficult to compare. In this case, we calculated relative responses in blink frequency by dividing a subject's high dose caprolactam eye blink frequency by their control blink frequency to yield a measure of relative response to caprolactam. Comparing relative blink increase (manual method) with absolute eye irritation increase at 1 hour of exposure, we found that responses are correlated (Spearman rho coefficient=0.54, $p=0.01$).

OEHHA used the same approach in applying the Spearman rho test for correlation between eye irritation score and odor score. Because the odor of caprolactam was apparent to the participants the subjective eye irritation score may be influenced by the odor, similar to what has been observed for other airborne chemicals with unpleasant odors (Dalton, 2003). As with eye irritation, odor response was calculated by subtracting control odor score from high dose odor score. At 1 hour of exposure, no correlation was found for absolute eye irritation score vs. absolute odor score

(Spearman rho coefficient=0.04, *p*=0.86). Finally, the Spearman rho test was also applied to relative eye blink frequency vs. absolute odor score at 1 hour of exposure. No correlation was observed (Spearman's rho coefficient=0.20, *p*=0.41). These results indicate that eye blink responses and eye irritation responses are correlated, but neither is correlated with odor.

No correlation was apparent between eye blink response and eye redness response (Spearman's rho =0.38, *p*=0.10), which was also calculated by taking the difference of an individual's high dose and control redness scores. However, this is not inconsistent with reports by other investigators. For example, Lang et al. (2008) observed eye redness at the same level of formaldehyde exposure that caused increased eye blink frequency in one exposure scenario (0.5 ppm formaldehyde with 1.0 ppm peaks), but did not observe eye redness in a similar scenario that caused increased eye blink rate (0.5 ppm formaldehyde with 1.0 ppm peaks + ethyl acetate as an odor masking agent). These data suggest eye blink frequency is a more consistent and sensitive indicator of eye irritation than eye redness.

Case Reports and Human Sensitization Studies

We identified three peer-reviewed published case reports of workers heavily exposed to caprolactam developing seizures and other severe symptoms. The exposure levels in these reports were not quantified, however the descriptions suggest that the exposures could be considerably higher than typical occupational exposures.

Tuma et al. (1981) reported the case of a 22-year-old man who developed dermatitis of the hands and feet, nausea and vomiting, leukocytosis, and "grand mal" (i.e., generalized tonic-clonic) seizures three days after being transferred to a section of a plastics plant that involved caprolactam use. The caprolactam dust coated his clothing and exposed areas of skin when he arrived at the hospital. Dermal inflammation was also noted on the buttocks and thighs. The authors did not indicate that any respiratory distress was present; a chest roentgenogram was reported to be normal. After a few days of observation in the hospital, the skin lesions manifested desquamation (peeling) and erythema (redness and swelling), although the other symptoms had cleared. A comprehensive neurological investigation identified no underlying organic CNS abnormalities, consistent with the seizures having been a consequence of the work-related caprolactam exposure.

In a second case report from South Korea translated from Korean by OEHHA, two young men were hospitalized with nausea, vomiting, dermatitis on the hands and feet, and tonic-clonic seizures following occupational exposure to caprolactam (Woo et al., 1998). The men had been packaging caprolactam, one for two days and the other for four days, working inside a caprolactam containment vessel. Laboratory testing documented leukocytosis and hyperglycemia. Brain CT scanning and EEG testing were reportedly normal. No further symptoms or seizures were seen over the two months after exposure. The authors concluded that the skin lesions and unexplained generalized tonic-clonic seizures in the men strongly indicated a causal effect of caprolactam intoxication.

In a third case report translated from Chinese by OEHHA, three workers in a Chinese plant were taken to a hospital emergency room with symptoms of dizziness, nausea and vomiting following handling or moving plastic bags containing chemical raw materials including caprolactam (Chen, 2002). Two of the workers (both of whom were working shirt-less) displayed tonic-clonic seizures, opisthotonus, froth around the mouth, upward-turned eyeballs, and a post-ictal altered mental status. There was no mention of any respiratory or dermal symptoms in the exposed workers. Physiological and hematological exams were generally normal. Urine caprolactam concentrations were stated to be 2.9-3.7 g/L, and 13.6-15.4 g of caprolactam was leached from the workers' clothing. Data on caprolactam metabolism indicate that only about 2% is excreted unmetabolized in urine. Although metabolism could be saturated with high exposure, the g/L amounts in the urine do not seem biologically plausible. More likely, the reported units of measure are incorrect. The colorimetric method used to estimate the urine caprolactam concentration appears to use mg/L as the unit of measure (Zhou, 1976), which would be a more reasonable caprolactam urine concentration in the workers in this case report.

Chen (2002) noted the two patients recovered and were released after a four-day hospitalization. It had been an extremely hot day (heat stroke was ruled out clinically) and the authors speculated that sweat facilitated the dermal absorption of caprolactam, particularly in the two workers that were shirt-less. The workers had a previous caprolactam exposure without reported symptoms.

These case reports suggest that neurotoxicity can be an important endpoint in humans heavily exposed to caprolactam. Moreover, dermal absorption can be an important exposure pathway and may also lead to dermatitis. Relevant to these human case reports, studies in rabbits show that concentrated caprolactam (50% aqueous caprolactam solution) placed on the skin can cause local irritation and be absorbed, leading to convulsions and death (Haskell Laboratory, 1950). Other animal studies with different exposure routes have also observed caprolactam-caused seizures (Goldblatt 1954; Gross 1984, reviewing the Eastern European literature). These animal studies will be discussed in greater detail in Section 5.3 to follow.

A review by Gross (1984) of the eastern European occupational studies conducted in the 1960s and 1970s suggests a significant number of workers may develop "hypersensitivity" to caprolactam. However, the methodology was not adequately described in these studies and there was co-exposure to other chemicals. Exposure studies conducted in the West, some of which were not peer-reviewed, indicated caprolactam solutions of 1-5% did not cause skin irritation or act as a skin sensitizing agent.

Goldblatt et al. (1954) applied a 5% aqueous caprolactam solution to the skin of the inner forearms of six normal persons (4 men, 2 women) as a patch test left in contact for 48 hours. Goldblatt et al. also applied a 5% caprolactam solution in either alcohol or olive oil to the same area on volunteers and allowed to dry. The process was repeated daily for four days. In all cases, no irritant effects were produced. The authors

concluded that caprolactam is not a skin irritant following these short-term exposures, and no evidence was found that it could act as a sensitizing or dermatitis agent.

In a study carried out in Haskell Laboratory in 1941 that was not published in a peer-reviewed journal, three human volunteers had a 1% aqueous solution of caprolactam applied to the skin (Haskell Laboratory, 1950). No skin irritation was reportedly produced. No other methods or descriptive information was provided.

In another non-peer-reviewed study (i.e., not published in a peer-reviewed journal) conducted in 1952-53 and recently reported to the U.S. EPA (2009a), a patch test was conducted in 204 human subjects to determine whether or not Nylon-6 containing 3-5% water-extractable caprolactam and dimers would produce primary skin irritation and/or sensitization in occupational exposures. No primary irritation or allergic sensitization was observed in the tested subjects. No other methods or descriptive information was provided. Animal data related to the question of sensitization from this study is summarized in Section 5.3.

In contrast to these negative human experimental studies there have been case reports consistent with caprolactam-related contact dermatitis. A worker with 29 years of experience in a Nylon-6 factory presented with an 18-month history of dermatitis (Aguirre et al., 1995). Patch testing with a 5% aqueous solution of caprolactam was positive for contact dermatitis. The lesions completely resolved following 2-month leave from work. In another case report, a woman developed dermatitis at a skin site where blue polyamide-6 suture thread had been removed following 10 days of placement following a dermatological procedure (Hausen, 2003). She had already undergone more than 40 similar procedures in the past. Patch testing was positive for caprolactam and the blue dye (acid blue 158) used in the thread. The patient, who was a hairdresser for 17 years, also had positive testing for ammonium persulfate and 2,5-diaminotoluene, two chemicals she was exposed to occupationally.

Occupational Exposure Limit Values for Caprolactam

The National Institute for Occupational Safety and Health (NIOSH) and the American Conference of Governmental Industrial Hygienists (ACGIH) currently have recommended occupational exposure limits for caprolactam (Table 4). The ACGIH (2003) recommends summing both aerosol and vapor forms of caprolactam together to determine the total airborne concentration. NIOSH has two caprolactam recommendations, one for the ‘dust’ (NIOSH, 1995a) to describe the solid or particulate form of caprolactam that may cause dermal irritation and another for the “vapor” (NIOSH, 1995b) that refers to both airborne aerosol and vapor forms. Both sets of recommended limit values by NIOSH and ACGIH are influenced by the published findings by Ferguson and Wheeler (1973) that reported no irritation of any kind, or any other signs and symptoms of discomfort and malaise, in workers below 7 ppm (32 mg/m^3).

Table 4. Summary of the ACGIH and NIOSH limit values for caprolactam

Agency	Occupational Exposure Value
ACGIH	TLV-TWA, 5 mg/m^3, as inhalable aerosol and vapor
ACGIH	TLV-STEL, no specific data on which to base a TLV-STEL
NIOSH	REL-TWA, 1 mg/m^3, dust
NIOSH	REL-TWA, 0.22 ppm (1 mg/m^3), vapor
NIOSH	REL-STEL, 3 mg/m^3, dust
NIOSH	REL-STEL, 0.66 ppm (3 mg/m^3), vapor

The ACGIH (2003) has recommended a combined caprolactam aerosol and vapor threshold limit value (TLV) of 5 mg/m^3 (1.08 ppm) as a time-weight average (TWA) for a normal 8-hr workday and a 40-hr workweek. The ACGIH (2003) did not find that sufficient data were available to recommend skin or sensitization notations.

NIOSH (1995b) has established a recommended exposure limit (REL) for caprolactam vapor of 0.22 ppm (1 mg/m^3) as a TWA for up to a 10-hr workday and a 40-hr workweek, and 0.66 ppm (3 mg/m^3) as a short-term exposure limit (STEL). These lower recommended values, compared to ACGIH values, are intended to prevent early signs of irritation in some workers. Based on available human exposure responses, primarily Ferguson and Wheeler (1973), NIOSH felt a sufficient margin of safety was warranted to prevent such outcomes due to caprolactam vapor.

For caprolactam dust, NIOSH (1995a) recommended an REL value of 1 mg/m^3, and a STEL of 3 mg/m^3. These exposure levels appear to be largely based on an unpublished letter to the ACGIH TLV committee in 1972 when occupational limits were being determined by that body for caprolactam (Ferguson and Wheeler, 1973; ACGIH, 2001). In this letter, airborne caprolactam dust was reported to be irritating to the skin of some individuals at 5 mg/m^3, but adequate protection was provided by a limit of 1 mg/m^3 particularly when this was combined with respirator use. From this description, it may be inferred that the exposure limits for caprolactam as airborne dust were designed to lead to lower deposition onto surfaces that facilitate direct dermal contact and dermal irritation. As stated previously, however, airborne vapors are likely to lead to surface condensation as well.

The U.S. Occupational Safety and Health Administration (OSHA) has promulgated a permissible exposure limit (PEL) neither for caprolactam vapor nor caprolactam dust. Cal/OSHA, however, in 1973 promulgated a PEL of 1 mg/m^3 and STEL of 3 mg/m^3 for caprolactam dust (Cal/OSHA, 2011). For the vapor form, the Cal/OSHA PEL is 5 ppm (20 mg/m^3) and the STEL, 10 ppm (40 mg/m^3). These occupational limit values were

likely adopted from earlier ACGIH (2001) limit values for caprolactam before they were revised by the ACGIH in 2003.

The ACGIH (2001) originally had higher exposure limits that mirrored Ferguson and Wheeler's conclusion that a worker threshold value of 5 ppm (23 mg/m^3) is recommended based on the absence of reported distress among those working at concentrations up to 7 ppm. The subsequent reduction of the ACGIH (2003) exposure limit to well below this worker threshold value suggests that that organization no longer accepted the findings by Ferguson and Wheeler (1973) in this regard.

5.2 Acute Toxicity to Infants and Children

No studies were located regarding acute toxicity to infants and children exposed to caprolactam. We found no studies of inhalation exposure to young or pregnant animals that could shed insight into acute toxicity in infants and children. In pregnant mice, oral delivery of radiolabeled caprolactam was rapidly absorbed from the stomach and freely distributed into all tissues, including the fetuses (Waddell et al., 1984). Some residual radioactivity was noted in the umbilical cord, amnion, and yolk sac. No radioactivity was retained in any other fetal tissues.

5.3 Acute Toxicity to Experimental Animals

Relatively few peer-reviewed studies of acute caprolactam exposure in experimental animals have been conducted. Acute inhalation, oral, and parenteral exposure studies are summarized below, including some non-peer-reviewed studies, to provide the full spectrum of effects resulting from acute intoxication from caprolactam exposure. Due to caprolactam's respiratory irritant action, dermal and inhalation sensitization studies are also reviewed. None of the sensitization studies was peer-reviewed. A summary of the animal toxicity findings, including acute and multi-day exposures, is presented in Table 5 at the end of this section.

The BASF Chemical Company conducted unpublished acute exposure studies in the 1960s and 70s that were reported by Ritz et al. (2002). In the rat, an oral LD_{50} of 1155 mg/kg is reported. Symptoms of acute intoxication were tonoclonic convulsions. In an acute toxicity study on rats and mice, the NTP (1982) administered caprolactam in corn oil by gavage to groups of five males and five females. The LD_{50} for male and female mice were 2070 and 2490 mg/kg, respectively. The LD_{50} for male and female rats were 1650 and 1210 mg/kg, respectively. No signs or symptoms of toxicity were discussed.

Goldblatt et al. (1954) observed 66% mortality in rats injected intraperitoneally with 800 mg/kg caprolactam with the appearance of delayed spasms. Lower non-fatal doses (500-600 mg/kg) resulted in tremors, apprehension, depression of temperature, and occasional chromodacryorrhea. Concentrations of 900 mg/kg and above proved fatal and resulted in epileptiform convulsions, salivation, and bleeding from the nares. Goldblatt et al. (1954) also injected rabbits intravenously with non-fatal doses of caprolactam ranging from 100 to 300 mg/kg. The effects were severe including

mydriasis, salivation, accelerated respiration, tremors, opisthotonic-like muscle contractions, and convulsions. The latter end-point has already been summarized previously in relation to human case reports of caprolactam-associated seizures.

Similar results were observed in the foreign toxicology literature (published mainly in Russian and German) of the 1950s and 1960s and reported in a review by Gross (1984). Caprolactam LD_{50} studies in experimental animals and exposure to high doses of caprolactam by intravenous and intraperitoneal injection produced tremors, epileptiform convulsions, salivation and bleeding from the nostrils. In an unpublished study by Haskell Laboratory (1950), an approximate lethal dose of 3375 mg/kg was observed in rats administered by gavage. Rats receiving 1500 mg/kg developed convulsions and some showed slight bleeding from the nose and mouth.

In an unpublished industrial study, four-hour exposure of rats to 5,250, 8,350, or 10,120 mg/m^3 caprolactam aerosol via a head-nose inhalation system resulted in eyelid closure, shallow to spasmodic breathing, and mild to strong defense reactions (BASF, 1985). After exposure, steppage gait, bloody nasal secretions, spasmodic breathing, marked tremor, and bloody lacrimation were observed. LC_{50}s of 9,600 and 7,080 mg/m^3 were recorded for male and female rats, respectively. In rats that died, general circulatory congestion, elevated hyperemia of the lung, moderate to severe fatty degeneration of the liver, and ischemic tubular nephrosis in the renal cortex were found. No additional deaths occurred after one day post-exposure and all surviving animals appeared normal 3 days post-exposure. Histopathological examination of the organs in surviving rats 14 days post-exposure was described as “unremarkable”.

In another unpublished[1] study, two rats exposed to a nominal particle caprolactam concentration expressed as 14,000 ppm (sic) for 17 min showed signs of general discomfort and inflammation around the eyes and nose (Haskell Laboratory, 1950). Note that particle exposures should be expressed in mg/m^3, which in this case would be approximately 65,000 mg/m^3. No gross pathology or micropathology was detected at sacrifice following a nine-day observation period.

The U. S. Consumer Product Safety Commission contracted a study of sensory and pulmonary irritation in Swiss-Webster mice exposed to compounds emitted from carpet and carpet-related products, including caprolactam (CPSC, 1996). The animals were placed in a head-only glass plethysmograph and exposed to 13.5 mg/m^3 caprolactam vapor, the highest attainable exposure concentration. The study protocol called for a one hour exposure, followed by a recovery period of 15 minutes in clean air, then exposure to the same concentration of caprolactam for another hour.

Sensory irritation was defined by a 12% or greater group decrease in the mean respiratory frequency, the minimum level of respiratory depression needed to classify an exposure as having a positive sensory irritation response (CPSC, 1996). By this approach, no measurable sensory irritation or reduction in respiratory rate was observed in the mice during the caprolactam exposure. However, the CPSC (1996) notes that measurable respiratory irritation in mice using this method usually occurs at levels 10 to 100 times higher than levels which would result in irritation in humans.

Inhalation and Dermal Sensitization/Irritation Studies

We consider sensitization here under acute exposure effects because the anamnestic response is manifested with an acute re-challenge, even though the process of sensitization itself may require repeated subacute or even chronic exposures.

In a skin absorption study, a 50% aqueous solution of caprolactam was applied to a shaved area between the shoulder blades of rabbits (Haskell Laboratory, 1950). The approximate lethal dose was 3375 mg/kg producing pathology similar to hypovolemic shock. Clinical observations included tremors, convulsions, and bleeding from the mouth and nose analogous to those observed in rats receiving oral doses. Edema and congestion of the skin at the site of application was noted, which may have increased dermal absorption as a result of skin damage. This study was not published in a peer-reviewed journal.

Gross (1984) reviewed the eastern European literature conducted in the 1970s concerning dermal sensitization studies in animals. It was claimed in these studies that both intracutaneous and dermal application of caprolactam in guinea pigs resulted in "sensitization." In the case of intracutaneous injections, the development of contact dermatitis was interpreted as indicative of successful sensitization. In one of two cases, it was claimed guinea pigs became sensitized to caprolactam by inhalation. However, other studies described below could not reproduce assertions of inhalation sensitization.

In an unpublished report submitted to U. S. EPA, groups of four male albino guinea pigs were exposed for 30 min on 5 consecutive days to 3, 10, or 30 mg/m^3 aerosols (1.5 micron) generated from a 15% aqueous caprolactam solution (U. S. EPA, 1994b; Rinehart et al., 1997). On day 19, 26, 33 and 40, animals were challenged for 30 min with 30 mg/m^3 caprolactam. Animals were monitored with whole-body plethysmography for indications of irritation and coughing, and pulmonary hypersensitivity was monitored using respiratory frequency, tidal volume, and airway constriction as criteria for effect. Caprolactam failed to induce immediate or delayed pulmonary hypersensitivity with this protocol, which has been positive for ovalbumin and trimellitic anhydride. In addition, there was no evidence of respiratory tract irritation at any exposure concentration.

In unpublished work carried out by the BASF Chemical Company, guinea pigs were exposed to repeated epicutaneous application (50% ether solution; 10 times) or intracutaneous injection (0.1% in physiological NaCl solution) (Ritz et al., 2002). Neither treatment caused local irritation or sensitization to the skin.

In an unpublished study carried out in 1941, a skin irritation test with a 66% aqueous solution of caprolactam was conducted in 10 albino guinea pigs (Haskell Laboratory, 1950). Initial application of the aqueous caprolactam solution to unbroken shaved skin resulted in erythema in one animal, faint erythema in two other animals, and negative results in the remaining 7 animals. The researchers concluded caprolactam produced only mild dermal irritation in the guinea pigs.

To further test for sensitization, a maximization test was conducted that consisted of a series of 6 treatments of a 66% aqueous solution of caprolactam to broken skin, or 6 intradermal injections of 0.1 ml of a 0.1% aqueous solution (Haskell Laboratory, 1950). This was followed by a rest period of two weeks, and then the 66% aqueous caprolactam solution was again applied to the unbroken skin at the same site as the original application. Seven of ten animals manifested dermal reactions indicating that sensitization had occurred. A final intradermal injection and application to broken skin likewise showed an increase in intensity of the reaction consistent with sensitization. Although the sensitization potential is limited by using an irritant concentration for challenge treatment, the researchers considered caprolactam should be considered a mild sensitizer on the basis of the strength of the reaction they observed.

In a similar (unpublished) maximization test protocol, 20 female guinea pigs received intradermal application of caprolactam (3.0% w/v) in water, or topical application of caprolactam (75% w/v) in water (Springborn Laboratories Inc., 1991). Challenge responses in the induced animals were compared to those of the controls. Blood samples were obtained prior to study initiation and following the challenge for evaluation of standard hematology parameters. Additionally, plasma histamine was determined for selected test and control animals following challenge. Based on the concurrent mild dermal reaction in the control group animals and the fading of reactions from 24 to 48 hours, caprolactam was not considered to be a contact sensitizer in that study.

In a Buehler test in rabbits, induction test animals were patched with 25% w/v caprolactam in water 3 times within 3 weeks (Springborn Laboratories Inc., 1991). In the challenge phase, the test group animals received 25% w/v caprolactam in water in a patch. Ten animals each were used in the challenge control and the rechallenge control groups. Dermal reaction was scored 24 and 48 hours after removal of the patch. Minimal dermal reaction was observed in both the test animals and negative control animals after the challenge as well as after the rechallenge. Mean dermal scores were also comparable between both groups. The skin sensitization potential of caprolactam was limited by using an irritant concentration for the challenge treatment. Therefore, caprolactam was not considered to be a contact sensitizer under the test conditions chosen. This study was also not published in a peer-reviewed journal.

In a dermal sensitization test by Rinehart et al. (1997), groups of 20 female albino guinea pigs were tested with 25% aqueous caprolactam solution using either the traditional modified Buehler or maximization test designs. Groups of 5 guinea pigs were treated with 5% DNCB (probably 1-Chloro-2,4-Dinitrobenzene) as a positive control. After the second challenge dose had been evaluated, blood samples were obtained for measurements of leukocytes, differential counts and plasma histamine levels. Neither test regimen showed positive results for animals treated with caprolactam. There were no body weight changes or any effects on hematologic components or plasma histamine levels caused by treatment with caprolactam. This study was reported in the journal only in abstract form.

A report of a skin sensitization test conducted on up to 6 guinea pigs and 4 dogs at the end of a subchronic inhalation exposure regimen was submitted to U. S. EPA (U. S.

EPA, 2009a). This study (not published in a peer-reviewed journal) was conducted in 1952-53 and only recently reported to the U.S. EPA. All animals were exposed to 444 mg/m^3 caprolactam as a fume (i.e., a solid suspension generated by heating caprolactam in air) 6 hrs/day for 43 exposures. Half of the guinea pigs and 3 of the dogs were then exposed to 1020 mg/m^3 on exposures 44 through 67 or 73. Observations were made one-hour, 24-hours, and 48 hours after patch application. Both guinea pigs and dogs acquired skin sensitization. No descriptive information was provided that clarify the severity of the response (although it was reported to be "mild").

In summary, acute caprolactam exposure in animals produced severe neurological effects. Caprolactam given orally by gavage, injected intravenously or intraperitoneally, or applied to the skin, can cause convulsions. Inhalation studies at lethal or near-lethal concentrations resulted in severe tremors. Dermal and inhalation sensitization test were mostly negative. Dermal sensitization has been noted in some studies, however, although interpretation of these is complicated by dermal irritation effects. A concern with the overall acute data is that most of these reports were not published in a peer-reviewed journal, and results were often insufficiently reported or published.

Table 5. Effects of Caprolactam Exposure in Experimental Animals

Species	Exposure	Response	Reference
Inhalation Studies (Detailed summaries in Section 5.3 and 6.3)			
Rats	65,000 mg/m^3, nominal exposure concentration, 17 min	Signs of general discomfort, eye and nose inflammation	Haskell Laboratory, 1950
Mice	13.5 mg/m^3, 2 hrs	RD_{50} study No measurable sensory irritation or reduction in respiratory rate	CPSC, 1996
Rats	0, 5250, 8350, 10,120 mg/m^3 for 4 hrs	LC_{50} = 9600 mg/m^3 (males) LC_{50} = 7080 mg/m^3 (females) Eyelid closure, spasmodic breathing, steppage gait, marked tremor, bloody eye and nasal secretions	BASF, 1985
Rats	Nominal exposure to 13,900 mg/m^3 for 2 hrs, then 1-2 hr exposures to 12,500 to 31,500 mg/m^3 on 5 successive days	General discomfort, eye and nasal inflammation during exposure. Slight lung edema and spleen congestion 3 days after exposure	Haskell Laboratory, 1950

Species	Exposure	Response	Reference
Guinea pigs	0, 3, 10, 30 mg/m^3 30 min/day for 5 days Challenge on day 19, 26, 33 and 40 with 30 mg/m^3 for 30 min	No indication of sensory irritation, coughing or pulmonary hypersensitivity as measured by whole body plethysmography	Rinehart et al., 1997
Guinea pigs	118-261 mg/m^3 for 7 hr/day for 7 days	Observed for irritant effects: Occasional cough seen	Goldblatt et al., (1954)
Guinea pigs	51 mg/m^3 5-8 hr/day for 26-30 days	Slight irritation of nasal and tracheal mucosa	Hohensee et al. (1951)
Dogs, guinea pigs, rats, rabbits	444 mg/m^3 6 hrs/day for 43 exposures, then 1020 mg/m^3 6 hr/day for 23 to 29 more exposures	Dogs: aggravated sores and eyes. Low blood pressure, tremors, weakness, coughing, dense froth around mouth at 1020 mg/m^3. Rabbits: slight corneal damage and eye irritation. Rats: no specific toxic findings Guinea pigs: nephritis	Conducted in 1952-53. Summarized in U.S. EPA, 2009a
Rats	0, 24, 70, 243 mg/m^3 5 days/wk, 13 wks	Treatment-related red facial stains, clear nasal discharge, moist rales, labored breathing. Nasal respiratory and olfactory mucosal lesions, and laryngeal tissue lesions.	Reinhold et al., 1998
Oral Gavage Studies (Detailed summaries in Section 5.3)			
Rats	No exposure dose information provided	LD_{50} = 1155 mg/kg Tonic-clonic convulsions	Summarized in Ritz et al., 2002
Rats & mice	Rats: 681, 1000, 1470, 2150 & 3160 mg/kg Mice: 1000, 1470, 2150, 3160 & 4640 mg/kg	Male rat LD_{50} = 1650 mg/kg Female rat LD_{50} = 1210 mg/kg Male mice LD_{50} = 2070 mg/kg Female mice LD_{50} = 2490 mg/kg Symptoms not described	NTP, 1982
Rats	Detailed exposure dose information not provided	LD_{50} = 3375 mg/kg 1500 mg/kg resulted in convulsions, bleeding from mouth and nose	Haskell Laboratory, 1950

Species	Exposure	Response	Reference
Dermal Toxicity and Sensitization Studies (Detailed summaries in Section 5.3)			
Rabbit	50% aqueous solution of caprolactam applied to shaved area of skin	LD_{50} = 3375 mg/kg Tremors, convulsions, bleeding from mouth and nose, skin damage at site of application	Haskell Laboratory, 1950
Guinea pigs	10 epicutaneous applications of 50% aqueous solution, or 10 intracutaneous injection of 0.1% aqueous solution	Neither treatment caused local irritation or sensitization to the skin	Summarized in Ritz et al., 2002
Guinea pigs	Maximization test with 6 skin applications of 66% aqueous solution, or 6 intradermal injections of 0.1% aqueous solution	Initial application produced erythema in some animals. Re-exposure of caprolactam by both methods after 2 wk rest period resulted in mild sensitization	Haskell Laboratory, 1950
Guinea pigs	Maximization test with 75% aqueous caprolactam solution	Dermal reaction in control animals. Challenge application produced no sensitization or increased plasma histamine	Springborn Laboratories Inc., 1991
Rabbits	Buehler patch test with 25% aqueous caprolactam solution	Comparable minimal dermal reaction in test and negative control animals after challenge and rechallenge	Springborn Laboratories Inc., 1991
Guinea pigs	Buehler and maximization tests with 25% aqueous caprolactam solution	After challenge and rechallenge, no positive results for sensitization, or change in body weight, plasma histamine, leukocytes or differential counts	Rinehart et al., 1997
Guinea pigs & dogs	Skin patch test following 43 exposures at 444 mg/m^3, and 23 to 29 additional exposure at 1020 mg/m^3	Observations made at one hour and 24 and 48 hours after patch application. Mild skin sensitization was observed. Caprolactam patch concentration not stated.	US EPA (2009)_ [an 8(a) submission to US EPA]

6 Chronic Toxicity of Caprolactam

6.1 Chronic Toxicity to Adult Humans

Occupational exposure to caprolactam is known to cause dermal, eye and upper respiratory tract irritation with acute or recurrent acute exposure, but occupational studies with prolonged caprolactam exposure in workers were considered by OEHHA to be inadequate for use as the basis of a chronic REL.

Gross (1984) summarized the early foreign literature regarding industrial exposure to caprolactam. With a few exceptions, the pertinent publications were Russian. These reports describe diverse complaints and abnormalities of the various organ systems in people exposed in factories producing nylon. The exposures in no instance were only to caprolactam. Exposure to caprolactam was commonly associated with exposure to dinyl oxides, such as diphenyl oxide. Other chemicals often associated with caprolactam exposures were cyclohexane, cyclohexanol, cyclohexanone, benzene, acetone, and trichloroethylene.

In a report from Germany translated from German by OEHHA, end of shift complaints by workers exposed to caprolactam at a factory included irritability, nervousness, heartburn, bloating, nose bleeds, upper airway inflammation, and dry and chapped lips and noses (Hohensee, 1951). Exposure included both the vapor and crystal, or dust, forms of caprolactam. Headache in response to the odor and unpleasant taste of the caprolactam vapor was also reported. All these symptoms subsided after a short (but unspecified duration) stay in fresh air. Factory inspection of the caprolactam concentration in the spinning room revealed a concentration of 61 mg/m^3, while the concentration in the laboratory room was 16-17 mg/m^3.

Although Ferguson and Wheeler (1973) were primarily focused on acute effects of airborne caprolactam exposure, the researchers also took 8-hr time-weighted average (TWA) measurements at two facilities and reviewed medical records. Other than dermal injuries resulting from direct contact to concentrated caprolactam solutions, no general health problems requiring medical follow-up were found in a review of medical records collected during the 18 years of plant operation. In addition, no worker had been removed or asked to be removed from exposure to caprolactam vapor for health reasons during plant operation.

At the caprolactam polymer plant, approximate 8-hr time-weighted average (TWA) air samples were collected from various locations in a work area over five days (Ferguson and Wheeler, 1973). The 8-hr TWA air concentrations of caprolactam vapor during working hours were 3.2 ppm (14.8 mg/m^3) with a range of 1.3 to 6.9 ppm (6.0 to 31.9 mg/m^3) at location 1, and 1.1 ppm with a range of <0.5 to 4.5 ppm (<2.3 to 20.8 mg/m^3) at location 2. Based on the percent time worked in specific locations of the caprolactam-contaminated rooms, the worker exposure durations were estimated to be about 15 to 45 min at location 1, and 1 to 4 hrs at location 2. At the caprolactam monomer plant, 8-hr TWA caprolactam vapor concentrations at various sites over a 3-week period were collected. The concentration of caprolactam sampled at various

worksite locations ranged from 0.2 to 17.6 ppm (0.9 to 81.5 mg/m^3). Worker exposure durations in the caprolactam-contaminated areas ranged from 10 min to almost 3 hrs.

From the 8-hr TWA data collected, Ferguson and Wheeler (1973) concluded that working atmospheric concentrations up to about 7 ppm (32 mg/m^3) at the caprolactam polymer plant generally resulted in no reported distress of interviewed workers in active and semi-active areas. This data supported their estimate of a worker irritant response threshold of 5 ppm (23 mg/m^3) based on the acute exposure portion of their study.

There are significant deficiencies in the Ferguson and Wheeler report that prevent it from use as the basis of an OEHHA chronic REL. As also noted by the U.S. EPA RfD/RfC Work Group, significant deficiencies included lack of information on the number of workers and the average duration and distribution of exposure (U. S. EPA, 1994b). Also, no historical air levels are given, all exposures are determined from area rather than personal samplers, and no attempt was made to reconstruct individual exposure histories.

Kelman (1986) conducted a clinical and occupational history of eight workers, seven of which were smokers, at a Nylon-6 manufacturing plant. Several of the workers (number not given) had complained of “some degree” of eye, nose, and throat irritation, although it was unclear from the study if the irritation was chronic in nature. All but one reported peeling of the skin on the hands. Five workers showed abnormal maximal expiratory flow volumes. The author considered the lung function tests unremarkable when the smoking history of the workers was taken into account. Blood and urine samples were collected for assessment of hematological, hepatic and renal functions. No evidence of systemic toxicity was found.

Exposure by Kelman (1986) was described as caprolactam vapor from heat-curing ovens, which subsequently condensed into a fume in the workplace air. Contact of the fume with cooler surfaces resulted in the formation of light feathery flakes. Average worker exposure was 4.8 years (range 9 months to 13 years) and mean atmospheric caprolactam dust concentrations at the time of the study were 84 mg/m^3 (range: 22-168 mg/m^3) for static samplers and 68 mg/m^3 (range: 6-131 mg/m^3) for personal samplers. Recovery of caprolactam vapor from distilled-water bubblers was considered negligible, which the authors interpreted as indicating exposure was limited to caprolactam dust. The caprolactam dose and exposure durations for individual workers were not provided in this study, and a characterization of the caprolactam particle sizes was not performed. The reference to formation of “light feathery flakes” suggests that some part of the caprolactam was in particle sizes too large to be inhaled and may not be relevant for inhalation exposure.

Billmaier et al. (1992) conducted an industrial exposure study of selected workers in two caprolactam plants, Chesterfield and Hopewell. Forty-nine workers were selected (27 smokers/ex-smokers) with 63 controls (workers not working in caprolactam areas, 42 smokers). The controls were matched to the exposed workers (all males) for age, race and smoking status. The workers selected had an average work exposure of 18.7 years (range: 8.2-31.7 years) against matched controls. The level of caprolactam in the

work areas was determined mainly by personnel monitoring. The monitoring method detected total caprolactam and did not differentiate between various states of the material. In operations where caprolactam or the polymer is heated and/or wet or in water solution, the airborne caprolactam was assumed to be in the vapor state [OEHHA notes that this description suggests much of the caprolactam may also be in aerosol form, especially at concentrations above the saturated vapor concentration of 13 mg/m^3]. The average concentrations from occasional monitoring over the previous 10 years at the Chesterfield plant averaged 4.5 mg/m^3 in the Polymer 25 area and 9.9 mg/m^3 in the Spinning 26 area (area monitoring only). Short term measurements of 15-59 minutes during specific plant operations that represented maximum short-term exposures to caprolactam ranged up to 34.8 mg/m^3. For the Hopewell plant, the levels were 4.2-7.8 mg/m^3 from occasional monitoring over 10 years, and an average of 17 mg/m^3 with a range of 2.3-30.8 mg/m^3 from short term measurements.

Pulmonary function tests were obtained by Billmaier et al. (1992) from all exposed and control workers. Pulmonary function tests began in 1978. "Nurses notes" used were from Chesterfield workers. These notes were obtained from workers who were ill, injured, had a physical examination or a return to work examination, or others over a period of 11 years. Only a few episodes of injury or illness were noted in the medical records that were specifically related to caprolactam exposure. One employee reported dermatitis on two separate occasions, and another employee reported dermal irritation following direct exposure to a lactam-containing solution. A third employee complained of eye irritation on one occasion and reportedly inhaled partially polymerized nylon flakes on another occasion, leading to nausea. No specific caprolactam exposure-related nose or throat symptomatology was reported. However, "symptoms" recorded in the notes may not have been assessed as this was optional.

There were no significant differences between exposed workers and their controls in the pulmonary function tests or lung function over the years (Billmaier et al., 1992). Wide differences were shown in the initial (using a Collins Eagle spirometer from 1980 to 1988) and last (using a Puritan Bennet spirometer which replaced the Collins Eagle spirometer) FEV_1/FVC ratios between smokers (n=21), ex-smokers (n=12) and non-smokers (n=7) but not between smokers and controls. The measurement of FEV_1/FVC ratios is sensitive to changes in lower airway function. The authors concluded that there would be differences in the FEV_1/FVC ratios between the exposed workers and their controls if they were present.

OEHHA notes several uncertainties with Billmaier et al. (1992) that preclude it from use as the basis of a chronic REL. Differences in the FEV_1/FVC ratios in smokers, ex-smokers and non-smokers may be due to the fact that tobacco smoke is inhaled deeply and reaches the lower airways. Caprolactam vapor may not be inhaled as deeply because it is a water soluble gas and will primarily deposit in the upper airways. Other toxicological studies summarized in this document indicate the endpoint for caprolactam exposure is the upper respiratory tract. Thus, FEV_1/FVC ratios may not be an effective measure of caprolactam effects. U.S. EPA (1994b) also notes that the spirometry performed was not in accordance with current guidelines and quality assurance procedures.

Another weakness in Billmaier et al. is that individual worker exposure histories could not be clearly determined due to high variability in caprolactam levels and changes in job responsibilities throughout the workday. As noted earlier, the irritation data from "nurses' notes" are probably unreliable and were apparently not collected systematically for all workers. Finally, the authors did not conduct a survey of the workers regarding sensory irritation symptoms or examine the upper respiratory tract for signs of inflammation.

Occupational studies of caprolactam workers have been conducted in China and were translated from Chinese into English by OEHHA. An occupational study of the health effects of caprolactam was conducted in 154 exposed workers at a Chinese caprolactam production plant (Li, 1996). The mean age of the exposed workers (111 men, 43 women) was 36.0 years (18 to 56 years of age), and the average working time at the facility was 15.7 years (1 to 22 years). The exposure group was divided into Extraction Section workers and Steaming and Packaging Section workers for assessment of health effects. Another 91 workers in the same plant but with no history of exposure (58 men, 33 women) was the control group. Their mean age was 38.1 years (17-55 years of age), and an average working time of 14.8 years (1 to 20 years).

Area air monitoring data for caprolactam over a ten year period from 1983 to 1992 were presented, with 19 to 28 samples collected per year for a total of 249 samples (Li, 1996). The concentration range over this time period was 0.5 to 110.0 mg/m^3 with a geometric mean of 9.2 mg/m^3. In the most recent year of sampling, 1992, the range was 0.6 to 6.5 mg/m^3 with a geometric mean of 2.0 mg/m^3. Statistically significant health effects and area air monitoring concentrations are presented in Table 6 for each work section of the facility. No air monitoring data were collected for the control group.

Table 6. Workplace caprolactam air concentrations and worker signs and symptoms of exposure.

Section	Geometric Mean and Concentration Range (mg/m^3)	Significant Health Effects Compared to Control Group
Extraction Section workers	11.8 (2.1 - 110.0) (n=92 air samples)	0.01<*p*<0.05: dizziness, insomnia, nosebleed, dermal lesions, reduced leukocytes P<0.01: nasal symptoms
Steaming Section workers	8.5 (0.5 – 98.6) (n=80 air samples)	0.01<*p*<0.05: nasal symptoms, dermal lesions, reduced leukocytes
Packaging Section workers	6.7 (0.5 – 38.6) (n=77 air samples)	

Health effects related to caprolactam exposure included dermal symptoms such as dry, smooth, cracked, scaling and peeling skin. Nasal symptoms included dryness, rhinitis and sinusitis. A reduction in leukocytes was observed, defined as <4.0x10^9/L. Li (1996) noted that workers that inhaled high concentrations of caprolactam experienced a sense of "tight chest". The author surmised that this symptom was possibly due to laryngeal

mucosal or tracheal/bronchial irritation resulting in contraction. Leukocyte classification, liver function, ECG, hemoglobin and urinalysis were considered normal in the exposed workers. The authors speculated that exposure to other chemicals in the factory did not have an impact on the health of the workers.

The occupational exposure study by Li (1996) provided a large cohort of exposed workers of sufficient exposure durations. However, the lack of personal air monitoring data make it problematic for OEHHA to establish a point of departure based on the geometric means presented. Historical air sampling for the previous 10 years is included in the paper, with the earlier years of sampling resulting in the highest exposures. Individual exposure histories including the earlier years of higher exposure would have been useful. Although the author indicated that co-exposure to other chemicals was not a concern, the caprolactam extraction process is known to include solvents such as benzene, toluene and chlorinated hydrocarbons (Ritz et al., 2002). Benzene is a known hematotoxic agent. The briefness of the report and the lack of a caprolactam air concentration for the control group are other deficiencies that prevent the study from use as the basis of a REL.

The health effects of caprolactam were investigated in workers at a different Chinese caprolactam production plant by Xu et al. (1997) (translated from Chinese by OEHHA). The mean age of the exposed workers (77 men, 48 women) was 29.3 years (20 to 57 years of age), and the average working time at the plant was 9.4 years (1 to 36 years). From the same plant, 120 workers (56 men, 64 women) with no history of poisoning or exposure to caprolactam dust were selected as the control group. Their mean age was 33.1 years (20 to 49 years of age), and an average working time of 12.6 years (1 to 28 years). The smoking rate for control males (55.36%) was slightly higher than the smoking rate for exposed males (43.06%). None of the women in the study smoked.

In the Xu et al., (1997) study, two air samples each were collected at four work stations with potential exposure to caprolactam. A dust sampler was used to collect caprolactam and measurement was by weighing the filter paper. The average air concentration of caprolactam at the work stations was 3.79 mg/m^3 (range: 0 to 7.93 mg/m^3). No air samples were collected in the control areas. Statistically significant (p<0.05) increases in insomnia, nausea and loss of appetite was reported by the exposed workers. Other questions (headache, dreams, stomach ache and back pain) were similar to controls.

Biochemical indicators of liver and kidney function and a peripheral blood micronucleus test were similar to control values (Xu et al., 1997). A peripheral blood lymphocyte chromosomal aberration test showed no difference from control values. However, exposed smokers showed a statistically significantly higher chromosomal aberration rate (2.50% vs. 1.36%, P<0.05) than smoking control group workers. No difference was seen between non-smoking exposed and control workers. The authors suggested a synergistic action for higher chromosomal aberration rate may exist with smoking and caprolactam exposure. In females, a higher rate of dysmenorrhea (i.e., painful menstruation) was observed in exposed vs. controls (37.5% vs. 17.5%, p<0.01). No

difference was seen between exposed and control groups regarding other menstrual disorders or pregnancy and delivery complications.

The study by Xu et al. (1997) did not ascertain sensory and dermal irritation, one of the most common complaints with industrial exposure to caprolactam in other studies. Air sampling collected particles (i.e., caprolactam dust), but not the vapor form of caprolactam that may have been present in the air. The dust sampler would reflect total airborne particulate matter, not just caprolactam. It was unclear from the report if the workers were exposed to other forms of particulate matter. The authors suggest some level of exposure to other chemicals used during the extraction process occurred, but was not measured. Historical air sampling data were not presented.

Another health study was conducted in a Chinese combined caprolactam production and Nylon-6 polymerization facility (Lan et al., 1998). In this report, the caprolactam concentration was purported to be below 5.6 mg/m^3 in each caprolactam work area, but how the air samples were collected and analyzed was not described nor was the mean and range of caprolactam concentrations presented. The authors reported statistically significant increases (p<0.01) in dizziness, headache, fatigue, insomnia, memory loss, loss of appetite, skin itching, and bleeding gums in the exposure group of 104 workers compared to a control group of 77 workers from a pharmaceutical factory. Dry nose was also present in the exposed group (0.01<p<0.05).

The workers in the Lan et al. (1998) study had an average work history of 4 years at the factory, and had an average age of 24.85 years. The control group had an average work history of 6 years and an average age of 30.20 years. Drinking and smoking histories were similar between the two groups. Other survey results, including liver function, blood tests, ECG, and chest x-ray, were normal in the exposed group. The authors indicated poor industrial hygiene among the workers likely resulted in both inhalation and dermal exposure to caprolactam.

Limited case reports of allergic contact dermatitis resulting from repeated exposure to caprolactam followed by an acute re-challenge response have been published. These have been summarized previously.

There are also data from a chronic oral human exposure protocol. In that study, investigating caprolactam as a weight-reducing agent, groups of obese patients received either placebo (n = 26), 3 g (n = 62) or 6 g (n = 28) of caprolactam daily as wafers or as tablets for 18 months (Riedl et al., 1963). The study participants were also instructed to eat a 1000-calorie weight-loss diet. The subjects receiving the placebo manifested no reduction in weight, while subjects treated with 3 and 6 gm caprolactam per day showed weight reductions averaging about 0.025 and 0.05 kg/day, respectively.

Those administered caprolactam showed minimal adverse effects other than weight loss. Of note, however, thirst was reported by one patient and a rash was observed in one patient. Factoring in body weights at the beginning of the study, average daily caprolactam intake of patients administered 3 g caprolactam daily was approximately 26 and 28 mg/kg body weight for males and females, respectively. The average daily

intake of patients administered 6 g caprolactam was approximately 52 and 56 mg/kg body weight for males and females, respectively.

Riedl *et al*. (1963) also investigated the effects of caprolactam on intermediary metabolism when obese patients were administered 1 g glucose per kg body weight. Caprolactam treatment was not clearly specified, but appeared to also consist of 3 or 6 g doses per day for at least two months prior to glucose loading. Blood lactic acid levels were reduced in those patients receiving caprolactam. Blood sugar and levels of citric acid and non-esterified fatty acids in blood were unaffected by caprolactam treatment.

A summary of the human exposure findings discussed in this document is presented in Table 7 below.

Table 7. Effects of Caprolactam in Humans

Caprolactam Exposure	Response	Reference
Exposure chamber studies and occupational surveys (Detailed summaries in Section 5.1 and 6.1		
20 participants, controlled chamber exposures to 0, 0.15, 0.5, 5 mg/m^3 for 6 hrs	Positive trend for eye blink and irritation with increasing concentration; increased eye blink and irritation at 5 mg/m^3. Positive trend for odor annoyance with increasing concentration; increased odor annoyance at 0.15 mg/m^3	Ziegler et al. 2008
5 non-acclimated workers, 46, 65, 116, 482 mg/m^3 from uncontrolled source for several minutes	Transient nasal and throat irritation in most or all participants at all concentrations. Eye irritation in 1 or 5 participants at 482 mg/m^3	Ferguson & Wheeler, 1973
61 mg/m^3 in spinning room, 16-17 mg/m^3 in laboratory.	Irritability, nervousness, heartburn, bloating, headache, nose bleeds, upper airway inflammation, dry and chapped lips and noses, unpleasant taste in the mouth.	Hohensee, 1951
8 workers, 4.8 yr mean exposure to mean of 68 mg/m^3 at time of study with personal samplers	Complaints of eye, nose and throat irritation from some workers, 7 of 8 workers had dermatitis	Kelman, 1986
49 workers, 63 controls 18.7 yr work history, 4.5 mg/m^3 and 9.9 mg/m^3 in 2 areas by occasional monitoring over 10 years	Reliance on nurse's notes, no formal interviews of workers. 3 reports of dermatitis, 1 report of occasional eye irritation with nausea from inhalation of caprolactam flakes.	Billmaier et al. 1986

Caprolactam Exposure	Response	Reference
154 workers, 91 controls 15.7 yr work history, area sampling over 10 yrs in 3 areas: geometric mean 11.8, 8.5, and 6.7 mg/m^3	Nasal dryness, rhinitis, sinusitis, nosebleed, dermatitis, dizziness, insomnia, reduced leukocytes.	Li, 1996
125 workers, 120 controls, 12.6 yr work history, mean of 3.79 mg/m^3 at time of study	Insomnia, nausea, loss of appetite, dysmenorrhea in female workers, increased peripheral blood lymphocyte chromosomal aberrations in smoking workers vs. control smoking workers.	Xu et al. 1997
Human case reports of occupational exposure (Detailed summaries in Section 5.1)		
1 worker, 3-days to unknown exposure, but caprolactam coated his clothing and skin	Dermatitis of hands and feet, nausea and vomiting, leukocytosis, tonic-clonic seizures.	Tuma et al. 1981
3 workers, unknown exposure, but caprolactam dust covered clothing	Dizziness, nausea and vomiting, tonic-clonic seizures, opisthotonus, brief coma. Caprolactam in urine: 2.9-3.7 g/L	Chen, 2002
2 workers, 2-4 day exposure to unknown concentration	Dermatitis of hands and feet, nausea and vomiting, leukocytosis, hyperglycemia, tonic-clonic seizures.	Woo et al. 1998
Sensitization studies and allergic contact dermatitis reports (Detailed summaries in Section 5.1)		
Patch test of 6 normal participants with 5% caprolactam solution, repeated daily for 4 days	No skin irritant effects produced	Goldblatt et al. 1954
1% aqueous caprolactam solution applied to 3 participants	No skin irritant effects produced	Haskell Laboratory, 1950
Patch test of 204 participants with 3-5% caprolactam solution	No skin irritant effects produced	Summarized by U.S. EPA, 2009
Worker with 29 yr experience at Nylon-6 factory	Presented with dermatitis for last 18 months; patch test with 5% aqueous caprolactam solution positive for allergic contact dermatitis.	Aguirre et al. 1995
Suture thread made of Nylon-6 used in patient that had undergone 40 operations for removing skin tumors	Patch testing with caprolactam solution positive for allergic contact dermatitis.	Hausen, 2003

6.2 Chronic Toxicity to Infants and Children

No toxicity studies were located regarding prolonged animal inhalation exposure to caprolactam beginning at a young age.

In an animal three-generation developmental study, reductions in body weight and food consumption were not found in first-generation (P_1) rats exposed to caprolactam in feed, but were observed in the second- (P_2) and third-generation (P_3) rats treated with caprolactam (Serota et al., 1988). The P_1 rats were young adults (approximately 6 weeks old) upon initiation of treatment. Since the P_2 and P_3 animals were exposed both *in utero* and through the early growth phase, the decreased body weights noted in the P_2 and P_3 animals were most likely due to the time in the life span at which treatment began.

6.3 Chronic Toxicity to Experimental Animals

Only a few peer-reviewed, multi-day inhalation studies were found in the literature, and no chronic inhalation studies have been performed. Only one comprehensive subchronic inhalation study by Reinhold et al. (1998) has been conducted and is assessed below. Multi-day inhalation and long-term oral studies are also reviewed, many of which were unpublished industry studies, in order to provide a more complete picture of toxic effects resulting from long-term exposure to caprolactam.

Reinhold et al. (1998) subchronic inhalation study

In a 13-week study, Sprague-Dawley CD rats (10/dose/sex) were exposed to caprolactam aerosol (mass median aerodynamic diameter = 3 µm; geometric standard deviation = 1.7) at a concentration of 0, 24, 70, and 243 mg/m^3 for 6 hours/day, 5 days/week (Reinhold et al., 1998). A second group of rats (10/dose/sex) was similarly exposed but euthanized following a 4-week clean air recovery period. Beginning the second week of exposure, treatment-related increases in respiratory (labored breathing) and secretory (nasal discharge) signs were noted in all groups during the caprolactam exposures (Table 7). The quantitative data presented in Table 7 were obtained from the original Huntingdon Life Sciences report by Hoffman (1997) from which the peer-reviewed Reinhold et al. (1998) study was derived. The number of animals exhibiting labored breathing decreased with time in the low- and mid-dose animals, and was not apparent in these two groups after the 36th exposure (approximately week 8 of exposure). Anywhere from 2 to 10 percent of the high exposure animals exhibited labored breathing up to the end of exposure at 13 weeks.

Detailed weekly physical exams noted an exposure-related trend toward an increased incidence of red staining (facial area), clear nasal discharge, and moist rales (Table 8). The incidence of moist rales was highest between the second and eighth week of exposure, where up to 9 out of 40 rats in the 243 mg/m^3 exposure group exhibited this symptom. None of the rats in the 24 mg/m^3 exposure group displayed moist rales during the weekly physical exams.

Table 8. Summary of Significant Findings from In-Life Physical Examinations and Daily Observations, Males and Females Combined[a]

	Exposure Group (mg/m^3)			
	0	**24**	**70**	**243**
In-life physical exam findings at week 13 # exhibiting condition out of 40 animals				
General animal condition within normal limits	21	14	8	0
Red facial stains	1	10	17	24
Clear nasal discharge	7	11	20	32
Moist rales	0	0	1	3
In-chamber observations, 6th to 26th exposure Percentage of animals exhibiting symptoms[b]				
Labored breathing	0	8.1	12.9	17

[a] The data in this Table was obtained from the Huntingdon Life Sciences industry report by Hoffman (1997)
[b] Animals exhibiting labored breathing presented as a mean percentage because the number of animals observed daily varied anywhere from 20 to 40 animals.

A neurotoxicity evaluation was conducted just prior to sacrifice based on a functional observational battery including tests for neuromuscular function and coordination, central nervous system activity and excitability, sensorimotor responses, and autonomic function. No evidence of neurotoxicity was observed based on these observational criteria.

At the 13-week terminal sacrifice, no evidence of ophthalmoscopic lesions, clinical pathology, organ weight changes, or macroscopic pathology was observed. Microscopic evaluation by Reinhold et al. observed treatment-related changes only in the nasoturbinal tissues and the larynx and are presented in Table 9. No apparent treatment-related microscopic changes were observed in other regions of the respiratory system including the trachea, main stem bronchi and lungs. Table 9 also shows the type of the nasal and laryngeal tissue lesions, and the pathologist grading of the severity of those lesions. The graded responses in males and females were similar, so the data were combined.

In the nasal region, respiratory epithelium showed a treatment-related increase in goblet cell hypertrophy/hyperplasia, and olfactory epithelium showed a treatment-related increase in incidence of intracytoplasmic eosinophilic material. In almost all of the control animals minimal changes were observed in the respiratory mucosa (19 of 20 rats), and minimal or slight changes were observed in the olfactory mucosa (17 of 20 rats). Thus, the increased severity of the nasal responses with increasing caprolactam concentration was superimposed on the low-level changes that were present in nearly all rat groups. In the larynx, no lesions were apparent in the control animals (Table 9). With caprolactam exposure, laryngeal tissues manifested a dose-related increased

incidence and severity of squamous or squamoid metaplasia or hyperplasia of the pseudostratified columnar epithelium covering the ventral seromucous gland. In five rats exposed to the highest caprolactam concentration of 243 mg/m^3, minimal laryngeal keratinization of the metaplastic epithelium was observed.

Table 9. Summary of Findings in Nasoturbinal and Laryngeal Tissues at the 13-Week Terminal Sacrifice, Males and Females Combined[a]

	Exposure Group (mg/m^3)			
	0	**24**	**70**	**243**
Number Examined	20	20	20	20
Nasal respiratory mucosa[b]				
No change[c]	1	0	0	0
Minimal	5	7	2	0
Slight	14	9	9	8
Moderate	0	4	9	12
Nasal olfactory mucosa[d]				
No change[c]	3	5	2	0
Minimal	17	13	10	3
Slight	0	2	6	3
Moderate	0	0	2	10
Moderately severe	0	0	0	4
Laryngeal tissue[e]				
No change[c]	20	15	8	0
Minimal	0	5	12	12
Slight	0	0	0	8

[a] Nasal and larynx lesions were categorically graded in Reinhold et al. on a scale from lowest to highest severity, as minimal, slight, moderate, or moderately severe. Further quantitative description and statistical analysis of the pathology findings was not presented.
[b] Goblet cell hypertrophy/hyperplasia
[c] Grade labeled "No Change" was not specifically presented in the original pathology tables of Reinhold et al., but was inferred by OEHHA as the number examined minus total number of animals exhibiting minimal or greater cellular changes
[d] Intracytoplasmic eosinophilic material in epithelial cells
[e] Squamous/squamoid, metaplasia/hyperplasia of the pseudostratified columnar epithelium covering the ventral seromucous gland.

Benchmark concentration (BMC) modeling was performed on the treatment-related upper respiratory tract endpoints shown in Table 8 using U.S EPA benchmark dose modeling software (2009c). Only the moderate grade or above lesions in each exposure group were used for BMC modeling of nasal respiratory mucosal lesions because these changes were of a higher severity grade than any of the control lesions (the latter were likely to be age-related). In other words, the moderate and above severity categories of nasal changes were designated as an exposure-related effect for the purposes of the BMC analysis. The resulting dichotomous dose-response input

data for BMC modeling for the 0, 24, 70 and 243 mg/m^3 exposure groups were: 0 out of 20 rats, 4 out of 20 rats, 9 out of 20 rats, and 12 out of 20 rats, respectively.

A similar approach was taken in modeling nasal olfactory mucosal changes, where minimal grade age-related lesions were found in the control animals (Table 9). Thus changes greater than minimal (slight, moderate, and moderately severe) were treated as the exposure outcome in BMC modeling. The resulting dichotomous dose-response data for the olfactory mucosa lesions in the 0, 24, 70 and 243 mg/m^3 exposure groups were: 0 out of 20 rats, 2 out of 20 rats, 8 out of 20 rats, and 17 out of 20 rats, respectively.

No age-related lesions were observed in laryngeal tissue of control animals (Table 9). For BMC modeling of laryngeal tissue change, therefore, minimal and slight grades of the non-keratinized lesions were combined for the analysis. The resulting dichotomous dose-response data for the laryngeal tissue lesions in the 0, 24, 70 and 243 mg/m^3 exposure groups were: 0 out of 20 rats, 5 out of 20 rats, 12 out of 20 rats, and 20 out of 20 rats, respectively.

$BMCL_{05}$s (the 95% lower confidence limit of the dose producing a 5% response rate) for the nasal respiratory and olfactory changes and the non-keratinized laryngeal tissue changes found at terminal sacrifice are shown in Table 10. The BMC modeling results used to derive the $BMCL_{05}$s is presented in Appendix A. The $BMCL_{05}$ is used as the point of departure for REL derivation. Using a $BMCL_{05}$ as a point of departure is particularly advantageous when exposure data does not clearly manifest a NOAEL, as is the case with the data from Reinhold et al. (1998) (Table 9). For each endpoint, the $BMCL_{05}$ was derived from the models that provided the best visual and statistical fit to the data, particularly in the low dose region of the line where the $BMCL_{05}$ resides. Following U.S. EPA guidelines, we chose the model with the lowest AIC (Akaike information criterion) in instances where various model fits to the data were similar.

Table 10. $BMCL_{05}$s for the toxic endpoints in the 13-week inhalation exposure study in rats (Reinhold et al., 1998).

Endpoint	$BMCL_{05}$ (model)	BMC_{05}[a] (mg/m^3)	*P* Value	AIC
Nasal respiratory mucosa lesions	3.6 mg/m^3 (log-logistic)	5.9	0.78	77.53
Nasal olfactory mucosa lesions	12 mg/m^3 (log-probit)	17	0.99	60.85
Laryngeal tissue lesions	2.8 mg/m^3 (multistage)	5.3	0.94	53.59

[a] The BMC_{05} represents the modeled concentration resulting in a 5% response rate for the endpoint

Table 11 displays the frequency of upper airway lesions present in the 4-week recovery group. The nasal mucosal changes related to injury due to caprolactam exposure (that is, at a level above that seen in controls) were still apparent in the two highest exposure groups, indicating incomplete recovery following 4-weeks in clean air. Moderate grade

goblet cell hypertrophy/hyperlasia in the nasal respiratory mucosa was still present in rats exposed to 70 and 243 mg/m^3. Moderate and moderately severe intracytoplasmic eosinophilic material was still present in the 70 and 243 mg/m^3 groups. In the laryngeal tissue, recovery of the high exposure group also was not complete at 4-weeks post-exposure. Only one rat in the mid-dose group showed squamous/squamoid metaplasia/hyperplasia of the pseudostratified columnar epithelium. Keratinization of the metaplastic epithelium was absent in the four-week recovery group.

Table 11. Incidences of microscopic findings of nasoturbinal and larynx lesions in the 4-week recovery group[a]

	Exposure Group (mg/m^3)			
	0	**24**	**70**	**243**
Number Examined	20	20	20	20
Nasal respiratory mucosa[b]				
No change[c]	1	2	2	1
Minimal	10	9	4	4
Slight	9	9	8	10
Moderate	0	0	6	5
Nasal olfactory mucosa[d]				
No change[c]	2	1	3	0
Minimal	15	17	10	1
Slight	2	2	3	4
Moderate	0	0	4	13
Moderately severe	0	0	0	2
Laryngeal tissue[e]				
No change[c]	20	20	19	17
Minimal	0	0	1	3

[a] Nasal and larynx lesions were categorically graded in Reinhold et al. on a scale from lowest to highest severity, as minimal, slight, moderate, or moderately severe. Statistical analysis of the pathology findings was not presented.
[b] Goblet cell hypertrophy/hyperplasia
[c] Grade labeled "No Change" was not presented in the original pathology tables of Reinhold et al., but was inferred by OEHHA as the number examined minus total number of animals exhibiting minimal or greater micropathological changes
[d] Intracytoplasmic eosinophilic material in epithelial cells
[e] Squamous/squamoid, metaplasia/hyperplasia of the pseudostratified columnar epithelium covering the ventral seromucous gland.

Reinhold et al. (1998) report that the background incidence of the nasoturbinal findings were considered by the pathologist to be within normal limits for test animals of this age and strain. The increase in incidence and severity of the nasoturbinal and squamous metaplastic/hyperplastic laryngeal findings in caprolactam-treated rats was stated to be a "localized adaptive response to a minimal irritant effect" and attributed to particulate

exposure rather than an adverse toxicological response to the test material in the nasal passages. The only toxicologically relevant finding considered by the authors due to caprolactam exposure was the keratinization in the larynx; they viewed 70 and 243 mg/m^3 as a NOAEL and a LOAEL, respectively.

Because the authors did not consider the nasal and laryngeal changes relevant for toxicological consideration, further review of these particular types of lesions by other pathologists is summarized below.

Increased goblet cell (i.e., mucous cell) hypertrophy/hyperplasia in respiratory mucosa has been frequently observed in the anterior nasal cavity of rodents in response to repeated inhalation of irritants (Renne et al., 2007; Renne et al., 2009). It develops from hypertrophic epithelium with typical goblet cells distended with secretory droplets. This region of the nose is one of the most sensitive sites in rodents due to high volume of air flow through the ventral aspect of the nasal cavity over the nasal and maxillary turbinates.

Eosinophilic deposits, or globules, like those found in the olfactory epithelial cells of the Reinhold et al. study occasionally have been described by other researchers in otherwise normal epithelium of untreated rats, but such changes are more frequently observed in aged animals (Morgan, 1991; Harkema et al., 2006; Renne et al., 2009). These deposits occur often in the epithelial sustentacular cells and are considered dilated endoplasmic reticulum containing proteinaceous material and not a dysplastic (i.e., abnormal) alteration. They have often been referred to as hyaline droplets or hyaline degeneration (Harkema et al., 2006). The lesion increases in severity and extent with age and exposure to specific irritants, such as dimethylamine and cigarette smoke (Morgan, 1991). The mechanism by which such lesions appear in aging rats, and the nature of the response to irritants, is not understood. Intracellular eosinophilic deposits also have been observed in other studies in nasal respiratory mucosa and in other respiratory tract epithelium (Morgan, 1991; Renne et al., 2009), but either was not found in the Reinhold et al. animals, or was found in comparable incidence and severity in rats from both control and exposure groups.

The region of the larynx investigated by Reinhold et al.(1998), the pseudostratified columnar epithelium on the ventral floor of the larynx at the base of the epiglottis, is especially sensitive to inhaled materials (Renne and Gideon, 2006; Renne et al., 2009). Squamous metaplasia as noted by Renne et al. (2009) may occur in association with acute and/or chronic inflammation or in the process of regeneration. Laryngeal squamous metaplasia has been characterized as a classic example of indirect metaplasia (Osimitz et al., 2007). Inhalation of an irritant damages sensitive respiratory or transitional epithelium, so that cells that proliferate to replace the lost cells produce a replacement epithelium that is better adapted to the new environment.

In an expert workshop to evaluate larynx squamous metaplasia, a similar conclusion was made. This type of epithelial change is a result of transformation of the pre-existing epithelium to a squamous epithelium, with or without keritinization (Kaufmann et al., 2009). The lesion was classified as the morphologic correlate of an adaptive process

from a more sensitive to a more resistant type of epithelium, which is indicative of local irritation. Focal, minor metaplastic changes that may also occasionally occur in control animals were considered "non-adverse", while moderate to severe squamous metaplasia should be considered adverse as it may be associated with dysfunction. In humans, this dysfunction may result in hoarseness and an altered coughing reflex. In the rats exposed to caprolactam, exposure to the low and mid-level concentrations resulted in only a "minimal" grading for larynx metaplasia (Table 9).

For an assessment of adversity (equivalent term to "toxicity"), Kaufman et al. (2009) felt it was more relevant to observe dysfunction of an organ or tissue (e.g., by test designed to measure mucociliary clearance). For the rats in the Reinhold et al. (1998) study, adverse effects were apparent in terms of treatment-related increases in labored breathing, nasal discharge, red staining of the facial area, clear nasal discharge, and moist rales that began after approximately 1-2 weeks of exposure.

An earlier paper by Osimitz et al. (2007) suggested that laryngeal squamous metaplasia should not be used as an endpoint for quantitative risk assessment, as it is well-differentiated, reversible, and generally lacking signs of progression. This, in the opinion of the workshop panel cited earlier (Kaufmann et al., 2009), was not an approach supported by data. All available information, according to that expert panel, should be carefully considered by the pathologist, including other related health effects that are evaluated as "adverse".

Other multi-day inhalation exposure studies

Several published and unpublished multi-day inhalation studies have been conducted with caprolactam. However, these studies generally lacked complete methodology descriptions and only provided brief overviews of their findings. As much pertinent data are summarized below as could be found for each of these studies.

Goldblatt et al., (1954) exposed three guinea pigs to 118 - 261 mg/m^3 caprolactam "dust" for 7 hr/day for 7 days and reported no adverse effects other than occasional cough. No other toxicological exams were apparently performed, other than observing for signs of irritation. The majority of the caprolactam particles formed for the study were reported to be below 5 µm in size.

Hohensee et al. (1951) exposed up to 10 guinea pigs to 51 mg/m^3 caprolactam 5-8 hr/day for 26-30 days. No external pathologic changes or evidence of convulsions were noted during the exposures. Pathological and histological examination of a few of the animals revealed compound-related slight inflammation of the nasal mucosa and tracheal mucosa. However, no information was provided on the nature or extent of the inflammation or whether controls were free of this involvement.

In a multi-day unpublished industry study, two rats were exposed to a nominal concentration reported as 3000 ppm (equivalent to 13,900 mg/m^3 caprolactam particles for 2 hrs, followed five days later by a series of five nominal 1 to 2 hr exposures ranging from 2700 to 6800 ppm (equivalent to12,500 to 31,500 mg/m^3 on successive days

(Haskell Laboratory, 1950). The exposure concentrations were expressed in ppm in the report. OEHHA notes that particle exposures should be expressed in mg/m^3 as shown above in parentheses. Nominal exposure entails calculating the loss of material to the gassing chamber when heated and the rate of air flow. No direct measurement of airborne caprolactam concentration is performed. Nominal estimates of air concentration often over-estimate actual air concentrations. General discomfort and inflammation around the eyes and nose were observed during the exposures. Gross and microscopic pathological examination three days following the last exposure showed slight lung edema and congestion of the spleen, but no pathology in any other organs.

In another unpublished study, 4 dogs, 6 guinea pigs, 6 rats and 2 rabbits were exposed subchronically to caprolactam fumes generated by heating the chemical in air. This study was conducted in 1952-53 and only recently reported to the U.S. EPA (2009a). The composition of the fume was not evaluated and it was unclear from the report if the exposures were nominal or dynamic exposures. The authors and laboratory conducting the experiment are not identified and the document was labeled 'company sanitized'. All animals were exposed to 444 mg/m^3 6 hrs/day for 43 exposures. Half of the guinea pigs, rat and rabbits, and 3 of the dogs, were then exposed to 1020 mg/m^3 on exposures 44 through 67 or 73.

In dogs, the fumes seemed to aggravate open sores and especially infections and soreness of the eyes (U. S. EPA, 2009a). Two of the four dogs displayed occasional muscle tremors during exposure to the low concentration of caprolactam. One of these dogs displayed severe muscle tremors, weakness, coughing with a dense white froth around the mouth when exposed to the high concentration during exposures 46 through 67. In all cases, the dogs were normal the next morning after exposure. One dog had a significant lowering of systolic pressure and pulse pressure but otherwise no other significant changes in weight, blood sugar, cholesterol, BUN, thymol turbidity or hematology. Gross pathology showed an indication of either acute duodenitis or gastroenteritis in two dogs, but it was suggested this was an aggravation of an existing gastro-intestinal disorder. Microscopic examination revealed no changes that were attributable to caprolactam.

The study reported that one rabbit of the two rabbits exposed showed slight corneal damage and both rabbits showed mild irritation of the conjunctiva in both eyes (U. S. EPA, 2009a). No gross or microscopic pathology was observed in the rats or rabbits. In guinea pigs, one of the six had a lung reaction to a foreign body and a kidney showed evidence of regeneration of tubules. Another guinea pig had nephritis. A third guinea pig displayed consolidation of the apex of the right lung. No other gross or microscopic changes were detected in the remaining 3 guinea pigs.

Long-term oral exposure studies

Although no chronic inhalation experimental animal exposure studies have been conducted for caprolactam, the NTP (1982) performed a chronic oral exposure study in rodents examining both cancer and noncancer endpoints. A comprehensive 90-day

oral exposure study in dogs is also summarized to provide toxicological information for a non-rodent species.

A two-year caprolactam carcinogenesis bioassay feeding study was conducted by the NTP (1982). Caprolactam was incorporated in the diet of male and female rats at concentrations of 3,750 ppm or 7,500 ppm, and in the diet of male and female mice at concentrations of 7,500 and 15,000 ppm. Mean body weights of all dosed groups were decreased compared to their respective control groups throughout the two-year study. Feed consumption was inversely related to dose in rats, but caprolactam in the feed of mice had no effect on feed consumption. Growth curves for rats and mice are presented in graphical form by the NTP, but statistical analysis on mean body weight gain and feed consumption was not performed. The NTP concluded that the dose-related decrements in mean body weight gains indicate that it is highly likely that animals in the study were receiving the maximum tolerated doses of caprolactam.

Histopathologic examination did not find any compound-related effects in nasal tissues, larynx, esophagus, stomach, or any other tissues and organs. Other than the dose-related decrements in feed consumption and body weight gains, the NTP concluded there was no evidence of nonneoplastic lesions associated with oral administration of caprolactam as demonstrated by histopathologic examination of rats and mice in this study. Table 12 presents the estimated range of daily caprolactam intake in feed, assuming 100% absorption, for each dose group during the study.

Table 12. Estimated range of daily intakea of caprolactam in mg/kg body weight during a two-year feeding study (NTP, 1982).

Species	Males		Females	
	Low Dose	High Dose	Low Dose	High Dose
Rat	210 - 400	400 - 670	260 - 370	440 - 670
Mouse	790 - 1100	2200 - 2400	1200 - 1800	3100 - 3900

[a] Caprolactam intake range for each dose group of each species was based on a week in the second year of the study with the lowest mg/kg body weight intake, and on week 4 feed consumption, the period of growth with the highest mg/kg body weight intake.

An unpublished 90-day oral exposure study in beagle dogs conducted by Burdock et al. (1984) resulted in analogous findings to those observed in rats and mice of the NTP study. Three groups of eight dogs (4 each per sex) were fed at dose levels of 0.1%, 0.5% and 1.0% caprolactam in the diet. These percentages of caprolactam in the diet were equivalent to an average of 32, 164 and 292 mg/kg/day caprolactam consumed by males, respectively, and 33, 158, 389 mg/kg/day caprolactam consumed by females, respectively. A control group of equal size was fed a basal diet only. At the conclusion of 90 days on study, the only significant finding was a slightly lower mean body weight of the 1.0% females compared to controls. A similar change was not observed for the males, and total food consumption was comparable between groups of both sexes. No difference from controls was observed in absolute or relative organ weights for any group. Gross and microscopic examination of tissues and organs revealed no

remarkable findings. Ophthalmologic findings were negative. No dose-related changes were observed for hematologic and serum chemistry samples.

7 Developmental and Reproductive Toxicity

Gross (1984) summarized the foreign language literature, almost exclusively in Russian, examining the gynecological effects of caprolactam in female workers. Most of this work was published between the 1950s and 1970s. Specific caprolactam exposure concentrations were not given, although in one instance concentrations between 100 to 400 mg/m^3 were reported during the charging and pouring of the melting tanks, and between 1 to 10 mg/m^3 at other times. The abnormalities found in excess over those of the control groups consisted of dysmenorrhea (i.e., painful menstruation) and menorrhagia (i.e., excessive uterine bleeding), oligomenorrhea (i.e., markedly reduced menstrual flow), and prolongation of the exfoliative phase. The obstetrical complications that were found to be excessive compared to those of controls consisted of post-partum hemorrhage, toxemia of pregnancy, premature birth, and inadequate uterine contractions during labor.

Gross (1984) noted that high temperatures, high humidity and noise level were likely contributory factors to the abnormalities described in female workers. Co-exposure to other chemicals, including dinyl oxides, benzene, cyclohexane, cyclohexanol, cyclohexanone, acetone, and trichloroethylene, were also possible contributory factors.

In a more recent investigation, a retrospective reproductive and development study was conducted in 312 female workers in a Chinese Nylon-6 manufacturing facility (Liu et al., 1988). This study was translated from Chinese into English by OEHHA. The workers were compared to a group of 302 female workers in a textile factory with no contact with caprolactam or other chemicals. The noise level in the two facilities was similar. All workers had worked in the facility for more than one year, and caprolactam exposure was said to be below 10 mg/m^3, except for a few measurements slightly higher than this level. Specific exposure concentrations were not provided. In the caprolactam-exposed women, primary infertility ($0.005<p<0.05$), and pregnancy hypertension ($p<0.005$) was greater compared to the control group. Other measures of pregnancy function and fetal development were similar to controls. Abnormal menstrual function, including abnormal menstrual cycles, was higher in the caprolactam-exposed female workers ($p<0.005$), although no difference was seen between the two groups regarding symptoms of dysmenorrhea.

No studies were located investigating the developmental and reproductive toxicity of caprolactam by the inhalation route in experimental animals.

In oral exposure studies, (Gad et al., 1987) dosed pregnant rats by gavage with caprolactam at 100, 500, or 1,000 mg/kg/day on gestation days 6-15. Increased maternal mortality was observed at the highest dose. A dose-related decrease in mean maternal body weight was observed with a statistically significant reduction ($p\leq0.05$) in total body weight at the highest dose level (a 10 and 11% reduction on gestational days 15 and 20, respectively). A statistically significant reduction ($p\leq0.05$) in mean weight

change was observed during the treatment period at the two highest doses (5.2 and 2.3% mean weight gain at the mid- and high-dose, respectively, compared to a 13.4% weight gain for the control group). Food consumption was reduced in the two highest dose groups. The mean incidence of resorptions was increased at the highest dose.

No dose-related skeletal anomalies or major malformations were noted among the offspring of any exposure group. An apparent dose-related increase in the mean number of skeletal variants per litter was observed, including incomplete ossification of the skull or vertebral column and the presence of extra ribs. However, no statistically significant difference in skeletal variation values between treated groups and the control group were noted.

Gad et al. (1987) also dosed pregnant rabbits by gavage with caprolactam at 50, 150, or 250 mg/kg/day on gestational days 6-28. Sixteen percent mortality and statistically significantly decreased overall maternal body weight gain were observed at the highest dose. Corrected weight gain (i.e., weight gain minus weight of gravid uterus) was statistically significantly lower (p<0.05) from day 6 to 29 of gestation in the 150 mg/kg group. Absolute maternal body weights were unaffected in this mid-dose group. Mean fetal weights were statistically significantly reduced (p<0.05) by 12% in each of the two highest dose groups compared to controls. The incidence of major malformations was unaffected by caprolactam treatment. Minor skeletal anomalies included an increased incidence of unilateral or bilateral thirteenth ribs in the highest dose group.

Gad et al. (1987) concluded that caprolactam given by gavage to two species up to levels that caused severe maternal toxicity did not support a finding of the compound causing either embyotoxicity or teratogenicity. Fetotoxicity was evidenced in rabbits by lower fetal weights at the two highest doses, and an increased incidence of 13^{th} ribs at the highest dose level.

In a multi-generation study, rats were given a 1,000, 5,000, or 10,000 mg caprolactam/kg diet (ppm) over three generations (Serota et al., 1988). Each generation was treated over a 10-week period. Consistently lower mean body weights and food consumption were observed in both P_2 and P_3 parental generations at 5,000 and 10,000 ppm, but body weights were unaffected in P_1 animals at all dose levels. The mean body weight changes were statistically significant (p≤0.05) in all high dose groups at all time points with weight reductions in both males and females ranging from 10 to 21% compared to controls. For mid-dose animals, a statistically significant change in mean body weight occurred only in P_2 males, a 13% reduction compared to controls, during week four of exposure.

Dose-related reductions of fetal body weights were observed in all filial generations. For example, statistically significant differences (p≤0.05) noted in F_{1a} and F_{1b} high dose groups (17 to 23% reductions compared to controls) and occasionally in mid-dose groups (11 to 14% reductions in F_{1b} offspring only compared to controls). Based on mean body weight and mean food consumption values at week 10 in P_1 females, caprolactam in the diet at 1000, 5000 and 10,000 ppm was equivalent to a daily dose of 700, 3500 and 5600 mg/kg caprolactam, respectively.

No treatment-related effect on gross appearance, gross pathology, survival rate or number of pups was observed. A slight increase in the severity of spontaneous nephropathy was observed on histopathologic examination of males in the high-dose group of the first parental generation.

Serota et al. (1988) concluded that caprolactam in the diet at the two highest exposures resulted in decreases in body weight in both pups and parental animals in utero through weaning. Similar effects on food consumption were also noted. Body weights were unaffected in P_1 animals at all dose levels, and reduced food consumption was observed only at week 10 in P_1 females. No effects were evident on reproductive performance or offspring survival, and only minimal kidney toxicity was observed in males at the highest dose level.

In other oral exposure studies, Salamone (1989) reported no sperm abnormality in male mice treated with 222, 333, 500, 750, or 1,125 mg/kg caprolactam by gavage daily for five days, although mortality was evident at the highest dose. Following the fifth gavage with 1,125 mg/kg caprolactam, the mice immediately became motionless. In four of the nine mice treated, this inactivity was followed 10 min later by racing around the cage and death within seconds. These deaths are probably related to the method of oral treatment because exposure of mice up to 2200 to 2400 mg/kg caprolactam in feed for two years by the NTP (1982) did not result in an increase in mortality. A similar study in male rats did not observe DNA damage to spermatocytes following an oral dose of 750 mg/kg caprolactam (Working, 1989).

The primary finding of the two developmental/reproductive toxicity oral exposure studies was that caprolactam may be fetotoxic due to reduced fetal body weight. Reductions in fetal weight in the gavage study occurred at the same dose levels that reductions in maternal food consumption and body weight occurred. Based on this gavage study, the concomitant reduction in both maternal body weight and fetal weight make it difficult for OEHHA to conclude that caprolactam is exclusively fetotoxic. However, body weights of P_1 rats in the multi-generation study were not reduced by caprolactam exposure yet resulted in reduced fetal weights in F_{1a} and F_{1b} offspring. This finding indicates a fetotoxic LOAEL of 5000 ppm caprolactam in feed, which is equivalent to a maternal daily dose of 3500 mg/kg. The calculated NOAEL is 700 mg/kg.

Assuming 100% pulmonary absorption, the NOAEL is equivalent to an air concentration of 2500 mg/m^3 (700 mg/kg x 70 kg body wt. / 20 m^3/day) in a route-to-route extrapolation. For comparison, brief human exposures to lower caprolactam concentrations in the range of 1900 to 5600 mg/m^3 (400 to 1200 ppm) have been characterized as extremely irritating, and subchronic exposures of rats to air concentrations as low as 24 mg/m^3 have resulted in labored breathing and nasal secretory discharge. Applying a 100-fold uncertainty factor (10-fold UF each for interspecies and intraspecies extrapolation) for extrapolation from an animal developmental study to human exposure would produce a proposed REL of 25 mg/m^3. The acute and chronic RELs of 50 µg/m^3 and 2.2 µg/m^3, respectively, are considerably lower than that derived from the oral multi-generation animal study.

8 These findings show that the oral dose at which fetotoxicity occurs is likely not relevant to air concentrations of caprolactam for REL derivation due to upper respiratory tract injury occurring at lower concentrations. The acute, 8-hour and chronic RELs developed in this document based on caprolactam air exposures would be protective for reproductive/developmental effects. Therefore, OEHHA is using pulmonary and sensory irritation endpoints for the caprolactam inhalation RELs.

8. REL Derivations

8.1 Derivation of the Acute Inhalation Reference Exposure Level (1-hour exposure)

As noted above, only two human studies exist that examined the acute sensory irritant effects in association with quantified concentrations of caprolactam. Because of limitations in the occupational study (Ferguson and Wheeler, 1973), an acute REL cannot be derived reliably from this study. The second acute exposure report was the chamber study by Ziegler et al. (2008). OEHHA applied a non-parametric test, Page's trend test, to the individual human data provided to us by Dr. Ziegler, as noted previously. We observed a statistically significant (p<0.05) dose-response relationship for eye blink frequency and subjective eye irritation at one hour of exposure. Both measures of sensory eye irritation were increased in subjects exposed to 5 mg/m^3 compared to the non-exposed group.

Walker et al. (2001) suggested that the increased rating of eye irritation and eye blink frequency with exposure to an irritant are manifestations of the same underlying event of ocular trigeminal nerve activation. Objective measures such as eye blink frequency are less susceptible to cognitive biases than subjective ratings of eye irritation. Eye blink frequency also had a more robust response at 5 mg/m^3 than eye irritation, indicating eye blink frequency is a more sensitive measure of caprolactam exposure. Thus, the acute REL is based on increased eye blink frequency, with eye irritation as supporting evidence for the REL.

Study	Ziegler et al., 2008
Study population	20 human adults: 10 male, 10 female
Exposure method	Whole body chamber
Exposure duration	1 hour
Critical effects	Increased eye blink frequency
LOAEL	5 mg/m^3
NOAEL	0.5 mg/m^3
Time adjusted exposure	0.5 mg/m^3 (irritant: no adjustment applied)
LOAEL uncertainty factor	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10
Cumulative uncertainty factor	10
Acute reference exposure level	0.05 mg/m^3 (0.011 ppm)

We applied a NOAEL/LOAEL approach to the statistically significant increase in eye blink frequency, rather than using benchmark modeling. BMC analysis using continuous model methodology could not fit the eye blink data to a model. Likely, the

low- and mid-exposure levels, with their slight increase in response over control values and large variances, were not different enough from the controls to provide a good curve fit with the available models. We did not apply a time adjustment to the NOAEL from Ziegler et al. since we used the exposure data collected at 1 hour of exposure.

Chemicals that have effects limited to the extrathoracic region (i.e., nose and larynx), including caprolactam, are not predicted to be much different kinetically in children compared to adults when dosimetric adjustments are made (OEHHA, 2008). Thus, no UF_{H-k} is applied for intraspecies toxicokinetic variation among individuals. Only normal individuals without allergic rhinitis or other respiratory symptoms were investigated by Ziegler et al. Thus, a UF_{H-d} of 10 is applied to the REL derivation to address the human variation in the intraspecies toxicodynamic response to respiratory irritants, including potential exacerbation of asthma in children and adults. The total UF = 10 applied to the NOAEL results in an acute REL = 0.05 mg/m^3.

Consistent findings of seizures in heavily exposed adult workers and in experimental animal studies merit concern for exposure in children, who may be more sensitive than adults to chemicals that have neurological effects. OEHHA believes an acute REL protective of eye irritant effects will be sufficient to protect children from the neurological effects, and that an additional UF beyond the cumulative intraspecies UF = 10 is not necessary. Worker exposure to unspecified high levels of caprolactam may produce tonic-clonic seizures, but the exposure levels necessary to cause this neurological effect are above estimated exposures in the range of 22 to 168 mg/m^3 that have resulted in dermal, eye and respiratory irritation in workers (Kelman, 1986; Ferguson and Wheeler, 1973; Hohensee, 1951). In rats, airborne exposure at g/m^3 levels resulted in tremors, while intraperitoneal injections of 900 mg/kg and above produced convulsions. The quantified exposure levels in these animal studies where these neurological effects were found were substantially higher than the NOAEL for eye irritation of 0.05 mg/m^3 derived from the work by Ziegler et al. (2008). The considerably lower NOAEL for eye irritation supports the application of a 10-fold intraspecies toxicodynamic UF as sufficient to protect children from any neurological effects resulting from acute caprolactam exposure.

Subjective eye irritation increased with increasing caprolactam exposure, although the irritation score did not rise sharply with exposure concentration (i.e., mean eye irritation scores were between 0 (not at all) and 1 (barely) for all caprolactam exposures). Typically, when the sensory irritant threshold is reached the graded response should rise steeply. Over a range of hardly more than one order of magnitude of concentration, sensory irritation may increase from “barely detectable” to “painful irritation” (Cain et al., 2006; Nielsen et al., 2007). Such a steep rise in the graded response was not apparent for caprolactam in this study, suggesting the sensory irritant threshold was not reached in all or most participants of the study, or that eye irritation is not as sensitive a measure of caprolactam exposure as eye blink frequency.

We also observed differences between the control group and the 5 mg/m^3 exposure group for total subjective symptom and complaint score (both with and without the odor subscore). We prefer to base a REL on an objective endpoint (i.e., eye blink frequency)

because the total symptom and complaint score are subjective measures and lacked independence for many of the questions. In other words, many of the questions discussed in Section 5.1 above were asking the same question in different ways.

8.2 Derivation of the 8-Hour Inhalation Reference Exposure Level

Study	Reinhold et al. 1998
Study population	Sprague-Dawley CD rats (10 animals/sex/group)
Exposure method	Discontinuous whole-body inhalation exposure to 0, 24, 70, and 243 mg/m^3 caprolactam aerosol
Critical effects	Upper airway lesions of nasal and laryngeal epithelium
LOAEL	24 mg/m^3
NOAEL	Not observed
$BMCL_{05}$	3 mg/m^3
Exposure continuity	6 hours per day, 5 days/week
Exposure duration	13 weeks
Average experimental exposure	1.607 mg/m^3 (3 mg/m^3 x 6/8 x 5/7)
Human equivalent concentration	0.402 mg/m^3 (for extrathoracic respiratory effects, RGDR = 0.25
LOAEL uncertainty factor	1 ($BMCL_{05}$ used as point of departure)
Subchronic uncertainty factor	2 (for 13 wk exposure in rodents) (see below)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	√10 (default: no toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	60
8-Hour reference exposure level	7 µg/m^3 (1.4 ppb)

The comprehensive subchronic exposure study in rats by Reinhold et al. (1998) is the basis of the 8-hour REL, resulting in a level of 7 µg/m^3 (rounded up to one significant figure from 6.70 µg/m^3), or 1.4 ppb (rounded to 2 significant figures to avoid large rounding errors because the first digit is a 1). The occupational studies of long-term exposure to caprolactam were considered by OEHHA to be inadequate for use as a point of departure for the 8-hour REL, which pertains to repeated 8 hr exposures, and the chronic REL (see Section 6.1 for reviews of the occupational studies).

In deriving 8-hr and chronic RELs, issues concerning the presence of unaccounted gas phase caprolactam in the Reinhold et al. study, and the phase of caprolactam that exists in the ambient air are considered. One of the uncertainties in the Reinhold et al. (1998) study is that the method used to measure the exposure levels in the chamber only captured the aerosol fraction, leaving any of the caprolactam that may have

partitioned into the vapor phase unmeasured. This could lead to an underestimation of the respirable caprolactam in the chamber. If the caprolactam concentration to which the rats were exposed was underestimated, it would mean that the point of departure for the chronic and 8-hour RELs could be too low. Therefore, OEHHA attempted to evaluate how much unmeasured vapor could have been present in the exposure chambers.

In the original Huntingdon Life Sciences report by Hoffman (1997) from which the peer-reviewed Reinhold et al. (1998) study was derived, it was stated that a very minor amount (<3 ppm) of unmeasured caprolactam vapor may have been present in the caprolactam aerosol atmospheres. The analysis of caprolactam by Reinhold et al. used gravimetric sampling to estimate the caprolactam concentrations in the test chambers. Air drawn from the chambers passed through glass-fiber filters mounted open-faced in a filter holder. The gravimetric concentration was calculated based on the weight of the filter papers before and after sample collection, and the known volume of air that passed through the filter papers. Any caprolactam vapor would have passed through the filter papers and not been measured.

There is supporting data that indicates the method used by Reinhold et al. to generate the caprolactam atmospheres produces predominantly caprolactam aerosol and dry particles, and that any additional amount of caprolactam vapor generation is small enough to be disregarded for REL derivation. Nau et al. (1984) investigated the effects of temperature and humidity on sample collection of airborne caprolactam aerosol and fume (a dry suspension resulting from condensation products). In their study, caprolactam was dissolved in water in a 1:1 to 1:0.2 solution (water to caprolactam) by weight and then aerosolized in a test chamber under different conditions of temperature and humidity. The chamber concentrations generated were between 2.7 and 40 mg/m^3. Reinhold et al. (1998) had used a similar method to generate caprolactam aerosols.

Caprolactam sample collection by Nau et al. (1984) consisted of the tandem utilization of a glass fiber filter followed by a XAD-2 resin tube to collect the particles. At the end of the sample train, two water impingers collected any caprolactam that escaped the filter and XAD-2 resin. Presumably, caprolactam gas and some very small caprolactam particles would pass through the glass fiber filter and would be captured in the XAD-2 resin tube or water impingers. Under exposure conditions similar to that used by Reinhold et al. (20-27°C, 21-74% relative humidity), only a mean total of 0.8% of the caprolactam was trapped by the XAD-2 resin tube and water impingers, with about 99% of the caprolactam trapped on the filter and filter support. These data show that estimating the 8-hour and chronic RELs based on the gravimetric concentrations estimated by Reinhold et al. are valid.

The other consideration to be addressed is the proportion of caprolactam found in the gas phase and the diameter range of caprolactam particles that would be found in the ambient air environment following release of caprolactam from an emission source. This information is important in determining the region of the respiratory tract that would be impacted by inhaled caprolactam. Little evidence could be found in the literature regarding the phase of caprolactam found in the environment, or the size of the particles

if they were in the solid phase. In an air quality study by Cheng et al. (2006) caprolactam aerosol was detected on $PM_{2.5}$ filters, but the level of caprolactam gas present in the air, or caprolactam particles greater than $PM_{2.5}$, was not measured.

In the Reinhold et al. study, the authors aerosolized an aqueous caprolactam solution for the exposures, which had an MMAD of 3.0 µm and a geometric standard deviation of 1.7 µm. The mammalian nose is an effective filter for a large fraction of particles above 1 µm in diameter (Stuart, 1984; Swift and Strong, 1996; Yeh et al., 1996). So it is not surprising that upper respiratory system was the target of caprolactam exposure.

At the level of the 8-hour and chronic RELs, it can be expected that a significant fraction of caprolactam would be in a gas phase because the RELs are considerably below the airborne saturation level of 13 mg/m^3. Very water-soluble gases, including caprolactam, also deposit in the upper respiratory tract (OEHHA, 2008). However, if very small caprolactam particles are released or formed in the ambient air, some may bypass the upper respiratory system when inhaled and deposit largely in the tracheobronchial regions, or even reach the alveoli. The available data in experimental animals indicate the upper respiratory system is the target of inhaled caprolactam, unless exposure to massive amounts of caprolactam occurs. No occupational studies have documented pulmonary dysfunction (i.e., lower respiratory tract) as a result of caprolactam exposure. Based on the limited data available, OEHHA concludes that both vapor and particle phase caprolactam predominantly deposits in the upper respiratory tract, and that using the most sensitive endpoint of upper respiratory tract lesions determined by Reinhold et al. (1998) should be considered protective of the lower respiratory tract as well.

The 8-hr REL derivation is based on a $BMCL_{05}$ = 3 mg/m^3 (rounded from 2.8 mg/m^3, see Table 9) for a pathology grading of minimal and slight increases in squamous/squamoid metaplasia/hyperplasia in the larynx of male and female rats exposed to caprolactam aerosol for 13 weeks (Reinhold et al., 1998). The $BMCL_{05}$ for exacerbation of changes to the respiratory and olfactory nasal mucosa resulted in essentially the same value (respiratory $BMCL_{05}$ = 4 mg/m^3) or was slightly greater (olfactory $BMCL_{05}$ = 12 mg/m^3).

Reinhold et al. (1998) regarded laryngeal keratinization of the metaplastic epithelium to be the primary adverse effect, resulting in a NOAEL of 70 mg/m^3. The other effects in the upper respiratory system were considered by the researchers to be normal adaptive responses to an irritant, which they did not consider a toxicological endpoint. However, OEHHA RELs include health protection against mild irritant/inflammatory effects. These types of mild inflammatory changes are primary endpoints of toxicity as indicated in the Revised Air Toxics Hot Spots Program Technical Support Document for the Derivation of Noncancer Reference Exposure Levels and RELs for Six Chemicals (OEHHA, 2008). The irritant-related microscopic changes in the upper respiratory tract, combined with the observations of respiratory irritation/inflammation (nasal discharge, moist rales, red staining around the face) and labored breathing in all caprolactam-treated groups presented in detail in the original Huntingdon Life Sciences report by Hoffman (1997), support the lack of an observed NOAEL in the principal study.

The $BMCL_{05}$ = 3 mg/m^3 was adjusted to an average experimental exposure of 1.6 mg/m^3 for eight-hour exposures, seven days/week. The concentration at the $BMCL_{05}$ is below the saturated vapor concentration of caprolactam of 13 mg/m^3 at 25ºC. Thus, a greater proportion of caprolactam may be in gaseous form rather than the aerosol form used in the study. Although no studies have been conducted comparing the potency of gaseous or aerosol forms of caprolactam, the evidence in this document indicates both forms are expected to have the same toxicological endpoints.

Given that the predominant form humans will be exposed to at the level of the RELs will likely be the gaseous form, a regional gas dose ratio (RGDR) approach will be used for the human equivalent concentration (HEC) adjustment. The RGDR of 0.25 was calculated using US EPA methodology (OEHHA, 2008) for extrapolation from rat and human exposure. The equation for gases with respiratory effects is:

$$RGDR = (MVa/MVh) / (SAa/SAh) \quad \text{Eq. 8-1}$$

Where:

MVa = animal minute volume
MVh = human minute volume
SAa = animal surface area for lung region of concern
SAh = human surface area for lung region of concern

Surface areas for the region of concern, the extrathoracic region, for rat (15 cm^2) and human (200 cm^2) were obtained from Table F.1.1 in OEHHA (2008). Minute volume for rats was calculated using Eq. 8-2 below, using intercept (b_0) and slope (b_1) values from Table F.1.2 in OEHHA (2008). Body weight (BW) for both male and female rats combined (0.323 kg) was averaged over the 13-week exposure duration from body weight tables in Reinhold et al. (1998) and the authors' unpublished industrial data (Hoffman, 1997).

$$\log_e(MV) = b_0 + b_1 \log_e(BW) \quad \text{Eq. 8-2}$$

Where

BW = 0.323 kg; b_0 = -0.578; b_1 = 0.821
$\log_e(MV) = -0.578 + 0.821 \log_e(0.323\ kg)$
MV = 0.222 L/min or 222 ml/min

For humans, an average adult male and female combined MV of 11,944 ml/min was estimated using breathing rate data from US EPA (2009b).

Thus:

RGDR = (222 ml/min/11,944 ml/min) / (15cm^2/200 cm^2)
RGDR = 0.0186 / 0.075
RGDR = 0.25

A subchronic UF = 2 was incorporated into the REL derivation for extrapolation from 13-week exposure in the rats to chronic exposure. Although 13 weeks of exposure is 12.5% of the 2-year life expectancy of rats, which would entail use of a subchronic UF = 1 for >12% of lifetime exposure, U.S. EPA (1994a) recommends using a subchronic adjustment factor for all 13-week studies regardless of species. OEHHA has typically used a subchronic UF = √10 for 13-week exposure studies in rats and mice. However, for rodent studies of this exposure duration a subchronic UF = 2 for upper respiratory irritants, when the resulting injury is considered mild, is appropriate.

The basis for using a subchronic UF = 2 was derived from the numerous rodent studies with formaldehyde (OEHHA, 2008). Comparison of 13-week exposure studies with studies of longer duration up to 2 years shows that the NOAELs and LOAELs for upper airway injury are often the same, with only a 2-fold difference between chronic and 13-week study NOAELs and LOAELs in some cases. The 2-fold lower NOAELs and LOAELs were often a result of the choice of the formaldehyde exposure concentration used in the studies.

The severity of the upper respiratory tract injury also supports a subchronic UF = 2. The pathology grading of the upper respiratory tract resulting from caprolactam exposure indicates only a mild increase in injury. The exacerbation by caprolactam exposure of normal nasal olfactory tissue degeneration was small, increasing from minimal to slight at the lowest dose of 24 mg/m^3. The laryngeal tissue damage caused by caprolactam was minimal, at best, at the low dose. Overall, only a few cases of moderately severe tissue injury were observed, occurring in the high concentration exposure group in olfactory tissue. In addition, all animals survived to the end of the study and the treatment-related labored breathing and nasal discharge generally decreased in incidence during the second half of the study.

We did not apply an interspecies toxicokinetic $UF_{A\text{-}k}$. Hybrid computational fluid dynamics and PBPK modeling for predicting nasal tissue dose metrics show that the predicted dose to the epithelium of the total nasal cavity following inhalation of an organic gas is similar, or slightly greater, in humans compared to rats (Frederick et al., 2001). Also, injury occurred to regions of the upper respiratory tract that are most sensitive to both chemical and mechanical irritants (primarily due to airflow characteristics) which indicates that caprolactam is primarily a direct-acting irritant, rather than a chemical requiring metabolic activation in nasal mucosa to cause tissue injury (Kilgour et al., 2000; Harkema et al., 2006; Renne et al., 2007; Kaufmann et al., 2009). In particular, Kaufmann et al. (2009) indicated the rat larynx may be more sensitive to inhaled irritant gases due to air flow characteristics than other species such as humans, monkeys and dogs. Therefore, the human equivalency concentration (HEC) adjustment for upper respiratory tract injury should also be sufficient for any residual interspecies toxicokinetics differences.

We applied a default interspecies $UF_{A\text{-}d}$ of √10 to compensate for the absence of data on pharmacodynamic differences between species. Specifically, only one comprehensive animal inhalation study in rats has been performed with caprolactam.

The toxicokinetic data for inspired upper respiratory irritants in humans suggest low interindividual variation and no dosimetry differences between adults and children (OEHHA, 2008). Thus, no UF_{H-k} is applied for intraspecies toxicokinetic variation among individuals.

While caprolactam is irritating to the upper respiratory tract, initiation or exacerbation of asthma by caprolactam has not been characterized. However, data summarized by OEHHA (2008) show asthmatics may be more sensitive to the effects of respiratory irritants than non-asthmatic individuals. Thus, an intraspecies toxicodynamic UF of 10 is applied to address the diversity in the human population, including children with asthma. Of equal concern are the consistent findings of seizures in heavily exposed adult workers and in experimental animal data. Children may be more sensitive than adults to chemicals that have neurological effects, and the finding of neurotoxicity in workers additionally supports the application of a 10-fold intraspecies toxicodynamic UF. Application of the cumulative UF = 60 to the human equivalent concentration of 0.402 mg/m^3 resulted in an 8-hour REL of 7 µg/m^3 (1.4 ppb) for caprolactam.

8.3 Derivation of the Chronic Inhalation Reference Exposure Level

Study	Reinhold et al. 1998
Study population	Sprague-Dawley CD rats (10 animals/sex/group)
Exposure method	Discontinuous whole-body inhalation exposure to 0, 24, 70, and 243 mg/m3 caprolactam aerosol
Critical effects	Upper airway lesions of nasal and laryngeal epithelium
LOAEL	24 mg/m^3
NOAEL	Not observed
$BMCL_{05}$	3 mg/m^3
Exposure continuity	6 hours per day, 5 days/week
Exposure duration	13 weeks
Average experimental exposure	0.536 mg/m^3 (3 mg/m^3 x 6/24 hr x 5/7 days)
Human equivalent concentration	0.134 mg/m^3 (for extrathoracic respiratory effects, RGDR = 0.25
LOAEL uncertainty factor	1 ($BMCL_{05}$ used as point of departure)
Subchronic uncertainty factor	2 (for13 wk exposure in rodents)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	√10 (default: no toxicodynamic data)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	60
Chronic reference exposure level	2.2 µg/m^3 (0.5 ppb)

The chronic REL is based on the same study as the 8-hr REL. The chronic REL derivation is the same as that used for the 8-hr REL, with the exception that the average experimental exposure is based on continuous, 24 hr/day exposure. The resulting human equivalent concentration is reduced to 0.134 mg/m^3. The application of uncertainty factors was the same for both 8-hr and chronic RELs, resulting in a chronic REL of 2.2 µg/m^3 (rounded to two significant figures from 2.23 µg/m^3 to avoid rounding errors because the first digit is a 2), or 0.5 ppb (rounded to one significant figure from 0.48 ppb).

8.4 Data Strengths and Limitations for Development of the REL

Significant strengths for the caprolactam RELs include: (1) the use of a well-conducted subchronic animal study with histopathological analysis, and (2) independent studies demonstrating comparable key irritant effects (nasal and throat irritation) in humans and experimental animals. Major areas of uncertainty are: (1) the lack of comprehensive human inhalation dose-response data for long-term exposures, (2) no inhalation developmental/reproduction toxicity data, although sufficient oral developmental/reproduction data exist (However, when converted to an air concentration, the level that causes fetotoxicity is greater than the level that results in severe pulmonary injury), (3) the absence of a NOAEL in the subchronic study, and (4) no chronic animal inhalation exposure studies.

9 References

ACGIH. (2001). Caprolactam. American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio. Online at: www.acgih.org.

ACGIH. (2003). Caprolactam. American Conference of Governmental Industrial Hygienists, Cincinnati, Ohio. Online at: www.acgih.org.

Aguirre A, Gonzalez Perez R, Zubizarreta J, Landa N, Sanz de Galdeano C and Diaz Perez JL (1995). Allergic contact dermatitis from epsilon-caprolactam. Contact Dermatitis 32(3): 174-5.

Ballistreri A, Garazzo D, Giuffrida M, Montaudo G, Filippi A, Guaita C, Manaresi P and Pilati F (1987). Fast atom bombardment mass spectrometry identification of oligomers contained in poly(epsilon-caprolactam) and poly(butylene isophthalate). Macromolecules 20: 1029-32.

BASF. (1985). Test report: Acute inhalation toxicity LC_{50}, 4 hours (rat): Evaluation of caprolactam (compound no. 84/217) as an aerosol liquid. Submitted by BASF Corporation, Parsippany, NJ, under TSCA Section 8(d) November 26, 1990

Billmaier DJ, Knowlden NF and Stidham DW (1992). Caprolactam: A study of current workers. Allied-Signal Inc., Morristown, NJ. EPA/OTS Doc #86-920001041. 1-16, A-1-A-9.

Bongard W (2000). Competitiveness of Nylon 6. Man-Made Fiber Year Book DSM Fibre Intermediates, Geleen, Netherlands: 14-15.

Bonifaci L, Frezzotti D, Cavalca G, Malauti E and Ravanetti GP (1991). Analysis of epsilon-caprolactam and its oligomers by high-performance liquid chromatography. J Chromatog 585: 333-36.

Bradley EL, Speck DR, Read WA and Castle L (2004). Method of test and survey of caprolactam migration into foods packaged in nylon-6. Food Addit Contam 21(12): 1179-85.

Burdock GA, Kulwich BA, Alsaker RD and Marshall PM (1984). Ninety Day Toxicity Study in Dogs with Caprolactam, Hazelton Laboratories America, Vienna, VA. In: Proceedings of a Symposium on an Industry Approach to Chemical Risk Assessment - Caprolactam and Related Compounds as a Case Study, May 15-17, 1984, Arlington VA. pp. 97-113. Hazelton Laboratories America, Vienna, VA.

Cain WS, Lee NS, Wise PM, Schmidt R, Ahn BH, Cometto-Muniz JE and Abraham MH (2006). Chemesthesis from volatile organic compounds: Psychophysical and neural responses. Physiol Behav 88(4-5): 317-24.

Cal/OSHA. (2011). Permissable Exposure Limits for Chemical Contaminants. California Occupational Safety and Health Administration, Online at: www.dir.ca.gov/title8/5155table_ac1.html.

Chen H (2002). A caprolactam poisoning report [in Chinese]. China Occup Med 29(2): 61.

Cheng Y, Li SM and Leithead A (2006). Chemical characteristics and origins of nitrogen-containing organic compounds in PM2.5 aerosols in the Lower Fraser Valley. Environ Sci Technol 40(19): 5846-52.

Cooper JE, Cooke DP, Dickson WA, Noell LT, Beal BM and Pirtle LS (1993). Petition to Delist Caprolactam Under Section 112(b) of the Clean Air Act. AlliedSignal Inc. Properties, Standards, and Use of Caprolactam. BASF Corporation, DSM Chemicals North America Inc. pp. 2-1 to 2-7.

CPSC. (1996). Sensory and Pulmonary Irritation Studies of Carpet System Materials and their Constituent Chemicals U.S. Consumer Product Safety Commission. Contract CPSC-C-94-1122. AQS Report No. 01890-06. Chapters 1 to 5 available at Internet address:http://www.cpsc.gov/LIBRARY/FOIA/FOIA98/os3519926D.pdf. Chapters 6 through Appendix B available at http://www.cpsc.gov/LIBRARY/FOIA/FOIA98/os/3512B0C.pdf.

Dalton P (2003). Upper airway irritation, odor perception and health risk due to airborne chemicals. Toxicology letters 140-141: 239-48.

Ferguson WS and Wheeler DD (1973). Caprolactam vapor exposures. Am Ind Hyg Assoc J 34(9): 384-9.

Frederick CB, Gentry PR, Bush ML, Lomax LG, Black KA, Finch L, Kimbell JS, Morgan KT, Subramaniam RP, Morris JB and Ultman JS (2001). A hybrid computational fluid dynamics and physiologically based pharmacokinetic model for comparison of predicted tissue concentrations of acrylic acid and other vapors in the rat and human nasal cavities following inhalation exposure. Inhal Toxicol 13(5): 359-76.

Gad SC, Robinson K, Serota DG and Colpean BR (1987). Developmental toxicity studies of caprolactam in the rat and rabbit. J Appl Toxicol 7(5): 317-26.

Gill K (2011). Written communication. Air Resources Board, California Environmental Protection Agency, Sacramento, CA.

Goldblatt MW, Farquharson ME, Bennett G and Askew BM (1954). Epsilon-Caprolactam. Br J Ind Med 11(1): 1-10.

Gross P (1984). Biologic activity of epsilon-caprolactam. Crit Rev Toxicol 13(3): 205-16.

Harkema JR, Carey SA and Wagner JG (2006). The nose revisited: a brief review of the comparative structure, function, and toxicologic pathology of the nasal epithelium. Toxicol Pathol 34(3): 252-69.

Haskell Laboratory (1950). Medical Research Project No. MR-173: Toxicity of Epsilon-Caprolactam. Report # HL-0022-50, Haskell Laboratory of Industrial Toxicology, Wilmington, Delaware.

Hausen BM (2003). Allergic contact dermatitis from colored surgical suture material: contact allergy to epsilon-caprolactam and acid blue 158. Am J Contact Dermat 14(3): 174-5.

Hegde RR, Dahiya A and Kamath MG (2004). Nylon Fibers. Online at: www.engr.utk.edu/mse/Textiles/Nylon%20fibers.htm.

Hodgson AT, Shendell DG, Fisk WJ and Apte MG (2004). Comparison of predicted and derived measures of volatile organic compounds inside four new relocatable classrooms. Indoor Air 14 Suppl 8: 135-44.

Hoffman GM. (1997). A 13-week inhalation toxicity study (with a 4-week recovery) of caprolactam (494-95A) in the rat via whole-body exposures. Huntingdon Life Sciences, East Millstone, NJ, Volume I to III, Study no.: 95-6095, IHF 3-95PA.

Hohensee F (1951). On the pharmacological and physiological effects of e-caprolactam. Faserforsch Textultech 2: 299-303.

HSDB (2006). Hazardous Substances Data Bank. Chemical/Physical Properties; Emergency Medical Treatment. National Library of Medicine, Bethesda, MD. Available at: http://toxnet.nlm.nih.gov.

IARC (1999). Caprolactam. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Re-evalualtion of Some Organic Chemicals, Hydrazine and Hydrogen Peroxide. World Health Organization, Lyon, France. 71 (part 2): 383-400.

IWMB (2003). Integrated Waste Management Board. Building Material Emissions Study. Sacramento, CA. Available at: www.ciwmb.ca.gov/Publications. pp. 1-15, 34-37, 62-63, 90-92, 164-184 (App. C), 305-308 (App. O).

Kaufmann W, Bader R, Ernst H, Harada T, Hardisty J, Kittel B, Kolling A, Pino M, Renne R, Rittinghausen S, Schulte A, Wohrmann T and Rosenbruch M (2009). 1st international ESTP expert workshop: "Larynx squamous metaplasia". A re-consideration of morphology and diagnostic approaches in rodent studies and its relevance for human risk assessment. Exp Toxicol Pathol 61(6): 591-603.

Kelman GR (1986). Effects of human exposure to atmospheric epsilon-caprolactam. Hum Toxicol 5(1): 57-9.

Kilgour JD, Simpson SA, Alexander DJ and Reed CJ (2000). A rat nasal epithelial model for predicting upper respiratory tract toxicity: in vivo-in vitro correlations. Toxicology 145(1): 39-49.

Kirk LK, Lewis BA, Ross DA and Morrison MA (1987). Identification of ninhydrin-positive caprolactam metabolites in the rat. Food Chem Toxicol 25(3): 233-239.

Krajnik V, Bozek P, Kondelikova J and Kralicek J (1982). High-performance liquid chromatography of 6-caprolactam and its cyclic oligomers present in polyamide 6. J Chromatog 240: 539-42.

Lan X-x, Bai T-j, Yang Z-r and Luo K-z (1998). Health status of workers exposed to caprolactam [in Chinese]. J China Ind Med 11(2): 115.

Lander J-A. (2002). Structure Development in Silicate-Layered Polymer Nanocomposites. A Thesis Submitted for the Degree of Doctor of Philosophy. Uxbridge, Middlesex: Wolfson Centre for Materials Processing, Brunel University.

Lang I, Bruckner T and Triebig G (2008). Formaldehyde and chemosensory irritation in humans: a controlled human exposure study. Regulatory toxicology and pharmacology : RTP 50(1): 23-36.

Li J (1996). Study of health effects of caprolactam on exposed workers [in Chinese]. Ind Hyg Occup Dis 22(2): 103-04.

Liu F-q, Bao Y-s and Zheng C-l (1988). Investigation on the effects of caprolactam on the maternal functions of female workers [in Chinese]. Chinese Journal of Industrial Hygiene and Occupational Diseases 6(2): 201-3.

Morgan KT (1991). Approaches to the identification and recording of nasal lesions in toxicology studies. Toxicol Pathol 19(4 Pt 1): 337-51.

Nau DR, Darr RW, Gad SC and Pai SV (1984). Validation Study of a Method for Monitoring Personnel Exposure to Caprolactam. In: Proceedings of a Symposium on an Industry Approach to Chemical Risk Assessment - Caprolactam and Related Compounds as a Case Study, May 15-17, 1984, Arlington VA. pp. 97-113.

Nielsen GD, Wolkoff P and Alarie Y (2007). Sensory irritation: risk assessment approaches. Regul Toxicol Pharmacol 48(1): 6-18.

NIOSH. (1995a). Occupational Safety and Health Guideline for Caprolactam Dust. National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services.

NIOSH. (1995b). Occupational Safety and Health Guideline for Caprolactam Vapor. National Institute for Occupational Safety and Health, U.S. Department of Health and Human Services.

NPG (2007). Nylon-6. Nylon-6 Promotional Group, Online at: www.npg-6.com.

NTP (1982). Carcinogenesis bioassay of caprolactam (CAS No. 105-60-2) in F344 rats and B6C3F$_1$ mice (feed study). National Toxicology Program, Technical Report Series No. 214. Online at: http://ntp-server.niehs.nih.gov.

OEHHA. (2008). Air Toxics Hot Spots Program Risk Assessment Guidelines. Technical Support Document for the Derivation of Noncancer Reference Exposure Levels. California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Oakland, CA. Online at: http://www.oehha.ca.gov/air/hot_spots/rels_dec2008.html.

OSHA. (1988). Caprolactam. Related Information: Chemical Sampling - Caprolactam (Dust), Caprolactam (Vapor). Occupational Safety and Health Administration, U.S. Department of Labor. Online at: http://www.osha.gov/dts/sltc/methods/partial/pv2012/2012.html.

Osimitz TG, Droege W and Finch JM (2007). Toxicologic significance of histologic change in the larynx of the rat following inhalation exposure: a critical review. Toxicol Appl Pharmacol 225(3): 229-37.

Reinhold RW, Hoffman GM, Bolte HF, Rinehart WE, Rusch GM, Parod RJ and Kayser M (1998). Subchronic inhalation toxicity study of caprolactam (with a 4-week recovery) in the rat via whole-body exposures. Toxicol Sci 44(2): 197-205.

Renne R, Brix A, Harkema J, Herbert R, Kittel B, Lewis D, March T, Nagano K, Pino M, Rittinghausen S, Rosenbruch M, Tellier P and Wohrmann T (2009). Proliferative and nonproliferative lesions of the rat and mouse respiratory tract. Toxicol Pathol 37(7 Suppl): 5S-73S.

Renne RA and Gideon KM (2006). Types and patterns of response in the larynx following inhalation. Toxicol Pathol 34(3): 281-5.

Renne RA, Gideon KM, Harbo SJ, Staska LM and Grumbein SL (2007). Upper respiratory tract lesions in inhalation toxicology. Toxicol Pathol 35(1): 163-9.

Riedl O, Hruskova J, Stuchlikova E, Komarkova A, Novotna Blazkova B, Tenorova M, Renner J, Spala M and Tvaroh F (1963). Treatment of obesity with caprolactam. Rev Czech Med 24: 167-82.

Rinehart WE, Alarie Y, Stock MF and Rush RE (1997). The sensitization potential of caprolactam. Int J Toxicol 16 (suppl 2): 22.

Ritz J, Fuchs H, Kieczka H and Moran WC (2002). Caprolactam. In: Ullmann's Encyclopedia of Industrial Chemistry. Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim. New York: 1-21.

Salamone MF (1989). Abnormal sperm assay tests on benzoin and caprolactam. Mutat Res 224(3): 385-9.

Serota DG, Hoberman AM, Friedman MA and Gad SC (1988). Three-generation reproduction study with caprolactam in rats. J Appl Toxicol 8(4): 285-93.

Springborn Laboratories Inc. (1991). Life Sciences Division, SLS Study No. 3224.7, April 25, 1991.

Stuart BO (1984). Deposition and clearance of inhaled particles. Environ Health Perspect 55: 369-90.

Swift DL and Strong JC (1996). Nasal deposition of ultrafine ^{218}Po aerosols in human subjects. J Aerosol Sci 27(7): 1125-32.

Tuma SN, Orson F, Fossella FV and Waidhofer W (1981). Seizures and dermatitis after exposure to caprolactam. Arch Intern Med 141(11): 1544-5.

U. S. EPA. (1994a). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. U.S. Environmental Protection Agency, EPA/600/8-90/066F.

U. S. EPA (1994b). Caprolactam (CASRN 105-60-2). U. S. Envoronmental Protection Agency. Integrated Risk Information System. Available at: http://www.epa.gov/iris. pp.1-13.

U. S. EPA. (2006). Inventory Update Reporting. Non-confidential 2006 IUR Records by Chemical, including Manufacturing, Processing and Use Information. Online at: http://cfpub.epa.gov/iursearch/2006_iur_natlcheminfo.cfm?id=2673.

U. S. EPA. (2009a). Epsilon-Caprolactam (CAS # 105-60-2). Letter of test results performed in 1952-53 filed with Office of Pollution Prevention and Toxics 8(e) coordinator, U.S. Environmental Protection Agency, Washington, DC, 8EHQ-09-17699, dated October 28, 2009.

U. S. EPA. (2009b). Metabolically derived human ventilation rates: A revised approach based upon oxygen consumption rates. U.S. Environmental Protection Agency, National Center for Environmental Assessment, Washington, DC; EPA/600/R-06/129F.

U. S. EPA. (2009c). Benchmark Dose Software, Version 2.1.1. National Center for Environmental Assessment. Online at: http://www.epa.gov/ncea/bmds.

Unger PD, Salerno AJ and Friedman MA (1981). Disposition of 14C-caprolactam in the rat. Food Cosmet Toxicol 19: 457-462.

Venema A, Henk van de Ven JFM, David F and Sandra P (1993). Supercritical fluid extraction of Nylon 6: An investigation into the factors affecting the efficiency of extraction of caprolactam and its oligomers. J High Resolution Chromatography 16: 522-24.

Vihola H, Laukkanen A, Valtola L, Tenhu H and Hirvonen J (2005). Cytotoxicity of thermosensitive polymers poly(N-isopropylacrylamide), poly(N-vinylcaprolactam) and amphiphilically modified poly(N-vinylcaprolactam). Biomaterials 26: 3055-64.

Waddell WJ, Marlowe C and Friedman MA (1984). The distribution of [^{14}C]caprolactam in male, female, and pregnant mice. Food Chem Toxicol 22(4): 293-303.

Walker JC, Kendal-Reed M, Utell MJ and Cain WS (2001). Human breathing and eye blink rate responses to airborne chemicals. Environ Health Perspect 109 Suppl 4: 507-12.

Wilke O, Jann O and Brodner D (2004). VOC- and SVOC-emissions from adhesives, floor coverings and complete floor structures. Indoor Air 14 Suppl 8: 98-107.

Wilkins CK, Wolkoff P, Gyntelberg F, Skov P and Valbjorn O (1993). Characterization of office dust by VOCs and TVOC release - Identification of potential irritant VOCs by partial least squares analysis. Indoor Air 3: 283-290.

Woo KH, Jung SJ, Park WS, Shin HR, Kim JS and Kim SW (1998). Two cases of convulsion associated with caprolactam [in Korean]. Korean J Occup Environ Med 10(1): 116-20.

Working PK (1989). Assessment of unscheduled DNA synthesis in Fischer 344 rat pachytene spermatocytes exposed to caprolactam or benzoin in vivo. Mutat Res 224(3): 365-8.

Xu Y-p, Ma B-l, Wang X-s, Wang M-s, Zhang L and Li Z (1997). Effects of low concentration caprolactam on worker health [in Chinese]. Chinese Journal of Industrial Medicine 10(5): 290-92.

Yeh H-C, Cuddihy RG, Phalen RF and Chang I-Y (1996). Comparisons of calculated respiratory tract deposition of particles based on the proposed NCRP model and the new ICRP66 model. Aerosol Sci Technol 25: 134-40.

Zaitsau DH, Paulechka YU, Kabo GJ, Kolpikau AN, Emel'yanenko VN, Heintz A and Verevkin SP (2006). Thermodynamics of the sublimation and of the vaporization of e-caprolactam. J Chem Eng Data 51: 130-35.

Zhou H-d (1976). Occupational poisoning tests (manual) [in Chinese]. People's Medical Publishing House, Beijing: 424-6.

Ziegler AE, Zimmer H and Triebig G (2008). Exposure study on chemosensory effects of epsilon-caprolactam in the low concentration range. Int Arch Occup Environ Health 81(6): 743-53.

Appendix A

Benchmark Concentration (BMC) Modeling of the Upper Respiratory Tract Lesions Resulting from Subchronic Caprolactam Exposure in Rats (Reinhold et al., 1998)

Pathologist-graded lesions in the nasal and laryngeal airways provided dose-response data for BMC modeling. Males and females were combined, given that no apparent gender differences were noted from the endpoint responses. US EPA does not as yet recommend an approved protocol for modeling categorical data such as lesion grades. So the Reinhold data were modeled as dichotomous data (i.e., all lesions of severity categories found in both control and treated animals were designated as not treatment-related; all lesions of severity categories found in treated animals that were greater than that found in control animals were designated as a treatment-related effect).

Per US EPA protocol, P-values ≤ 0.1 indicate that the model is a poor fit to the data and recommended not to be used for BMC determination. US EPA also states that P-values identify those models that are consistent with the experimental results, but should not be compared among the various models. The Akaike Information Criterion (AIC) can be used to compare models. Generally, the model with the lowest AIC is considered the preferential model, although other considerations such as model fit to the low end of the concentration curve where the $BMCL_{05}$ resides are also taken into account. Three regions of the upper respiratory tract (nasal olfactory mucosa, nasal respiratory mucosa and laryngeal epithelial tissue) at sacrifice provided dose response data on which to run BMC modeling:

1) **Incidence of epithelial intracytoplasmic eosinophilic material in nasal olfactory mucosa**

Minimal-grade lesions were present in most animals in the control group. Caprolactam exposed animals exhibited slight, moderate and moderately severe lesions, which we considered exposure-related. The P-values and AIC indicate the log-probit and log-logistic models show the best fit to the data, particularly at the low end of the dose-response curve where the BMC_{05} lies (models highlighted in bold in Table A-1). The BMC_{05} are nearly identical for these two models but the log-probit has a smaller range between the MLE and the BMC_{05} suggesting greater confidence with this model at the low concentration end where the $BMCL_{05}$ resides, even though the log-logistic model fit produces a slightly smaller AIC. Thus, the log-probit model was chosen as the basis for the point of departure for this endpoint.

Table A-1. Benchmark concentration results modeling incidence (slight, moderate and moderately severe grades combined) of epithelium-intracytoplasmic eosinophilic material in nasal olfactory mucosa.

Method	$BMCL_{05}$	BMC_{05}	P-value	AIC
Probit	18	25	0.11	66.457
Log-probit (slope restricted ≥ 1)	**12**	**17**	**0.99**	**60.845**
Logistic	18	26	0.097	66.925
Log-logistic(slope restricted ≥ 1)	**5.6**	**16**	**0.99**	**60.834**
Weibull (power ≥ 1)	5.3	11	0.90	61.038
Gamma (power ≥ 1)	5.3	12	0.93	60.984
Quantal-linear	5.2	7.2	0.90	59.495
Quantal-quadratic	29	36	0.055	67.148
Multistage (2^{nd} degree)	5.3	8.8	0.85	61.180

Figure A-1: Log-probit model fit to nasal olfactory mucosa lesion incidence data

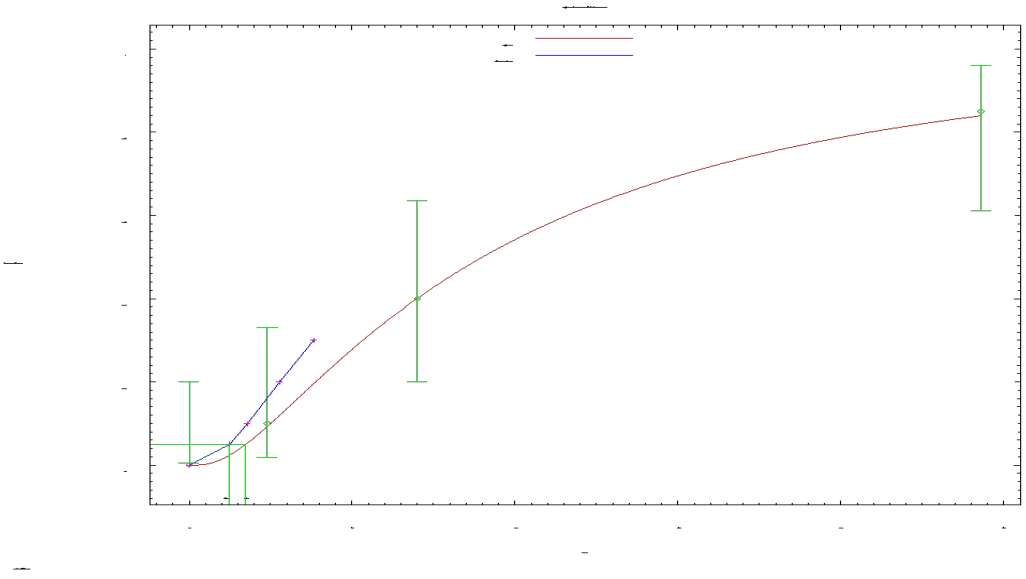
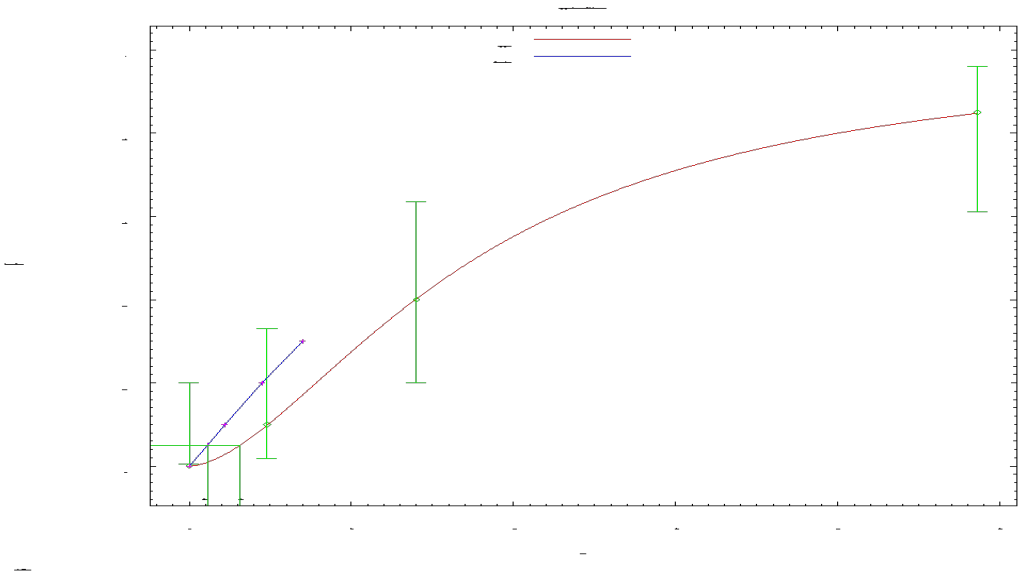


Figure A-2: Log-logistic model fit to nasal olfactory mucosa lesion incidence data



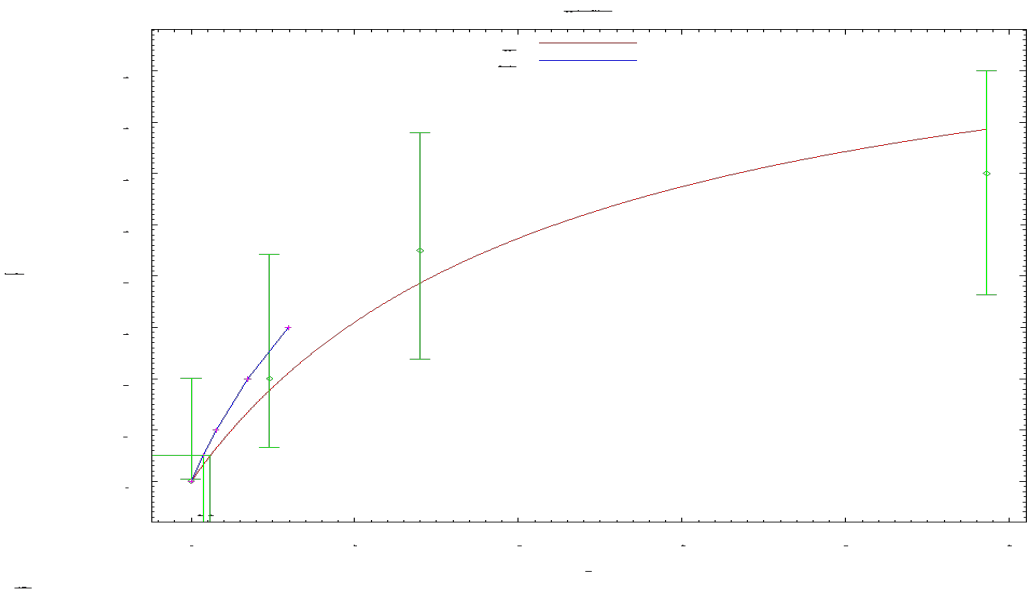
2) Incidence of goblet cell hypertrophy/hyperplasia in respiratory nasal mucosa

Minimal and slight lesions of the nasal respiratory epithelium were present in control animals. Moderate-grade lesions were present in caprolactam-exposed animals and considered exposure-related. Models in Table A-2 with P-values <0.10 are not considered a good fit to the data. Among the remaining models, the log-logistic model (shown in bold in Table A-2) had the lowest AIC and best fit to the data, thus we chose the $BMCL_{05}$ from the log-logistic model as the point of departure for this endpoint.

Table A-2: BMC results modeling moderate-grade incidence of goblet cell hypertrophy/hyperplasia in respiratory nasal mucosa.

Method	$BMCL_{05}$	BMC_{05}	P-value	AIC
Probit	21	29	0.024	88.321
Log-probit (slope restricted ≥ 1)	15	24	0.026	86.384
Logistic	22	31	0.022	88.643
Log-logistic (slope restricted ≥ 1)	**3.6**	**5.9**	**0.78**	**77.525**
Weibull (power ≥ 1)	6.8	9.4	0.21	80.650
Gamma (power ≥ 1)	6.8	9.4	0.21	80.650
Quantal-linear	6.8	9.4	0.21	80.650
Quantal-quadratic	45	59	0.017	88.507
Multistage (2^{nd} degree)	6.8	9.4	0.21	80.650

Figure A-3: Log-logistic model fit to nasal respiratory mucosa lesion incidence data



3) Incidence of squamous/squamid metaplasia/hyperplasia of laryngeal tissue

Minimal and slight severity levels present in caprolactam-exposed animals were considered exposure-related. The model that provided a low AIC, best p-value and best fit to the data at the low concentration end of the curve was the multistage model (Table A-3). Although the quantal-linear model produced a lower AIC, the line fit to the data point at the low end of the curve (24 mg/m^3) was not quite as good as that provided by the multistage model (Figures A-4 and A-5). Thus, we chose the multistage model as the basis for the point of departure for this endpoint.

Table 3. Benchmark concentration results modeling incidence of (minimal and slight combined) squamous/squamid metaplasia/hyperplasia of laryngeal tissue

Method	$BMCL_{05}$	BMC_{05}	P-value	AIC
Probit	8.3	12.6	0.33	56.43
Log-probit (slope restricted ≥ 1)	6.7	11.9	0.52	55.20
Logistic	8.9	13.6	0.29	56.92
Log-logistic (slope restricted ≥ 1)	3.9	10.7	0.44	55.80
Weibull (power ≥ 1)	2.7	7.2	0.84	53.85
Gamma (power ≥ 1)	2.7	7.8	0.79	54.06
Quantal-linear	**2.6**	**3.6**	**0.82**	**52.92**
Quantal-quadratic	12.2	14.9	0.33	54.27
Multistage (2nd degree)	**2.8**	**5.3**	**0.94**	**53.59**

Figure A-4: Multistage model fit to laryngeal lesion incidence data

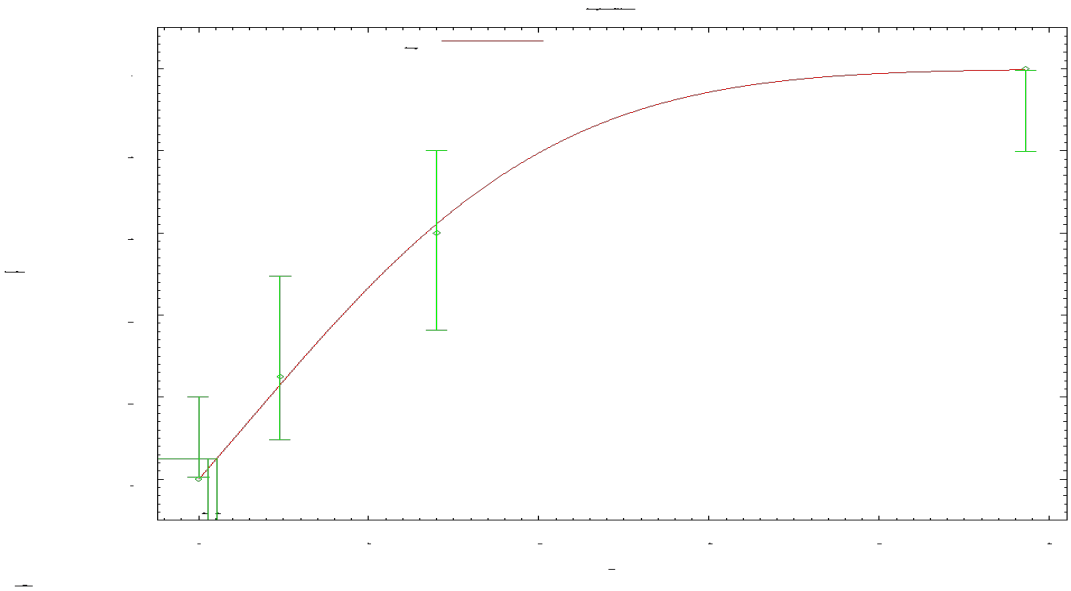
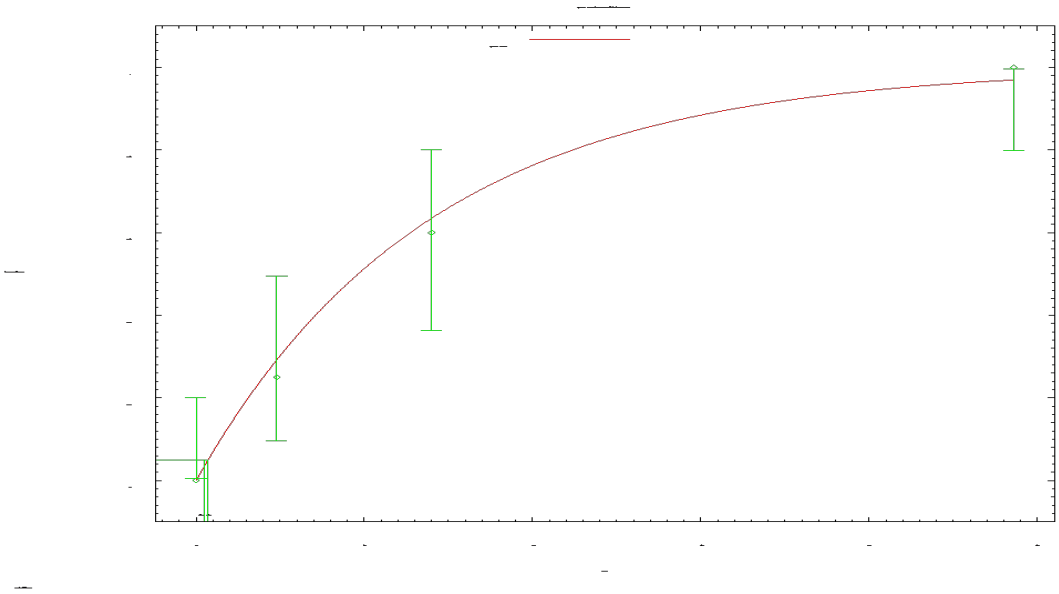


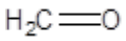
Figure A-5: Quantal linear model fit to laryngeal lesion incidence data



Formaldehyde Reference Exposure Levels

(Methanal, oxomethane, methylene oxide)

CAS 50-00-0



1. Summary

The non-cancer adverse health effects of formaldehyde are largely a manifestation of its ability to irritate mucous membranes. As a result of its solubility in water and high reactivity, formaldehyde is efficiently absorbed into the mucus layers protecting the eyes and respiratory tract where it rapidly reacts, leading primarily to localized irritation. Acute high exposure may lead to eye, nose and throat irritation, and in the respiratory tract, nasal obstruction, pulmonary edema and dyspnea. Prolonged or repeated exposures have been associated with allergic sensitization, respiratory symptoms (coughing, wheezing, shortness of breath), histopathological changes in respiratory epithelium, and decrements in lung function. Children, especially those with diagnosed asthma, may be more likely to show impaired pulmonary function and symptoms than are adults following chronic exposure to formaldehyde. The studies reviewed for this document include those published through the Spring of 2008.

1.1 Formaldehyde Acute REL

Reference Exposure Level	**55 µg/m³ (44 ppb)**
Critical effect(s)	Mild and moderate eye irritation
Hazard Index target(s)	Eye irritation

1.2 Formaldehyde 8-Hour REL

Reference Exposure Level	**9 µg/m³ (7 ppb)**
Critical effect(s)	Nasal obstruction and discomfort, lower airway discomfort, and eye irritation
Hazard Index target(s)	Respiratory

1.3 Formaldehyde Chronic REL

Reference Exposure Level	**9 µg/m³ (7 ppb)**
Critical effect(s)	Nasal obstruction and discomfort, lower airway discomfort, and eye irritation
Hazard Index target(s)	Respiratory

2. Physical & Chemical Properties (ATSDR, 1999)

Description	Colorless gas
Molecular formula	CH_2O
Molecular weight	30.03 g/mol
Density	0.815 g/L @ -20° C
Boiling point	-19.5° C
Melting point	-92° C
Vapor pressure	3883 mm Hg @ 25° C
Flashpoint	300° C
Explosive limits	7% - 73%
Solubility	soluble in water, alcohol, ether and other polar solvents
Odor threshold	0.05-0.5 ppm
Metabolites	formic acid
Conversion factor	1 ppm in air = 1.24 mg/m^3 @ 25° C

3. Occurrence and Major Uses

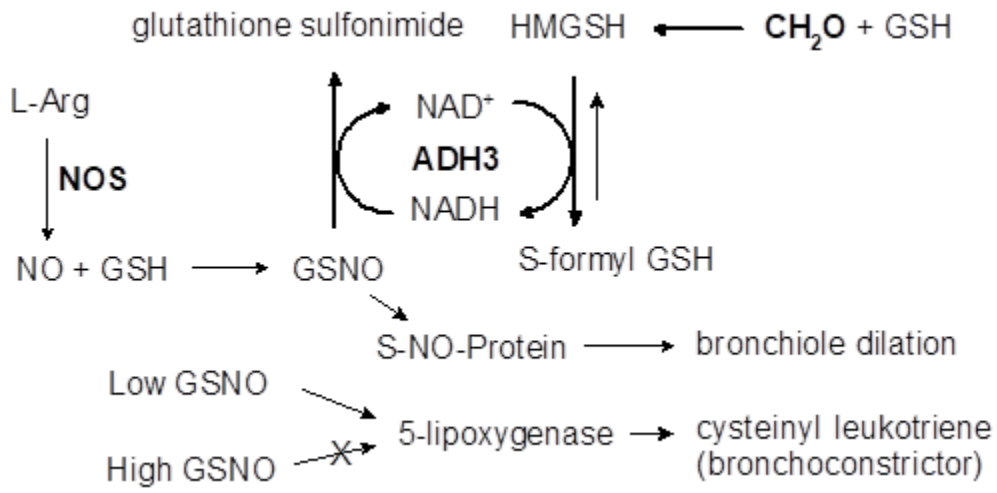
Formaldehyde has four major applications: as an intermediate in the manufacture of melamine, polyacetal, and phenolic resins; as an intermediate in the production of industrial chemicals; as a bactericide or fungicide; and as a component in the manufacture of end-use consumer products. Phenol-formaldehyde resins are used in the production of plywood, particleboard, foam insulation, and a wide variety of molded or extruded plastic items. Formaldehyde is also used as a preservative, a hardening and reducing agent, a corrosion inhibitor, a sterilizing agent, and in embalming fluids. Indoor sources include upholstery, permanent press fabrics, carpets, pesticide formulations, urea-formaldehyde foam insulation, and cardboard and paper products. Outdoor sources include emissions from fuel combustion (motor vehicles), industrial fuel combustion (power generators), oil refining processes, and other uses (copper plating, incinerators, etc.). The largest portion of outdoor ambient formaldehyde results from photochemical oxidation of a number of reactive organic gases in the atmosphere (CARB, 2006). According to the California Toxics Inventory (CARB, 2005a), the mean statewide ambient level of formaldehyde in 2004 was 2.69 ppb, with the highest levels (3.76 ppb) reported for the South Coast Air Basin. The California Air Resources Board (CARB) reported statewide emissions of 20,251 tons from stationary and mobile sources (CARB, 2005b).

4. Metabolism

Inhaled formaldehyde reacts rapidly at the site of contact and is efficiently absorbed in the respiratory tract. A portion of the formaldehyde entering the fluid layer covering the respiratory epithelium, the respiratory tract lining fluid (RTLF), is reversibly hydrated to methylene glycol. Among other components, the RTLF is rich in antioxidants including glutathione (Cross et al., 1994) with which formaldehyde may reversibly react to form *S*-hydroxymethylglutathione. Both the hydrated and unreacted formaldehyde may be absorbed into the epithelial layer where there is further opportunity for formaldehyde to bind to glutathione. This glutathione conjugate in turn is oxidized to *S*-formylglutathione by formaldehyde dehydrogenase. Hydrolysis of *S*-formylglutathione yields formate and glutathione. Formic acid may be eliminated in urine and feces, or dehydrogenated to CO_2 and exhaled. The presence of glutathione and formaldehyde dehydrogenase in epithelial cells of the respiratory tract varies with location and influences the amount of formaldehyde reaching the blood. While glutathione-bound formaldehyde is rapidly metabolized, free formaldehyde in cells can form DNA-protein cross-links (Franks, 2005).

Formaldehyde dehydrogenase (ADH3), although central to the metabolism of formaldehyde, has a broad specificity that includes the structurally related compound, S-nitrosoglutathione (GSNO), an endogenous bronchodilator and reservoir of nitric oxide (NO) activity (Jensen et al., 1998). In cultured cells, formaldehyde appears to trigger ADH3-mediated GSNO reduction by enzyme-bound cofactor recycling (Staab et al., 2008). As shown in Figure 1, the *S*-hydroxymethylglutathione (HMGSH) formed spontaneously from formaldehyde and glutathione is oxidized by ADH3 with the formation of NADH that may then participate in the ADH3-mediated reduction of GSNO (Thompson and Grafstrom, 2008). (Because of its participation in this reaction, ADH3 is also known as GSNO reductase.) This reductive pathway results in low levels of GSNO that in turn stimulate the production and activity of 5-lipoxygenase, the rate-limiting enzyme in the synthesis of powerful bronchoconstrictors, the cysteinyl leukotrienes. On the other hand, high levels of GSNO inhibit this enzyme and thus the synthesis of the bronchoconstrictors (Zaman et al., 2006). Up-regulation of the degradation of GSNO has been demonstrated in mouse lung following inhalation of formaldehyde (Yi et al., 2007), while low levels of GSNO in the lungs have been associated with severe asthma attacks in children (Gaston et al., 1998) and airway hyperactivity in mice (Que et al., 2005). These results suggest that the potential association of formaldehyde exposure with asthma-like respiratory symptoms is in part due to its effects on NO via the enhanced degradation of GSNO. Nitric oxide has multiple functions in the lungs, from its participation in the regulation of airway and vascular tone to mucin secretion and mucociliary clearance (Reynaert et al., 2005). The dysregulation of NO by formaldehyde helps to explain the variety and variability in the toxic manifestations following formaldehyde inhalation.

Figure 4 Formaldehyde Driven Reduction of GSNO



Oxidation of the glutathione conjugate of formaldehyde, HMGSH, by ADH3 generates NADH that drives the reduction of GSNO, also by ADH3, thereby reducing the nitric oxide available for bronchiole dilation. Low GSNO levels stimulate, but high GSNO levels inhibit 5-lipoxygenase production of cysteinyl leukotriene.

5. Acute Toxicity of Formaldehyde

The acute effects of formaldehyde exposure appear to be largely a result of its irritant properties. However, some individuals experience symptoms following acute exposures that are a result of previous sensitization following acute high formaldehyde exposure, or long term low level exposures. For this reason, some of the studies included in this section describe manifestations of toxicity in which acute exposure was the precipitating event but in which the contribution of previous exposures or sensitization is unknown. Sensitization manifests as heightened responsiveness and may be of an immunological nature with the development of formaldehyde-specific IgE or IgG (e.g. Thrasher et al. 1987). Alternatively, heightened responsiveness may be neurologically mediated with involvement of the hypothalamic/pituitary/adrenal axis (Sorg et al., 2001a,b). In addition, genetic variation among individuals in the alcohol dehydrogenases mentioned above affects individual responses to formaldehyde. This is especially germane to studies in which the effects include symptoms such as bronchoconstriction and airway hyperreactivity, and in which there is unexpected individual variation.

Many of the studies described in this document have evaluated the relationship between formaldehyde inhalation and clinically-diagnosed asthma or asthma-like symptoms. Asthma is a chronic disease of airway obstruction resulting in variable airflow that has classically been considered to involve both airway inflammation and airway hyperresponsiveness. Asthma manifests as a characteristic cough, wheeze, and shortness of breath due to spasmodic contractions of the bronchi and mucus hypersecretion. These symptoms may or may not reflect an underlying allergic response. As shown in the study by Que et al. (2005), the hyperresponsiveness and

the inflammation are not necessarily coupled. Although the RELs presented in this document are not based on studies that used asthma as the critical endpoint, uncertainty factors were applied in the REL estimates to explicitly consider the potential of formaldehyde to cause or exacerbate asthma-like wheeze and cough symptoms, especially in asthmatic children. We have therefore included discussion of recent work that provides a biochemical mechanism by which formaldehyde exposure is linked to at least one symptom of asthma, bronchoconstriction. The bronchoconstrictive effects of formaldehyde exposure may be partially responsible for the lower airway discomfort reported in the study upon which the 8-hour and chronic RELs are based.

5.1 Acute Toxicity to Adult Humans

In small human studies, exposure to formaldehyde (1-3 ppm) has resulted in eye and upper respiratory tract irritation (Weber-Tschopp et al., 1977; Kulle et al., 1987). Most people cannot tolerate exposures to more than 5 ppm formaldehyde in air; above 10-20 ppm symptoms become severe and shortness of breath occurs (Feinman, 1988). High concentrations of formaldehyde may result in nasal obstruction, pulmonary edema, choking, dyspnea, and chest tightness (Porter, 1975; Solomons and Cochrane, 1984).

A few human case studies report severe pulmonary symptoms. A medical intern with known atopy and exposure to reportedly high (but unspecified) levels of formaldehyde over a period of 1 week developed dyspnea, chest tightness, and edema, following a subsequent 2 hour exposure to formaldehyde (Porter, 1975). Five workers exposed to formaldehyde from newly installed urea-formaldehyde chipboard in a poorly ventilated basement experienced intolerable eye and upper respiratory tract irritation, choking, marked dyspnea, and nasal obstruction (Solomons and Cochrane, 1984). However, the concentration of formaldehyde and the contribution of other airborne chemicals were unknown in both reports.

Numerous acute controlled and occupational human exposure studies have been conducted with both asthmatic and normal subjects to investigate formaldehyde's irritative and pulmonary effects (Frigas et al., 1984; Sheppard et al., 1984; Sauder et al., 1986; Schachter et al., 1986; Kulle et al., 1987; Sauder et al., 1987; Schachter et al., 1987; Witek et al., 1987; Uba et al., 1989; Harving et al., 1990; Akbar-Khanzadeh et al., 1994). Short exercise sessions during exposure on a bicycle ergometer were included in some of the studies. Concentrations of formaldehyde in the human exposure studies ranged as high as 3 ppm for up to 3 hours. The major findings in these studies were mild to moderate eye and upper respiratory tract irritation typical of mild discomfort from formaldehyde exposure.

Chemosensory irritation and subjective symptoms following exposure to formaldehyde at concentrations relevant to the workplace were examined by Lang et al. (2008) in 11 male and 10 female volunteers. Each subject was exposed for 4 hours to a randomized sequence of ten exposure conditions. These included exposures at concentrations of 0, 0.15, 0.3 and 0.5 ppm, exposures at 0.3 and 0.5 ppm that included four transient peak exposures at 0.6 and 1.0 ppm, respectively, and exposures in the presence of 10 ppm ethyl acetate of 0, 0.3, 0.5, and 0.5 ppm with 1.0 ppm peaks.

Objective measures of irritation included conjunctival redness, blinking frequency, nasal flow resistance, pulmonary function, and reaction times. The participant's subjective evaluation of physical and mental wellbeing was assessed by questionnaire before, during and after each day's exposure. To assess the potential influence of personality traits on subjective responses, each subject's positive or negative affectivity was evaluated with PANAS (Positive and Negative Affectivity Schedule) that consists of 10 positive affects (interested, excited, strong, enthusiastic, proud, alert, inspired, determined, attentive, and active) and 10 negative affects (distressed, upset, guilty, scared, hostile, irritable, ashamed, nervous, jittery, and afraid). Participants are asked to rate items on a scale from 1 to 5, based on the strength of emotion where 1 = "very slightly or not at all," and 5 = "extremely". Subjective ratings of eye irritation and olfactory symptoms were significantly higher than control at 0.3 ppm. However, when negative affectivity (anxiety) was included as a covariate, eye and olfactory irritation at this exposure level were no longer significant. Conjunctival irritation and blinking frequency, objective measures of irritation, were significantly elevated only with exposure to 0.5 ppm with peaks of 1.0 ppm ($p < 0.05$). The authors considered this level to be a LOAEL. However, at 0.5 ppm without 1.0 ppm peaks, conjuctival irritation and blinking were not significantly increased so this was considered a NOAEL for these effects. There were no statistically significant changes in nasal resistance, pulmonary function or reaction time. While there were large inter-individual differences in complaints or reports of wellbeing, there were no significant treatment effects. This study identified eye irritation as the most sensitive endpoint, with personality traits, such as negative affectivity, as a modifying factor.

In a human irritation study by Weber-Tschopp et al. (1977), 33 subjects were exposed to formaldehyde at concentrations ranging from 0.03-3.2 ppm (0.04-4.0 mg/m^3) for 35 minutes. Thresholds were 1.2 ppm (1.5 mg/m^3) for eye and nose irritation, 1.7 ppm (2.1 mg/m^3) for eye blinking, and 2.1 ppm (2.6 mg/m^3) for throat irritation.

Kulle et al. (1987) exposed nonasthmatic humans to up to 3.0 ppm (3.7 mg/m^3) formaldehyde in a controlled environmental chamber for 3 hours. Significant dose-response relationships were seen with odor and eye irritation (Table 5.1) as ranked on symptom questionnaires as none, mild, moderate or severe. Irritation was assessed in this manner prior to exposure, at the end of exposure, and again 24 hour after exposure.

Table 5.1 Mean Symptom Difference (t180-t0) ± SE with Formaldehyde* (from Kulle et al., 1987)

	Formaldehyde conc. (ppm)				P value
	0.0	1.0	2.0	3.0	
Odor sensation	0.00 ± 0.00	0.22 ± 0.15	0.44 ± 0.18	1.00 ± 0.29	<0.0001
Nose/throat irritation	0.00 ± 0.00	0.11 ± 0.11	0.33 ± 0.17	0.22 ± 0.15	0.054
Eye irritation	0.00 ± 0.00	0.44 ± 0.24	0.89 ± 0.26	1.44 ± 0.18	<0.0001
Chest discomfort	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.11	0.00 ± 0.00	0.62
Cough	0.00 ± 0.00	0.11 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.11
Headache	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.11	0.33

*Presence and severity of symptoms scored as: 0 = none; 1 = mild (present but not annoying); 2 = moderate (annoying); 3 = severe (debilitating). Data from Table II.

At 0.5 ppm for 3 hours, none of 9 subjects had eye irritation. At 1.0 ppm, 3 of 19 subjects reported mild eye irritation and one experienced moderate irritation. At 2.0 ppm, 6 subjects reported mild and 4 reported moderate eye irritation. Measured nasal flow resistance was increased at 3.0 ppm but not at 2.0 ppm (2.5 mg/m^3). With respect to the lower respiratory tract, there were no significant decrements in pulmonary function nor increases in methacholine induced bronchial reactivity as a result of 3-hour exposures to 0.5-3.0 ppm (0.6-3.7 mg/m^3) formaldehyde at rest or during exercise, including 24 hours post exposure.

Eleven healthy subjects and nine patients with formalin skin sensitization were exposed to 0.5 mg/m³ (0.4 ppm) formaldehyde for 2 hours (Pazdrak et al., 1993). Nasal lavage was performed prior to and 5 to 10 minutes, 4 hours, and 18 hours after exposure. Rhinitis was reported and increases in the number and proportion of eosinophils, elevated albumin and increased protein levels were noted in nasal lavage fluid 4 and 18 hours after exposure. No differences were found between patients with skin sensitization and healthy subjects.

In a study by Green et al. (1987), volunteer asthmatic and normal subjects exposed to formaldehyde displayed decrements in pulmonary function. Exposure to 3 ppm formaldehyde for 1 hour resulted in clinically significant reductions of forced expiratory volume in one second (FEV_1) (defined as > 20% or more) and FEV_1/forced vital capacity (FVC) (ratio 70% or less) in 5 individuals in the study (2 of 16 asthmatics, 2 of 22 normal subjects, and one clinically normal subject with hyperactive airways). Of these individuals, 3 had reductions of FEV_1 of 20% or more during exposure. One of 22 asthmatics had a greater than 20% reduction in FEV_1 (-25.8%) at 17 minutes into exposure following a 15 minute moderate exercise session (minute ventilation [V_E] = 30-40 l/min), which, according to the authors, was low enough to prevent exercise-induced bronchospasm. One of 22 normal subjects also exhibited a greater than 20% clinically significant reduction in FEV_1 (-24.4%) and in FEV_1/FVC, which occurred at 47 minutes into exposure to 3 ppm formaldehyde. These reductions occurred following a second 15- minute heavy-exercise session (V_E = 60-70 l/min) near the end of the 1 hour exposure period. A third asymptomatic “normal” subject with hyperactive airways had a clinically significant reduction of FEV_1 (-20.5%) at 17 minutes, following the first heavy exercise session. This subject exhibited occult airway hyperactivity and was excluded from analysis with the other exposure groups due to his respiratory condition. Subjects exhibiting reductions in FEV_1 of greater than 20% following exposure also exhibited FEV_1/FVC ratios of less than 70%. However, none of the subjects in the study exhibited a clinically significant reduction of 50% or greater in airway conductance (SG_{aw}) during exposure to 3 ppm formaldehyde.

Kriebel et al. (2001) conducted a subchronic epidemiological study of 38 anatomy class students who, on average, were exposed to a geometric mean of 0.70 ± 2.13 ppm for 2 hours per week over 14 weeks. After class, eye, nose and throat irritation was significantly elevated compared with pre-laboratory session exposures, with a one unit increase in symptom intensity/ppm of formaldehyde. Peak expiratory flow (PEF) was found to decrease by 1%/ppm formaldehyde during the most recent exposure. Changes in PEF and symptom intensity following formaldehyde exposure were most

pronounced during the first weeks of the semester but attenuated with time, suggesting partial acclimatization.

Rhinitis and a wide range of respiratory symptoms can result from exposure to formaldehyde. Some studies have reported that workers exposed to low concentrations may develop severe prolonged asthma attacks after prior exposure; this suggests that they may have become sensitized (Feinman, 1988). However, in adults, an association between formaldehyde exposure and allergic sensitization through IgE- and IgG-mediated mechanisms has been observed only inconsistently (Thrasher et al., 1987; Krakowiak et al., 1998; Wantke et al., 2000; Kim et al., 2001).

Formaldehyde provocation of human subjects, occupationally exposed to formaldehyde and suffering from respiratory symptoms such as wheezing, shortness of breath, or rhinitis, occasionally resulted in pulmonary function decrements (2 to 33% response rate) consistent with immediate, delayed, or both immediate and delayed bronchoconstriction (Hendrick and Lane, 1977; Wallenstein et al., 1978; Burge et al., 1985; Nordman et al., 1985). While some of the concentrations of formaldehyde that elicited a positive response following provocation tests (6 to 20.7 ppm) were quite high, the authors of these studies suggested that formaldehyde-induced bronchial hyperreactivity is due to specific sensitization to the gas. However, none of these studies was able to detect antibodies to formaldehyde which would support that sensitization to formaldehyde occurs through an immunologic pathway. Alternatively, the wheezing and shortness of breath may be related to the formaldehyde-stimulated depletion of the bronchodilator, GSNO, in the airways.

In controlled studies with asthmatics from urea-formaldehyde insulated homes, formaldehyde concentrations equal to or greater than those found in indoor environments have not resulted in hematologic or immunologic abnormalities. These tests include: blood count and differential, erythrocyte sedimentation rate; lymphocyte subpopulations (E-rosetting, T3, T4, T8, B73.1, Fc receptor positive lymphocytes and large granular lymphocytes); lymphocyte response to phytohemagglutinin and formalin-treated red blood cells; serum antibody against the Thomsen-Friedenrich RBC antigen and against formalin-RBC; and natural killer, interferon-boosted natural killer, and antibody-dependent cell-mediated cytotoxicity (Pross et al., 1987). While six of the studies cited above reported decrements in lung function associated with short-term formaldehyde exposure among at least some of the asthmatic subjects, a number of other exposure studies of patients with asthma have failed to demonstrate that exposure to formaldehyde results in onset or aggravation of the patients' asthmatic symptoms (Sheppard et al., 1984; Sauder et al., 1987; Harving et al., 1990; Krakowiak et al., 1998).

The effects of formaldehyde on asthmatics may be dependent on previous, repeated exposure to formaldehyde. Burge et al. (1985) found that 3 out of 15 occupationally exposed workers challenged with formaldehyde vapors at concentrations from 1.5 ppm to 20.6 ppm for brief durations exhibited late asthmatic reactions. Six other subjects had immediate asthmatic reactions likely due to irritant effects. Asthmatic responses (decreased PEF, FVC, and FEV_1) were observed in 12 occupationally-exposed workers

challenged with 2.0 ppm (2.5 mg/m^3) formaldehyde (Nordman et al., 1985). Similarly, asthmatic responses were observed in 5 of 28 hemodialysis workers occupationally exposed to formalin and challenged with formaldehyde vapors (concentration not measured) (Hendrick and Lane, 1977). In asthmatics not occupationally exposed to formaldehyde, Sheppard et al. (1984) found that a 10-minute challenge with 3 ppm formaldehyde coupled with moderate exercise did not induce significant changes in airway resistance or thoracic gas volume.

Gorski et al. (1992) evaluated the production of active oxygen species by neutrophils in 18 persons exposed to 0.5 mg/m^3 formaldehyde for 2 hours. All 13 subjects who had allergic contact dermatitis (tested positive to formaldehyde in skin patch) exhibited significantly higher chemiluminescence of granulocytes isolated from whole blood 30 minutes and 24 hours post-exposure than the individuals who were not formaldehyde sensitive. Thus, the immune cellular response of skin-sensitized individuals to an inhalation exposure to formaldehyde indicates increased production of active oxygen species. This is consistent with increasing evidence that endogenous or exogenous reactive oxygen and reactive nitrogen species are responsible for the airway inflammation of asthma (Sugiura and Ichinose, 2008).

In addition to its effects on the respiratory tract, the irritant properties of formaldehyde also manifest as ocular irritation. In an anatomy dissecting laboratory, formaldehyde levels were found to peak at 0.62 ppm, with a gradual decrease to 0.11 ppm. Formaldehyde-related irritation of the eyes, nose, throat, airways and skin was reported by 59% of the students. These effects were significantly ($p < 0.001$) higher among wearers of contact lenses compared with students without glasses or wearing glasses (Tanaka et al., 2003). The ability of contact lenses to trap and concentrate volatile compounds, and to extend the exposure time by limiting the eye's normal self-cleansing, may make contact lens wearers more susceptible to ocular exposure and irritation by formaldehyde.

5.2 Acute Toxicity to Infants and Children

No studies of the effects of acute exposure to formaldehyde in children or young experimental animals were located. However, as noted above for adults, there is evidence that following acute exposure to formaldehyde, asthmatics and others previously sensitized to formaldehyde may be more likely to show respiratory symptoms such as wheezing, shortness of breath, rhinitis, and/or decrements in pulmonary function consistent with immediate and/or delayed bronchoconstriction (Nordman et al., 1985; Burge et al., 1985; Hendrick and Lane, 1977; Wallenstein et al., 1978). Furthermore, some asthmatics may respond with significant reductions in lung function due to the irritant effects on asthma, sensitized or not. Additionally, the depletion of the endogenous bronchodilator, GSNO, following formaldehyde exposure may be particularly important in children. Gaston et al. (1998) compared concentrations of tracheal S-nitrosothiol concentrations in eight asthmatic children in respiratory failure with those of 21 non-asthmatic children undergoing elective surgery. In asthmatic children, the metabolism of GSNO was accelerated and the mean S-nitrosothiol concentrations significantly lower compared to normal children (65 ± 45 vs 502 ± 429

nmol/l). Thus asthmatic children, with low levels of GSNO, are expected to be unusually vulnerable to any further depletion of GSNO caused by formaldehyde.

The potential association between formaldehyde exposure and asthma is of special concern for children since, as noted in OEHHA (2001): "*OEHHA considers asthma to impact children more than adults. Children have higher prevalence rates of asthma than do adults (Mannino et al., 1998). In addition, asthma episodes can be more severe due to the smaller airways of children, and result in more hospitalizations in children, particularly from the ages of 0 to 4 years, than in adults (Mannino et al., 1998).*" Thus children, particularly asthmatic children, may be at greater risk from acute exposure to formaldehyde.

5.3 Acute Toxicity to Experimental Animals

Acute exposures of experimental animals to formaldehyde are associated with changes in pulmonary function (decreased respiratory rate, increased airway reactivity and resistance) at low concentrations, while pulmonary edema and death have been reported at high concentrations. Neurochemical and neurobehavioral changes have also been observed.

In 72 rats exposed to approximately 600-1,700 mg/m^3 (500-1,400 ppm) formaldehyde vapor for 30 minutes, the LC_{50} was found to be 1,000 mg/m^3 (800 ppm) (Skog, 1950). The first deaths did not occur until 6 hours after cessation of exposure. Respiratory difficulty lasted several days after exposure and the last of 49 rats died after 15 days of purulent bronchitis and diffuse bronchopneumonia. Three weeks following exposure, histological examinations of the 23 surviving animals revealed bronchitis, pulmonary microhemorrhages, and edema. No changes were seen in other organs.

A multispecies study by Salem and Cullumbine (1960) showed that a 10-hour exposure to 15.4 ppm (19 mg/m^3) formaldehyde vapor killed 3 out of 5 rabbits, 8 of 20 guinea pigs, and 17 of 50 mice. The report stated that formaldehyde exposure resulted in delayed lethality.

Alarie (1981) determined the 10 minute LC_{50} for formaldehyde in mice to be 2,162 ppm (95% confidence interval, 1,687-2,770 ppm). The post-exposure observation period was 3 hours. From the concentration mortality graph provided in the report, an MLE_{05} and BC_{05} of 1,440 ppm and 778 ppm, respectively, could be estimated for a 10-minute formaldehyde exposure. However, as indicated in the previous reports, delayed deaths occur with formaldehyde which suggests that the 3-hour post-exposure observation period used in this study may not have been long enough.

In other lethality studies, Nagornyi et al.(1979) determined a 4-hour formaldehyde LC_{50} in rats and mice to be 588 mg/m^3 (474 ppm) and 505 mg/m^3 (407 ppm), respectively. However, the raw data for this study were not included in the report. Horton et al. (1963) observed that a 2-hour exposure of mice to 0.9 mg/l (900 mg/m^3) formaldehyde resulted in deaths from massive pulmonary hemorrhage and edema, but a 2 hour exposure to 0.14 mg/l (140 mg/m^3) did not produce signs of "substantial distress."

Swiecichowski et al., (1993) exposed groups of five to seven guinea pigs to 0.86, 3.4, 9.4, 31.1 ppm (1.1, 4.2, 11.6, 38.6 mg/m^3) formaldehyde for 2 hours, or to 0.11, 0.31, 0.59, 1.05 ppm (0.14, 0.38, 0.73, 1.30 mg/m^3) formaldehyde for 8 hours. An 8-hour exposure to levels greater than or equal to 0.3 ppm ($\geq$ 0.4 mg/m^3) formaldehyde was sufficient to produce a significant increase in airway reactivity. Similar effects occurred after greater than 9 ppm (> 11 mg/m^3) formaldehyde for the 2-hour exposure group. Formaldehyde exposure also heightened airway smooth muscle responsiveness to acetylcholine (or carbachol) *ex vivo*. No inflammation or epithelial damage was seen up to 4 days after exposure. The researchers suggest that duration of exposure is important to the induction of airway hyperreactivity and that prolonged (8-hour), low-level exposures may generate abnormal physiologic responses in the airways not detectable after acute (2-hour) exposures.

Male F-344 rats, 7-9 weeks old, were exposed to 0.5, 2, 6 or 15 ppm formaldehyde for 6 hours per day for 1 to 4 days (Monteiro-Riviere and Popp, 1986). Effects noted in the rat nasal respiratory epithelium with 0.5 or 2 ppm were limited to altered cilia with occasional wing-like projections on the ends of the ciliary shafts. Effects noted at 6 ppm for 1 day were autophagic vacuoles in some basal cells, neutrophils in the basal and suprabasal layers, and hypertrophy of goblet and ciliated cells. Loss of microvilli in ciliated cells was noted at all exposure concentrations.

Rats were exposed to 0, 5, 10 or 20 ppm formaldehyde for 3 hours per day on 2 consecutive days (Boja et al., 1985). Decreased motor activity and neurochemical changes in dopamine and 5-hydroxytryptamine neurons were reported.

The effects of formaldehyde inhalation on open-field behavior in mice were examined by Malek et al. (2004) 2 and 24 hours after a single 2-hour exposure to 0, 1.1, 2.3 or 5.2 ppm. Two hours after exposure there were significant decreases in rearing and in several measures of exploratory behavior, with evidence of dose-dependence in all dose groups compared with controls. At 24 hours, there were still significant differences between dosed and control mice but the dose-dependence was no longer evident.

Nielson et al. (1999) analyzed the breathing patterns of Balb/c mice exposed to 0.2-13 ppm formaldehyde and found a concentration-dependent decrease in respiratory rate of 32.9%/log concentration. In the range of 0.3-4.0 ppm, the decrease in respiratory rates was attributable to sensory irritation. Above 4.0 ppm, bronchoconstriction also contributed to the decreased breathing rate. The authors suggest a NOEL of 0.3 ppm for these effects in mice.

Amdur (1960) exposed groups of 4 to 18 guinea pigs to formaldehyde at 0.05, 0.31, 0.58, 1.22, 3.6, 11.0, or 49 ppm formaldehyde for one hour. Resistance to flow and lung compliance were calculated from measures of intrapleural pressure, tidal volume, and rate of flow to the lungs at the end of exposure and one hour later. Resistance and compliance were significantly different from the control level for the 0.31 ppm exposure ($p<0.05$) and increasingly significant at higher concentrations. One hour later, only the 49 ppm exposure remained significant ($p<0.01$). In addition, the tracheas of groups of 6 to 10 guinea pigs were cannulated and exposed for one hour to 0.90, 5.2, 20, or 50 ppm

formaldehyde, and 1.14 or 3.6 ppm formaldehyde with 10 mg/m^3 sodium chloride. With the protective effect of the trachea bypassed, the resistance and compliance changed substantially. The addition of sodium chloride further enhanced the effect, including a significant effect after one hour for the 1.14 ppm formaldehyde exposure. These results show that formaldehyde that reaches the lungs has a marked effect on airways resistance and compliance in addition to an effect on the upper airways.

Riedel et al. (1996) studied the influence of formaldehyde exposure on allergic sensitization in guinea pigs. Three groups of guinea pigs (12/group) were exposed to clean air or two different formaldehyde concentrations (0.13 and 0.25 ppm) over five consecutive days. Following exposure, the animals were sensitized to allergen by inhalation of 0.5% ovalbumin (OA). Three weeks later the animals were subjected to bronchial provocation with OA and specific anti-OA-IgGl (reaginic) antibodies in serum were measured. In another group of six animals, the respiratory tract was examined histologically for signs of inflammation directly after the end of formaldehyde or clean air exposure. In the group exposed to 0.25 ppm formaldehyde, 10/12 animals were found to be sensitized to OA (positive reaction on specific provocation) vs. 3/12 animals in the control group ($P < 0.01$). Furthermore, compressed air measurements of specific bronchial provocation and serum anti-OA-antibodies were significantly higher in the 0.25 ppm formaldehyde group than in controls. The median for compressed air measurement was 0.35 ml for the formaldehyde-exposed group vs. 0.09 ml for the controls ($p< 0.01$), indicating increased bronchial obstruction. The median for the anti-OA-IgGl measured in the formaldehyde-exposed group was 13 vs. less than 10 EU in the controls, ($p < 0.05$), indicating enhanced sensitization. In the group exposed to 0.13 ppm formaldehyde, no significant difference was found compared to the control group. Histological examination found edema of the bronchial mucosa, but there was no sign of inflammation of the lower airways in formaldehyde-exposed guinea pigs. The investigators concluded that short-term exposure to a low concentration of formaldehyde (0.25 ppm) can significantly enhance sensitization to inhaled allergens in the guinea pig.

As described in Section 5, the main formaldehyde-metabolizing enzyme, ADH3, also reduces the endogenous bronchodilator GSNO. To examine the role of GSNO and ADH3 (known in this study as GSNO reductase, GSNOR) in airway tone and asthma, Que et al. (2005) used wild type mice and mice with a targeted deletion of GSNOR ($GSNOR^{-/-}$). Following a challenge with allergen (ovalbumin), GSNOR activity in bronchoalveolar lavage fluid from wild type mice increased significantly ($p < 0.05$) compared to buffer (PBS) controls, while as expected, no GSNOR activity was detected in the $GSNOR^{-/-}$ mice with either treatment. Levels of S-nitrosothiols (SNO) were assayed in homogenates of lung tissues from both types of mouse and found to be barely detectable with PBS treatment. However, after ovalbumin challenge, SNO levels were significantly higher ($p < 0.02$) in $GSNOR^{-/-}$ mice compared to wild type, indicating metabolism of SNOs by GSNOR under “asthmatic-like” conditions in wild type mice. Metabolism of GSNO results in a loss of bronchodilation capacity. Deletion of GSNOR had no effect on NO generation by NO synthase as there were no differences between wild type and $GSNOR^{-/-}$ mice in nitrate or nitrite levels regardless of treatment. To investigate the effects of deletion of GSNOR on airway hyper-responsivness, pulmonary

resistance was measured at baseline (PBS) and after methacholine challenge, with and without ovalbumin treatment. At baseline, there was no difference among mouse types and treatments, while at higher methacholine levels (100-1000 µg/kg), pulmonary resistance was found to be significantly lower ($p < 0.001$) in $GSNOR^{-/-}$ mice than in wild type, presumably due to higher GSNO levels that enhance bronchodilation. Importantly, ovalbumin caused a marked increase in airway responsiveness in wild type mice but had little effect in $GSNOR^{-/-}$ mice. This indicates that GSNOR regulates basal airway tone as well as hyper-responsiveness to both allergen challenge and bronchoconstrictor agonists. It is also noteworthy that the total number and composition of leukocytes, levels of interleukin-13 and total serum IgE were comparable between wild type and $GSNOR^{-/-}$ mice. This indicates that protection from asthma in the $GSNOR^{-/-}$ mice is not a result of a suppressed response to allergen, and that SNOs, especially GSNO, can preserve airway patency in the presence of inflammation. Thus the inflammatory response is not linked to hyper-responsiveness as long as adequate levels of GSNO are maintained.

A connection between formaldehyde and the activity of GSNOR described in the study above by Que et al., was outlined by Thompson and Grafstrom (2008) and supported by Yi et al. (2007) and Staab et al. (2008). In the study by Yi and associates, groups of 6 mice were exposed to formaldehyde at 0, 1, or 3 mg/m^3 continuously for 72 hours. Following exposure, lungs were isolated to allow measurement of GSNOR mRNA levels by RT-PCR, and enzymatic activity with GSNO. Formaldehyde at 3 mg/m^3 significantly increased the numbers of GSNOR transcripts compared to control (0.58 vs 0.4 GSNOR/ß actin; $p < 0.05$), while GSNOR reduction of GSNO showed a significant dose-dependent increase with formaldehyde concentration ($p < 0.01$). The stimulation of GSNO reduction by formaldehyde was also observed by Staab et al. (2008) in an in vitro study using recombinant human GSNOR. In this study, GSNO levels in buccal carcinoma cells were reduced in a dose-dependent fashion following a 1 hour exposure to formaldehyde in the 1-5 mM range with significance at 5 mM ($p < 0.05$). The results from this study support a model in which formaldehyde (as the glutathione conjugate, HMGSH) is oxidized by GSNOR (ADH3) in the presence of high levels of NAD^+, producing NADH. This process was found to be accelerated by high levels of GSNO. GSNO is in turn reduced with the oxidation of NADH to form glutathione sulfonimide. Formaldehyde thus depletes cellular SNO (in the form of GSNO) which results in dysregulation of NO signaling pathways.

6. Chronic Toxicity of Formaldehyde

6.1 Chronic Toxicity to Adult Humans

Formaldehyde primarily affects the mucous membranes of the upper airways and eyes. Exposed populations that have been studied include embalmers, residents in houses insulated with urea-formaldehyde foam, anatomy class students, histology technicians, wood and pulpmill workers, and asthmatics. A number of studies describing these effects have been briefly summarized below. For the sake of brevity, only the studies that best represent the given effects are presented. Formaldehyde is also a recognized carcinogen (IARC, 2006), however, this document will address only its non-carcinogenic properties.

In the study chosen for determination of the 8-hour and chronic RELs, nasal obstruction and discharge, and frequency of cough, wheezing, and symptoms of bronchitis were reported in 66 workers in a formaldehyde production plant exposed for 1-36 years (mean = 10 years) to a mean concentration of 0.21 ppm (0.26 mg/m^3) formaldehyde (Wilhelmsson and Holmstrom, 1992). All workers were exposed almost exclusively to formaldehyde, the concentrations of which were measured in the ambient air of the worksite with personal sampling equipment. Referents consisted of 36 office workers in a government office with exposure to a mean concentration of 0.07 ppm (0.09 mg/m^3) formaldehyde, and no industrial solvent or dust exposure. Symptom data, collected by questionnaire, were separated into general and work-related, and allowed identification of individuals with atopy and mucosal hyperreactivity. The critical effects from chronic exposure to formaldehyde in this study included nasal obstruction, lower airway discomfort, and eczema or itching. The frequency of reported lower airway discomfort (intermittent cough, wheezing, or symptoms of chronic bronchitis) was significantly higher among formaldehyde-exposed vs non-exposed workers (44 vs 14%; $p < 0.01$) (Table 6.1). Work-related nasal discomfort also was significantly higher in the formaldehyde group (53%) compared with the referent group (3%; $p < 0.001$). Similarly, work-related eye discomfort was 20% in the formaldehyde group but nonexistent among referents. The significant increase in symptoms of nasal discomfort in exposed workers did not correlate with total serum IgE antibody levels. However, two exposed workers, who complained of nasal discomfort, had elevated IgE levels. The investigators concluded that formaldehyde can induce nonspecific nasal hypersensitivity.

Table 6.1.1 Symptoms of Formaldehyde Exposure vs Reference Group (from Wilhelmsson and Holmstrom, 1992)

	Formaldehyde	Reference	Rate difference	
	% (n=66)	% (n=36)	%	95% CI
General nasal discomfort	67	25	42	24-60
Workplace nasal discomfort	53	3	50	37-63
General lower airway discomfort	44	14	30	14-47
Workplace lower airway discomfort	33	3	28	15-40
General eye discomfort	24	6	18	6-36
General skin discomfort	36	11	25	10-41

In a cross-sectional study supportive of these results, Edling et al. (1988) reported histopathological changes in nasal mucosa of workers (n=75) occupationally exposed to formaldehyde (one wood laminating plant) or formaldehyde plus wood dust (two particle board plants). Ambient formaldehyde measurements in these three composite wood processing plants between 1975 and 1983 gave a time-weighted average (TWA) of 0.1-1.1 mg/m^3 (0.08- 0.89 ppm) with peaks of up to 5 mg/m^3 (4 ppm). The exposed workers were compared on the basis of medical and work histories, clinical examinations and nasal biopsies to 25 workers selected with regard to age and smoking habits but without occupational formaldehyde exposure.

Based on the histories, there was a high frequency of eye and upper airway symptoms among workers. Nasal symptoms (running nose and crusting) associated with formaldehyde exposure were reported in 60% of the workers, while 75% complained of lacrimation. Clinical examinations revealed grossly normal nasal mucosa in 75% of the cases while 25% had swollen or dry changes, or both, to the nasal mucosa. Histological examination (Table 6.2) revealed that only 3 of the 75 formaldehyde-exposed workers had normal, ciliated pseudostratified epithelium. Squamous metaplasia was reportedly observed in 59, while 6 showed mild dysplasia, and in 8 there was loss of ciliated cells and goblet cell hyperplasia. The histological grading showed a significantly higher score for nasal lesions among workers with formaldehyde exposure when compared with the referents (2.9 versus 1.8; $p < 0.05$). Exposed smokers had a higher, but non-significant, score than ex-smokers and non-smokers.

While the mean exposure time was 10.5 years (range 1-39 yr), there was no discernable difference among histology scores as a function of years of employment. The histology scores were also not different between workers in the particle board plants, exposed to both formaldehyde and wood dust, and workers in the laminate plant with exposure only to formaldehyde. The authors thus attribute the pathological changes in the nasal mucosa and the other adverse effects to formaldehyde alone in the 0.1-1.1 mg/m^3 range.

Table 6.1.2 Distribution of Histological Characteristics Associated with Formaldehyde Exposure (from Edling et al., 1988)

Histological characteristic	Grading score	Point score	Workers	%
Normal respiratory epithelium	0	0	3	4
Loss of ciliated cells	1	1	8	11
Mixed cuboidal/squamous epithelium, metaplasia	2	2	24	32
Stratified squamous epithelium	3	3	18	24
Keratosis	4	4	16	21
Budding of epithelium	add 1	5	0	0
Mild or moderate dysplasia	6	6	6	8
Severe dysplasia	7	7	0	0
Carcinoma	8	8	0	0

Histological changes in the nasal mucosa of formaldehyde-exposed workers were also reported by Boysen et al. (1990). In this study, nasal biopses were collected from 37 workers with 5 or more years of occupational formaldehyde exposure (0.5 - > 2 ppm) and compared with age-matched, unexposed controls who otherwise had similar environmental exposures and smoking habits. Histological changes in the nasal epithelium were scored as indicated in Table 6.1.3.

Table 6.1.3 Types of Nasal Epithelia and Scoring (from Boysen et al., 1990)

Types of epithelia	Histological score
Pseudostratified columnar	0
Stratified cuboidal	1
Mixed stratified cuboidal/stratified squamous	2
Stratified squamous, non-keratinizing	3
Stratified squamous, keratinizing	4
Dysplasia	5

As shown by the histological scoring in Table 6.1.4 below, metaplastic changes in the nasal epithelium were more pronounced in the formaldehyde-exposed workers although this difference did not reach statistical significance.

Table 6.1.4 Histological Scores of Nasal Epithelia

	Histological score							
	No	**0**	**1**	**2**	**3**	**4**	**5**	**Mean**
Exposed	37	3	16	5	9	1	3	1.9
Controls	37	5	17	10	5	0	0	1.4

Rhinoscopical examination revealed hyperplastic nasal mucosa in 9 of 37 formaldehyde-exposed workers but in only 4 of the controls. In addition, the incidence of subjective nasal complaints was significantly ($p < 0.01$) higher in the exposed group. While the small size of this study, and the small amount of the nasal mucosa accessible to biopsy limited its ability to detect formaldehyde- related histopathology, the results are consistent with the histopathologies reported by Edling et al. above.

In another occupational health study (Grammer et al., 1990), 37 workers, who were exposed for an unspecified duration to formaldehyde concentrations in the range of 0.003 to 0.073 ppm, reported ocular irritation. However, no significant serum levels of IgE or IgG antibodies to formaldehyde-human serum albumin were detected.

Kerfoot and Mooney (1975) reported that estimated formaldehyde exposures of 0.25-1.39 ppm evoked numerous complaints of upper respiratory tract and eye irritation among seven embalmers at six different funeral homes. Three of the seven embalmers in this study reportedly had asthma. Levine et al. (1984) examined the death certificates of 1477 Ontario undertakers. Exposure measurements taken from a group of West Virginia embalmers were used as exposure estimates for the embalming

process, ranging from 0.3-0.9 ppm (average 1-hour exposure) and 0.4-2.1 ppm (peak 30-minute exposure). Mortality due to non-malignant diseases was significantly elevated due to a two-fold excess of deaths related to the digestive system. The authors suggest increased alcoholism could have contributed to this increase.

Ritchie and Lehnen (1987) reported a dose-dependent increase in health complaints (eye and throat irritation, and headaches) in 2000 residents living in 397 mobile and 494 conventional homes. Complaints of symptoms of irritation were noted at concentrations of 0.1 ppm formaldehyde or above. Similarly, Liu et al. (1991) found that exposure to 0.09 ppm (0.135 mg/m^3) formaldehyde exacerbated chronic respiratory and allergy problems in residents living in mobile homes.

Employees of mobile day-care centers (66 subjects) reported increased incidence of eye, nose and throat irritation, unnatural thirst, headaches, abnormal tiredness, menstrual disorders, and increased use of analgesics as compared to control workers (Olsen and Dossing, 1982). The mean formaldehyde concentration in these mobile units was 0.29 ppm (0.43 mg/m^3) (range = 0.24 - 0.55 mg/m^3). The exposed workers were exposed in these units for a minimum of 3 months. A control group of 26 subjects in different institutions was exposed to a mean concentration of 0.05 ppm (0.08 mg/m^3) formaldehyde.

Occupants of houses insulated with urea-formaldehyde foam insulation (UFFI) (1726 subjects) were compared with control subjects (720 subjects) for subjective measures of irritation, measures of pulmonary function (FVC, FEV_1, FEF_{25-75}, FEF_{50}), nasal airway resistance, odor threshold for pyridine, nasal cytology, and hypersensitivity skin-patch testing (Broder et al., 1988). The mean length of time of exposure to UFFI was 4.6 years. The mean concentration of formaldehyde in the UFFI-exposed group was 0.043 ppm, compared with 0.035 ppm for the controls. A significant increase in symptoms of eye, nose and throat irritation was observed in subjects from UFFI homes, compared with controls. No other differences from control measurements were observed.

Alexandersson and Hedenstierna (1989) evaluated symptoms of irritation, spirometry, and immunoglobulin levels in 34 wood workers exposed to formaldehyde over a four-year period. Exposure to 0.4 - 0.5 ppm formaldehyde resulted in significant decreases in FVC, FEV_1, and FEF_{25-75}. Removal from exposure for four weeks allowed for normalization of lung function in the non-smokers.

Kriebel et al. (2001) conducted a subchronic epidemiological study of 38 anatomy class students who, on average, were exposed to a geometric mean of 0.70 ± 2.13 ppm formaldehyde for two hours per week over fourteen weeks. After class, eye, nose and throat irritation was significantly elevated compared with pre-laboratory session exposures, with a one unit increase in symptom intensity/ppm formaldehyde. Peak respiratory flow (PEF) was found to decrease by 1%/ppm formaldehyde during the most recent exposure. Changes in PEF and symptom intensity following formaldehyde exposure were most pronounced during the first week of the semester but attenuated with time, suggesting partial acclimatization.

Histology technicians (280 subjects) were shown to have reduced pulmonary function, as measured by FVC, FEV_1, FEF_{25-75}, and FEF_{75-85}, compared with 486 controls (Kilburn et al., 1989). The range of formaldehyde concentrations was 0.2 - 1.9 ppm, volatilized from formalin preservative solution.

Malaka and Kodama (1990) investigated the effects of formaldehyde exposure in plywood workers (93 exposed, 93 controls) exposed for 26.6 years, on average, to 1.13 ppm (range = 0.28 - 3.48 ppm). Fifty-three smokers were present in both exposed and control groups. Exposure assessment was divided into three categories: high (> 5 ppm), low (< 5 ppm), and none (reference group). Subjective irritation and pulmonary function tests were performed on each subject, and chest x-rays were taken of ten randomly selected volunteers from each group. Respiratory symptoms of irritation were found to be significantly increased in exposed individuals, compared with controls. In addition, exposed individuals exhibited significantly reduced FEV_1, FEV_1/FVC, and forced expiratory flow rate at 25% through 75% of FVC ($FEF_{25-75)}$, compared with controls. Forced vital capacity was not significantly reduced. Pulmonary function was not found to be different after a work shift, compared to the same measurement taken before the shift. No differences in chest x-rays were observed between exposed and control workers.

Occupational exposure to formaldehyde concentrations estimated to be 0.025 ppm (0.038 mg/m^3) for greater than six years resulted in complaints by 22 exposed workers of respiratory, gastrointestinal, musculoskeletal, and cardiovascular problems, and in elevated formic acid excretion in the urine (Srivastava et al., 1992). A control group of twenty seven workers unexposed to formaldehyde was used for comparison. A significantly higher incidence of abnormal chest x-rays was also observed in formaldehyde-exposed workers compared with controls.

Chemical plant workers (70 subjects) were exposed to a mean of 0.17 ppm (0.26 mg/m^3) formaldehyde for an unspecified duration (Holmstrom and Wilhelmsson, 1988). Compared with 36 control workers not exposed to formaldehyde, the exposed subjects exhibited a higher frequency of eye, nose, and deep airway discomfort. In addition, the exposed subjects had diminished olfactory ability, delayed mucociliary clearance, and decreased FVC.

Alexandersson et al. (1982) compared the irritant symptoms and pulmonary function of 47 carpentry workers exposed to a mean concentration of formaldehyde of 0.36 ppm (range = 0.04 - 1.25 ppm) with 20 unexposed controls. The average length of employment for the exposed workers was 5.9 years. Symptoms of eye and throat irritation as well as airway obstruction were more common in exposed workers. In addition, a significant reduction in FEV_1, FEV_1/FVC, and MMF was observed in exposed workers compared with controls.

Horvath et al. (1988) compared subjective irritation and pulmonary function in 109 workers exposed to formaldehyde with similar measures in a control group of 254 subjects. The formaldehyde concentrations for the exposed and control groups were 0.69 ppm (1.04 mg/m^3) and 0.05 ppm (0.08 mg/m^3), respectively. Mean formaldehyde

concentration in the pre-shift testing facility and the state (Wisconsin) ambient outdoor - formaldehyde level were both 0.04 ppm (0.06 mg/m^3). Duration of formaldehyde exposure was not stated. Subjects were evaluated pre- and post work-shift and compared with control subjects. Significant differences in symptoms of irritation, FEV_1, FEV_1/FVC ratio, FEF_{50}, FEF_{25}, and FEF_{75} were found when comparing exposed subjects' pre- and post work-shift values. However, the pre-workshift values were not different from controls.

The binding of formaldehyde to endogenous proteins creates haptens that can elicit an immune response. Chronic exposure to formaldehyde has been associated with immunological hypersensitivity as measured by elevated circulating IgG and IgE autoantibodies to human serum albumin (Thrasher et al., 1987). In addition, a decrease in the proportion of T-cells was observed, indicating altered immunity. Thrasher et al. (1990) later found that long-term exposure to formaldehyde was associated with autoantibodies, immune activation, and formaldehyde-albumin adducts in patients occupationally exposed, or residents of mobile homes or of homes containing particleboard sub-flooring. The authors suggest that the hypersensitivity induced by formaldehyde may account for a mechanism for asthma and other health complaints associated with formaldehyde exposure.

An epidemiological study of the effects of formaldehyde on 367 textile and shoe manufacturing workers employed for a mean duration of 12 years showed no significant association between formaldehyde exposure, pulmonary function (FVC, FEV_1, and PEF) in normal or asthmatic workers, and occurrence of specific IgE antibodies to formaldehyde (Gorski and Krakowiak, 1991). The concentrations of formaldehyde did not exceed 0.5 ppm (0.75 mg/m^3).

Workers (38 total) exposed for a mean duration of 7.8 years to 0.11 - 2.12 ppm (mean = 0.33 ppm) formaldehyde were studied for their symptomatology, lung function, and total IgG and IgE levels in the serum (Alexandersson and Hedenstierna, 1988). The control group consisted of 18 unexposed individuals. Significant decrements in pulmonary function, FVC ($p < 0.01$) and FEV_1 ($p < 0.05$)) were observed, compared with the controls. Eye, nose, and throat irritation was also reported more frequently by the exposed group. No correlation was found between duration of exposure, or formaldehyde concentration, and the presence of IgE and IgG antibodies.

As described in section 5.1, chronic or repeated exposure to formaldehyde may influence the response of asthmatics to acute or short-term challenges. In the study by Burge et al. (1985) late asthmatic reactions were noted in 3 out of 15 occupationally exposed workers after short-duration exposure to 1.5 – 20.6 ppm formaldehyde. Similarly, among workers with occupational exposure to formaldehyde, asthmatic responses (decreased PEF, FVC, and FEV_1) were reported in 12 workers challenged with 1.67 ppm (2.5 mg/m^3) formaldehyde (Nordman et al., 1985) and in 5 of 28 hemodialysis workers following challenge with formaldehyde vapors (concentration not measured) (Hendrick and Lane, 1977). In contrast, Sheppard et al. (1984) found that in asthmatics not occupationally exposed to formaldehyde, a 10-minute challenge with 3 ppm formaldehyde coupled with moderate exercise did not induce significant changes in

airway resistance or thoracic gas volume. Thus individuals with chronic formaldehyde exposure may be at greater risk for adverse responses to acute exposures. These individuals may have been sensitized immunologically, as in the cases of elevated circulating antibodies, or rendered neurologically hyperresponsive, following repeated or chronic exposures to formaldehyde (Sorg et al., 2001a,b).

6.2 Chronic Toxicity to Infants and Children

There are few studies that compare the effects of chronic formaldehyde exposure on children versus adults. Among those that do there is evidence that children are more susceptible to the adverse effects of chronic exposure. Krzyzanowski et al. (1990) assessed chronic pulmonary symptoms and function in 298 children (6-15 years of age) and 613 adults (> 15 years of age) in relation to measured formaldehyde levels in their homes. Information on pulmonary symptoms and doctor-diagnosed asthma and chronic bronchitis was collected by questionnaire. Pulmonary function was assessed as peak expiratory flow rates (PEFR) measured up to four times a day. The prevalence of chronic respiratory symptoms in children was not related to formaldehyde levels measured in tertiles (< 40, 41-60, > 60 ppb). However, doctor-diagnosed asthma and chronic bronchitis were more prevalent in houses with elevated formaldehyde (p for trend < 0.02). This effect was driven by the high disease prevalence observed in homes with kitchen formaldehyde levels >60 ppb, and was especially pronounced among children with concomitant exposure to environmental tobacco smoke (Table 6.2.1). By comparison, in adults, while the prevalence rates of chronic cough and wheeze were somewhat higher in houses with higher formaldehyde, none of the respiratory symptoms or diseases was significantly related to formaldehyde levels.

Table 6.2.1 Prevalence Rate (per 100) of Diagnosed Bronchitis and Asthma in Children with Formaldehyde (from Krzyzanowski et al., 1990)

	Formaldehyde (ppb)			**P value**
Bronchitis	**≤ 40 (N)**	**41-60 (N)**	**>60 (N)**	**X^2 trend**
Household mean	3.5 (258)	17.2 (29)	9.1 (11)	<0.02
Main room mean	3.2 (253)	15.6 (32)	9.1 (11)	<0.01
Bedroom mean	3.8 (262)	16.0 (25)	9.1 (11)	<0.04
Subject's bedroom	4.7 (256)	6.7 (30)	11.1 (9)	>0.35
Kitchen	3.5 (255)	0 (22)	28.6 (21)	<0.001
No ETS	4.3 (141)	0 (12)	10.0 (10)	>0.40
ETS	1.9 (106)	0 (10)	45.5 (11)	<0.001
Asthma				
All children	11.7 (256)	4.2 (24)	23.8 (21)	<0.03
No ETS	8.5 (142)	8.3 (12)	0 (10)	>0.50
ETS	15.1 (106)	0 (12)	45.5 (11)	<0.05

In a random effects model, Krzyzanowski et al. (1990) reported that lung function (PEFR) in children, but not adults, was significantly decreased by formaldehyde (coefficient ± SE: -1.28 ± 0.46 vs 0.09 ± 0.27). Measurements of PEFR in the morning suggested that children with asthma (n = 4) were more severely affected than healthy children (coefficient ± SE: -1.45 ± 0.53 vs 0.09 ± 0.15) (Table 6.2.2). Compared to

children, the effects of formaldehyde on pulmonary function in adults were smaller, transient, limited to morning measurements, and generally most pronounced among smokers exposed to the higher levels of formaldehyde. These studies suggest that children may be more susceptible to the effects of chronic formaldehyde exposure on lung function than are adults.

Table 6.2.2 Relation of PEFR (L/min) to Indoor Formaldehyde (from Krzyzanowski et al., 1990)

Factor	Child coefficient ± SE	Adult coefficient ± SE
HCHO house mean	-1.28 ± 0.46	0.09 ± 0.27
Morning vs bedtime	- 6.10 ± 3.0	-5.90 ± 1.10
HCHO bdrm mean/morning	0.09 ± 0.15	-0.07 ± 0.04
HCHO bdrm mean/morning/asthma	-1.45 ± 0.53	

Among studies of children only, a case-control study by Rumchev et al. (2002) examined risk factors for asthma among young children (6 mo- 3 yr). Cases included children with clinically-diagnosed asthma, and controls were children of the same age group without such a diagnosis. Formaldehyde levels were measured in the homes, once in summer and once in winter. Questionnaires were used to assess potential risk factors for asthma and to collect parental reports of respiratory symptoms characteristic of asthma (cough, shortness of breath, wheeze, runny nose, trouble breathing, and hay fever) in their children. Formaldehyde levels were higher in the homes of children exhibiting respiratory symptoms. Estimates of the relative risk for clinically-diagnosed asthma (odds ratios) were adjusted for measured indoor air pollutants, relative humidity, temperature, atopy, family history of asthma, age, gender, socioeconomic status, pets, smoke exposure, air conditioning, and gas appliances. Compared with children exposed to < 8 ppb, children in homes with formaldehyde levels > 49 ppb had a 39% higher risk of asthma ($p < 0.05$) after adjusting for common asthma risk factors.

Franklin et al. (2000) measured exhaled nitric oxide (eNO) levels in 224 children 6-13 years of age as an indicator of inflammation of the lower airways following chronic low-level formaldehyde exposure in the home. While there was no effect of formaldehyde on lung function measured by spirometry, eNO was significantly higher in children from homes with average formaldehyde levels ≥ 50 ppb compared with those from homes with levels ≤ 50 ppb (15.5 ppb eNO vs 8.7; $p = 0.02$).

Garrett et al. (1999) examined the association between formaldehyde levels at home (median 15.8 $\mu g/m^3$; maximum 139 $\mu g/m^3$) and atopy and allergic sensitization in 148 children, 7-14 years of age. The risk of atopy increased by 40% with each 10 $\mu g/m^3$ increase in bedroom formaldehyde. Two measures of allergic sensitization to twelve common environmental allergens, the number of positive skin prick tests and maximum wheal size, both showed linear associations with increasing maximum formaldehyde exposure levels. After adjusting for parental asthma and allergy, there was no evidence of an association between asthma in the children and formaldehyde levels. However, these data do suggest that formaldehyde levels commonly found in homes can enhance sensitization of children to common aeroallergens.

Of the numerous, primarily occupational, studies in adults, the NOAEL and LOAEL are 17 μg/m^3 (14 ppb) and 101 μg/m^3 (81 ppb), respectively, after adjustment for exposure continuity. These values are based on data on nasal and eye irritation as observed in Wilhelmsson and Holstrom (1992), and histological lesions in the nasal cavity as documented in Edling et al. (1988). However, studies in children, including the Krzyzanowski study above, indicate adverse health impacts in children at concentrations as low as 30 ppb. Wantke et al. (1996) reported that formaldehyde-specific IgE and respiratory symptoms were reduced when children transferred from schools with formaldehyde concentrations of 43 to 75 ppb to schools with concentrations of 23 to 29 ppb. While these human studies are not entirely consistent with each other, and there is potential for confounding in each, nevertheless, taken together, they suggest that children may be more sensitive to formaldehyde toxicity than adults.

A potential role for formaldehyde, GSNO and its metabolizing enzyme, GSNOR, in asthma is described in Section 5 above. The activity of GSNOR tends to be higher, and the levels of GSNO lower, in the lungs of asthmatics compared to non-asthmatics. This connection prompted Wu et al. (2007) to investigate whether genetic variation in GSNOR is associated with childhood asthma and atopy. The study group included 532 children, aged 4 to 17 with clinically diagnosed asthma, and their parents. Seven single nucleotide polymorphisms (SNPs) in GSNOR were genotyped in DNA extracted from lymphocytes to examine the relationship between common haplotypes and asthma. Atopy was determined with skin prick tests using a collection of 25 aeroallergens. Two of the GSNOR SNPs were associated with increased risk of asthma, but none was associated with atopy. Whereas a lower risk for asthma was associated with one (RR 0.77; 95% CI 0.61-0.97) or two (RR 0.66; 95% CI 0.44-0.99) copies of the minor A allele of SNP rs1154404, homozygosity for the major T allele of this SNP carried an increased risk of asthma. Homozygosity for the minor allele of SNP re28730619 also carried an increased risk of asthma (RR 1.60; 95% CI 1.13-2.26; p = 0.0077). In the haplotype analysis, children with the most common GSNOR haplotype (GTCGG), that contained the major T allele of rs1154404 and the minor G allele of rs28730619, were at increased risk of childhood asthma. These results thus suggest that variants in GSNOR genotype influence childhood asthma susceptibility.

It should be noted that while term neonates have high levels of reduced glutathione in the fluid lining the lungs, these levels drop rapidly after birth. However, among premature infants, glutathione levels are typically substantially below those of term infants (Grigg et al., 1993) and adults (Reise et al., 1997). As a result of low levels of a critical component of formaldehyde metabolism, glutathione, these infants may be at increased risk from formaldehyde exposure.

6.3 Chronic Toxicity to Experimental Animals

Studies of the effects of chronic formaldehyde exposure in experimental animals tend to focus on lesions in the upper respiratory tract and the hyperplastic or metaplastic changes observed in the respiratory epithelium. Systemic effects, such as changes in body or organ weight, or blood chemistry, appear to be secondary to the effects of the

olfactory irritation on feeding behavior. There is also evidence that repeated or long-term exposure to formaldehyde may cause neurologically-based hyperresponsiveness to formaldehyde (Sorg et al., 2001a) and altered expression of stress hormones (Sorg et al., 2001b).

In studies examining respiratory effects, Fischer-344 rats and B6C3F1 mice (120 animals/sex) were exposed to concentrations of 0, 2.0, 5.6, or 14.3 ppm formaldehyde vapor for 6 hours/day, 5 days/week for 24 months (Kerns et al., 1983). The exposure period was followed by up to six months of non-exposure. Interim sacrifices were conducted at 6, 12, 18, 24, 27, and 30 months. Both male and female rats in the 5.6 and 14.3 ppm groups demonstrated decreased body weights over the two-year period. At the 6 month sacrifice, the rats exposed to 14.3 ppm formaldehyde had non-neoplastic lesions of epithelial dysplasia in the nasal septum and turbinates. As the study progressed, epithelial dysplasia, squamous dysplasia, and mucopurulent rhinitis increased in severity and distribution in all exposure groups. In mice, cumulative survival decreased in males from 6 months to the end of the study. Serous rhinitis was detected at 6 months in the 14.3 ppm group of mice. Metaplastic and dysplastic changes were noted at 18 months in most rats in the 14.3 ppm group and in a few mice in the 5.6 ppm exposure group. By 24 months, the majority of mice in the 14.3 ppm group had metaplastic and dysplastic changes associated with serous rhinitis, in contrast to a few mice in the 5.6 ppm group and a few in the 2 ppm group (exact number not given).

Woutersen et al. (1989) exposed male Wistar rats (60 animals/group) 6 hours/day for 5 days/week to 0, 0.1, 1.0 and 10 ppm formaldehyde vapor for 28 months. Compound-related nasal lesions of the respiratory and olfactory epithelium were observed only in the 10 ppm group. In the respiratory epithelium, the lesions consisted of rhinitis, squamous metaplasia and basal cell/pseudoepithelial hyperplasia. In the olfactory region, the lesions included epithelial degeneration and rhinitis. No differences in behavior or mortality were noted among the various groups. However, growth retardation was observed in the 10 ppm group from day 14 onwards. In a parallel study, male Wistar rats were exposed to 0, 0.1, 1.0 and 10 ppm formaldehyde for 3 months followed by a 25-month observation period. Compound-related histopathological changes were found only in the noses of the 10 ppm group and comprised increased incidence of squamous metaplasia of the respiratory epithelium, and rhinitis.

In a chronic exposure study that primarily investigated aspects of nasal tumor development, Monticello et al. (1996) examined nasal cavities of male F-344 rats (0-10 ppm, 90 animals/group; 15 ppm, 147 animals) following exposure to 0, 0.7, 2, 6, 10, and 15 ppm formaldehyde for 6 hours/day, 5 days/week for 24 months. Treatment-related decreases in survival were apparent only in the 15 ppm group. Nasal lesions at the two highest doses included epithelial hypertrophy and hyperplasia, squamous metaplasia, and a mixed inflammatory cell infiltrate. Lesions in the 6 ppm group were minimal to absent and limited to focal squamous metaplasia in the anterior regions of the nasal cavity. No formaldehyde-induced lesions were observed in the 0.7 or 2 ppm groups.

Kamata et al. (1997) exposed 32 male F-344 rats/group to gaseous formaldehyde at 0, 0.3, 2, and 15 ppm 6 hours/day, 5 days/week for up to 28 weeks. A room control, non-exposed group was also included in the study. Five animals per group were randomly selected at the end of the 12, 18, and 24 months, and surviving animals at 28 months were sacrificed for full pathological evaluation. Behavioral effects related to sensory irritation were evident in the 15 ppm group. Significant decreases in food consumption, body weight and survival were also evident in this group. No exposure-related hematological findings were observed. Biochemical and organ weight examination revealed decreased triglyceride levels and absolute liver weights at the highest exposure, but was likely related to reduced food consumption. Abnormal histopathological findings were confined to the nasal cavity. Inflammatory cell infiltration, erosion or edema of the nasal cavity was evident in all groups, including controls. Significantly increased incidence of non-proliferative (squamous cell metaplasia without epithelial cell hyperplasia) and proliferative lesions (epithelial cell hyperplasia with squamous cell metaplasia) were observed in the nasal cavities beginning at 2 ppm. In the 0.3 ppm group, a non-significant increase in proliferative nasal lesions (4/20 animals) were observed in rats that were either sacrificed or died following the 18th month of exposure.

Rusch et al. (1983) exposed groups of 6 male cynomolgus monkeys, 20 male or female rats, and 10 male or female hamsters to 0, 0.2, 1.0, or 3.0 ppm (0, 0.24, 1.2, or 3.7 mg/m^3) formaldehyde vapor for 22 hours/day, 7 days/week for 26 weeks. There was no treatment-related mortality during the study. In monkeys, the most significant findings were hoarseness, congestion and squamous metaplasia of the nasal turbinates in 6/6 monkeys exposed to 2.95 ppm. There were no signs of toxicity in the lower exposure groups. In the rat, squamous metaplasia and basal cell hyperplasia of the nasal epithelia were significantly increased in rats exposed to 2.95 ppm. The same group exhibited decreased body weights and decreased liver weights. In contrast to monkeys and rats, hamsters did not show any signs of response to exposure, even at 2.95 ppm.

Kimbell et al. (1997) exposed male F-344 rats ($\leq$ 6/group) to 0, 0.7, 2, 6, 10, and 15 ppm 6 hr/day, 5 days/week for 6 months. Squamous metaplasia was not observed in any regions of the nasal cavity in any of the control, 0.7, or 2 ppm groups. However, the extent and incidence of squamous metaplasia in the nasal cavity increased with increasing dose beginning at 6 ppm.

In subchronic studies, Wilmer et al. (1989) found that intermittent (8 hours/day, 5 days/week) exposures of rats to 4 ppm formaldehyde for 13 weeks resulted in significant histological changes in the nasal septum and turbinates. In contrast, continuous exposure of rats for 13 weeks to 2 ppm formaldehyde did not produce significant lesions. This study revealed the concentration dependent nature of the nasal lesions caused by formaldehyde exposure. Zwart et al. (1988) exposed male and female Wistar rats (50 animals/group/sex) to 0, 0.3, 1, and 3 ppm formaldehyde vapor for 6 hr/day, 5 days/week for 13 weeks. Compound related histopathological nasal changes varying from epithelial disarrangement to epithelial hyperplasia and squamous metaplasia were found in the 3 ppm group, and were restricted to a small area of the

anterior respiratory epithelium. These changes were confirmed by electron microscopy and were not observed in other groups.

Woutersen et al. (1989) exposed rats (20 per group) to 0, 1, 10, or 20 ppm formaldehyde 6 hours/day, 5 days/week for 13 weeks. Rats exposed to 20 ppm displayed retarded growth, yellowing of the fur, and significant histological lesions in the respiratory epithelium. Exposure to 10 ppm did not affect growth, but resulted in significant histological lesions in the respiratory tract. No effects on specific organ weights, blood chemistries, liver glutathione levels, or urinalysis were detected at any level. No significant adverse effects were seen at the 1.0 ppm exposure level.

Appelman et al. (1988) found significant nasal lesions in rats (20 per group; 0, 0.1, 1.0, or 10.0 ppm) exposed to 10 ppm formaldehyde 6 hours/day, 5 days/week for 52 weeks, but exposure to 1.0 ppm or less for this period did not result in nasal histological lesions. However, the rats exposed to formaldehyde displayed decreased body weight in all groups compared with controls.

Apfelbach and Weiler (1991) determined that rats (5 exposed, 10 controls) exposed to 0.25 ppm (0.38 mg/m^3) formaldehyde for 130 days lost the olfactory ability to detect ethyl acetate odor.

Maronpot et al. (1986) exposed groups of 20 mice to 0, 2, 4, 10, 20, or 40 ppm formaldehyde 6 hours/day, 5 days/week, for 13 weeks. Histological lesions in the upper respiratory epithelium were seen in animals exposed to 10 ppm or greater. Exposure to 40 ppm was lethal to the mice.

A six-month exposure of rats to 0, 0.5, 3, and 15 ppm formaldehyde (3 rats per group) resulted in significantly elevated total lung cytochrome P450 in all formaldehyde-exposed groups (Dallas et al., 1989). The degree of P450 induction was highest after 4 days exposure and decreased slightly over the course of the experiment.

A series of studies have addressed the effects of long-term repeated exposures to formaldehyde on altered functioning of the hypothalamic-pituitary-adrenal (HPA) axis (Sorg et al., 2001b) and on neurobehavioral changes in rats (Sorg et al., 2001a). To study formaldehyde's effects on the HPA, Sorg et al. (2001b) measured corticosterone levels in the trunk blood of male Sprague-Dawley rats 20 or 60 min following acute chamber exposures to air or formaldehyde (0.7 or 2.4 ppm). All groups showed increased corticosterone levels above naive basal levels at 20 min followed by a return to baseline by 60 min, with no differences between treatment groups. A second experiment assessed the effects of repeated formaldehyde exposure (1 h/day, 5 days/week for 2 or 4 weeks) on basal corticosterone levels and those after a final challenge. Basal corticosterone levels were increased above naive values after 2 week exposure to air or 0.7 ppm formaldehyde. By 4 weeks, corticosterone levels in the air group returned to naive values, but remained elevated in the 0.7 ppm formaldehyde group. There were no differences in basal corticosterone levels among either 2.4 ppm exposed groups. After a final air or formaldehyde challenge, the 2 and 4 week air and 0.7 ppm formaldehyde groups had elevated corticosterone levels similar to their acute

response, while in the 2 and 4 week 2.4 ppm formaldehyde groups, corticosterone levels were higher than their acute response levels, indicating enhanced reactivity of the HPA axis to subsequent formaldehyde. It thus appears that repeated low-level formaldehyde exposure alters HPA axis functioning and the release of stress hormones. Since glucocorticoids may stimulate or inhibit the synthesis of surfactant-associated proteins in the lung (Liley et al., 1988), the alteration of HPA function may represent another pathway by which formaldehyde affects pulmonary function. For example, the pulmonary surfactants that regulate surface tension in the lungs are in turn regulated by surfactant-associated proteins. Reports of lower airway discomfort associated with chronic formaldehyde exposure may be related to the altered release or activity of these surfactant-associated proteins in the lung.

In another study of the effects of formaldehyde and the hypothalamus-pituitary-adrenal (HPA) axis, Sari et al. (2004) exposed female C3H/He mice to formaldehyde (0, 80, 400, 2000 ppb) by inhalation for 16 h/day, 5 days/week, for 12 weeks. Immunocytochemistry was used to examine corticotropin releasing hormone (CRH)-immunoreactive (ir) neurons in the hypothalamus, and adrenocorticotropin hormone (ACTH)-ir cells in the pituitary. RT-PCR was used to quantify ACTH rnRNA in the pituitary. Two groups of female mice were exposed, one of which comprised control mice with no allergen exposure. The other group was made allergic by injection of ovalbumin and alum prior to exposure to formaldehyde. Animals in the second group were further exposed to aerosolized ovalbumin as a booster four times during the exposure period. In the non-allergic group, formaldehyde caused a dose-dependent increase in the number of CRH-ir neurons with a similar pattern of increases in ACTHir cells and ACTH mRNA. The allergic mice showed an increase in basal levels of all these markers of HPA activity, and were responsive to the lowest concentration of formaldehyde. Thus at low levels of exposure, allergen and formaldehyde exposure exacerbate each other's effects on the stress response of the HPA.

7. Developmental and Reproductive Toxicity

In humans there are few data on the association of teratogenicity or adverse reproductive effects with formaldehyde exposure. Existing data do not suggest that formaldehyde, by inhalation or oral routes, produces significant teratogenic or reproductive effects (ATSDR, 1999).

A developmental toxicity study on formaldehyde was conducted by Martin (1990). Pregnant rats (25 per group) were exposed to 0, 2, 5, or 10 ppm formaldehyde for 6 hours/day, during days 6-15 of gestation. Although exposure to 10 ppm formaldehyde resulted in reduced food consumption and body weight gain in the maternal rats, no effects on the number, viability or normal development of the fetuses were seen. In addition, Saillenfait et al. (1989) exposed pregnant rats (25 per group) to 0, 5, 10, 20, or 40 ppm formaldehyde from days 6 - 20 of gestation. Maternal weight gain and fetal weight were significantly reduced in the 40 ppm exposure group. No significant fetotoxicity or teratogenic defects were observed at formaldehyde levels that were not also maternally toxic.

Evidence of embryotoxicity was reported by Kitaeva et al. (1990) in embryos of rats that had been exposed to formaldehyde by inhalation 4 h/d, 5 d/wk for 4 months. At 1.5 mg/m^3, but not at 0.5 mg/m^3, there was a significant increase in the proportion of degenerate embryos. By comparison, the bone marrow cells of the mothers appeared to be more sensitive to formaldehyde as shown by significant increases in the numbers of cells with aberrations, and the numbers of chromosomes with aberrations and aneuploidy at both dose levels.

In the context of developmental susceptibility to formaldehyde exposure, as noted above, the respiratory tract lining fluid (RTLF) protecting the lungs is often lower in glutathione levels than is the RTLF of adult lungs (Reise et al., 1997). This is especially true in premature infants who later develop chronic lung disease (Grigg et al., 1993). As glutathione is central to the lungs' antioxidant defenses, and is involved in the metabolism of inhaled formaldehyde, this relative deficiency may make the neonate's and infant's developing lungs more susceptible to toxic insult. It should be noted that ascorbate is also an important component of the lung's antioxidant defense, especially when glutathione levels are depressed (Jain et al., 1992). In healthy lungs, ascorbate helps to maintain glutathione levels. However, as is the case for glutathione, ascorbate levels in RTLF drop during the first week following birth (Vyas et al., 2001), potentially adding to the neonate's susceptibility to glutathione-reactive substances. Indeed, alterations in lung development following early life air toxicant exposure has been shown for environmental tobacco smoke (Wang and Pinkerton, 2007) and ozone (Plopper et al., 2007). Whether early life exposure to formaldehyde has similar effects on lung development remains to be demonstrated. However, there is concern that allergen exposure can modulate trophic interactions of conducting airway epithelial and interstitial wall components (Finkelstein and Johnston, 2004) and alter postnatal development of the airways (Plopper et al., 2007). This, coupled with the ability of formaldehyde to enhance the immune response to proteins/allergens with which it binds (Thrasher et al., 1987, 1990), may render developing lungs more susceptible to formaldehyde exposure. If evidence of such developmental effects associated with formaldehyde exposure becomes available, a re-evaluation of the REL for formaldehyde may be necessary.

8. Derivation of Reference Exposure Levels

8.1 Formaldehyde Acute Reference Exposure Level

Study	Kulle et al., 1987
Study population	19 nonasthmatic, nonsmoking humans
Exposure method	Whole body to 0.5-3.0 ppm
Exposure continuity	Single exposure per concentration
Exposure duration	3 hr
Critical effects	mild and moderate eye irritation
LOAEL	1 ppm
NOAEL	0.5 ppm
Benchmark concentration	0.44 ppm
Time-adjusted exposure	not applied
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	not applied
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	10
Reference Exposure Level	**55 µg/m^3 (44 ppb)**

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document).

Kulle et al (1987) was chosen as the critical study for the determination of the acute REL as it used a sensitive endpoint, eye irritation. It featured human subjects showing significant ($p < 0.05$) responses with short-term exposures to a range of formaldehyde concentrations, and the data permitted the use of a benchmark concentration (BMC) approach. As described in the technical support document, OEHHA recommends the use of the BMC approach whenever the available data support it as the BMC method provides a more statistically sound estimate of the point of departure in the REL determination.

The proposed acute REL was based on a $BMCL_{05}$ for eye irritation, estimated using log-probit analysis (Crump, 1984). The $BMCL_{05}$ is defined as the 95% lower confidence limit of the concentration expected to produce a response rate of 5%. The resulting

$BMCL_{05}$ from this analysis was 0.44 ppm (0.53 mg/m^3) formaldehyde. The endpoint of eye irritancy appears to be more a function of formaldehyde concentration rather than duration of exposure (Yang et al., 2001), so no time correction factor was applied. An uncertainty factor (UF_{H-k}) of 1 was used since sensory irritation is not expected to involve large toxicokinetic differences among individuals. Although the toxicological endpoint is eye irritation, the REL should protect against all possible adverse effects. The respiratory irritant effect, with documented potential to exacerbate asthma, is clearly an effect with the potential to differentially impact infants and children. In addition, the ability of formaldehyde to exacerbate the immune response to aeroallergens is of especial concern during development of the lungs. The toxicodynamic component of the intraspecies uncertainty factor UF_{H-d} is therefore assigned an increased value of 10 to account for potential asthma exacerbation. These considerations are applied equally to the acute, 8-hour and chronic REL.

As noted in Section 5.1, contact lens wearers appear to be at greater risk for ocular irritation with formaldehyde exposure. However, since contact lens users, and infants and children are generally mutually exclusive groups, it is expected that with the ten-fold toxicodynamic UF_{H-d} described above, the acute REL should be adequately protective of these individuals as well.

8.2 Formaldehyde 8-Hour Reference Exposure Level

Study	Wilhelmsson and Holmstrom, 1992
Study population	66 chemical plant workers
Exposure method	Discontinuous occupational exposure
Exposure continuity	8 hr/day, 5 days/week (assumed)
Exposure duration	10 years (average); range 1-36 years
Critical effects	Nasal obstruction and discomfort, lower airway discomfort, and eye irritation.
LOAEL	Mean 0.26 mg/m^3 (range 0.05 – 0.6 mg/m^3) (described as exposed group)
NOAEL	Mean of 0.09 mg/m^3 (described as control group of office workers)
Benchmark concentration	not derived
Time-adjusted exposure	0.09 mg/m^3 (time adjustment not applied)
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	1 (NOAEL observed)
Subchronic uncertainty factor (UFs)	not applied
Interspecies Uncertainty Factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF_{H-k})	1 (site of contact; no systemic effects)
Toxicodynamic (UF_{H-d})	10 (asthma exacerbation in children)
Cumulative uncertainty factor	10
Reference Exposure Level	**9 µg/m^3 (7 ppb)**

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the Technical Support Document).

The 8-hour REL is based on the occupational study by Wilhelmsson and Holmstrom (1992). This study evaluated the effects of formaldehyde on the upper airways of adult human subjects exposed to a mean formaldehyde concentration of 0.26 mg/m^3 during the work day compared with a referent group exposed to 0.09 mg/m^3. The critical effects in this study included nasal obstruction and discomfort, lower airway discomfort, and eye irritation. A NOAEL and a LOAEL may be derived from these data but no other dose-response information was provided. This study included only adults, but there is evidence that children may be more susceptible to long term exposures to formaldehyde than are adults. Thus, in the absence of child-specific data, an intraspecies uncertainty factor of 10 for toxicodynamic variability and developmental susceptibility was applied.

For comparison, the 8-hour REL of 9 µg/m³ is similar to the value of 10 µg/m^3 based on increased pulmonary resistance in guinea pigs following an 8 hr exposure to 0.11 – 1.05 ppm formaldehyde (Swiecichowski et al., 1993). The NOAEL of 0.59 ppm in guinea pigs was adjusted to a Human Equivalent Concentration (HEC) of 0.49 ppm with a regional gas dose ratio (RGDR) of 0.826. Use of the HEC adjustment entails an interspecies uncertainty factor of 6, while an intraspecies uncertainty factor of 10 addresses toxicodynamic variability.

Study	Swiecichowski et al., 1993
Study population	25-35 adult male guinea pigs
Exposure method	Whole body exposure
Exposure continuity	
Exposure duration	8 hr
Critical effects	Increased specific pulmonary resistance
LOAEL	1.0 ppm
NOAEL	0.59 ppm
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	0.49 ppm (610 µg/m^3) (0.59 * RGDR 0.826 for pulmonary effects)
LOAEL uncertainty factor (UF_L)	1 (default: NOAEL observed)
Subchronic uncertainty factor (UFs)	not applied
Interspecies Uncertainty Factor	
Toxicokinetic (UF_{A-k})	6 (with HEC adjustment)
Toxicodynamic (UF_{A-d})	1 (with HEC adjustment)
Intraspecies Uncertainty Factor	
Toxicokinetic (UF_{H-k})	1 (no systemic effect)
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	60
Reference Exposure Level	**10 µg/m³ (8 ppb)**

8.3 Formaldehyde Chronic Reference Exposure Level

Study	Wilhelmsson and Holmstrom, 1992 supported by Edling et al., 1988
Study population	66 human chemical plant workers
Exposure method	Discontinuous occupational exposure
Exposure continuity	8 hr/day, 5 days/week (assumed)
Exposure duration	10 years (average); range 1-36 years
Critical effects	Nasal obstruction and discomfort, lower airway discomfort.
LOAEL	Mean 0.26 mg/m^3 (range 0.05 – 0.6 mg/m3) (described as exposed group)
NOAEL	Mean of 0.09 mg/m^3 (described as control group of office workers)
Benchmark concentration	not derived
Time-adjusted exposure	0.09 mg/m^3 for NOAEL group
Human Equivalent Concentration	not applied
LOAEL uncertainty factor (UF_L)	not applied
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (no systemic effects)
Toxicodynamic (UF_{H-d})	10 (potential asthma exacerbation in children)
Cumulative uncertainty factor	10
Reference Exposure Level	**9 µg/m^3 (7 ppb)**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

The study by Wilhelmsson and Holmstrom (1992) was selected for development of the chronic REL as it investigated long-term exposure to formaldehyde relatively free of other confounding exposures. From this study it was possible to determine both a NOAEL and a LOAEL. Since this study included only adults, a combined intraspecies uncertainty factor of 10 for toxicodynamic variability was applied to account for the possibly greater susceptibility of children with long term exposures to formaldehyde.

The susceptibility of young children was examined in a study by Rumchev et al. (2002) that compared children (mean age 25 mo) with a clinical diagnosis of asthma to children without this diagnosis. The LOAEL used (60 µg/m^3) represents the formaldehyde level at which the authors found a statistically elevated risk for asthma-related respiratory symptoms. For this comparison, the NOAEL was taken to be 30 µg/m^3, the lower end of the NOAEL range. Intraspecies uncertainty factors of 3.16 for potential

toxicodynamic variability and 1 for toxicokinetic differences give a cumulative uncertainty factor of 3.16 for an inhalation chronic REL of 10 µg/m^3 (8 ppb), similar to the chronic REL calculated from the critical study.

Study	Rumchev et al., 2002
Study population	88 asthmatic children (mean age 25 mo); 104 nonasthmatic controls (mean age 20 mo)
Exposure method	Ambient in home
Exposure continuity	Continuous assumed
Exposure duration	range 0.5-3 years
Critical effects	Parent-reported respiratory symptoms (cough, shortness of breath, wheeze, trouble breathing)
LOAEL	60 µg/m^3
NOAEL	30 µg/m^3 (lower limit of NOAEL range)
Benchmark concentration	not derived
Time-adjusted exposure	not applied
Human Equivalent Concentration	30 µg/m^3
LOAEL uncertainty factor (UF_L)	1
Subchronic uncertainty factor (UFs)	not applied
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default, human study)
Toxicodynamic (UF_{A-d})	1 (default, human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	1 (study performed in children)
Toxicodynamic (UF_{H-d})	√10 (inter-individual variation)
Cumulative uncertainty factor	√10
Reference Exposure Level	**10 µg/m^3 (8 ppb)**

The Rumchev study supports an association with exposure to formaldehyde and the observation of asthma symptoms (cough, shortness of breath, wheeze, trouble breathing) in children. However, it was not selected for REL development due to the difficulties in distinguishing asthma from other wheezing conditions in the clinical diagnoses in such a young population. There are additional uncertainties associated with the exposure continuity, and the possibility of observational and/or recall bias in the parental reports of respiratory symptoms characteristic of asthma.

For comparison with the chronic REL of 9 µg/m^3 (7 ppb) presented above, Table 8.3.1 below presents a summary of potential formaldehyde RELs based on chronic and subchronic animal studies originally presented in OEHHA (2000). The toxicological endpoint was nasal lesions, consisting principally of rhinitis, squamous metaplasia, and dyplasia of the respiratory epithelium.

The most striking observation is the similarity of potential RELs among the rat chronic studies (exposures $\geq$ 26 weeks) that contain a NOAEL. The range of RELs from these animal studies, 1.5 – 24.9 ppb, includes the proposed REL (7 ppb) based on a human

study. Another related observation is that the NOAEL and LOAEL are similar among all the studies, regardless of exposure duration. The NOAEL and LOAEL are generally in the range of 1 - 4 ppm and 1 – 10 ppm, respectively, with the exception of the study by Kamata et al. (1997) that may be due to the absence of a dose level between 2 and 0.3 ppm. It is also of interest that the studies of Rusch et al (1983) indicate that monkeys and rats are of about the same sensitivity. In addition, the results of the Rusch studies suggest that, at least for the endpoint of squamous metaplasia, formaldehyde concentration is more important than the total dose since these animals, receiving more continuous exposure, exhibited the same adverse effects seen in studies using more intermittent exposures.

ATSDR has estimated minimum risk levels (MRLs), defined as “an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure” (ATSDR, 1999). For formaldehyde inhalation exposures they describe as “acute” (≤ 14 days), the MRL is 40 ppb based on a LOAEL of 0.4 ppm from a study by Pazdrak et al. (1993), and a 9-fold uncertainty factor (3 for use of a LOAEL; 3 for intraspecies variability). This exposure period is much longer than the acute REL of one hour, but the acute REL represents possibly repeated exposures. The MRL for an “intermediate” exposure period of 15-364 days is 30 ppb based on a NOAEL of 0.98 ppm for clinical signs of nasopharyngeal irritation and lesions in the nasal epithelium in monkeys (Rusch et al., 1983). A chronic MRL (≥ 365 d) of 8 ppb was developed based on damage to nasal epithelium in chemical factory workers (Holmstrom et al., 1989). This number is similar to the chronic REL of 7 ppb reported here. The MRLs are more similar to the chronic RELs developed by OEHHA in that they assume continuous exposure over the specified time period rather than regular but periodic exposures, as assumed for the 8-hour RELs considered above. For 8-hr exposures, NIOSH (1988) suggested a TWA 8-hr REL of 16 ppb based on sensory irritation.

8.4 Formaldehyde as a Toxic Air Contaminant

Formaldehyde was identified by the ARB as a toxic air contaminant (TAC) in accordance with sections 39660-39662 of the California Health and Safety Code on March 12, 1992 (Title 17, California Code of Regulations, section 93001)(CCR, 2007). In view of the differential impacts on infants and children identified in Section 6.2, OEHHA recommends that formaldehyde be listed as a toxic air contaminant which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

Table 8.3.1. Summary of Chronic and Subchronic Formaldehyde Studies in Experimental Animals

Study	Animal	Duration	Exposure	LOAEL ppm	NOAEL ppm	Time adj	DAF	LOAEL UF	UFak	UFad	UFhk	UFhd	UFsc	Cum UF	REL ppb	REL µg/m3
Woutersen 89	rat	28 mo	6 h 5 d	9.8	1	0.179	0.148	1	1	3.16	1	10	1	30	4.9	6.1
Kerns 83	rat	24 mo	6 h 5 d	2	n/a	0.357	0.296	6	1	3.16	1	10	1	200	1.5	1.8
Monticello 96	rat	24 mo	6 h 5 d	6.01	2.05	0.366	0.304	1	1	3.16	1	10	1	30	10.1	12.6
Kamata 97	rat	24-28 mo	6 h 5 d	2	0.3	0.054	0.044	1	1	3.16	1	10	1	30	1.5	1.8
Appelman 88	rat	52 wk	6 h 5 d	9.4	1	0.179	0.148	1	1	3.16	1	10	1	30	4.9	6.1
Rusch 83	rat	26 wk	22 h 7d	2.95	0.98	0.898	0.746	1	1	3.16	1	10	1	30	24.9	30.8
Kimbell 97	rat	26 wk	6 h 5 d	6	2	0.357	0.296	1	1	3.16	1	10	1	30	9.9	12.3
Wilmer 89	rat	13 wk	8 h 5 d	4	2	0.238	0.198	1	1	3.16	1	10	1	30	6.6	8.2
Woutersen 87	rat	13 wk	6 h 5 d	9.7	1	0.179	0.148	1	1	3.16	1	10	1	30	4.9	6.1
Zwart 88	rat	13 wk	6 h 5 d	2.98	1.01	0.180	0.15	1	1	3.16	1	10	1	30	5.0	6.2
Kerns 83	mouse	24 mo	6 h 5 d	5.6	2	0.357	0.296	1	2	3.16	1	10	1	60	4.9	6.1
Maronpot 86	mouse	13 wk	6 h 5 d	10.1	4.08	0.729	0.605	1	2	3.16	1	10	1	60	10.1	12.5
Rusch 83	monkey	26 wk	22 h 7d	2.95	0.98	0.898	not used	1	2	2	1	10	1	40	22.5	27.8

9. References

Akbar-Khanzadeh F, Vaquerano MU, Akbar-Khanzadeh M and Bisesi MS (1994). Formaldehyde exposure, acute pulmonary response, and exposure control options in a gross anatomy laboratory. Am J Ind Med 26(1): 61-75.

Alarie Y (1981). Toxicological evaluation of airborne chemical irritants and allergens using respiratory reflex reactions. . Proceedings of the inhalation toxicology and technology symposium.Ann Arbor Sciences, Inc. 207-231. Kalamazoo, MI, October 23-24, 1980.

Alexandersson R and Hedenstierna G (1988). Respiratory hazards associated with exposure to formaldehyde and solvents in acid-curing paints. Arch Environ Health 43(3): 222-7.

Alexandersson R and Hedenstierna G (1989). Pulmonary function in wood workers exposed to formaldehyde: a prospective study. Arch Environ Health 44(1): 5-11.

Alexandersson R, Hedenstierna G and Kolmodin-Hedman B (1982). Exposure to formaldehyde: effects on pulmonary function. Arch Environ Health 37(5): 279-84.

Amdur MO (1960). The response of guinea pigs to inhalation of formaldehyde and formic acid alone and with a sodium chloride aerosol. Int J Air Pollut 3: 201-20.

Apfelbach R and Weiler E (1991). Sensitivity to odors in Wistar rats is reduced after low-level formaldehyde-gas exposure. Naturwissenschaften 78(5): 221-3.

Appelman LM, Woutersen RA, Zwart A, Falke HE and Feron VJ (1988). One-year inhalation toxicity study of formaldehyde in male rats with a damaged or undamaged nasal mucosa. J Appl Toxicol 8(2): 85-90.

ATSDR. (1999). *Toxicological profile for formaldehyde*. Atlanta, GA: Agency for Toxic Substances and Disease Registry

http://www.atsdr.cdc.gov/toxprofiles/tp111.pdf.

Boja JW, Nielsen JA, Foldvary E and Truitt EB, Jr. (1985). Acute low-level formaldehyde behavioural and neurochemical toxicity in the rat. Prog Neuropsychopharmacol Biol Psychiatry 9(5-6): 671-4.

Boysen M, Zadig E, Digernes V, Abeler V and Reith A (1990). Nasal mucosa in workers exposed to formaldehyde: a pilot study. Br J Ind Med 47(2): 116-121.

Broder I, Corey P, Brasher P, Lipa M and Cole P (1988). Comparison of health of occupants and characteristics of houses among control homes and homes insulated with urea formaldehyde foam. III. Health and house variables following remedial work. Environ Res 45(2): 179-203.

Burge PS, Harries MG, Lam WK, O'Brien IM and Patchett PA (1985). Occupational asthma due to formaldehyde. Thorax 40(4): 255-60.

CARB (2005a). Annual Statewide Toxics Summary - Formaldehyde. Sacramento, CA. http://www.arb.ca.gov/adam/toxics/statepages/hchostate.html.

CARB. (2005b). *The California Almanac of Emissions and Air Quality - 2005 Edition*. California Air Resources Board. http://www.arb.ca.gov/aqd/almanac/almanac05/almanac05.htm.

CARB. (2006). *The California Almanac of Emissions and Air Quality - 2006 Edition*. California Air Resources Board. http://www.arb.ca.gov/aqd/almanac/almanac06/almanac2006all.pdf.

CCR (2007). California Code of Regulations Section 93001 Hazardous Air Pollutants Identified as Toxic Air Contaminants. Sacramento, CA: California Office of Administrative Law. 8-20-07. http://ccr.oal.ca.gov/linkedslice/default.asp?SP=CCR-1000&Action=Welcome.

Cross CE, van der Vliet A, O'Neill CA, Louie S and Halliwell B (1994). Oxidants, antioxidants, and respiratory tract lining fluids. Environ Health Perspect 102 Suppl 10: 185-91.

Crump KS (1984). A new method for determining allowable daily intakes. Fundam Appl Toxicol 4(5): 854-71.

Dallas CE, Badeaux P, Theiss JC and Fairchild EJ (1989). The influence of inhaled formaldehyde on rat lung cytochrome P450. Environ Res 49(1): 50-9.

Edling C, Hellquist H and Odkvist L (1988). Occupational exposure to formaldehyde and histopathological changes in the nasal mucosa. Br J Ind Med 45(11): 761-5.

Feinman SE (1988). Formaldehyde sensitivity and toxicity. Boca Raton (FL): CRC Press Inc.

Finkelstein JN and Johnston CJ (2004). Enhanced sensitivity of the postnatal lung to environmental insults and oxidant stress. Pediatrics 113(4 Suppl): 1092-6.

Franklin P, Dingle P and Stick S (2000). Raised exhaled nitric oxide in healthy children is associated with domestic formaldehyde levels. Am J Respir Crit Care Med 161(5): 1757-9.

Franks SJ (2005). A mathematical model for the absorption and metabolism of formaldehyde vapour by humans. Toxicology and Applied Pharmacology 206(3): 309-320.

Frigas E, Filley WV and Reed CE (1984). Bronchial challenge with formaldehyde gas: lack of bronchoconstriction in 13 patients suspected of having formaldehyde-induced asthma. Mayo Clin Proc 59(5): 295-9.

Garrett MH, Hooper MA, Hooper BM, Rayment PR and Abramson MJ (1999). Increased risk of allergy in children due to formaldehyde exposure in homes. Allergy 54(4): 330-7.

Gaston B, Sears S, Woods J, Hunt J, Ponaman M, McMahon T and Stamler JS (1998). Bronchodilator S-nitrosothiol deficiency in asthmatic respiratory failure. Lancet 351(9112): 1317-9.

Gorski P and Krakowiak A (1991). Formaldehyde--induced bronchial asthma--does it really exist? [Abstract]. Pol J Occup Med Environ Health 4(4): 317-20.

Gorski P, Tarkowski M, Krakowiak A and Kiec-Swierczynska M (1992). Neutrophil chemiluminescence following exposure to formaldehyde in healthy subjects and in patients with contact dermatitis. Allergol Immunopathol (Madr) 20(1): 20-3.

Grammer LC, Harris KE, Shaughnessy MA, Sparks P, Ayars GH, Altman LC and Patterson R (1990). Clinical and immunologic evaluation of 37 workers exposed to gaseous formaldehyde. J Allergy Clin Immunol 86(2): 177-81.

Green DJ, Sauder LR, Kulle TJ and Bascom R (1987). Acute response to 3.0 ppm formaldehyde in exercising healthy nonsmokers and asthmatics. Am Rev Respir Dis 135(6): 1261-6.

Grigg J, Barber A and Silverman M (1993). Bronchoalveolar lavage fluid glutathione in intubated premature infants. Arch Dis Child 69(1 Spec No): 49-51.

Harving H, Korsgaard J, Pedersen OF, Molhave L and Dahl R (1990). Pulmonary function and bronchial reactivity in asthmatics during low-level formaldehyde exposure. Lung 168(1): 15-21.

Hendrick DJ and Lane DJ (1977). Occupational formalin asthma. Br J Ind Med 34(1): 11-8.

Holmstrom M and Wilhelmsson B (1988). Respiratory symptoms and pathophysiological effects of occupational exposure to formaldehyde and wood dust. Scand J Work Environ Health 14(5): 306-11.

Holmstrom M, Wilhelmsson B and Hellquist H (1989). Histological changes in the nasal mucosa in rats after long-term exposure to formaldehyde and wood dust. Acta Otolaryngol 108(3-4): 274-83.

Horton AW, Tye R and Stemmer KL (1963). Experimental carcinogenesis of the lung. Inhalation of gaseous formaldehyde or an aerosol of coal tar by C3H mice. J Natl Cancer Inst 30: 31-43.

Horvath EP, Jr., Anderson H, Jr., Pierce WE, Hanrahan L and Wendlick JD (1988). Effects of formaldehyde on the mucous membranes and lungs. A study of an industrial population. JAMA 259(5): 701-7.

IARC. (2006). Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol; Summary of data reported and evaluation. International Agency for Research on Cancer. Lyon, France

Jain A, Martensson J, Mehta T, Krauss AN, Auld PA and Meister A (1992). Ascorbic acid prevents oxidative stress in glutathione-deficient mice: effects on lung type 2 cell lamellar bodies, lung surfactant, and skeletal muscle. Proc Natl Acad Sci U S A 89(11): 5093-7.

Jensen DE, Belka GK and Du Bois GC (1998). S-Nitrosoglutathione is a substrate for rat alcohol dehydrogenase class III isoenzyme. Biochem J 331 (Pt 2): 659-68.

Kamata E, Nakadate M, Uchida O, Ogawa Y, Suzuki S, Kaneko T, Saito M and Kurokawa Y (1997). Results of a 28-month chronic inhalation toxicity study of formaldehyde in male Fisher-344 rats. J Toxicol Sci 22(3): 239-54.

Kerfoot EJ and Mooney TF (1975). Formaldehyde and paraformaldehyde study in funeral homes. Am Ind Hyg Assoc J 36(7): 533-7.

Kerns WD, Pavkov KL, Donofrio DJ, Gralla EJ and Swenberg JA (1983). Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. Cancer Res 43(9): 4382-92.

Kilburn KH, Warshaw R and Thornton JC (1989). Pulmonary function in histology technicians compared with women from Michigan: effects of chronic low dose formaldehyde on a national sample of women. Br J Ind Med 46(7): 468-72.

Kim CW, Song JS, Ahn YS, Park SH, Park JW, Noh JH and Hong CS (2001). Occupational asthma due to formaldehyde. Yonsei Medical Journal 42(4): 440-445.

Kimbell JS, Gross EA, Richardson RB, Conolly RB and Morgan KT (1997). Correlation of regional formaldehyde flux predictions with the distribution of formaldehyde-induced squamous metaplasia in F344 rat nasal passages. Mutat Res 380(1-2): 143-54.

Kitaeva LV, Kitaev EM and Pimenova MN (1990). [The cytopathic and cytogenetic sequelae of chronic inhalational exposure to formaldehyde on female germ cells and bone marrow cells in rats]. Tsitologiia 32(12): 1212-6.

Krakowiak A, Gorski P, Pazdrak K, Ruta U, Wantke F, Focke M, Hemmer W, Tschabitscher M, Gann M, Tappler P, Gotz M and Jarisch R (1998). Airway response to formaldehyde inhalation in asthmatic subjects with suspected respiratory formaldehyde sensitization. Formaldehyde and phenol exposure during an anatomy dissection course: a possible source of IgE-mediated sensitization? Am J Ind Med 33(3): 274-81.

Kriebel D, Myers D, Cheng M, Woskie S and Cocanour B (2001). Short-term effects of formaldehyde on peak expiratory flow and irritant symptoms. Arch Environ Health 56(1): 11-8.

Krzyzanowski M, Quackenboss JJ and Lebowitz MD (1990). Chronic respiratory effects of indoor formaldehyde exposure. Environ Res 52(2): 117-25.

Kulle TJ, Sauder LR, Hebel JR, Green DJ and Chatham MD (1987). Formaldehyde dose-response in healthy nonsmokers. Japca 37(8): 919-24.

Lang I, Bruckner T and Triebig G (2008). Formaldehyde and chemosensory irritation in humans: A controlled human exposure study. Regul Toxicol Pharmacol 50(1): 23-36.

Levine RJ, Andjelkovich DA and Shaw LK (1984). The mortality of Ontario undertakers and a review of formaldehyde-related mortality studies. J Occup Med 26(10): 740-6.

Liley HG, White RT, Benson BJ and Ballard PL (1988). Glucocorticoids both stimulate and inhibit production of pulmonary surfactant protein A in fetal human lung. Proc Natl Acad Sci U S A 85(23): 9096-100.

Liu KS, Huang FY, Hayward SB, Wesolowski J and Sexton K (1991). Irritant effects of formaldehyde exposure in mobile homes. Environ Health Perspect 94: 91-4.

Malaka T and Kodama AM (1990). Respiratory health of plywood workers occupationally exposed to formaldehyde. Arch Environ Health 45(5): 288-94.

Malek FA, Moritz KU and Fanghanel J (2004). Effects of a single inhalative exposure to formaldehyde on the open field behavior of mice. Int J Hyg Environ Health 207(2): 151-8.

Mannino DM, Homa DM, Pertowski CA, Ashizawa A, Nixon LL, Johnson CA, Ball LB, Jack E and Kang DS (1998). Surveillance for asthma--United States, 1960-1995. MMWR CDC Surveill Summ 47(1): 1-27.

Maronpot RR, Miller RA, Clarke WJ, Westerberg RB, Decker JR and Moss OR (1986). Toxicity of formaldehyde vapor in B6C3F1 mice exposed for 13 weeks. Toxicology 41(3): 253-66.

Martin WJ (1990). A teratology study of inhaled formaldehyde in the rat. Reprod Toxicol 4(3): 237-9.

Monteiro-Riviere NA and Popp JA (1986). Ultrastructural evaluation of acute nasal toxicity in the rat respiratory epithelium in response to formaldehyde gas. Fundam Appl Toxicol 6(2): 251-62.

Monticello TM, Swenberg JA, Gross EA, Leininger JR, Kimbell JS, Seilkop S, Starr TB, Gibson JE and Morgan KT (1996). Correlation of regional and nonlinear formaldehyde-induced nasal cancer with proliferating populations of cells. Cancer Res 56(5): 1012-22.

Nagornyi PA, Sudakova Zh A and Shchablenko SM (1979). [General toxic and allergic action of formaldehyde]. Gig Tr Prof Zabol(1): 27-30.

Nielsen GD, Hougaard KS, Larsen ST, Hammer M, Wolkoff P, Clausen PA, Wilkins CK and Alarie Y (1999). Acute airway effects of formaldehyde and ozone in BALB/c mice. Hum Exp Toxicol 18(6): 400-9.

NIOSH. (1988). *Current Intelligence Bulletin 50: Carcinogenic effects of exposure to diesel exhaust*. Centers for Disease Control.

Nordman H, Keskinen H and Tuppurainen M (1985). Formaldehyde asthma--rare or overlooked? J Allergy Clin Immunol 75(1 Pt 1): 91-9.

OEHHA. (2000). *The Air Toxics Hot Spots Program Risk Assessment Guidelines Part III: Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Levels*. OEHHA. http://www.oehha.ca.gov/air/chronic_rels/pdf/relsP32k.pdf.

Olsen JH and Dossing M (1982). Formaldehyde induced symptoms in day care centers. Am Ind Hyg Assoc J 43(5): 366-70.

Pazdrak K, Gorski P, Krakowiak A and Ruta U (1993). Changes in nasal lavage fluid due to formaldehyde inhalation. Int Arch Occup Environ Health 64(7): 515-9.

Plopper CG, Smiley-Jewell SM, Miller LA, Fanucchi MV, Evans MJ, Buckpitt AR, Avdalovic M, Gershwin LJ, Joad JP, Kajekar R, Larson S, Pinkerton KE, Van Winkle LS, Schelegle ES, Pieczarka EM, Wu R and Hyde DM (2007). Asthma/allergic airways disease: does postnatal exposure to environmental toxicants promote airway pathobiology? Toxicol Pathol 35(1): 97-110.

Porter JA (1975). Letter: Acute respiratory distress following formalin inhalation. Lancet 2(7935): 603-4.

Pross HF, Day JH, Clark RH and Lees RE (1987). Immunologic studies of subjects with asthma exposed to formaldehyde and urea-formaldehyde foam insulation (UFFI) off products. J Allergy Clin Immunol 79(5): 797-810.

Que LG, Liu L, Yan Y, Whitehead GS, Gavett SH, Schwartz DA and Stamler JS (2005). Protection from experimental asthma by an endogenous bronchodilator. Science 308(5728): 1618-21.

Reise JA, Taylor GW, Fardy CH and Silverman M (1997). Glutathione and neonatal lung disease. Clin Chim Acta 265(1): 113-9.

Reynaert NL, Ckless K, Wouters EF, van der Vliet A and Janssen-Heininger YM (2005). Nitric oxide and redox signaling in allergic airway inflammation. Antioxid Redox Signal 7(1-2): 129-43.

Riedel F, Hasenauer E, Barth PJ, Koziorowski A and Rieger CH (1996). Formaldehyde exposure enhances inhalative allergic sensitization in the guinea pig. Allergy 51(2): 94-9.

Ritchie IM and Lehnen RG (1987). Formaldehyde-related health complaints of residents living in mobile and conventional homes. Am J Public Health 77(3): 323-8.

Rumchev KB, Spickett JT, Bulsara MK, Phillips MR and Stick SM (2002). Domestic exposure to formaldehyde significantly increases the risk of asthma in young children. Eur Respir J 20(2): 403-8.

Rusch GM, Clary JJ, Rinehart WE and Bolte HF (1983). A 26-week inhalation toxicity study with formaldehyde in the monkey, rat, and hamster. Toxicol Appl Pharmacol 68(3): 329-43.

Saillenfait AM, Bonnet P and de Ceaurriz J (1989). The effects of maternally inhaled formaldehyde on embryonal and foetal development in rats. Food Chem Toxicol 27(8): 545-8.

Salem H and Cullumbine H (1960). Inhalation toxicities of some aldehydes. Toxicol Appl Pharmacol 2: 183-7.

Sari DK, Kuwahara S, Tsukamoto Y, Hori H, Kunugita N, Arashidani K, Fujimaki H and Sasaki F (2004). Effect of prolonged exposure to low concentrations of formaldehyde on the corticotropin releasing hormone neurons in the hypothalamus and adrenocorticotropic hormone cells in the pituitary gland in female mice. Brain Research 1013(1): 107-116.

Sauder LR, Chatham MD, Green DJ and Kulle TJ (1986). Acute pulmonary response to formaldehyde exposure in healthy nonsmokers. J Occup Med 28(6): 420-4.

Sauder LR, Green DJ, Chatham MD and Kulle TJ (1987). Acute pulmonary response of asthmatics to 3.0 ppm formaldehyde. Toxicol Ind Health 3(4): 569-78.

Schachter EN, Witek TJ, Jr., Brody DJ, Tosun T, Beck GJ and Leaderer BP (1987). A study of respiratory effects from exposure to 2.0 ppm formaldehyde in occupationally exposed workers. Environ Res 44(2): 188-205.

Schachter EN, Witek TJ, Jr., Tosun T, Leaderer BP and Beck GJ (1986). A study of respiratory effects from exposure to 2 ppm formaldehyde in healthy subjects. Arch Environ Health 41(4): 229-39.

Sheppard D, Eschenbacher WL and Epstein J (1984). Lack of bronchomotor response to up to 3 ppm formaldehyde in subjects with asthma. Environ Res 35(1): 133-9.

Skog E (1950). A toxicological investigation of lower aliphatic aldehydes. Acta Pharmacol 6: 299-318.

Solomons K and Cochrane JW (1984). Formaldehyde toxicity. Part I. Occupational exposure and a report of 5 cases. S Afr Med J 66(3): 101-2.

Sorg BA, Bailie TM, Tschirgi ML, Li N and Wu W-R (2001b). Exposure to repeated low-level formaldehyde in rats increases basal corticosterone levels and enhances the corticosterone response to subsequent formaldehyde. Brain Research 898(2): 314-320.

Sorg BA, Tschirgi ML, Swindell S, Chen L and Fang J (2001a). Repeated formaldehyde effects in an animal model for multiple chemical sensitivity. Ann NY Acad Sci 933(Role of Neural Plasticity in Chemical Intolerance): 57-67.

Srivastava AK, Gupta BN, Bihari V, Gaur JS, Mathur N and Awasthi VK (1992). Clinical studies of employees in a sheet-forming process at a paper mill. Vet Hum Toxicol 34(6): 525-7.

Staab CA, Alander J, Brandt M, Lengqvist J, Morgenstern R, Grafstrom RC and Hoog JO (2008). Reduction of S-nitrosoglutathione by alcohol dehydrogenase 3 is facilitated by substrate alcohols via direct cofactor recycling and leads to GSH-controlled formation of glutathione transferase inhibitors. Biochem J 413(3): 493-504.

Sugiura H and Ichinose M (2008). Oxidative and nitrative stress in bronchial asthma. Antioxid Redox Signal 10(4): 785-97.

Swiecichowski AL, Long KJ, Miller ML and Leikauf GD (1993). Formaldehyde-induced airway hyperreactivity in vivo and ex vivo in guinea pigs. Environ Res 61(2): 185-99.

Tanaka K, Nishiyama K, Yaginuma H, Sasaki A, Maeda T, Kaneko SY, Onami T and Tanaka M (2003). [Formaldehyde exposure levels and exposure control measures during an anatomy dissecting course]. Kaibogaku Zasshi 78(2): 43-51.

Thompson CM and Grafstrom RC (2008). Mechanistic considerations for formaldehyde-induced bronchoconstriction involving S-nitrosoglutathione reductase. J Toxicol Environ Health A 71(3): 244-8.

Thrasher JD, Broughton A and Madison R (1990). Immune activation and autoantibodies in humans with long-term inhalation exposure to formaldehyde. Arch Environ Health 45(4): 217-23.

Thrasher JD, Wojdani A, Cheung G and Heuser G (1987). Evidence for formaldehyde antibodies and altered cellular immunity in subjects exposed to formaldehyde in mobile homes. Arch Environ Health 42(6): 347-50.

Uba G, Pachorek D, Bernstein J, Garabrant DH, Balmes JR, Wright WE and Amar RB (1989). Prospective study of respiratory effects of formaldehyde among healthy and asthmatic medical students. Am J Ind Med 15(1): 91-101.

Vyas JR, Currie A, Dunster C, Kelly FJ and Kotecha S (2001). Ascorbate acid concentration in airways lining fluid from infants who develop chronic lung disease of prematurity. Eur J Pediatr 160(3): 177-84.

Wallenstein G, Rebohle E, Bergmann I, Voigt U and Schneider WD (1978). [Occupational diseases of the respiratory system due to chemical substances with potential allergen effects]. Dtsch Gesundheitsw 33(24): 1119-23.

Wang L and Pinkerton KE (2007). Air pollutant effects on fetal and early postnatal development. Birth Defects Res C Embryo Today 81(3): 144-54.

Wantke F, Demmer CM, Tappler P, Gotz M and Jarisch R (1996). Exposure to gaseous formaldehyde induces IgE-mediated sensitization to formaldehyde in school-children. Clin Exp Allergy 26(3): 276-80.

Wantke F, Focke M, Hemmer W, Bracun R, Wolf-Abdolvahab S, Gotz M, Jarisch R, Gotz M, Tschabitscher M, Gann M and Tappler P (2000). Exposure to formaldehyde and phenol during an anatomy dissecting course: sensitizing potency of formaldehyde in medical students. Allergy 55(1): 84-87.

Weber-Tschopp A, Fischer T and Grandjean E (1977). [Irritating effects of formaldehyde on man (author's transl)]. Int Arch Occup Environ Health 39(4): 207-18.

Wilhelmsson B and Holmstrom M (1992). Possible mechanisms of formaldehyde-induced discomfort in the upper airways. Scand J Work Environ Health 18(6): 403-7.

Wilmer JW, Woutersen RA, Appelman LM, Leeman WR and Feron VJ (1989). Subchronic (13-week) inhalation toxicity study of formaldehyde in male rats: 8-hour intermittent versus 8-hour continuous exposures. Toxicol Lett 47(3): 287-93.

Witek TJ, Jr., Schachter EN, Tosun T, Beck GJ and Leaderer BP (1987). An evaluation of respiratory effects following exposure to 2.0 ppm formaldehyde in asthmatics: lung function, symptoms, and airway reactivity. Arch Environ Health 42(4): 230-7.

Woutersen RA, van Garderen-Hoetmer A, Bruijntjes JP, Zwart A and Feron VJ (1989). Nasal tumours in rats after severe injury to the nasal mucosa and prolonged exposure to 10 ppm formaldehyde. J Appl Toxicol 9(1): 39-46.

Wu H, Romieu I, Sienra-Monge JJ, Estela Del Rio-Navarro B, Anderson DM, Jenchura CA, Li H, Ramirez-Aguilar M, Del Carmen Lara-Sanchez I and London SJ (2007). Genetic variation in S-nitrosoglutathione reductase (GSNOR) and childhood asthma. J Allergy Clin Immunol 120(2): 322-8.

Yang X, Zhang YP, Chen D, Chen WG and Wang R (2001). Eye irritation caused by formaldehyde as an indoor air pollution--a controlled human exposure experiment. Biomed Environ Sci 14(3): 229-36.

Yi C, Ke K, Xiaohua L and Xu Y (2007). Up-regulation of GSNO reductase in mice lungs by formaldehyde inhalation. Bioinform Biomed Engineering 6-8: 294-297.

Zaman K, Hanigan MH, Smith A, Vaughan J, Macdonald T, Jones DR, Hunt JF and Gaston B (2006). Endogenous S-nitrosoglutathione modifies 5-lipoxygenase expression in airway epithelial cells. Am J Respir Cell Mol Biol 34(4): 387-93.

Zwart A, Woutersen RA, Wilmer JW, Spit BJ and Feron VJ (1988). Cytotoxic and adaptive effects in rat nasal epithelium after 3-day and 13-week exposure to low concentrations of formaldehyde vapour. Toxicology 51(1): 87-99.

Manganese and Compounds Reference Exposure Levels

1. Summary

Acute inhalation of high levels of manganese results in a nonspecific pulmonary edema, while chronic manganese inhalation leads to a characteristic neurotoxicity known as manganism with strong similarities to Parkinson's disease. Manganism is characterized by motor deficits (dystonia, altered gait, fine tremor, generalized rigidity) and may include psychiatric disturbances. At low manganese levels and in the absence of frank manganism, subtle deficits in cognitive and neurobehavioral functions have been reported in both adults and children. Neurodevelopmental deficits have been associated with early life exposure to excessive manganese and include impaired intellectual performance and behavioral disinhibition. The studies described in this document include those published through the Spring of 2008. The RELs below are applicable to all respirable inorganic manganese compounds.

1.1 Manganese Acute REL

An acute REL for manganese was not developed at this time.

1.2 Manganese 8-Hour REL

Reference Exposure Level	**0.17 µg/m^3**
Critical effect(s)	Impairment of neurobehavioral function in humans
Hazard index target	Nervous system

1.3 Manganese Chronic REL

Reference Exposure Level	**0.09 µg/m^3**
Critical effect(s)	Impairment of neurobehavioral function in humans
Hazard index target(s)	Nervous system

2. Physical and Chemical Properties

Table 2.1 Manganese and Manganese Species*

Molecular Formula	*Synonyms*	*Molecular Weight*	*CAS Reg. No.*
Mn	elemental manganese; colloidal manganese; cutaval	54.94 g/mol	7439-96-5
MnO	manganese oxide; manganese monoxide; manganosite	70.94 g/mol	1344-43-0
MnO_2	manganese dioxide; black manganese oxide	86.94 g/mol	1313-13-9
Mn_3O_4	manganese tetroxide; trimanganese tetraoxide; manganomanganic oxide	228.82 g/mol	1317-35-7
$MnCl_2$	manganese chloride; manganese dichloride; manganous chloride	125.84 g/mol	7773-01-5
$MnSO_4$	manganese sulfate	151.00 g/mol	7787-85-7
$KMnO_4$	potassium permanganate	158.03 g/mol	7724-64-7

Description	Lustrous, gray-pink metal (Mn); green (MnO), black (MnO_2) or pink ($MnCl_2$, $MnSO_4$), purple ($KMnO_4$) crystals; brownish-black powder (Mn_3O_4)
Molecular formula	see Table 2.1
Molecular weight	see Table 2.1
Density (in g/cm^3)	7.21-7.4 (Mn – depending on allotropic form); 5.43-5.46 (MnO); 4.88 (Mn_3O_4); 2.977 @ 25°C ($MnCl_2$)
Boiling point	2095°C (Mn); not available (MnO); not available (Mn_3O_4); 1190°C ($MnCl_2$); 850°C ($MnSO_4$)
Melting point	1246°C (Mn); 1839°C (MnO); 1567°C (Mn_3O_4); 650°C ($MnCl_2$) (CRC, 2005); 700°C ($MnSO_4$)
Vapor pressure	1 torr @ 1292°C (Mn); non-volatile at room temperature (Mn_3O_4); not available (MnO; $MnCl_2$)
Solubility	Sol. in dil. acids and aq. solns. of Na- or K-bicarbonate (Mn); sol. in NH_4Cl, insol. in H_2O (MnO); insol. in H_2O, HNO_3, or cold H_2SO_4 (MnO_2 (Merck, 1976)); insol. in H_2O, sol. in HCl (Mn_3O_4); 72.3 g/100 ml H_2O @ 25°C ($MnCl_2$); sol in 1 part H_2O ($MnSO_4$); 72.3 g/100 ml H_2O ($KMnO_4$)
Conversion factor	Not applicable (dusts or powders)

3. Occurrence and Major Uses

Metallic manganese is used in the manufacturing of steel, carbon steel, stainless steel, cast iron, and superalloys to increase hardness, stiffness, and strength (HSDB, 2006). Manganese chloride is used in dyeing, disinfecting, batteries, and as a paint drier and dietary supplement. Manganese oxide (MnO) is used in textile printing, ceramics, paints, colored glass, fertilizers, and as food additives and supplements. Manganese dioxide is used in batteries and may also be generated from the welding of manganese alloys. Use of manganese-containing welding rods is a major source of occupational exposure to welders. Manganese tetroxide may be generated in situations where other oxides of manganese are heated in air (NIOSH, 2005). Manganese is also released into the air during the erosion of manganese-containing rock and alloys. Relatively high levels of manganese have been measured in subways (428 ng/m^3 vs 9.7 ng/m^3 ambient), presumably from the frictional erosion of manganese-containing steel (Crump, 2000). As methylcyclopentadienyl manganese tricarbonyl (MMT), manganese has found use as an octane enhancer in some unleaded gasolines and is released during fuel combustion as manganese sulfate, phosphate, and oxides. Manganese exposure may also be significant among farm workers using the fungicide Maneb (manganese ethylene-bis-dithiocarbamate).

Manganese is present in ambient air as particles, often associated with other metals or organic material. The size of these particles depends on their source, history and contents. For example, Singh et al. (2002) compared the metal contents and size distributions of particles at two sites in the Los Angeles Basin, Downey, which is in the vicinity of downtown Los Angeles and Riverside, 70 km east of Los Angeles. In Downey, 7% of the manganese was in ultrafine particles ≤ 0.1 µm, 38% was in fine particles of 0.35-1.0 µm, and less than 20% in the coarse (PM 2.5-10 µm) fraction. By comparison, in Riverside, less than 2% of the manganese was in ultrafine PM (≤ 0.1 µm) 8% in the 0.35-1.0 range, while nearly 80% was in the 2.5-10 µm fraction (Table 3.1).

Table 3.1 Particle Size and Manganese Distribution

Particle size (µm)	Downey	Riverside
2.5-10	18%	77%
1-2.5	21%	12%
0.35-1	38%	8%
0.1-0.35	16%	1.5%
≤ 0.1	7%	1.3%

In contrast to Riverside, manganese measured in other urban settings tends to be mainly in the respirable fine fraction. In urban aerosols in Seville, Spain, manganese was found predominantly in fine particles < 0.61 µm (44%), with smaller amounts in coarse particles (> 10 µm, 17.8%; 4.9-10 µm, 18.3%) (Espinoza et al., 2001). A bimodal distribution of sizes was also found for manganese-containing aerosols in

Tihany, Hungary. The bulk of the manganese was found bound to organic matter and silicates in particles of approximately 0.118 µm, with smaller amounts in the 1.4-2.8 µm range (Hlavay et al., 1998). In cities in which the gasoline contains MMT, manganese is also predominantly in the fine (PM 2.5) fraction (Pellizzari et al., 2001). Since combustion is associated with the production of ultrafines, more research on airborne ultrafine manganese particles in areas where MMT is present in the gasoline is warranted

The 2004 annual statewide emissions of manganese reported in the most recent California Toxics Inventory (CARB, 2005a) were estimated to be 1,055 tons. For 2002, the mean statewide ambient level was 31.5 ng/m^3.

4. Metabolism / Toxicokinetics

Environmental manganese can enter the body primarily by oral and inhalation routes. Dermal absorption of manganese is insignificant through intact skin; however, broken skin would obviously allow more access to manganese (e.g., potassium permanganate) and other poorly dermally absorbed compounds. Parenteral exposures have occurred through parenteral feeding and more recently i.v. drug abuse, leading to human disease. Manganese is an essential element normally absorbed from the intestinal tract as part of the diet. It is estimated that 2 to 5% of ingested manganese is retained in the adult body (Andersen et al., 1999). Retention can be up to 41% in breast-fed infants, and 20% in formula-fed infants (Dorner et al., 1989). Manganese absorption is increased (along with iron absorption) when there is a deficiency of iron in the diet (Davis et al., 1992). Ascorbic acid, calcium and phosphorus also affect manganese utilization (ibid).

As part of the normal manganese homeostatic mechanism, high levels of dietary manganese diminish absorption from the intestinal tract. Manganese appears to be absorbed from the gut largely in the divalent form, with approximately 80% of absorbed manganese subsequently bound in plasma to ß$_1$-globulin and albumin (Foradori et al., 1967). These manganese-protein complexes are efficiently removed from the blood by the liver and returned to the gut in bile for elimination, thus establishing an entero-hepatic circuit for manganese. In the blood, unbound manganese may be converted by ceruloplasmin to the trivalent cation which is then bound by transferrin. Transferrin-manganese complexes are much less efficiently removed by the liver and thus survive first pass elimination to circulate throughout the body (Gibbons et al., 1976). In the brain, transferrin receptors in the capillary beds may mediate uptake in regions with efferents to the nucleus accumbens and the caudate putamen. Other mechanisms also appear to contribute to brain uptake of manganese including a divalent metal transporter (DMT-1), and a less well-defined non-saturable mechanism. From these sites, manganese is thought to move by neuronal transport to the pallidum, thalamic nuclei, and substantia nigra, areas involved with motor control and movement (Aschner et al., 2005). While at normal plasma levels, manganese enters the brain mainly across the capillary epithelium, at elevated levels of manganese in the blood, transport across the choroid plexus becomes more prominent (Aschner, 2000).

The mechanisms mentioned above are thought to apply generally to the transport of manganese across the blood brain barrier of adults. However, in the fetus and neonate, the blood brain barrier is characterized as having greater permeability to many substances, including manganese, and a different distribution of molecular transporters (Erikson et al., 2007b). In mice following a single parenteral administration of $MnCl_2$ on postnatal days 7, 14, or 42, maximum retention of manganese in the brain occurred 24 hours after exposure and was 3.5%, 2.5%, and 0.3%, respectively, of the administered dose. For manganese administered on day 0, the maximum brain concentration (2.9%) occurred 43 days later, suggesting a lack of perinatal homeostatic control (Valois and Webster, 1989). The approximately ten-fold higher brain levels following dosing on days 0-14 compared with day 42 indicate a more rapid and extensive uptake of manganese from the blood in neonates compared with adults. The drop in maximum brain levels between days 14 and 42 is thought to reflect attainment of adult blood brain barrier function on day 21.

Manganese may be introduced directly into the blood during parenteral feeding or during injection of illicit designer drugs contaminated with permanganate. As with the inhalation route described below, parenteral administration of manganese avoids first pass clearance of manganese by the liver, and may result in high exposure of all organs to manganese.

Manganese exposure via the pulmonary route leads to more rapid absorption with higher efficiency, and with greater transfer to the brain compared with other routes (Drown et al., 1986; Roels et al., 1997). In experiments in 3-month old rats, Roels et al. (1997) used intratracheal instillation as a surrogate for inhalation for comparison with the oral route (gavage). Intratracheal instillation of $MnCl_2$ (1.22 mg/kg, once weekly for four weeks) raised the steady state manganese levels 68% in blood, 205% in the striatum, 48% in the cortex, and 27% in the cerebellum compared to controls. By gavage, a much higher dose of $MnCl_2$ (24.3 mg/kg) was required to achieve the same blood levels (68%). However, by this route, manganese levels in the striatum and cerebellum were not affected, and levels in the cortex were raised by only 22% (Table 4.1). In animals given a single intratracheal dose of $MnCl_2$ (1.22 mg/kg bw), blood manganese levels peaked within 30 min at 7,050 ng/100 ml. This was followed by a gradual decline but blood levels remained elevated over controls for at least 24 hours. By comparison, the single oral administration of 24.3 mg $MnCl_2$/kg bw resulted in a five-fold lower peak blood level of 1,660 ng/100 ml after one hour, followed by a return to control levels in 12 hours. Thus, compared to ingestion, inhalation of a relatively water soluble form of manganese leads to a rapid increase in blood levels that remain higher for longer, and results in higher brain manganese levels.

Table 4.1 Increase in Tissue Manganese by Route and Chemical Form

Chemical Form and Route	Increase in Tissue Manganese (%)			
	Blood	Striatum	Cortex	Cerebellum
$MnCl_2$ Intratracheal (1.2 mg/kg)	68	205	48	27
$MnCl_2$ Gavage (24.3 mg/kg)	68	0	22	0
MnO_2 Intratracheal (1.2 mg/kg)	41	48	34	31
MnO_2 Gavage (24.3 mg/kg)	0	0	0	0

Using the same exposure protocol with the less soluble MnO_2, intratracheal instillation raised manganese levels 41% in blood, 48% in striatum, 31% in cerebellum, and 34% in cortex. By contrast, neither blood nor brain levels were increased following oral exposure (Table 4.1). As with $MnCl_2$, Mn blood levels following intratracheal MnO_2 reached a higher peak value (1,760 ng Mn/100 ml; 200% increase) than that achieved after gavage (900 ng/100 ml; 27% increase). Blood levels rose more slowly than with $MnCl_2$, starting at 48–72 hr after intratracheal instillation and peaking at 168 hr. By gavage, blood levels rose gradually to peak at 144 hr (Roels et al., 1997). In these studies, the solubility of the manganese complexes influenced the rate of absorption by either route, but in both cases inhalation resulted in substantially higher blood and brain levels.

In a further demonstration of the dependence of tissue distribution on oxidation state and route of exposure, Reaney et al. (2006) exposed 8-month old rats to 0, 2, or 6 mg/kg Mn(III)-pyrophosphate or $Mn(II)Cl_2$ intraperitoneally (i.p.) for five weeks. Significantly higher blood manganese levels were seen with Mn(III) vs equimolar Mn(II). A dose-dependent increase in brain manganese was observed, with Mn(III) producing levels that were 25% higher than following Mn(II). This may be related to the higher blood levels of manganese achieved with Mn(III) vs Mn(II) via the i.p. route. Examination of the striatum, globus pallidus, thalamus, and cerebral cortex by PIXE (particle induced x-ray emission; an x-ray fluorescence technique) revealed no differences in the distribution of manganese across these brain regions. There were, however, differences among regions in response to the concentration and oxidation state of the manganese. In the globus pallidus, the highest cumulative dose (90 mg/kg) of both forms of manganese increased GABA levels compared to controls (15-30%, $p = 0.037$). By contrast, dopamine levels in globus pallidus at this dose were increased by 60% with Mn(III), but decreased by 40% with Mn(II). The mechanism behind this differential effect is not clear but suggests that manganese oxidation states are important in manganese toxicity.

Drown and colleagues studied the distribution of soluble $^{54}MnCl_2$ and insoluble $^{54}Mn_3O_4$ after instillation into the adult rat lung (Drown et al., 1986). Initially the soluble form of manganese distributed more rapidly from the lung to the peripheral tissues than did the insoluble form. After two weeks the rates of distribution of the two forms became almost equal. Manganese (^{54}Mn) reached higher concentrations in the liver, kidney, and gastrointestinal tissues, but persisted longer in the heart, brain, and bone. The manganese was eliminated mainly in bile with very little elimination in urine.

The influence of solubility on tissue distribution was examined with aerodynamically similar aerosols of three manganese compounds of differing solubilities ($MnPO_4$, $MnSO_4$, and Mn_3O_4) in rats following inhalation (6 hr/day, 14 days) at 0, 0.03, 0.3, and 3.0 mg/m^3. At comparable dose levels, animals exposed to $MnSO_4$ had lower lung manganese levels than those exposed to either Mn_3O_4 (Dorman et al., 2001) or $MnPO_4$ (Vitarella et al., 2000), suggesting more rapid pulmonary clearance of the most soluble form. Consistent with this observation, after exposure to 3 mg/m^3, manganese levels in the olfactory bulb and striatum were highest with $MnSO_4$, followed by Mn_3O_4, then $MnPO_4$. As observed by Drown et al. (1986) and Roels et al. (1997) for intratracheal instillation, the more soluble forms of manganese accumulate in the brain more quickly following inhalation than do the less soluble compounds (Normandin et al., 2004).

For humans with occupational and/or environmental exposures, the main route of exposure is via inhalation. In both cases the manganese is usually in the form of particulates of various sizes. Manganese deposited in the lung can be absorbed directly into the blood stream, or can migrate (by mucociliary transport) into the upper respiratory tract and then be swallowed for possible absorption in the GI tract. In experimental animals, inhaled manganese may be transported via olfactory nerves directly to the brain following absorption from nasal passages (Brenneman et al., 2000; Dorman et al., 2002a; Elder et al., 2006; Dorman et al., 2006a). Neither pulmonary nor gastrointestinal absorption is required for this route of exposure, and the blood-brain barrier is bypassed. Evidence for absorption of particulate manganese oxide from the nose and transport to the brain was provided by Elder et al. (2006) in rats. Manganese concentrations in the olfactory bulb increased 3.5-fold following 12 days of intranasal instillation of ultrafine manganese oxide particles (3-8 nm) in both nares. With occlusion of the right nostril and instillation in the left naris, manganese accumulated almost exclusively in the left olfactory bulb. In this experimental paradigm, instillation of either the soluble manganese chloride or the insoluble manganese oxide particles (solubilization rate 1-1.5% per day) in the patent naris resulted in comparable levels of manganese in the ipsilateral side of the olfactory bulb. This, in conjunction with the observation that an increase in manganese in the olfactory bulb was detectable within 30 minutes of the instillation, suggests that particulate rather than dissolved manganese was the form transported to the brain. While instillation is not inhalation, this study does indicate absorption of manganese ultrafine particles occurs across the nasal epithelium with direct transport to the brain.

The deposition and uptake of manganese from the upper and lower airways is also influenced by particle size. Rats with nose-only exposure to MnO_2 aerosols of 1.3 and 18 µm mass median aerodynamic diameters (MMAD) showed higher levels of manganese in the lungs and olfactory bulbs following 15 days of inhalation exposure to the smaller (1.3 µm) versus larger (18 µm) particles (Fechter et al., 2002). Thus, while there was greater deposition of large particle manganese in the nasal passages compared with small particles, uptake from the nose was more efficient with the smaller particles. In addition, for the smaller particles, the lungs were a larger reservoir for more continuous systemic uptake of inhaled manganese. Thus the evaluations of potential toxicity from inhaled manganese must consider not only the chemical form of manganese, but also the particle size as important determinants of the toxicokinetics.

This may be particularly relevant to combustion products of MMT in which particle size is of 0.2-0.4 µm (Ter Haar et al., 1975). The inhalation toxicity of manganese-containing particles in this size range has received less attention than the larger fine and coarse particles.

More evidence for direct nose to brain transport in primates was provided by magnetic resonance imaging (MRI) studies in adult rhesus monkeys exposed by inhalation to manganese sulfate for 6 hours/day, 5 days/week for 13 weeks (Dorman et al., 2006a). Increases in signal intensity on T1-weighted images of various brain regions were well correlated with manganese levels measured upon necropsy. Increases in mean pallidal manganese concentrations of approximately 1.7-, 2.7-, and 6-fold over air-exposed controls were seen following exposure to $MnSO_4$ at 0.19, 0.97, and 4.55 mg/m^3, respectively. The particles at these three concentrations had MMAD of 1.73, 1.12, and 2.12 µm, respectively (1.04, 1.07, 1.12 µm geometric mean diameter (GMD), respectively). As expected, much higher increases in manganese concentrations were observed in olfactory epithelium, bulb, tract, and cortex. Lower but statistically significant ($p < 0.05$) increases were also observed in the putamen, white matter, and cerebellum. This study provided no evidence for translocation of manganese from the olfactory bulb to other brain regions, consistent with uptake from the blood as the source of manganese in the globus pallidus. However, the resolution of the MRI used in this study did not allow visualization of individual nerve tracts to rule out direct transfer of manganese between brain regions. Nevertheless, this study provides evidence for axonal transport from the nasal epithelium at least as far as the olfactory bulb in primates.

The major route of excretion of manganese is via bile, although a lesser amount is excreted via urine (Davis et al., 1993). That the liver maintains homeostasis of manganese can be seen by the fact that patients with cirrhosis of the liver accumulate abnormally high levels of manganese in their brains, especially in the globus pallidus (Rose et al., 1999). Similarly, rats that have a liver bypass also show high levels of manganese in the brain, especially in the globus pallidus (Rose et al., 1999).

Neonatal humans do not excrete manganese for the first two to three weeks of life. The intestinal barrier to manganese absorption is also immature in premature and neonatal infants (Cawte, 1985).

The toxicokinetics of manganese may also influence and be influenced by other metals. There is evidence that manganese uptake from the intestinal tract (Mena, 1974; Erikson et al., 2002), lungs (Brain et al., 2006), and nose (Thompson et al., 2007), is enhanced by iron deficiency. Rats rendered anemic by periodic bleeding absorbed significantly higher amounts of manganese (Brain et al., 2006). In each of these studies, brain levels of manganese were increased by iron deficiency. Based on data from NHANES III, the prevalence of iron deficiency among infants 1-2 years of age was 9%, and 9-11% among adolescent girls and women of child-bearing age. For comparison, the prevalence among teenage boys and men less than 50 years of age was 1%. Among older children and adults over 50 years of age, the prevalence of iron deficiency did not exceed 7% (Looker et al., 1997). Inasmuch as iron deficiency is a widespread condition

that disproportionately affects the young (Beard et al., 2001), children represent a more susceptible population.

Manganese may exist in eleven different valence states (-3 to +7) and may participate in a variety of oxidation-reduction reactions as a pro- or anti-oxidant. In biological systems, while the divalent (Mn^{2+}) and trivalent (Mn^{3+}) forms are most abundant, the trivalent form predominates in many tissues and appears to be responsible for manganese's pro-oxidant properties, possibly by its participation in Fenton-type reactions (HaMai et al., 2001). In vitro studies attempting to emulate conditions in the brain have shown that Mn^{3+}, but not Mn^{2+} (both as pyrophosphate), oxidizes dopamine, DOPA (a dopamine precursor), norepinephrine, and epinephrine to quinones and other products, with reduction of Mn^{3+} to $Mn(OH)_2$ (Archibald and Tyree, 1987). Polymerization of these catecholamine-derived quinones to form neuromelanin, from which the substantia nigra derives its name, is an O_2^--generating, auto-oxidative process that in turn enhances oxidation of Mn^{2+} to Mn^{3+}, thus increasing cellular oxidative stress.

The role of oxidative stress in manganese toxicity has been inferred in part from changes in cellular markers of oxidative stress upon exposure to high levels of manganese. As a result of participation in reactions with reactive oxygen species (ROS), levels of GSH in manganese-exposed cells decrease. This decrease may also reflect binding of GSH by manganese. Where metals are involved in the generation of ROS, metallothionein levels typically rise. Other markers, such as glutamine synthetase and tyrosine hydroxylase, are used due to their sensitivity to cellular oxidation states. In the hypothalamus of rats, subchronic inhalation exposure to $MnSO_4$ (0.03, 0.3, 3.0 mg/m^3) has been associated with a decrease in GSH and an increase in metallothionein mRNA, while the olfactory bulb experienced an increase in glutamine synthetase (Dobson et al., 2003). Similarly, subchronic inhalation of $MnSO_4$ (0.06, 0.3, 1.5 mg/m^3) by rhesus monkeys resulted in decreased tyrosine hydroxylase, glutamate transporter-1, glutamate/aspartate transporter, and glutamine synthetase (Erikson et al., 2007a). These changes reflect exposure to oxidative stress that impairs neurotransmitter synthesis, while increased metallothionein mRNA in all the brain regions examined (caudate, cerebellum, frontal cortex, globus pallidus, olfactory cortex, putamen) is a cellular response to ameliorate the effects of reactive oxygen species. Many of these effects were significantly different from controls starting at the lowest manganese concentration tested (0.06 mg/m^3). While these studies of manganese have focused on the role of metal-induced oxidative stress, the ability of manganese to bind to sulfhydryl groups, exemplified by GSH, suggests the possibility that manganese may also bind protein sulfhydryls. Indeed, the depletion of both protein- bound and non-protein sulfhydryls by manganese has been demonstrated in rat brains (Shukla and Chandra, 1977), and the possibility that such interactions alter the structure/function of key proteins has been theorized to represent an important mode of manganese toxicity (Martin, 1986).

The ability of manganese to readily bind thiol groups can enhance cellular susceptibility to oxidative stress by directly depleting GSH levels. However, manganese may also lower GSH levels indirectly by enhancing the autooxidation of cysteine, the rate limiting

precursor to glutathione (Wang and Cynader, 2001). In addition to making it unavailable for glutathione synthesis, the autooxidation of cysteine generates free radicals that are cytotoxic. Manganese III may oxidize cellular thiols, such as GSH and cysteine, to thiyl radicals (Wariishi et al., 1989). These may in turn participate in the oxidation of critical cellular components or signalling molecules as exemplified by the oxidation of dopamine by the cysteinyl thiyl radical (Shen and Dryhurst, 1998).

The effects of manganese on the markers of oxidative stress described above show an age and sex dependency. Juvenile (8 weeks) male and female rats, and senescent males (18 mo) breathed atmospheres containing $MnPO_4$ at 0.099 or $MnSO_4$ at 0.01, 0.098, or 0.478 mg Mn/m^3 (1.85, 1.92, 2.03 µm MMAD) for 6 hr/day, 5 day/wk for 13 weeks (Erikson et al., 2004). In young males, but not females or senescent males, there was an increase in glutamine synthetase levels in the hippocampus, but a decrease in the hypothalamus with both forms of manganese that was significant ($p < 0.05$) with exposure to $MnPO_4$. With exposure to the medium dose of $MnSO_4$, female and old, but not young, male rats showed significant decreases in glutamine synthetase levels in the hippocampus. Total GSH levels significantly decreased in the olfactory bulb of young males, but increased in females. In the striatum, GSH levels were significantly decreased in females and old males at all doses of $MnSO_4$, but were largely unchanged in young males. This is interesting in light of the observation by Dorman et al. (2004) that neither old age nor gender influenced delivery of manganese to the striatum. The decrease of GSH in the striatum in aged rats may be a result of the age-related loss of dopaminergic neurons, while the effect in females is suggested to be related to differences in levels of sex hormones between males and females. These data indicate that toxicokinetic and toxicodynamic characterizations of manganese must take age and gender into account.

That the chemical form and oxidation state of manganese is critical to its toxicokinetics is evident from the foregoing. No less critical to the toxicokinetics is the presence of manganese on or as nanoparticles versus free Mn_3O_4 in solution. The ability of manganese to cause oxidative stress in cultured human lung epithelial cells was assessed by measurement of reactive oxygen species (ROS) (Limbach et al., 2007). Nanoparticles (20-75 nm) of pure manganese, or silica doped with 0.5 and 1.6 wt % manganese, were suspended in culture medium at 30 ppm for comparison with the more soluble Mn_3O_4 at comparable concentrations. Compared with pure silica nanoparticles, particles doped with as little as 1.6 wt% manganese increased ROS in cultured cells by 2,500% while the free Mn_3O_4 increased ROS by only 400%. For comparison, in similar experiments Co_3O_4 particles were less than half as potent. In cell-free culture medium, ROS production was not different between the particles and the dissolved salt. This suggests that it is the dissolved metal, not the particles per se, that is responsible for the ROS generation, and that the nanoparticles function to facilitate manganese uptake by the cell.

5. Acute Toxicity of Manganese

Acute inhalation exposure to high levels of manganese as its oxides is associated with pulmonary edema and impaired function (Shiotsuka, 1984). The very small body of literature on acute toxicity includes two animal experiments involving acute exposures by inhalation. One is a two-hour exposure of 200 female CD-1 mice to manganese oxide (Mn_3O_4) aerosols (Adkins et al., 1980) that resulted in a NOAEL of 2.9 mg/m^3 based on respiratory effects (edema). The other is a 24 hr exposure of guinea pigs to 22 mg/m^3 MnO_2 (Bergstrom, 1977) that examined the effects of manganese exposure on pulmonary leukocytes, macrophages, and the clearance of bacteria from the lungs. However, since no dose response in lung wet:dry weight ratios was observed, no LOAEL was reported in the Adkins study, and the Bergstrom study employed a single exposure level. The accumulation of manganese in brain structures following acute inhalation exposure (Newland et al., 1987; Brenneman et al., 2000; Dorman et al., 2002a), and following intranasal instillation (Gianutsos et al., 1997) has been described; however, the toxicological consequences of these exposures were not reported. Neurobehavioral effects have been observed in mice following acute subcutaneous injections (Dodd et al., 2005), and in rats after a single oral administration of manganese (Shukakidze et al., 2003), but it is not clear how these routes of exposure compare to inhalation. No studies of acute manganese inhalation were located that demonstrated a dose-response or evaluated other toxicological endpoints.

6. Chronic Toxicity of Manganese

6.1 Chronic Toxicity to Adult Humans

Exposure of humans to manganese by inhalation leads to a suite of neurological effects called “manganism” (Lucchini et al., 1999). Frank manganism is a progressive disease that involves symptoms similar to those of Parkinson’s disease. Manganism is characterized by altered gait, fine tremor and occasionally psychiatric disturbances. The psychiatric disturbances are seldom seen in Parkinson’s disease, although dementia sometimes occurs late in this disease. Despite their similarities, the symptoms of manganism and Parkinson’s disease differ somewhat (Barbeau, 1984; Calne et al., 1994). Both manganism and Parkinson’s disease involve generalized bradykinesia and widespread rigidity. However, tremor is less frequent and dystonia more frequent in manganism. Manganism is also distinguished by a propensity to fall backward, failure to achieve a sustained therapeutic response to levodopa, and failure to detect a reduction in fluorodopa uptake by positron emission tomography (Calne et al., 1994). In Parkinsonism, the damage appears to be confined to the substantia nigra, whereas in manganism the damage is more widespread, involving other parts of the basal ganglia (Huang et al., 1998).

Manganese accumulates in certain brain structures, especially the extrapyramidal system. Structures rich in dopaminergic neurons show a heightened sensitivity to manganese toxicity. Within these tissues, manganese is found preferentially in mitochondria where it disrupts oxidative phosphorylation and mitochondrial function

(Gavin et al., 1999). Cytochrome c, released from damaged mitochondria, leads to apoptosis and loss of neurons (Malecki, 2001). Trivalent manganese can promote the formation of reactive oxygen species (HaMai et al., 2001) that can cause oxidative stress, which in turn has been shown to lead to apoptosis of neurons in the rat brain (Dobson et al., 2003). While individuals exposed to massive amounts of manganese show frank neurological symptoms as in the Groote Eylandt studies (Kilburn, 1987) and the industrial workers studies, individuals exposed to lesser amounts of manganese show more subtle neurological deficits in neurobehavioral tasks (Wennberg et al., 1992; Lucchini et al., 1999).

Adverse effects may occur at manganese exposure levels that are too low to cause frank manganism. Lucchini and his co-workers studied a group of 61 Italian ferroalloy workers who had been exposed to low levels of manganese dust by inhalation (Lucchini et al., 1999). These workers did not exhibit the frank signs of manganism, but they did exhibit subtler neurofunctional changes. The workers were exposed to a "current overall value" of 54 µg Mn per m^3 air at the time of the study, and an estimated average of 70.83 µg Mn dust/m^3 per year over an average 15.17 years of exposure. Earlier exposures were higher. In order to obtain a measure of cumulative exposure the investigators calculated a "cumulative exposure index" (CEI) for each worker based on their exposure history in the factory. For the purposes of analysis, workers were separated into three CEI groups of low CEI (< 500 µg/m^3 x yrs), mid CEI (500-1800 µg/m^3 x yrs), and high CEI (> 1800 µg/m^3 x yrs). The CEIs correlated positively with blood manganese levels. The workers were subjected to symptom questionnaires and neurobehavioral and neurophysiological testing for the purpose of finding whether neurological effects correlated with cumulative exposure. In multiple regression analyses, positive correlations were found between the log of the CEI and the following tests of the Swedish Performance Evaluations System: finger tapping in the dominant ($R = 0.32$, $p = 0.01$) and non-dominant ($R = 0.32$, $p = 0.01$) hands, Symbol Digit ($R = 0.33$, $p = 0.01$) and Digit Span ($R = 0.44$, $p = 0.004$). The moderate but significant correlation coefficients reported in this study suggest that manganese is an important contributor to these effects but likely not the only one. In addition, these results demonstrate that subtle neurological changes are taking place in workers exposed to relatively low levels of manganese in the absence of frank manganism. To identify safe exposure levels, the authors took the geometric mean of the mid CEI group (1113 µg/m^3 x yrs) and divided this by the geometric mean exposure time (11.51 yrs) to derive a value of 96.71 µg/m^3. When the low CEI group is used as the control group, this value represents the LOAEL for the observed neurobehavioral symptoms.

A battery of neurofunctional tests was also employed by Mergler et al. (1994) to document early nervous system dysfunction among workers with long-term (mean 16.7 yr) manganese exposure in a ferromanganese and silicomanganese alloy plant. Subjects ($n = 115$) were matched by age, educational level, and number of children to workers in the same geographical region but without exposure to metals or other neurotoxicants in the workplace. The test batteries assessed motor function (range, speed, stability, grip strength, manual dexterity, graphomotor) and sensory function (visual acuity, chromatic discrimination, contrast sensitivity, olfactory and vibrotactile threshold). A third battery assessed speech initiation and regulation, attention,

concentration and memory, cognitive flexibility, and affect. Environmental manganese levels were measured with personal monitors for total dust and manganese content (0.014 – 11.48 mg Mn/m^3), while stationary monitors measured manganese in respirable (0.001 – 1.273 mg/m^3) and nonrespirable dust. Manganese measured in blood was higher among manganese workers (1.03 vs 0.68 µg/100 ml, $p < 0.0001$), while urine levels were not significantly different.

On the tests of motor functions, the performance of manganese-exposed workers was significantly worse than controls ($p < 0.001$), with the greatest differences associated with tests requiring rapid, alternating, coordinated movements. While in the context of speech initiation and regulation there were no overall differences between groups, the manganese-exposed workers took significantly longer on the second trial of the digit naming test ($p = 0.05$) and made more errors ($p < 0.001$). Cognitive flexibility was also worse with manganese exposure ($p < 0.002$). Attention, concentration, and memory functions were similar between groups. In the comparison of mood states, manganese workers displayed significantly more tension ($p < 0.01$), anger ($p = 0.01$), fatigue ($p < 0.001$), and confusion ($p = 0.01$) than controls. The cross-sectional nature of this study precludes assigning symptoms of manganese toxicity to specific environmental levels of manganese since levels in the plant varied widely both spatially and likely historically. However, there was a strong association between blood levels of manganese and subtle neurobehavioral deficits.

Whether the neurobehavioral effects associated with occupational exposure to manganese are permanent or transitory has been the subject of several follow-up studies of occupational cohorts. Occupational exposure at the ferroalloy plant featured in the study above by Mergler et al. (1994) ceased with its closure in 1990. Fourteen years later, 69 of the original workers and 68 referents were re-examined with many of the same assessment tools used in the 1994 study (Bouchard et al., 2007a). After controlling for age, education, alcohol consumption, and smoking, manganese workers performed significantly worse on tests of motor function (Luria Motor Scale) than did referents in both the initial ($p < 0.001$) and follow-up ($p < 0.05$) evaluations, although the differences at follow-up were not as striking. The motor deficits that persisted between the initial and follow-up studies included slowing of simple and alternate movements, and poorer quality of form drawing. Deficits in hand steadiness observed in the initial study remained but were less pronounced at follow-up. These deficits were significantly associated with increasing levels of cumulative manganese exposure ($p < 0.05$). Although in the initial study, several tests of cognitive function showed significant deficits among the exposed workers, these differences were no longer evident at follow-up. In general, measures of mood states showed improvement over time. However, compared to referents, manganese workers tended to report more feelings of anger and hostility in both the initial and follow-up studies ($p < 0.1$). Feelings of confusion and bewilderment, while not different between groups in the initial study, were significantly more pronounced among manganese workers ($p < 0.05$) at follow-up and significantly associated with cumulative exposure ($p < 0.01$). In a neuropsychiatric profile, former manganese workers were significantly ($p < 0.05$) more likely to experience feelings of anger and depression than the referents (Bouchard et al., 2007b). In addition to the effects on the nervous system, in the present study former manganese workers had a

ten-fold increase in risk of respiratory problems. These two studies suggest that with cessation of manganese exposure, there is improvement in some neurological functions, but deficits in others remain.

While studies of the effects of manganese have tended to emphasize occupational exposures, a similar constellation of neurobehavioral effects has been found in a community study from which those with occupational exposures were excluded. Mergler et al. (1999) assessed nervous system functions in 273 individuals (151 women, 122 men) randomly selected from those living in proximity to a former manganese production plant in Southwest Quebec. A battery of tests similar to those of the occupational study above (Mergler et al., 1994) was used to profile nervous system function in relation to blood manganese levels. Motor skills and coordination, learning and recall, visual perception and speed, verbal naming, and cognitive flexibility were assessed. Blood manganese levels ranged from 2.5 to 15.9 µg/l, with results stratified by blood levels (low < 7.5 µg/l vs high >7.5 µg/l), age and sex. Elevated blood manganese (> 7.5 µg/l) was associated with poorer upper limb coordination ($p = 0.04$) and deficits in learning and recall, which was stronger in men ($p = 0.002$) than in women ($p = 0.04$). These deficits were more pronounced in older subjects with elevated blood manganese. The neurobehavioral effects reported here were observed at blood manganese levels lower than those of the occupational study above (0.75 µg/ 100 ml vs 1.03 µg/100 ml). The authors thus suggest that "manganese neurotoxicity can be viewed as a continuum of dysfunction, with early, subtle changes at lower exposure levels, progressing to more severe neurological disorders at the high exposure levels that have been observed in mining, industry and agriculture."

Male workers (n = 92, plus 101 matched controls) in an alkaline battery plant in Belgium exposed to manganese dioxide dust were the subjects of a cross-sectional epidemiological study (Roels et al., 1992). Total manganese concentrations and manganese dust were measured in the workers' breathing zones with personal samplers. Lifetime integrated respirable dust levels (LIRD) ranged from 0.04 to 4.43 mg Mn/m^3 * year, with a geometric mean of 0.793 mg Mn/m^3 * year. The average age of control and exposed groups was 30 years with a mean manganese exposure time of 5.3 years (0.2 to 17.7 years) for the latter group. In exposed workers, the geometric mean levels of blood and urine manganese (corrected for creatinine) were significantly higher ($p < 0.001$) than in controls. The subjects were also evaluated for neurobehavioral function, lung function, and hematological parameters. There were no significant differences in respiratory symptoms between those exposed and controls, and hematological parameters were in the normal range for both groups. In neurobehavioral tests, significant decrements in performance were found in exposed workers on tests for visual reaction time ($p < 0.001$), five measures of eye-hand coordination ($p < 0.005$), and in two of three tests of hand tremor ($p < 0.03$). The data for individuals in this study were used in a BMD analysis to calculate the 8-hr and chronic RELs.

In 1999, Roels et al. (1999) published a follow-up study of the cohort in the Roels et al. (1992) study described above. During the course of the present study, covering the years 1988-1995, the cohort dropped from 92 to 34 workers. Three neurobehavioral

assessments were made. Eye-hand coordination was tested yearly with an orthokinesimeter. Starting in 1991, yearly assessments were also made of visual reaction time, and hand steadiness with a hole tremormeter. Respirable manganese dust exposure was measured in a manner similar to that of the 1992 study using personal air monitors. Three levels of exposure (low, medium, and high), with average exposures from 1987-1992 of 400, 600, and 2,000 µg Mn/m^3, respectively, were compared with unexposed controls in a nearby chemical plant. After 1992, there was a substantial decline in manganese levels with the mean manganese levels dropping by the end of the study to 119, 181, and 744 µg/m^3 for the low, medium and high groups, respectively. In the low exposure group, the test of eye-hand coordination showed improvement with the decreasing manganese levels, and results were normal by the end of the study, while the effects in the higher exposure groups persisted. The time courses of the hand steadiness and visual reaction time tests showed no improvement and suggested irreversible impairment. Similarly, in neurobehavioral assessments of workers who had ceased manganese exposure, eye-hand coordination significantly improved, but deficits in hand steadiness and visual reaction time remained. These studies suggest that some of the neurological deficits improve when manganese exposure decreases, while others may be permanent.

Another follow-up among workers in the same plant covered by Roels et al., 1992 and 1999 was conducted by Crump and Rousseau (1999) for the years 1985-1996. This study covered 213 workers including 114 of the 140 originally tested by Roels. In this study the metric for manganese exposure was blood and urine levels, neither of which was associated with memory or eye-hand coordination tests. There were, however, marginally significant associations between manganese levels and poorer hand steadiness ($p = 0.05$). As in the Roels et al. (1999) follow-up, some neurobehavioral deficits improved with time and lower manganese levels; others appeared to be more permanent.

Welding in confined spaces represents a setting in which significant occupational inhalation exposure to manganese may occur. In an evaluation of 43 welders working on the San Francisco Bay Bridge, Bowler et al. (2007) documented decrements on neurological, neurophysiological, and pulmonary tests associated with exposure to a time-weighted average manganese dust level of 0.11-0.46 mg/m^3 for an average of 16.5 months. Manganese blood levels exceeded 10 µg/l in 43% of the workers. Multiple regression analyses against blood manganese and/or the individual's cumulative exposure index (CEI) revealed significant inverse dose-effect relationships with IQ ($p \leq 0.05$), executive function ($p \leq 0.03$), sustaining concentration and sequencing ($p \leq 0.04$), verbal learning ($p \leq 0.01$), working memory ($p \leq 0.04$), and immediate memory ($p \leq 0.02$) after adjustment for demographics and years of welding before working on the Bay Bridge. Spirometric measurements, taken at three time points, indicated declining lung function with manganese exposure. The first time point was after working on the bridge for an average of 1.5 months, the second after 10.8 months, and the third after 20.9 months. Between the first and third time points, measures of lung function decreased: 7% for FEV_1, 2% for FVC, and 21,2% for the FEV_1:FVC ratio ($p < 0.05$). In tests of mood and affect, the levels of clinical depression and anxiety among welders were greater than two standard deviations above the normative mean.

Neuropsychological tests of parameters characteristic of parkinsonism found that tremor was present 39-90% of the time on three different tests, postural sway was increased in about half of the welders, and motor dexterity and speed were impaired 52-95% of the time. Additional symptoms reported by the welders that showed significant negative correlations with the CEI included sexual function ($p < 0.05$), fatigue ($p < 0.05$), depression ($p < 0.01$), and headache ($p < 0.05$). Compared to test norms, olfaction was impaired in 88% of the welders. This study suffers from the absence of an unexposed control group for comparison. However, all welders were prescreened during the hiring process to ensure good health and fitness. Blood levels of copper, iron, and lead were also measured and considered to be in the normal range. This, coupled with the significant correlations between both blood and air manganese levels and the physiological and neurological decrements, strongly implicates manganese as a causative agent.

That an association between exposure to welding fumes and symptoms of neurotoxicity may be due to manganese was corroborated by magnetic resonance imaging (MRI) detection of characteristic bilateral hyperintense T1-weighted signals in the globus pallidus of eight welders referred for neurological assessment (Josephs et al., 2005). Among the six cases with multiple MRI follow-up scans, the intensity of the MRI signal among the four for whom manganese exposure was discontinued either remained the same (1 case) or faded (3 cases), indicating a loss of pallidal manganese. In the remaining two with continued exposure, the signal remained the same or increased in intensity. All cases presented multiple symptoms characteristic of manganism including postural tremors, reduced arm swing, ataxia, altered gait, multifocal myoclonus, and cognitive impairment. In addition, several cases reported irritability, memory loss, headaches, slurred speech, and reduced sexual drive. All cases had elevated or high normal serum manganese levels. This constellation of symptoms in association with manganese exposure and characteristic MRI images suggests a role for welding fumes in the development of manganism. Substantially similar symptomologies have been reported elsewhere in case studies of welders (Sadek et al., 2003). However, it is important to note that welding fumes are a mixture of metals, many of which are also neurotoxic and may contribute to the reported neurological symptoms.

The neurotoxic effects of exposure to welding fumes may be accompanied by pulmonary damage. Clara cells lining the airways normally secrete Clara cell protein (CC16) that has anti-inflammatory properties. Pulmonary damage that includes the Clara cells results in decreased recovery of CC16 in bronchoalveolar lavage fluid, as well as an increase in serum levels of CC16. The former effect is presumably due to reduced production by the affected Clara cells, while the latter is attributed to damage to the bronchoalveolar/blood barrier (Hermans et al., 1999). In ship welders, measures of serum CC16 levels, blood, urine and air manganese levels, pulmonary function (vital capacity), and subclinical neurological effects (EEG and visual evoked potential (VEP) were compared with unexposed controls (Halatek et al., 2005; 2008). During examinations that assessed both subjective and objective neurological status, 66% of the 59 workers reported subjective central nervous system symptoms, while 29% had abnormal VEP results and 41% had abnormal EEGs. Among welders showing neurological symptoms, blood manganese was significantly elevated (12.2 vs 6.1 µg/l, p

< 0.05) while vital capacity was significantly depressed (84.5%, $p < 0.05$) (Halatek et al., 2008). Multiple linear regression analysis revealed strong partial correlations between abnormal VEP and EEG, and both blood manganese (0.72, $p = 0.03$) and an index of cumulative manganese exposure (0.66, $p = 0.01$) (Halatek et al., 2005). Levels of CC16 were significantly correlated (0.82, $p = 0.015$) with abnormal VEP, EEG and CNS symptoms. The CC16 levels were significantly lower (9.6 µg/l, $p < 0.05$) among the younger welders who had fewer years of exposure (3 years) but higher blood manganese levels (13.7 µg/l), abnormal VEP and EEG results, and depressed vital capacity (83%). The authors suggest that the elevated CC16 levels indicate that welding fume exposure compromises pulmonary function, including that of the bronchoalveolar barrier. This in turn facilitates manganese access to the blood and brain, with the attendant subclinical neuropathological changes.

As mentioned in the study above, in addition to neurotoxicity, manganese inhalation may lead to symptoms of pulmonary toxicity. Indeed, the incidence of respiratory disease is higher among manganese-exposed workers than those not exposed (Boojar and Goodarzi, 2002). In a case report, Wittczak et al. (2008) present the study of a 42-year-old non-smoking welder with suspected occupational asthma. At admission, the patient presented with a recurrent nonproductive cough, and dyspnea with wheezing that usually occurred after 30-60 minutes of welding. Compared with non-work days, on the days the patient was exposed to welding fumes, he exhibited a greater than 20% variability in his peak expiratory flow rate (PEFR). Histamine challenge revealed significant bronchial hyperreactivity (PC_{20} = 0.5 mg/ml). In contrast to a placebo inhalation challenge (1% KCl solution), five minutes following a challenge with 0.1% MnCl solution, dyspnea occurred and forced expiratory volume (FEV1) dropped 45% . At one hour, FEV1 was 55% below resting levels, and only recovered to 35% below resting levels by 24 hours. At 4 and 24 hours post-exposure, changes in the proportions of eosinophils (8% and 10% resp.) and basophils (1% and 3%, resp.) were observed in induced sputum. None of these effects was observed in non-exposed controls after similar challenges. This constellation of symptoms and sensitivity to manganese challenge supports a role for manganese in occupational asthma.

Another occupational study of lower exposures was done in Sweden (Wennberg et al., 1992). In this study workers had been exposed for a year or more to manganese dust at mean concentrations of 0.18 mg/m^3 at one smelter, and 0.41 mg/m^3 at another. They were compared to workers at similar industrial plants without manganese exposure via a suite of neurological tests, including electroencephalogram, brainstem auditory evoked potential, event related auditory evoked potential, and diadochokinesometry (a test of the subject's ability to rotate a handle rapidly). Of these tests, the only one that produced significantly different results in the exposed subjects was the diadochokinesometry. The manganese-exposed workers were unable to rotate the handle as quickly as the control workers. This is interpreted as evidence of a "preclinical" effect of low-level manganese exposure.

A major study of non-industrial human exposures is the study of the natives of Groote Eylandt, a large island off the coast of Australia. The inhabitants of this island are Australian Aborigines. The island is so rich in manganese that the environment has

been described as a “manganese ecology” (Kilburn, 1987). The inhabitants are exposed by virtually all routes of exposure, but especially by ingestion of food and water high in manganese. Kilburn studied the natives of Groote Eylandt and compared them to a control group of Australian Aborigines living in another part of Australia. This paper does not quantitate the manganese exposures or body levels of manganese in the study population, and it would be difficult to quantitate exposures in this complex environmental situation. Kilburn reports certain congenital abnormalities, such as deformations of the foot (talipes equinovarus), closed anus (imperforate anus), and anorectal malformations, and neurobehavioral problems, including progressive muscle wasting (amyotrophy) and failure of muscle coordination (ataxia), that apparently occur with greater frequency in the islanders than in the control groups, but these could also be due to genetic factors present in this small population. Indeed all of the problems were seen in just two pedigrees. A likely interpretation would be that the adverse health effects observed reflect gene-environment interactions.

Exposure to excess manganese may occur via the parenteral route, especially in individuals receiving total parenteral nutrition (TPN). Unfortunately, patients receiving TPN are often those with liver damage and/or gastrointestinal disorders, both of which compromise the hepatobiliary circuit by which the body regulates retention of dietary manganese. Manganese intoxication in these cases typically manifests as confusion, dysarthria, rigidity, gait disturbances, and hypokinesia, and is generally confirmed by marked hyperintensity of the globus pallidus by magnetic resonance imaging (MRI) (Ono et al., 1995; Nagatomo et al., 1999). Symptomatic improvement and reversion to more normal T1-weighted MRI images following discontinuation of manganese supplementation support the diagnosis of manganism. While the parenteral route is thus involved in the increased risk of hypermanganesaemia during TPN, it is also involved in the recent rise in cases of manganism in adults associated with long-term intravenous use of illicit designer drugs. Sikk et al. (2007) described four cases of young adults presenting with symptoms of manganism (including impaired postural control, unsteady gait, manual dysfunction) in conjunction with long-term (7 months – 8 years) repeated use of the psychostimulant ephedrone. In two cases, drug use started as young as 17 and 19 years of age. The synthesis of ephedrone involved the oxidation of pseudoephedrine with potassium permanganate, which remained in the injected solution. Based on the drug injection history of two of the cases, and an analysis of the manganese content of a similarly synthesized ephedrone preparation, the authors estimate a total body burden of manganese corresponding to 900 and 500 mg/kg body weight compared to a normal body burden of 10-20 mg/kg (Schroeder et al., 1966). Cranial MRI of two individuals with exposures and symptoms similar to those described by Sikk et al. revealed hyperintense patterns in the globus pallidus indicating an abnormally high accumulation of manganese (Meral et al., 2007).

6.2 Chronic Toxicity to Infants and Children

Manganese is an essential nutrient, but it has toxic effects if exposure is excessive or prolonged, especially if exposure is by the inhalation route. A number of studies have reported correlations between early life exposure to excessive manganese and symptoms of impaired neurodevelopment as revealed on neurobehavioral tests and in

poorer academic performance. In a prospective study of the neurobehavioral effects of *in utero* exposure to manganese, Takser et al. (2003) reported an inverse correlation between cord blood manganese at birth and three subscales of psychomotor development (McCarthy scales of children's abilities) measured at three years of age (n = 126): attention (partial r = -0.33, $p < 0.01$), nonverbal memory (partial r = -0.28, $p < 0.01$), and hand skills (partial r = -0.22, $p < 0.05$). The adverse effects of manganese on neurodevelopment in these children persisted after adjustment for gender and maternal education, although the effects of manganese on hand skills were only observed in boys. Similarly, Collipp et al. (1983) used a battery of tests, including cognitive and projective tests, psycho-educational evaluation, speech, language and hearing evaluations, and social services evaluations, to identify 16 children who were hyperkinetic and exhibited learning disabilities. In comparison with 44 normal children of the same age, significantly elevated levels of hair manganese (0.434 µg/g; measured at 8 years of age) were reported in children with learning disabilities and hyperactivity compared with normal children (0.268 µg/g) ($p < 0.05$). An association between poorer performance in school and elevated hair manganese (1.242 µg/g) has also been observed among children in China compared with children with more normal manganese levels (Zhang et al., 1995).

Wasserman et al. (2006) reported adverse effects of manganese in 10-year old children (n = 142) in Bangladesh who had been exposed to manganese in their drinking water (< 200, 200-499, 500-999, > 1,000 µg/l). Comparing the lowest and highest dose groups (< 200 vs. > 1,000 µg/l), significant decrements in intellectual function at 9.5-10.5 years of age were revealed in scores on the Wechsler Intelligence Scale for Children-III with increasing daily intake of manganese (full scale, $p < 0.0001$; performance, $p < 0.0001$; verbal, $p < 0.02$). The scores of children with intermediate manganese exposures were also lower than those of the lowest dose group, but not significantly so. In this study, confounding by co-exposure to arsenic was limited by including only children whose drinking water contained < 10 µg As/l. Scores were adjusted for maternal education and intelligence, house type, television, child height and head circumference. Blood levels of manganese, arsenic and lead were also determined and added to the core model. In this case, only blood lead was correlated with decreased intellectual performance. However, in a simultaneous analysis of water manganese, water arsenic, and blood lead, the negative association between manganese water levels and intellectual function test scores remained (Full-Scale ß = -4.56, $p < 0.01$; Performance ß = -3.82, $p < 0.01$).

The uptake of metals into developing teeth provides a record of gestational exposure to manganese. In multiple regression analyses, after controlling for lead, high levels of manganese incorporated into teeth during the 20th week of gestation were positively correlated with behavioral disinhibition at 36 months of age (R = 0.48, $p < 0.01$) and, at 54 months, with impulsive errors on the Mirsky Continuous Performance Test (R = 0.48, $p < 0.01$) and the Children's Stroop Test (R = 0.38, $p < 0.01$). Positive correlations with manganese were also seen in ratings made by both parents and teachers of externalizing and attention problems on the Child Behavior Checklist in the 1st (R = 0.40 – 0.47, $p < 0.05$) and 3rd grades (R = 0.38 – 0.48, $p < 0.05$), and in the 3rd grade with the teachers' ratings on the Disruptive Behavior Disorders Scale (R = 0.44, $p < 0.05$),

ADHD (R = 0.48, p < 0.01), and hyperactivity – impulsivity (R = 0.55, p < 0.01). In contrast, manganese levels in tooth enamel formed in the 62-64th week of gestation (i.e., postnatally) were correlated only with teachers' reports of externalizing behaviors in the 1st (R= 0.40, p < 0.05) and 3rd grades (R = 0.57, p < 0.01). It thus appears that high prenatal manganese exposure may adversely affect behaviors expressed postnatally. There was, however, no correlation between tooth manganese and cognitive ability as measured on the Woodcock-Johnson Psycho-Educational Battery (Ericson et al., 2007).

Subtle neurobehavioral effects were seen in a case report of a 10-year old boy exposed for five years to elevated manganese in the family's drinking water (Woolf et al., 2002). The boy's hair manganese was high (3,091 ppb vs normal reference < 260 ppb), as was that of his 16 year-old brother (1,988 ppb). Neuropsychological tests on the 10 year-old revealed intact global cognitive skills but striking deficits in visual and verbal memory (< 20th percentile in the Wide Range Assessment of Visual-motor Abilities). No obvious neurobehavioral problems were noted for either the parents or the older sibling.

As with adults, children receiving long-term parenteral nutrition are at greater risk of hypermanganesaemia. This is especially problematic since it is often the premature infants that require TPN. Among infants receiving parenteral nutrition containing supplemental manganese, MRI scans revealed bilaterally symmetrical hyperintense signals in the globus pallidus associated with movement disorders (dystonia and abnormal posturing) (Fell et al., 1996), and in basal ganglia, brainstem, and cerebellum associated with seizures (Komaki et al., 1999). In one infant these effects developed within eight months (Fell et al., 1996). While an abnormally high T1-weighted MRI signal suggests high brain manganese levels, especially when removal of manganese results in gradually diminishing signal intensity, it should be noted that not all patients with elevated brain manganese develop overt neurological symptoms. Kafritsi et al. (1998) reported on siblings receiving parenteral nutrition for 63 and 23 months that resulted in elevated blood manganese levels of 323 nmol/l and 516 nmol/l, respectively (normal = 73-210 nmol/l) and hyperintense signals in the globus pallidi. The signal intensity reverted to normal following cessation of manganese supplementation with no evidence of abnormal neurological development at three years of follow up. Whether either child had subclinical effects or effects that will manifest only later in life is unknown.

6.3 Animal Studies of Chronic Toxicity

Animal studies of the toxic effects of chronic manganese exposure have focused on altered neurobehavior and the effects of manganese on the associated brain structures. These studies indicate that differences in age at exposure, route, and chemical form of the metal are critical to the distribution of manganese, and the type and extent of the adverse effects.

Early life exposure to air borne manganese may occur by multiple routes: in utero via the mother, and perinatally via milk ingestion and inhalation. To examine the effects of these exposures on tissue concentrations of manganese in adult and young rats, adult

male and female rats were exposed to $MnSO_4$ (0.05, 0.5, or 1 mg Mn/m^3; 1.05 µm GMD) or air 28 days prior to breeding, for up to 14 days during the mating period, during gestation days 0-19, and from one day post-partum through postnatal day (PND) 18 (Dorman et al., 2005a). Exposures were for 6 hr/day, 7 days/week. While these exposures did not affect maternal brain, lung, pancreas, or liver weights, the high dose (1 mg/m^3) was associated with decreased brain weights in pups on PND 14, female pups on PND 19, and male pups on PND 45. Measurements of manganese in the striatum, cerebellum, and olfactory bulb of neonatal rats on PND 19 showed statistically significant and dose-dependent increases relative to controls. On PND 18, maternal olfactory bulb, striatum, and cerebellum also had significantly ($p < 0.05$) elevated manganese from exposures to 0.5 mg/m^3 and above. Measurements made 27 days following exposure cessation on PND 45 showed that all tissue manganese levels had returned to control values. However, some pups on PND 45 still showed decreased brain weights suggesting that high early-life manganese exposure may result in prolonged alterations in brain size. By PND 63, all brain weights were at control values, but whether structural or functional deficits were present was not determined in this study.

Some consequences of early life exposure to manganese may not manifest until later in life. This appears to be the case for prenatal exposure to the manganese-containing fungicide Maneb (manganese ethylene-bis-dithiocarbamate), followed later in life by exposure to the pesticide paraquat and the development of symptoms of manganism. It has been reported previously (Thiruchelvam et al., 2000) that mice treated with Maneb twice a week for six weeks showed reduced motor activity immediately after treatment, with recovery of function in 24 hours. This effect was not seen with paraquat alone but was enhanced by co-exposure to paraquat. In these mice, co-exposure reduced tyrosine hydroxylase and dopamine transporter immunoreactivity in dorsal striatum. Similarly, only the combined Maneb/paraquat exposure decreased striatal tyrosine hydroxylase protein levels, caused reactive gliosis in dorsal-medial but not ventral striatum, and reduced tyrosine hydroxylase immunoreactivity and cell counts in the substantia nigra but not ventral tegmental area.

Barlow et al. (2004) extended these observations to early life susceptibility with the treatment of pregnant mice with Maneb, paraquat, or saline on gestation days 10-17. As adults, these mice received challenge exposures for eight days to either Maneb or paraquat on postnatal days 45-55. Prior to the challenge exposures, locomotor activity was evaluated but no significant differences among groups were found. On the eighth day of challenge exposures, locomotor activity was depressed in all animals exposed to Maneb as adults, but recovered to control levels within one week of the last challenge exposure, except for males prenatally exposed to Maneb and subsequently exposed to paraquat as adults. These males showed a 95% reduction in locomotor activity, while similarly exposed females showed no effects. In the context of dopaminergic neurochemistry, neither prenatal exposure to Maneb alone nor adult exposure to paraquat alone caused significant change in either gender. However, compared to male controls and paralleling the locomotor effects, males receiving Maneb prenatally and paraquat as adults had 50% lower striatal dopamine levels, 35% lower 3,4-dihydroxyphenylacetic acid (DOPAC, a dopamine metabolite) levels, and 40% greater

dopamine turnover. In the substantia nigra pars compacta, these males showed a loss of tyrosine hydroxylase-positive neurons of 30% compared with saline-treated males ($p < 0.001$), 30% compared with males receiving Maneb prenatally and saline as adults ($p < 0.001$), and 21% compared with males receiving paraquat as adults only ($p < 0.05$). The reduction in tyrosine hydroxylase positive neurons only occurred with Maneb followed by paraquat, not following Maneb alone. These results suggest that prenatal exposure to Maneb causes damage to the nigrostriatal region of the male brain that is only revealed in adulthood following another neurotoxic insult in the form of paraquat. While these experiments do not demonstrate that it is the manganese in Maneb that is responsible for the observed neurotoxicity, the types of toxicity described are similar to those observed for other manganese compounds. These experiments also do not address the potentially enhanced neurotoxicity associated with more continuous exposure to manganese as Maneb during prenatal to adult development. However, long-term exposure to Maneb among adult farm workers has been associated with the development of symptoms of Parkinson's Disease, characteristic of manganism (Ferraz et al., 1988; Meco et al., 1994). It should also be noted that while this experimental design emphasized the neurotoxicity of the sequential exposure to Maneb, then paraquat, it is possible that the deleterious effects of exposure to other neurotoxic substances during development or adulthood would also be enhanced by early life exposure to manganese-containing pesticides.

The relative sensitivity of neonatal and adult CD rats to manganese-induced neurotoxicity was studied by administering manganese dichloride orally to rats at doses of 0, 25, and 50 mg/kg per day (Dorman et al., 2000). Adults and pups were dosed for 21 consecutive days, and then were evaluated with behavioral tests such as pulse elicited startle response amplitude, and in terms of manganese levels in striatum, hippocampus, hindbrain, and cortex. Neonatal rats exposed at the highest level of manganese showed a statistically significant increase in amplitude of acoustic startle response. They also showed increases in brain levels of manganese. The results suggest that neonates may be at greater risk for manganese-induced neurotoxicity when compared to adults receiving high oral levels of manganese. The authors state that there are known pharmacokinetic processes that may relate to the increase in brain manganese concentration in neonatal rats including increased manganese absorption from the juvenile gastrointestinal tract, an incompletely formed blood-brain barrier, and a virtual absence of excretory mechanisms until weaning.

The effects of manganese inhalation on levels of the metal in various tissues has been explored in Rhesus monkeys. In a study by Dorman et al. (2006b), Rhesus monkeys, 20-24 months of age, inhaled manganese sulfate (60, 300 or 1,500 $\mu g/m^3$; 1.04, 1.07, 1.12 µm; GMD, respectively) 6 hours per day, 5 days per week for 13 weeks. At termination, tissue manganese levels were significantly ($p < 0.05$) elevated in all tissues examined, except testes, in animals exposed to the highest dose (1,500 $\mu g/m^3$). Even at the lowest dose (60 $\mu g/m^3$), manganese levels were significantly elevated in four of the eight brain regions examined, globus pallidus, putamen, white matter and cerebellum (Table 6.3.1). For comparison, the table includes manganese levels in these same brain regions reported below by Schneider et al. (2006) and Guilarte et al. (2006) for monkeys displaying neurobehavioral toxicity. To facilitate comparison with

human occupational studies, an annual dose is also presented showing the calculated air concentration had the dose levels been spread over a period of one year. In the study by Roels et al (1992), used for the development of the REL (Section 8), a similar measure of the annualized exposure was calculated as the lifetime integrated respirable dose (LIRD). In the Roels study, neurotoxicity was observed in individuals with LIRDs in the range of 60 to 3,715 µg*yr/m^3, a range that overlaps the concentrations used by Dorman that resulted in brain levels associated with neurotoxicity by Schneider and Guilarte. Although the brain levels of manganese were not measured in workers showing neurotoxicity in the Roels study, these studies in primates provide support that the air concentrations to which the workers were exposed was sufficient to result in brain manganese levels with which neurotoxicity is associated in primates.

Table 6.3.1 Manganese Levels in Primate Brain After Inhalation or IV Exposure

	Dorman et al., 2006			Schneider et al., 2006/
	Inhalation level			Guilarte et al., 2006
	60 µg/m^3	**300 µg/m^3**	**1,500 µg /m^3**	**3.26-4.89 mg Mn/kg***
Caudate	0.47 µg/g	0.69 µg/g	1.72 µg/g	1.18 µg/g
Putamen	0.58	0.75	1.81	1.50
Globus pallidus	0.80	1.28	2.94	3.30
White matter	0.25	0.39	0.87	0.57
Annual dose**	**15 µg*yr/m^3**	**75 µg*yr/m^3**	**375 µg*yr/m^3**	

* Neurotoxicity reported in monkeys with the indicated brain Mn levels.
* Roels reported neurotoxicity at an overlapping range: LIRD = 60 - 3,715 µg*yr/m^3

Neurobehavioral effects may be preceded by changes in brain chemistry. Such changes were studied in four female rhesus monkeys exposed in an inhalation chamber to 30 mg/m^3 respirable manganese dust for five hours/day, five days/week (Bird et al., 1984). After two years the animals were sacrificed and compared to unexposed controls. The exposed monkeys showed decreased dopamine in the caudate and globus pallidus, as well as a 60 to 80 percent increase in manganese levels in the basal ganglia of the brain. However, the exposed monkeys did not exhibit any of the movement disorders that are characteristic of Parkinson's disease.

In another study of the effects of manganese inhalation on neurotransmitters in rhesus monkeys (20-24 months old), Struve et al. (2007) found that subchronic (13 wk) exposure to manganese sulfate resulted in statistically significant increases in mean manganese concentrations in the pallidus and putamen at 0.06, 0.3, and 1.5 mg/m^3 (MMAD 1.73, 1.89, 2.12 µm) , and in the caudate at ≥ 0.3 mg/m^3. Marginally statistically significant ($p < 0.1$) changes in neurotransmitter levels were seen only at the highest manganese concentration (1.5 mg/m^3) in the globus pallidus for GABA and 5-HIAA, and in the caudate for norepinephrine. This is consistent with the suggestion that manganese neurotoxicity derives in part from dis-regulation of GABA-ergic neurons (Fitsanakis et al., 2006), possibly related to the observed decreases in tyrosine hydroxylase and glutamine synthetase by manganese (Erikson et al., 2007a).

The distribution of manganese in primate brain, and its neurobehavioral and cognitive effects in 5-6 year old Cynomolgus macaques following weekly intravenous injection of $MgSO_4$ (10-15 $MgSO_4$ or 3.26-4.89 mg Mn/kg) for 39 weeks was investigated by Guilarte and associates. Neurobehavior, as rated on a modified Parkinsonian symptoms scale, activity levels measured with an activity monitor, and fine motor skills, assessed as the number of errors while trying to retrieve objects from wells of different sizes, all showed significant decrements ($p < 0.05$) at the end of the experiment compared with baseline (Guilarte et al., 2006a). Over this same period, stereotypical or compulsive-like behaviors, such as licking/biting fingers and grooming, significantly increased in frequency with manganese exposure ($p < 0.01$) (Schneider et al., 2006). The levels of manganese were significantly ($p < 0.05$) elevated in exposed monkeys compared to controls in the globus pallidus (3.3 µg/g tissue), caudate (1.18 µg/g), putamen (1.50 µg/g), and frontal white matter (0.57 µg/g) (Table 6.3.1). Imaging studies were performed at 128 days and 157 days after the start of manganese exposure, and included T-1 weighted magnetic resonance imaging (MRI), magnetic resonance spectroscopy (H-MRS), and positron emission tomography (PET). As assessed by PET, manganese decreased the ability of amphetamine to stimulate dopamine release in the striatum, apparently without the loss of dopaminergic terminals. The authors speculate that the inhibition of dopamine release may alter the excitability of nigrostriatal dopaminergic neurons and/or may alter dopamine compartmentalization. The former case may contribute to the behavioral symptoms while, in the latter case, the probability of dopamine oxidation and consequent neuronal damage may be increased (Guilarte et al., 2006a). Neuronal loss or dysfunction in these monkeys was suggested by a change in brain metabolites with chronic manganese exposure. Specifically, significant decreases in the N-acetylaspartate: creatinine ratio in parietal cortex ($p = 0.028$), and a near significant ($p = 0.055$) decrease in the white matter were observed.

Concern for the consequences of exposure to the combustion products of methylcyclopentadienyl manganese tricarbonyl (MMT) has fueled investigation of the bioaccumulation and neurobehavioral effects following subchronic exposure to manganese as the free metal, conjugates of sulfate and phosphate, and a mixture of the two conjugates. In a collection of related studies (Normandin et al., 2002; Salehi et al., 2003; Normandin et al., 2004; Tapin et al., 2005), young adult rats were exposed to aerosolized manganese or its conjugates for 6 hours/day, 5 days/week for 13 weeks at target levels of 30, 300, and 3,000 µg/m^3 (<1.55 – 6 µm MMAD). Following exposure, locomotor activity over a 36 hr period was recorded as resting time, distance traveled, and total ambulatory count. The animals were then sacrificed and the manganese levels in various tissues and brain regions measured. These studies consistently showed significant ($p < 0.05$) dose-dependent increases in manganese in the lungs for all forms of the metal. The highest dose of the manganese conjugates, separately or mixed, resulted in significantly ($p < 0.05$) elevated levels in all tissues except the liver, reflecting the liver's role in manganese homeostasis. The sulfate and phosphate conjugates were better assimilated into all extra-pulmonary tissues than was the un-conjugated metal (Normandin et al., 2004). Uptake of manganese into brain tissue was more efficient from the combined sulfate and phosphate exposure than from exposure to metallic manganese or the phosphate conjugate alone, presumably due to the higher solubility of the sulfate conjugate. This difference was reflected in the significantly ($p <$

0.05) lower ambulatory count for the animals exposed to the conjugate mixture compared to controls (Normandin et al., 2004). For rats exposed to $MnSO_4$ alone, the total distance traveled in the locomotor studies increased at all manganese concentrations concomitant with an increase in total resting time, suggesting shorter bursts of activity. These rats also showed a dose-dependent decrease in total ambulatory counts over 36 hours, as well as a dose-dependent loss of cells in the globus pallidus and caudate putamen (Tapin et al., 2005). Similarly, the highest exposure to the Mn sulfate/phosphate mixture produced a significant increase in motor activity and a significant decrease in total ambulatory counts (Saleh et al., 2003). In contrast, no behavioral changes were noted with exposure to the phosphate conjugate (Normandin et al., 2002). Collectively these studies suggest that behavioral neurotoxic effects are associated with inhalation of manganese conjugates and that the sulfate conjugate is more toxic than the phosphate or metallic forms, consistent with its greater solubility.

In addition to neurotoxicity, pulmonary dysfunction may be associated with inhalation of manganese. In a subchronic chamber study, young, male rhesus monkeys were exposed to manganese sulfate at 0.06, 0.3, or 1.5 mg Mn/m^3 for 6 hours/day, 5 days/week, for 65 exposure days (Dorman et al., 2005b). The MMADs of the particles in these aerosols were 1.73, 1.89, and 2.12, respectively (1.04, 1.07, 1.12 µm GMD, respectively). Another set of monkeys, exposed to 1.5 mg Mn/m^3 by this regimen, was held for 45 or 90 days prior to evaluation. A third set was exposed to 1.5 mg Mn/m^3 and evaluated after 15 or 30 days of exposure. The evaluations included histopathological assessments of the lungs, and measurements of the manganese content of lungs and olfactory epithelium. Manganese levels were significantly elevated ($p < 0.05$) in olfactory epithelium with all exposures, and in lungs at exposures of 0.3 mg/m^3 and above. In animals exposed to 1.5 mg/m^3 and evaluated after 15 or 33 days, significantly ($p < 0.05$) elevated manganese levels were found in both olfactory epithelium and lungs, however, these levels returned to control levels 45 and 90 days after exposure was discontinued. Significant bronchiolitis and alveolar duct inflammation was seen only in the animals exposed to 1.5 mg Mn/m^3, but these effects were apparently reversible as they were no longer present at 45 and 90 days post exposure. Increased bronchus-associated-lymphoid tissue was also observed only with the highest exposure. Thus the inflammatory changes in small airways and increased manganese lung concentrations were only associated with the highest exposure levels used (1.5 mg Mn/m^3), and were apparently reversible following cessation of exposure, suggesting that the lungs are a less sensitive target for manganese toxicity than is the central nervous system.

The pulmonary toxicity of manganese, as measured by induction of an inflammatory response, is relatively high compared with a number of the transition metals found in particulate pollution. Intratracheal instillation of the sulfates of copper, vanadium, nickel, iron, zinc, and manganese (0.1, 1.0 µmol/kg) was used to assess the relative inflammatory potential of these metals in rats. Bronchoalveolar lavage (BAL) at 4, 16 and 48 hours following exposure provided the medium in which markers of inflammation were measured (Rice et al., 2001). Lactate dehydrogenase activity and total protein levels in the lavage fluid, used as general indicators of toxicity, showed copper at 1

µmol/kg to be the most toxic at all time points, followed by nickel and manganese. Whereas with copper LDH activity peaked at 16 hours post-exposure, then declined by 48 hours, manganese-induced LDH activity was significantly ($p < 0.05$) elevated at all time points and continued to increase with time. Similarly, the numbers of leukocytes recovered from BAL fluid were highest for manganese compared with the other metals at 16 and 48 hours. At these same time points, neutrophilia was seen at the low dose only with copper, while at the higher dose, manganese was the most potent. Significant eosinophilia was observed for manganese, copper, iron, and nickel at 16 and 18 hours, but eosinophil numbers were the highest with manganese by two to three-fold. Lymphocyte levels were not elevated by metal treatment except with low-dose copper at 16 hours, and high dose manganese at 48 hours. Thus manganese and copper were found to be the most proinflammatory of the metals tested but presumably by different signaling pathways. The effects with copper tended to appear earlier and at lower exposure levels, while manganese was more effective at stimulating the appearance of immune cells at a higher dose and later time points.

6.4 Dietary Exposure to Manganese

Newborns and infants may be exposed to more manganese in their diets than are adults. Infant formulas based on cow's milk have about 16 times more manganese than human milk (Dorner et al., 1989). Soy based formulas have even higher levels of manganese – about 40 times the manganese of human milk (Tran et al., 2002a; Tran et al., 2002b). Formula usage can lead to significantly elevated body burdens of manganese. For example, the hair manganese in normal infants at birth was reported to be 0.19 µg/g hair and, in breast-fed infants, increased to 0.330 µg/g at four months of age. By comparison, hair manganese levels in infants on a formula diet reached 0.965 µg/g at six weeks of age, and 0.685 µg/g at four months (Collipp et al., 1983). In addition, infants can have a less varied diet than adults and may consume more of certain foods that are high in manganese (e.g., sweet potatoes, 2.6 mg/cup; spinach, 1.8 mg/cup; oatmeal, 1.4 mg/cup; (NWU, 2006)).

6.5 Nutritional Requirement

Manganese is an essential nutrient involved in the formation of bone, and in amino acid, cholesterol, and carbohydrate metabolism (FNB, 2004). It is required in a number of metalloenzymes, including arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and superoxide dismutase (FNB, 2004). Levels of manganese in adult tissues are maintained at stable levels by homeostatic mechanisms that involve regulation of both uptake and excretion (Aschner and Aschner, 2005). Manganese homeostasis is not maintained in newborn infants, and it is not clear how long it takes for it to develop (FNB, 2004); homeostasis in mice takes 17 to 18 days to become effective (Fechter, 1999). Rat pups born to manganese-exposed mothers (dosed with 2000 ppm Mn in drinking water throughout pregnancy and for 11 days of lactation) have seven times the manganese (whole body) as controls (Kostial et al., 2005). By weaning (11 days after birth) the manganese concentration in both groups is virtually the same, indicating that in rat pups manganese homeostasis may begin shortly after birth and become effective by weaning (Kostial et al., 2005).

Adequate intakes (AI) of manganese have been established by the Food and Nutrition Board of the Institute of Medicine (FNB, 2004). They are given in Table 6.5.1 below. This table also contains tolerable upper intake levels (UL) for manganese consumption. It is of note that in many cases the UL is not very far above the AI level. For children one to three years of age the UL is less than twice the AI.

The AI for infants 0 to 6 months was set based on the amount of manganese in human milk and the average amount of milk consumed. There are no reports of nursing infants showing any symptoms of manganese deficiency (FNB, 2004). The AI for infants 7 to 12 months of age is based on the manganese content of a typical diet including human milk and other foods. This AI is much higher than the one for infants 0 to 6 months because the manganese content of other foods is generally much higher than the manganese content of human milk (FNB, 2004).

Table 6.5.1 Adequate Intakes and Tolerable Upper Intake Levels for Manganese for Different Age Groups

Group	Adequate Intake (AI) (mg/day)	Tolerable Upper Intake Level (UL) (mg/day)
Infants, 0-6 months	0.003	“not possible to establish”
Infants, 7-12 months	0.6	“not possible to establish”
Children, 1-3 years	1.2	2
Children, 4-8 years	1.5	3
Boys, 9-13 years	1.9	6
Boys, 14-18 years	2.2	9
Girls, 9-13 years	1.6	6
Girls, 14-18 years	1.6	9
Men, 19 to >70 years	2.3	11
Women, 19 to >70 years	1.8	11
Pregnant women, 14-18 yrs	2	9
Pregnant women, 19-50 yrs	2	11
Lactating mothers, 14-18 years	2.6	9
Lactating mothers, 19-50 years	2.6	11

6.6 Potential for Differential Effects in Children

Infants and children may be more susceptible than adults to manganese toxicity for the following toxicodynamic and toxicokinetic reasons:

1. As noted in the previous section, manganese exposures in childhood are associated with impaired neurodevelopment including decrements in intellectual function. Thus a major toxicodynamic factor that differs between adults and children, namely development of the central nervous system, presents hypersensitive targets for toxicity in the developing infant and child.
2. Early life manganese exposures may predispose to manifestations of neurological damage in adulthood following subsequent exposure to environmental toxicants (Barlow et al., 2004).
3. Newborns absorb and retain more manganese from the gastrointestinal tract than do adults (Dorner et al., 1989; Davis et al., 1993).
4. The liver of newborns has not yet developed the ability to maintain safe levels of manganese in the bloodstream and brain tissues by excreting excess manganese in the bile, i.e., homeostasis of manganese has not yet developed (Miller et al., 1975).
5. Some infant formulas and foods are high in manganese. Soy formula may contain 200-300 µg Mn/l compared with 6 µg Mn/l for breast milk (Tran et al., 2002a,b). High dietary intake, combined with inhalation exposure, may put infants at greater risk of manganese toxicity.
6. The newborn's brain is still developing, myelination is incomplete, and the blood-brain barrier is not fully formed (Chan et al., 1992). These conditions facilitate manganese uptake into the central nervous system and increase the risk of attaining toxic levels.
7. Modeling of the inhalation dosimetry of particles (0.001-10 µm), comparing infants (3 mo) and adults, in four regions of the respiratory tract (extra-thoracic, tracheo-upper bronchi, bronchiolar, pulmonary), suggests that differences in the dose per unit surface area between neonates and adults are dependent on particle size and respiratory tract region (Ginsberg et al., 2005). These differences are most pronounced in the pulmonary and bronchiolar regions for ultrafine particles in the 0.1 to 0.001 µm range where neonates experience a 2-4 fold higher particle dose. In addition, infants and young children experience overall higher deposition of particles than adults.
8. Manganese absorption from the nose (Thompson et al., 2007), lungs (Brain et al., 2006), and intestinal tract (Erikson et al., 2002) is enhanced by low iron levels, a condition more prevalent among children than adults. Uptake directly from the nose or from the lungs bypasses first pass through the liver.

7. Developmental and Reproductive Toxicity

While data are scarce on the developmental effects of perinatal manganese exposure in humans, rats exposed to supplemental manganese (50, 250, 500 µg/day) beginning at birth show decreased dopamine in the striatum and poorer performance on behavioral tests (Tran et al., 2002b). This is consistent with studies examining manganese levels in various brain regions following developmental exposure. Female rats were exposed to $MnCl_2$ in drinking water (10 mg/ml) from the time of mating through weaning. The female offspring were similarly treated until sacrifice at 5, 10, 22, or 120 days postpartum (Chan et al., 1992). These time points represented the early postnatal period (day 5), the period of active myelination (days 10- 22), and sexual maturation (days 22-120). As shown in Table 7.1, manganese levels in all regions of the 5-day-old brain, except the cerebellum, are significantly elevated relative to unexposed controls. During the period of myelination (days 10-22), the manganese concentrations decreased. However, compared with controls, the concentrations in the treated rats were 2-13-fold higher, with the greatest difference in the striatum, followed by the midbrain. The differences in levels between groups decreased through sexual maturation. These data suggest that manganese distribution in the developing brain is heterogeneous and age-dependent, with the striatum and midbrain as potentially more susceptible regions for metal accumulation with high exogenous exposure.

Table 7.1 Brain Regional Mn Concentrations (µg/g wet wt.)
fr. Chan et al. (1992)

Brain Region		Postnatal Age (days) 5	10	22	120
Hypothalamus	Control	2.50 ± 0.33[c]	0.52 ± 0.05[c]	0.42 ± 0.01[c]	0.30 ± 0.03
	Mn	4.52 ± 0.72[c,,e]	0.99 ± 0.11[c,e]	2.23 ± 0.20[c,e]	1.11 ± 0.22[e]
Cerebellum	Control	5.73 ± 0.28[c]	3.97 ± 0.19[c]	0.47 ± 0.16	0.38 ± 0.05
	Mn	4.95 ± 0.95[c]	6.16 ± 0.11[c,e]	1.32 ± 0.09[b,e]	0.94 ± 0.07[e]
Pons & medulla	Control	9.56 ± 1.16[c]	4.73 ± 0.50[c]	0.42 ± 0.01[b]	0.35 ± 0.04
	Mn	13.86 ± 0.53[c,e]	5.00 ± 0.28[c]	1.46 ± 0.05[b,e]	1.07 ± 0.23[e]
Striatum	Control	12.05 ± 0.10[c]	1.78 ± 0.10[c]	0.12 ± 0.02[c]	0.24 ± 0.03
	Mn	12.86 ± 0.54[c,d]	3.72 ± 0.13[c,e]	1.57 ± 0.24[b,e]	1.13 ± 0.24[e]
Midbrain	Control	1.96 ± 0.27[c]	1.51 ± 0.08[c]	0.19 ± 0.04[c]	0.38 ± 0.07
	Mn	6.43 ± 0.51[c,e]	2.49 ± 0.03[c,e]	2.15 ± 0.04[b,e]	1.35 ± 0.32[e]
Cerebral cortex	Control	0.85 ± 0.20[c]	1.15 ± 0.10[c]	0.19 ± 0.04[b]	0.34 ± 0.07
	Mn	4.42 ± 0.21[c,e]	2.56 ± 0.05[c,e]	1.39 ± 0.16[c,e]	0.62 ± 0.16[d]

[a] Values are the means ± SD of 6-10 female rats [b] $P < 0.05$ compared to day 120 by ANOVA
[c] $P < 0.01$ compared to day 120 by ANOVA [d] $P < 0.01$ compared to age-matched controls by t-test
[e] $P < 0.001$ compared to age-matched controls by t-test

In children on long-term parenteral nutrition resulting in blood manganese levels of 615-1840 nmol/l (vs reference range of 72-210 nmol/l), elevated manganese levels have been seen in globus pallidus and subthalamic nuclei (Fell et al., 1996), suggesting an enhanced potential for neurological damage. This is consistent with the decrements in

intellectual function in children exposed to manganese in drinking water reported by Wasserman et al. (2006).

The effects of manganese on reproduction in humans have been reported in epidemiological studies of workers with occupational exposure to manganese. The results have been mixed with Gennart et al. (1992) reporting no effect on fertility among workers exposed to a median manganese dust level of 0.71 mg/m^3, while those exposed to 0.07-8.61 mg/m^3 (geometric mean 0.94 mg/m^3) in a study by Lauwerys et al. (1985) sired a statistically significant lower number of children during the period of paternal exposure. However, workers in the Gennart et al. study were exposed to the relatively insoluble manganese oxide and had mean urine manganese levels of 0.82 µg/g creatinine. By comparison, the workers in the study by Lauwerys et al. were exposed to the more soluble manganese salts in addition to the oxide, and had mean urinary manganese levels of 4.37 µg/g creatinine. Thus the differences in the effects of manganese on reproduction reported in these two studies may be due to the significant differences in manganese exposures.

Adverse changes in reproductive parameters and behaviors have been seen in studies of rodents exposed to high levels of manganese. In immature female rats (23 days old), manganese (1-25 µg $MnCl_2$) introduced into the third ventricle of the brain significantly and dose-dependently stimulated the release of luteinizing hormone (LH). This effect was apparently at the level of the hypothalamus as pretreatment with the LH releasing hormone (LHRH) receptor antagonist, acyline, prior to manganese exposure blocked the release of LH (Pine et al., 2005). These authors further reported that serum LH, follicle stimulating hormone, and estradiol were all elevated by 29 days of age in rats that had received $MnCl_2$ by gavage starting on postnatal day 12. In these animals manganese altered the timing of reproductive events resulting in a significantly ($p < 0.001$) earlier onset of puberty as measured by vaginal opening.

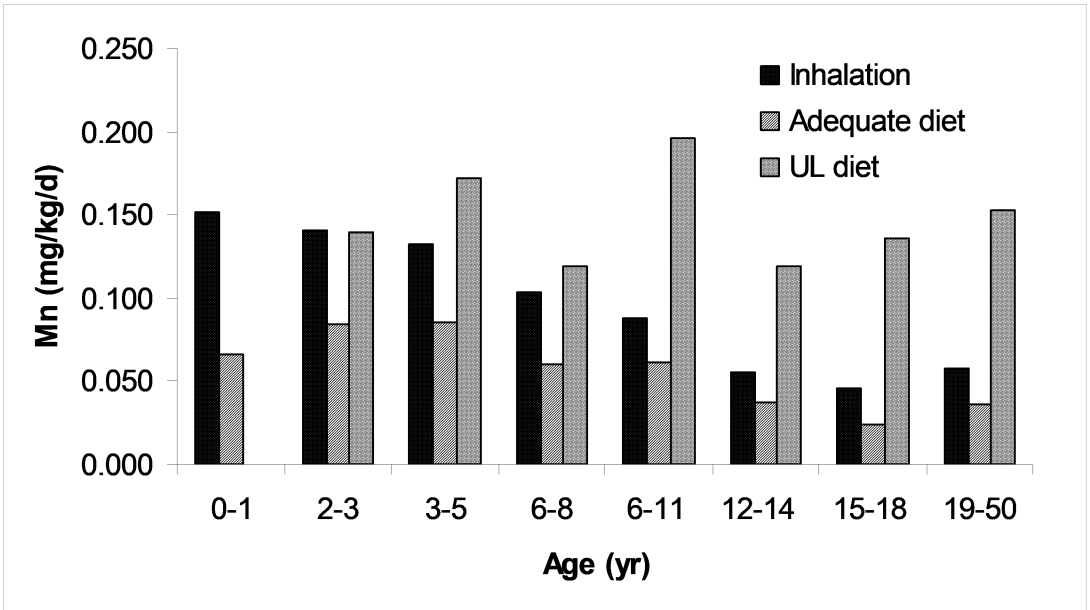
In adult male rats, exposure to 1,000 ppm manganese sulfate in drinking water for 12 weeks significantly suppressed sexual performance compared with controls as measured by prolonged ejaculatory latencies ($p < 0.001$), and increased post-ejaculatory intervals ($p < 0.05$). Displays of aggressive behaviors (lateralizations, boxing bouts, and fights with stud males) were also reduced ($p < 0.001$). The extent to which the altered behaviors represent neurological effects versus effects on testes and androgen production is not clear. However, among females mated to the manganese-treated males, the total number of resorptions was significantly increased ($p < 0.025$), suggesting a testicular effect. This is supported by a significant ($p < 0.001$) reduction in absolute and relative testes weights, and absolute seminal vesicle weights among manganese-exposed males (Bataineh et al., 1998). An effect of manganese on male reproductive organs was also investigated in mice following 43 days of oral manganese acetate (7.5 – 30 mg/kg/d) (Ponnapakkam et al., 2003). Unlike the study with rats above, there was no significant change in testicular weight or pathology with manganese exposure in the mice. Nor was there evidence of abnormal mating behavior. However, epididymal weights were significantly lower ($p < 0.05$) and there was a significant ($p < 0.001$) dose-dependent decrease in sperm number and motility.

The available data suggest that manganese is a reproductive toxicant in animals (both males and females) albeit at relatively high doses. Neurobehavioral toxicity manifests at levels encountered in the environment (Wasserman et al., 2006). Whether this decrement in intellectual function represents a true developmental effect with permanent consequences is not clear.

8. Derivation of Reference Exposure Levels

The determination of safe exposure levels to manganese is complicated by its status as an essential nutrient. However, as described above, inhalation of manganese results in a qualitatively and quantitatively different exposure compared to oral intake, with inhalation resulting in more rapid uptake and higher blood and brain levels. While dietary manganese levels moderate intestinal absorption of manganese, there appears to be no effect of dietary manganese on the pharmacokinetics of inhaled manganese (Dorman et al., 2002b). To provide perspective on potential manganese exposure from inhalation relative to the suggested upper limits for age-dependent dietary intake, we compared the potential manganese internal dose from inhalation with that from the recommended dietary levels for the various age groups indicated (Fig. 8.1). For this comparison, we used an air level of manganese of 0.215 mg Mn/m^3, the average level of respirable manganese dust to which workers were exposed in an occupational study of the effects of manganese exposure (Roels et al., 1992). It thus represents a high but real world exposure level associated with neurotoxicity. Adequate dietary intake and the recommended upper limit levels, above which toxic effects may be observed, are those set by the Food and Nutrition Board (FNB, 2004). The data for inhalation represent the amount of manganese taken up by the different groups, age-adjusted for average breathing rates per kilogram body mass per day (OEHHA, 2000; Arcus-Arth and Blaisdell, 2007). For this example, we assumed that uptake from the lungs is 100% of the inhaled manganese, and that absorption from dietary intake is 41% for 0-1 yrs, 10% for 2-3 yrs, and 5% for all other ages. The inhaled manganese becomes the age-specific breathing rate multiplied by the air concentration. We compared the inhaled dose to the internal dose of manganese expected from an adequate diet, and from intake at the upper limit. This analysis suggests that among neonates and children through age 8, the manganese uptake from inhalation of this high level of manganese would substantially exceed the adequate dietary amount and may approach or exceed the levels beyond which toxicity may be expected. Therefore, compared with adults at the same exposure level, a child's manganese inhalation is a larger proportion of the maximum recommended levels. This is due to the higher breathing rates, lower body mass, and greater absorption of manganese by children. Thus, for comparable air exposures, children are more at risk for exceeding safe levels than are adults.

Figure 8.5 Internal Manganese Dose from Inhalation and Diet by Age



Age- and breathing rate-adjusted internal manganese dose following chronic inhalation of 0.215 mg/m^3 manganese (Inhalation), consumption of recommended minimum daily intake (Adequate diet), or upper limit level beyond which toxic effects may be observed (UL diet). Data expressed as mg Mn/kg body weight/day.

8.1 Manganese Acute Reference Exposure Level

Acute Reference Exposure Levels (RELs) are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document (TSD)). Pulmonary damage and inflammation are the principal endpoints associated with acute inhalation exposure to manganese. However, at present the database is insufficient to allow the development of an acute REL for manganese based on inhalation studies. No studies were located that reported dose-response data for acute inhalation exposures, nor was it possible to determine both LOAELs and NOAELs from the available data.

8.2 Manganese 8-Hour Reference Exposure Level

Study	Roels et al., 1992
Study population	92 workers in a battery plant
Exposure method	Inhalation of workplace air
Exposure continuity	
Exposure duration	8 hr/day, 0.2-17.7 yr (mean 5.3 yr)
Critical effects	Impaired neurobehavior: visual reaction time, eye-hand coordination, hand steadiness
NOAEL	not observed
Benchmark concentration	72 μg/m^3
Time-adjusted exposure	51 μg/m^3 (72*5/7)
LOAEL uncertainty factor (UF_L)	Not applicable
Subchronic uncertainty factor (UFs)	√10 (default 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10 (greater absorption and lung deposition in children)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children to neurotoxicity)
Cumulative uncertainty factor	300
Reference Exposure Level	**0.17 μg/m^3**

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the TSD).

The proposed 8-hr REL for manganese is 0.17 μg/m^3 based on impairment of neurobehavioral function in humans in the occupational study of Roels et al. (1992) described in Section 6.1. Data on the lifetime integrated exposure to respirable dust (LIRD) for each of the 92 workers and whether or not their response scores were abnormal on each of the three tests (visual reaction time, eye-hand coordination, and hand steadiness), were originally compiled by Dr. Roels, and were provided to OEHHA by Dr. J. Michael Davis of the US EPA. Abnormal scores were defined as those values that exceeded the 95th percentile value of the parameter as estimated from the cumulative frequency curves of the control group as in Roels et al. (1982). The LIRD was estimated for each worker based on the current airborne manganese concentration characteristic of each job multiplied by the number of years the worker had spent in that job. The result for each job held by an individual worker was then summed to obtain the LIRD for the individual. In our analysis, using US EPA's BMDS version 1.4.1b software, the LIRD (in μg/m^3 x yr), divided by the total years of manganese exposure for each individual, was compared with his performance on each of the three tests (scored as 0 if the test was normal and 1 if abnormal). In this treatment, the group size is 1. Eye-hand

coordination (EHC) and hand steadiness (HST) are the two most critical endpoints; the incidence of changes in the visual reaction time in the study population was consistently lower. For both these endpoints, the best fitting models were the Probit and Logistic models; the quality of fit was not distinguishable on statistical grounds. The lower 95% confidence bound benchmark confidence level ($BMCL_{05}$) for the Probit fit to the EHC data was selected as the most health protective value among these relatively closely spaced results.

Eye Hand Coordination (EHC)					
Model	BMC_{05}	$BMCL_{05}$	BMC/BMCL	P for fit	AIC
Probit	97	**72**	1.4	0.3797	93.9124
Logistic	105	78	1.4	0.3874	93.8307
Hand Steadiness (HST)					
Model	BMC_{05}	$BMCL_{05}$	BMC/BMCL	P for fit	AIC
Probit	162	115	1.4	0.2201	68.2214
Logistic	175	125	1.4	0.2389	67.9531

This analysis yielded a $BMCL_{05}$ of 72 µg Mn/m^3. A time correction was applied since the workers in the study were only exposed 5 days per week, whereas the 8-hour REL is designed to protect against daily exposures, as noted in the TSD. A cumulative UF of 300 was applied, comprising a $\sqrt{10}$ for subchronic to chronic conversion (average exposure duration = 5.3 yr; Section 4.4.6 of the TSD), and 10 each for intraspecies toxicokinetic ($UF_{H\text{-}k}$) and toxicodynamic ($UF_{H\text{-}d}$) uncertainty, resulting in an 8-hour REL of 0.17 µg Mn/m^3. This REL is based on healthy adult male workers with adjustments for the potentially greater susceptibility of children. The intraspecies $UF_{H\text{-}k}$ of 10 was chosen in part to reflect the 3-4-fold greater deposition of inhaled particulates in the 1-10 µm size range in the lungs of neonates relative to adults exposed to similar particulate levels in ambient air (Ginsberg et al., 2005). In addition, neonates and infants more efficiently absorb and retain manganese than do adults (Dorner et al., 1989).

A $UF_{H\text{-}d}$ of 10 is used to address the expectation that the still-developing brain of newborn and infant children is more sensitive to the effects of manganese and that injuries to the nervous system during development are anticipated to have lasting effects.

The development of these RELs is based on a benchmark concentration analysis of data from an occupational study as described above. An alternative approach involves the use of physiologically-based pharmacokinetic (PBPK) models, the development of which in rats has been described in a recent series of papers by workers at the Chemical Industry Institute of Toxicology , now the Hamner Institutes for Health Sciences (Teeguarden et al., 2007a; Teeguarden et al., 2007b; Teeguarden et al., 2007c); (Nong et al., 2008). While these papers represent significant progress in the modeling of manganese pharmacokinetics, they have yet to be extended to and validated in humans or non-human primates. For this reason, they were not used for the estimation of these REL values.

8.3 Manganese Chronic Reference Exposure Level

Study	Roels et al., 1992
Study population	92 workers in a battery plant
Exposure method	Inhalation of workplace air
Exposure continuity	
Exposure duration	8 hr/day, 0.2-17.7 yr (mean 5.3 yr)
Critical effects	Impaired neurobehavior: visual reaction time, eye-hand coordination, hand steadiness
NOAEL	not observed
Benchmark concentration	72 µg/m^3
Time-adjusted exposure	26 µg/m^3 (72 µg/m^3 x 10/20 x 5/7)
LOAEL uncertainty factor (UF_L)	Not applicable
Subchronic uncertainty factor (UFs)	√10 (default 8-12% of lifetime)
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10 (greater absorption and lung deposition in children)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children to neurotoxicity)
Cumulative uncertainty factor	300
Reference Exposure Level	**0.09 µg/m^3**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from continuous lifetime exposures (see Section 7 in the Technical Support Document).

The proposed chronic REL for manganese of 0.09 µg/m^3 is based on impairment of neurobehavioral function in humans in the occupational study of Roels et al. (1992). The benchmark dose approach was used, as described above for the 8-hour REL, to derive a $BMCL_{05}$ of 72 µg/m^3. This corresponded to a time-adjusted concentration of 26 µg/m^3 (based on an 8 hour TWA occupational exposure to 10 m^3 manganese-contaminated air per day out of 20 m^3 total air inhaled per day over 5 days/week).

A cumulative UF of 300 was used, comprising a √10 for subchronic to chronic conversion (average exposure duration = 5.3 yr; Section 4.4.6 of the TSD), and 10 each for intraspecies toxicokinetic (UF_{H-k}) and toxicodynamic (UF_{H-d}) uncertainty. This REL is based on healthy adult male workers with adjustments for the potentially greater susceptibility of children. The intraspecies UF_{H-k} of 10 was chosen in part to reflect the 3-4-fold greater deposition of inhaled particulates in the 1-10 µm range in the lungs of neonates relative to adults exposed to similar particulate levels in ambient air (Ginsberg et al., 2005). In addition, neonates and infants more efficiently absorb and retain manganese than do adults (Dorner et al., 1989). It should also be noted that the effects

reported in the Roels study were to a relatively insoluble form of manganese, MnO_2. As shown in Table 4.1 above, exposures to similar levels of the more soluble $MnCl_2$ by the same route result in higher manganese brain levels.

A UF_{H-d} of 10 is used to address the expectation that the still-developing brain of newborn and infant children is more sensitive to the effects of manganese and that injuries to the nervous system during development are anticipated to have lasting effects. This REL was developed with specific consideration of the potentially greater susceptibility of children to manganese neurotoxicity. For comparison, the RfC for chronic manganese inhalation developed by the US EPA is 0.05 μg/m^3 (U.S.EPA, 1993) and is also based on Roels et al. (1992).

As described in Section 6.3 above, the studies by Dorman et al. (2006), Guilarte et al. (2006) and Schneider et al. (2006) report comparable post-controlled exposure manganese levels in the same regions of the brains of Rhesus monkeys, albeit using different experimental protocols. Based on the inhalation data from Dorman et al., it is possible to extrapolate an air manganese concentration at which the neurotoxicity reported by Guilarte and Schneider would have been expected. After adjustment for the differences in exposure duration, the projected air manganese levels from Dorman corresponding to the toxicity-associated region-specific brain levels reported by Guilarte are 75 μg/m^3 inhaled concentration for the caudate, 98 μg/m^3 for putamen, 150 μg/m^3 for globus pallidus, and 73 μg/m^3 for white matter. While the differences in the exposure regimens among these studies precludes using them to derive a REL, it is relevant and supportive that this range of exposure subsumes the LOAEL for neurotoxic effects in humans reported by Lucchini et al. (1999) of 97 μg Mn/m^3, and the point of departure for the REL based on the Roels et al. study of 72 μg Mn/m^3. Thus, these studies of non-human primates support the effect level upon which the REL is based.

8.4 Manganese as a Toxic Air Contaminant

In view of the potential for higher exposure in children than adults coupled with a lower ability to regulate manganese, and enhanced neurodevelopmental susceptibility leading to differential impacts in infants and children identified in Section 6.2.1, OEHHA recommends that manganese be identified as a toxic air contaminant which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

9. References

Adkins B, Jr., Luginbuhl GH and Gardner DE (1980). Acute exposure of laboratory mice to manganese oxide. Am Ind Hyg Assoc J 41(7): 494-500.

Andersen ME, Gearhart JM and Clewell HJ, 3rd (1999). Pharmacokinetic data needs to support risk assessments for inhaled and ingested manganese. Neurotoxicology 20(2-3): 161-71.

Archibald FS and Tyree C (1987). Manganese poisoning and the attack of trivalent manganese upon catecholamines. Arch Biochem Biophys 256(2): 638-50.

Arcus-Arth A and Blaisdell RJ (2007). Statistical distributions of daily breathing rates for narrow age groups of infants and children. Risk Anal 27(1): 97-110.

Aschner JL and Aschner M (2005). Nutritional aspects of manganese homeostasis. Mol Aspects Med 26(4-5): 353-62.

Aschner M (2000). Manganese: brain transport and emerging research needs. Environ Health Perspect 108 Suppl 3: 429-32.

Aschner M, Erikson KM and Dorman DC (2005). Manganese dosimetry: species differences and implications for neurotoxicity. Crit Rev Toxicol 35(1): 1-32.

Barbeau A (1984). Manganese and extrapyramidal disorders (a critical review and tribute to Dr. George C. Cotzias). Neurotoxicology 5(1): 13-35.

Barlow BK, Richfield EK, Cory-Slechta DA and Thiruchelvam M (2004). A fetal risk factor for Parkinson's disease. Dev Neurosci 26(1): 11-23.

Bataineh H, Al-Hamood MH and Elbetieha AM (1998). Assessment of aggression, sexual behavior and fertility in adult male rat following long-term ingestion of four industrial metals salts. Hum Exp Toxicol 17(10): 570-6.

Bergstrom R (1977). Acute pulmonary toxicity of manganese dioxide. Scand J Work Environ Health 3 Suppl 1: 1-41.

Bird ED, Anton AH and Bullock B (1984). The effect of manganese inhalation on basal ganglia dopamine concentrations in rhesus monkey. Neurotoxicology 5(1): 59-65.

Boojar MM and Goodarzi F (2002). A longitudinal follow-up of pulmonary function and respiratory symptoms in workers exposed to manganese. J Occup Environ Med 44(3): 282-90.

Bouchard M, Mergler D, Baldwin M, Panisset M, Bowler R and Roels HA (2007a). Neurobehavioral functioning after cessation of manganese exposure: a follow-up after 14 years. Am J Ind Med 50(11): 831-40.

Bouchard M, Mergler D, Baldwin M, Panisset M and Roels HA (2007b). Neuropsychiatric symptoms and past manganese exposure in a ferro-alloy plant. Neurotoxicology 28(2): 290-7.

Bowler RM, Roels HA, Nakagawa S, Drezgic M, Diamond E, Park R, Koller W, Bowler RP, Mergler D, Bouchard M, Smith D, Gwiazda R and Doty RL (2007). Dose-effect relationships between manganese exposure and neurological, neuropsychological and pulmonary function in confined space bridge welders. Occup Environ Med 64(3): 167-77.

Brain JD, Heilig E, Donaghey TC, Knutson MD, Wessling-Resnick M and Molina RM (2006). Effects of iron status on transpulmonary transport and tissue distribution of Mn and Fe. Am J Respir Cell Mol Biol 34(3): 330-7.

Brenneman KA, Wong BA, Buccellato MA, Costa ER, Gross EA and Dorman DC (2000). Direct olfactory transport of inhaled manganese ((54)MnCl(2)) to the rat brain: toxicokinetic investigations in a unilateral nasal occlusion model. Toxicol Appl Pharmacol 169(3): 238-48.

Calne DB, Chu NS, Huang CC, Lu CS and Olanow W (1994). Manganism and idiopathic parkinsonism: similarities and differences. Neurology 44(9): 1583-6.

CARB. (2005a). *California Toxics Inventory for 2004*. California Air Resources Board. http://www.arb.ca.gov/toxics/cti/cti.htm.

Cawte J (1985). Psychiatric sequelae of manganese exposure in the adult, foetal and neonatal nervous systems. Aust N Z J Psychiatry 19(3): 211-7.

Chan AW, Minski MJ, Lim L and Lai JC (1992). Changes in brain regional manganese and magnesium levels during postnatal development: modulations by chronic manganese administration. Metab Brain Dis 7(1): 21-33.

Collipp PJ, Chen SY and Maitinsky S (1983). Manganese in infant formulas and learning disability. Ann Nutr Metab 27(6): 488-94.

CRC (2005). CRC Handbook of Chemistry and Physics. Boca Raton (FL): CRC Press.

Crump KS (2000). Manganese exposures in Toronto during use of the gasoline additive, methylcyclopentadienyl manganese tricarbonyl. J Expo Anal Environ Epidemiol 10(3): 227-39.

Crump KS and Rousseau P (1999). Results from eleven years of neurological health surveillance at a manganese oxide and salt producing plant. Neurotoxicology 20: 273-86.

Davis CD, Malecki EA and Greger JL (1992). Interactions among dietary manganese, heme iron, and nonheme iron in women. Am J Clin Nutr 56(5): 926-32.

Davis CD, Zech L and Greger JL (1993). Manganese metabolism in rats: an improved methodology for assessing gut endogenous losses. Proc Soc Exp Biol Med 202(1): 103-8.

Dobson AW, Weber S, Dorman DC, Lash LK, Erikson KM and Aschner M (2003). Oxidative stress is induced in the rat brain following repeated inhalation exposure to manganese sulfate. Biol Trace Elem Res 93(1-3): 113-26.

Dodd CA, Ward DL and Klein BG (2005). Basal ganglia accumulation and motor assessment following manganese chloride exposure in the C57BL/6 mouse. Intl J Toxicol 24(6): 389-397.

Dorman DC, Brenneman KA, McElveen AM, Lynch SE, Roberts KC and Wong BA (2002a). Olfactory transport: a direct route of delivery of inhaled manganese phosphate to the rat brain. J Toxicol Environ Health A 65(20): 1493-511.

Dorman DC, McElveen AM, Marshall MW, Parkinson CU, James RA, Struve MF and Wong BA (2005a). Tissue manganese concentrations in lactating rats and their offspring following combined in utero and lactation exposure to inhaled manganese sulfate. Toxicol Sci 84(1): 12-21.

Dorman DC, McManus BE, Marshall MW, James RA and Struve MF (2004). Old age and gender influence the pharmacokinetics of inhaled manganese sulfate and manganese phosphate in rats. Toxicol Appl Pharmacol 197(2): 113-24.

Dorman DC, Struve MF, Gross EA, Wong BA and Howroyd PC (2005b). Sub-chronic inhalation of high concentrations of manganese sulfate induces lower airway pathology in rhesus monkeys. Respir Res 6: 121.

Dorman DC, Struve MF, James RA, Marshall MW, Parkinson CU and Wong BA (2001). Influence of particle solubility on the delivery of inhaled manganese to the rat brain: manganese sulfate and manganese tetroxide pharmacokinetics following repeated (14-day) exposure. Toxicol Appl Pharmacol 170(2): 79-87.

Dorman DC, Struve MF, Marshall MW, Parkinson CU, James RA and Wong BA (2006b). Tissue Manganese Concentrations in Young Male Rhesus Monkeys following Subchronic Manganese Sulfate Inhalation. Toxicol Sci 92(1): 201-10.

Dorman DC, Struve MF, Vitarella D, Byerly FL, Goetz J and Miller R (2000). Neurotoxicity of manganese chloride in neonatal and adult CD rats following subchronic (21-day) high-dose oral exposure. J Appl Toxicol 20(3): 179-87.

Dorman DC, Struve MF and Wong BA (2002b). Brain manganese concentrations in rats following manganese tetroxide inhalation are unaffected by dietary manganese intake. Neurotoxicology 23(2): 185-95.

Dorman DC, Struve MF, Wong BA, Dye JA and Robertson ID (2006a). Correlation of brain magnetic resonance imaging changes with pallidal manganese concentrations in

Rhesus monkeys following subchronic manganese inhalation. Toxicol Sci 92(1): 219-227.

Dorner K, Dziadzka S, Hohn A, Sievers E, Oldigs HD, Schulz-Lell G and Schaub J (1989). Longitudinal manganese and copper balances in young infants and preterm infants fed on breast-milk and adapted cow's milk formulas. Br J Nutr 61(3): 559-72.

Drown DB, Oberg SG and Sharma RP (1986). Pulmonary clearance of soluble and insoluble forms of manganese. J Toxicol Environ Health 17(2-3): 201-12.

Elder A, Gelein R, Silva V, Feikert T, Opanashuk L, Carter J, Potter R, Maynard A, Ito Y, Finkelstein J and Oberdorster G (2006). Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ Health Perspect 114(8): 1172-8.

Ericson JE, Crinella FM, Clarke-Stewart KA, Allhusen VD, Chan T and Robertson RT (2007). Prenatal manganese levels linked to childhood behavioral disinhibition. Neurotoxicol Teratol 29(2): 181-7.

Erikson KM, Dorman DC, Lash LH and Aschner M (2007a). Manganese inhalation by rhesus monkeys is associated with brain regional changes in biomarkers of neurotoxicity. Toxicol Sci 97(2): 459-66.

Erikson KM, Dorman DC, Lash LH, Dobson AW and Aschner M (2004). Airborne manganese exposure differentially affects end points of oxidative stress in an age- and sex-dependent manner. Biol Trace Elem Res 100(1): 49-62.

Erikson KM, Shihabi ZK, Aschner JL and Aschner M (2002). Manganese accumulates in iron-deficient rat brain regions in a heterogeneous fashion and is associated with neurochemical alterations. Biol Trace Elem Res 87(1-3): 143-56.

Erikson KM, Thompson K, Aschner J and Aschner M (2007b). Manganese neurotoxicity: A focus on the neonate. Pharmacol Ther 113(2): 369-77.

Espinoza A, Rodriguez M, Barragan de la Rosa F and Sabchez J (2001). Size distribution of metals in urban aerosols in Seville (Spain). Atmosph Environ 35(14): 2595-2601.

Fechter LD (1999). Distribution of manganese in development. Neurotoxicology 20(2-3): 197-201.

Fechter LD, Johnson DL and Lynch RA (2002). The relationship of particle size to olfactory nerve uptake of a non-soluble form of manganese into brain. Neurotoxicology 23(2): 177-83.

Fell JM, Reynolds AP, Meadows N, Khan K, Long SG, Quaghebeur G, Taylor WJ and Milla PJ (1996). Manganese toxicity in children receiving long-term parenteral nutrition. Lancet 347(9010): 1218-21.

Ferraz HB, Bertolucci PH, Pereira JS, Lima JG and Andrade LA (1988). Chronic exposure to the fungicide maneb may produce symptoms and signs of CNS manganese intoxication. Neurology 38(4): 550-3.

Fitsanakis VA, Au C, Erikson KM and Aschner M (2006). The effects of manganese on glutamate, dopamine and gamma-aminobutyric acid regulation. Neurochem Int 48(6-7): 426-33.

FNB (2004). Dietary Reference Intake Tables: Elements Table. Food and Nutrition Board, Institutes of Medicine (IOM, 2004). http://www.iom.edu/Object.File/Master/7/294/Webtableminerals.pdf.

Foradori AC, Bertinchamps A, Gulibon JM and Cotzias GC (1967). The discrimination between magnesium and manganese by serum proteins. J Gen Physiol 50(9): 2255-66.

Gavin CE, Gunter KK and Gunter TE (1999). Manganese and calcium transport in mitochondria: implications for manganese toxicity. Neurotoxicology 20(2-3): 445-53.

Gennart JP, Buchet JP, Roels H, Ghyselen P, Ceulemans E and Lauwerys R (1992). Fertility of male workers exposed to cadmium, lead, or manganese. Am J Epidemiol 135(11): 1208-19.

Gianutsos G, Morrow GR and Morris JB (1997). Accumulation of manganese in rat brain following intranasal administration. Fundam Appl Toxicol 37(2): 102-5.

Gibbons RA, Dixon SN, Hallis K, Russell AM, Sansom BF and Symonds HW (1976). Manganese metabolism in cows and goats. Biochim Biophys Acta 444(1): 1-10.

Ginsberg GL, Foos BP and Firestone MP (2005). Review and analysis of inhalation dosimetry methods for application to children's risk assessment. J Toxicol Environ Health A 68(8): 573-615.

Guilarte TR, Chen MK, McGlothan JL, Verina T, Wong DF, Zhou Y, Alexander M, Rohde CA, Syversen T, Decamp E, Koser AJ, Fritz S, Gonczi H, Anderson DW and Schneider JS (2006a). Nigrostriatal dopamine system dysfunction and subtle motor deficits in manganese-exposed non-human primates. Exp Neurol 202(2): 381-90.

Halatek T, Sinczuk-Walczak H and Rydzynski K (2008). Early neurotoxic effects of inhalation exposure to aluminum and/or manganese assessed by serum levels of phospholipid-binding Clara cells protein. J Environ Sci Health A Tox Hazard Subst Environ Eng 43(2): 118-24.

Halatek T, Sinczuk-Walczak H, Szymczak M and Rydzynski K (2005). Neurological and respiratory symptoms in shipyard welders exposed to manganese. International Journal of Occupational Medicine and Environmental Health 18(3): 265-274.

HaMai D, Campbell A and Bondy SC (2001). Modulation of oxidative events by multivalent manganese complexes in brain tissue. Free Radic Biol Med 31(6): 763-8.

Hermans C, Knoops B, Wiedig M, Arsalane K, Toubeau G, Falmagne P and Bernard A (1999). Clara cell protein as a marker of Clara cell damage and bronchoalveolar blood barrier permeability. Eur Respir J 13(5): 1014-21.

Hlavay J, Polyak K, Molnar A and Meszaros E (1998). Determination of the distribution of elements as a function of particle size in aerosol samples by sequential leaching. Analyst 123: 859-863.

HSDB. (2006). *Manganese compounds*. National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~YuJ7Vy:2.

Huang CC, Chu NS, Lu CS, Chen RS and Calne DB (1998). Long-term progression in chronic manganism: ten years of follow-up. Neurology 50(3): 698-700.

Josephs KA, Ahlskog JE, Klos KJ, Kumar N, Fealey RD, Trenerry MR and Cowl CT (2005). Neurologic manifestations in welders with pallidal MRI T1 hyperintensity. Neurology 64(12): 2033-9.

Kafritsa Y, Fell J, Long S, Bynevelt M, Taylor W and Milla P (1998). Long-term outcome of brain manganese deposition in patients on home parenteral nutrition. Arch Dis Child 79(3): 263-5.

Kilburn CJ (1987). Manganese, malformations and motor disorders: findings in a manganese-exposed population. Neurotoxicology 8(3): 421-9.

Komaki H, Maisawa S, Sugai K, Kobayashi Y and Hashimoto T (1999). Tremor and seizures associated with chronic manganese intoxication. Brain Dev 21(2): 122-4.

Kostial K, Blanusa M and Piasek M (2005). Regulation of manganese accumulation in perinatally exposed rat pups. J Appl Toxicol 25(2): 89-93.

Lauwerys R, Roels H, Genet P, Toussaint G, Bouckaert A and De Cooman S (1985). Fertility of male workers exposed to mercury vapor or to manganese dust: a questionnaire study. Am J Ind Med 7(2): 171-6.

Limbach LK, Wick P, Manser P, Grass RN, Bruinink A and Stark WJ (2007). Exposure of engineered nanoparticles to human lung epithelial cells: influence of chemical composition and catalytic activity on oxidative stress. Environ Sci Technol 41(11): 4158-63.

Looker AC, Dallman PR, Carroll MD, Gunter EW and Johnson CL (1997). Prevalence of iron deficiency in the United States. JAMA 277(12): 973-6.

Lucchini R, Apostoli P, Perrone C, Placidi D, Albini E, Migliorati P, Mergler D, Sassine MP, Palmi S and Alessio L (1999). Long-term exposure to "low levels" of manganese oxides and neurofunctional changes in ferroalloy workers. Neurotoxicology 20(2-3): 287-97.

Malecki EA (2001). Manganese toxicity is associated with mitochondrial dysfunction and DNA fragmentation in rat primary striatal neurons. Brain Res Bull 55(2): 225-8.

Martin R (1986). Bioinorganic chemistry of metal ion toxicity. In: Metal ions in biological systems: Concepts on metal ion toxicity. Sigel H. a. S., A. CRC Press. 20: 22-62.

Meco G, Bonifati V, Vanacore N and Fabrizio E (1994). Parkinsonism after chronic exposure to the fungicide maneb (manganese ethylene-bis-dithiocarbamate). Scand J Work Environ Health 20(4): 301-5.

Mena I (1974). The role of manganese in human disease. Ann Clin Lab Sci 4(6): 487-91.

Meral H, Kutukcu Y, Atmaca B, Ozer F and Hamamcioglu K (2007). Parkinsonism caused by chronic usage of intravenous potassium permanganate. Neurologist 13(2): 92-4.

Merck (1976). The Merck Index. Rahway, NJ: Merck and Co.

Mergler D, Baldwin M, Belanger S, Larribe F, Beuter A, Bowler R, Panisset M, Edwards R, de Geoffroy A, Sassine MP and Hudnell K (1999). Manganese neurotoxicity, a continuum of dysfunction: results from a community based study. Neurotoxicology 20(2-3): 327-42.

Mergler D, Huel G, Bowler R, Iregren A, Belanger S, Baldwin M, Tardif R, Smargiassi A and Martin L (1994). Nervous system dysfunction among workers with long-term exposure to manganese. Environ Res 64(2): 151-80.

Miller ST, Cotzias GC and Evert HA (1975). Control of tissue manganese: initial absence and sudden emergence of excretion in the neonatal mouse. Am J Physiol 229(4): 1080-4.

Nagatomo S, Umehara F, Hanada K, Nobuhara Y, Takenaga S, Arimura K and Osame M (1999). Manganese intoxication during total parenteral nutrition: report of two cases and review of the literature. J Neurol Sci 162(1): 102-5.

Newland MC, Cox C, Hamada R, Oberdorster G and Weiss B (1987). The clearance of manganese chloride in the primate. Fundam Appl Toxicol 9(2): 314-28.

NIOSH. (2005). *NIOSH Pocket Guide to Chemical Hazards; Manganese tetroxide (as Mn)*. Centers for Disease Control. http://www.cdc.gov/niosh/npg/npgd0381.html

Nong A, Teeguarden JG, Clewell HJ, 3rd, Dorman DC and Andersen ME (2008). Pharmacokinetic modeling of manganese in the rat IV: assessing factors that contribute to brain accumulation during inhalation exposure. J Toxicol Environ Health A 71(7): 413-26.

Normandin L, Ann Beaupre L, Salehi F, St -Pierre A, Kennedy G, Mergler D, Butterworth RF, Philippe S and Zayed J (2004). Manganese distribution in the brain and neurobehavioral changes following inhalation exposure of rats to three chemical forms of manganese. Neurotoxicology 25(3): 433-41.

Normandin L, Carrier G, Gardiner PF, Kennedy G, Hazell AS, Mergler D, Butterworth RF, Philippe S and Zayed J (2002). Assessment of bioaccumulation, neuropathology, and neurobehavior following subchronic (90 Days) inhalation in Sprague-Dawley rats exposed to manganese phosphate. Toxicol. Appl. Pharmacol. 183(2): 135-145.

NWU. (2006). Nutrition Fact Sheet: Manganese. Retrieved 8-17-07, from http://www.feinberg.northwestern.edu/nutrition/factsheets/manganese.html.

OEHHA. (2000). *The Air Toxics Hot Spots Program Risk Assessment Guidelines Part IV: Technical Support Document for Exposure Assessment and Stochastic Analysis*. Air Toxicology and Epidemiology Section, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

Ono J, Harada K, Kodaka R, Sakurai K, Tajiri H, Takagi Y, Nagai T, Harada T, Nihei A, Okada A and et al. (1995). Manganese deposition in the brain during long-term total parenteral nutrition. JPEN J Parenter Enteral Nutr 19(4): 310-2.

Pellizzari ED, Clayton CA, Rodes CE, Mason RE, Piper LL, Fort B, Pfeifer G and Lynam D (2001). Particulate matter and manganese exposures in Indianapolis, Indiana. Journal Expo Anal Environ Epidemiol 11(6): 423-40.

Pine M, Lee B, Dearth R, Hiney JK and Dees WL (2005). Manganese acts centrally to stimulate luteinizing hormone secretion: a potential influence on female pubertal development. Toxicol Sci 85(2): 880-5.

Ponnapakkam TP, Bailey KS, Graves KA and Iszard MB (2003). Assessment of male reproductive system in the CD-1 mice following oral manganese exposure. Reprod Toxicol 17(5): 547-51.

Reaney SH, Bench G and Smith DR (2006). Brain accumulation and toxicity of Mn(II) and Mn(III) exposures. Toxicol Sci 93(1): 114-124.

Rice TM, Clarke RW, Godleski JJ, Al-Mutairi E, Jiang NF, Hauser R and Paulauskis JD (2001). Differential ability of transition metals to induce pulmonary inflammation. Toxicol Appl Pharmacol 177(1): 46-53.

Roels H, Lauwerys R, Buchet JP, Bernard A, Barthels A, Oversteyns M and Gaussin J (1982). Comparison of renal function and psychomotor performance in workers exposed to elemental mercury. Int Arch Occup Environ Health 50(1): 77-93.

Roels H, Meiers G, Delos M, Ortega I, Lauwerys R, Buchet JP and Lison D (1997). Influence of the route of administration and the chemical form (MnCl2, MnO2) on the absorption and cerebral distribution of manganese in rats. Arch Toxicol 71(4): 223-30.

Roels HA, Eslava MIO, Ceulemans E, Robert A and Lison D (1999). Prospective study on the reversibility of neurobehavioral effects in workers exposed to manganese dioxide. Neurotoxicology 20: 255-272.

Roels HA, Ghyselen P, Buchet JP, Ceulemans E and Lauwerys RR (1992). Assessment of the permissible exposure level to manganese in workers exposed to manganese dioxide dust. Br J Ind Med 49(1): 25-34.

Rose C, Butterworth RF, Zayed J, Normandin L, Todd K, Michalak A, Spahr L, Huet PM and Pomier-Layrargues G (1999). Manganese deposition in basal ganglia structures results from both portal-systemic shunting and liver dysfunction. Gastroenterology 117(3): 640-4.

Sadek AH, Rauch R and Schulz Paul E. (2003). Parkinsonism due to manganism in a welder. Department of Neurology, Baylor College of Medicine, Houston, Texas 77030, USA. United States

Salehi F, Krewski D, Mergler D, Normandin L, Kennedy G, Philippe S and Zayed J (2003). Bioaccumulation and locomotor effects of manganese phosphate/sulfate mixture in Sprague-Dawley rats following subchronic (90 days) inhalation exposure. Toxicol. Appl. Pharmacol. 191(3): 264-271.

Schneider JS, Decamp E, Koser AJ, Fritz S, Gonczi H, Syversen T and Guilarte TR (2006). Effects of chronic manganese exposure on cognitive and motor functioning in non-human primates. Brain Res 1118(1): 222-31.

Schroeder HA, Balassa JJ and Tipton IH (1966). Essential trace metals in man: manganese. A study in homeostasis. J Chronic Dis 19(5): 545-71.

Shen XM and Dryhurst G (1998). Iron- and manganese-catalyzed autoxidation of dopamine in the presence of L-cysteine: possible insights into iron- and manganese-mediated dopaminergic neurotoxicity. Chem Res Toxicol 11(7): 824-37.

Shiotsuka R. (1984). Inhalation toxicity of manganese dioxide and magnesium oxide-manganese dioxide mixture. NTIS ADA-148868. Inhalation Toxicology Facility, Medical Dept., Brookhaven National Laboratory, Uptown, NY. Fort Detrick, MD

Shukakidze A, Lazriev I and Mitagvariya N (2003). Behavioral impairments in acute and chronic manganese poisoning in white rats. Neurosci Behav Physiol 33(3): 263-7.

Shukla GS and Chandra SV (1977). Levels of sulfhydryls and sulfhydryl-containing enzymes in brain, liver and testis of manganese treated rats. Arch Toxicol 37(4): 319-25.

Sikk K, Taba P, Haldre S, Bergquist J, Nyholm D, Zjablov G, Asser T and Aquilonius SM (2007). Irreversible motor impairment in young addicts--ephedrone, manganism or both? Acta Neurol Scand 115(6): 385-9.

Singh M, Jaques P and Sioutas C (2002). Size distribution and diurnal characteristics of particle-bound metals in source and receptor sites of the Los Angeles basin. Atmosph Environ 36(10): 1675-1689.

Struve MF, McManus BE, Wong BA and Dorman DC (2007). Basal ganglia neurotransmitter concentrations in rhesus monkeys following subchronic manganese sulfate inhalation. Am J Ind Med 50(10): 772-8.

Takser L, Mergler D, Hellier G, Sahuquillo J and Huel G (2003). Manganese, monoamine metabolite levels at birth, and child psychomotor development. Neurotoxicology (Amsterdam) 24(4-5): 667-674.

Tapin D, Kennedy G, Lambert J and Zayed J (2005). Bioaccumulation and locomotor effects of manganese sulfate in Sprague-Dawley rats following subchronic (90 days) inhalation exposure. Toxicol Appl Pharmacol 26(3): 351-9.

Teeguarden JG, Dorman DC, Covington TR, Clewell HJ, 3rd and Andersen ME (2007a). Pharmacokinetic modeling of manganese. I. Dose dependencies of uptake and elimination. J Toxicol Environ Health A 70(18): 1493-504.

Teeguarden JG, Dorman DC, Nong A, Covington TR, Clewell HJ, 3rd and Andersen ME (2007b). Pharmacokinetic modeling of manganese. II. Hepatic processing after ingestion and inhalation. J Toxicol Environ Health A 70(18): 1505-14.

Teeguarden JG, Gearhart J, Clewell HJ, 3rd, Covington TR, Nong A and Andersen ME (2007c). Pharmacokinetic modeling of manganese. III. Physiological approaches accounting for background and tracer kinetics. J Toxicol Environ Health A 70(18): 1515-26.

Ter Haar GL, Griffing ME, Brandt M, Oberding DG and Kapron M (1975). Methylcyclopentadienyl manganese tricarbonyl as an antiknock: composition and fate of manganese exhaust products. J. Air Pollut. Control Assoc. 25(8): 858-860.

Thiruchelvam M, Richfield EK, Baggs RB, Tank AW and Cory-Slechta DA (2000). The nigrostriatal dopaminergic system as a preferential target of repeated exposures to combined paraquat and maneb: implications for Parkinson's disease. J Neurosci 20(24): 9207-14.

Thompson K, Molina RM, Donaghey T, Schwob JE, Brain JD and Wessling-Resnick M (2007). Olfactory uptake of manganese requires DMT1 and is enhanced by anemia. FASEB J 21(1): 223-30.

Tran TT, Chowanadisai W, Crinella FM, Chicz-DeMet A and Lonnerdal B (2002a). Effect of high dietary manganese intake of neonatal rats on tissue mineral accumulation, striatal dopamine levels, and neurodevelopmental status. Neurotoxicology 23(4-5): 635-43.

Tran TT, Chowanadisai W, Lonnerdal B, Le L, Parker M, Chicz-Demet A and Crinella FM (2002b). Effects of neonatal dietary manganese exposure on brain dopamine levels and neurocognitive functions. Neurotoxicology 23(4-5): 645-51.

U.S.EPA. (1993). Manganese (CASRN 7439-96-5) Reference concentration for chronic inhalation exposure (RfC). http://www.epa.gov/IRIS/subst/0373.htm Retrieved 3/28/06, from http://www.epa.gov/IRIS/subst/0373.htm.

Valois AA and Webster WS (1989). Retention and distribution of manganese in the mouse brain following acute exposure on postnatal day 0, 7, 14 or 42: an autoradiographic and gamma counting study. Toxicology 57(3): 315-28.

Vitarella D, Wong BA, Moss OR and Dorman DC (2000). Pharmacokinetics of inhaled manganese phosphate in male Sprague-Dawley rats following subacute (14-day) exposure. Toxicol Appl Pharmacol 163(3): 279-85.

Wang XF and Cynader MS (2001). Pyruvate released by astrocytes protects neurons from copper-catalyzed cysteine neurotoxicity. J Neurosci 21(10): 3322-31.

Wariishi H, Valli K, Renganathan V and Gold MH (1989). Thiol-mediated oxidation of nonphenolic lignin model compounds by manganese peroxidase of Phanerochaete chrysosporium. J Biol Chem 264(24): 14185-91.

Wennberg A, Hagman M and Johansson L (1992). Preclinical neurophysiological signs of parkinsonism in occupational manganese exposure. Neurotoxicology 13(1): 271-4.

Wittczak T, Dudek W, Krakowiak A, Walusiak J and Palczynski C (2008). Occupational asthma due to manganese exposure: a case report. Int J Occup Med Environ Health 21(1): 81-3.

Woolf A, Wright R, Amarasiriwardena C and Bellinger D (2002). A child with chronic manganese exposure from drinking water. Environ Health Perspect 110(6): 613-616.

Zhang G, Liu D and He P (1995). [Effects of manganese on learning abilities in school children]. Zhonghua Yu Fang Yi Xue Za Zhi 29(3): 156-8.

Mercury Reference Exposure Levels

(Hg^0 Elemental; Quicksilver)

CAS 7439-97-6

1. Summary

Elemental mercury exposures adversely affect several organ systems. The effects of acute, high level inhalation exposures first appear in the lungs as pulmonary dysfunction, possibly followed by respiratory failure leading to death. At lower levels of exposure, the kidneys and brain, especially the developing brain, are more sensitive targets. Short term maternal exposure to mercury vapor during pregnancy may result in long lasting neurobehavioral effects in the offspring, an effect upon which the acute REL is based. Chronic, low level exposures also adversely affect the central nervous system and manifest as motor deficits (tremors, unsteady gait, performance decrements), mood changes (irritability, nervousness), poor concentration, short-term memory deficits, tremulous speech, blurred vision, paresthesia, and decreased nerve conduction. Renal and cardiovascular functions are also impaired with long term exposure. This REL focuses on inhalation exposures. There is a large body of literature on methylmercury poisoning as well as the toxicology of ingested mercury. Much of the latter is reviewed in OEHHA's documentation of the Public Health Goal for drinking water (OEHHA, 1999)

1.1 Mercury Acute REL

Reference Exposure Level	**0.6 µg Hg/m³ (0.07 ppb Hg^0)**
Critical effect(s)	CNS disturbances in offspring
Hazard Index target(s)	Nervous system

1.2 Mercury 8-Hour REL

Reference Exposure Level	**0.06 µg Hg/m³ (0.007 ppb Hg^0)**
Critical effect(s)	Impairment of neurobehavioral functions in humans
Hazard Index target(s)	Nervous system

1.3 Mercury Chronic REL

Reference Exposure Level	**0.03 µg Hg/m³ (0.004 ppb Hg^0)**
Critical effect(s)	Impairment of neurobehavioral functions in humans
Hazard Index target(s)	Nervous system

2. Physical & Chemical Properties - Elemental Mercury

Description	Silver-white, heavy, mobile, odorless, liquid metal
Molecular formula	Hg^0
Molecular weight	200.59 g/mol
Density	13.53 g/cm^3 @ 25° C
Boiling point	356.73° C
Melting point	-38.7° C
Vapor pressure	2 x 10^{-3} mm Hg @ 25° C
Solubility	soluble in nitric acid, to some extent in lipids, and up to 0.28 µmol in water @ 25° C
Odor threshold	odorless
Conversion factor	1 ppm in air = 8.34 mg/m^3 @ 25° C

3. Occurrence and Major Uses

Mercury and mercury-containing compounds are widely used in diverse applications. Thermometers, barometers and thermostats take advantage of mercury's uniform temperature-dependent volume expansion over a broad temperature range. It is used in mercury arc and fluorescent lamps, as a catalyst in oxidation of organic compounds, in the extraction of gold and silver from ores, and as a cathode in electrolysis. It is also used in pulp and paper manufacturing, as a component of batteries, in dental amalgams, and in the manufacture of switching devices such as oscillators, the manufacture of chlorine and caustic soda, as a lubricant, and as a laboratory reagent. To a lesser extent mercury has been used as a grain fumigant, in pharmaceuticals, agricultural chemicals, and as a preservative (ACGIH, 1986).

The annual statewide emissions of mercury from mobile, stationary and natural sources reported in the California Toxics Inventory for 2004 were estimated to be 18 tons (CARB, 2005a). Statewide ambient levels of mercury in 2002 were 1.7 ng/m^3 (CARB, 2005b). Mercury emitted in the metallic form is slowly oxidized in the atmosphere to the ionic mercurous and mercuric (+1 and +2) forms, which are much more soluble in water. These forms dissolve in raindrops and are deposited onto land and water. Much of this precipitation enters sediment of streams or other water bodies, where it is converted to methylmercury and can be accumulated by fish. Thus human exposure to air-borne mercury may be direct, via inhalation, and indirect, through a diet containing contaminated fish. For the purposes of evaluating a Reference Exposure Level, however, we focus on studies of inhalation exposure to mercury.

4. Metabolism / Toxicokinetics

Inhalation exposure to mercury is usually to vapors of the elemental form. However, combustion processes may also emit mercury salts (chlorides and oxides). Thus inhalation exposure to these forms also occurs. Exposure to the inorganic forms of mercury, the mercurous and mercuric salts, also occurs via the oral route. However,

absorption from the intestinal tract is much less efficient (2-38%) than from the lungs (70-80%) (ATSDR, 1999). To protect against oral exposure to inorganic mercury via drinking water, OEHHA (1999) has developed a public health goal (PHG) of 0.0012 mg/L (1.2 ppb) as a level of exposure expected to pose no significant health risk to individuals consuming the water on a daily basis. The difference between the PHG and the REL values reported in this document in part reflects differences in the toxicokinetics by the different routes of exposure. For inhalation exposure to mercury vapor, modeling based on human and experimental animal studies suggests that approximately 80% of inhaled mercury is deposited in the respiratory tract, of which about 70% is rapidly absorbed into the blood with a half-time of around 1 min. The remainder is absorbed more slowly with half-times of 8 hr to 5 days (Leggett et al., 2001). Absorption is markedly decreased if the breathing is done only through the mouth (Teisinger and Fiserova-Bergerova, 1965). It is not clear whether this difference is related to the direct uptake of mercury from nasal passages but mercury is known to be transported via olfactory nerves directly to the brain (Tjalve and Henriksson, 1999). In the blood, elemental mercury (Hg^0) may be oxidized by catalase and peroxidase to the more toxic inorganic forms. Cellular membranes and the blood-brain barrier are readily permeable to Hg^0, but much less so to the inorganic forms. Residual Hg^0 in the blood may enter target cells and be oxidized to the mercuric form intracellularly, effectively trapping it in the cells. The biological half-life of mercury in the human head is reported to be 21 days, and 64 days in the kidney (Hursh et al., 1976). Mercury is eliminated in urine, feces and exhaled air.

Mercury exerts its toxicity through several mechanisms mainly related to the high affinity of the mercuric ion for sulfhydryl groups. By binding to non-protein sulfhydryls such as glutathione and N-acetyl cysteine, mercury alters intracellular thiol status, thus promoting oxidative stress and lipid peroxidation. Mercury interacts with the mitochondrial electron transport chain resulting in increased H_2O_2. There is a concomitant depletion of mitochondrial glutathione, depolarization of the inner mitochondrial membrane, and increased susceptibility of the mitochondrial membrane to peroxidation. Mitochondrial function is thus impaired and oxidative stress increased (Lund et al., 1993). In addition to mercury's pro-oxidant effects, the binding of mercury by sulfhydryl-containing proteins disrupts a broad range of critical cellular functions such as microtubule polymerization (Yole et al., 2007), DNA transcription (Rodgers et al., 2001), glutamine synthesis (Allen et al., 2001), and calcium homeostasis (Yole et al., 2007). These effects may lead to cell dysfunction and death, an effect that is exacerbated by mercury's ability to promote auto-immune responses (Rowley and Monestier, 2005). Indeed, among genetically susceptible individuals, much of the renal pathology associated with mercury exposure has been attributed to auto-antibodies to renal proteins (Hua et al., 1993). Disruption of cellular processes during development can have severe and long-lasting effects. This is especially true during the growth and organization of the central nervous system as it is critically dependent on cell division and neuronal migration. These processes in turn depend on microtubule polymerization which is powerfully inhibited by both the mercuric ion and methylmercury.

5. Acute Toxicity of Mercury

5.1 Acute Toxicity to Adult Humans

The respiratory tract is the first organ system affected in cases of acute inhalation poisoning (Levin et al., 1988). Acute exposure to Hg^0 can lead to shortness of breath within 24 hours and a rapidly deteriorating course leading to death due to respiratory failure (Kanluen and Gottlieb, 1991; Asano et al., 2000). In a case report, Kanluen and Gottlieb (1991) observed four individuals from a private home where silver was being smelted from dental amalgam containing an unknown amount of Hg^0. All individuals died 9-23 days post-exposure from respiratory distress despite treatment with dimercaprol, a mercury chelator. Autopsy revealed acute lung injury characterized by necrotizing bronchiolitis with edema, emphysema, and obliteration of alveolar spaces with extensive interstitial fibrosis. The concentrations of mercury to which the individuals were exposed and the duration of exposure are not known.

Central nervous system (CNS) effects such as tremors or increased excitability are sometimes seen in cases of acute accidental exposures (Netterstrom et al., 1996). Long-term effects from a single exposure to Hg^0 were reported in 6 male workers exposed to an estimated concentration of 44 mg Hg/m³ for a period of several hours (McFarland and Reigel, 1978). Long-term CNS effects included nervousness, irritability, lack of ambition, and loss of sexual drive for several years. Shortness of breath also persisted for years in all cases. Acute inhalation exposure to Hg^0 vapors from broken thermometers resulted in generalized skin eruptions in 15 individuals (Nakayama et al., 1983). The doses and durations of exposure were not estimated.

A similar symptomatology was reported by Sexton et al. (1978) following the spillage of 100-300 ml of elemental mercury in two mobile homes that exposed 11 people to mercury vapor for one to two months. Following one to two weeks of exposure, the most intensely exposed residents, three teenage girls, reported the onset of anorexia, painful mouth, abdominal cramps, mild diarrhea, bleeding gingiva, irritated eyes, insomnia, difficulty concentrating and general restlessness. Prior to the girls' hospitalization, changes in academic performance, handwriting and personality were noted by the girls' teachers. A similar constellation of symptoms including intention tremor was subsequently observed in the other eight exposed residents. Skin rashes of varying severity were also seen among five of the residents. Blood mercury levels ranged from 183 to 620 ng/ml (normal is < 5 ng/ml). The highest air mercury level measured in one of the vacated and sealed trailers was 1.0 mg/m^3 five months after the initial spill. Neurological exams at two to four months following termination of exposure were normal for eight of the residents. However, at four months, two of the intensely exposed girls still showed neurological abnormalities as manifested in difficulties copying simple diagrams, and abnormal electroencephalograms.

The acute effects of inhalation exposure to mercury may be compounded by simultaneous dietary intake of methylmercury. The use of mercury amalgamation in the recovery of gold in the Amazon Basin has resulted in locally elevated mercury levels both in indoor air in gold shops (250-40,600 ng/m^3), and in ambient urban air (20-5,800

ng/m^3) (Hacon et al., 1995), thus increasing the opportunities for both acute and long-term exposures. At the same time, gold extraction activities have caused mercury contamination of waterways resulting in a concomitant increase in methylmercury in the diet from the consumption of contaminated fish (Cordier et al., 1998). Adverse neurological and otological effects have been associated with elevated blood mercury levels in both adults and children in this environment (Counter et al., 1998).

Predisposing Conditions for Mercury Toxicity

Medical: Persons with preexisting nervous system disorders or kidney diseases might be at increased risk of mercury toxicity. Also at higher risk are persons previously sensitized to mercury (Lerch and Bircher, 2004), and those with genetic susceptibililty to mercury-induced hypersensitivity (Warfvinge et al., 1995). Developing organisms (fetuses and infants) are especially susceptible to the neurotoxicity of mercury (USEPA, 1997).

Other: People who consume significant amounts of fish from areas with advisories for daily fish intake due to mercury contamination may be more susceptible to the chronic toxicity of airborne mercury due to existing body burden.

5.2 Acute Toxicity to Infants and Children

The data regarding the toxic effects of acute exposure of children to Hg^0 are largely limited to case reports with little or no information on actual exposure levels. In children who inhale high levels of toxic Hg^0 vapors, pulmonary dysfunction is the primary cause of mortality. For example, autopsy of a 4-month-old child who died following acute exposure to Hg^0 vapors revealed pulmonary and general edema, nephrotic degeneration, ventricular dilation, and a greyish, necrotic appearance in the digestive mucosa (Campbell, 1948). In another case study, severe interstitial pneumonitis, erosion of the bronchial epithelium, membrane lesions of the alveoli and alveolar ducts, and significantly elevated Hg in the kidneys and liver were documented by Matthes et al. (1958) following the deaths of three children aged 4, 20, and 30 months from acute Hg^0 vapor exposure in the home. Cases of CNS disturbances, including irritability, insomnia, malaise, anorexia, fatigue, ataxia, and headache have been reported in children exposed to vapor from spilled elemental mercury in their homes (Florentine and Sanfilippo, 1991).

5.3 Acute Toxicity to Experimental Animals

As reported for humans, acute inhalation exposure of experimental animals to high levels of mercury is associated with pulmonary toxicity. However, the effects of mercury inhalation following short term exposure have also been examined in the context of neurotoxicity, notably neurobehavioral effects, and mercury deposition and distribution in the nervous system, as well as pathological changes in various organs.

Pathological changes in lung tissues similar to those reported in humans (edema, fibrosis, and necrosis of alveolar epithelium and hyaline membranes) were observed by Livardjani et al. (1991) in rats exposed to 26 mg (3.1 ppm) Hg/m³ for 1 hour, or 27 mg

(3.2 ppm) for 2 hours. A dose-dependence of lung pathology and mortality was reported. No mortality was observed during the subsequent 15 days following the 1 hour exposure, while 50% mortality and more severe lesions were seen during the first 5 days following the 2 hour exposure.

In a study of pulmonary effects of mercury inhalation, as well as the possible role of metallothionein (MT), Yoshida et al. (1999) exposed both MT-null and wild-type mice to 6.6 - 7.5 mg/m^3 (0.79 - 0.90 ppm) mercury vapor for 4 hours on 3 consecutive days. Examination of the lungs 24 hours after exposure revealed severe congestion, atelectasis (incomplete expansion of the lung), and mild hemorrhage of the alveoli in MT-null mice, along with 60% mortality. Among wild-type mice, these pulmonary effects were much less severe, pulmonary MT expression was markedly increased, and no lethality was observed. Mercury was found bound to MT in the lungs of wild-type, but not in MT-null mice. MT thus appears to ameliorate the effects of mercury inhalation.

The neurobehavioral manifestations in the offspring of mice with maternal exposure to mercury vapor during pregnancy suggest damage to motor control and learning centers. In the study upon which the acute REL derivation is based, Danielsson et al. (1993) exposed pregnant rats (12 per group) by inhalation to 1.8 mg/m³ (0.22 ppm) of Hg^0 vapor for 1 hour/day (0.07 mg/kg/d) or 3 hours/day (0.2 mg/kg/d) during gestational days 11-14 and 17-20. The dose level was selected to avoid maternal toxicity. Tests of motor activity (locomotion, rearing, rearing time, total activity) in the offspring at 3 months of age revealed significant dose-dependent deficits compared to controls ($p < 0.01$). When tested at 14 months of age, the hypoactivity seen at 3 months was no longer apparent and, in the 0.2 mg/kg/d dose group, was replaced with significant hyperactivity (Table 5.3.1).

Table 5.3.2 Effects of Prenatal Metallic Mercury on Motor Activity

		3 months			14 months		
		Control	**0.07**	**0.2**	**Control**	**0.07**	**0.2**
Activity	**Day**	(SEM)	mg/kg/d	mg/kg/d	(SEM)	mg/kg/d	mg/kg/d
Locomotion	1	2785 (135)	2141 (104)*	2212 (135)*	1862 (119)	1289 (167)	1767 (127)
	2	2069 (127)	1432 (119)*	1385 (143)*	1194 (111)	1218 (104)	1512 (119)
	3	1719 (175)	1663 (191)	1090 (135)*	1162 (111)	915 (135)	1369 (119)
Rearing	1	404 (25)	321 (25)*	338 (25)*	204 (22)	143 (20)	210 (27)
	2	312 (29)	190 (20)*	161 (25)*	87 (22)	110 (28)	123 (22)
	3	247 (29)	238 (18)	157 (32)*	84 (18)	98 (25)	106 (18)
Rearing time	1	431 (19)	243 (20)*	232 (22)*	159 (21)	78 (24)	167 (26)
	2	269 (21)	138 (23)*	160 (24)*	66 (19)	99 (24)	114 (23)
	3	212 (21)	179 (23)	138 (21)*	87 (17)	76 (22)	138 (24)
Total activity	1	4854 (271)	3836 (318)*	3979 (302)*	3565 (302)	2435 (223)*	3151 (271)
	2	3804 (223)	2737 (239)*	2817 (350)*	2308 (255)	2324 (302)	3151 (334)*
	3	3183 (318)	3183 (350)	2132 (318)*	2228 (255)	2069 (271)	2546 (2711)

*p<0.01 Data estimated from Danielsson et al. (1993) Figure 1.

Significant learning deficits (swim maze performance) were observed in the 0.2 mg/kg/d-exposed, but not the lower-exposure rats tested at 15 months of age ($p < 0.05$) (Table 5.3.2). The brain concentrations of mercury in the 0.2 mg/kg/d dose group (0.012 mg/kg) were 2.5-fold higher than in the 0.07 mg/kg/d dose group (0.005 mg/kg), and 12-fold higher than in the control group (0.001 mg/kg).

Table 5.3.3 Prenatal Metallic Mercury and Learning Deficits

		7 months			15 months		
		Control	**0.07**	**0.2**	**Control**	**0.07**	**0.2**
Morris maze	**Day**		mg/kg/d	mg/kg/d		mg/kg/d	mg/kg/d
	1	53	48	46	42	40	29
	2	30	41	26	29	21	13*

*p<0.01 Data estimated from Danielsson et al. (1993) Figure 3.

These data indicate adverse effects of mercury exposure on the developing brain, but it is not clear at what nervous tissue levels effects first manifest.

To evaluate mercury deposition in neurons at low exposure levels, Pamphlett and Coote (1998) exposed female BALB/c mice to mercury vapor at 25 μg/m^3 (0.003 ppm) for 2-20 hr, or to 500 μg/m^3 (0.06 ppm) for 5-240 min. At 25 μg/m^3, mercury was first found in the perikarya of scattered large motor neurons in the lateral anterior horn of the spinal cord after 12 hr of exposure. Exposure at this level for 16 and 20 hr resulted in labeling of most of the large neurons of this area. By comparison, mercury was found in renal tubular epithelium after only 2 hr of exposure. Mice that survived longer than 6 weeks showed no mercury in the renal epithelia while mercury persisted in the brainstem motor neurons up to 30 weeks. At the higher dose of 500 μg/m^3, mercury labeling of spinal motor neurons was seen after only 30 min. The doses that resulted in mercury uptake into mouse motor neurons in these experiments are similar to those that workers in mercury-using occupations may receive in the course of a few hours. While the toxicological significance of the observed mercury labeling was not addressed in these mice, the accumulation of mercury in the motor neurons is consistent with the behavioral alterations reported above.

The effects of short term, high level exposure to mercury are not limited to pulmonary and nervous tissues. Severe cellular degeneration and necrosis were observed in the kidneys, brain, colon, and heart tissue of 2 rabbits exposed for 4 hours to 29.7 mg Hg/m³ (3.6 ppm) (Ashe et al., 1953). Exposure of rabbits to 31.3 mg Hg/m³ (3.8 ppm) for 1 hour resulted in moderate pathological changes (unspecified), but no necrosis, in the brain and kidney. In contrast, heart and lung tissues showed mild pathologic changes (Ashe et al., 1953). Increased duration (6 hours/day for 5 days) of exposure at this concentration was lethal.

6. Chronic Toxicity of Mercury

6.1 Chronic Toxicity to Adult Humans

This section briefly summarizes a large body of literature on mercury toxicity, emphasizing studies of inhalation exposure useful in the development of the 8-hr and chronic reference exposure levels. The reader is referred to OEHHA (1999) for more information on measuring toxicity by the oral route of exposure. The effects of chronic exposure to mercury vapor have been known for centuries and are most pronounced in the central nervous system. Toxic effects include tremors (mild or severe), unsteady gait, irritability, poor concentration, short-term memory deficits, tremulous speech, blurred vision, performance decrements, paresthesia, and decreased nerve conduction (Smith et al., 1970; Langolf et al., 1978; Fawer et al., 1983; Piikivi et al., 1984; Albers et al., 1988; Kishi et al., 1993). While some motor system disturbances can be reversed upon cessation of exposure, memory deficits may be permanent (Kishi et al., 1993). Studies have shown effects such as tremor and decreased cognitive skills in workers exposed to approximately 25 μg/m^3 (0.003 ppm) mercury vapor (Piikivi et al., 1984; Piikivi and Hanninen, 1989; Piikivi and Toulonen, 1989) (see discussion below).

The kidney is also a sensitive target organ of mercury toxicity. Effects such as proteinuria, proximal tubular and glomerular changes, albuminuria, glomerulosclerosis, and increased urinary N-acetyl-β-glucosaminidase have been seen in workers exposed

to approximately 25-60 μg/m^3 (0.003 - 0.007 ppm) mercury vapor (Roels et al., 1982; Bernard et al., 1987; Barregard et al., 1988; Piikivi and Ruokonen, 1989).

Chronic exposure to mercury vapors has also resulted in cardiovascular effects such as increased heart rate and blood pressure (Piikivi, 1989; Fagala and Wigg, 1992; Taueg et al., 1992), and in leukocytosis and neutrophilia (Fagala and Wigg, 1992).

A number of other studies with similar exposure levels also found adverse psychological and neurological effects in exposed versus unexposed individuals. Fawer et al. (1983) measured intention tremor with an accelerometer attached to the third metacarpal of the right hand in 26 male workers (mean age of 44 years) exposed to low concentrations of mercury vapor. The men worked either in a chloralkali plant (n = 12), a fluorescent tube manufacturing plant (n = 7), or in acetaldehyde production (n = 7). Twenty-five control subjects came from different parts of the same plants and were not occupationally exposed to mercury. The average exposure as measured by personal air sampling was 0.026 mg/m^3 (0.003 ppm) and the average duration of exposure was 15 years. The measurements of intention tremor were significantly higher in exposed workers than in controls ($p = 0.011$). Using the average exposure as a LOAEL and adjusting for occupational ventilation rates and workweek, the resultant LOAEL is 0.009 mg/m^3 (0.001 ppm).

Piikivi and Tolonen (1989) studied the effects of long-term exposure to mercury vapor on electroencephalograms (EEGs) of 41 chloralkali workers exposed for a mean of 15.6 years as compared to 41 matched controls. EEGs were analyzed both qualitatively and quantitatively. In the qualitative analysis, EEGs were interpreted visually with classification of normality and abnormality based on a previously established scale that separated focal, generalized and paroxysmal disturbances into four classes (normal, or mildly, moderately, or severely disturbed). Exposed workers, who had blood mercury levels of 11.6 μg/L, tended to have an increased number of EEG abnormalities and brain activity was found to be significantly lower than matched controls ($p < 0.001$). The abnormalities were most prominent in the parietal cortex, but absent in the frontal cortex. The authors used a conversion factor calculated by Roels et al. (1989) to extrapolate from blood mercury levels of 12 μg/L to an air concentration of 25 μg/m^3 (0.003 ppm).

Another study by Piikivi (1989) examined subjective and objective symptoms of autonomic dysfunction in the same 41 chloralkali workers described above. The exposed workers had mean blood levels of 11.6 μg/L corresponding to a TWA exposure of 25 μg Hg/m^3 in air (Roels et al., 1987). The workers were tested for pulse rate variation in normal and deep breathing, the Valsalva maneuver, vertical tilt, and blood pressure responses during standing and isometric work. The only significant difference in subjective symptoms was an increased reporting of palpitations in exposed workers. The objective tests demonstrated an increase in pulse rate variations at 30 μg Hg/m^3 (0.006 ppm; extrapolated from blood levels based on methods of Roels et al. (1987)), which is indicative of autonomic reflex dysfunction.

Piikivi and Hanninen (1989) studied subjective symptoms and psychological performance on a computer-administered test battery in 60 chloralkali workers exposed to approximately 25 µg/m^3 mercury vapor for a mean of 13.7 years. The subjective symptoms, evaluated by questionnaire, included the frequency or intensity of memory disturbances, difficulties concentrating, sleep disorders, and hand tremors. In addition a mood scale was used to evaluate tension, depression, anger, fatigue, and confusion. The psychomotor tests included finger tapping, eye-hand coordination, symbol digit substitution, pattern comparison, and a continuous performance test. Memory and learning effects were captured on tests of associate learning, associate memory, pattern memory, and serial digit learning. A statistically significant increase in subjective symptoms of sleep disturbance and memory disturbance was noted in the exposed workers ($p < 0.001$), as were increased anger, fatigue and confusion ($p < 0.01$). There were no differences in objective measures of memory, learning, or motor abilities, with the exception of poorer eye-hand coordination ($p < 0.001$).

A study by Ngim et al. (1992) assessed neurobehavioral performance in a cross-sectional study of 98 dentists exposed to a TWA concentration of 14 µg Hg/m^3 (range 0.7 to 42 µg/m^3) compared to 54 controls with no history of occupational exposure to mercury. Exposed dentists were matched to the control group for age, amount of fish consumption, and number of amalgam fillings. Air concentrations were measured with personal sampling badges over typical working hours (8-10 hours/day) and converted to a TWA. Blood samples were also taken (average 9.8 µg/L). The average concentration in air was estimated at 23 µg Hg/m^3 when the methods of Roels et al. (1987) were used. The average duration in this study of dentists was only 5.5 years, shorter than the above studies. The performance of the dentists was significantly worse than controls on a number of neurobehavioral tests measuring motor speed (finger tapping), visual scanning, visuomotor coordination and concentration, visual memory, and visuomotor coordination speed ($p < 0.05$). These neurobehavioral changes are consistent with central and peripheral neurotoxicity commonly observed in cases of chronic mercury toxicity.

Liang et al. (1993) investigated workers in a fluorescent lamp factory with a computer-administered neurobehavioral evaluation system and a mood-inventory profile. The cohort consisted of 88 individuals (19 females and 69 males) exposed for at least 2 years prior to the study. Exposure was monitored with area samplers and ranged from 8 to 85 µg Hg/m^3 across worksites. The average level of exposure was estimated at 33 µg Hg/m^3 and the average duration of exposure was estimated at 15.8 years. The exposed cohort performed significantly worse than the controls on tests of finger tapping, mental arithmetic, two digit searches, switching attention, and visual reaction time ($p < 0.05$-0.01). The effects on performance persisted after controlling for chronological age as a confounding factor.

6.2 Chronic Toxicity to Infants and Children

A number of case studies indicate that long-term exposure to Hg^0 in children is associated with severe arterial hypertension, acrodynia, seizures, tachycardia, anxiety, irritability and general malaise (Sexton et al., 1978; Torres et al., 2000). These

symptoms are consistent with the brain and kidneys as the principal target organs for Hg^0. By comparison, for methylmercury (MeHg), the brain is the most toxicologically relevant organ. An extensive literature supports the association between chronic MeHg exposure and neurological and developmental deficits in children (Choi, 1989; Harada, 1995; Grandjean et al., 1999). Unlike inorganic mercury, both Hg^0 and MeHg easily cross cell membranes, the blood brain barrier, and the placenta (Ask et al., 2002). Intracellular oxidation of Hg^0 and the slower demethylation of MeHg both lead to the mercuric ion that binds cellular macromolecules, trapping it within the cell and contributing to the toxicity associated with exposures to the respective forms. While the complete mechanisms of toxicity for the two forms are not well understood and are likely not identical, there are important similarities. Methylmercury and the mercuric ion formed from Hg^0 avidly bind to protein sulfhydryls and may inactivate enzymes. Disruption of protein synthesis has been reported after exposure to either Hg^0 or MeHg, although the former is the more powerful inhibitor (NAS, 2000). The neurotoxic effects observed in adult rats following *in utero* exposure to Hg^0, MeHg, or both, are reportedly similar with MeHg potentiating the effects of Hg^0 (Fredriksson et al., 1996). Given the high susceptibility of children to MeHg and the apparent similarities in mechanisms with Hg^0, children are expected to be more susceptible to Hg^0 toxicity as well.

There is a considerable body of evidence from human poisoning episodes that mercury exposure in utero and postnatally results in developmental neurotoxicity (McKeown-Eyssen et al., 1983; Grandjean et al., 1994; Harada, 1995; Grandjean et al., 1997). Thus, infants and children are susceptible subpopulations for adverse health effects from mercury exposure. These effects fall into several general categories: 1) effects on neurological status (Castoldi et al., 2001); 2) age at which developmental milestones are achieved (Marsh et al., 1979); 3) infant and preschool development (Kjellstrom et al., 1986; Kjellstrom et al., 1989); 4) childhood development (age 6 and above) (Grandjean et al., 1997); and 5) sensory or neurophysiological effects (Murata et al., 1999). These studies and others are extensively reviewed by the U.S. EPA (2000) and the NAS (2000)

Whereas MeHg and elemental mercury readily cross the blood-brain barrier and the placental barrier, the mercuric ion (Hg^{2+}) does not readily cross these barriers. However, in fetuses and neonates mercuric species concentrate more in the brain because the blood-brain barrier is incompletely formed. Methylmercury and elemental mercury are lipophilic and are distributed throughout the body. In adults mercuric species accumulate more in the kidney. However, in neonates mercuric species do not concentrate in the kidneys but are more widely distributed to other tissues (NAS, 2000). It is possible that the increased distribution of mercuric species to the brain in fetuses and neonates accounts for some of the sensitivity of the brain to mercury during these developmental periods. The sensitivity of the fetal brain might also be due to the high proportion of dividing and differentiating cells during neuronal development in the fetal and neonatal periods. These dividing cells may be more sensitive to damaging effects of mercury-protein complexes. Furthermore, neurodevelopment is a “one-way street”. Disruption along the route results in permanent deficits. Methylmercury can also alter the relative levels of thyroid hormones to which the fetus is exposed and upon which normal neurodevelopment depends.

In addition to prenatal and postnatal dietary exposure, neonates may receive added postnatal dietary exposure to mercuric species and MeHg from breast milk (Drexler and Schaller, 1998; Sundberg et al., 1999). Animal data suggest that suckling rats retain a higher percentage of ingested organic mercury than do adults, with much higher concentrations in the brain (Kostial et al., 1978). School children can be accidentally exposed to elemental mercury which is a curiosity and an attractive nuisance (George et al., 1996; Lowry et al., 1999). Younger children may also be exposed when elemental mercury is spilled on floors and carpets where they are more active.

6.3 Chronic Toxicity to Experimental Animals

Studies of the effects of mercury in experimental animals generally employ mercury levels in excess of those to which humans are exposed in most settings, thus limiting their ability to model the consequences of long-term, low level exposures. To address this issue, and to test for a role of metallothionein (MT) in mitigating mercury's effects ,Yoshida et al. (2004) exposed wild type and MT-null mice to mercury vapor at 0.06 mg/m^3 (0.007 ppm), 8 hr/day for 23 weeks. Neurobehavioral effects in open field and passive avoidance tests were evaluated at 12 and 23 weeks, and brain levels of mercury were determined. Mercury levels in the brains of mice were 0.66 and 0.97 µg/g tissue for MT-null and wild type, respectively. For comparison, the authors cite human brain mercury levels ranging from 0.3 µg/g in dental personnel to 33 µg/g in retired mercury miners. Mercury-exposed mice showed enhanced motor activity that was statistically significant for both strains at 12 weeks ($p < 0.01$), and for the MT-null mice at 23 weeks ($p < 0.05$). In a learning task (passive avoidance of an electric shock), there were no significant differences between controls and either strain of mouse at 12 weeks of exposure. However, after 23 weeks of exposure, MT-null, but not wild type mice, showed significantly less avoidance than controls ($p < 0.05$) suggesting impaired long-term memory. These data suggest that long-term mercury exposure that results in brain levels of mercury comparable to those seen in occupationally-exposed humans, causes changes in neurobehavior, an effect that is exacerbated by low levels of MT. For comparison, Fawer et al (1983) reported increased intention tremor in human workers exposed to an average of 0.003 ppm for an average of 15 years (Section 6.1).

There is a substantial body of work delineating the neurotoxic effects of MeHg exposure on animals exposed in utero. A comparison between mercury vapor and MeHg, separately and in concert, was conducted in rats. Fredriksson et al. (1996) exposed pregnant rats to MeHg by gavage (2 mg/kg/d during days 6-9 of gestation), and metallic mercury (Hg^0) vapor by inhalation (1.8 mg/m^3 (0.22 ppm) for 1.5 h per day during gestation days 14-19), or both. Controls received the combined vehicles for each of the two treatments. The dose by inhalation was approximately 0.1 mg Hg^0/kg/day. No differences were observed among groups in clinical observations and developmental markers up to weaning. Tests of behavioral function, performed at 4-5 months of age, included spontaneous motor activity, spatial learning in a circular bath, and instrumental maze learning for food reward. Offspring of dams exposed to Hg^0 showed hyperactivity over all three measures of motor activity: locomotion, rearing and total activity. This effect was enhanced in the animals of the MeHg + Hg^0 group. Compared to either the control or MeHg groups in the swim maze test, rats in the MeHg + Hg^0 and Hg^0 groups

took longer to reach a submerged platform whose location they had learned the previous day. Similarly, both the MeHg + Hg^0 and Hg^0 groups showed more ambulations and rearings in the activity test prior to the learning trial in the enclosed radial arm maze. During the learning trial, these same animals showed longer latencies and made more errors in acquiring the food reward. Generally, the results indicated that prenatal exposure to Hg^0 caused alterations to both spontaneous and learned behaviours, suggesting some deficit in adaptive functions. In these experiments, exposure to MeHg was not observed to alter these functions but rather appeared to potentiate the effects of Hg^0.

The similarities in the effects of MeHg and Hg^0 imply similar targets in the brain, which appears to be the case. Pregnant squirrel monkeys were exposed to mercury vapor (0.5 or 1 mg/m^3 (0.06 or 0.12 ppm)) for 4 or 7 hours per day starting in the fifth to the seventh week of gestation and generally ending between 18 and 23 weeks of gestational age (Warfvinge, 2000). The concentration of mercury was found to be higher in maternal (0.80-2.58 µg/g tissue) than in offspring (0.20-0.70 µg/g) brains, but with similar cerebellar distributions. In this study, mercury was localized mainly to Purkinje cells and Bergmann glial cells, similar to the distribution seen after MeHg exposure. The nuclei affected in these and other studies are part of the motor system.

In rats exposed to mercury vapor at ~1 mg/m^3 (0.12 ppm) for 6 h/d, 3 d/wk for 5 wk (low dose), or 24 h/d, 6 d/wk for 5 wk (high dose), an exposure duration-dependent loss of Purkinje cells and proliferation of Bergmann glial cells were observed (Hua et al., 1995). Whereas mercury accumulated to a higher degree in kidney compared to brain, the mercury level in kidney only increased 17% (90 to 105 µg/g tissue) from low to high doses, while that of the brain increased 608% (0.71 to 5.03 µg/g). These neuropathological changes were observed at the same mercury doses as this group reported previously for kidney autoimmune disease (Hua et al., 1993). The brain is a more sensitive target for mercury toxicity in part due to its greater ability to concentrate the metal.

7. Developmental and Reproductive Toxicity

Occupational exposure to mercury vapor has been associated with reproductive problems in a number of epidemiological studies. In a study of 418 dental assistants, Rowland et al. (1994) reported that the fecundability of the women with high exposure to dental amalgams was 63% (95% CI 42-96%) of that reported for the dental assistants with no amalgam exposure. Similarly, in a Chinese study by Yang et al. (2002), there was a significantly higher prevalence of abdominal pain (OR 1.47, 95% CI 1.03: 2.11) and dysmenorrhea (OR 1.66, 95% CI 1.07; 2.59) among female factory workers exposed to ambient mercury vapor (0.001-0.200 mg/m^3) compared with factory workers without mercury exposure. In another study of female factory workers exposed to mercury vapors, the frequency of adverse birth outcomes, especially congenital anomalies, was higher among those exposed to mercury levels at or substantially lower than 0.6 mg/m^3 (Elghany et al., 1997).

The adverse effects of elemental mercury exposure have also been demonstrated in animal models. In rats, elemental mercury readily crosses the placental barrier and accumulates in the fetus following inhalation (Morgan et al., 2002). Pregnant rats exposed by inhalation to 1.8 mg/m³ of metallic mercury for 1 hour or 3 hours/day during gestation (days 11 through 14 plus days 17 through 20) bore pups that displayed significant dose-dependent deficits in behavioral measurements 3-7 months after birth compared to unexposed controls (Danielsson et al., 1993). Behaviors measured included spontaneous motor activity, performance of a spatial learning task, and habituation to the automated test chamber. The pups also showed dose-dependent, increased mercury levels in their brains, livers, and kidneys 2-3 days after birth.

Morgan et al. (2002) exposed pregnant rats for 2 hr per day to 1, 2, 4, or 8 mg/m^3 mercury vapor during gestation days (GD) 6-15, and found a dose-dependent distribution of mercury to all maternal and fetal tissues. Adverse effects on resorptions, postnatal litter size and neonatal body weights were only observed at the highest mercury dose, which was also maternally toxic. It is of interest to note that following cessation of maternal exposure on GD 15, the mass of the fetal brain and its content of mercury both increased 10-fold. Thus the fetal brain continued to accumulate mercury eliminated from maternal tissues. This suggests that the period of fetal exposure is longer than that of maternal exposure, and may affect more neurodevelopmental stages than the timing of the maternal exposure would suggest.

Mercury and mercury compounds, including inorganic forms, are listed under California Proposition 65 (Cal/EPA, Safe Drinking Water and Toxic Enforcement Act of 1986) as developmental toxins. It should be noted that there is substantial evidence in humans of the developmental toxicity of methylmercury exposure. However, this REL summary is meant to be applied to elemental and inorganic mercury, and thus we are not describing methylmercury toxicity in depth in this document.

8. Derivation of Reference Exposure Levels

8.1 Mercury Acute Reference Exposure Level

Study	Danielsson et al., 1993
Study population	groups of 12 pregnant rats
Exposure method	inhalation of metallic mercury vapors
Exposure continuity	
Exposure duration	1 hour per day
Critical effects	CNS disturbances in offspring
LOAEL	1.8 mg/m³
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	
Human Equivalent Concentration	n/a
LOAEL uncertainty factor (UF_L)	10 (default; severe effect, no NOAEL)
Subchronic uncertainty factor (UFs)	
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	√10 (default, animal study)
Toxicodynamic (UF_{A-d})	10 (greater human vs rat susceptibility)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (default: critical study in young)
Toxicodynamic (UF_{H-d})	√10 (default: critical study in young)
Cumulative uncertainty factor	3000
Reference Exposure Level	**0.6 µg Hg/m³ (0.07 ppb Hg^0)**

Acute Reference Exposure Levels are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document (TSD)).

In the absence of acute inhalation studies in humans, the study by Danielsson et al. (1993) was selected as the critical study since it used a sensitive endpoint, neurotoxicity, in a highly susceptible, developmental stage. Maternal rats were exposed by inhalation to 1.8 mg/m³ of metallic mercury vapor for 1 hour/day or 3 hours/day during gestation. The offspring displayed significant dose-dependent deficits in behavior 3-7 months after birth compared to controls. The default uncertainty factor of 10 is applied for the use of a LOAEL for moderate to severe effects in the absence of a NOAEL.

A default interspecies uncertainty factor of √10 for toxicokinetic (UF_{A-k}) variability was used, while a larger interspecies UF_{A-d} of 10 for toxicodynamic differences was used to reflect the potentially greater developmental susceptibility of humans versus rats. This is based, in part, on Lewandowski et al. (2003) who used a comparative approach to analyze in vivo and in vitro data on the responses of neuronal cells of rats, mice, and humans to MeHg. Their analysis suggests that humans may be up to 10-fold more sensitive to MeHg than are rats. Application of Lewandowski's analysis assumes that

the human and rat responses to elemental mercury are comparable with those to MeHg. The study by Fredriksson et al. (1996) (above) supports this assumption for neurobehavioral effects. A greater susceptibility of humans to adverse neurobehavioral effects following early-life exposures compared with experimental animals has also been seen with other metals, especially lead. For example, Schwartz (1994) reported no evidence for a threshold for neurobehavioral effects in children with blood lead levels of 1 µg/dL compared with less than 15 µg/dL in primates (Gilbert and Rice, 1987) and less than 20 µg/dL in rats (Cory-Slechta et al., 1985).

Since the critical study involved early life exposures, the default intraspecies toxicodynamic uncertainty factor (UF_{H-d}) of $\sqrt{10}$ was employed to account for individual variability. The intraspecies toxicokinetic uncertainty factor of $\sqrt{10}$ reflects the absence of data in young humans, but also the lack of reason to expect major age differences, at least in the short-term kinetics. The resulting acute REL was 0.6 µg/m^3 (0.07 ppb).

This REL is developed for metallic mercury vapor but would be expected to be protective for inhalation of mercury salts. Although mercury salts have no significant vapor pressure under normal atmospheric conditions, they are of concern as hazards if aerosolized or produced during combustion. Animals exposed to mercury vapor inhalation had ten-fold higher brain mercury levels than animals exposed to a similar amount of injected inorganic mercury (mercuric nitrate) (Berlin et al., 1969); however the relationship between kinetics of mercury vapor and mercuric salts has not been extensively studied and may be complex, and dependent on the route, level and timing of exposure.

8.2 Mercury 8-Hour Reference Exposure Level

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 of the Technical Support Document).

The half life of elimination of mercury in humans following a single inhalation exposure of 14-24 min. was 21 days from the head, 64 days from the kidney, and 58 days from the body as a whole (Hursh et al., 1976). Urinary elimination among workers occupationally exposed for several years had an elimination half life of 55 days (Sallsten et al., 1994). Thus, since mercury is only slowly eliminated, the intervals between daily 8-hr exposures, and between weeks are not long enough for the elimination of significant amounts of the metal and it will accumulate in the body with repeated exposure. In view of this bioaccumulative property of mercury exposure in humans, it was considered necessary to use the same study and derivation (in terms of exposure for seven vs only five days per week) for the 8-hour REL as for the chronic REL described below. However, the exposure duration adjustment used in this case reflects a repeated exposure of 8 hours per day with an activity-related air intake of 10 m^3 per day (i.e. half that assumed for a 24-hour period for the chronic REL). As a result, the time-adjusted exposure is twice that for the chronic REL. This adjustment reflects the expectation that activity levels, and hence breathing rates, will be higher during the

exposure period than during the remaining 16 hours. The increased breathing rate enhances mercury inhalation during the 8 hour exposure period.

Study	Piikivi and Hanninen (1989); Fawer et al. (1983); Piikivi and Tolonen (1989); Piikivi (1989); Ngim et al. (1992)
Study population	Humans (236)
Exposure method	Inhalation of workplace air
Exposure continuity	8 hours per day, 5 days/week
Exposure duration	13.7 to 15.6 years
Critical effects	Neurotoxicity as measured by: intention tremor; memory and sleep disturbances; decreased performance on neurobehavioral tests (finger tapping, visual scan, visuomotor coordination, visual memory); decreased EEG activity
LOAEL	25 µg/m^3 (3 ppb)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	18 µg/m^3 for LOAEL group (25 x 5/7)
LOAEL uncertainty factor (UF_L)	10 (default, severe effect, no NOAEL)
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (default for inter-individual variability)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children and their developing nervous systems)
Cumulative uncertainty factor	300
Reference Exposure Level	**0.06 µg Hg/m^3 (0.007 ppb Hg^0)**

The studies chosen for determination of the 8-hr REL examined neurotoxicity in humans as a sensitive endpoint following long-term exposures. They all point to a LOAEL of approximately 25 µg/m^3 (3 ppb) with a time-adjusted value of 18 µg/m^3 (25 x 5/7). In the absence of a NOAEL, we applied an uncertainty factor of 10, the default with neurotoxicity considered a moderate to potentially severe effect. The critical study was conducted in humans and was not a subchronic study so no interspecies or subchronic uncertainty factors were applied. To allow for interindividual variability and to specifically account for greater susceptibility among children, an overall intraspecies uncertainty factor of 30 was applied with a toxicokinetic factor (H-k) of √10 to reflect interindividual variability, and a toxicodynamic factor of 10 that reflects the higher susceptibility of the developing nervous system. The cumulative uncertainty is 300, and the resultant 8-hour REL is thus 0.06 µg Hg/ m^3 (0.007 ppb Hg °).

8.3 Mercury Chronic Reference Exposure Level

Study	Piikivi and Hanninen (1989); Fawer et al. (1983); Piikivi and Tolonen (1989); Piikivi (1989); Ngim et al. (1992)
Study population	Humans (236)
Exposure method	Inhalation of workplace air
Exposure continuity	8 hours per day (10 m^3/workday), 5 days/week
Exposure duration	13.7 to 15.6 year
Critical effects	Neurotoxicity as measured by: intention tremor; memory and sleep disturbances; decreased performance on neurobehavioral tests (finger tapping, visual scan, visuomotor coordination, visual memory); decreased EEG activity
LOAEL	25 µg/m^3 (3 ppb)
NOAEL	not observed
Benchmark concentration	not derived
Time-adjusted exposure	9 µg/m^3 for LOAEL group (25 x 10/20 x 5/7)
LOAEL uncertainty factor (UF_L)	10 (default, severe effect, no NOAEL)
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1 (default: human study)
Toxicodynamic (UF_{A-d})	1 (default: human study)
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10 (default for inter-individual variability)
Toxicodynamic (UF_{H-d})	10 (greater susceptibility of children and their developing nervous systems)
Cumulative uncertainty factor	300
Reference Exposure Level	**0.03 µg Hg/m^3 (0.004 ppb Hg^0)**

The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from chronic exposures (see Section 7 in the Technical Support Document).

To calculate the chronic REL, studies were chosen that examined a sensitive endpoint (neurotoxicity) in humans following long-term exposures. They all point to a LOAEL of approximately 0.025 mg/m^3 (3 ppb). When adjusted for worker ventilation and workweek exposure, the LOAEL becomes 9 µg/m^3 (25 µg/m^3 x 10 m^3/20 m^3 x 5 d/7 d). In the absence of a NOAEL, we applied an uncertainty factor of 10, the default with neurotoxicity considered a moderate to potentially severe effect. The critical study was conducted in humans and was not a subchronic study so no interspecies or subchronic uncertainty factors were applied. To allow for interindividual variability and to specifically account for greater susceptibility among children, an overall intraspecies

uncertainty factor of 30 was applied with a toxicokinetic factor (H-k) of √10 to reflect interindividual variability, and a toxicodynamic factor of 10 that reflects the higher susceptibility of the developing nervous system. The cumulative uncertainty is 300, and the resultant chronic REL is thus 0.03 µg Hg/ m^3 (0.004 ppb Hg °).

The U.S.EPA (1995) based its RfC of 0.3 µg/m^3 (0.04 ppb) on the same study but used an intraspecies uncertainty factor of 3, a LOAEL uncertainty factor of 3 and included a Modifying Factor (MF) of 3 for database deficiencies (lack of developmental and reproductive toxicity data). This modifying factor was not used by OEHHA since allowance was made via the UF_{H-d} for the known sensitivity of children to the neurodevelopmental impacts of mercury.

It is noteworthy that none of the above studies discussed in sufficient detail a dose-response relationship between mercury vapor inhalation and the toxic effects measured. Because none of the studies mention a level below which toxic effects were not seen (a NOAEL), the extrapolation from a LOAEL to a NOAEL should be regarded with caution. Secondly, one study (Ngim et al., 1992) demonstrated neurotoxic effects from mercury inhalation at an exposure level slightly above the other studies, but for a shorter duration. It is possible that mercury could cause neurotoxic effects after a shorter exposure period than that reported in the study used in derivation of the chronic REL.

As mentioned above, OEHHA (1999) has developed a PHG for inorganic mercury in drinking water of 0.0012 mg Hg/L (1.2 ppb) as a level of exposure expected to be without significant health risk from daily water consumption. This value was based on data from a 1993 study by the National Toxicology Program that supported a NOAEL of 0.16 mg Hg/kg-day for renal toxicity in rats with chronic oral exposure. Application of the cumulative uncertainty factor of 1,000 (10 for use of a subchronic study, and 10 each for inter- and intraspecies variability) used in the PHG derivation, gives an oral REL of 0.16 µg Hg/kg-day. This value is several-fold higher than the chronic REL developed above for inhalation of elemental mercury, and reflects the greater ease with which elemental mercury (vs. inorganic mercury) penetrates membranes, especially when exposure is via inhalation versus the oral route.

8.4 Mercury as a Toxic Air Contaminant that Disproportionately Impacts Children

In view of the differential impacts on infants and children identified in Section 6.2.1, and the possibility of direct (inhalation) and indirect exposure (through a diet containing aquatic animals contaminated with methylmercury), OEHHA recommends that elemental mercury be identified as a toxic air contaminant (TAC) which disproportionately impacts children under Health and Safety Code, Section 39699.5.

9. References

ACGIH (1986). Documentation of the Threshold Limit Values and Biological Exposure Indices. Cincinnati (OH): American Conference of Governmental Industrial Hygienists.

Albers JW, Kallenbach LR, Fine LJ, Langolf GD, Wolfe RA, Donofrio PD, Alessi AG, Stolp-Smith KA and Bromberg MB (1988). Neurological abnormalities associated with remote occupational elemental mercury exposure. Ann Neurol 24(5): 651-9.

Allen JW, Mutkus LA and Aschner M (2001). Mercuric chloride, but not methylmercury, inhibits glutamine synthetase activity in primary cultures of cortical astrocytes. Brain Res 891(1-2): 148-57.

Asano S, Eto K, Kurisaki E, Gunji H, Hiraiwa K, Sato M, Sato H, Hasuike M, Hagiwara N and Wakasa H (2000). Acute inorganic mercury vapor inhalation poisoning. Pathol Int 50(3): 169-74.

Ashe W, Largent E, Dutra F, Hubbard D and Blackstone M (1953). Behavior of mercury in the animal organism following inhalation. Ind. Hyg. Occup. Med. 17: 19-43.

Ask K, Akesson A, Berglund M and Vahter M (2002). Inorganic mercury and methylmercury in placentas of Swedish women. Environ Health Perspect 110(5): 523-6.

ATSDR. (1999). Toxicological Profile for Mercury. U.S. Department for Human Health Services. Atlanta, GA

Barregard L, Hultberg B, Schutz A and Sallsten G (1988). Enzymuria in workers exposed to inorganic mercury. Int Arch Occup Environ Health 61(1-2): 65-9.

Berlin M, Fazackerley J and Nordberg G (1969). The uptake of mercury in the brains of mammals exposed to mercury vapor and to mercuric salts. Arch Environ Health 18(5): 719-29.

Bernard AM, Roels HR, Foidart JM and Lauwerys RL (1987). Search for anti-laminin antibodies in the serum of workers exposed to cadmium, mercury vapour or lead. Int Arch Occup Environ Health 59(3): 303-9.

Campbell J (1948). Acute mercurial poisoning by inhalation of metallic vapour in an infant. Can Med Assoc J 58: 72-75.

CARB (2005a). Annual Statewide Toxics Summary - mercury http://www.arb.ca.gov/aqd/toxics/statepages/hgstate.html. Sacramento, CA.

CARB. (2005b). *California Toxics Inventory for 2004*. California Air Resources Board. http://www.arb.ca.gov/toxics/cti/cti.htm.

Castoldi AF, Coccini T, Ceccatelli S and Manzo L (2001). Neurotoxicity and molecular effects of methylmercury. Brain Res Bull 55(2): 197-203.

Choi BH (1989). The effects of methylmercury on the developing brain. Prog Neurobiol 32(6): 447-70.

Cordier S, Grasmick C, Paquier-Passelaigue M, Mandereau L, Weber JP and Jouan M (1998). Mercury exposure in French Guiana: levels and determinants. Arch Environ Health 53(4): 299-303.

Cory-Slechta DA, Weiss B and Cox C (1985). Performance and exposure indices of rats exposed to low concentrations of lead. Toxicol Appl Pharmacol 78(2): 291-9.

Counter SA, Buchanan LH, Laurell G and Ortega F (1998). Blood mercury and auditory neuro-sensory responses in children and adults in the Nambija gold mining area of Ecuador. Neurotoxicology 19(2): 185-96.

Danielsson BR, Fredriksson A, Dahlgren L, Gardlund AT, Olsson L, Dencker L and Archer T (1993). Behavioural effects of prenatal metallic mercury inhalation exposure in rats. Neurotoxicol Teratol 15(6): 391-6.

Drexler H and Schaller KH (1998). The mercury concentration in breast milk resulting from amalgam fillings and dietary habits. Environ Res 77(2): 124-9.

Elghany NA, Stopford W, Bunn WB and Fleming LE (1997). Occupational exposure to inorganic mercury vapour and reproductive outcomes. Occup Med (Lond) 47(6): 333-6.

Fagala GE and Wigg CL (1992). Psychiatric manifestations of mercury poisoning. J Am Acad Child Adolesc Psychiatry 31(2): 306-11.

Fawer RF, de Ribaupierre Y, Guillemin MP, Berode M and Lob M (1983). Measurement of hand tremor induced by industrial exposure to metallic mercury. Br J Ind Med 40(2): 204-8.

Florentine MJ and Sanfilippo DJ, 2nd (1991). Elemental mercury poisoning. Clin Pharm 10(3): 213-21.

Fredriksson A, Dencker L, Archer T and Danielsson BR (1996). Prenatal coexposure to metallic mercury vapour and methylmercury produce interactive behavioural changes in adult rats. Neurotoxicol Teratol 18(2): 129-34.

George L, Scott FE, Cole D, Siracusa L, Buffett C, Hunter W and Zinkewich R (1996). The mercury emergency and Hamilton school children: a follow-up analysis. Can J Public Health 87(4): 224-6.

Gilbert SG and Rice DC (1987). Low-level lifetime lead exposure produces behavioral toxicity (spatial discrimination reversal) in adult monkeys. Toxicol Appl Pharmacol 91(3): 484-90.

Grandjean P, Weihe P and Nielsen JB (1994). Methylmercury: significance of intrauterine and postnatal exposures. Clin Chem 40(7 Pt 2): 1395-400.

Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sorensen N, Dahl R and Jorgensen PJ (1997). Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 19(6): 417-28.

Grandjean P, White RF, Nielsen A, Cleary D and de Oliveira Santos EC (1999). Methylmercury neurotoxicity in Amazonian children downstream from gold mining. Environ Health Perspect 107(7): 587-91.

Hacon S, Artaxo P, Gerab F, Yamasoe MA, Campos RC, Conti LF and De Lacerda LD (1995). Atmospheris mercury and trace elements in the region of Alta Floresta in the Amazon basin. Water, Air, and Soil Pollution 80(1-4): 273-283.

Harada M (1995). Minamata disease: methylmercury poisoning in Japan caused by environmental pollution. Crit Rev Toxicol 25(1): 1-24.

Hua J, Brun A and Berlin M (1995). Pathological changes in the Brown Norway rat cerebellum after mercury vapour exposure. Toxicology 104(1-3): 83-90.

Hua J, Pelletier L, Berlin M and Druet P (1993). Autoimmune glomerulonephritis induced by mercury vapour exposure in the Brown Norway rat. Toxicology 79(2): 119-29.

Hursh JB, Cherian MG, Clarkson TW, Vostal JJ and Mallie RV (1976). Clearance of mercury (HG-197, HG-203) vapor inhaled by human subjects. Arch Environ Health 31(6): 302-9.

Kanluen S and Gottlieb CA (1991). A clinical pathologic study of four adult cases of acute mercury inhalation toxicity. Arch Pathol Lab Med 115(1): 56-60.

Kishi R, Doi R, Fukuchi Y, Satoh H, Satoh T, Ono A, Moriwaka F, Tashiro K and Takahata N (1993). Subjective symptoms and neurobehavioral performances of ex-mercury miners at an average of 18 years after the cessation of chronic exposure to mercury vapor. Mercury Workers Study Group. Environ Res 62(2): 289-302.

Kjellstrom T, Kennedy P, Wallis S, Stewart A, Friberg L, Lind B, Wutherspoon T and Mantell C. (1989). Physical and mental development of children with prenatal exposure to mercury from fish. Stage 2: Interviews and psychological tests at age 6. Report 3642. National Swedish Environmental Protection Board. Solna, Sweden

Kjellstrom T, Kennedy S, Wallis S and Mantell C. (1986). Physical and mental development of children with prenatal exposure to mercury from fish. Stage I: Preliminary tests at age 4. Report #3080. National Swedish Environmental Protection Board. Solna, Sweden

Kostial K, Kello D, Jugo S, Rabar I and Maljkovic T (1978). Influence of age on metal metabolism and toxicity. Environ Health Perspect 25: 81-6.

Langolf GD, Chaffin DB, Henderson R and Whittle HP (1978). Evaluation of workers exposed to elemental mercury using quantitative tests of tremor and neuromuscular functions. Am Ind Hyg Assoc J 39(12): 976-84.

Leggett RW, Munro NB and Eckerman KF (2001). Proposed revision of the ICRP model for inhaled mercury vapor. Health Phys 81(4): 450-5.

Lerch M and Bircher AJ (2004). Systemically induced allergic exanthem from mercury. Contact Dermatitis 50(6): 349-53.

Levin M, Jacobs J and Polos PG (1988). Acute mercury poisoning and mercurial pneumonitis from gold ore purification. Chest 94(3): 554-6.

Lewandowski TA, Ponce RA, Charleston JS, Hong S and Faustman EM (2003). Effect of methylmercury on midbrain cell proliferation during organogenesis: potential cross-species differences and implications for risk assessment. Toxicol Sci 75(1): 124-33.

Liang YX, Sun RK, Sun Y, Chen ZQ and Li LH (1993). Psychological effects of low exposure to mercury vapor: application of a computer-administered neurobehavioral evaluation system. Environ Res 60(2): 320-7.

Livardjani F, Ledig M, Kopp P, Dahlet M, Leroy M and Jaeger A (1991). Lung and blood superoxide dismutase activity in mercury vapor exposed rats: effect of N-acetylcysteine treatment. Toxicology 66(3): 289-95.

Lowry LK, Rountree PP, Levin JL, Collins S and Anger WK (1999). The Texarkana mercury incident. Tex Med 95(10): 65-70.

Lund BO, Miller DM and Woods JS (1993). Studies on Hg(II)-induced H2O2 formation and oxidative stress in vivo and in vitro in rat kidney mitochondria. Biochem Pharmacol 45(10): 2017-24.

Marsh DO, Myers GJ, Clarkson TW, Amin-Zaki L, Tikriti S and Majeed MA (1979). Fetal methylmercury poisoning: clinical and toxicological data on 29 cases. Ann Neurol 7(4): 348-53.

Matthes FT, Kirschner R, Yow MD and Brennan JC (1958). Acute poisoning associated with inhalation of mercury vapor; report of four cases. Pediatrics 22(4 Part 1): 675-88.

McFarland RB and Reigel H (1978). Chronic mercury poisoning from a single brief exposure. J Occup Med 20(8): 532-4.

McKeown-Eyssen GE, Ruedy J and Neims A (1983). Methyl mercury exposure in northern Quebec. II. Neurologic findings in children. Am J Epidemiol 118(4): 470-9.

Morgan DL, Chanda SM, Price HC, Fernando R, Liu J, Brambila E, O'Connor RW, Beliles RP and Barone S, Jr. (2002). Disposition of inhaled mercury vapor in pregnant rats: maternal toxicity and effects on developmental outcome. Toxicol Sci 66(2): 261-73.

Murata K, Weihe P, Renzoni A, Debes F, Vasconcelos R, Zino F, Araki S, Jorgensen PJ, White RF and Grandjean P (1999). Delayed evoked potentials in children exposed to methylmercury from seafood. Neurotoxicol Teratol 21(4): 343-8.

Nakayama H, Niki F, Shono M and Hada S (1983). Mercury exanthem. Contact Dermatitis 9(5): 411-7.

NAS. (2000). Toxicological Effects of Methyl Mercury. National Academy of Sciences. Washington D.C.

Netterstrom B, Guldager B and Heeboll J (1996). Acute mercury intoxication examined with coordination ability and tremor. Neurotoxicol Teratol 18(4): 505-9.

Ngim CH, Foo SC, Boey KW and Jeyaratnam J (1992). Chronic neurobehavioural effects of elemental mercury in dentists. Br J Ind Med 49(11): 782-90.

OEHHA. (1999). Public Health Goal for Inorganic Mercury in Drinking Water. Office of Environmental Health Hazard Assessment. California Environmental Protection Agency. Scramento, CA

Pamphlett R and Coote P (1998). Entry of low doses of mercury vapor into the nervous system. Neurotoxicology 19(1): 39-47.

Piikivi L (1989). Cardiovascular reflexes and low long-term exposure to mercury vapour. Int Arch Occup Environ Health 61(6): 391-5.

Piikivi L and Hanninen H (1989). Subjective symptoms and psychological performance of chlorine-alkali workers. Scand J Work Environ Health 15(1): 69-74.

Piikivi L, Hanninen H, Martelin T and Mantere P (1984). Psychological performance and long-term exposure to mercury vapors. Scand J Work Environ Health 10(1): 35-41.

Piikivi L and Ruokonen A (1989). Renal function and long-term low mercury vapor exposure. Arch Environ Health 44(3): 146-9.

Piikivi L and Tolonen U (1989). EEG findings in chlor-alkali workers subjected to low long term exposure to mercury vapour. Br J Ind Med 46(6): 370-5.

Rodgers JS, Hocker JR, Hanas RJ, Nwosu EC and Hanas JS (2001). Mercuric ion inhibition of eukaryotic transcription factor binding to DNA. Biochem Pharmacol 61(12): 1543-50.

Roels H, Lauwerys R, Buchet JP, Bernard A, Barthels A, Oversteyns M and Gaussin J (1982). Comparison of renal function and psychomotor performance in workers exposed to elemental mercury. Int Arch Occup Environ Health 50(1): 77-93.

Rowland AS, Baird DD, Weinberg CR, Shore DL, Shy CM and Wilcox AJ (1994). The effect of occupational exposure to mercury vapour on the fertility of female dental assistants. Occup Environ Med 51(1): 28-34.

Rowley B and Monestier M (2005). Mechanisms of heavy metal-induced autoimmunity. Mol Immunol 42(7): 833-8.

Sallsten G, Barregard L and Schutz A (1994). Clearance half life of mercury in urine after the cessation of long term occupational exposure: influence of a chelating agent (DMPS) on excretion of mercury in urine. Occup Environ Med 51(5): 337-42.

Schwartz J (1994). Low-level lead exposure and children's IQ: a meta-analysis and search for a threshold. Environ Res 65(1): 42-55.

Sexton DJ, Powell KE, Liddle J, Smrek A, Smith JC and Clarkson TW (1978). A nonoccupational outbreak of inorganic mercury vapor poisoning. Arch Environ Health 33(4): 186-91.

Smith RG, Vorwald AJ, Patil LS and Mooney TF, Jr. (1970). Effects of exposure to mercury in the manufacture of chlorine. Am Ind Hyg Assoc J 31(6): 687-700.

Sundberg J, Jonsson S, Karlsson MO and Oskarsson A (1999). Lactational exposure and neonatal kinetics of methylmercury and inorganic mercury in mice. Toxicol Appl Pharmacol 154(2): 160-9.

Taueg C, Sanfilippo DJ, Rowens B, Szejda J and Hesse JL (1992). Acute and chronic poisoning from residential exposures to elemental mercury--Michigan, 1989-1990. J Toxicol Clin Toxicol 30(1): 63-7.

Teisinger J and Fiserova-Bergerova V (1965). Pulmonary retention and excretion of mercury vapors in man. Ind Med Surg 34: 580-4.

Tjalve H and Henriksson J (1999). Uptake of metals in the brain via olfactory pathways. Neurotoxicology 20(2-3): 181-95.

Torres AD, Rai AN and Hardiek ML (2000). Mercury intoxication and arterial hypertension: report of two patients and review of the literature. Pediatrics 105(3): E34.

U.S.EPA. (2000). Reference Dose for Mercury. External Review Draft. NCEA-S-0930. National Center for Environmental Assessment.

USEPA. (1995). *Mercury, elemental Reference concentration for chronic inhalation exposure (RfC)* http://www.epa.gov/iris/subst/0370.htm.

USEPA. (1997). Mercury Study Report to Congress. Health Effects of Mercury and Mercury Compounds (Vol V.). Office of Air Quality Planning and Standards; Office of Research and Development.

Warfvinge K (2000). Mercury distribution in the neonatal and adult cerebellum after mercury vapor exposure of pregnant squirrel monkeys. Environ Res 83(2): 93-101.

Warfvinge K, Hansson H and Hultman P (1995). Systemic autoimmunity due to mercury vapor exposure in genetically susceptible mice: dose-response studies. Toxicol Appl Pharmacol 132(2): 299-309.

Yang JM, Chen QY and Jiang XZ (2002). Effects of metallic mercury on the perimenstrual symptoms and menstrual outcomes of exposed workers. Am J Ind Med 42(5): 403-9.

Yole M, Wickstrom M and Blakley B (2007). Cell death and cytotoxic effects in YAC-1 lymphoma cells following exposure to various forms of mercury. Toxicology 231(1): 40-57.

Yoshida M, Satoh M, Shimada A, Yasutake A, Sumi Y and Tohyama C (1999). Pulmonary toxicity caused by acute exposure to mercury vapor is enhanced in metallothionein-null mice. Life Sci 64(20): 1861-7.

Yoshida M, Watanabe C, Satoh M, Yasutake A, Sawada M, Ohtsuka Y, Akama Y and Tohyama C (2004). Susceptibility of metallothionein-null mice to the behavioral alterations caused by exposure to mercury vapor at human-relevant concentration. Toxicol Sci 80(1): 69-73.

Nickel and Nickel Compounds, including Nickel Oxide. Reference Exposure Levels

1 Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). OEHHA developed a Technical Support Document (TSD) in response to this statutory requirement that describes acute, 8 hour and chronic reference exposure levels (RELs) and was adopted in December 2008. The TSD presents methodology reflecting the latest scientific knowledge and techniques, and in particular explicitly includes consideration of possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, chapter 731, statutes of 1999, Health and Safety Code Sections 39669.5 *et seq.*). These guidelines have been used to develop acute, 8-hour and chronic RELs for nickel and nickel compounds. The nickel RELs are applicable to the chemicals listed in Table 4 below, with the exception of nickel carbonyl because of its unique toxicity. In addition, nickel oxide has a separate chronic REL.

Table 4. Nickel and Common Compounds

Molecular Formula	Molecular Weight	Synonyms	CAS Registry Number
Ni	58.69	elemental nickel nickel metal	7440-02-0
NiO	74.69	nickel oxide green nickel monoxide nickel(II) oxide	1313-99-1
Ni_2O_3	165.36	nickel oxide black	
$Ni(OH)_2$	92.71	nickel hydroxide nickelous hydroxide	12054-48-7
$NiCl_2$	129.6	nickel chloride nickel dichloride	7718-54-9
$NiSO_4$	154.75	nickel sulfate nickelous sulfate	7786-81-4
$NiSO_4 \cdot 6H_2O$	262.85	nickel sulfate hexahydrate	10101-97-0
$NiCO_3$	118.7	nickel carbonate carbonic acid nickel(2+) salt nickelous carbonate	3333-67-3
Ni_3S_2	240.2	nickel subsulfide trinickel disulfide Heazlewoodite	12035-72-2

Table 4. Nickel and Common Compounds

Molecular Formula	Molecular Weight	Synonyms	CAS Registry Number
NiS	90.8	nickel sulfide nickel monosulfide Millerite	11113-75-0
$Ni(NO_3)_2 \cdot 6H_2O$	290.8	nickel nitrate hexahydrate	13478-00-7
$Ni(O_2CCH_3)_2$	178.8	nickel acetate	373-02-4
$Ni_3(CO_3)(OH)_4$	304.1	nickel carbonate hydroxide	12607-70-4
$Ni(CO)_4$	170.7	nickel carbonyl	13463-39-3

Nickel causes a variety of non-carcinogenic toxic effects including occupational contact dermatitis, occupational asthma, and reproductive toxicity in humans. Studies in experimental animals exhibit immune suppression, nephrotoxicity, pneumotoxicity, perinatal mortality and altered gene expression. The most sensitive effects appear to be in the lung and immune system. Descriptions of toxicokinetics, standard acute and chronic toxicity, immunotoxicity and reproductive toxicity appear below in Sections 4 to 8. Selection of key studies and derivation of RELs are presented in Section 9. Other observations on toxic effects and related studies which are important in defining the overall toxicity profile of nickel and its compounds, but do not contribute to the derivation of the RELs are described in Appendix A. The findings suggest that nickel be identified as a toxic air contaminant which may disproportionately impact children, pursuant to Health and Safety Code, Section 39669.5(c). The key values are summarized below.

1.1 Acute Toxicity (for a 1-hour exposure)

Inhalation reference exposure level **0.2 µg Ni/m³**
Critical effect(s) Immune system
Hazard Index target(s) Immune system

1.2 8-Hour REL (for repeated 8-hour exposures)

Inhalation reference exposure level **0.06 µg Ni/m³**
Critical effect(s) Lung lesions, immunotoxicity
Hazard Index target(s) Respiratory system; immune system

1.3 Chronic REL Nickel and Nickel Compounds (except NiO)

Inhalation reference exposure level **0.014 µg Ni/m³**
Critical effect(s) Lung, nasal epithelial and lymphatic pathology in male and female rats
Hazard index target(s) Respiratory system; hematopoietic system

1.4 Chronic REL Nickel Oxide

Inhalation reference exposure level	**0.02 µg Ni/m^3**
Critical effect(s)	Lung pathology in male and female mice
Hazard index target(s)	Respiratory system

1.5 Chronic Oral REL Nickel and Nickel Compounds

Oral Reference exposure level	**0.011 mg Ni/kg-day**
Critical effect(s)	Perinatal mortality in rats
Hazard index targets(s)	Developmental system

2 Physical and Chemical Properties (HSDB, 1994 except as noted)

Description	Ni metal: silvery metal NiO: black crystals $NiCl_2$: yellow deliquescent crystals (U.S.EPA, 1985)
Density	8.9 g/cm^3 (Ni) 2.07 g/cm^3 ($NiSO_4 \cdot 6H_2O$) 6.67 g/cm^3 (NiO)
Boiling point	2730°C (Ni)
Melting point	1455°C (Ni); 1030°C ($NiCl_2$)
Vapor pressure	not applicable for dust
Flashpoint	not applicable
Explosive limits	Nickel dust or powder is flammable (CDTSC, 1985).
Solubility	Elemental nickel, nickel subsulfide, and nickel oxide are insoluble in water, but are soluble in dilute nitric, hydrochloric, and sulfuric acids. The chloride and sulfate forms of nickel are water-soluble.
Odor threshold	odorless
Metabolites	Ni^{2+}
Oxidation states	0, +1, +2, +3 (Von Burg, 1997)

2.1 Physicochemical Properties Affecting Toxicity

Aerosols, liquid or solid particulate matter (PM) suspended in air are present in the atmosphere as a result of dust storms, forest and grass fires, vegetation, sea spray, vehicular and industrial emissions, and atmospheric chemical reactions (Rostami, 2009). Anthropogenic activities account for about 10% of atmospheric aerosols.

The toxicity of inhaled aerosols depends upon the extent of deposition in the head or extra-thoracic region, upper and lower airways of the lung (bronchi and alveoli), chemical composition, and subsequent fate, including clearance. The deposition of airborne particles depends on physical properties, the size or diameter of the particle

(and distribution thereof), the concentration, and the density. The clearance of deposited particles depends on location of deposition, solubility, and the mass deposited or burden. In general deposited PM is more rapidly cleared from the upper airways (tracheobroncheal region, TB) than from the pulmonary region (alveoli) and soluble particles are more rapidly cleared than insoluble particles. Removal of particles from the alveoli may require engulfment by alveolar macrophages. Several computational models are available for the prediction of airway deposition and clearance (Jarabek et al., 2005; Brown et al., 2005; Rostami, 2009).

In the inhalation studies described and analyzed in this document nickel particles are usually described as having a mass median aerodynamic diameter (MMAD) in µm, a geometric standard deviation (for lognormal size distribution), and a particle density in g/cm^3. All three parameters and the aerosol concentration are required inputs in the Multiple Path Particle Dosimetry (MPPD2) model used to assess airway deposition in the calculation of chronic RELs. The model was also used in deposition and clearance mode to estimate nickel particle retention over various timed simulations for age-specific human models (µg Ni/day/m^2 alveolar surface area). However, retention estimates are less certain than deposition values since they depend on factors other than size, particularly solubility of the various nickel compounds in the lung surface layers.

Emissions of nickel particles from facilities subject to risk assessments under the Air Toxics Hot Spots program will vary in size and distributional characteristics. These characteristics are not necessarily reported in the emissions inventory, which forms the basis of the site-specific risk assessments. Thus, there is an implicit assumption that the size distributions are similar enough to those used in the toxicity studies that form the basis of the Reference Exposure Level.

In the studies used as a basis for the chronic RELs, animals were exposed to particle size distributions more or less centered on 2.5 µm mean diameter. CARB (2009) estimated that for 2010, $PM_{2.5}$ from stationary sources comprised about 15% of $PM_{2.5}$ emissions from all sources and about 38% of PM from stationary sources. Kleeman and Cass (1999) concluded that $PM_{2.5}$ from various stationary sources ranged from 11 to 50% of total PM emissions (tons/day), the remainder essentially was PM_{10}.

Linak et al. (2000) evaluated particle size distributions (PSDs) and elemental partitioning with three coal types and residual fuel oil combusted in three different systems simulating process and utility boilers. Uncontrolled PM emissions from the three coals ranged from 3800 to 4400 mg/m^3 compared to 90 to 180 mg/m^3 for fuel oil. The mass and composition of particles between 0.03 and >20µm in aerodynamic diameter showed that PM for the combustion of these fuels produced distinctive bimodal and trimodal PSDs. The trace element concentrations (µg/g) in emitted PM size fractions indicated that Ni was somewhat higher in the <2.5µm fraction than in the >2.5µm fraction: Western Kentucky coal, 110/86.2; Montana coal, 41.5/29.3; Utah coal, 109/39.4; and high sulfur No.6 oil, 8000/2270, respectively.

Krudysz et al. (2008) investigated spatial variation of PM in an urban area impacted by local and regional PM sources. Weekly size-segregated (<0.25, 0.25-2.5, and >2.5 µm)

PM samples were collected in the winter of 2005 in the Long Beach, California area. Coefficients of divergence analyses were conducted for size-fractionated PM mass, organic and elemental carbon, sulfur and 18 other metals and trace elements. For most metal species the highest concentrations were present in the coarse particles (>2.5µm), followed by the 0.25 to 2.5 µm fraction with significantly lower concentrations in the <0.25µm fraction. However, vanadium, nickel, cadmium, zinc and lead concentrations were highest in the <0.25µm and 0.25 to 2.5µm fractions. Nickel concentrations in the three fractions were approximately 2 ng/m^3, <0.25µm; 1 ng/m^3, 0.25-2.5µm; and 1.5 ng/m^3, >2.5µm (their Fig. 4).

On this basis we think that the particle size distributions used in the animal studies are a reasonable surrogate for $PM_{2.5}$ and PM_{10} emitted from stationary and possibly mobile sources.

The aqueous solubility of nickel compounds has a significant effect on their uptake and tissue distribution. In rodent studies with several water soluble and insoluble compounds, the water soluble compounds (e.g., $NiSO_4$, $NiCl_2$, $Ni(NO_3)_2$) were generally found in 10 to 100 fold higher concentrations in lung, liver, kidney, heart, brain and blood than the water insoluble compounds (e.g., NiS, Ni_3S_2, NiO). Insoluble compounds have solubility <0.01 mol/L, soluble compounds have solubility >0.1 mol/L and slightly soluble compounds range between 0.01 and 0.1 mol/L. Insoluble nickel compounds have solubility products that range from about 1×10^{-9} to 1×10^{-31} (Table 5).

Table 5. Aqueous Solubility and Solubility Products of Nickel Compounds

Name	Formula	Solubility g/L @ 20ºC	Ksp @ 25ºC
Soluble Compounds			
Nickel chloride	$NiCl_2$	553	
Nickel nitrate hexahydrate	$Ni(NO_3)_2•6H_2O$	600	
Nickel sulfate hexahydrate	$NiSO_4•6H_2O$	400	
Nickel acetate tetrahydrate	$Ni(CH_3CO_2)_2•4H_2O$	270 @ 0ºC	
Insoluble Compounds			
Nickel carbonate	$NiCO_3$	90 mg/L	6.6×10^{-9}
Nickel hydroxide	$Ni(OH)_2$		2.0×10^{-15}
Nickel sulfide	NiS		3.0×10^{-19}
Nickel sulfide α	NiS		4.0×10^{-20}
Nickel sulfide β	NiS		1.3×10^{-25}
Nickel arsenate	$Ni(AsO_4)_2$		3.1×10^{-26}
Nickel cyanide	$Ni(CN)_2$		1.7×10^{-9}
Nickel ferrocyanide	$Ni_2[Fe(CN)_6]$		1.3×10^{-15}
Nickel oxalate	NiC_2O_4		4.0×10^{-10}
Nickel iodate	$Ni(IO_3)_2$		4.7×10^{-5}
Nickel phosphate	$Ni_3(PO_4)_2$		4.7×10^{-32}

Sources:
http://chemed.chem.wisc.edu/chempaths/Table-of-Some-Solubility-Products-at-25ºC;
http://www.csudh/oliver/chemdata/data-ksp.htm;
http://www.ktf-split.hr/periodni/en/abc/kpt.html;
Occupational Health Guide for Nickel Metal and Soluble Nickel Compounds, National Institute for Occupational Safety and Health, September, 1978.

3 Major Uses or Sources of Exposure

The most common airborne exposures to nickel compounds are to insoluble nickel compounds such as elemental nickel, nickel sulfide, and the nickel oxides from dusts and fumes. Contributions to nickel in the ambient air are made by combustion of fossil fuels, nickel plating, and other metallurgical processes. The most common oxidation state of nickel is the divalent (Ni(II) or Ni^{2+}) form (U.S.EPA, 1985). Elemental nickel is a malleable, silvery-white metal that is highly resistant to strong alkali. Because of its corrosion resistance, about 40% of nickel is used in the production of stainless steel, permanent magnets, and other alloys that require resistance to extremes of temperature or stress (U.S.EPA, 1985). About 20% of nickel is produced as nickel sulfate and hydroxide used in electroplating baths, batteries, textile dyes, and catalysts (U.S.EPA, 1985, Von Burg, 1997). Nickel dust or powder is flammable (CDTSC, 1985). Nickel carbonyl also is volatile. However, because of its unique toxicity relative to the inorganic nickel compounds, this REL is not applicable to nickel carbonyl.

3.1 Air

The primary stationary source categories that emit nickel into ambient air in California are fuel combustion, nickel alloy manufacture, cement production, asbestos mining and milling, municipal waste sludge incineration, iron and steel foundries, secondary metal recovery, cooling towers, coal gasification petroleum processing, and electroplating. Also nickel has been detected in vehicular exhaust, tobacco smoke, and indoor smoke from home-heating and cooking fuels (CARB, 1991). The United States Environmental Protection Agency (U.S. EPA, 1986) estimated that particles found in ambient air as a result of oil combustion might contain nickel in the form of nickel sulfate, with smaller amounts of nickel oxide and complex metal oxides containing nickel. The majority of the nickel in the atmosphere is thought to be associated with human activities. Up to one-third of atmospheric nickel could come from windblown dusts, forest fires and volcanic emissions (CARB, 1991). The annual average ambient air concentration of nickel as measured by the air monitoring network operated by the California Air Resources Board and local air districts in 2002 was 4.5 ± 4.1 SD ng/m^3 (CARB, 2008). This value is quite similar to the values reported for earlier years 1992 to 2001 (CARB, 2008). Recent data from the south coast air basin (SCAQMD, 2008) show average sampled concentrations of nickel in total suspended particulate of around 6 ng/m^3. The highest individual area was West Long Beach at about 11 ng/m^3 possibly resulting from increased shipping activity at the ports since nickel is naturally present in bunker fuel used in ships. Some additional recent studies of nickel in ambient air are listed in Table 6. In general concentrations range from 2 to 9 ng Ni/m^3. Besides ambient and occupational exposures, nickel has been measured in mainstream cigarette smoke in concentrations higher than other metals such as copper, cadmium and iron: 0.2-0.51, 0.19, 0.07-0.35, and 0.042 µg/m^3, respectively (IARC, 1986).

Table 6. Atmospheric Nickel Concentrations and Dry Deposition Rates in Some Recent Studies

Study	Region	No. sites sampled	Analyte(s)	Sample period	Ni, ng/m^3	Deposition Rate, µg Ni/d/m^2
Agrawal et al., 2009	Los Angeles, CA Air Basin	10	Metals, $PM_{2.5}$	2 years	3-7.5	N.A.
Armami et al., 2009	Los Angeles, CA, Long Beach	6	Metals, $PM_{2.5-10}$ $PM_{0.25-2.5}$ $PM_{0.25}$QUF	7 weeks	2-8 5-9 4-9	N.A.
Lim et al., 2006	Los Angeles, CA	7	Metals, PM_{6-11} PM_{11-20}	24 hr x 4 seasons	9.2	9.4
Polidori et al., 2009	So. Calif indoor and outdoor retirement communities	4	Metals, $PM_{<0.25}$ $PM_{0.25-2.5}$ $PM_{2.5-10}$	2 x 6 weeks/site	4 indoor 5 outdoor S.Gabriel	N.A.
Sabin et al., 2006	Los Angeles, CA. I-405 highway proximity	4 10-400m	Metals, $PM_{<6}$ PM_{6-11} PM_{11-20} PM_{20-29} $PM_{>29}$	3 weeks, 8AM- 5 PM 300,000 vehicles/day	10	1-3
Sabin et al., 2008	So. Calif coast, Santa Barbara to San Diego	8	Metals	3months 10/site		0.21-5.4
Hays et al., 2011	Raleigh, NC I-440 highway	1 20m	Metals, $PM_{2.5-10}$ $PM_{0.1-2.5}$ $PM_{0.1}$	2 months, 125,000 vehicles/d	0.7 1.1 0.2	N.A.
Bell et al., 2010	Connecticut, and Mass. Low birth weight in 76,788 infants of exposed mothers	4	Metals, $PM_{2.5}$	Weekly averages for 39 week gestation period	3.1±1.5	N.A.

3.2 Soil

Nickel occurs naturally in the Earth's crust at an average concentration of 0.0086% (86 ppm) (Duke, 1980). The nickel content of soil can vary widely depending on local geology. Both the southeastern United States and southern Quebec can have nickel

concentrations greater than 1000 ppm due to local ultramafic rock, which is rich in nickel. Typical nickel soil concentrations range from 4 to 80 ppm (ATSDR, 2005). A soil survey by the U.S. Geological Survey throughout the U.S. reported concentrations from <5 to 700 ppm, with a geometric mean of 13.0 ± 2.31. Nickel ranked 15th among 50 elements included in the study (Shacklette and Boerngen, 1984). Auto emissions can also raise the level of nickel in soil. Lagerwerff and Sprecht (1970) found nickel concentrations from 0.9 to 7.4 ppm in roadside soils. The concentrations were lower at greater distances from the road and at greater soil depths. Munch (1993) found 32 ppm Ni in soil lying directly at the roadside edge of a busy forest road (3200 vehicles/day) in Germany. Haal et al. (2004) reported nickel roadside soil concentrations of 12 to 33 ppm 5 to 15 m from the roads in Tallinn, Estonia. They noted that while lead had decreased over the past decade, Zn and Ni had doubled.

3.3 Water

Nickel enters groundwater and surface water via dissolution of rocks and soils, from atmospheric deposition, from biological decay, and from waste disposal. Nickel compounds are relatively soluble in water and usually exist as nickel ions in aqueous environments. Uncontaminated surface freshwater and seawater usually contain low concentrations of nickel (<0.3 µg/L, Barceloux, 1999). The nickel concentration of fresh surface water has been reported to average between 15 and 20 µg/L (Grandjean, 1984; ATSDR, 2005). The nickel concentration in groundwater is normally less than 20 µg/L (U.S.EPA, 1986), and levels appear similar in raw, treated, and distributed municipal water.

Elevated nickel in drinking water may result from corrosion of nickel-containing alloys used in valves and other components in the water distribution system as well as from nickel-plated faucets. Tap water that is used for drinking purposes generally contains nickel at concentrations ranging from 0.55 to 25 µg Ni/L in the United States (ATSDR, 2005; FDA 2000; O'Rourke et al. 1999; Thomas et al. 1999). Nickel concentrations in tap water measured in the Total Diet Study 1991–1999 ranged from 0 to 0.025 mg Ni/kg (0–25 µg Ni/L) with a mean value of 0.002 mg/kg (2 µg Ni/L) (FDA 2000). Analysis of data obtained during 1995 - 1997 from the National Human Exposure Assessment Study (NHEXAS) yielded median concentrations of nickel in tap water (used as drinking water) of 4.3 µg Ni/L (10.6 µg Ni/L, 90th percentile) in the Arizona study and 4.0 µg Ni/L (11 µg Ni/L, 90th percentile) in the U.S. EPA Region 5 (Illinois, Indiana, Michigan, Minnesota, Ohio, and Wisconsin) study (O'Rourke et al., 1999; Thomas et al., 1999). In a 1969–1970 survey of 969 water supplies in the United States representing all water supplies in eight metropolitan areas and one state (2,503 samples), 21.7% of samples had concentrations <1 µg Ni/L, 43.2% of the samples contained between 1 and 5 µg Ni/L, 25.6% of the samples contained between 6 and 10 µg Ni/L, 8.5% of the samples contained between 11 and 20 µg Ni/L, and 1% had levels >20 µg Ni/L (NAS 1975).

Nickel has been detected in California drinking water sources. According to the monitoring data collected by the California Department of Health Services (DHS) between 1984 and 1997, the highest, average and median concentrations of nickel in

water were 540 µg/L, 26 µg/L, and 17.9 µg/L, respectively (DHS, 1998). The detection limit for the purposes of reporting for nickel is 10 µg/L (10 ppb).

3.4 Food

Terrestrial plants take up nickel from soil mainly via the roots. The amount of uptake depends on the concentration in soil, soil pH, organic matter content and the type of plant. The nickel concentration in most natural vegetation ranged from 0.05 to 5.0 mg Ni/kg dry weight (dw) (NRC, 1975). Some food sources such as chocolate, nuts, beans, peas, and grains are relatively rich in nickel.

There have been several studies regarding nickel content in an average diet (ATSDR, 2005). Current information on the dietary intake of nickel in the United States is based on data gathered from the NHEXAS study. Nickel concentrations were measured in duplicate diet samples, which, in combination with study participant's estimates of food and water intake, were used to determine both the overall concentration of nickel in combined solids and liquids in the total diet and the average nickel intake of study participants. In the U.S. EPA Region 5 (Illinois, Indiana, Michigan, Minnesota, Ohio, and Wisconsin) study, the mean and median concentrations of nickel in combined dietary solids and liquids were 47 and 43 µg Ni/kg, respectively (Thomas et al., 1999).

Calamarie et al. (1982) showed that nickel is not likely to accumulate in fish. They exposed rainbow trout (*Salmo gairdneri*) to nickel contaminated water at 1.0 mg Ni/L for 180 days and found 2.9 mg Ni/kg wet weight in liver, 4.0 mg/kg in kidneys, and 0.8 mg/kg in muscle. Initial study values for these tissues were 1.5, 1.5, and 0.5 mg Ni/kg, respectively.

Myron et al. (1978) studied nickel levels in meals sampled from the University of North Dakota and from a hospital. The average nickel concentration of the student meals ranged from 0.19 to 0.29 µg Ni/g dry weight and for the hospital meals from 0.21 to 0.41 µg Ni/g dry weight. Based on the nine diets examined, the authors estimated an average daily dietary intake of 168 $\pm$ 11 µg nickel. This value is similar to those estimated in other studies (ATSDR, 2005).

4 Toxicokinetics

4.1 Absorption

Ishimatsu et al. (1995) demonstrated that the absorption fraction of orally administered nickel compounds in rats was closely related to their water solubility. They administered eight nickel compounds and nickel metal. The solubilities in saline solution were in the following order: [$Ni(NO_3)_2$ > $NiCl_2$ > $NiSO_4$] >> [NiS > Ni_3S_2] > [NiO (black) > Ni (metal) > NiO (green)]. The insoluble nickel metal and nickel oxides ranged from 0.01 to 0.09% absorbed. The absorption of the slightly soluble nickel subsulfide and nickel sulfide was 0.5% to 2.1% and the soluble nickel compounds (sulfate, nitrate and chloride) ranged from 10 to 34 percent. In rats administered $NiCl_2$, $NiSO_4$, and NiS 84-87% of recovered nickel was detected in the kidneys. Lesser kidney ratios were found for Ni_3S_2, $Ni(NO_3)_2$, NiO(B) and Ni(M): 76%, 73%, 62%, and 51%, respectively. However, NiO(G) showed greater recovery from liver than kidney.

Ho and Furst (1973) reported that gavage administration of rats with $^{63}NiCl_2$ in 0.1N HCl led to 3 to 6 percent absorption of the labeled nickel, independent of dose level (4, 16, and 64 mg Ni/kg body weight (bw)). One day after administration 94 to 97 percent of the dose was excreted in the feces and 3 to 6 percent in the urine. Nielsen et al. (1993) administered $^{57}NiCl_2$ at 3 to 300 µg Ni/kg bw by gavage to male mice, and estimated that intestinal absorption ranged from 1.7 to 7.5 percent of administered dose.

Nickel is absorbed in the gastrointestinal (G.I.) tract of humans either as free ions or as complexes. The degree of uptake or bioavailability depends on the vehicle (water or food) and has ranged from 1% to 40% in several studies (Table 7).

Cronin et al. (1980) reported that ingestion of a soluble nickel compound during fasting by a group of female subjects resulted in urinary elimination of four to 20 percent of the dose. Sunderman et al. (1989) found that about 40 times more nickel was absorbed from the G.I. tract when nickel sulfate was given to human volunteers in drinking water (27 $\pm$ 17 %, mean $\pm$ SD) than when it was given in food (0.7 $\pm$ 0.4 %). Sunderman et al. (1989) also reported that absorption fraction was independent of dose at 12, 18, or 50 µg Ni/kg bw.

Table 7 Absorption of Ingested Nickel in Humans from Bioavailability Studies (Diamond et al., 1998; ATSDR, 2005)

Study	Number of subjects	Vehicle	Duration	Fasting status	Absorption (% of Dose)
Nielsen et al., 1999	8	Water plus scrambled eggs	Acute	Fasted	25.8 to 2.5
Patriarca et al., 1997	4	Water	Acute	Fasted	29-40
Sunderman et al., 1989	8	Water	Acute	Fasted	29.3
Sunderman et al., 1989	8	Food	Acute	Fasted	1.8
Cronin et al. , 1980	5	Capsule plus 100 mL water	Acute	Fasted	12-32
Christensen & Lagassoni, 1981	8	Capsule	Acute	With meal	5.7
Gawkrodger et al., 1986	3	Capsule	Acute	With meal	2.7, 2.8
Menne et al., 1978	6	Capsule	Acute	Not fasted	2.2 (women)
Menne et al., 1978	7	Capsule	Acute	Not fasted	1.7 (men)
Horak & Sunderman, 1973	10-50	Food	Chronic	Not fasted	1.0
McNeeley et al., 1972	19	Food & water	Chronic	Not fasted	1.6
McNeeley et al., 1972	20	Food	Chronic	Not fasted	1.2

Solomons et al. (1982) and Nielson et al. (1999) reported similar results. They found that plasma nickel concentrations in five fasted human subjects were significantly elevated when they were given nickel sulfate (5 mg Ni) in drinking water with a peak level of about 80 µg Ni/L at three hours after oral administration. When five mg Ni (as nickel sulfate) were administered in whole cow-milk, coffee, tea, orange juice, or Coca Cola®, the rise in plasma Ni was significantly suppressed with all but the Coca Cola®. By four days after administration, 26% of a dose given in water was excreted in urine and 76% in feces. When the nickel dose was given in food, 2% was excreted in the urine and the balance in feces. The elimination half-life for absorbed nickel averaged 28 ± 9 hours (Sunderman et al., 1989).

Solomons et al. (1982) showed that plasma nickel levels of subjects who consumed a typical Guatemalan meal with 5 mg nickel or a North American breakfast with 5 mg nickel were only about 5 to 20 percent of that which resulted from the consumption of 5 mg nickel in water. Nielsen et al. (1999) administered nickel in drinking water

(12 µg Ni/kg bw) to eight fasted volunteers at different time intervals, with standardized portions of scrambled eggs. They found that the highest fraction of nickel dose (25.8%) excreted in urine was observed when the scrambled eggs were taken four hours prior to nickel in drinking water. A much lower fraction of nickel dose (2.5%) was excreted when the nickel was mixed into the eggs or when the drinking water was taken together with the eggs (3.4%).

Patriarca et al. (1997) studied nickel metabolism in humans using the stable isotope ^{62}Ni (98.83%, as metal). Four healthy adult subjects (two women and two men) were fasted overnight and administered 10 µg ^{62}Ni/kg bw in water. Blood samples were drawn in fixed intervals and the total daily output of urine and feces was collected for the first five days after dose ingestion. ^{62}Ni was measured in plasma, urine and feces by isotope dilution using ^{61}Ni and plasma-mass spectrometry. Fecal excretion of ^{62}Ni averaged 66.9 ± 4.9 % of administered dose with an absorbed fraction of 33.1 ± 4.9 %. Urinary excretion over five days ranged from 51% to 82% (mean ± SD= 65.2 ± 13.4 %) of absorbed dose. Plasma ^{62}Ni peaked between 1.5 and 2.5 hours after ingestion with concentrations ranging between 269 and 344 nM; ^{62}Ni was rapidly cleared from the plasma but was still detectable at 96 hr post ingestion (< 32 nM). The authors reported no evidence of biliary excretion or enterohepatic circulation of ^{62}Ni as indicated by the appearance of secondary peaks in plasma or urinary nickel concentrations. Also the elimination of ^{62}Ni in feces followed the same pattern as the fecal marker (radio-opaque pellets) indicating that biliary excretion is very low or absent in humans, albeit with a limited number of subjects.

Nickel has been reported as an essential element in several animal species. Signs of Ni deficiency include depressed growth and reduced hematocrit (Nielsen, 1996). In the case of human nutrition the essentiality of Ni has yet to be established (IOM, 2001).

Animal models have been used to estimate the inhalation absorption of water-soluble and water-insoluble nickel compounds. English et al. (1981) administered nickel chloride and nickel oxide intratracheally to rats and reported greater than 50% of the soluble nickel chloride was cleared from the lungs within three days. Most of the nickel was excreted in the urine. In contrast, the water-insoluble nickel oxide persisted in the lung for more than 90 days, and the nickel was excreted equally in urine and feces.

Valentine and Fisher (1984) administered slightly soluble nickel subsulfide intratracheally to mice and observed the pulmonary clearance to have two distinct components with initial and final half-lives of 1.2 and 12.4 days, respectively. The excretion of the chemical (measured as ^{63}Ni) was 60% in the urine and 40% in the feces. Similar findings were reported by Finch et al. (1987) who observed that the pulmonary clearance of intratracheally administered nickel subsulfide in mice was biphasic with clearance half-lives of two hours and 119 hours for initial and final phases, respectively.

Tanaka et al. (1985) exposed male Wistar rats to NiO aerosols of mass median aerodynamic diameter (MMAD) and geometric standard deviation (gsd) of 1.2 µm, 2.2 gsd and 4.0 µm, 2.0 gsd. The average exposure concentration was 0.6 mg/m^3 or

70 mg/m^3 and total exposure time was 140 hours. Some rats were sacrificed after exposure while others were kept for 12 and 20 months prior to sacrifice. The biological half-lives of NiO deposited in the lungs based on the assumption of first order clearance kinetics were 11.5 and 21 months for 1.2 and 4.0 µm MMAD aerosols, respectively. The relation used was $T_{50} = -0.301/\log(1- f)$, where f , the clearance ratio, was selected as 0.002 or 0.001 depending on fit to the experimental data.

Following a single 70-minute inhalation exposure of rats to green nickel oxide (^{63}NiO; 9.9 mg Ni/m^3; AMAD 1.3 µm, 2.0 gsd), the fraction of the inhaled material deposited in the total respiratory tract was 0.13, with 0.08 deposited in the upper respiratory tract and 0.05 deposited in the lower respiratory tract (Benson et al. 1994). During the 180 days post-exposure, nickel was not detected in extra-respiratory tract tissues.

Tanaka et al. (1988) studied the biological half-life of amorphous NiS aerosols in exposed rats. The rats were exposed to a NiS aerosol with MMAD of 4.0 µm (gsd = 2.0) and either a single four hr exposure of 107 mg/m^3 or repeated 8.8 mg/m^3 for 7 hr/day, 5 days/week for one month. After exposure, the nickel contents in lung, liver, kidney, spleen, blood and urine were measured. In sharp contrast to the findings with NiO (above), NiS was rapidly cleared from lung tissue following a four-hour exposure with a half-life of 20 hours (f = 0.57). Repeated exposures of NiS at lower concentration showed no accumulation of NiS in the lung and similar clearance kinetics following the final exposure (their Fig. 2).

Following a single 120 minute inhalation exposure of rats to nickel subsulfide ($^{63}Ni_3S_2$; 5.7 mg Ni/m^3 AMAD 1.3 µm, gsd 1.5), the fraction of inhaled material deposited in the upper respiratory tract was similar to that observed for nickel oxide (0.14 in the total respiratory tract, 0.09 in the upper respiratory tract, and 0.05 in the lower respiratory tract). In contrast to nickel from nickel oxide, nickel from nickel subsulfide was detected in the blood, kidneys, and carcass between 4 and 24 hours after the exposure (Benson et al., 1994).

Data in rats and mice indicate that a higher percentage of less-soluble nickel compounds was retained in the lungs for a longer time than soluble nickel compounds (Benson et al. 1987, 1988; Dunnick et al. 1989; Tanaka et al. 1985) and that the lung burden of nickel decreased with increasing particle size (≤ 4 µm) (Kodama et al. 1985a, 1985b). Nickel retention was six times (mice) to 10 times (rats) greater in animals exposed to less-soluble nickel subsulfide compared to soluble nickel sulfate (Benson et al. 1987, 1988).

The lung burdens of nickel generally increased with increasing exposure duration and increasing levels of the various nickel compounds (Dunnick et al. 1988, 1989). From weeks 9 to 13 of exposure, lung levels of nickel sulfate and nickel subsulfide remained constant while levels of nickel oxide continued to increase (Dunnick et al. 1989). Slow clearance of nickel oxide from the lungs was also observed in hamsters (Wehner and Craig 1972). Approximately 20% of the inhaled concentration of nickel oxide was retained in the lungs at the end of exposure for two days, three weeks, or three months. The retention was not dependent on the duration of exposure or exposure

concentration. By 45 days after the last exposure to nickel oxide (two-day exposure), 45% of the initial lung burden was still present in the lungs (Wehner and Craig 1972).

Workers occupationally exposed to nickel have higher lung burdens of nickel than the general population. Dry weight nickel content of the lungs at autopsy was 330 ± 380 µg/g in roasting and smelting workers exposed to less-soluble compounds, 34 ± 48 µg/g in electrolysis workers exposed to soluble nickel compounds, and 0.76 ± 0.39 µg/g in unexposed controls (Andersen and Svenes 1989). In an update of this study, Svenes and Andersen (1998) examined 10 tissue samples taken from different regions of the lungs of 15 deceased nickel refinery workers; the mean nickel concentration was 50 µg/g dry weight. Nickel levels in the lungs of cancer victims did not differ from those of other nickel workers (Kollmeier et al. 1987; Raithel et al. 1989).

Nickel levels in the nasal mucosa are higher in workers exposed to less-soluble nickel compounds relative to soluble nickel compounds (Torjussen and Andersen 1979). These results indicate that, following inhalation exposure, less-soluble nickel compounds remain deposited in the nasal mucosa. Higher serum nickel levels have been found in occupationally exposed individuals compared to non-exposed controls (Angerer and Lehnert 1990; Elias et al. 1989; Torjussen and Andersen 1979). Serum nickel levels were found to be higher in workers exposed to soluble nickel compounds compared to workers exposed to less-soluble nickel compounds (Torjussen and Andersen 1979). Concentrations of nickel in the plasma, urine, and hair were similar in nickel-sensitive individuals compared to non-sensitive individuals (Spruit and Bongaarts 1977).

Serita et al. (1999) evaluated pulmonary clearance and lesions in rats after a single inhalation of ultrafine metallic nickel (Uf-Ni, 20 nm average particle diameter). Wistar rats (sex unspecified) were exposed to 0.15 (Low), 1.14 (Medium), or 2.54 (High) mg Uf-Ni/m^3 for five hours. Groups of five rats per dose group were sacrificed at 0 hr and 1, 3, 7, 14, and 21 days post exposure. The amount of nickel in the lung accumulated in a dose-dependent manner (1.4, 10.1, 33.5 µg Ni/lung, respectively). The half times for nickel in the lung averaged about 32 days and appeared independent of initial dose.

4.2 Distribution

Several studies of nickel administered to rodents via the oral route show that nickel was mainly concentrated in the kidneys, liver, and lungs, and the absorbed nickel was excreted primarily in the urine (Borg and Tjalve, 1988; Jasim and Tjalve, 1984, 1986a, 1986b; Dieter et al., 1988). Nielsen et al. (1993) showed that retention and distribution of nickel in mice was dependent on the route of administration. As shown in Table 8, Nielsen et al. (1993) showed that 20 hours after nickel administration, deposition in body tissues resulting from intraperitoneal (i.p.) injection was much greater than that observed after gavage administration.

Table 8. Median Nickel Body Burden and Contents of Major Organs in Mice as Percentage of Administered Dose (from Nielsen et al., 1993)*.

Tissue	Gastric Intubation	Intraperitoneal Injection
Liver	0.0439 (0.046)[a]	0.255 (0.044)[b]
Kidneys	0.029 (0.030)	1.772 (0.306)
Lungs	<0.010 (0.010)	0.114 (0.020)
Carcass	0.106 (0.111)	3.164 (0.546)
Stomach	0.014 (0.015)	<0.010 (0.002)
Intestine	0.762 (0.799)	0.490 (0.084)
Total body burden	0.954 (1.0)	5.794 (1.0)

*Note: a) Measurements made 20 hr after oral dose of 10 µmol Ni/kg bw. b) Measurements made 20 hr after intraperitoneal injection of 1.0 µmol Ni/kg bw. Values in parentheses are ratios of relative tissue burden over total body burden.

Ishimatsu et al. (1995) evaluated the distribution of various nickel compounds in rat organs 24 hours after oral administration. Male Wistar rats (10 weeks old, 8/compound) were administered the nickel compounds by gavage as 10 mg of Ni dissolved in a 5% starch saline solution. The animals were sacrificed at 24 hr after dosing and organs and blood taken for Ni determination. Selected results are presented in Table 9. The kidney stands out as the major site of nickel deposition. This table also demonstrates the high bioavailability of soluble nickel compounds compared to poorly soluble compounds.

Obone et al. (1999) measured the accumulation of nickel in tissues of rats exposed to $NiSO_4$ in drinking water for 13 weeks. Accumulation in all organs examined was observed to increase with increasing dose level. The order of accumulation compared to the control was kidneys > testes > brain > spleen > lung = heart= liver (Table 10).

Absorbed nickel is unlikely to exist as free ionic Ni^{2+}, but rather as nickel complexes. Sunderman and Oskarsson (1991) noted that in humans absorbed nickel is transported by binding to a metalloprotein (nickeloplasmin), albumin, and ultra-filterable ligands, such as small polypeptides and L-histidine. Van Soestbergen and Sunderman (1972) administered nickel chloride (as ^{63}Ni) to rabbits by intravenous injection at 0.24 mg Ni/kg bw. They found that between two and 24 hr after injection, approximately 90% of serum ^{63}Ni was bound to proteins (e.g., albumin) with molecular weights greater than 10,000 and the remaining label was bound to small organic molecules such as short peptides and amino acids.

Table 9. Mean Nickel Concentrations in Rat Organs 24 Hours after Oral Administration (adapted from Ishimatsu et al., 1995)*

Ni Compound	Lung µg/g	Liver µg/g	Kidney µg/g	Heart µg/g	Brain µg/g	Blood µg/mL
NiO (Green)	0.04	0.02	0.03	0.03	0.03	0.03
Ni metal	0.18	0.04	0.31	0.04	0.02	0.02
NiO (Black)	0.08	0.04	0.32	0.04	0.02	0.05
Ni_3S_2	0.17	0.07	1.2	0.04	0.02	0.05
NiS	0.34	0.11	6.4	0.60	0.04	0.21
$NiSO_4$	2.5	0.57	25.5	0.47	0.04	0.28
$NiCl_2$	3.7	0.53	28.7	1.2	0.18	0.31
$Ni(NO_3)_2$	6.3	1.1	32.6	2.4	0.15	2.25
Control	0.04	0.03	0.03	0.03	0.02	0.03

* Note 8 animals/compound; 10 mg Ni oral dose by gavage

Table 10. Mean Nickel Concentrations in Rat Organs after 13 Weeks Exposure to $NiSO_4$ in Drinking Water (µg Ni/g tissue, Obone et al., 1999)*

Treatment NiSO4	Liver	Kidney	Spleen	Heart	Lungs	Brain	Testis
0%	1.58	1.39	1.51	1.60	1.22	1.59	1.50
0.02%	1.60	1.88	1.85	1.74	1.60	1.68	1.85
0.05%	1.63	3.45	1.86	1.83	1.95	1.77	2.05
0.1%	2.08	5.48	2.26	2.12	2.11	2.78	2.84

*Note: Values are means of three different experiments. Measurements made 24 hr after termination of exposure.

Chelation of Ni^{2+} by organic compounds has a significant effect on the cellular uptake, absorption, and distribution of Ni^{2+} (Sakar, 1984; Nierborer et al., 1984; Borg and Tjalve, 1988; Hopfer et al., 1987). Nierborer et al. (1984) studied cellular uptake of Ni^{2+} in human B-lymphoblasts, human erythrocytes and rabbit alveolar macrophages. They observed that addition of L-histidine or human serum albumin at physiological concentrations to the cell cultures reduced Ni^{2+} uptake by up to 70%. The concentration of Ni^{2+} used in the study was 7×10^{-8} M (4.1 µg/L); it was comparable to serum nickel levels observed in workers occupationally exposed to nickel.

Rezuke et al. (1987) measured nickel concentrations in human postmortem samples in seven to 10 adults. In decreasing order the mean and range in µg Ni/kg dry weight in the tissue specimens were: lung 173 (71-371); thyroid 141 (41-240); adrenal 132 (53-

241); kidney 62 (19-171); heart 54 (10-110); liver 50 (11-102); brain 44 (20-65); spleen 37 (9-95); and pancreas 34 (7-71). In five specimens of bile, nickel concentrations averaged 2.3 ± 0.8 µg/L (range 1.5-3.3 µg/L). These values differ markedly from the distribution of Ni in the rat noted in Table 10 above. The relatively high Ni burden in the human lung and low burden in the human kidney may indicate significantly more inhalation exposure in humans and/or significant differences in the chemical state of nickel absorbed in laboratory rodent versus human environmental exposures.

Nickel has been shown to cross the human placenta; it has been found in both fetal tissue (Schroeder et al., 1962) and the umbilical cord blood serum (McNeely et al., 1971). Similar findings have been reported in animal studies. Szakmary et al. (1995) administered a single gavage dose of 5.4, 11.3, or 22.6 mg Ni/kg bw as nickel chloride to pregnant rats. Twenty-four hours after exposure, nickel levels in fetal blood were raised from 10.6 (control) to 14.5, 65.5, and 70.5 µg/L for the low, medium, and high dose groups, respectively. Jacobsen et al. (1978) observed that when pregnant mice were given a single i.p. injection of ^{63}Ni chloride (0.14 mg/kg bw) on day 18 of gestation, passage of ^{63}Ni from mother to fetus was rapid and concentrations in fetal tissues were generally higher than those in the dam.

The distribution of nickel chloride in pregnant and lactating rats following its injection has been studied by a number of authors (Dostal et al., 1989; Mas et al., 1986; Sunderman et al., 1978). Half-lives of nickel in whole blood following i.p. treatment of pregnant and non-pregnant rats were similar (3.6–3.8 hours), while the half-life for nickel in fetal blood was 6.3 hours following treatment on gestation days 12 or 19 (Mas et al., 1986). Intramuscular injection of nickel chloride (12 mg Ni/ kg/day) into pregnant and non-pregnant rats resulted in a greater accumulation of nickel in the pituitary of pregnant rats (Sunderman et al. 1978).

Tallkvist et al. (1998) evaluated the olfactory transport and subcellular distribution of $^{63}Ni^{2+}$ solution instilled intra-nasally in rats (4 µg/nostril). Cellular fractionation was conducted at one day, one week and three weeks after exposure. Of the $^{63}Ni^{2+}$ present in the olfactory epithelium, 60% to 70% was present in the supernatant, whereas in the olfactory bulb and the basal hemisphere about 70% - 80% of the nickel was bound to particulate cellular constituents. Gel filtration of the cytosol indicated that the $^{63}Ni^{2+}$ eluted with a molecular weight of about 250, identical to that obtained with histidine. Also, in olfactory tissues $^{63}Ni^{2+}$ was partly present in the cytosol associated with a 25,000 molecular weight component. The authors conclude that: 1) nickel is transported in the primary olfactory neurons via slow axonal transport; (2) the metal is bound to both soluble and particulate cytosolic constituents; and (3) the metal also shows this subcellular distribution in other parts of the olfactory system. The authors also note that neuronal transport of nickel was about 20 times slower than cadmium ($^{109}Cd^{2+}$) or manganese ($^{54}Mn^{2+}$) studied earlier.

Schwerdtle and Hartwig (2006) evaluated the subcellular distribution of $NiCl_2$ and black NiO in human lung A549 cells exposed for 20 and 24 hr, respectively. Cells treated with $NiCl_2$ at 0, 50, 100, 250, or 500 µM exhibited dose-dependent uptake of Ni into the cytoplasm and nuclei. Intracellular Ni concentrations in cytoplasm were about 10, 20,

50, 275, and 550 µM, respectively. Concentrations in the nuclei were much lower at about 5, 10, 15, 40, and 110 µM, respectively. Cells treated with black NiO at 0, 0.2, 0.5, 1.0, and 2.0 µg NiO/cm^2 showed a similar pattern of intracellular distribution with greater relative concentrations in the nuclei. For cytoplasmic distribution the Ni concentrations were about 5, 110, 150, 240, and 450 µM, respectively. For nuclear distribution the Ni concentrations were about 2, 60, 70, 125, and 230 µM, respectively. The authors concluded that particulate Ni(II) exhibits greater toxicity due to its longer retention times rather than a different MOA which still involves Ni(II) ions as the direct or indirect genotoxicant.

4.3 Excretion

Nickel burden in humans does not increase with age. A majority of nickel absorbed from environmental media and diet is rapidly excreted via the urine. Solomons et al. (1982) found that nickel in water was quickly absorbed and excreted by humans; they estimated a biological half-life of about eight hours. Hogetveit et al. (1978) reported that elevated levels of nickel were detected in urine samples collected from workers exposed to soluble or insoluble nickel through inhalation.

The kinetics of nickel elimination in humans and animals appear to be similar. Onkelinx et al. (1973) injected nickel chloride i.v. to rats and rabbits and followed the nickel in plasma over time. Elimination profiles were similar in both species with early and later phases of elimination from plasma exhibiting first-order kinetics with half-lives of 6 and 50 hr for rats and 8 and 83 hr for rabbits, respectively.

Sweat and milk are also possible excretion routes for absorbed nickel in humans. Hohnadel et al. (1973) observed that, in sauna bathers, the mean concentrations of nickel in the sweat from healthy men and women were significantly higher than the mean concentrations in urine. Several studies have demonstrated that excretion of nickel in human milk is quite low and should be considered a minor route of excretion in lactating women (Feeley et al., 1983; Mingorance and Lachica, 1985). Casey and Neville (1987) reported a mean nickel concentration of 1.2 ± 0.4 µg/L in 46 human milk samples from 13 women during the first month of lactation with an average estimated daily infant intake of 0.8 µg Ni. Krachler et al. (2000) measured trace elements in 27 human milk samples and found a median nickel concentration of 0.79 µg/L (range < 0.13-6.35 µg/L).

Graham et al. (1978) measured the clearance of $NiCl_2$ aerosol in mice exposed to 644 µg Ni/m^3 for two hours. Immediately following exposure and at 24 hr intervals thereafter the mice were sacrificed, their lungs and spleens were removed and weighed, and nickel concentrations were determined by atomic absorption spectroscopy. Clearance of nickel from the lung followed first-order kinetics with a fitted curve of Y = 7.569exp (-0.291t), where Y is µg Ni/g dry weight lung and t is days post exposure. The spleen did not exhibit a significant uptake of nickel following exposure.

Koizumi et al. (2004) measured the urinary excretion of nickel nitrate hexahydrate in rats by inductively coupled plasma argon emission spectroscopy (ICPAES). Male

Wistar rats received single oral doses of 0, 0.005, 0.01, 0.025, 0.05, 0.075, 0.1, 0.125, 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 10.0, 20.0, and 50.0 mg $Ni(NO_3)_2 \cdot 6H_2O$/kg bw. Five animals were used for analysis at each dose level. The 24-hr urinary excretion of nickel was observed to fit the relation $Y = 62.68X^{0.8527}$, R = 0.9488, where Y is the excreted Ni in µg and X is the oral dose in mg/kg bw. The proportion of total nickel elimination decreased from 25% at 0.01 mg/kg to about 5% at 0.1 mg/kg and higher doses. Urological analysis of markers of renal toxicity, N-acetyl-β-D-glucosamine (NAG), β_2-microglobulin, urine albumin, and urine protein, showed no indication of toxicity at any dose level used.

Dostal et al. (1989) showed that milk is an excretion pathway of nickel chloride in rodents. Daily subcutaneous injections of lactating rats with 3 or 6 mg Ni/kg bw for four days raised nickel levels in milk from < 2 µg/L to 513 ± 54 and 1030 ± 66 µg/L, respectively. They also showed that nickel treatment significantly changed the composition of milk by increasing the milk solids (42%) and lipids (110%) and decreasing milk protein (29%) and lactose (61%).

Oyabu et al. (2007) studied the biopersistence of inhaled NiO particles in the rat lung. Thirty male Wistar rats were exposed to NiO particles (geometric mean diameter = 139±12 nm, average exposure concentration = $1.0 \pm 0.5 \times 10^5$ particles/m^3) for six hr/day for four weeks. At four days and one and three months after inhalation, a group of 10 rats was sacrificed and the NiO particles deposited in the lung determined by chemical analysis. The retained Ni particle content of the lung decreased exponentially with a calculated half time of 62 days.

Oliveira et al. (2000) studied urinary nickel excretion in 10 workers from a galvanizing plant using $NiSO_4$, a soluble nickel compound, and 10 control subjects. Personal air monitors were used with 0.8 µm filters (OSHA method). No other particle size information was provided. Nickel airborne levels varied between 2.8 and 116.7 µg/m^3. Pre- and post-shift urinary Ni levels were taken on five consecutive workdays. Post-shift values ranged from 4.5 to 43.2 µg Ni/g creatinine. A significant correlation was observed between urinary and airborne nickel (r = 0.96, P ≤ 0.001) with the relation urinary Ni (µg/g creatinine) = 6.00 + 0.43(airborne Ni, µg/m^3). No differences were observed with respect to different workdays.

Yokota et al. (2007) studied the urinary elimination of nickel and cobalt in relation to airborne exposures in a battery plant. The workers were exposed to nickel hydroxide, metallic cobalt, and cobalt oxyhydroxide. Nickel in the air was several fold higher than cobalt and positively correlated ($r^2 = 0.958$). Cobalt in air and post-shift urine gave a regression equation of $Co\ (\mu g/L)_{urine} = 15.8 + 243.8\ Co\ (mg/m^3)_{air}$ with a poor correlation coefficient (r = 0.491). No correlation was found between Ni in air and post-shift urine [$Ni\ (\mu g/L)_{urine} = -17.3 + 7.33\ Ni\ (mg/m^3)_{air}$, r = 0.272, P = 0.15]. The authors note that the workers were using respiratory protection which presumably reduced inhalation exposure to $Ni(OH)_2$. They also note discrepancy with treatment of Ni inhalation by the DFG (Deutsche Forschungsgemeinschaft, 2005) which gives the relations for airborne nickel exposure and urinary nickel for water-soluble and water-insoluble compounds. For soluble nickel compounds including the hydroxide, acetate, chloride, sulfate and

similar salts they give Ni $(\mu g/L)_{urine}$ = 10 + 600 Ni$(mg/m^3)_{air}$. For insoluble nickel compounds including the metal, oxide, carbonate, sulfide, and sulfidic ores they give Ni $(\mu g/L)_{urine}$ = 7.5 + 75 Ni$(mg/m^3)_{air}$. The authors argue that $Ni(OH)_2$ should be treated as an insoluble compound with respect to urinary excretion rather than a soluble one.

Afridi et al. (2006) measured metal content in biological samples from 56 production workers (PW) and 35 quality control workers (QCW) of a steel mill and 75 unexposed normal controls (all male, age range 25-55 yr). For nickel in scalp hair the PW showed the highest Ni concentration of 13.76 ± 4.48 µg Ni/g with QCW lower at 9.02 ± 2.64 µg Ni/g. These values were significantly higher than the non-occupationally exposed controls at 5.25 ± 1.46 µg Ni/g hair ($P < 0.02$). Surprisingly the mean lead values were quite similar at 16.21, 10.33, and 6.84 µg Pb/g hair, respectively. Urine concentrations were also measured and showed lesser, but also significant, differences i.e. 9.47, 7.62, and 6.31 µg Ni/L urine, respectively.

Ohashi et al. (2006) evaluated selected urinary metals in 1000 women in the general Japanese population. The geometric mean concentration for nickel was 2.1 µg Ni/L or 1.8 µg Ni/g creatinine. Unlike copper and manganese both nickel and cobalt showed no substantial age dependency for urinary excretion.

4.4 Physiological Models

Onkelinx et al. (1973) conducted a kinetic analysis of $^{63}Ni^{2+}$ clearance in rats and rabbits following a single intravenous injection of $^{63}NiCl_2$ (specific activity 5.9 µCi/µg Ni). In both species $^{63}Ni^{2+}$ was rapidly cleared from plasma or serum during the first two days, and more slowly after two days. The blood elimination data were best described by the bi-exponential relations:

Rats: $S = 226 \exp[-0.11t] + 0.57 \exp[-0.014t]$ for 17 µg Ni/rat (82 µg Ni/kg bw);

Rabbits: $S = 1165 \exp[-0.092t] + 4.95 \exp[-0.0084t]$ for 816 µg Ni/rabbit (240 µg Ni/kg bw);

where S is the plasma concentration of Ni^{2+} (µg/L) and t is the time after injection (hr). A two-compartment model derived from the data successfully predicted serum or plasma concentrations of Ni^{2+} in animals receiving continuous infusions or repeated daily injections of $^{63}NiCl_2$.

Sunderman et al. (1989) developed a model to predict nickel absorption, serum levels, and excretion following oral exposure to nickel in water and food. The model was developed based on two experiments in humans in which serum nickel levels and urinary and fecal excretion of nickel were monitored for two days before and four days after eight subjects were given an oral dose of nickel as nickel sulfate (12, 18, or 50 µg Ni/kg bw) in water or in food. The data were then analyzed using a four-compartment toxicokinetic model consisting of Gut, Serum, Urine and Tissues. Two inputs of nickel, the single oral dose, in which uptake was considered to be a first-order process, and the baseline dietary ingestion of nickel, in which uptake was considered to be a pseudo-zero order process were used. Model parameters were determined for the model from the two experiments. No further model validation (i.e. with independent data) was

described. A sample model code implemented in Berkeley Madonna software is given in Appendix B.1 for a single 50 µg Ni/kg bw dose in water.

Uthus (1999) proposed a 16-compartment biokinetic model to describe the uptake and metabolism of orally administered $^{63}NiCl_2$. The compartments were either in groups representing the GI tract, Blood, Liver or Body, or individual for Urine and Feces. Transfer of Ni mass between compartments was governed by first order rate constants. Oral dosing of female Sprague-Dawley rats with 0.84 µg ^{63}Ni (10.7 µCi) resulted in seven day cumulative urinary and fecal excretions of 2.46% and 97.5% of dose, respectively. For liver, peak ^{63}Ni radiolabel occurred within 30 min of dosing and reached 0.09% of dose. Peak radiolabel in kidney was 0.04% of dose and in bone 0.001% of dose. The model predicts 2.54% and 96.4% of dose excretions for urine and feces, respectively. Retention of Ni in grouped organs was predicted to amount to 0.34% seven days after dosing. Model code for a single oral dose is provided in Appendix B.

Franks et al. (2008) describe a mathematical model of the in vitro keratinocytes response to chromium or nickel exposure. The model tracks the interaction between metal ions (in both intra- and extra-cellular states) and their effect on the viability of keratinocytes and the release of the pro-inflammatory cytokine interleukin-1α (IL-1ά). The model is intended to describe a monolayer of freshly isolated keratinocytes (10 µm), which has been grown to confluence and dosed with media containing, e.g., 0.01 to 10,000 µM $NiCl_2$ for 24 hours. The metal ion is assumed to be in equilibrium between extracellular concentration (A_c) and intracellular concentration (A_i), with the latter inducing the cytokine response. The volume fraction of keratinocytes is (n) and the amount of metal associated with the cell is given by (nA_i). The volume fraction of keratinocytes in the system is described by $dn/dt = - K_d n$, where $K_d = \delta_{ni}A_i + \delta_n$. This accounts for death due to the toxic effects of the intracellular ion ($\delta_{ni}A_i$) and the net birth and natural death of cells (δ_n). Control experiments indicated that 80% of cells were still alive after 24 hr, indicating that $\delta_n > 0$. The model assumes: (1) an exchange between extra- and intracellular metal ions; (2) cell death releases metal ions to the extracellular region; and (3) partitioning between extra- and intracellular states according to a partition coefficient (μ_n). The main equations for extra- and intracellular metal ions, respectively, are as follows:

$$d/dt((1 - n)A_c) = -k_n n(\mu_n A_c - A_i) + K_d n A_i;$$

$$d/dt(nA_i) = k_n n(\mu_n A_c - A_i) - K_d n A_i.$$

Keratinocytes with metal bound to them release a variety of chemokines and cytokines, in particular IL-1ά, release of the latter is described in the model by:

$$d/dt((1 - n)c) = \beta_{cn} n + \beta_{ci} n A_i - \delta_c(1 - n)c;$$

where β_{cn} is the rate of cytokine release by unaffected cells, β_{ci} is the rate of cytokine release by affected cells, δc is the rate of natural decay of cytokines in the media, and c is the concentration of IL-1ά. In comparing model predictions to experimental data for nickel the authors report no apparent relationship between nickel dose and IL-1ά

release except a decrease at high nickel concentrations (> 100 μM). Good agreement between model predictions and existing experimental data was observed. An example implementation of the Franks et al. model in Berkeley Madonna code is given in the Appendix B.2. Approximate parameter values obtained by curve fitting to experimental data were: δ_{ni} =6.3 x 10^{-5}/μM-d; β_{ci} = 0/day; K_n = 320/day; μ_n = 2.2 unitless; δ_n = 0.21/day; δ_c = 3.2/day; β_{cn} = 1.5 x 10^{-3}/μM-d. The initial concentration of cells (n_0) was estimated to be 0.0165 and the molecular mass of IL-1α was 17.7 kDa.

The only PBPK models for nickel compounds identified in the published literature were those of Menzel et al. (1987) and Menzel (1988). These rat models are interesting but few details were provided by the authors and they would be difficult to reproduce. An example of what an alternative nickel PBPK model might look like is given in Appendix B.4. This example is based in part on the manganese rat PBPK model of Teeguarden et al. (2007). The model was adjusted for nickel using data from Ishimatsu et al. (1995), Benson et al. (1994) and Tanaka et al. (1985). The model represents six perfused tissues: upper and lower respiratory tracts, bone, liver, kidneys, and muscle. Each of these tissues has a shallow tissue pool in rapid equilibrium with blood and a deep tissue store connected to the shallow tissue by transfer rate constants. Exchange of nickel between the shallow tissue pools and venous blood is controlled by tissue/blood partition coefficients (Ishimatsu et al., 1995). Absorption of airborne nickel oxide includes transport of deposited nickel into shallow tissue pools and mechanical removal from respiratory surfaces to the gastro-intestinal tract. The model includes fecal, urinary and biliary excretion of absorbed or ingested nickel. Comparisons of model predictions with observed data of Tanaka et al. (1985) for prolonged exposures to NiO aerosol were good for lung tissue Ni concentrations at high and low exposure concentrations and for liver and kidney concentrations at high exposure concentration (Appendix B.4).

Hack et al. (2007) describe a physiological model of the intracellular dosimetry of inhaled nickel. The model consists of seven intracellular compartments of the tracheobronchial epithelial cell: Cytoplasm, Cytoplasmic Proteins, Vacuolar Particles, Perinuclear Cytoplasm, Perinuclear Cytoplasmic Proteins, Nucleus, and Nuclear Proteins. Extracellular compartments consist of Surface Particles, GI Tract, Ionic Ni in Mucus, and Venous Blood. The model accepts the deposited dose into the mucous layer following inhalation of nickel particles or aerosols.

Particulate nickel compounds are either cleared from the mucous layer by mucociliary action, dissolved into Ni^{2+} ions, or taken up by the cells. Phagocytosis of nickel particles, such as Ni_3S_2 or crystalline NiS, results in the formation of a vacuole in which nickel particles are encased and ultimately dissolved. Extracellular dissolution of soluble nickel compounds results in the release of ionic nickel, which enters the cell via divalent ion transport systems (e.g., magnesium). Both influx and efflux of nickel ions are described by saturable Michaelis-Menten kinetics. Once in the cytoplasm nickel ions may bind with cytosolic proteins or diffuse through the cytoplasm to the perinuclear cytoplasm. Once there, nickel ions may bind reversibly to perinuclear proteins, enter the nucleus and bind to nuclear proteins. Model processes are mostly modeled with first order rate constants for forward and reverse directions. An exception is the Michaelis-Menten kinetics for influx and efflux of Ni from mucous to cytoplasm to

venous blood. In this respect the Hack et al. model resembles a biokinetic model. Model parameters were based mostly on published values. An example of this model implemented in Berkeley Madonna code is given in Appendix B.3.

The model for uptake of $NiCl_2$ by cultured pneumocytes predicted steady state concentrations better than the rate of uptake where the model underpredicted intracellular levels in the first half hour after exposure (data of Saito and Menzel, 1986). Model comparisons with the data of Costa et al. (1981) gave good observed/predicted ratios (O/P) of 1.57 to 0.94 for Ni_3S_2 in the nucleus (nmol/mg protein), 0.65 for $NiCl_2$ in the whole cell, 0.3 for $NiCl_2$ in the cytoplasm, and 0.5 for $NiCl_2$ in the nucleus (all nmol/mg protein). With the data of Abbracchio et al. (1982) agreement was more variable for O/P: $NiCl_2$ in the nucleus, 2.5 to 5.7; $NiCl_2$ in cytoplasm, 0.18; crystalline NiS in the nucleus 0.96 to 3.5; crystalline NiS in the particulate fraction 1.02; crystalline NiS in the cytoplasmic fraction 1.10.

Hsieh et al. (1999a) proposed a dosimetric model of nickel deposition and clearance from the rat lung. The model was developed using lung burden data from the National Toxicology Program (NTP) studies of nickel sulfate (NTP, 1996c), nickel subsulfide (NTP, 1996b), and nickel oxide (NTP, 1996a) and earlier models (Yu and Xu, 1986). The model consists of a single alveolar compartment. Deposited particles are removed from the lung by two principal mechanisms: (1) mechanical clearance via mucociliary transport; and (2) clearance by dissolution. For moderately soluble Ni_3S_2 particles both mechanisms are operable. The lung burden buildup in the alveolar region of the rat lung is described by the following equations:

$$dM_i/dt = r_i - \lambda_i M_i \qquad (1);$$

$$r_i = C_i \times \eta\, MV \qquad (2);$$

$$\lambda_i = a_i \times \exp[-b_i\{m_s/m_{s0})^{ci}] \qquad (3);$$

where M is the mass burden, i indicates the particular nickel compound, r is the deposition rate, λ_i is the total alveolar clearance rate coefficient, η is the alveolar deposition fraction, C_i is the air concentration, MV is the minute ventilation, a_i, b_i, c_i are compound specific clearance rate coefficient constants, m_s = M/S in which M is the lung mass burden and S is the total alveolar surface area ($m_s = 5.38 \times 10^3$ cm^2 for rats), and m_{s0} = 1 mg/cm^2 is the dimensional constant introduced to normalize m_s. For $NiSO_4$, the a, b, c parameter values were 10.285, 17.16, and 0.105, respectively. For Ni_3S_2, the a, b, c parameter values were 0.00768, -20.135, and 0.266, respectively. For NiO, the a, b, c parameter values were 0.0075, 300, and 0.95, respectively.

Hsieh et al. (1999b) modified the rat model to develop a model of deposition and clearance of nickel in humans. Deposition rates were calculated for six scenarios: nose-breathing at rest, nose-breathing at light work, nose breathing at moderate work, mouth breathing at rest, mouth breathing at light work, and mouth breathing at moderate work. The clearance rate coefficient constants for humans were modified from the rat values. For nickel oxide, clearance rate coefficient constant *a* was estimated to be 1/7.6 times the rat value; constants *b* and *c* were assumed to be the same as rats. For nickel subsulfide, clearance is due to mechanical transport and dissolution; the clearance rate

coefficient constant *a* was estimated to be the sum of the clearance rate coefficient constant *a* for insoluble nickel (nickel oxide) and the difference between the clearance coefficient constant for nickel oxide and for nickel subsulfide for rats. For nickel sulfate, clearance rate coefficient constants in humans were assumed to be the same as in rats.

Hsieh et al. (1999c) developed a model for deposition, clearance and retention kinetics in the respiratory tract for inhaled nickel compounds in the mouse. The nickel compounds studied were NiO (green), Ni_3S_2, and $NiSO_4 \cdot 6H_2O$. The approach and equations for alveolar deposition and clearance are similar to those given above for the rat (Hsieh et al., 1999a). In this case the compound specific clearance coefficients a, b, c were: NiO, 0.0085, 180, 0.95; Ni_3S_2, 0.011, -9.293, 0.266; and $NiSO_4$, 10.285, 15.78, 0.105, respectively. The model predictions were compared with experimental data for the normalized lung burden metric (Ni-lung burden/g lung/unit concentration) and the calculated results did not always show good agreement. Because of lower deposition rates and faster clearance rates, mice have lower lung burdens than rats when exposed to the same concentrations of NiO or $NiSO_4$ particles. For Ni_3S_2, the lung burden/gram of lung in mice can be lower or higher than in rats depending upon exposure concentration and duration.

The Yu et al. (2001) modification of the model was used to predict lung burdens in nickel refinery workers and comparison with measured lung Ni burdens in deceased refinery workers showed good agreement between predicted and measured values. The model treats the alveolar region of the human lung as a single compartment. The kinetic expressions governing the change in mass with time in this compartment for NiO, Ni_3S_2 and $NiSO_4$ are as follows:

$$dM_{NiO}/dt = r_{NiO} - \lambda_{NiO} M_{NiO};$$

$$dM_{Ni3S2}/dt = r_{Ni3S2} - \lambda_{Ni3S2} M_{Ni3S2};$$

$$dM_{NiSO4}/dt = r_{NiSO4} - \lambda_{NiSO4} M_{NiSO4};$$

where M is the mass burden, r is the deposition rate and λ is the total alveolar clearance rate coefficient (/day) over all clearance pathways. For a given concentration, r in the above expressions is equal to concentration x alveolar deposition fraction (η) x minute ventilation (MV). The clearance rate coefficients are based on extrapolation from rat data, e.g.

$$\lambda_{NiO} = 0.00099 \exp[-300(V/V_{AM})^{0.95}] \text{ (/day)};$$

where V is the total volume of Ni compounds retained in the lung (mm^3) and $V_{AM} = 1.75 \times 10^4$ mm^3 is the total alveolar macrophage volume in humans. When the dosimetry model is applied to worker exposure, three additional factors are incorporated in the model: inhalability, mixed breathing mode, and clearance rate coefficient of a Ni compound mixture. The inhalability expression is based on the recommendation of the International Commission on Radiological Protection (ICRP ,1994):

$$\eta_{inhalability} = 1\text{-}0.5 \times (1\text{-}(7.6 \times 10^{-4} d_a^{2.8} + 1)\text{-}1 + 1.0 \times 10^{-5} U^{2.75} \exp(0.055 d_a);$$

where d_a is the aerodynamic diameter of the particle in μm and U is the wind speed in m/s, usually taken to be zero for workplace calculations. Deposition rates are calculated for three different ventilations: at rest, light work, and moderate work.

This modified dosimetry model was applied to the data on lung Ni burden for 39 workers reported by Andersen and Svenes (1989). Since particle sizes were not measured in the study, values from the same facility measured by Vincent (1996) were used. Particle sizes ranged from 42 to 62 μm MMAD for roasting and smelting and 1.4 to 51 μm MMAD for electrowinning work areas. These values are much greater than the 2 to 3 μm MMAD used in the chronic rat inhalation study. The correspondence of observed vs. predicted lung burdens for the two work areas are presented by the authors (their Figs. 1 and 2) but no statistical correlations were provided. Nevertheless several points fall on or close to the 1:1 correlation line generally supporting their claim of good agreement.

5 Acute Toxicity

5.1 Acute Toxicity Summary

Studies of acute toxicity of nickel and compounds are summarized in Table 11 and Table 12. The acute toxicity of inhaled nickel compounds is affected by their solubility and particle size distribution. Similar toxic effects were seen in both exposed humans and experimental animals, primarily lung lesions, decreased lung function and immunotoxicity. The immunotoxicity endpoint appears to form the best basis for deriving an acute reference exposure level.

5.2 Acute Toxicity to Humans

A group of seven metal plating workers with occupational asthma were evaluated for atopy and pulmonary function challenge in response to inhalational challenge with nickel sulfate hexahydrate and other metals (Cirla et al., 1985). Three of the asthmatics tested positive for the presence of nickel-specific IgE antibodies. Positive reactions to skin testing with nickel were found in 3 of the asthmatic workers who also had dermatitis. Six out of the seven asthmatics exhibited significantly decreased FEV_1 (> 15%) when exposed to 0.3 mg/m^3 nickel sulfate aerosol for 30 minutes. Control challenges with other metal salts did not reveal similar deficits in FEV_1. No particle size information was provided by the authors.

The study by Cirla et al. (1985) has been used in previous analyses of nickel health effects by OEHHA and U.S. EPA, but was considered inadequate for the present purpose. Other studies of acute toxicity to humans are reported in Table 11 below.

Soluble nickel compounds appear to be the greatest concern for acute health effects. The soluble forms of nickel are absorbed as Ni^{2+} (Coogan *et al.*, 1989). Divalent nickel competes with copper for binding to serum albumin and is systemically transported in this way (Sunderman, 1986). The kidneys, lungs, and placenta are the principal organs for systemic accumulation of nickel (Sunderman, 1986). In contrast to the long half-life of the insoluble forms of nickel in the nasal mucosa, the elimination half-life of Ni^{2+} in the plasma is 1-2 days in mice (Nieboer *et al.*, 1988).

A two-year-old child died after accidentally ingesting an oral dose of approximately 570 mg/kg bw of nickel sulfate (Daldrup et al., 1983). Cardiac arrest occurred four hours after the ingestion, and the child died eight hours after the accident. Webster (1980, cited in Norseth, 1984) reported nickel intoxication in a group of 23 dialysis patients. The source of nickel was plated stainless steel in a water heater tank. The concentration of nickel was approximately 250 µg/L in the dialysate. This level was much higher than those found in five other dialysis units (average 3.6 µg/L, range 2.5 to 4.5 µg/L). Symptoms observed included nausea, weakness, vomiting, headache and palpitations.

Remission was relatively rapid, occurring in three to 13 hours after cessation of dialysis. Sunderman et al. (1988) report on an episode of 32 workers in an electroplating plant

accidentally drinking water containing $NiSO_4$ and $NiCl_2$ with a concentration of 1.63 g Ni/L. Twenty workers experienced nausea, vomiting, abdominal discomfort, diarrhea, giddiness, lassitude, headache, cough and shortness of breath, which lasted for a few hours to several days. Nickel intakes were estimated at between 0.5 and 2.5 g. Serum concentrations ranged from 13 to 1340 µg Ni/L and urine concentrations from 0.15 to 12 mg Ni/g creatinine. Elimination half times ranged from 27 hr with induced diuresis to 60 hr in non-induced subjects.

Nickel fumes from high nickel alloy welding (mean concentration = 440 µg Ni/m³, range = 70-1,100 µg Ni/m³) caused complaints of upper respiratory irritation and headache in welders exposed for 4 weeks (Akesson and Skerfving, 1985).

Exposure to nickel in occupational settings causes dermatitis and asthma in some individuals with repeated exposures (Davies, 1986). The nickel ion, bound to proteins in the dermis, acts as an antigen eliciting a type IV (delayed type) hypersensitivity response. This response, mediated by T-lymphocytes, causes dermal hypersensitivity. This hypersensitivity can be diagnosed by patch testing (Menne and Maibach, 1989).

Phillips et al. (2010) re-examined a case report of a 38-year-old healthy male who inhaled nanoparticles of nickel while spraying nickel onto bushes for turbine bearings using a metal arc process. The spraying process lasted about 90 minutes and the subject was observed to remove a protective half face mask during the spraying process. The subject complained of feeling unwell and went home and the next day he complained of cough, shortness of breath, and a tight chest. Four days after exposure he was admitted to the hospital and was tachypneic, pyrexial and cyanosed. He was treated with supplemental oxygen and antibiotics but died of respiratory failure 13 days after exposure (official cause of death was adult respiratory distress syndrome, ARDS). Nickel nanoparticles (< 25 nm) were identified in lung macrophages using transmission electron microscopy. High levels of nickel were measured in the subject's urine (780 µg/L) and his kidneys showed evidence of tubular necrosis. In addition, there was hematuria and proteinuria also indicative of kidney toxicity. The updated examination supports the idea that inhaled nickel can be absorbed systemically and affect other organs.

Table 11. Summary of Acute Nickel Toxicity in Humans

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Dalrup *et al*., 1983	$NiSO_4$	Accidental ingestion of a single dose, ca. 570 mg/kg	Mortality at 8 hr via cardiac arrest	2-yr old child.
Webster, 1980	Ni^{2+} form not specified	Dialysis patients, 250 µg Ni/L in dialysis fluid, N = 23	Nausea, weakness, vomiting, headache, palpitations	Remission in 3-13 hr after cessation of dialysis.
Sunderman *et al*., 1988	$NiSO_4$ $NiCl_2$	Accidental ingestion of contaminated drinking water with 1.63 g Ni/L N = 20 electroplating workers	Nausea, vomiting, abdominal discomfort, diarrhea, giddiness, lassitude, headache, cough, shortness of breath	Ni intake estimates 0.25 to 2.5 g. Serum concentrations 13-1340 µg Ni/L, urine concentrations 0.15-12 mg Ni/g creatinine
Cirla *et al*., 1985	$NiSO_4$ aerosol	Metal plating workers with occupational asthma. N = 7, 0.3 mg Ni/m^3 x 30 min	6/7 had FEV_1 reductions > 15%	No particle size information, 3/7 positive for Ni-specific IgE antibodies.
Phillips *et al*., 2010	Ni nanoparticles, <25 nm diameter	Occupational exposure during spraying 90 min	ARDS, respiratory failure and death 13 days after exposure	Ni nanoparticles found in lung macrophages, high levels of Ni in urine (780 µg/L) and kidneys. Evidence of kidney tubular necrosis.

Note: ARDS = adult respiratory distress syndrome; FEV1 = forced expiratory volume 1 second.

5.3 Acute Toxicity to Experimental Animals

Ishihara et al. (2002) studied inflammatory responses and mucus secretion in rats with acute bronchiolitis induced by nickel chloride inhalation. Male Wistar-jcl strain SPF rats at age 10 weeks were exposed (5 animals/group) via whole body to aerosols of nickel chloride with an ultrasonic nebulizer 5 hours/day for 5 days. The average concentrations of the aerosols were 0.85 mg Ni/m^3 in day one and 0.24 mg Ni/m^3 during days two to five. Following exposure the animals were given clean air on days six to eight prior to sacrifice. The nickel aerosols had a MMAD of 1.8 µm with a gsd of 1.6. The measured inflammatory biomarkers were total protein concentration, total elastolytic activity, α1-antitrypsin, and β-glucuronidase activity. Sialic acid and fucose were measured as mucus components. Also measured were soluble L-selectin, cytokine-induced neutrophil chemoattractant (CINC) and growth-regulated gene products (GRO). Total protein concentrations, total elastolytic activity, trypsin inhibitory capacity, β-glucuronidase, fucose, and sialic acid in bronchoalveolar lavage fluid (BALF) were significantly greater than control ($P < 0.05$ vs. control, $N = 5$) at day 3 to day 8 time points following nickel exposure. CINC/GRO and soluble L-selectin were significantly increased at days 3-6 and days 5-6, respectively. The extent of lung tissue injury was scored by histopathological observations. There was no exfoliation of the airway epithelium found on exposure day five when bronchiolitis developed. The data indicate that nickel chloride inhalation caused an acute inflammatory response with hypersecretion of mucus, which cleared in one month.

The data of Ishihara et al. were analyzed using benchmark dose methodology. Doses were calculated as mg Ni^{2+} inhaled with the average body weight of 0.289 kg and the relation Inhalation in rats (m^3/day) = 0.105 x (bodyweight, kg/0.113)$^{2/3}$. Adequate model fits ($P \geq 0.1$) were obtained for continuous benchmark doses at the one standard deviation point with linear, power or polynomial models. The 95% lower bounds on the benchmark doses for a one standard deviation change in the respective endpoints ($BMDL_{1SD}$) were 5.5 µg (linear, $P = 0.132$), for total cells/µL BALF; 18.6 µg (power, $P= 0.156$), for total protein mg/mL BALF; 50 µg (polynomial, $P = 0.19$), for total elastolytic activity as nmol succinyl trialanine *p*-nitroanilide hydrolyzed/hr/mL BALF; and 13.5 µg (power, $P = 0.34$) for sialic acid µg/mL BALF. For the five hours/day times five days inhaled exposure volume of 0.2 m^3, the $BMDL_{1SD}$ equivalent concentrations for the four endpoints would be 27.5, 93, 250, and 67.5 µg/m^3, respectively. These values appear significantly lower than the BMDL of 165 µg/m^3 for inhibition of antibody production in mice (data of Graham et al., 1978) but are consistent with the more extensive exposure protocol (5hr/day x 5 days).

It has been shown that water-soluble nickel compounds are more acutely toxic than the less soluble compounds by ingestion. The single dose oral LD_{50}'s in rats for less-soluble NiO and Ni_3S_2 were > 3000 mg Ni/kg bw, while the oral LD_{50}'s for the more soluble $NiSO_4$ and nickel acetate ranged from 39 to 141 mg

Ni/kg bw in rats and mice (Mastromatteo, 1986; Haro et al., 1968). Soluble nickel compounds appear to be more toxic by intraperitoneal (i.p.) injection than by intramuscular (i.m.) or subcutaneous (s.c.) injections. Acute LD_{50} values for $NiCl_2$ in rats were 5 mg Ni/kg bw by i.p. injection, 23 mg Ni/kg bw by i.m. injection, and 25 mg Ni/kg bw by s.c. injection (Knight et al., 1991).

Jia et al. (2010) conducted a mechanistic study of nickel-induced olfactory impairment. Male mice were intranasally instilled with $NiSO_4$ or saline followed by ATP, purinergic receptor antagonists, or saline. The olfactory epithelium was examined histologically and with immunochemistry 1 to 7 days postinstillation. Doses of 0, 0.1, 0.5, or 2.5 mg/kg body weight showed both time and dose dependence in nasal toxicity indicated by decreases in thicknesses of the ectoturbinate 2 and endoturbinate 2 regions. Decreases in thickness ranged up to 30 and 15 µm at the top doses, respectively. No effects were seen in the nasal septum. Reductions in thickness were due to sustentacular cell loss measured by terminal dUTP nick-end labeling (TUNEL) staining at 1-day postinstillation and caspase-3-dependent apoptosis of olfactory sensory neurons at 3-days postinstillation. An increase in cell proliferation was observed by BrdU incorporation at 5 and 7 days postinstillation. Treatment with purinergic receptor antagonists reduced cell proliferation whereas exogenous ATP significantly increased cell proliferation. The authors conclude that ATP has neuroproliferative and neuroprotective roles in normal and injured olfactory epithelium.

Subcutaneous injections of 10 mg/kg nickel chloride have been shown to increase prolactin secretion in rats one day following administration (Clemons and Garcia, 1981). However, an earlier study showed that prolactin secretion in rats is specifically inhibited for 30 minutes following intravenous exposure to 100 µg Ni^{2+} as $NiCl_2$ (LaBella *et al*., 1973).

Donskoy et al. (1986) found that s.c. injection of 125 to 750 µmol Ni/kg to male Fischer 344 rats resulted in acute hepatic toxicity within 24 hr as evidenced by enhanced lipid peroxidation, microvesicular steatosis, and increased serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT). The latter serum enzymes were significantly increased about two-fold by the low dose of 125 µmol Ni/kg compared to control animals ($P < 0.05$, $N = 14$).

Subacute (12-day) inhalation exposures (5 days/week, 6 hours/day) of 10 mice to nickel, as 10 mg Ni_3S_2/m^3 (AMAD = 1.3µm, gsd = 1.5), caused 100% mortality (Benson *et al*., 1987). Two of 10 rats also died from this exposure. Although no effect was seen on natural killer cell activity in these animals, lesions in the nasal and lung epithelium and in bronchial lymph node were observed. Pathology revealed emphysematous changes in the lungs of rats exposed to 5 or 10 mg Ni_3S_2/m^3, and fibrosis in mice exposed to 5 mg Ni_3S_2/m^3. Atrophy of lymphoid tissues, including spleen, thymus, and bronchial lymph nodes, was observed in mice and rats exposed to 5 or 10 mg Ni_3S_2/m^3.

Nickel distributes preferentially to the lungs and kidneys following intratracheal administration of $NiCl_2$ to rats (Carvalho and Ziemer, 1982). The electrophilic Ni^{2+} ion is reported to be the causative agent of nephrotoxicity in rats; it binds to intracellular nucleophiles in kidney tissue such as guanine, adenine, and glutathione two hours following intraperitoneal exposure to 10 mg Ni/kg as $NiCO_3$ (Ciccarelli and Wetterhahn, 1984).

Toya et al. (1997) evaluated the effects of single and repeated intratracheal instillations of nickel fumes (about 10 nm in diameter), Ni_2O_3 (2.0 µm geometric mean diameter and 1.69 gsd) and NiO (2.2µm geometric mean and 1.68 gsd) powders, all dispersed in saline and sonicated immediately prior to instillation, in the Sprague-Dawley rat. The LD_{50} of nickel fumes was estimated to be 38.2 mg/kg bw. Body weight gain was retarded by single doses of 13.0 mg Ni_2O_3/kg, 14.3 mg Ni fumes/kg, or 13.0 mg NiO/kg compared to controls. The lung lesions induced by a single nickel exposure were characterized by goblet cell hyperplasia, perivascular inflammatory cells and edema in the alveolar space. Nickel fumes and Ni_2O_3 produced goblet cell hyperplasia, focal granuloma, and inflammatory cells in the alveolar space but NiO did not produce lesions. Repeated instillations of nickel fumes (5.9 mg/kg-d for four days to one week) produced a secretion of proteinaceous materials in the alveolar space. The authors note that although the Ni fumes were composed of about 3% Ni_2O_3 and the remainder NiO, its toxicity was greater on a weight basis than Ni_2O_3 administered alone. They speculate that the difference in toxicity was due to the presence of ultrafine particles in the Ni fumes.

Serita et al. (1999) studied lesions formed in rat lungs after a single five hour inhalation exposure to agglomerated ultrafine metallic nickel (Uf-Ni) with an initial 20 nm average particle diameter and an exposure aerosol of MMAD = 1.3 µm, and geometric standard deviation (gsd) = 1.54. Sixty to 80 Wistar rats per dose group (sex unspecified) were exposed to 0.15 (Low), 1.14 (Medium), or 2.54 (High) mg Uf-Ni/$m^3$for five hours. Five animals /dose group were sacrificed at 0 hr and 1, 3, 7, 14, and 21 days post exposure. The Low group also had sacrifices at 28, 56, and 84 days post exposure. The toxicological findings included: (1) a significant increase in lung weight in the Medium and High groups; (2) accumulation of foamy alveolar macrophages (AM) and debris of burst AM which may restrict pulmonary ventilation; (3) degenerated AM indicating alveolar lipoproteinosis which was aggravated for up to four weeks in the High group; and (4) acute calcification of the degenerated AM possibly related to a disruption of Ca^{2+} ion transport by solubilized Ni^{2+} ion. This study indicates a LOAEL of 1.14 mg Ni/m^3 and a NOAEL of 0.15 mg Ni/m^3 for a single five-hour exposure to metallic nickel. However, as the authors point out, if half of the amount of Ni deposited in the lung in the Low group were carried over to the next day, the amount of deposition after 30 days at 5hr/d would exceed the single exposure deposition for the High group. Therefore, it is difficult to accept 0.15 mg/m^3 as a true NOAEL applicable to repeated exposure scenarios.

Prows and Leikauf (2001) studied the genetic determinants underlying the susceptibility to acute nickel-induced lung injury in sensitive and resistant mouse strains. The mice were exposed 6 times over one year in an inhalation chamber to air containing 152 ± 12 μg Ni/m^3 (0.2 μm MMAD) generated from 50 mM (10^{-3} M) $NiSO_4$•$6H_2O$ (duration of individual exposures not given). Quantitative trait loci (QTL) analysis of 307 backcross mice generated from the sensitive A/J and resistant C57BL/6J mouse strains identified a significant linkage on chromosome 6 (designated *Aliq4*) and suggestive linkages on chromosomes 1 and 12. Analysis of phenotypic extreme responders to nickel-induced lung injury, including 55 most sensitive (survival times ≤ 66 hr) and 54 most resistant (survival times ≥ 112 hr) backcross mice, identified possible linkages on chromosomes 1, 6, 8, 9, and 12, which explained 62% of the genetic variance in the extreme phenotypic cohort. Comparing mean survival times of backcross mice with similar haplotypes gave an allelic combination of four QTLs that could account for the survival differences. The QTL intervals on chromosomes 6 and 12 were previously identified with ozone sensitivity. Candidate genes for chromosome 6 locus include *Tbxas1* (thromboxane A synthase 1), *Aqp1* (aquaporin-1), *Crhr2* (corticotropin releasing hormone receptor-2), *Sftpb* (surfactant-associated protein-B), *Pecam* (platelet/endothelial cell adhesion molecule), and *Tgfa* (TGF-α). The results suggest that relatively few genes might be important for irritant-induced acute lung injury. In a subsequent study (Prows et al., 2003) examined gene expression in sensitive and resistant strains (see Appendix A, Section A3.2)

Nishi et al. (2009) evaluated the effects of NiO nanoparticles on inflammation and chemokine expression in rats exposed intratracheally. The mass median diameter of NiO agglomerates suspended in distilled water was 26 nm (8.41 nm weighted average surface primary diameter and 104.6 m^2/g specific surface area). The particle size distribution of the sample nanoparticles was determined by a dynamic light scattering technique (diameter range ca. 10 to 60 nm). Male Wistar rats were exposed to 0.1 mg (0.33 mg/kg) or 0.2 mg (0.66 mg/kg) followed by sacrifice at 3 days, 1 week, and 1, 3, and 6 months following a single instillation. Control animals received intratracheal instillation of distilled water. Cytokine-induced neutrophil attractant-1 (CINC-1), CINC-2αβ, and CINC-3 in lung tissue and BALF were determined by measurement of protein by ELISA. Both CINC-1 and CINC-2αβ were elevated from day 3 to 3 months in lung tissue and from 3 to 6 months in BALF. CINC-3 was elevated on day 3 both in lung tissue and BALF, then decreased. Total cell and neutrophil counts in BALF were increased from day 3 to 3 months. In lung tissue, infiltration of neutrophils and alveolar macrophages was seen from day 3 to 6 months in alveoli. Dose-responses were observed for total cells at 1, 3 , and 6 months; CINC-1 in lung at 3 days, 1 week and 3 months and in BALF at 3 days, and 6 months; CINC-2 at 3 days, 1 week, and 3 months in lung and 3 days, 1 month and 6 months in BALF; and CINC-3 at 3 days and 1 week in lung and 3 days, 1 week and 1 month in BALF. The data indicate the involvement of CINC in NiO nanoparticle induced lung injury.

Singla et al. (2006) found that acute oral administration of $NiSO_4$ (50 mg/kg-d x 7 days) to rats affected the structural and functional integrity of the intestine. The activities of the brush border enzymes maltase ($P < 0.05$), lactase ($P < 0.05$), alkaline phosphatase ($P < 0.05$) and leucine amino peptidase ($P < 0.05$) were increased in purified brush borders from Ni-treated rats compared to controls. Alternatively, sucrase, trehalase ($P < 0.01$) and glutamyl transpeptidase ($P < 0.05$) were reduced in nickel fed animals compared to controls. Kinetic analysis of alkaline phosphatase and sucrase indicated that quantity of enzymes (Vmax) was altered by nickel exposure rather than their activity (Km). Regional analysis indicated that the changes in enzyme activity were mainly located in the villus tip and mid villus regions, rather than the crypt base. The authors conclude that acute feeding of nickel affects the development of various brush border enzymes along the crypt-villus axis of the rat intestine.

Table 12. Summary of Acute Nickel Toxicity in Animals

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Mastromatteo, 1986	NiO, Ni_3S_2	Single dose oral toxicity in mice and rats.	LD_{50}s > 3000 mg/kg.	
Haro *et al*., 1986	$NiSO_4$, Ni acetate	Single dose oral toxicity in mice and rats.	LD_{50}s 39 to 141 mg/kg.	
Knight *et al*., 1991	$NiCl_2$	Intraperitoneal (i.p.), intramuscular (i.m.) and subcutaneous (s.c.) injection in rats.	Acute LD_{50}s: i.p. = 5 mg/kg i.m. = 23 mg/kg; s.c. = 25 mg/kg	
Donskoy *et al*., 1986	$NiCl_2$	s.c. injection of 125-750 µmol Ni/kg in male F344 rats.	Acute hepatic toxicity within 24 hr.	Enhanced lipid peroxidation at 750 µmol Ni/kg, 4-fold increase in thiobarbituric acid chromogens in hepatic cytosol.
Benson *et al*., 1987	Ni_3S_2 AMAD = 1.3 µm, gsd = 1.5	Inhalation exposure 6hr/d. 5d/wk, 12 days, 5 or 10 mg/m^3 to mice and rats	Mortality 100 % in mice, 20% in rats	Lesions in nasal and lung epithelium and lymph nodes. Atrophy of lymphoid tissues incl. spleen, thymus, bronchial lymph in mice and rats at both doses.
Graham *et al*. 1975, 1978	$NiCl_2$, 99% < 3 µm	Inhalation of 0, 100, 250, 375, 490 µg Ni/m^3 in mice for 2 hr. Challenge with sheep RBC.	Immunotoxicity, decrease in splenic antibody formation with linear dose response.	BMDL based on loss of 100 hemolytic plaques per 10^6 spleen cells.
Condevaux *et al*., 2001	$NiCl_2$	Natural killer (NK) cell activity with 0, 1, 10, 100 µg Ni/mL monkey and rat cells *in vitro*	NK activity: in monkey 34-42% ↓: in rat 22-24% ↓.	

Table 12. Summary of Acute Nickel Toxicity in Animals

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Haley *et al*., 1987, 1990	Ni_3S_2 NiO	Intratracheal instillation of 0.06 µmol Ni/g lung in monkey and mice	Impaired pulmonary macrophage phagocytic function.	Secondary increase in NK cell-mediated killing of target AM cells.
Adkins *et al*., 1979	$NiCl_2$, 86-96% < 1.4µm, 99% < 3µm	Host resistance in 80-120 mice/dose group: 0, 289, 369, 499 µg Ni/m^3 x 2 hr.	Response to experimental infection with *Streptococcus pyogenes* 24 hr after Ni dose gave increase in mortality	BMDL of 365 µg Ni/m^3 for a doubling of mortality increase (3.74 to 7.4%). Supporting study for aREL.
Toya *et al*., 1997	Ni_2O_3, NiO powders and Ni fumes in particulate suspensions	Intratracheal instillation in rats: 23 -30 /group. Ni_2O_3 1.4, 13 mg/kg; NiO 13 mg/kg; Ni fumes 3.8, 14.3 mg/kg and repeated 4 x 5.9 mg/kg in 8 weeks.	Mortality of Ni fumes LD_{50} = 38.2 mg/kg. Reduced BW gain at 13 mg/kg Ni_2O_3, 14 mg/kg Ni fumes, and 13 mg/kg NiO.	Lung lesion induced by single exposures of Ni fumes and Ni_3O_2 but not NiO.
Serita *et al*., 1999	Ultrafine Ni 20 nm MMAD = 1.3 µm, gsd = 1.54	Single inhalation study in rats, 60-80 rats/dose. 0.15, 1.14, 2.54 mg/m^3 for 5 hr. Rats sacrificed at intervals up to 84 days	Lung lesions observed at 1.14 mg Ni/m3	Lung weight ↑ Foaming alveolar macrophages (AM) ↑ Degenerated AM with alveolar lipoproteinosis ↑ Acute calcification of degenerated AM ↑
Ishihara *et al*., 2002	$NiCl_2$ aerosol MMAD = 1.8µm, gsd = 1.6	Inhalation in male rats 5hr/d x 5 d 0.85 (d1) to 0.24 (d2-5) mg Ni/m^3.	Inflammatory biomarkers in BALF: total protein concentration ↑; Total elastolytic activity ↑; trypsin inhibitory capacity ↑; β-glucuronidase ↑.	Fucose ↑; sialic acid ↑; L-selectin ↑ BMDLs estimated for total cells, total protein, total elastolytic activity and sialic acid.

Table 12. Summary of Acute Nickel Toxicity in Animals

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Nishi *et al*., 2009	NiO nanoparticles MMAD = 26 nm, 10-60 nm distribution by light scattering.	Intratracheal instillation, inflammation and chemokine expression in rats. 0.1 or 0.2 mg single instillation sacrifice 3d to 6 mo.	Cytokine –induced neutrophil attractant 1(CINC-1) ↑ and CINC-2αβ ↑ in BALF at both doses $P < 0.01$ vs. control.	CINC-3 ↑ at 3 days then decreased.
Singla *et al*., 2006	$NiSO_4$	Oral exposure in rats 50 mg/kg-d x 7 days.	Functional integrity of intestine, activity of brush border enzymes: maltase ↑, lactase ↑, alkaline phosphatase ↑, leucine amino peptidase ↑ all $P < 0.05$.	Sucrose ↓ $p < 0.01$, trehalase ↓$p < 0.01$, glutamyl trans-peptidase↓ $p < 0.05$.
Jia *et al*.,2010	$NiSO_4$	Intranasal instillation in mice, 0, 0.1, 0.5, 2.5 mg/kg bw. Histological and immuno-chemical analysis at 1-7 d post treatment.	Nasal toxicity time and dose dependent decreases in thickness of ectoturbinate 2 and endoturbinate II regions.	$BMDL_{05}$ estimates of 10.6 to 24.3 µg Ni/kg bw for endoturbinate II data set.

Note: AM = alveolar macrophages; AMAD = activity median aerodynamic diameter; MMAD = mass median aerodynamic diameter ARDS = adult respiratory distress syndrome; BMDL 95% lower bound on a specific response level (e.g. BMDL05 = lower bound on a 5% response); BALF = bronchial alveolar lavage fluid; CI = 95% confidence interval; NK = natural killer;; ↑ = increase; ↓ = decrease.

5.4 Predisposing Conditions for Nickel Toxicity

Medical: Asthmatics or atopic individuals may be especially at risk for developing nickel-induced asthma (Cirla *et al*., 1984). Cigarette smokers may receive greater nickel exposure, since cigarette smoke contains nickel (Menden et al., 1972; Smith et al., 1997; Reprotext, 1999; Torjussen et al., 2003). These authors report about 0.5 to 2.5% of cigarette tobacco nickel appeared in the particulate phase of mainstream smoke. Additionally, a review of the literature on nickel toxicity showed that Ni^{2+} causes vasoconstriction in animals and humans, which may potentiate the effects of a primary ischemic lesion in the cardiovascular system (U.S.EPA, 1985).

Chemical: In rats, rabbits, and dogs, one mg/kg nickel chloride antagonizes the cardiac arrhythmia induced by digoxin by competing with calcium at cardiac membrane sites (Prasad *et al*., 1980). The implications of this effect for persons with congestive heart failure have not been investigated.

6 Reproductive and Developmental Toxicity

6.1 Reproductive and Developmental Toxicity Summary

Studies of reproductive and developmental toxicity of nickel and compounds are summarized below. Human studies (Table 13) of workers exposed to nickel compounds by the inhalation route suggest increased incidence of spontaneous abortions in females and spermatotoxicity in males. In experimental animals (Table 14), no inhalation studies were identified. But oral exposures resulted in spermatotoxicity in mice and rats involving both induction of mutation and endocrine disruption, and reduced reproduction in rats (both sexes exposed separately and together). Nickel-exposed mice and rats also exhibited significantly increased perinatal mortality.

Although reproductive and developmental effects are a substantial source of concern, none was selected as the basis of any of the inhalation RELs. The animal studies used less relevant routes of exposure. The human studies involved fairly high exposures, where these were quantified. The inhalation-based acute, 8-hour and chronic RELs derived in this document are at least 50-fold lower than the chronic oral REL which is based on perinatal mortality in rats (see section 9.8).

6.2 Human Studies

Chashschin et al. (1994) reported that an increase in spontaneous abortions was observed among 290 women (15.9%) who worked in a nickel hydrometallurgy refining plant in Russia, compared with 336 female construction workers without any occupational nickel exposure as controls (8.5%). The workers were exposed to primarily nickel sulfate at 0.11 to 0.31 mg Ni/m^3, but no particle size information was provided. In the same study, the authors also noted a statistically significant increase in structural malformations among offspring born to 356 workers (16.9%) compared to 342 controls (5.8%). They reported relative risks were 2.9 for all kinds of defects, 6.1 for cardiovascular system defects, and 1.9 for musculoskeletal defects. They noted heavy manual activity and heat stress as potential confounders. No confidence intervals or other statistical analyses were provided by the authors.

Benoff et al. (2000) studied the effects of metal ions on human sperm mannose receptor expression, a biomarker of spermatotoxicity. Exposure of human sperm to Ni(II) had a biphasic effect with a low concentration of 4.21 nM Ni(II) stimulating the mannose receptor expression ($P < 0.01$) and higher concentrations of 421 nM and 42 µM Ni(II) decreasing expression ($P < 0.014$).

Danadevi et al. (2003) studied semen quality of Indian welders occupationally exposed to nickel and chromium. Fifty-seven workers from a welding plant in South India and 57 controls were monitored. Blood nickel and chromium concentrations (oxidation states unspecified) were determined by inductively

coupled plasma mass spectrometry (ICP-MS). World Health Organization criteria were employed in analyzing semen samples. The nickel and chromium blood concentrations for 28 exposed workers were 123±35 and 131±53 µg/L, respectively. The control levels (N = 27) were much lower at 16.7±5.8 and 17.4±8.9 µg/L, respectively. Sperm concentrations were 14.5±24.0 x 10^6/mL for exposed workers vs. 62.8±43.7 x 10^6/mL in the controls. Rapid linear sperm motility was decreased in the exposed subjects compared to controls and there was a significant positive correlation between the percentage of sperm tail defects and blood nickel in exposed workers (R = 0.485, P = 0.036). These investigators also report a negative correlation of sperm concentration with blood chromium in exposed workers (R = -0.424, P = 0.025).

Vaktskjold et al. (2006) investigated the incidence of genital malformations in newborns of women nickel-refinery workers. In this register-based cohort study, data about pregnancy outcome and occupation were obtained from the Kola Birth Registry, covering the township of Monˇcegorsk in Northwestern Russia. The reference population comprised delivering non-Ni-exposed women from Monˇcegorsk. Nickel exposure was characterized by using as a guideline the water-soluble Ni subfraction of the inhalable aerosol fraction obtained by personal monitoring for nickel- and copper-refinery workers and/or measured urinary-Ni concentrations. The following exposure categories were assigned according to the occupation the delivering woman had at the time of becoming pregnant: background, observed urinary Ni concentration < 10 µg/L; low, <70 µg/L; high, ≥ 70 µg/L, which roughly corresponds to airborne exposure concentrations ≥160 µg/m^3 of the water-soluble inhalable subfraction. This registry and exposure classifications were also used in the other studies by Vaktskjold et al. described below. The association of the outcome with assigned exposure ratings was analyzed with a logistic regression model, adjusted for parity, maternal malformation, exposure to solvents and infection in early pregnancy. There was no association between nickel exposure and genital malformations in this study. The odds ratio (OR) for nickel-exposed women delivering a newborn with a genital malformation was 0.81(95% C.I. 0.52-1.26) and that for undescended testicle was 0.76(95% C.I. 0.40-1.47). The study is limited by few cases in the higher exposure groups.

Vaktskjold et al. (2007) evaluated the possible association between nickel exposure in early pregnancy and the delivery of small-for gestational-age (SGA) newborns. Live births and stillbirths after at least 28 weeks' gestation from the Kola Birth Registry were considered. The study population consisted of 22,836 births and SGA was defined as birth weight below the tenth percentile for the gestational age in the source population. There were 2,096 (9.2%) newborns defined as SGA. The mothers of 10.6 percent of the SGA and 13 percent of the reference infants were employed at jobs with Ni exposure above the background level. The unadjusted odds ratio (OR) for an SGA birth per unit increase in exposure category was 0.79 (95% C.I. = 0.68-0.91) and the adjusted OR (Model 1) was 0.84 (95% C.I. = 0.75-0.93). The authors concluded that the maternal exposures to water-soluble nickel in the first part of pregnancy did not increase

the risk of an SGA newborn without trisomy in the study population. (The marginal decrease in OR for SGA with exposure category, which was reduced by adjustment, was not considered biologically significant.)

Vaktskjold et al. (2008a) studied the incidence of musculoskeletal defects in the offspring of women occupationally exposed to nickel in early pregnancy, based on the Kola registry and exposure categories described above. In total, the study population consisted of 22,965 births. Three hundred and four infants (13.3/1000 births; 95% C.I. 11.9-14.7) were diagnosed with isolated musculoskeletal defects(s) at birth. The adjusted odds ratio for the association between maternal exposure to nickel and the observed defects was 0.96 (95% C.I. 0.76-1.21). The authors concluded that despite the high incidence of defects there was no apparent association with maternal nickel exposure.

Similarly, Vaktskjold et al. (2008b) studied the incidence of spontaneous abortion among nickel-exposed female refinery workers. A case-control study involved women employed in nickel-exposed work areas in early pregnancy. Each pregnancy record was assigned a categorical nickel exposure rating according to occupation at pregnancy onset. The guidelines were the water-soluble Ni subfraction of the inhalable aerosol fraction obtained by personal monitoring for nickel- and copper-refinery workers and/or measured urinary-Ni concentrations. The cut-off between low and high exposure levels was 70 µg Ni/L urine corresponding to about 160 µg Ni/m^3 of the water soluble sub-fraction. The unadjusted OR for the association between maternal Ni exposure and spontaneous abortion was 1.38 (95% C.I. 1.04-1.84), and the adjusted OR was 1.14 (95% C.I. 0.95-1.37). Adjustments included previous induced abortion, previous delivery, solvent or paint exposure, heavy lifting, and maternal age >34 years. Addition of maternal smoking did not significantly change the OR, 1.15(0.96-1.39). The authors concluded that no statistical association was established; however they note that the findings do not exclude the possibility of a weak excess risk.

Table 13. Summary of Human Reproductive or Developmental Toxicity of Nickel

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Chashschin *et al*., 1994	$NiSO_4$	Airborne exposure of female refinery workers, 0.11 to 0.31 mg Ni/m^3, N = 290 *vs*. 336 controls	Spontaneous abortion (SA): 15.9% in exposed vs.8.5% in controls. No confidence interval (CI).	Significant increase in structural malformations (16.9%) vs. 5.8% in controls. RR = 2.9 for all defects, 1.9 for musculoskeletal defects. No CI's.
Benoff *et al*., 2000	Ni^{2+}	In vitro treatment of human spermatozoa: 4.21 nM, 421 nM, 42 µM Ni^{2+}.	Mannose receptor expression a biomarker for spermatotoxicity. 4.21 nM ↑, 421 nM ↓, 42 µM ↓	P < 0.014 for observed decreases in mannose receptor expression.
Danadevi *et al*., 2003	Ni, Cr	Semen quality in Indian welders. N = 57 exposed *vs* 57 controls	Sperm concentrations 14.5 ± 24.0 x 10^6/mL in exposed vs. 62.8 ± 43.7 x 10^6/mL in controls.	Linear sperm motility ↓, sperm tail defects ↑. Effects correlate with blood Ni.
Vaktsjold *et al*., 2006	Ni, water soluble inhalable	Female Ni refinery workers. Urinary Ni < 10µg/L control, < 70 µg/L low exposure group, ≥ 70 µg/L high exposure group	Genital malformations: OR = 0.81 (95% CI = 0.52-1.26); undescended testicle OR = 0.76 (95% CI = 0.75-0.93)	Few cases in high exposure group. Only 103 newborns diagnosed with one or more malformation (44.5/10,000)
Vaktsjold *et al*., 2007	Ni, water soluble inhalable	Female Ni refinery workers. Urinary Ni < 10µg/L control, < 70 µg/L low exposure group, ≥ 70 µg/L high exposure group.	Small for gestational age (SGA) newborns: unadjusted OR = 0.79(95%CI = 0.68-0.91); adjusted OR = 0.84(95%CI = 0.75-0.93)	SGA = birth weight below 10^{th} percentile. 2096 (9.2%) defined as SGA

Table 13. Summary of Human Reproductive or Developmental Toxicity of Nickel

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Vaktsjold *et al*., 2008a	Ni, water soluble inhalable	Female Ni refinery workers. Urinary Ni < 10µg/L control, < 70 µg/L low exposure group, ≥ 70 µg/L high exposure group.	Musculoskeletal (MS) defects: adjusted OR = 0.96 (95% CI = 0.76-1.21)	No Ni-induced MS defects despite high incidence: 304 (13.3/1000).
Vaktsjold *et al*., 2008b	Ni, water soluble inhalable	Female Ni refinery workers. 474 cases, 4571 controls. Urinary Ni < 10µg/L control, < 70 µg/L low exposure group, ≥ 70 µg/L high exposure group	Spontaneous abortion (SA): unadjusted OR 1.38 (95% CI = 1.04-1.84); adjusted OR = 1.14 (95% CI = 0.95-1.37).	Possible weak effect or increased excess risk in early pregnancy.

Note: CI = 95% confidence interval; MS = musculoskeletal; OR = odds ratio; SA = spontaneous abortion; SGA = small for gestational age; ↑ = increase; ↓ = decrease.

6.3 Animal Studies

Animal studies of the developmental and reproductive toxicity of nickel compounds are summarized in Table 14.

The studies of NiPERA (2000a,b) showing perinatal mortality in nickel treated rats were selected as the basis of the chronic oral REL. The details of the derivation are given in section 9.8.

NiPERA (2000a) sponsored a one-generation reproduction study in Sprague-Dawley rats with nickel sulfate hexahydrate. Eight animals per sex/dose group were administered 0, 10, 20, 30, 50 or 75 mg/kg-d by daily aqueous gavage to the F0 parental animals and selected F_1 offspring. Dosing of the F0 animals began two weeks prior to mating and dosing of F_1 offspring began on PND 22. All doses were given at constant volume of 10 mL/kg. Both F_0 and F_1 animals were examined for indications of toxicity. Experimental endpoints for F_0 animals included clinical observations, body weights, food and water consumption, mating, parturition, lactation and offspring growth and viability. Experimental endpoints for selected F_1 animals included survival, clinical observations and body weight during the F_1 dosing phase. All F_0 and F_1 animals were subjected to gross necropsy examination at time of death or terminal sacrifice. For the F_0 animals post-implantation loss (implantation scar count minus the number of live pups on Day 0) was significantly increased at the 30, 50, and 75 mg/kg-d dose levels and increased at the 10 and 20 mg/kg-d levels (mean loss values: 0.4 control; 2.6; 1.5; 2.3 ($P<0.05$); 2.7 ($P<0.01$); 4.8 ($P<0.01$)). For F_1, pup viability was significantly decreased at all dose levels except 50 mg/kg-d compared to the control (dead/live: 1/128 control; 12/100; 10/106; 10/92; 4/89; 23/80 all $P < 0.01$ except 50 mg/kg-d). For this study a LOAEL of 10 mg/kg-d equivalent to 2.1 mg Ni/kg-d was identified.

NiPERA (2000b) sponsored a two-generation reproduction study in Sprague-Dawley rats with nickel sulfate hexahydrate. Twenty-eight animals per sex per group were administered 0, 0.22, 0.56, 1.12, or 2.23 mg Ni/kg-d by aqueous gavage. The animals were exposed from ten weeks prior to mating for F_0, through gestation, and until PND 21 (13 weeks to delivery of F_1 offspring). Exposure for F_1 was from *in utero*, during lactation, through development from PND 22 to about PND 92 (a minimum of 70 days of treatment). In contrast to the one-generation study the F0 animals showed no statistically significant effects of nickel administration on implantation and post-implantation losses. Statistically significant differences in F_0 organ weights consisted of decreased absolute and relative liver weights in the high dose males, decreased absolute brain weight in the mid dose females, and increased relative liver weight in 0.56, 1.12 and 2.23 mg/kg-d group females. The investigators did not consider these organ weight effects to be toxicologically meaningful. The percent of dead pups/total in the respective dose groups were: 2.2 (control); 3.7; 2.2; 2.1; 4.2 ($P = 0.105$ vs.

control by one-sided Fisher exact test). For this study a NOAEL of 2.23 mg Ni/kg-d was identified by the authors.

Schroeder and Mitchener (1971) conducted a three-generation reproduction study in Long-Evans rats administered drinking water containing five mg Ni/L (0.43 mg Ni/kg-d, U.S.EPA, 1988). Five pairs of rats were randomly selected at the time of weaning, placed in separate cages and given nickel in drinking water *ad libitum*. The rats were allowed to breed for up to nine months of age or longer. At weaning, pairs were randomly selected from the first, second and third litter (F_1) and allowed to breed and to produce the F_2 generation. Pairs were likewise selected at random from the F_2 litters to breed the F_3 generation. They observed that all nickel-exposed animals in the three generations gave birth to litters that exhibited significantly increased perinatal mortality ($P < 0.0001$), and there was a significantly increased number of "runts" in the first ($P < 0.025$) and third ($P < 0.0001$) generations. The study suffers from small sample size, and the fact that matings were not randomized and that the males were not rotated.

Ambrose et al. (1976) studied the effects of dietary administration of nickel sulfate hexahydrate in a three-generation study in rats. Male and female rats in the parent generation were exposed to 0, 250, 500, or 1000 ppm nickel, starting at 28 days of age. Mating was initiated after 11 weeks of nickel exposure. Rats in the first, second and third generations were also given the same diet as the parent generation. At each mating, 20 females from each dose level were transferred to individual breeding cages and mated with a male from the same dietary nickel level. The authors did not observe any adverse effect on fertility, pregnancy maintenance, or postnatal survival of offspring in the three generations. They did report a dose-dependent decrease in the number of siblings weaned per litter averaging 8.1, 7.2, 6.8, and 6.4, respectively. Weanling body weight was clearly affected at the top dose level averaging 73% of the controls. The study suffers from small sample size and the use of pups rather than litters as the unit of comparison.

In a two-generation study (RTI, 1988), nickel chloride was administered in drinking water to male and female CD rats (30/sex/dose) at dose levels of 0, 50, 250, or 500 ppm (mg Ni^{2+}/L) for 90 days before breeding. A significant decrease in the P_0 maternal body weight was observed at the highest dose level. A significant decrease in live pups/litter and average pup body weight versus controls was also seen at the 500 ppm level in the F_{1a} generation. Similar effects were seen in the F_{1b} litters of P_0 dams exposed to the 500 ppm dose level. Increased pup mortality and decreased live litter size were also observed in the 50 and 250 ppm dose groups in the F_{1b} litters. These latter findings are questionable due to increased temperature and humidity experienced by the F_{1b} litters, which could have influenced the observed effects (Edwards, 1986). F_{1b} males and females were randomly mated on postnatal day (PND) 70 and their offspring were evaluated through PND 21. The 500 ppm dose level caused a significant body weight depression of both mothers and pups, and increased neonatal mortality. The 250 ppm dose level produced transient depression of

maternal weight gain and water intake during gestation of the F_{2b} litters. A significant increase in short ribs was observed in the 50 ppm dose group, but since this was not seen in the higher doses, it is not considered to be biologically significant.

Kakela et al. (1999) evaluated the effect of $NiCl_2$ administered in drinking water on reproduction in Wistar rats. Four groups of six female rats were exposed to 10-100 ppm Ni^{2+} for up to 100 days prior to conception and through gestation and lactation. Two groups of male rats were exposed to 30 ppm Ni^{2+} for 28 and 42 days prior to conception and one group of males and females were exposed to 30 ppm Ni^{2+} for 28 days prior to conception. Exposure was continued for the females through lactation. The males were sacrificed at conception. When males were exposed to Ni^{2+} both the number of pregnancies and the number of pups born were reduced. The control value for gestation index (number of live pups per dam) was 10.2 ± 1.5 SE versus 2.7 ± 1.4 ($P < 0.01$) for 28 day exposures and 7.8 ± 2.0 for 42 day exposures. The litter sizes were 9.2 ± 1.5, 1.3 ± 0.9 ($P < 0.01$), and 6.2 ± 2.0, respectively. Females exposed to 100 ppm Ni^{2+} 14 days prior to conception also gave reduced litters: 4.0 ± 1.0 ($P < 0.05$). Histological examination of testes in nickel-exposed rats revealed shrinkage of the seminiferous tubules and decreased number of basal spermatogonia. When both parents were exposed to nickel, pup mortality during lactation was high.

Administration of 25 µmol Ni/kg-d for 5 days only marginally affected mating efficiency of males (75% vs. 80-90% in controls). No significant difference was seen in the total number of implantations among pregnancies resulting from nickel-treated males. Total implantations/litter from nickel-treated males ranged from 10.9 to 11.4. However there was a marked decrease in the number of live implantations among the nickel animals during weeks 1 to 3. The mean incidence of dead implantations during these three weeks was 1.9, 3.2, and 2.2, respectively (all $P < 0.05$ vs. control). These values compare with those for a single 100 mg/kg dose of cyclophosphamide, a dominant lethal mutagen, of 5.3, 6.33, and 3.6, respectively (all $P < 0.001$ vs. control). The percentage of dead implantations/litter expressed as a percentage of total implants for weeks 1, 2, and 3 were: control, 8.69, 8.03, 10.9; nickel, 16.5 ($P < 0.05$), 28.00 ($P < 0.001$), 19.64 ($P < 0.001$); cyclophosphamide, 60.27, 55.86, 35.00 (all $P < 0.002$). The results clearly suggest a specific Ni-induction of dominant lethal-type mutations.

Table 14. Summary of Animal Reproductive and Developmental Toxicity of Nickel

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Schroeder and Mitchener, 1971	Ni in drinking water	3-generation reproduction study in rats, 0.43 mg Ni/kg-d	Increased perinatal mortality in all generations.	Small sample size, mating not randomized, males not rotated.
Ambrose *et al*., 1976	$NiSO_4 \cdot 6H_2O$	3-generation study in rats: 0, 250, 500, 1000 ppm Ni	Reproductive effects: dose-dependent decreases in number of siblings/litter, 8.1, 7.2, 6.8, 6.2, respectively.	Small sample size, use of pups rather than litters as unit of comparison.
RTI, 1988	$NiCl_2$	2-generation study in rats, 30/sex/dose. 0, 50, 250, 500 ppm Ni in drinking water for 90 d before breeding.	Reproductive effects: P_0 maternal BW ↓, live pups/litter ↓, avg. pup weight in F_{1a} and F_{1b} ↓.	500 ppm considered significant BW ↓ in mothers and pups, and increased neonatal toxicity.
Kakela *et al*., 1999	$NiCl_2$	Reproduction in female rats 10, 30, 100 ppm Ni, and 30 ppm Ni in male rats.	Reproductive effects: females exposed t o100 ppm Ni 14 d prior to conception gave reduced litters. Males exposed gave reduced number of pregnancies and number of pups born.	Histology of males showed shrinkage of the seminiferous tubules and decreased number of basal spermatogonia. Perinatal mortality seen when both parents were exposed.
NiPERA, 2000a	$NiSO_4 \cdot 6H_2O$	1-generation reproduction study in rats, 8/sex/dose group, 0, 10, 20, 30, 50, or 75 mg/kg-d, aqueous gavage.	Reproductive effects: F_1 pup viability significantly decreased at all dose levels except 50 mg/kg-d.	LOAEL = 10 mg/kg-d ≈2.1 mg Ni/kg-d.

Table 14. Summary of Animal Reproductive and Developmental Toxicity of Nickel

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
NiPERA, 2000b	$NiSO_4 \cdot 6H_2O$	2-generation reproduction study in rats 28/sex/dose group; 0, 0.22, 0.56, 1.12, 2.23 mg Ni/kg-d.	Reproductive effects: % dead pups/total: 2.2, 3.7, 2.2, 2.1, 4.2 (P = 0.105), respectively. Suggestion of a specific Ni-induced dominant lethal mutation.	Decreased absolute brain weight in mid dose females, increase in relative liver weight at 0.56, 1.12, or 2.23 mg/kg-d.
Smith *et al.,* 1993	$NiCl_2$	2-generation reproduction study in rats 34 females/dose group; 0, 10, 50, 250 ppm Ni in drinking water for 11 weeks prior to mating.	Reproductive effects: maternal weight gain reduced. Increased perinatal mortality in G1 250 ppm (P<0.01) and in G2 10 ppm (P< 0.03); 50 ppm (P< 0.076), 250 ppm (P < 0.01).	LOAEL = 10 ppm ≈ 1.3 mg Ni/kg-d.
Slotkin & Seidler, 2008	$NiCl_2$	Neurodevelopmental cell model. PC12 pheochromocytoma cells treated with 30 µM $NiCl_2$, 5-8 cell cultures examined at 24, 72 hr post treatment.	Gene expression: Tryptophan hydroxylase (*tph*) ↓, vesicular monoamine transporter (*slc6a4*) ↑. Ni reduced net expression of 5HT receptor genes.	Significant decrements in *htr1d*, *htr2a*, and *htr3b*. Evidence of toxic action on specific neurotransmitter pathways.
Pandey *et al*., 1999	$NiSO_4$	Oral exposure of adult male mice 0, 5, 10 mg/kg bw, 5d/week x 35 days.	Dose-dependent decreases in absolute and relative weights of testes, epididymides, seminal vesicle, and prostate gland at 5 mg/kg-d (except epididymides) and 10 mg/kg-d.	Sperm motility ↓; Sperm concentration ↓. Altered marker testicular enzymes: γ-glutamyl trans-peptidase ↑; sorbitol dehydrogenase ↓; LDH ↑. All effects at 10 mg/kg-d, P < 0.05.

Table 14. Summary of Animal Reproductive and Developmental Toxicity of Nickel

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Pandey and Srivastava, 2000	$NiSO_4$ $NiCl_2$	Oral exposure of young male mice, 6/dose group, 0, 5, 10, or 20 mg/kg-d x 5d/week x 35 days.	Same effects as above for reproductive tissue weights and sperm concentration and motility. Abnormal sperm head, neck and tail morphology with higher doses of either compound.	Curved neck, curved, bent, round, loop, or folded tails with higher doses of $NiSO_4$ or $NiCl_2$. $BMDL_{1SD}$ (motility decrease) = 2.91 mg/kg-d $NiSO_4$; $BMDL_{1SD}$ (sperm abnormality) = 0.46 mg/kg-d $NiSO_4$; 0.34 mg/kg-d $NiCl_2$.
Xie *et al*., 1995	$NiCl_2•6H_2O$	Male ICR mice i.p. doses of 0, 0.5, 1.0, 3.0, or 5.0 mg Ni/kg bw. Mice sacrificed 24 hr post treatment and 5.0 mg/kg 7 days post treatment.	Dose dependent increases in testicular LPO, Ni, Ca, Fe ($P<0.05$, N=5). Lesser increases in Cu, Zn.	Testicular weight decrease 0.65% bw to 0.4% bw ($P<0.05$, N=5).
Das & Dasgupta, 1997	$NiSO_4•6H_2O$	Adult male rats fed normal or protein restricted diets dosed with 20mg/kg i.p. on alternate days for 20 days.	Testicular steroido-genesis: $NiSO_4$ significantly reduced 3β-hydroxysteroid dehydrogenase (HSD) and 17β-HSD in both dietary regimes.	Significant recovery seen 15 days after cessation of nickel treatment.
Forgacs *et al*., 1998	$NiSO_4•7H_2O$	Primary Leydig cell cultures with exposures *in vivo* or *in vitro*. Mice dosed s.c with $NiSO_4$ 0, 10, 20, 40 mg/kg bw every 3 days x 4.	Dose-dependent depression of human chorionic gonadotropin (hCG)-stimulated testosterone over 48 hr in cultured testicular interstitial cells.	*In vitro* exposure of 48 hr cultures of hCG-stimulated testicular interstitial cells to 0, 62.5, 125, 250, 500, and 1000 μM $NiSO_4$. Testosterone production 100, 105, 78*, 56*, 32*, 18*%, respectively (* $P < 0.05$, N = 7).

Table 14. Summary of Animal Reproductive and Developmental Toxicity of Nickel

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Doreswamy *et al*., 2004	$NiCl_2$	Testicular oxidative stress in male mice 0, 12.5, 25, or 50 µmol $NiCl_2$/kg-d i.p. for 3-5 days. Mice sacrificed 24 hr after last dose.	LPO increased in: testicular homogenate (10-25%); mitochondria (20-45%); microsomes (25-60%); epididymal sperm (8-25%).	Antioxidant enzymes ↑: GSH peroxidase (8-26%); GST (15-26%); catalase (10-25%). Double stranded DNA↓ in testis and epididymal spermatozoa.

Note: BMDL 95% lower bound on a specific response level (e.g. BMDL05 = lower bound on a 5% response); G1,G2 = first and second generations; GSH = glutathione; GST = glutathione sulfotransferase; hCG = human chorionic gonadotropin; HSD = hydroxysteroid dehydrogenase; LDH = lactate dehydrogenase; LPO = lipid peroxidase; ↑ = increase; ↓ = decrease.

There are several reports of teratogenicity and other reproductive effects in laboratory animals exposed to nickel (Ambrose et al., 1976; Schroeder and Mitchener, 1971; RTI, 1987; Smith et al., 1993). Mice exposed during pregnancy to $NiCl_2$ by intraperitoneal injection bore offspring with numerous fetal malformations and skeletal anomalies. In addition there were increased fetal resorption rates and decreased fetal weights (Lu *et al*., 1979). Woollam (1972) showed that nickel acetate, when injected intraperitoneally into pregnant hamsters, caused significant fetal mortality at 25 mg/kg.

Intravenous exposure of pregnant rats to 11 mg Ni/kg caused increased fetal mortality and a 16% incidence of fetal malformations including anopthalmia, cystic lungs, and hydronephrosis (Sunderman et al., 1983). Temporary hyperglycemia was seen in pregnant rats exposed intraperitoneally to $NiCl_2$ at four mg/kg (Mas *et al*., 1985). The authors proposed that this hyperglycemia was a mechanism for teratogenicity.

Sunderman et al. (1978) administered nickel chloride (16 mg Ni/kg) to Fischer rats by intramuscular (i.m.) injection on day eight of gestation. The body weights of fetuses on day 20 of gestation and of weanlings four to eight weeks after birth were reduced. No congenital anomalies were found in fetuses from nickel-treated dams, or in rats that received 10 i.m. injections of 2 mg Ni/kg as nickel chloride twice daily from day 6 to day 10 of gestation.

Diwan et al. (1992) showed that intraperitoneal (i.p.) injection of nickel acetate to pregnant F344/NCr rats caused early mortality in the offspring. They administered four i.p. injections of nickel acetate (2.6 mg Ni/kg) on days 12, 14, 16, and 18 of gestation and reported that all offspring died within 72 hr after birth.

Smith et al. (1993) administered nickel chloride in drinking water at 0, 10, 50, or 250 ppm (0, 1.3, 6.8, or 31.6 mg/kg-d) to 34 female Long-Evans rats per group for 11 weeks before mating and subsequently during two sequential gestations (G1, G2) and lactation (L1, L2) periods. Pups were observed until weaning and breeder males were unexposed. Dams were rested for two weeks after weaning of the first litters before initiating the second breeding. During this time exposure to nickel was continuous. The animals were 6-7 months old when they produced their second litters. Throughout the study, there were no overt clinical signs of toxicity in any of the dose groups. Reproductive performance was unaltered by nickel exposure although maternal weight gain was reduced during G1 in the mid and high dose groups. The most significant finding was the increased frequency of perinatal death (Table 15). The authors reported that the proportion of dead pups per litter was significantly increased at the highest dose level in G1 ($P \leq 0.01$) and at the low ($P \leq 0.03$) and high ($P \leq 0.01$) dose levels in G2. The mid dose level in G2 was also increased ($P = 0.076$). Overall there was a dose related increase in perinatal mortality in both segments of the study. The authors concluded that 10 ppm $NiCl_2$ (1.3 mg Ni/kg-d) represented the LOAEL in the study.

Table 15. Reproductive Outcomes of Breeding Female Rats Exposed to Nickel Chloride in Drinking Water (Smith *et al*., 1993).

Concentration of nickel in water ppm Ni (No. females)	Sperm positive females	No. viable litters	Average no. of pups per litter (live and dead)	No. of litters with dead pups at birth	Total dead pups on post natal day 1(% dead pups per litter)
G1, L1					
0 (34)	29	25	12.9	5	5 (1.7)
10 (34)	30	25	12.2	5	9 (3.1)
50 (34)	30	24	11.7	0	0 (0)
250 (34)	32	27	13.2	11	35*** (13.2)**
G2, L2					
0 (29)	28	23	10.6	2	2 (1.0)
10 (29)	28	22	12.5	7	11** (4.3)**
50 (30)	29	24	13.3	6	16* (4.6)
250 (31)	31	25	11.3	10	22*** (8.8)***

Note: Significant levels, pairwise comparison to control: * $0.05 < P \leq 0.10$; ** $0.01 < P \leq 0.03$; *** $0.001 < P \leq 0.01$.

Slotkin and Seidler (2008) evaluated the effects on Ni^{2+} in a neurodevelopmental cell model. Neurodifferentiating rat PC12 pheochromocytoma cells were treated with 30 µM $NiCl_2$. The cell cultures were examined 24 and 72 hr after the start of exposure with five to eight independent cultures at each time point. Unlike organophosphorus (OP) agents studied with this system, nickel reduced expression of tryptophan hydroxylase (*tph*) and enhanced vesicular monoamine transporter (*slc6a4*). Nickel exposure reduced the net expression of serotonin (5HT) receptor genes more effectively than did diazinon or dieldrin. Significant decrements were seen for receptor genes *htr1d*, *htr2a* and *htr3b*. The authors conclude that the results provide "evidence connecting the direct, initial mechanisms of toxicant action on specific neurotransmitter pathways with their long-term effects on synaptic function and behavior."

Male rat reproductive toxicity (damage to epididymal tubules and abnormal spermatozoa) was observed following a single subcutaneous dose of 5 mg Ni/kg as Ni_3S_2 (Hoey, 1966). Benson et al. (1987) showed that mice and rats exposed to 5 or 10 mg Ni_3S_2/m^3 displayed degeneration of testicular germinal epithelium after 12 days exposure (6 hours/day, 5 days/week).

Pandey et al. (1999) administered $NiSO_4$ orally to adult male mice at 0, 5 or 10 mg/kg bw for 5 days/week for 35 days. Significant dose-dependent decreases were observed in absolute and organ-to-body weight ratios of testes, epididymides, seminal vesicles, and prostate gland. Also observed were decreases in sperm motility and count. Significant alterations of marker testicular enzymes were seen: γ-glutamyl transpeptidase, 28.76, 35.23, and 38.44*; sorbitol dehydrogenase, 7.88, 6.00, and 4.04*; and lactate dehydrogenase, 194, 237, 244*, respectively (* P<0.05, N=10, all activities nmol/min/mg protein).

Pandey and Srivastava (2000) reported spermatotoxic effects of nickel in mice. Young male mice (25 ± 5 g), six/dose group were administered 0, 5, 10, or 20 mg/kg bw of $NiSO_4$ or $NiCl_2$ orally by gavage in 0.2 mL distilled water five days/week for 35 days. The animals were sacrificed on day 36 and the testes, epididymides, seminal vesicles and prostate glands were removed and weighed. No overt toxicity was observed. The absolute and relative weights of testes, epididymides, seminal vesicles and prostate gland were significantly decreased at the top dose of 20 mg/kg bw. Dose-dependent reductions in sperm motility were observed at 10 and 20 mg/kg bw with nickel sulfate and nickel chloride (P <0.05). Dose-dependent decreases in sperm count were also seen with both nickel compounds but were statistically significant only at the top dose with $NiSO_4$. There was a significant increase in abnormal sperm including abnormalities of the head, neck and tail region. Curved neck and curved, bent, round, loop and folded tail were seen at both higher doses with $NiSO_4$ and $NiCl_2$. A continuous benchmark dose analysis of the sperm motility and sperm count data gave only one adequate fit, namely decrease in motility with $NiSO_4$ treatment ($BMDL_{1SD}$ = 2.91 mg/kg bw, linear model, P = 0.22). A similar analysis of sperm abnormality data gave adequate fits for both compounds: $NiSO_4$, $BMDL_{1SD}$ = 0.46 mg/kg, polynomial model, P = 0.97; and $NiCl_2$, $BMDL_{1SD}$ = 0.34 mg/kg, polynomial model, P = 0.12.

Xie et al. (1995) evaluated the effects of chelating agents on testicular toxicity in mice caused by acute nickel exposure. Male ICR mice were injected intraperitoneally with $NiCl_2 \cdot 6H_2O$ at doses of 0, 0.5, 1.0, 3.0, or 5.0 mg Ni/kg bw and sacrificed 24 hr after injection. Nickel administration resulted in dose-dependent increases in testicular lipid peroxidation (LPO), and Ni, calcium (Ca) and iron (Fe) concentrations (all P < 0.05, N=5). Lesser increases in testicular copper (Cu) and zinc (Zn) were also seen. Treatment with 5.0 mg Ni/kg and seven days observation showed increasing LPO with a peak at two days after Ni administration followed by a gradual decrease. Testicular weight decreased from about 0.65% of body weight to 0.4% over the same period (P < 0.05, N = 5). Among five chelating agents tested *meso*-2, 3-dimercaptosuccinic acid (DMSA) and *N*-benzyl-D-glucaminedithiocarbamate (BGD) were the most effective in removing nickel from the testes, protecting against LPO and Ni-induced sterility.

Das and Dasgupta (1997) treated male Wistar rats with 20 mg $NiSO_4$/kg bw by intraperitoneal injection on alternate days for 20 days. Significant decreases were observed in testicular weight, lactate dehydrogenase, and protein

concentration and increases in testicular glycogen and cholesterol (all $P < 0.05$, $N = 6$). The differences from control animals were generally enhanced in parallel groups fed a protein-restricted diet with or without nickel sulfate administration.

Forgacs et al. (1998) evaluated the effects of Ni(II) on testosterone production of mouse Leydig cells in vitro following repeated in vivo or in vitro exposures. CFLP mice were injected s.c. (four treatments every three days) with 0, 10, 20, or 40 mg $NiSO_4 \cdot 7H_2O$/kg bw. Human chorionic gonadotropin (hCG)-stimulated testosterone response was reduced by Ni-treatment in 48 hr cultures of testicular interstitial cells from treated animals in a dose-dependent manner (100 (control), 88%, 80%*, and 59%*, respectively (* $P < 0.05$, $N = 4$)). Direct nickel effects were assessed in 48 hr cultures of hCG-stimulated testicular interstitial cells exposed to 0, 62.5, 125, 250, 500, or 1000 µM $NiSO_4$. Testosterone production relative to hCG control was 100% (control), 105%, 78%*, 56%*, 32%*, and 18%* respectively (* $P < 0.05$, $N = 7$). Cytotoxicity was assessed by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay following exposure and cell viability remained above 80% at all doses. The data indicate that the effect of nickel on the Leydig cell testosterone production is time and concentration dependent, and is not due to cytotoxicity.

Das and Dasgupta (2000) treated male Wistar rats with 20 mg $NiSO_4$/kg bw by intraperitoneal injection on alternate days for 20 days. Significant decreases in cauda epididymal sperm count and sperm motility were observed following treatment ($P < 0.05$). In addition decreases were seen in testicular DNA, RNA, and total protein concentrations ($P < 0.05$). The authors conclude that $NiSO_4$ is a likely gonadotoxicant that adversely affects the expression of genetic information via reduced nucleic acids and protein. In a subsequent study using a similar protocol in male rats Das and Dasgupta (2002) found that nickel treatment significantly reduced the activities of two testicular steroidogenic enzymes, 3β- and 17β-hydroxy steroid dehydrogenases (HSD), and plasma testosterone concentration. 3β-HSD was reduced from 8.97 ± 0.18 in control normal protein diet rats to 6.57 ± 0.23 units/mg ($P < 0.05$) in normal diet plus $NiSO_4$. For 17β-HSD the reduction was from 6.50 ± 0.29 to 5.10 ± 0.21 units/mg protein ($P < 0.05$), respectively. Plasma testosterone was reduced from 3.27 ± 0.06 to 2.43 ± 0.10 ng/mL ($P < 0.05$), respectively. Increases in testicular cholesterol and ascorbic acid were observed in the same groups of rats. Some reversibility of the effects was seen when treated animals were fed a normal diet during a withdrawal period.

Doreswamy et al. (2004) treated adult male CFT-Swiss mice with 0, 12.5, 25, or 50 µmol $NiCl_2$/kg bw/d by single i.p. injection for three or five treatments. The mice were sacrificed 24 hr after the final dose and evaluated for biochemical endpoints, DNA damage and fragmentation and at 1, 2, 3, and 5 weeks from the beginning of treatment for sperm head abnormalities. No clinical signs of toxicity were observed at any administered dose. Dose-dependent increases in lipid peroxidation were seen with whole testicular homogenates (10-25%), mitochondrial fractions (20-45%), microsomal fractions 25-60%), and epididymal

sperm (8-25%). Antioxidant enzymes were similarly increased: glutathione peroxidase (8-26%); glutathione *S*-transferase (15-26%); and catalase (10-25%). Nickel treatment also resulted in a dose-dependent decrease in double stranded DNA (ds-DNA) in the testis and in epididymal spermatozoa. For testis the proportion of ds-DNA was 83% (control), 80%, 65% ($P < 0.05$), and 62% ($P < 0.05$), respectively. For epididymal sperm the values were 90%, 85%, 82% ($P < 0.01$), and 80% ($P < 0.01$), respectively. Agarose gel electrophoresis of genomic DNA, visualized by ethidium bromide fluorescence, showed DNA damage at 6.25, 12.5, 25.0 and 50 µmol Ni/kg-d for three days. Caudal sperm counts did not differ from the control. However, nickel treatment induced a significant dose-dependent increase in the percentage of abnormal sperm, mainly amorphous heads, balloon heads, and hammerheads.

7 Chronic Toxicity

7.1 Chronic Toxicity Summary

Studies of human chronic toxicity of nickel and compounds, and also studies with human cells *in vitro,* are summarized in Table 16 and Table 17. Animal studies are summarized in Table 18. The most important toxic effect seen in both nickel-exposed humans and experimental animals by inhalation is pneumotoxicity. In humans exposed occupationally this is expressed as nickel-induced asthma, pulmonary fibrosis, decreased lung function (FEV_1), and increased lung abnormalities revealed by radiography. In experimental animals adverse lung effects included inflammatory lesions, macrophage hyperplasia, alveolar proteinosis, and fibrosis (rats only), in addition to bronchial lymph node hyperplasia and nasal epithelial atrophy. Numerous other adverse effects at the cellular level were also seen contributing to cytotoxicity, genetic toxicity, immunotoxicity, and other metal-induced toxicity (Beyersmann and Hartwig, 2008; Rana, 2008). However, the most sensitive adverse effects (occurring at lower doses/exposures) were seen in the lung.

7.2 Human Studies

A number of studies indicate that occupational inhalation exposure to nickel aerosols can result in development of asthma specific to nickel. Davies (1986) found 3 cases of asthma among 53 nickel-plating workers without a history of asthma prior to employment. Novey et al. (1983) described biphasic metal-specific bronchial responses in an individual metal-plating worker exposed to nickel and chromium salts. In another case, immunological studies conducted in a 24-year old man showed nickel-specific antibodies in the serum after several weeks of working in a nickel-plating shop using nickel sulfate (McConnell et al., 1973). Dermatitis was observed on exposed areas of his skin, and pulmonary function, measured by FEV_1 with and without isoproterenol challenge, was significantly impaired compared with a control subject and normal values. This worker reported dyspnea, non-productive cough, chest-tightness, and wheezing as symptoms during the work period.

Fernandez-Nieto et al. (2006) reported results obtained from four patients with work-related asthma due to exposure to metallic salts. Two subjects came from factories where potassium dichromate and nickel sulfate were used for electroplating, another worked in a cement factory (potassium dichromate), and one was a welder exposed to metal fumes, including nickel and chromium. All the patients had bronchial hyperresponsiveness (BH) to methacholine, which increased 24 hr after challenge with metal salts. Airway hyperresponsiveness to methacholine was assessed as the provocative concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}). The methacholine inhalation test was performed the day before the antigen challenge and again 24 hr after challenge. A two-fold or greater reduction of the PC_{20} compared to baseline value was

considered a significant change. Nickel sulfate challenge of subject 1 (electro-plating) elicited a BH response at a methacholine concentration of 10 mg/mL and in subject 2 (cement) of 0.1 mg/mL. Twenty-four hours after nickel challenge, the PC_{20} for subject 1 was 0.15 mg/mL.

Although asthma has been described in the above studies, occupational inhalation of nickel dusts has not been found to be associated with pulmonary fibrosis although an increase in irregular lung opacities was observed by Muir et al. (1993) with exposures ≥ five years in 149 nickel sinter plant workers. Pang et al. (1996) observed slight but not statistically significant increased relative risk of mortality due to non-malignant diseases of the respiratory system in nickel platers exposed to $NiCl_2$ and $NiSO_4$. The relative risk with adjustment for age, period of follow up, and year starting nickel work was 1.59 (95% CI, 0.58 to 4.36). The study suffers from low numbers (248 subjects total) and relatively brief soluble nickel exposures (mean = 2.1 yr, median 0.86 yr). An occupational epidemiology report by Broder et al. (1989) found no significant effects on pulmonary function in relation to nickel exposure in a nickel smelter.

Moulin et al. (2000) conducted a mortality study of 4898 stainless steel workers exposed to metallic alloys including nickel. Among the non-malignant endpoints included, no significant increases in standardized mortality ratios (SMRs) for chronic bronchitis, pneumoconiosis or other respiratory system effects were seen. Huvinen et al. (2002) studied 284 workers in a ferrochromium and stainless steel plant. Long-term workers (average 23 years) exposed to low levels of dusts and fumes containing molybdenum (0.3 $\mu g/m^3$), nickel (1.8 $\mu g/m^3$) and chromium (4.7 $\mu g/m^3$) did not show evidence of respiratory disease detectable by lung function tests or chest radiography. Similarly, Egedahl et al. (2001) studied mortality experience among employees at a hydrometallurgical nickel refinery and fertilizer complex in Alberta, Canada. A total of 1649 males who worked continuously for at least 12 months during the years 1954 to 1978 were followed for an additional 17 years. Exposure with this refining process involves nickel metal rather than soluble nickel or sulfides. The observed deaths due to respiratory disease were less than expected (SMR = 36, C.I. 13 to 79).

Berge and Skyberg (2003) reported evidence of increased radiographic lung abnormalities with increased exposure to soluble or sulfidic nickel, albeit with a relatively small number of cases (47/1046) and relatively mild effects. Exposure factors for 1046 refinery workers were, mean ± SD: total Ni, 5.59 ± 11.73; soluble Ni, 1.43 ± 2.23; sulfidic Ni, 0.55 ± 1.19; oxidic Ni, 3.09 ± 8.54; and metallic Ni, 0.52 ± 1.35 $(mg/m^3)yr$. For quantal dose response analysis the following mean exposures were used for sulfidic nickel: 0.03 (254 subjects), 0.27 (237), 1.03 (282), and 4.32 $(mg/m^3)yr$ (263). For soluble nickel the mean exposures were: 0.01 (264), 0.08 (237), 0.33 (282), and 1.73$(mg/m^3)yr$ (263). Pulmonary fibrosis was defined as a median reading of International Labor Organization (ILO) score ≥ 1/0. For soluble nickel exposure the crude odds ratio for pulmonary fibrosis was 4.34 (95% CI, 1.75 to 10.77). The risk adjusted for age, smoking, asbestos, and sulfidic nickel was 2.24 (95% CI, 0.82 to 6.16) with a dose-response. The

corresponding values for sulfidic nickel were crude 5.06 (95% CI, 1.70 to 15.09) and adjusted, as above except for substituting soluble nickel for sulfidic nickel, 2.04 (95% CI, 0.54 to 7.70). The prevalence values for pulmonary fibrosis and both soluble and sulfidic cumulative nickel exposure (their Tables 5 and 6) were acceptably fit by the multistage model. For soluble nickel a $BMDL_{01}$ (1 % excess risk) of 0.35 (mg Ni/m^3)-yr was obtained ($\chi^2 = 2.21$, P = 0.33). For sulfidic nickel the $BMDL_{01}$ was 0.19 (mg Ni/m^3-yr, $\chi^2 = 3.91$, P = 0.14). Dose responses on the adjusted data sets were not fit as well by the model as were the crude data. For example the soluble nickel gave a $BMDL_{01}$ of 0.69 ($\chi^2 = 3.11$, P = 0.08) when adjusted for smoking, age, asbestos and sulfidic Ni (g-adjustment) and a $BMDL_{01}$ of 0.56 (mg/m^3)-yr ($\chi^2 = 1.72$, P = 0.42) when adjusted for age, smoking and asbestos only (f-adjustment). For sulfidic nickel no BMD or BMDL could be calculated from the g-adjusted data sets and with f-adjustment the $BMDL_{01}$ was 0.34 (mg Ni/m^3)-yr ($\chi^2 = 4.16$, P = 0.125). As the authors note, the data are not strong but there is a measureable dose response for cumulative nickel exposure and pulmonary fibrosis. The mean and median exposure periods were 21.8 and 21.9 years, respectively.

Sivulka et al. (2007) reviewed the literature on nickel exposure and non-malignant respiratory disease and suggested that the failure to observe frank lung toxicity in exposed nickel workers may be related to the particle size to which the workers were exposed. The authors point out that in rat studies showing lung lesions, exposures have been to respirable-sized particles (< 4 μm diameter) whereas occupational exposures constitute largely non-respirable larger diameter particles.

Table 16. Summary of Chronic Nickel Toxicity in Humans

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Fernandez-Nieto *et al*., 2006	$NiSO_4$ $K_2Cr_2O_7$	Work-related asthma in electroplating and cement workers (N=4). Bronchial hyper-responsiveness (BH) to methacholine.	Specific inhalation challenge to reduce concentration of methacholine to cause a 20% reduction of FEV_1 (PC20) = increased BH. Both Ni and Cr gave positive responses.	Positive IgE determination for Cr and Ni was found in one subject. Skin tests negative for Cr and Ni.
Pang *et al*., 1996	$NiCl_2$ $NiSO_4$	Nickel platers, N = 248, exposure mean = 2.1 yr, median = 0.86 yr.	Mortality due to non-malignant diseases of the respiratory tract. Adjusted RR = 1.59 (95% CI = 0.58-4.36).	Low numbers and brief exposures.
Moulin *et al*., 2000	Metallic alloys incl. Ni	Steel workers N = 4898	Non-malignant endpoints, chronic bronchitis, pneumo-coniosis, and other respiratory system effects: no significant increases in SMRs.	
Berge & Skyberg, 2003	Soluble Ni Sulfidic Ni	Nickel refinery workers 47/1046, mean soluble Ni for exposure categories, 0.03, 0.27, 1.03, and 4.32 $(mg/m^3)yr$; mean sulfidic Ni 0.01, 0.08, 0.33, 1.73 $(mg/m^3)yr$.	Radiographic lung abnormalities indicative of pulmonary fibrosis (PF). Soluble Ni adjusted OR = 2.24 (95% CI = 0.82-6.16); sulfidic Ni adjusted OR = 2.04 (95% CI = 0.54-7.70).	Unadjusted data: soluble Ni $BMDL_{01}$ = 0.35 $(mg/m^3)yr$; sulfidic Ni $BMDL_{01}$ = 0.19 $(mg/m^3)yr$. Adjusted data: 0.56 and 0.34 $(mg/m^3)yr$, respectively. Data are weak but there is a measureable dose-response.

Table 16. Summary of Chronic Nickel Toxicity in Humans

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Jensen *et al.,* 2004	$NiSO_4 \bullet 6H_20$	Lymphocyte subpopulations and cytokine profiles in Ni-sensitive (N = 33) and normal (N=19) subjects. Ni-sensitive (7-10/group): 0, 0.3, 1.0, 4.0 mg Ni; controls (9-10/group) 0, 4.0 mg Ni.	PBMC isolated from blood 24 hr after Ni treatment for analysis. Ni-sensitive had significantly higher fractions of lymphocytes in their blood: $CD3^+$-type (P = 0.0035); $CD4^+$-type (P = 0.000095); $CD8^+$-type (P = 0.000007).	
Yoshioka *et al*., 2007	Ni, Cd	Ni-Cd battery workers, N = 66	Ni in urine, Cd in blood, 8-OH-G in urine. Creatinine adjusted 8-OH-G correlated with age, Ni-U, Cd-B.	Combined effects of Ni and Cd not additive. Data suggest that Ni is the main stressor increasing 8-OH-G in urine.

Note: BMDL 95% lower bound on a specific response level (e.g. $BMDL_{01}$ = lower bound on a 1% response); 8-OH-G = 8-hydroxyguanine.

Yoshioka et al. (2007) studied the urinary excretion of 8-hydroxyguanine (8-OH-G), an oxidative stress marker, in nickel-cadmium battery workers. Sixty-six subjects (64 male and two female) provided urine and blood samples. The levels of cadmium in blood (Cd-B) and nickel in urine (Ni-U) were determined by graphite furnace atomic absorption spectroscopy. 8-OH-G in urine was analyzed by high performance liquid chromatography-electrochemical detector system. Creatinine-adjusted 8-OH-G was significantly correlated with age, Ni-U, and Cd-B in univariate analysis, while multivariate analysis revealed that Ni-U and Cd-B were significantly independent variables positively correlated with 8-OH-G. The data were analyzed for mixture toxicity. The subjects were divided into groups based on median concentration of Ni-U and Cd-B (2.86 µg/g creatinine and 0.23 µg/dL, respectively). Subjects with high Ni-U/high Cd-B (Group 4) had the highest levels of 8-OH-G (21.7, 2.0, GM, GSD), followed by those with high Ni-U/low Cd-B (11.5, 1.6, Group 3), those with low Ni-U/high Cd-B (Group 2, 8.9, 1.9) and those with low Ni-U/low Cd-B (Group 1, 8.5, 1.5). The p values of Student's t-tests between Group 1 and Group 2, 3, and 4 were 0.847, 0.050, and < 0.001, respectively. The combined effect of Cd and Ni on the urinary excretion of 8-OH-G departed from additivity. The results indicate that nickel exposure was the primary stressor resulting in increased production and excretion of 8-OH-G.

Carroll and Wood (2000) exposed monolayer cultures of human keratinocytes and fibroblasts to nickel sulfate at concentrations above 0.001 M. Cytotoxicity to both cell types was 50% based on decreased viability. ^{35}S-methionine labeling followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting with specific monoclonal antibodies indicated an increased synthesis of heat shock protein 90 (Hsp90) in keratinocytes at concentrations above 10^{-5} M and induction of heat shock protein 72 (Hsp72) above 10^{-4} M. For fibroblasts increased induction of Hsp90 was seen at all concentrations tested and a dose-related increase was observed for Hsp72. The results indicate a stress response to the toxic effects of nickel ions at fairly low concentrations.

Cell lines derived from monkey kidney (COS-7), human lung tumors (A549), or human liver tumors (HepG2) were cultured for four days with 0, 100, 200, or 400 µM Ni Cl_2. Nickel treatment decreased growth rates in all cell lines after four days in a dose dependent manner. In HepG2 cells GRP96 expression was significantly enhanced at 400 µM Ni(II) ($P < 0.05$) whereas Hsp72 and Hsp73 were significantly suppressed ($P < 0.01$). COS-7 cells showed a similar pattern. GRP96 was over-expressed in A549 cells at 400 µM Ni(II) and Hsp73 was moderately increased.

Au et al. (2006) studied the cytotoxicity of nickel(II) in human T-lymphocyte Jurkat cells in vitro. Jurkat cells were incubated with 0, 1, 10, or 100 µg/mL Ni^{2+} (compound unspecified: 100 µg/mL Ni^{2+} = 1.7mM) for 24 hours. The treatment reduced cell viability and proliferation in a dose-dependent manner. Cell viability

was reduced by 35% at 100 µg Ni/mL. A significant decrease in cell proliferation was also seen at 100 µg Ni/mL. Nickel(II) at 10 µg Ni/mL induced expression of caspase-3, but not at 100 µg Ni/mL. Cells incubated at 100 µg Ni/mL showed fragmented nuclei. Enumeration of Hoechst 33258-stained cells showed that Ni^{2+} at 100 µg/mL induced 16% of the cells to undergo apoptosis. In contrast the lower Ni concentrations were indistinguishable from the control. The authors note that the onset of apoptosis by metal ions may be due to a disruption in cell signaling, DNA damage, or changes in cell constituents such as Ca^{2+}.

M'Bemba-Meka et al. (2006) exposed isolated human lymphocytes to solubilized Ni_3S_2 in vitro to assess cytotoxicity. Lymphocyte suspensions were exposed to 0, 0.25, 0.50, 0.75, 1.0, 1.5, or 2.0 mM Ni_3S_2 for 3-4 hr and to 2.0 mM Ni_3S_2 for 30, 60, 90, 120, 180 or 240 min. Cell viability was assessed by trypan blue exclusion. Nickel(II) treatment resulted in both concentration- and time-dependent lymphocyte death. Significant increases in cell death were seen at 0.75 mM Ni_3S_2 for 4 hr and 1.0 mM Ni_3S_2 for 2 hr ($P < 0.05$). Increased production of H_2O_2 and superoxide anion (O_2^-), lipid peroxidation and depletion of cellular sulfhydryl contents were induced by 1 mM Ni_3S_2. Nickel-induced lymphocyte death was significantly prevented by pretreatment with scavengers of reactive oxygen species (catalase, superoxide dismutase, dimethylthiourea/mannitol, deferoxamine or glutathione/*N*-acetylcysteine). Co-treatment with cyclosporin A inhibited Ni_3S_2-induced disturbances of mitochondrial membrane potential ($\Delta\Psi m$), and significantly prevented Ni_3S_2-induced cell death ($P < 0.05$ vs. Ni_3S_2 alone treatment). Lymphocyte death was also significantly reduced by treatment with Ca^{2+} channel blockers (diltiazem, nifedipine, and verapamil) and intracellular Ca^{2+} antagonists (dantrolene, cyclosporin A, and ruthenium red). Treatment of lymphocytes with 1 mM Ni_3S_2 alone increased intracellular Ca^{2+} about three fold over three hours. The authors interpret the findings as indicative of an activation of cell death signaling pathways involving generation of reactive oxygen species (ROS) and oxidative stress, loss of mitochondrial membrane potential, and disruption of cellular calcium homeostasis.

Guan et al. (2007) also studied the toxicity of nickel(II) in human T-lymphocyte Jurkat cell line. The cells were exposed to 0, 20, 40, 60, or 80 µg Ni/mL $NiCl_2$ for 0, 6, 12, or 24 hr and viability measured by trypan blue staining assay. Viability was less than 10% when cells were incubated for 24 hr at 80 µg Ni/mL. Treated cells exhibited morphological changes and chromosomal condensation indicative of apoptosis. The apoptotic fraction increased in a dose- and time-dependent manner. After incubation with nickel(II) for 6 hr the concentration of NO increased linearly from ca. 0.9 (control) to 3.7 µM (80 µg Ni/mL) (monitored by release of NO_2^-/NO_3^- into the cell culture medium). Nickel(II) treatment was also observed to dissipate mitochondrial membrane potential and down regulate bcl-2 mRNA after 12 hr exposure at 60 µg Ni/mL possibly modulating Ni-induced cell apoptosis. The authors speculate that a key process in the immune cellular response to nickel(II) is nickel induced apoptosis mediated by a mitochondrial pathway associated with NO.

Ke et al. (2007) studied fluorescent tracking of nickel ions in human cultured cells. Water-insoluble nickel compounds such as NiS and Ni_3S_2 were shown in vitro to enter cells by phagocytosis. Using a dye that fluoresces when intracellular Ni^{2+} ion binds to it, the authors showed that both soluble and insoluble nickel compounds elevated Ni ions in the cytoplasm and nuclear compartments. However, soluble nickel compounds were more readily removed than the insoluble nickel compounds. Within 10 hours after $NiCl_2$ removal from the culture medium, Ni ions disappeared from the nucleus and were not detected in the cells by 16 hours. Insoluble Ni_3S_2 yielded Ni ions that persisted in the nucleus after 16 hours and were detected in the cytoplasm even after 24 hours following Ni removal.

Trombetta et al. (2005) evaluated the toxic effects of nickel in a three dimensional model of human epithelium (RHE) reconstituted from TR146 cells derived from a human squamous cell carcinoma of the buccal mucosa. The RHE cultures were exposed for 72 hr to eight concentrations of $NiCl_2$ ranging from 0.05 to 7.6 mM. Cell viability, assessed by the MTT assay, was significantly reduced at Ni(II) concentrations greater than 1.3 mM. Similarly the release of prostaglandin E2 and interleukin-6 into the culture medium was also significantly increased above 1.3 mM Ni(II). However no change was seen in interleukin-8 release at any nickel concentration. In addition to cytokines the effect of nickel on glutathione (GSH) was also measured. Nickel induced a non statistically significant reduction in GSH from 2.392 nmol/cm^2 in control cultures to 2.151 nmol/cm^2 at 7.6 mM Ni(II). By contrast an increase in tissue oxidized glutathione (GSSG) was seen at all nickel concentrations and was statistically significant above 0.7 mM ($P < 0.05$). Total tissue glutathione (GSH + GSSG) appeared to increase compared to controls after nickel exposure. The ratio of GSH/GSSG was significantly reduced at all nickel concentrations tested ($P < 0.05$). The results indicate that nickel exposures that are not toxic enough to affect cell viability or inflammatory cytokine release can affect cellular redox equilibrium. The authors also observed an increase in vacuolized cells and apoptotic cells in tissue cultures at all Ni concentrations ≤ 0.7 mM without evidence of cellular necrosis. Thus low “non-toxic” nickel exposure may modify cellular effectors of apoptosis.

Davidson et al. (2005) reported that $^{63}NiCl_2$ interfered with cellular iron homeostasis in human lung A549 cell cultures. Soluble nickel was observed to block the uptake of iron into transferrin-bound iron and non-transferrin-bound iron (NTBI) leading to cellular ferritin accumulation. Since excessive iron is toxic to cells, such nickel-induced blockage might be expected to lead to cytotoxicity. Nickel also decreased the binding of Von Hippel-Landau (VHL) protein to hypoxia inducible factor 1α (HIF-1α) possibly by competing for iron sites on prolyl hydroxylases. Prolyl hydroxylases 1-3 hydroxylate the ODD (oxygen-dependent degradation) domain in HIF-1α. VHL can bind to hydroxylated proline residues in the ODD domain of HIF-1α and target it for degradation. When the prolyl hydroxylases are not functional, no hydroxylation of proline residues occurs and VHL will not bind.

Cheng et al. (2003) quantified gene expression in microarrays with cDNA chips (ca. 8000 cDNAs) after exposure of human peripheral lung epithelial cells to nickel(II). Cultured human lung epithelial HPL1D cells were exposed for 24 hr to non-cytotoxic (50, 100, or 200 µM) or cytotoxic (400, 800, or 1600 µM) Ni^{2+} concentrations. Cytotoxicity was assessed by loss of cell adhesion in 70% confluent cultures after 24 hr Ni-exposure. The data set comprising 868 genes was filtered to select only those 113 genes, which showed a ≥ 2-fold change in expression at one or more of the three nontoxic nickel concentrations. Most of the genes impacted by low nickel concentrations were related to gene transcription, protein synthesis and stability, cytoskeleton, signaling, metabolism, cell membrane, and extracellular matrix.

Gazel et al. (2008) evaluated transcriptional profiles in Ni(II) treated human epidermal keratinocytes using DNA microarrays. Reconstructed human epidermis (RHE) was exposed to 11 µM $NiSO_4$ for 30 min or 6 hr. Microarray analysis showed that 134 genes were affected by Ni(II) exposure: 97 genes were induced and 37 genes were suppressed. The functional categories of affected genes indicated that Ni(II) inhibits apoptosis, promotes cell cycle and induces synthesis of extracellular matrix proteins and proteases. Ni also regulates secreted signaling proteins, inducing vascular endothelial growth factor (VEGF), amphiregulin (AREG), placental growth factor (PGF), prostate differentiation factor (GDF15), and bone marrow stromal cell antigen 2 (BST2), while suppressing IL-18, galectin-3 (LGALS3), and lipopolysaccharide-induced TNF-α Factor (LITAF). Interestingly no Ni(II) effects were seen in epidermal differentiation genes.

Ouyang et al. (2009) studied the effect of nickel compounds on the cell cycle in human lung carcinoma A549 cells in vitro. $NiCl_2$ at doses from 0.25 to 1.0 mM were found equivalent to 0.25 to 2 µg NiS/cm^2 in the activation of transcription factor NFkB and HIF-1α, and induction of TNF-α and CAP43 gene expression. Growth of A549 cells was significantly inhibited by 0.25 mM $NiCl_2$ but only marginally inhibited by NiS at 2.0 µg/cm^2. Nickel sulfide also failed to significantly inhibit human bronchial epithelial cell line HCCBE-3 or mouse skin epidermal cell line C141. Exposure to $NiCl_2$, but not NiS, caused a significant inhibition of cell growth and G1/G0 cell cycle arrest concomitant with a marked down-regulation of cyclin D1 in A549 cells. The down-regulation is due to protein degradation rather than inhibition of transcription. The degradation of cyclin D1 is a ubiquitination- and proteosome-dependent process, but how soluble nickel initiates or regulates this process is unknown. Effects on other cell cycle regulatory proteins were also evaluated, namely cyclin E and p21. Nickel had no effect on cyclin E while both nickel compounds increased the amounts of p21.

Rossman (2009) has criticized the use of dyes, particularly Trypan Blue in the assessment of cytotoxicity when used close to the time of exposure. These methods give better results (close to results with clonal survival) when used about three days after exposure; otherwise cytotoxicity may be significantly underestimated.

Table 17. Summary of Studies with Human Cells *in vitro*

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Au *et al*., 2006	Ni^{2+}	Human T-lymphocytes Jurkat cells in vitro: 0, 1, 10, 100 µg Ni/mL for 24 hr.	Cell viability ↓ 35% at 100 µg/mL; caspase-3 ↑ at 10 µg/mL; fragmented nuclei at 100 µg/mL.	16% of cells induced to undergo apoptosis at 100 µg Ni/mL.
M'Bemba-Meka *et al*., 2006	Ni_3S_2	Isolated human lymphocytes in vitro: 0, 0.25, 0.50, 0.75, 1.0, 1.5, or 2.0 mM $Ni_3S_2$for 30 min to 6 hr	Concentration and time-dependent lymphocyte death. Significant death increases at 0.75 mM for 4 hr and 1.0 mM for 2hr ($P < 0.05$)	At 1.0 mM Ni_3S_2: H_2O_2 ↑; O_2^-↑, lipid peroxidation ↑; cellular sulfhydryl ↓.
Guan *et al*., 2007	$NiCl_2$	Human T-lymphocytes Jurkat cells in vitro: 0, 20, 40, 60, or 80 µg Ni/mL for 0, 6, 12, 24 hr.	Viability < 10% at 80 µg/mL-24 hr. After 6 hr Ni treatment NO increased from 0.9 (0) to 3.7 µM(80 µg Ni/mL)	Morphological changes and chromosome condensation indicative of apoptosis in Ni-treated cells, dose- and time-dependent.
Trombetta *et al*., 2005	$NiCl_2$	Human oral epithelium model (RHE) from TR-146 cells exposed to 0, 0.05, 0.1, 0.3, 0.7, 1.0, 1.3, 3.3, or 7.6 mM Ni for 72 hr.	Cell viability reduced at >1.3 mM Ni, prostaglandin E2 and IL-6 increased at < 1.3 mM Ni.	No changes in IL-8 noted, GSH decreased but not significantly, GSSG increased at all concentrations, $P< 0.05$ at ≥ 0.7 mM Ni.

Table 17. Summary of Studies with Human Cells *in vitro*

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Davidson *et al.*, 2005	$^{63}NiCl_2$ $FeSO_4$	Human A549 cells in culture. Cells treated with 1 mM $NiCl_2$, 500 µM $FeSO_4$, 500 µM $FeSO_4$ + 500 µM $NiCl_2$; 500 µM $FeSO_4$ + 1 mM $NiCl_2$ for 24 hr.	Ni^{2+} taken up by cells via divalent metal ion transporter 1 (DMT1). Ni blocked transferrin-dependent and transferrin independent Fe binding and led to increased cellular ferritin accumulation.	Ni decreased binding of Von Hippel-Landau (VHL) protein to HIF-1α, indicating a decrease in prolyl hydroxylase activity, affecting HIF-1α signaling pathway.
Gazel *et al.*, 2008	$NiSO_4$	Human epidermal keratinocytes (RHE) exposed to 11 µM Ni SO_4 for 30 min or 6 hr. cDNA micro array analysis of gene expression.	134 genes affected, 97 induced, 37 suppressed. Apoptosis suppressed, cell cycle and protein synthesis induced.	Factors induced: VEGF, AREG, PGF, GDF-15, BST2. Factors suppressed IL-18, LGALS3, LITAF.
Ouyang et al., 2009	$NiCl_2$ NiS	Human lung carcinoma cells A549 in vitro. 0.25 to 1.0 mM $NiCl_2$, 0.25 to 2.0 µg NiS/cm^2.	Inhibition of cell growth and G1/G0 cell cycle arrest by $NiCl_2$ at 0.25 and 0.5 mM but not NiS at 2 µg/cm^2. $NiCl_2$ also caused a marked decrease in cyclin D1 protein, NiS effect was marginal.	$NiCl_2$ and NiS doses were equivalent in activation of NFκB and HIF-1α and induction of TNF-α and CAP43 gene expression.

Afridi et al. (2010) evaluated the association between trace toxic elements zinc (Zn), cadmium (Cd), nickel (Ni) and lead (Pb) in biological samples of scalp hair, blood, and urine of 457 smoker and nonsmoker hypertensive patients and 369 referent males, residents of Hyderabad, Pakistan. Of the hypertensive subjects 297 were smokers and 160 were nonsmokers. The metal concentrations were measured by atomic absorption spectroscopy. Mean values of Cd, Ni and Pb were significantly higher in hair, blood and urine of both smoker and nonsmoker hypertensive patients than in referents ($P < 0.001$). Zinc was lower in hair and blood but higher in urine of hypertensive subjects versus referents.

The levels of Ni in scalp hair samples of nonsmoker and smoker referents were lower 6.1 ± 1.5 and 7.85 ± 0.95 µg/g, respectively than in hypertensives 12.2 ± 1.48 and 15.7 ± 0.96 µg/g, respectively. The excretion of Ni in hypertensive subjects was higher than in referents ($P < 0.0002$). The amount of nickel in tobacco ranges from 0.64 to 1.15 mg/g and the higher Ni in hair of hypertensive smokers may be due in part to Ni inhaled from smoking. The reduced Zn and higher exposure to toxic metals as a result of smoking may be synergistic with other risk factors associated with hypertension. Chronic Toxicity to Experimental Animals

Studies of chronic toxicity in animals are summarized in Table 18. The principal target site identified in these studies is the lung.

Both chronic RELs for nickel and nickel compounds (except NiO) and for NiO were based on lung toxicity seen in NTP (1994c, $NiSO_4$) and NTP (1994a, NiO). These are large studies involving several interim evaluations and relatively large numbers of mice and rats of both sexes. The critical effect for the 8-hour REL was also based on lung toxicity seen in NTP (1994c). See sections 9.4 amd 9.5 for details of these derivations.

A two-year inhalation study of nickel oxide (MMAD = 2.8 µm, gsd = 1.87, density = 7.45 g/cm^3) in rats and mice (65 per sex, per group) was conducted by the National Toxicology Program (NTP, 1994a). In the first study, rats were exposed to 0, 0.62, 1.25, or 2.5 mg nickel oxide/m^3 (0, 0.5, 1.0, or 2.0 mg Ni/m^3) 6 hours/day, 5 days/week for 104 weeks. In addition to the carcinogenic effects of nickel oxide, a number of non-cancerous lesions were observed, particularly in the lungs. The incidence of inflammatory pigmentation in the alveoli was significantly greater in all exposed groups, compared to controls. The severity of the lesions reportedly increased with increasing exposure. Atypical alveolar hyperplasia was also seen in all exposed groups. Lymphoid hyperplasia in the bronchial lymph nodes was observed in males and females exposed to 1 mg Ni/m^3 or greater at 7 and 15 months and the incidence generally increased with increasing concentration at the end of the 2-year study. Females had an increased incidence of adrenal medullary hyperplasia at all exposures of nickel oxide. Body weights were significantly lower in the groups exposed to 2.0 mg Ni/m^3 for both sexes, and in males exposed to 1.0 mg Ni/m^3.

A companion study on nickel oxide in mice conducted by NTP showed similar lung inflammatory changes as seen in the rats, in addition to pigmentation of the alveolar region at all exposure concentrations, compared with controls (NTP, 1994a). The mice were exposed to 0, 1.0, 2.0, or 3.9 mg Ni/m^3. Bronchial lymph-node hyperplasia was also evident in all nickel-exposed animals. Body weights were slightly but significantly lower in the 3.9 mg Ni/m^3 group, compared with controls.

A continuous exposure of rats (20 - 40 per group) to 0, 60, or 200 µg Ni/m^3 as nickel oxide for two years resulted in severe pulmonary damage and premature mortality so that carcinogenesis could not be evaluated (Glaser *et al.*, 1986). Pulmonary alveolar proteinosis and septal fibrosis were observed in the animals exposed to nickel. Only one rat per group survived the nickel exposures to the end of the experiment.

The NTP (1994c) studied the chronic non-cancer and carcinogenic effects of nickel sulfate hexahydrate (MMAD = 2.50 µm, gsd = 2.38, density = 2.07 g/cm^3) on rats and mice. Rats were exposed to 0, 0.12, 0.25, or 0.5 mg $NiSO_4$/m^3 (0, 0.03, 0.06, or 0.11 mg Ni/m^3) for 6 hours/day, 5 days/week for 16 days to 104 weeks. Interim evaluations were made at 16 days and 13 weeks, and 7 and 15 months. Chronic effects of nickel exposure in rats included inflammatory lesions in the lung, lung macrophage hyperplasia, alveolar proteinosis, and fibrosis, in addition to bronchial lymph node hyperplasia and nasal epithelial atrophy. The above effects were seen at exposures of 0.06 mg Ni/m^3 or greater and at interim evaluations from 13 weeks. Histological details of these effects are quoted from the NTP report:

> "The incidences of chronic active inflammation, macrophage hyperplasia, alveolar proteinosis, and fibrosis were markedly increased in male and female rats exposed to 0.25 and 0.5 mg/m^3. Chronic active inflammation consisted of multifocal, minimal to mild accumulations of macrophages, neutrophils, and cell debris within alveolar spaces, frequently subjacent to pleural surfaces (Plate 1). Macrophage hyperplasia was of minimal to mild severity and consisted of macrophages (usually with abundant pale vacuolated cytoplasm) within alveolar spaces. The source of these macrophages was probably the intravascular pool of circulating monocytes. Proteinosis consisted of minimal to mild amounts of eosinophilic granular or globular homogeneous pale, acellular, proteinaceous material within alveolar spaces (Plate 2). Fibrosis included increased connective tissue and collagen involving alveolar septae within the parenchyma and subjacent to the pleura and focal solid sclerotic areas either subjacent to the pleura or at the tips of the lung lobes. Focal alveolar epithelial hyperplasia was slightly increased in 0.5 mg/m^3 female rats. Focal alveolar epithelial hyperplasia was a discrete cluster of of alveoli lined by low cuboidal or low columnar cells."

Mice were exposed to a similar regimen that included 0, 0.06, 0.11, and 0.22 mg Ni/m^3 as nickel sulfate hexahydrate (NTP, 1994c). Similar pulmonary, lymphatic and nasal changes were observed in the mice as with the rats. Fibrosis was not reported, but an increased incidence of interstitial infiltration and alveolar proteinosis were observed at exposures of 0.11 mg Ni/m^3 or greater. No clinical findings or hematological effects were observed, but body weights were significantly depressed in all groups of nickel-exposed female mice. The body weights of males were reduced only in the group exposed to 0.22 mg Ni/m^3.

A two-year study on the effects of nickel subsulfide (MMAD = 2.54 µm, gsd = 2.1, density = 5.82 g/cm^3) in rats and mice was conducted by NTP (1994b). Rats (52-53 per sex per group) were exposed to 0, 0.15, or 1 mg Ni_3S_2/m^3 (0, 0.11, or 0.73 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. Body weights were lowered in rats exposed to 0.73 mg Ni/m^3 compared with controls. Lung inflammation, alveolar hyperplasia, macrophage hyperplasia, and pulmonary fibrosis were observed with a significantly increased incidence at both nickel concentrations. Female rats exposed to nickel had significantly increased adrenal medullary hyperplasia. In addition to the pulmonary lesions, nasal inflammation and olfactory epithelial atrophy were observed in both sexes exposed to 0.73 mg Ni/m^3.

In the second phase of the NTP study (NTP, 1994b), mice were exposed to 0, 0.6, or 1.2 mg Ni_3S_2/m^3 (0, 0.44, or 0.88 mg Ni/m^3) for 6 hours/day, 5 days/week for 104 weeks. The same pathological lesions were observed in the lung and nasal passages as in the rats in the above study. These lesions were evident at both the 0.44 mg Ni/m^3 and the 0.88 mg Ni/m^3 concentrations. The adrenal medullary hyperplasia seen in female rats was not observed in the mice.

It should be noted that although the non-neoplastic lung effects seen in the animal studies discussed above were relatively mild similar effects in humans may be serious or even fatal. For example pulmonary alveolar proteinosis (PAP) is a rare clinical condition first described by Rosen et al. (1958) with some 410 cases reported through 2002 (Seymour and Presneill, 2002). The syndrome is characterized by alveolar accumulation of surfactant components with minimal interstitial inflammation or fibrosis. PAP has a variable clinical course from spontaneous resolution to death with pneumonia or respiratory failure (Seymour and Presneill, 2002). Kitamura et al. (1999) have identified idiopathic pulmonary alveolar proteinosis (I-PAP) with an autoimmune disease. Neutralizing antibody against granulocyte/macrophage colony stimulating factor (GM-CSF) was found in all specimens of BALF from 11 I-PAP patients but not in 2 secondary PAP patients, 53 normal subjects and 14 patients with other lung diseases. A possible immunological mechanism in human alveolar proteinosis is consistent with the nickel-induced immunotoxicity and pneumotoxicity seen in the rodent studies.

An exposure of rats to either 0 or 0.97 mg Ni_3S_2/m^3 (0 or 0.71 mg Ni/m^3) for 6 hours/day, 5 days/week for 78-80 weeks resulted in decreased body weight, hyperplasia, metaplasia, and neoplasia in the lungs (Ottolenghi *et al.,* 1974).

Rats and mice (10 per group) were exposed to nickel sulfate, nickel subsulfide, or nickel oxide six hours/day, five days/week, for 13 weeks (Dunnick *et al.*, 1989). Exposure-related increases in lung weight and histological lesions were observed in both species for all nickel exposures. Histological lesions included inflammatory changes, fibrosis, and alveolar macrophage hyperplasia. Nasal lesions were also observed in animals treated with nickel sulfate or nickel subsulfide. Lung weight changes were observed at exposures of 0.05 mg Ni/m^3 or greater in female rats. Macrophage hyperplasia in the alveolar region was observed at concentrations as low as 0.02 mg Ni/m^3. Additional inflammatory lesions in the lungs were observed at 0.1 mg Ni/m^3.

Early studies on the chronic non-cancer effects of metallic nickel dust were complicated by early mortality and cancer in guinea pigs and rats (Hueper, 1958).

Tanaka et al. (1988) exposed male Wistar rats (five/dose group) to green NiO aerosols (MMAD = 0.6 µm) for 7 hr/day, 5 days/week for up to 12 months. The average exposure concentration was either 0.3 mg/m^3 or 1.2 mg/m^3. For histopathological examination, rats were sacrificed at 3, 6, and 12 months of exposure and 8 months following a 12-month exposure. The nickel content of rat lungs was as high as 2.6 mg and 0.6 mg after 12 months exposure at the high and low concentrations, respectively. Higher incidence of lesions in exposed compared to control animals was seen for pneumonia in all exposure durations at low and/or high exposure concentrations and for bronchiolar metaplasia and adenomatosis for 12 months exposure at the low and/or high exposure concentrations.

Obone et al. (1999) evaluated the effects of $NiSO_4 \cdot 6H_2O$ (0, 44.7, 111.75, or 223.5 mg Ni/L) in drinking water of male Sprague-Dawley rats exposed for 13 weeks. Alkaline phosphatase activity in bronchoalveolar lavage fluid (BALF) was significantly decreased at all dose levels compared to the control animals (8/dose group, $P < 0.05$). No significant changes were seen in the activities of alkaline phosphatase, acid phosphatase, or lactate dehydrogenase in lung tissues after 13 weeks exposure. However, a significant increase in BALF proteins was seen at 111.8 and 223.5 mg Ni/L $NiSO_4$ in drinking water ($P<0.05$).

McDowell et al. (2000) exposed C57BL/6 mice to $NiSO_4 \cdot 6H_2O$ aerosol in a steel inhalation chamber. The particulate aerosol had a MMAD of 0.22 µm and a gsd of 1.85 with a chamber concentration of 110 ± 26 $µg/m^3$. The mice were exposed for 0 (control), 3, 8, 24, 48, or 96 hr before sacrifice and assessment of the progression of lung injury by microarray analysis with murine complementary DNAs. Lung polyadenylated mRNA was isolated, reverse transcribed, and fluorescently labeled. Samples from exposed mice (Cy5 labeled) were

competitively hybridized against samples from unexposed, control mice (Cy3 labeled) to microarrays containing 8734 murine cDNAs. Of the > 8700 genes analyzed, 17 were differentially expressed at 3 hr and 255 at 96 hr. The overall pattern of gene expression with increasing lung injury was indicative of oxidative stress, hypoxia, cell proliferation and extracellular matrix repair, followed by a decrease in surfactant proteins.

Oller et al. (2008) evaluated the effects of inhaled nickel metal powder in a chronic study in Wistar rats. The animals (50/sex/dose group) were exposed by whole-body inhalation to 0, 0.1, 0.4 and 1.0 mg Ni/m^3 nickel metal powder (MMAD = 1.8 µm, gsd = 2.4) for six hr/day, five days/week for up to 24 months. High mortality in the 1.0 mg Ni/m^3 dose group resulted in earlier termination of exposures in this group. No NOAEL was observed. Non-respiratory treatment-related histopathological lesions were a granular brown pigment in the kidneys, extramedullary hematopoiesis in the spleen and hypercellularity of sternum and femoral bone marrows, all in both sexes. Respiratory tract lesions included alveolar proteinosis, alveolar histiocytosis, chronic inflammation, bronchiolar-alveolar hyperplasia and bronchial lymph node infiltrate. Nearly all of these effects exhibited dose-responses in both sexes.

A benchmark dose analysis of the data in Oller et al. (2008, their Table 5B) for the sum of moderate and severe incidences of respiratory tract lesions is summarized in Table 19. $BMDL_{05}$ values ranged from 1 to 12 µg Ni/m^3. A similar analysis of non-respiratory tract lesions (not shown) gave $BMDL_{05}$ values ranging from 8 µg Ni/m^3 (female spleen) to 27 µg Ni/m^3 (male kidney). An average dosimetric adjustment factor (DAF) of 0.395 was derived from Multipath Particle Deposition (MPPD) model (v.2) airway deposition calculations for the rat and average of human age groups (3 months to 21 years) exposed continuously to 0.1 mg Ni/m^3. The human equivalent concentration (HEC) is calculated as Rat Concentration x DAF.

At the 78-week evaluation significant increases ($P < 0.01$) were seen in mean red blood cell count (RBC), hemoglobin levels (Hb) and hematocrit values (HCT) at 0.1 and 0.4 mg Ni/m^3 in males and at 0.4 mg Ni/m^3 in females. These findings were suggested by the study authors as possibly resulting from hypoxia secondary to lung injury, however, they note that similar increases were seen in another study of oral nickel sulfate hexahydrate exposure when no lung injury was observed (Heim et al., 2007). Also, a direct effect of nickel on gene expression of erythropoietin has been reported (e.g. Salnikow et al. 2000). A continuous benchmark dose analysis was conducted on the blood effects data (Oller et al., 2008, Table 3). For male rats the $BMDL_{1SD}$ values for RBC, Hb and HCT averaged 1.9 µg/m^3 and, for females, averaged 3.1 µg/m^3. All the individual data sets were well fit visually by the polynomial model although there were insufficient degrees of freedom to do a fitness test (data not shown).

Ogami et al. (2009) evaluated the toxicity of different sizes of nickel oxide particles following intratracheal instillation in rats. Two sizes of NiO were used: a

fine sized NiO with a median diameter of 0.8 μm (nNiOm), and micrometer sized NiO with a median diameter of 4.8 μm (NiO). The particle distributions were bimodal (NiO) or trimodal (nNiOm) with lower or higher peaks than the median, respectively. The pathological effects were compared with crystalline silica (SiO_2, geometric mean diameter 1.6 μm, gsd = 2.0) and TiO_2 (geometric mean diameter 1.5 μm, gsd = 1.8) particles. The particles (2.0 mg) were suspended in 0.4 mL saline and instilled into Wistar rats (10 weeks old, 25 animals/group) along with a saline only control group. Animals were sacrificed at three days, one week, one month, three and six months after particle instillation. At autopsy 50 mL of bronchoalveolar lavage fluid (BALF) were obtained by injecting saline into the right lung of each animal. Total cells and polymorphonuclear leukocytes (PMN) in BALF were recovered and counted.

The number of total cells in BALF in the nNiOm group was significantly higher than the control and the other particle treatments at all time periods except SiO_2 at 6 mo when comparable values were seen (all $P < 0.01$). NiO showed a gradual increase in total cells with a significant difference at 6 mo ($P < 0.05$). The PMN percentages in BALF were significantly higher than controls for nNiOm and SiO_2 for all time periods, although nNiOm decreased over time (40% to10%) while SiO_2 increased (40% to 65%) (all $P < 0.01$). TiO_2 also showed a significant increase at three days only (25%, $P < 0.05$). The inflammation area rate by the point counting method showed a gradual increase for nNiOm with significant increases vs. controls at all time points with a peak at 3 mo ($P < 0.01$). SiO_2 also increased gradually showing the highest value at 6 mo ($P < 0.01$). No significant differences were seen for the NiO or TiO_2 groups. The results suggest that submicrometer nano-nickel oxide is significantly more toxic to the lung than micrometer-sized nickel oxide. The observed effects were similar in qualitative and quantitative respects to those caused by similar administration of crystalline silica but apparently less persistent.

Lu et al. (2009a) evaluated several short-term in vitro assays for predicting the potential of metal oxide nanoparticles including NiO to cause pulmonary inflammation. The assays were intrinsic free radical generation, extracellular oxidative activity, cytotoxicity to lung epithelial cells, hemolysis, and inflammation in rat lungs via intratracheal instillation. Twelve nanoparticle species (NPs) ranging from 2-4 nm (Al_2O_3, alumina 1) to 300 nm (Alumina 3) were included in the study. The nickel oxide was characterized as 10-20 nm in size, 92 m^2/g in surface area, and 5.4 mg/500 cm^2 in mass (their Table 1, we calculate as 0.54 mg/500 cm^2). Intrinsic free radical generation (IFR) was assessed by electron paramagnetic resonance with surface area doses of 1,500 and 3,000 cm^2/mL. Only NiO, CeO_2, Co_3O_4 and carbon black (CB) showed significant increases in IFR over control ($P < 0.05$). Oxidative potential was measured with a cell-free dichlorofluorescein assay and significant fluorescence intensity over control was observed only for NiO, Co_3O_4, and CB ($P< 0.05$). Cytotoxicity was assessed by incubating alveolar A549 cells with NPs at different surface area doses (9.4 – 300 cm^2/mL) for 24 hr and measuring lactate dehydrogenase (LDH) release in cell lysates. There were clear positive LDH dose-responses for NiO, Co_3O_4 and

CB. Linear dose-dependent hemolytic activity in fresh human venous blood was observed for NiO, CeO_2, and alumina 2. Lung inflammation in vivo was assessed by intratracheal instillation of NPs at 500 cm^2/mL in rats and measuring polymorphonuclear neutrophil (PMN) numbers in BALF 24 hr after instillation. Only NiO and alumina 2 were significantly inflammogenic at the dose employed. Of the assays evaluated, only blood hemolysis gave a correct prediction of lung inflammatory activity for 12/13 NPs (CeO_2, false positive). NiO gave the strongest positive response in all five assays and gave the largest inflammation response *in vivo* (total PMN).

Table 18. Summary of Chronic Nickel Toxicity in Animals

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Tanaka et al., 1988	NiO (green) aerosols MMAD = 0.6 µm	Male Wistar rats 5/dose group: 0, 0.3, 1.2 mg/m^3, 7 hr/d, 5 d/wk, 3, 6, 12 mo.	Ni content of organs and lung histopathology. Lung Ni 0.6 and 2.6 mg, respectively. Lung lesions at 12 mo: pneumonia at 0.3 mg/m^3; bronchitis with atypical gland hyperplasia, bronchiolar metaplasia, adenomatosis at 1.2 mg/m^3.	After an 8 mo clearance period following a 12 mo exposure, no lung lesions were observed in the low dose group. Lower incidences of pneumonia, bronchitis and bronchiolar metaplasia were seen at the high dose. No adenomatosis was seen in this group.
NTP, 1994a	NiO MMAD = 2.8 µm, gsd = 1.87, density = 7.45 g/cm^3	2-Year Inhalation study in rats, 65/sex/dose group 0, 0.62, 1.25, 2.5 mg NiO/m^3 0, 0.5, 1.0, 2.0 mg Ni/m^3, 6hr/d, 5d/wk, 104 weeks.	Lung lesions: dose-dependent atypical alveolar epithelial hyperplasia in males and females. Chronic inflammation of the lung in most animals exposed ≥ 7 mo.	Pigmentation in the alveoli of exposed rats. Pigmentation in bronchial lymph nodes similar to lung except 0.62 mg/m^3 animals at 7 mo.
NTP, 1994a	NiO MMAD = 2.8 µm, gsd = 1.87, density = 7.45 g/cm^3	2-Year Inhalation study in mice 65/sex/dose group: 0, 1.25, 2.5, or 5.0 mg NiO/m^3, 0, 1.0, 2.0, 3.9 mg Ni/m^3, 6hr/d, 5d/wk, 104 weeks.	Lung lesions: Chronic inflammation increased with exposure in males and females at ≥ 7mo. At 2 yr incidences of chronic inflammation, alveolar epithelial hyperplasia and proteinosis most severe in high dose mice.	Pigment in the lungs increased with exposure conc. at ≥ 7 mo. Lymphoid hyperplasia dose- and time-related increases in males and females. Lung Ni burdens at 15 mo 331 to 2258 µg/g lung (dose- and time-dependent)

Table 18. Summary of Chronic Nickel Toxicity in Animals

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
NTP, 1994b	Ni_3S_2 MMAD = 2.54µm, gsd = 2.1, density = 5.82 g/cm^3	2-Year Inhalation study in rats 52-53/sex/dose group: 0, 0.15, or 1.0 mg Ni_3S_2/m^3, 0, 0.11 or 0.73 mg Ni/m^3, 6hr/d, 5d/wk, 104 weeks.	Lung lesions: inflammation, alveolar hyperplasia, macrophage hyperplasia, pulmonary fibrosis. Body weights lowered at high dose.	Females had significantly increased adrenal medullary hyperplasia. Nasal inflammation and olfactory epithelial atrophy seen in both sexes at 0.73 mg Ni/m^3.
NTP, 1994b	Ni_3S_2 MMAD = 2.54µm, gsd = 2.1, density = 5.82 g/cm^3	2-Year Inhalation study in mice, 52-53/sex/dose group: 0, 0.6, or 1.2 mg Ni_3S_2/m^3, 0, 0.44 or 0.88 mg Ni/m^3, 6hr/d, 5d/wk, 104 weeks.	Lung lesions: inflammation, alveolar hyperplasia, macrophage hyperplasia, pulmonary fibrosis.	Nasal inflammation and olfactory epithelial atrophy seen in both sexes at 0.88 mg Ni/m^3.
NTP, 1994c	$NiSO_4$•$6H_2O$ MMAD = 2.50µm, gsd = 2.38, density = 2.07 g/cm^3	2-Year Inhalation study in rats 52-53/sex/dose group: 0, 0.12, 0.25 or 0.5 mg $NiSO_4$/m^3, 0, 0.03, 0.06 or 0.11 mg Ni/m^3, 6hr/d, 5d/wk, 104 weeks.	Lung, lymph nodes and nasal lesions: active pulmonary inflammation, macrophage hyperplasia, alveolar proteinosis, fibrosis, lymph node hyperplasia, olfactory epithelial atrophy.	
NTP, 1994c	$NiSO_4$•$6H_2O$ MMAD = 2.50µm, gsd = 2.38, density = 2.07 g/cm^3	2-Year Inhalation study in mice 60-61/sex/dose group: 0, 0.25, 0.5 or 1.0 mg $NiSO_4$/m^3, 0, 0.06, 0.11 or 0.22 mg Ni/m^3, 6hr/d, 5d/wk, 104 weeks.	Lung lesions: Chronic active inflammation, bronchialization, macrophage hyperplasia, interstitial infiltration, alveolar proteinosis, at high dose in both sexes and in females at 0.11 mg Ni/m^3.	

Table 18. Summary of Chronic Nickel Toxicity in Animals

Study	Compound	System	Toxic Endpoint	Comments / other effects observed.
Glaser et al., 1986	Ni (form and size unspecified)	2-Year Inhalation Study in rats 20-40/dose group: continuous exposure 0, 60, 200 µg Ni/m^3.	Severe pulmonary damage and mortality. Pulmonary alveolar proteinosis, septal fibrosis.	Only 1 Ni-exposed rat survived for 2 yr.
Obone *et al*., 1999	$NiSO_4 \cdot 6H_2O$	13-week drinking water study in rats 8 rats /dose group: 0, 0.02, 0.05, 0.1% or 0, 44.7, 111.75, 223.5 mg Ni/L dw.	Alkaline phosphatase activity in BALF significantly decreased at all dose levels ($P < 0.05$).	Blood: total proteins ↓; plasma albumins ↓; globulins ↓; plasma glutamate-pyruvate transaminase ↓ all $P < 0.05$ at high dose.
Oller et al., 2008	Ni metal powder MMAD = 1.8µm gsd = 2.4	2-Year inhalation study in rats 50/sex/dose group: 0, 0.1, 0.4, 1.0 mg Ni/m^3, 6hr/d, 5 d/wk, 24 mo	Respiratory tract lesions: alveolar proteinosis, alveolar histiocytosis, chronic inflammation, bronchial alveolar hyperplasia, bronchial lymph node infiltrate, most effects in both sexes.	High mortality at 1 mg Ni/m^3, granular brown pigment in kidneys, extramedullary hematopoiesis in spleen, hypercellularity in sternum and femoral bone marrows.
Ogami et al., 2009	NiO 0.8µm MMAD (nNiOm) 4.8 µm MMAD (NiO)	Intratracheal instillation in rats: 5/dose group examined at 3d, 1 wk, 1 mo, 3 mo, 6 mo post treatment, single 2 mg doses.	BALF: significantly increased total cells, % PMN and inflammation at all time points with nNiOm vs. controls ($P < 0.01$) and for total cells at 6 mo with NiO ($P < 0.05$).	

Note: AM = alveolar macrophages; MMAD = mass median aerodynamic diameter; BALF = bronchial alveolar lavage fluid; PMN = polymorphonuclear lymphocytes; ↑ = increase; ↓ = decrease.

Table 19. Benchmark Dose Analysis of Respiratory Tract Lesions Induced by Nickel Metal Inhalation in Wistar Rats (Data of Oller et al. 2008).*

Lung Lesion Observed	Incidence at 0, 0.1, 0.4 mg/m^3	X^2	P	BMD_{05} mg/m^3	$BMDL_{05}$ mg/m^3	$BMDL_{05}$ µg/m^3 Continuous*
Male						
Proteinosis	0/50, 19/50, 40/50	0.35	0.83	0.012	0.0095	1.7
Histiocytosis	0/50, 7/50, 17/50	0.69	0.71	0.045	0.0326	5.8
Inflammation	0/50, 1/50, 22/50	0.34	0.84	0.12	0.07	12.5
Hyperplasia	1/50, 3/50, 9/50	0	1.0	0.12	0.069	12.3
Lymph node infiltrate	0/34, 4/37, 9/42	1.16	0.56	0.073	0.0475	8.5
Female						
Proteinosis	0/50, 22/50, 38/54	0	1.0	0.0077	0.0053	0.95
Inflammation	0/50, 10/50, 23/54	0	1.0	0.021	0.012	2.1
Lymph node infiltrate	0/39, 4/42, 9/44	0.88	0.64	0.078	0.051	9.1

*Note: All dose responses fit with the multistage-quadratic model of BMDS v 1.4.1c; values are for rats adjusted for continuous exposure (values multiplied by 6/24 x 5/7) but not for human equivalent concentrations. X^2 and P are the goodness of fit statistics. An acceptable fit has a P value ≥ 0.1)

Morimoto et al. (1995) studied the effects of nickel oxide (green) (MMAD = 2.7 µm, gsd = 2.3) on the production of tumor necrosis factor (TNF) by alveolar macrophages of rats exposed in vitro and in vivo. For in vivo exposure five male Wistar rats (nine weeks old) were exposed to 11.7 ± 2.0 mg NiO/m^3 for 8 hr/day, 5days/week, for 4 weeks along with five unexposed control animals. Bronchoalveolar lavage was performed and recovered alveolar macrophages were assayed for TNF production. Nickel oxide exposure produced a three-fold higher concentration of TNF produced by macrophages from exposed animals compared to controls ($P < 0.01$). In addition acid phosphatase and lactate dehydrogenase (LDH) release from macrophages were also significantly greater ($P<0.01$) than controls, both indicators of cytotoxicity.

Shiao et al. (1998) investigated the effects of nickel acetate on cell cycle, apoptosis and p53 expression in Chinese hamster ovary (CHO) cells in vitro. CHO cells were grown for 72 hours in medium containing 0, 40, 80, 160, 240, 320, 480, or 640 µM nickel(II) acetate. DNA fragmentation, representative of

apoptosis, was examined by gel electrophoresis. The distribution of cells in various stages of the cell cycle was determined by DNA flow cytometry and p53 expression by the Western blotting technique. DNA fragmentation was seen at nickel concentrations ≥ 160 µM. The proportion of cells at S-phase declined in a Ni^{2+} concentration-dependent manner above 160 µM (33% to 12%). The decline was accompanied by an increase in the proportion of G_2/M phase cells (9% to 26%). Expression of p53 was not affected by nickel exposure. The authors conclude that these cellular responses were most likely induced by a common effector(s) that cause G_2/M arrest and concurrent apoptosis. P53 protein is apparently not responsible for the effects seen but nickel(II) up-regulates other proteins, which may be involved.

Gurley et al. (1983) studied the toxicity to CHO cells in vitro of particulate Ni_5As_2, one of a number of nickel arsenides formed during oil shale retorting. The Ni_5As_2 particles (examined by electron microscopy) ranged in size from 0.14 to 9.40 µm with 1.8% >2µm, 75% 0.23 to 1.0 µm, and 94% 0.18 to 1.40µm. The insoluble Ni_5As_2 powder was suspended in culture medium with the cells at concentrations of 0, 10, 25, 50, and 100 µM Ni_5As_2 (assuming complete solubility of the powder). At 10 µM Ni_5As_2 the growth rate doubling time was increased from 16.5 hr (control) to 40 hr. At 100 µM Ni_5As_2 growth was completely inhibited. Cell cycle analysis showed that at Ni_5As_2 concentrations ≥ 50µM cells accumulated in the G2 +M phases. Cells treated for 24 hr with 25 µM Ni_5As_2 and transferred to nickel arsenide free medium completely recovered viability but grew at a slower than control rate. Cells similarly treated at 50 or 75 µM nickel arsenide had survivals of only 61% and 25%, respectively.

Takahashi et al. (1999) studied the cytotoxicity of two types of NiO (black and green) and five intermediate types prepared by calcinations of black NiO at 600-1000ºC. The NiO forms varied in Ni and O content, color and X-ray diffractometric pattern. They also varied in water solubility from NiO(B) at 6-7µg/mL to 1-3 µg/mL for calcined forms and 0.5-1.5 for NiO(G). Cytotoxicity was assessed with rat alveolar macrophages obtained from female Sprague-Dawley rats aged 12-16 weeks and CHO cells cultured in vitro. The viability of rat alveolar macrophages exposed to NiO at 800 µg/mL for 18, 42 and 72 hr showed the greatest toxicity for NiO(B) followed by NiO(600ºC) and NiO(800ºC). CHO cells exposed to 50, 100, or 200 µg/mL of each nickel oxide for 24 hr exhibited a dose and compound related decrease in cell proliferation from NiO(B) to NiO(G) with the calcined forms in order of temperature. The authors conclude that water solubility, which is inversely related to calcination temperature, modulates the cytotoxicity of NiO particles.

Clemens and Landolph (2003) evaluated the cytotoxicity and cell transformation of mouse embryo cells by samples of nickel refinery dust containing different concentrations of nickel arsenide and pure nickel arsenide. Mouse embryo C3H/T101/2 cells (200/dose) were treated with 0, 0.5, 1.0, 2.5, 5.0 or 7.5 µg/mL. The dust samples were composed largely of NiO and $Cu_2Ni_8O_{10}$ with 25% Ni_5As_2 in dust sample 1 and 2.5% Ni_5As_2 in dust sample 2. After treatment for 48 hr the

cells were recovered and assayed for survival. For each treatment the average survival fraction was plotted to determine the 50 percent lethal concentration (LC_{50}) value. Dust sample 1 and nickel arsenide gave an identical LC_{50} value of 2.4 µg/mL, whereas dust sample 2 with less Ni_5As_2 gave a slightly lower LC_{50} of 1.7 µg/mL. Although the dust sample appeared to be more cytotoxic than the other samples, the reverse was true in parallel chromosome aberration and cell transformation assays.

Nickel chloride induced lactate dehydrogenase (LDH) release and lipid peroxidation (LPO) in rat renal cortical slices in vitro in a concentration- (0 to 2.0 mM) and time- (0 to 4 hr) dependent manner (Chakrabarti and Bai, 1999). Both $NiCl_2$-induced LDH release and LPO were significantly prevented by glutathione and dithiothreitol, suggesting that $NiCl_2$-induced renal cell injury is partially dependent on thiols. Superoxide dismutase partially reduced the $NiCl_2$-induced LDH release without affecting LPO and glutathione, whereas catalase did not affect such LDH release and LPO. Dimethylthiourea and DMSO completely prevented $NiCl_2$-induced LPO, but only partially reduced LDH release. Deferoxamine prevented $NiCl_2$-induced renal cell injury without affecting LPO and without significantly reducing Ni^{2+} uptake by the renal cortex, suggesting that nickel chelation is not important in prevention of cell injury. $NiCl_2$-induced loss of cellular glutathione was significantly prevented by thiols and deferoxamine, but not by superoxide dismutase or dimethylthiourea. The results suggest that LPO was not related to $NiCl_2$-induced lethal renal cell injury. Renal cell injury was more likely the result of the induction of the Fenton reaction, generating hydroxyl radicals.

The effects of nickel chloride on the expression patterns of stress proteins in rat organs and human and monkey cell lines was studied by Hfaiedh et al. (2005). Three-month old female Wistar rats were injected i.p. with 4 mg $NiCl_2$/kg bw for 1, 3, 5, or 10 days. Rat kidneys, liver and ovaries were cut into small pieces, sonicated briefly in lysis buffer, and 5000 x g (30 min) supernatants collected and frozen until use. Relative protein expression in total organ extracts was measured for three proteins, namely, cytosolic Hsp72 and Hsp73, and the reticulum-associated GRP94. In kidney, nickel induced significant increases ($P < 0.01$) in GRP96 and Hsp73 at ≥ 3 days of treatment (GRP96) and at 3 and 5 days (Hsp73). Hsp72 was significantly suppressed at all days of treatment ($P < 0.05$). Few effects were noted in liver or ovary. Dietary restriction (1 month 50%) did not significantly alter the results. The authors infer that Ni-induced GRP94 over-expression in kidney and in cell lines could be mediated by hypoxic stress at the cellular level.

The effects of nickel ions on reductive amination and oxidative deamination activities of bovine liver glutamate dehydrogenase (GDH) were studied kinetically by UV spectroscopy (Ghobadi et al., 2007). The fact that Ni^{2+} ions have the capacity to enhance binding of NADH (reduced nicotinamide adenine dinucleotide) to the enzyme was confirmed by an electrochemical method. Ni^{2+} decreased the K_m for NADH from 0.083 mM (control) to 0.053 mM at 200 µM

$NiCl_2$. The NADPH (reduced nicotinamide adenine dinucleotide phosphate) K_m was similarly decreased (0.077 to 0.036 mM, respectively). Lineweaver-Burk plots with respect to alpha-ketoglutarate and ammonium ions indicated substrate and competitive inhibition patterns in the presence of nickel ion, respectively. Adenosine diphosphate (ADP) at 0.2 mM protected inhibition caused by nickel. The observations are explained by the authors in terms of formation of a nickel-NADH complex with a higher affinity for binding to the regulatory site in GDH, than in the absence of nickel. (The K_m is the Michaelis or affinity constant for Michaelis-Menten enzyme kinetics defined by the rectangular hyperbola, reaction velocity $V = V_{max} \times S/(K_m + S)$ where V_{max} is the maximum reaction rate (e.g., mg/hr), S is the substrate concentration (mg/L) and K_m is the concentration at $V_{max}/2$.)

Lu et al. (2009b) studied the mechanisms of cytotoxicity of Ni(II) ions based on gene expression profiles. Mouse fibroblast cells (L-929) were cultured in medium with 0, 100, 200, 300, 400, or 500 µM $NiCl_2 \cdot 6H_2O$ for 24, 48, or 72 hours. Cytotoxicity was assessed by methylthiazoltetrazolium (MTT) assay. Ni-induced cytotoxicity was dose- and time-dependent. After 72 hr, cell viability was reduced from 100% (control) to 36.1% at 500 µM. Gene expression was assessed by cDNA microarray analysis of cells treated with 200 µM Ni(II) for 24, 48, or 72 hr. Twenty up-regulated and 19 down-regulated genes were differentially expressed in all three exposure periods. Gene ontology analysis showed that the Ni-affected genes represented biological processes (e.g., development- 7%, cellular process-36%, physiological process-38%), molecular function (e.g., binding-52%, catalytic activity-24%, signal transducer-6%), and cellular components (cell-48%, protein complex-8%, organelle-36%). Specifically the down-regulation of the *Hsp90aa1* gene affected the processes associated with cell adhesion, cell morphogenesis, regulation of cell proliferation, and regulation of cell migration. Overall the results showed broad effects on gene expression even when no obvious cytotoxicity was evident (i.e., 91.5% viability at 200 µM Ni(II), 24 hr). Ni(II) has extensive effects on cells by inhibiting cell proliferation and differentiation, through inducing cell apoptosis, affecting cell development and influencing cholesterol metabolism.

Rubanyi and Kovach (1980) observed the effects of $NiCl_2$ on contractility, NADH-fluorescence, O_2-consumption and total coronary resistance (TCR) of isolated perfused rat hearts. Ni^{2+} at 1 mM abolished contractability, reduced O_2 consumption, increased TCR and caused a biphasic NADH-fluorescence response. Inhibition of cardiac contractability was dose-dependent in the Ni^{2+} concentration range 10^{-7} to 10^{-3} M, in the presence of 1.3 mM Ca^{2+}. The amplitude of TCR elevation reached its maximum at 10^{-6} M Ni^{2+}. Koller et al. (1982) reported Ni-induced coronary vasoconstriction in dog heart in situ in the presence of the selective Ca-antagonist verapamil. Verapamil abolished the coronary blood flow (CBF) and basal conductance (BC) decreasing the effect of low doses of Ni^{2+} (0.02-0.2 mg/kg). Higher doses of $NiCl_2$ increased CBF and BC in the presence of verapamil. The authors conclude that trace amounts of exogenous $NiCl_2$ induce coronary vasoconstriction in the dog heart in situ by enhancing Ca^{2+} influx into vascular smooth muscle cells.

Golovko et al. (2003) studied the possible role of the Na-Ca exchange (NCX) in arrhythmogenesis in isolated rat heart atrial preparations using microelectrodes. In preparations with low beating frequency (~48/min) a partial inhibition of NCX by 0.3 mM Ni(II) was observed to cause a single early afterdepolarization (EAD) at 15 min. In preparations with a high beating frequency (~84/min) 0.3 mM Ni(II) did not cause EAD, but at a higher concentration of 0.5 mM a single EAD was observed. The authors conclude that Ca2+ overload due to partial block of NCX may contribute to the development of atrial tachyarrhythmias.

Wellenius et al. (2002) studied the effects of Boston residual oil fly ash (ROFA, 3 mg/m^3) in a rat model for myocardial infarction. The ROFA was reported to produce arrhythmias, ECG abnormalities, and decreases in heart rate variability (HRV). Increased arrhythmias, decreased heart rates, and hypothermia were seen in monocrotaline-treated Sprague-Dawley rats exposed to 15 mg/m^3 ROFA (Watkinson et al., 2000). The same concentration of ROFA in spontaneously hypertensive (SH) rats caused cardiomyopathy, monocytic cell infiltration, and increased expression of cardiac cytokines IL-6 and TGF-β. ROFA-exposed SH rats also exhibited ECG abnormalities compared to air-exposed rats. Inhalation of 50 $\mu g/m^3$ of oxides or sulfates of Ni or V for 3 hr/d for 3 consecutive days in old dogs with preexisting cardiac abnormalities showed no acute changes in cardiovascular function (Muggenburg et al. 2003). However, in a different study $NiSO_4$ (>1.2 mg/m^3, 6hr/d, for 4 days) caused delayed bradycardia, hypothermia, and arrhythmogenesis in rats (Campen et al., 2001).

Lippmann et al. (2009) evaluated the cardiovascular effects of nickel in ambient air in a mouse model of atherosclerosis. Six week old *ApoE*$^{-/-}$ mice were implanted with electrocardiograph (ECG) transmitters three weeks prior to the initiation of exposure. Ten-second ECG, heart rate (HR), activity, and body temperature were sampled every 5 minutes. Six mice were exposed to 10 times concentrated air particulate matter (CAPs, with 43 to 174 ng Ni/m^3) or filtered air for 6 hr/d, 5 days/ week, for 6 months. Six control mice were sham exposed to

the same protocol. To estimate the effects of exposure on HR and heart rate variability (HRV), generalized additive models (GAMs) were used to fit the nonlinear trends of chronic and acute effects. Of the four metals evaluated in the GAM for acute HR effect only nickel was a significant CAP component (β = 3.321 ± 1.628SE, P = 0.041). Similarly for acute HRV only nickel was significant (β = 0.044 ± 0.016SE, P = 0.005). The authors note the paucity of mechanistic studies on the cardiovascular effects of Ni but also note nickel's effects on signaling pathways that may have an adverse cumulative effect on vascular function.

Kang et al. (2011) found that inhaled nickel hydroxide nanoparticles exacerbated atherosclerosis in hyperlipidemic, apoprotein E-deficient (ApoE-/-) mice exposed to 0 or 79 μg Ni/m^3, via whole body inhalation, for 5 hr/day, for either 1 week or 5 months. The nanoparticles of $Ni(OH)_2$ induced significant oxidative stress and inflammation in the pulmonary and extrapulmonary regions. These effects were indicated by up-regulated levels of antioxidant enzyme and inflammatory cytokine genes, increased mitochondrial DNA damage in the aorta, significant signs of inflammation in BALF, and alterations in lung histopathology. After 5 month's exposure the nickel nanoparticles exacerbated the progression of atherosclerosis in the ApoE-/- mouse model.

8 Immunotoxicity

8.1 Immunotoxicity Summary

Contact dermatitis is a widespread disease and, in the western hemisphere, nickel sensitization is the most common single cause of contact allergy (Lisby, 1999b). The mechanism underlying nickel-induced allergy is still incompletely understood. As noted in the papers described below most research has focused on T cell activation in Ni-allergic patients. Systemic contact dermatitis in humans has been used to study inflammatory skin disease occasionally seen as a flare-up of previous dermatitis or as de novo dermatitis when sensitized individuals are exposed to the hapten orally, transcutaneously, intravenously or by inhalation. Studies of immunological mechanism of Ni-induced disease have tried to determine if effects are elicited primarily via activation of CD4+ and/or CD8+ T cells of the type 1 or type 2 or even type 0 cytokine profile subsets (Jensen et al., 2004). The likely involvement of MAPK and possibly other signaling pathways in the disease process has added another level of complexity. The potential role of nickel in airborne particulate matter (PM2.5)-induced human respiratory disease may also have an immunological mechanism.

8.2 Human Immunotoxicity Studies

Dermal exposure to nickel and nickel alloys has long been known to cause dermatitis in both nickel workers and the general population. A number of studies indicated that oral exposure of nickel could aggravate nickel dermatitis in people who are sensitive to nickel. Christensen and Möller (1975) found that oral administration of nickel (approximately 5 mg) in diet worsen hand eczema in nickel-allergic patients. In a clinical trial, Kaaber et al. (1978) reduced the nickel dose to 2.5 mg and observed flaring of hand dermatitis in 13 of the 28 patients with chronic nickel dermatitis. A similar finding was reported by Veien et al. (1983); they observed that 26 patients had flare-ups following oral challenge with nickel compounds (2.5 mg nickel in a capsule). The conditions of some of the patients improved when they were placed on a low-metal allergen diet for four to six weeks (Kaaber et al., 1978; Veien et al., 1983).

Cronin et al. (1980) gave groups of five fasting female patients that had hand eczema a gelatin lactose capsule containing nickel, together with 100 ml of water. Three doses were used, 2.5 mg, 1.25 mg, and 0.6 mg nickel as nickel sulfate. After administration of nickel, the fast was continued for a further hour, at which time the patient was given a cup of coffee; thereafter, normal meals were taken. Assuming a female body weight of 62 kg (OEHHA, 2000b, p10-4) and the lowest dose that aggravated nickel dermatitis of 0.6 mg, we estimate a LOAEL of 9.7 µg Ni/kg bw.

Nielsen et al. (1999) studied the aggravation of nickel dermatitis in people by giving them an oral dose of soluble nickel. Twenty nickel-sensitized women and 20 age-matched controls, both groups having vesicular hand eczema of the

pompholyx type, were given a single dose of nickel in drinking water (3 µg/mL or 12 µg Ni/kg bw). All patients fasted overnight and fasting was maintained for another 4 hours after the nickel administration. Nielsen et al. (1999) reported that nine of 20 nickel-allergic eczema patients experienced aggravation of hand eczema after nickel administration, and three also developed a maculopapular exanthema. No exacerbation was seen in the control group. From the results of this study, we identified a LOAEL of 12 µg Ni/kg bw for the nickel-sensitized women.

A number of human studies have shown that oral administration of low levels of soluble nickel over a long period of time may reduce nickel contact dermatitis. Sjovall et al. (1987) orally administered 0, 5 or 0.5 mg nickel per day to a group of patients allergic to nickel. After six weeks, they found evidence of reduced sensitization in patients exposed to 5 mg/day but not to 0.5 mg/day. Santucci et al. (1988) gave a single oral dose of 2.2 mg Ni to 25 nickel-sensitized women and found that 22 reacted to the treatment. After a 15-day rest period, the subjects were given gradually increasing doses under the following schedule: 0.67 mg Ni/day for one month, 1.34 mg Ni/day for the second month, and 2.2 mg Ni/day for the third month. In the last phase of the testing, 3/17 of the subjects had flare-ups even at the lowest dose. The other 14 subjects, however, did not respond to the highest dose, even though they had responded to that dose in the initial testing.

Boscolo et al. (1999) evaluated systemic effects of ingested nickel on the immune system of nickel-sensitized women. Twenty-eight women were administered 10 mg of $NiSO_4$. Group A consisted of 19 non-atopic Ni-sensitized or nine non-allergic women. After Ni ingestion non-allergic and 12 Ni-sensitized women were asymptomatic (non-responders, group B) while seven Ni-sensitized women showed a flare up of urticaria and/or eczema (responders, group C). Before Ni treatment, groups B and C showed higher values of blood CD19+ (280 for both groups, vs. 150 pg/mL for Group A, $P < 0.05$) and CD5--CD19+ (235 for B,183 for C, vs. 113 pg/mL for A, $P < 0.05$). Group C also showed higher serum interleukin (IL) 2 (538 vs. 483) and lower serum IL-5 (296 vs. 445, $P < 0.05$) than Group A. Four hours after Ni ingestion, group C showed a significant increase in serum IL-5 (+53.7%, $P <0.05$). Twenty-four hours after treatment, group A showed a significant reduction in blood CD4+-CD45RO- “virgin” cells and an increase of CD8+ lymphocytes, while group C showed a marked decrease in total blood lymphocytes and CD3+(-41.5%), CD4+-CD45RO-(-46.5%), CD4+-CD45RO+(-35.6%), CD8+(-34.6%), CD19+(-28.8%), and CD-CD19+(-20.8%) cell subsets (all $P <0.05$ by Kruskall-Wallis test and/or Wilcoxon matched-pairs signed-rank test). Overall the results suggest that Ni ingestion induces a change in immune response from a TH-1 like pattern to a TH-0 like pattern in responder patients with systemic symptoms, as indicated by elevated serum IL-2 and IL-5 during the test.

Rietschel et al. (2008) studied trends in nickel sensitivity in 25,626 North American subjects over the period 1992 to 2004. The data exhibited a steady

increase in nickel sensitivity indicated by patch test from 14.5% in 1992 to 18.8% in 2004 ($P < 0.0001$). Females were 1.1 to 1.2 times more likely to be allergic in the late (2001-2004) group compared to the early group (1992-1995) with a relative risk (RR) = 1.2, 95% C.I. 1.10-1.28, $P < 0.0001$, or the middle group (1996-2000) $P = 0.0011$. Younger males and females (≤ 18 yr) showed significantly higher sensitivity compared to older subjects, i.e. 14.1% (55/389) vs. 6.1% (536/8839) in males and 32.4% (177/546) vs. 21.4% (3385/15,821) in females. The cause of increased sensitivity is unclear but seems indicative of increased population exposures to nickel possibly related to body piercing (Nielsen et al., 1993; Meijer et al., 1995).

Mann et al. (2010) conducted a cross-sectional study of airborne nickel exposure and nickel sensitization in 309 6-year old children from three towns in North Rhine Westphalia, Germany (about 100 subjects from each town). Two of the towns were in the proximity of steel mills (Duisburg and Dortmund) and one was in a rural area (Borken). Ambient air quality data and Lagrangian dispersion modeling were used to estimate individual annual average air concentrations. Assessment of internal nickel exposure was accomplished by analysis of morning urine samples by electro-thermal atomic absorption spectroscopy. Nickel content of drinking water was also analysed as a potential confounder. Nickel sensitization was measured with a dermatological patch test. A weak but significant correlation ($r = 0.256$, 95% CI = 0.137-0.375, $P < 0.001$) was observed between nickel concentration in ambient air and urine using Pearson correlation of log-transformed values. A comparison of the nickel concentrations in ambient air between sensitized and non-sensitized children shows an association of nickel sensitization prevalence with exposure to nickel for Duisburg (Mann-Whitney test: $P = 0.094$). A similar association was not seen in Dortmund. Overall, nickel levels in urine of sensitized children were higher than non-sensitized children ($P < 0.001$). Children who had urinary Ni or ambient air Ni below the median showed a higher prevalence of Ni sensitization than children with both levels below the median (X^2-test: $P = 0.109$). The authors conclude that nickel in ambient air might be a risk factor for nickel sensitization, but a larger study is necessary.

Lisby et al. (1999a) observed nickel-induced activation of T cells in individuals with negative patch test to nickel sulfate. Eighteen subjects (8 males and 10 females, aged 27-54 years) were included in the study. Maximum T cell proliferation was seen after seven days of in vitro stimulation of isolated peripheral blood mononuclear cells (PBMC) with $NiSO_4$. Nickel sulfate concentrations above 1.0 mM were toxic to the cells by trypan blue exclusion. At concentrations between 0.1 and 100 µM a dose-dependent stimulation of PBMC was seen in 16 of the 18 subjects. Maximum stimulation occurred between 1 and 100 µM $NiSO_4$ with the mean maximum stimulation index (SI) of 7.1, range 1.4-21.8 ($P < 0.0005$). Similar results were obtained with $NiCl_2$ ($N = 3$, mean SI = 13, range 8.0-20.2). The functional capacity of Ni-inducible T cells was assessed by cytokine release from PBMC from Ni-allergic and Ni-nonallergic individuals. T cells from both allergic and nonallergic subjects released interferon-γ (IFN-γ) but

no significant difference was observed between the two groups in the concentrations of IFN-γ released after 72 hr stimulation with $NiSO_4$. Umbilical cord mononuclear cells (UCMC) were used as a model for unexposed individuals. When incubated with 10^{-10} to 10^{-4} M $NiSO_4$ these cells showed no cell proliferation compared to controls. The authors note that: "even if the observed T cell reactivity towards Ni by itself does not result in the development of clinical disease, such a T cell reactivity may add to the reactivity of other T cells with other allergen specificity resulting in the development of overt clinical disease."

In a follow-up study, Lisby et al. (1999b) found that the proliferative response in Ni-nonallergic individuals was mainly confined to T cells within the CD4+ subset. Also in contrast to the conventional recall antigen tetanus toxoid, $NiSO_4$ stimulated both naïve and memory CD4+ T cells. Preincubation of monocytes/macrophages but not T cells with $NiSO_4$ resulted in subsequent T cell proliferation. The results suggest that T cells in Ni-nonallergic individuals are capable of recognizing nickel or nickel-modified peptides.

Buchvald and Lundeberg (2004) investigated the in vitro responses of peripheral blood mononuclear cells (PBMCs) to nickel stimulation in groups of atopic and nonatopic patients with nickel allergic contact dermatitis (ACD). ACD is dependent on cell-mediated immune responses mediated by type-1 T lymphocytes whereas atopic dermatitis (AD) occurs via sustained activation of type-2 subsets of T cells. Ten subjects each with nonatopic nickel ACD, nickel ACD + concomitant AD, AD but no contact allergy, and healthy controls provided PBMCs that were stimulated with $NiSO_4$, phytohemagglutinin (PHA), or tetanus toxoid (TT). Ni-induced lymphocyte DNA synthesis in PBMC cultures was measured with [^{3}H] thymidine incorporation and expressed as a stimulation index (SI). The SI for controls averaged about one, for AD about two, for ACD about 20 and for ACD+AD about two. IL-2 secretion (pg/mL) averaged about 1, 1, 50, and 10, respectively. IL-5 secretion (pg/mL) averaged about 10, 10, 175, and 25, respectively. The results indicated that PBMCs of nickel-allergic subjects with concomitant AD exhibited impaired in vitro proliferative and secretory responses to nickel but not to the mitogen PHA or the recall antigen TT. There was a statistically significant correlation between the amounts of IL-2 and IL-5 secreted by Ni-stimulated lymphocytes of the ACD+AD subjects. The authors speculate that IL-5 may play a role in the development of ACD.

Moed et al. (2004) determined the identity of nickel-responding T cell subsets in five nickel-allergic subjects and four controls. The T cell subsets were isolated from peripheral blood mononuclear cells (PBMCs) and their proliferative capacity, type-1 or type-2, measured by IFN-γ or IL-5 release, and phenotypical marker expression were assessed after nickel treatment with 50 µM $NiSO_4$. The authors found that only CD4+ CLA+ CD45RO+ and not CD8+ T cells proliferated and produced both type-1 and type-2 cytokines in response to nickel. Cells with the marker CLA in combination with CD4+, CD45RO+, or CD69 are increased after nickel stimulation. Analysis of nickel-reactive cells for expression of distinct

chemokine receptors showed that proliferative capacity and cytokine production were confined to subsets expressing CXCR3 and CCR4 but not CCR6. A subset of T cells expressing CLA+ and CXCR3, CCR4 and CCR10 increased in response to allergen. The authors conclude that Ni-reactive T cells are characterized as CD4+ CLA+ memory cells, which express chemokine receptors CXCR3, CCR4, and CCR10, but not CCR6. The lack of Ni-induced IFN-γ or IL-5 release from CD8+ T-cell fractions suggests that they play no significant role in nickel allergy.

Jensen et al. (2004) similarly characterized lymphocyte subpopulations and cytokine profiles in PBMCs of Ni-sensitive individuals after nickel exposure. Thirty-three Ni-sensitive individuals were randomly divided into four groups of 7-10 each and orally challenged with 0, 0.3, 1.0, or 4.0 mg nickel given as $NiSO_4 \cdot 6H_2O$. Nineteen healthy controls were randomly divided into two groups and orally challenged with 0 or 4.0 mg Ni. Blood samples were obtained 24 hr after Ni-exposure and PBMCs isolated for analysis. Ni-sensitive individuals had significantly higher fractions of lymphocytes in their peripheral blood than the healthy controls (mean percent): $CD3^+$ $CD45RO^+$ CLA^+ cells (12.5 vs. 8.5, P = 0.0035); $CD4^+$ $CD45RO^+$ CLA^+ cells (21.2 vs. 12.2, P = 0.000095); and $CD8^+$ $CD45RO^+$ CLA^+ cells (6.1 vs. 1.6, P = 0.000007).

The Ni-sensitive subjects were divided into two groups based on cutaneous response following oral exposure (responders N = 13, non-responders N = 20). A dose-response reaction was observed among nickel-sensitive subjects. Both responders and non-responders had significantly higher fractions of $CD3^+$ $CD45RO^+$ CLA^+ lymphocytes before challenge than the healthy controls (P = 0.014 and 0.049, respectively). After challenge this was significant only for the non-responders (P = 0.025). Both Ni-sensitive groups showed significantly higher fractions of $CD4^+$ $CD45RO^+$ CLA^+ cells before and after Ni-challenge (P < 0.001). Responders had the highest fraction of $CD8^+$ $CD45RO^+$ CLA^+ before and after Ni-challenge [7.7 vs. 1.6 (P = 0.022) and 6.5 vs. 1.6 (P = 0.0014), respectively]. Only those individuals that responded to Ni-challenge with 4 mg Ni had significantly elevated levels of IL-5 in the serum (P = 0.025) and a smaller non-significant increase in IL-10. No differences in the levels of IL-2, IL-4, IFN-γ, or TNF-α were observed before or after challenge. Overall the results indicate that $CD8^+$ $CD45RO^+$ CLA^+ T-lymphocytes and T lymphocytes with the type 2 cytokine profile are involved in systemic contact dermatitis associated with nickel exposure.

Minang et al. (2006a) investigated the effect of IL-10 on Ni-induced Th-1(IFN-γ) and Th-2-type (IL-4 and IL-13) cytokine responses in human peripheral blood mononuclear cells (PBMC). PBMC from 15 Ni-allergic and 8 control donors were stimulated with nickel and the frequency of cytokine-producing cells and cytokine concentrations analyzed by enzyme-linked immunospot (ELISpot) and enzyme-linked immunosorbent assay (ELISA). PBMC suspensions of 2.5×10^5 cells with or without 50 µM $NiCl_2 \cdot 6H_2O$ were incubated with different concentrations of recombinant rIL-10 (0 to 25 ng/mL). Nickel-PBMC showed significantly higher

levels of endogenous IL-10 compared to control PBMC. The mean increase in IL-10 induced by Ni(II) was 33.1 pg/mL and 2.2 pg/mL in the Ni-PBMC and control PBMC, respectively. Addition of rIL-10 to Ni-PBMC reduced the levels of Ni-induced IL-13, and IFN-γ. The mean levels of IFN-γ were reduced by 40% to 71% using 0.2 and 1 ng/mL of rIL-10. No effects of rIL-10 were seen in the control PBMC. The results suggest that IL-10 may play a role in vivo in counteracting the allergic reactions mediated by Th-1-type reactions. In a follow-up study the authors observed similar mixed Th1- and Th2-type cytokine profiles in allergic subjects with cobalt(II), chromium(Cr III and VI), palladium(Pd II) and gold(Au I and III). In terms of the optimal dose for induction of cytokines IL-2, IL-4 and IL-13 the order of effectiveness was: Cr(VI), 0.5 μM > Au(III), 2 μM > Au(I), 25 μM > Ni(II) ~ Co(II), 50 μM > Cr(III) ~ Pd(II), 100 μM.

8.3 Studies on Cells in vitro.

Zeromski et al. (1995) measured the effects of Ni_3S_2 (median particle size ≥ 30 μm) or $NiSO_4$ on human lymphocytes in vitro. Blood was obtained from a blood bank and peripheral mononuclear cells (PBMCs) from normal donors were cultured for 24 hr at 0, 0.01, 0.02, or 0.04 mM Ni. Following culture, the immuno-phenotype of the cells was determined by indirect immunofluorescence, using monoclonal antibodies to major differentiation antigens of PBMCs, and their natural killer (NK) activity toward K562 target cells. Ni_3S_2 had a marked inhibitory effect on the PBMCs consisting of a decreased number of CD4-positive cells at 0.02 and 0.04 mM Ni and a fall of NK (CD56-positive) cell number at all concentrations tested. $NiSO_4$ induced a significant 30 percent decrease in the CD4 phenotype of T cells at 0.04 mM ($P < 0.05$ vs. control). The inhibitory effects noted by both nickel compounds could be prevented by co-treatment with magnesium acetate. Ni or Mg salts did not affect CD3, CD8, CD20, or CD11a cell populations.

Caicedo et al. (2007) investigated the metal ion-induced DNA damage, apoptosis, necrosis and proliferation in a human CD4+ T-helper lymphocyte (Jurkat) cell line. Cell suspensions with 1×10^6 cells were incubated for 48 hr with 0, 0.05, 0.5, 1.0, or 5.0 mM metal ion as chlorides. The results indicated that the metal ions did not preferentially induce Jurkat T-lymphocyte DNA damage prior to other forms of toxicity indicated by apoptosis and/or necrosis. In terms of the average concentration (of the four endpoints) required to induce a significant adverse effect, the metals were ranked as follows: V(III), 0.29 mM; Ni(II), 1.41 mM; Co(II), 2.65 mM; Cu(II), >2.65 mM; Nb(V), >2.75 mM; Mo(V), >2.87 mM; Zr(II), >3.875 mM; Be(II), >4 mM; Cr(III), >5 mM; Al(III), >5 mM; and Fe(III), >5 mM. Vanadium (III) and nickel (II) stand out as the more toxic of the metal ions surveyed on average. In terms of cytotoxicity only cobalt (II) and niobium (V) were more toxic (0.5 mM) than vanadium (1.0 mM) and nickel (5.0 mM).

Miyazawa et al. (2008) studied the role of the mitogen-activated protein kinase (MAPK) signaling pathway in the activation of dendritic-type THP-1 cells by nickel

sulfate. Nickel and other low molecular weight allergens induce contact hypersensitivity via a cell-mediated delayed-type immune response. In the induction phase these compounds or haptens first make contact with dendritic cells (DCs) in the skin, including Langerhans cells (LCs). Activated DCs migrate to regional lymph nodes and trigger the allergen-specific T-cell response with expression of stimulatory molecules (e.g., CD86 and CD54) and the production of several stimulatory cytokines (e.g., IL-1β). Human myeloid cell lines (THP-1, U937 and MUTZ-3) are good surrogates of DCs and have a high capacity to induce tumor necrosis factor (TNF-ά) release and CD86, CD54 and CD40 expression following allergen treatment. THP-1 cells (1×10^6) were cultured for one hour in one mL of culture medium with either 170 µg/mL $NiSO_4$ or 5 µg/mL 2,4-dinitrochlorobenzene (DNCB). Some experiments included 0.03 to 3 µM of the p38 MAPK inhibitor SB203580. Nickel sulfate and DNCB induced phosphorylation of p38 and extracellular signal-regulated kinase (ERK). Inhibition of p38 MAPK activation selectively blocked DNCB-induced TNF-ά release, but not $NiSO_4$. Alternatively, inhibition of ERK pathways selectively suppressed $NiSO_4$-induced TNF-ά but not DNCB-induced release. The authors conclude that the two allergens activate p38 MAPK and ERK, and stimulate TNF-ά release via different signal transduction pathways.

Boisleve et al. (2005) demonstrated that in immature human $CD34^+$-derived DC, three MAPK pathways (ERK, p38MAPK, and JNK) participated in the expression of CD83, CD86 and CCR7 molecules induced by $NiSO_4$. In contrast, following TNF-α stimulation, only p38 MAPK was involved in CD83 and CCR7 expression. ERK inhibited DC maturation while JNK had no effect. The authors also demonstrated that inhibition of the MAPK pathways did not suppress $NiSO_4$-induced down-regulation of the adhesion molecule E-cadherin and the specific LC protein, langerin, suggesting that other signaling pathways may be involved.

Goebeler et al. (1993) evaluated the effects of sensitizing agents (2,4-dinitro-benzenesulfonic acid, metal salt haptens) on endothelial adhesion molecule expression. Endothelial surface molecules play a role in leukocyte recruitment to sites of inflammation. Using flow cytometry and an enzyme-linked immunosorbent assay, $NiCl_2$ and to a lesser extent $CoCl_2$ were observed to up-regulate intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1(ELAM-1, E-selectin) expression on cultured human umbilical vein endothelium. The other substances tested showed no effects including $AlCl_3$, $CrCl_3$, $K_2Cr_2O_7$, $MnCl_2$, $CuCl_2$, $ZnCl_2$ and dinitrobenzenesulfonate. Induction of adhesion molecules by $NiCl_2$ required de novo mRNA and protein synthesis and could be blocked by kinase inhibitor H-7. Neutralizing antibodies to IL-1 did not block Ni(II) up-regulation indicating independence of an IL-1-dependent autocrine mechanism. In a separate analysis of foreskin specimens in organ culture, $NiCl_2$ up-regulated microvascular ELAM-1 expression ($2.06 \pm 0.31_{SEM}$ in control *vs*. $3.25 \pm 0.27_{SEM}$ with 0.7mM $NiCl_2$: $P < 0.01$). The authors speculate on the importance of the findings with regard to nickel induced contact allergies.

Schmidt et al. (2010) reported that Ni(II) (form not specified) triggered an inflammatory response by directly activating human Toll-like receptor 4 (TLR4). The response was specific to humans and absent in mouse TLR4. Studies with mutant TLR4 proteins showed that the non-conserved histidines 456 and 458 of human TLR4 are required for Ni(II) activation but not by the natural ligand polysaccharide. Transgenic expression of human TLR4 in TLR4 deficient mice allowed efficient sensitization to Ni(II). The results suggest site-specific human TLR4 inhibition as a potential therapy for contact hypersensitivity.

Gao et al. (2010) studied the interaction of microbial stimuli and nickel to amplify the release of inflammatory and immune-modulating cytokines in cultured human lung fibroblasts (HLF). $NiSO_4$ and MALP-2(*M. fermentans*-derived macrophage-activating lipopeptide-2) induced synergistic increases in IL-6 gene expression. HLF were exposed to 200 µM $NiSO_4$ and/or 600 pg/mL MALP-2. The combined treatment increase in IL-6 mRNA was about 20-fold versus 5-fold for individual treatments over 30 hr. Nickel and MALP-2, alone or together, led to rapid and transient phosphorylations of $ERK_{1/2}$ and JNK/SAPK. P38 phosphorylation was seen only after prolonged treatment with both agents together. PI3K-dependent Akt phosphorylation was unchanged by Ni and/or MALP-2 treatment. IL-6 induced by Ni/MALP-2 was partially dependent on the activity of HIF-1α and COX-2. IL-6 was also partially sensitive to the inhibition of $ERK_{1/2}$, p38, and PI3K signaling. Protein kinase inhibitors had little or no effect on Ni/MALP-2-induced accumulation of HIF-1α protein, however, COX-2 expression and, especially, PGE_2 production were suppressed. The authors conclude that Ni/MALP-2 interactions involve multiple protein kinase pathways ($ERK_{1/2}$, p38, PI3K) that modulate events downstream from early accumulation of HIF-1α gene expression to COX-2 derived autocrine products like PGE_2.

Fugitive fly ash derived from the combustion of residual fuel oil (ROFA) containing nickel has been used to study the effects of metal-containing PM. The toxicity of ROFA and other PM involves initiation of inflammatory cascades within the lung (Gao et al., 2010). It is possible that these effects may play a role in human disease caused by nickel bearing PM.

Carter et al. (1997) exposed normal human bronchial epithelial (NHBE) cells for 2 or 24 hr to 0, 5, 50, or 200 µg/mL residual oil fly ash (ROFA). The ionizable metal content of the ROFA was mainly vanadium (185 mg/g), nickel (37.5 mg/g) and iron (35.5 mg/g). Concentrations of inflammatory cytokines IL-8, IL-6 and TNF-α, as well as mRNA coding for these cytokines were measured using ELISA and RT-PCR methods. Incubation of cells for 2 hr stimulated the accumulation of IL-8 protein and mRNA in a dose-dependent manner. Significant increase of IL-8 mRNA was seen in 2 hr with 5 µg/mL ROFA. ROFA induction of IL-6 was similar to that of IL-8. ROFA induction of TNF-α was not as marked, with cells requiring 50 or 200 µg/mL for 2 hr to elicit a significant increase. Cytokine induction by ROFA was inhibited by inclusion of either the metal chelator deferoxamine (1.0 mM) or the free radical scavenger dimethylthiourea (1.0 mM). On this basis the

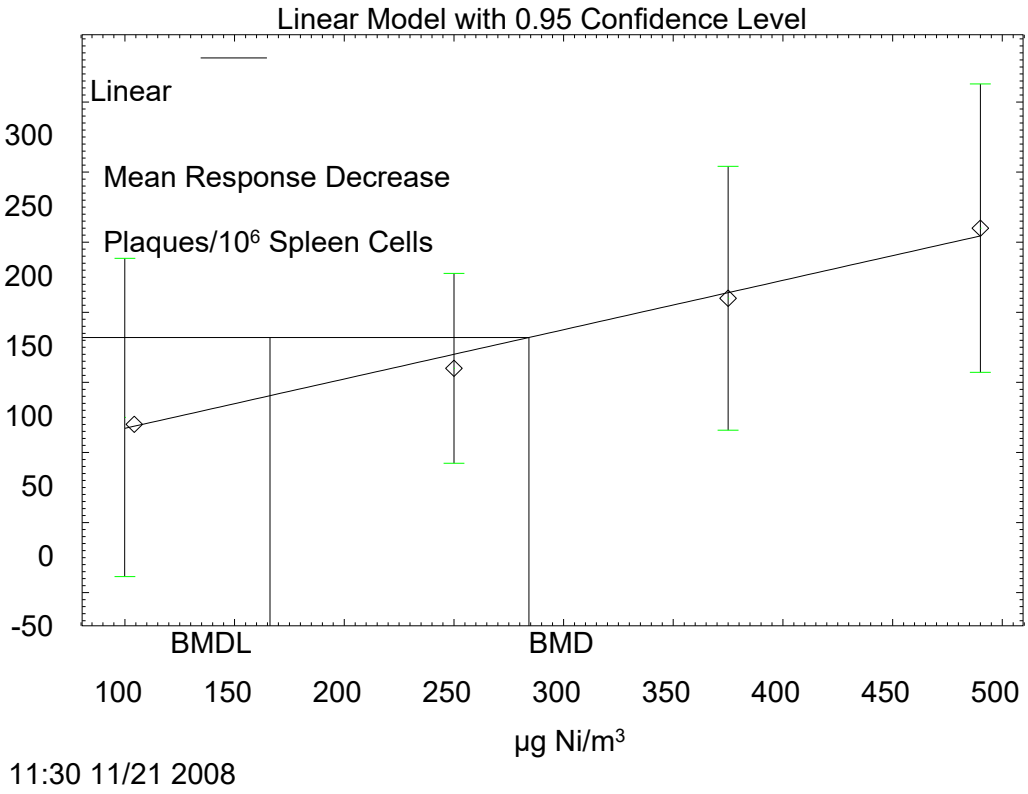
authors concluded that the ROFA-induced cytokine production by the human airway cells was metal-dependent.

8.4 Immunotoxicity Studies in Experimental Animals

Both the critical study and the supporting study for the derivation of the acute REL for nickel compounds exhibited immunotoxicity endpoints in experimental animals. The study of Graham et al. (1978) on inhibition of antibody production was the critical study for the aREL and was a supporting study for the 8-hour REL. Adkins et al. (1979) showing increased mortality in nickel-treated animals subjected to experimental infection was the supporting study for the aREL. The details of the derivations are given in sections 9.3 and 9.4 below.

Studies by Graham et al. (1975, 1978) indicate that the immune system is a sensitive target for acute nickel toxicity showing inhibition of antibody production against sheep erythrocytes. These authors used a hemolytic plaque technique to determine the number of specific antibody-producing spleen cells. Six-week old SPF female Swiss mice (14-29 per group) were exposed by inhalation to 0, 100, 250, 375, or 490 µg Ni/m³ as $NiCl_2$ (99% of particles were ≤ 3µm in diameter, exposure values were estimated from their Fig. 3) for two hours. The exposed animals showed a significant decrease in splenic antibody-forming cells following a challenge with a T-lymphocyte dependent antigen (Graham *et al*., 1978). A linear dose-response was observed with a negative linear regression of Y = -34.9 - 0.347X, where Y is the number of hemolytic plaques formed/10^6 spleen cells and X is the exposure concentration in µg Ni/m^3. The results indicate a LOAEL of 250 µg Ni/m^3 and a potential NOAEL of 100 µg Ni/m^3. Unfortunately this study is short on details and the NOAEL is not considered as reliable as the LOAEL (no control values are given). We analyzed the data in the Graham et al. (1978, Figure 3) with a continuous benchmark dose approach. The extrapolated background from their Fig. 3 is approximately -40 plaques/10^6 cells. Using a criterion of -100 plaques/10^6 cells as a significant effect (a reduction of more than double the background), we obtained a good fit to a linear model (P = 0.95) with a benchmark dose (BMD) for a 100 plaque loss of 284 µg Ni/m^3 and a 95% lower confidence limit on BMD (BMDL) of 164.6 µg Ni/m^3 (Figure 6). The latter value is used as the point of departure in the derivation of a potential 8-hour REL (Section 9.4).

Figure 6. Continuous Benchmark Dose Analysis of Decrease in Plaques/10^6 Spleen Cells vs. µg Ni/m^3, 2 Hours Exposure of Female Swiss Mice. BMD and BMDL are for a 100 Plaque Decrease (data from Graham et al. 1978, their Fig. 3).



A host-resistance study by Adkins *et al*. (1979) showed that mice (80-120 per group) exposed to inhaled soluble nickel aerosols for two hours in the form of $NiCl_2$ or $NiSO_4$ (particle sizes 86 to 96% <1.4µm, 99% <3.0µm) were significantly more susceptible to mortality from streptococcal bacterial infection. The concentrations of nickel that showed these effects were 499 µg Ni/m³ ($NiCl_2$) and 455 µg Ni/m³ ($NiSO_4$). No significant change in mortality was seen with exposure to 369 µg Ni/m³ as $NiCl_2$. The data for percentage mortality difference from control for the post $NiCl_2$ treatment infection interval of 24 hr (their Table 1) was analyzed by the benchmark dose method. Using a doubling of the mortality percentage as the benchmark (i.e., 3.74 to 7.5%) a BMDL of 365 µg Ni/m^3 was obtained with the power model and unequal variances (doses of 0, 289, 369, and 499 µg Ni/m^3). This value is about twice the BMDL obtained with the Graham et al. (1978) data shown above but for a more severe endpoint.

Some of the immunologic effects of nickel in exposed rodents in vivo are summarized in Table 20.

Table 20. Immunologic Effects of Nickel Compounds Observed in Rodent Studies (NTP, 1996a)

Nickel Compound	Species/Route	Chemical treatment	Response	Reference
Cell-mediated immunity				
Nickel chloride	CBA/J mice, intramuscular	Single injection, 18 mg/kg bw	Reduced T-lymphocyte proliferation	Smialowicz et al., 1984
Nickel sulfate	B6C3F1 mice female, oral	Up to 4,000 mg/kg-d for 23 weeks	Depressed spleen lymphoproliferative response to LPS (no effect on NK activity; PFC assay; mitogen response in spleen cells; resistance to *Listeria* challenge)	Dieter et al., 1988
Nickel sulfate	Sprague-Dawley rats, oral, drinking water 13 weeks	0, 0.02, 0.05, 0.1%$NiSO_4$•$6H_2O$, or 0, 44.7, 11.75, 223.5 mg Ni/L	Increase of CD4+ and CD8+ T-cells and decrease of CD4/CD8 ratio	Obone et al. 1999.
Humoral immunity				
Nickel chloride	CBA/J mice, intramuscular	Single injection, 18 mg/kg bw	Reduced antibody response to T-cell dependent sheep red blood cells	Smialowicz et al., 1984
	Swiss albino mice, intramuscular	3-12 µg Ni/kg bw followed by immunization with sheep red blood cells	Depressed antibody formation	Graham et al., 1975
	Swiss mice, inhalation	2-hour inhalation exposure at 250 µg/m^3	Depressed antibody response to sheep red blood cells	Graham et al., 1978
Nickel acetate	Sprague-Dawley rats, intraperitoneal	11 mg/kg bw immunized with *E. coli* bacteriophage	Depressed circulating antibody response	Figoni and Treagan, 1975
Macrophage function				
Nickel chloride	CBA/J mice, intramuscular	Single injection, 18 mg/kg bw	No effect on phagocytic capacity of peritoneal macrophages	Smialowicz et al., 1984

Table 20. Immunologic Effects of Nickel Compounds Observed in Rodent Studies (NTP, 1996a)

Nickel Compound	Species/Route	Chemical treatment	Response	Reference
Natural killer cell activity				
Nickel chloride	CBA/J and C57BL/6J mice, intramuscular	Single injection, 18 mg/kg bw	Depressed NK activity against Yac-1 murine lymphoma cells	Smialowicz et al., 1984, 1985, 1986
Host resistance				
Nickel chloride and nickel oxide	CD mice and Sprague-Dawley rats, inhalation	0.5 mg/m^3 for 2 hours	Enhanced respiratory infection by *Streptococcus*	Adkins et al., 1979

A similar suppression in antibody-forming cells was seen in mice (10-12/dose group) exposed intramuscularly to 0, 3.09, 6.17, 9.25, or 12.34 µg Ni/g body weight as $NiCl_2$ or $NiSO_4$ (Graham *et al*., 1975, 1978). Statistically significant decreases in plaque production ($P < 0.05$ vs. control by William's test) were seen at 9.25 µg Ni/m^3 with $NiCl_2$ and at 3.09 µg Ni/m^3 with $NiSO_4$ (Graham et al., 1975). Similar exposures with NiO showed no decreases at any dose. A linear dose-response was given for $NiCl_2$ of $Y = -2.64 – 0.028X$, where Y is the log10 of plaques/10^6 cells and X is the i.m. dose of µg Ni/g bw.

Condevaux et al. (2001) compared the effects of morphine and nickel chloride on natural killer (NK) cell activity in vitro in rats and in the cynomolgus monkey. The NK cells were exposed to either $NiCl_2$ at 0, 1, 10, or 100 µg/mL or morphine at 0, 0.01, 1, or 1000 nM. There were statistically significant decreases in NK cell activity at the highest concentrations of nickel or morphine. The magnitudes of the decreases were greater in the monkey than in the rat, i.e. for $NiCl_2$ the decreases were 34.4-42.2% in monkey and 21.6-24.3% in rat. Morphine hydrochloride induced decreases of 59.1-68% in the monkey and 23.7-34.7% in the rat.

Haley *et al*. (1987) showed that male cynomolgus monkeys, exposed to intratracheal Ni_3S_2 (particle size not stated) at a delivered dose of 0.06 µmol Ni/g lung tissue, had impaired pulmonary macrophage phagocytic function and increased NK cell activity. Mice also exhibited impairment of pulmonary macrophage function in addition to decreases in antibody-forming spleen cells with inhalation exposure to Ni_3S_2 or NiO (Haley *et al*., 1990). Natural killer cell activity measured by splenic cytotoxic activity to tumor cells as well as by clearance of melanoma tumors *in vivo* was suppressed in two strains of mice exposed to intramuscular injections of 18.3 mg Ni/kg as $NiCl_2$ as compared to controls (Smialowicz *et al*., 1985).

Smialowicz et al. (1984, 1985) injected nickel chloride i.m. in mice and found a significant reduction in a variety of T-lymphocytes and natural killer cell-mediated immune functions. They also demonstrated that suppression of natural killer cell activity could be detected with *in vitro* and *in vivo* assays and that reduction of natural killer cell activity was not associated with either a reduction in spleen cellularity or the production of suppressor cells. Their findings confirmed those reported by other investigators on the immunosuppressive effects of nickel compounds on circulating antibody titers to T_1 phage in rats (Figoni and Treagan, 1975), on antibody response to sheep erythrocytes (Graham et al., 1975), on interferon production in vivo in mice (Grainer et al., 1977), and on the susceptibility to induced pulmonary infection in mice following inhalation of nickel chloride (Adkins et al., 1979).

Haley *et al.* (1990) found that exposure of mice to nickel sulfate, nickel subsulfide, or nickel oxide resulted in various immunological effects. Mice were exposed to 0, 0.11, 0.45, or 1.8 mg Ni/m^3 as Ni_3S_2 (MMAD = 2.4 µm, gsd = 2.2); 0.47, 2.0, or 7.9 mg Ni/m^3 as NiO (MMAD = 2.8 µm, gsd = 1.8); and 0.027, 0.11, and 0.45 mg Ni/m^3 as $NiSO_4$ (MMAD = 2.3 µm, gsd = 2.4) for 6 hours/day, 5 days/week for 13 weeks. Nickel exposures consistently decreased splenic antibody-forming cell (AFC) responses, with significant decreases occurring at 1.8 mg Ni/m^3 as nickel subsulfide. In contrast, AFC responses in the lung-associated lymph nodes were consistently increased, indicating a possible indirect influence of inflammatory mediators released in the lung on local lymph nodes.

Rabbits (8 nickel exposed and 8 controls) exposed to 0.24 mg Ni/m^3 as nickel chloride (MMAD = 0.5-1.0 µm, cut off at ≤7.0µm) 6 hours/day, 5 days/week for 4 weeks exhibited significantly decreased macrophage lysozyme activity in pulmonary lavage fluid and in macrophage cultures, compared with control animals (Lundborg and Camner, 1984). Similar exposures of rabbits to chlorides of cadmium, cobalt, or copper did not reduce lysozyme activity.

Obone et al. (1999) evaluated the bioaccumulation and toxicity of nickel sulfate in rats following 13 weeks of oral exposure. Adult male Sprague-Dawley rats (8/dose group) were given 0, 0.02%, 0.05% and 0.1% nickel sulfate, i.e. 0, 44.7, 111.75, and 223.5 mg Ni/L, in their drinking water for 13 weeks. Measurements of splenic lymphocyte subpopulations following exposure to 0.05% $NiSO_4$ showed significant increases in absolute numbers of T-cells, CD4+ and CD8+. Statistically significant increases in CD8+ and decrease in the ratio of CD4/CD8 were observed at all dose levels. Significant increases in both the absolute number and percentage of thymocyte CD8+ cell populations were also seen at all dose levels. The findings indicate a LOAEL of approximately 7.0 mg/kg-d for immunotoxicity ($C = 0.1*W^{0.7377}$ L/d, W = 0.185 kg rats; U.S. EPA, 1988).

Harkin et al. (2003) studied immunosuppression in Sprague-Dawley rats following i.p. administration of 0 (vehicle), 0.12, 0.36, 1.1, or 3.3 mg $NiCl_2$/kg bw. Nickel chloride suppressed T-lymphocyte proliferation and Th-1 (IFN-γ) and Th-2

(IL-10) cytokine production in a dose- and time-dependent manner. In addition, $NiCl_2$ inhibited production of the pro-inflammatory cytokine TNF-ά and increased the production of the anti-inflammatory cytokine IL-10 from lipopolysaccharide (LPS) stimulated cultures. Three of the cytokine data sets from Harkin et al. (2003) were subjected to continuous benchmark dose analysis (their Figure 2 (a), (b), and (c)). All the data sets were fit by the Hill model with P values greater than 0.22 (P ≥ 0.1 adequacy of fit criterion). For concanavalin-A (Con-A) stimulated Th1:IFN-γ, the $BMDL_{1SD}$ was 0.18 mg Ni^{2+}/kg bw. For Con A-stimulated Th2:IL-10, the $BMDL_{1SD}$ was 0.14 mg Ni^{2+}/kg bw. LPS-stimulated TNF-ά gave a $BMDL_{1SD}$ of 0.17 mg Ni^{2+}/kg bw. The similarity of the quantitative dose responses for nickel-induced cytokine suppression may indicate a common mode of action. The authors reported that the minimum plasma concentrations of nickel required to provoke immunosuppression are in the range 209 to 585 ng/mL. In the kinetic portion of the study a 3.3 mg/kg $NiCl_2$ dose provoked immunological changes that were maximal one hour following administration. The data demonstrate that $NiCl_2$ suppresses T-cell function and promotes an immunosuppressive macrophage phenotype in rats.

Roberts et al. (2009) studied the metal components of residual oil fly ash (ROFA) on pulmonary host defense in rats. The soluble fraction of ROFA contained Ni, Fe, Al and Zn. Sprague-Dawley rats were intratracheally instilled with 55.7 µg/rat ($NiCl_2$), 32.7 µg/rat ($FeSO_4$), 46.6 µg/rat ($Al_3(SO_4)_2$), 8.69 µg/rat ($ZnCl_2$), or a combination of all metals. Rats were also instilled with mixtures without a specific metal e.g., Mix-No Ni. Prior to infection with *Listeria monocytogenes* (5 x 10^4 cells) soluble nickel alone or in metal mixture produced no more lung injury than saline controls. Following infection nickel-treated animals had increased bacterial lung burden and body weight decrease. Ni alone and in mixtures increased reactive oxidants in the lung and was most important in suppressing T-cell activity following infection. Weight decreases in the mixes without Fe or Al indicate that iron and aluminum may act antagonistically to nickel. Overall the authors conclude that soluble Ni is the primary metal involved in the increased susceptibility to infection observed in rats exposed to the soluble metals of ROFA.

9 Derivation of Reference Exposure Levels

9.1 Introduction

The toxic effects of chemicals are of varying types and degrees of severity. Toxic effects from airborne substances may be due to exposure via the skin, eyes, and upper and lower respiratory tract. Systemic effects, such as hemolysis or central nervous system injury, may result from absorption of material through the lungs, and, to a lesser extent, through the skin. For a toxic endpoint to be considered due to acute exposure, the effects do not have to be observed immediately. Rather, the effects may be observed hours to days following the acute exposure. OEHHA has chosen to adopt U.S.EPA's general definition of adverse effects as "a biochemical change, functional impairment, or pathologic lesion that negatively affects the performance of the whole organism, or that reduce an organism's ability to respond to an additional challenge" (U.S.EPA, 2007). In assessing the dose-response relationship for non-cancer toxicological endpoints and developing RELs, the objective is to define concentrations of chemicals at or below which no adverse health effects are anticipated in the general human population, including sensitive subpopulations, over the specified exposure duration (1 hour, eight hours or chronic).

In selecting the critical and supporting studies upon which to base the RELs a number of factors are considered. Firstly, human studies are preferred if they are of sufficient quality in terms of endpoint relevance, numbers of subjects, dose response, study design etc. Most often we rely on animal studies, which generally are more available and have better dosimetry data than human studies. Here we look for the most sensitive effect in the most sensitive sex and species. We favor studies that provide a dose response that we can analyze with either quantal or continuous data yielding a BMDL or 95% lower confidence bound on a specific response level, usually 5%. This approach uses all the available data and is generally superior to the traditional approach of identification of a NOAEL or LOAEL, which is more influenced by dose selection (spacing), does not consider sample size and does not use information from the higher doses. When a BMD analysis is not possible, the NOAEL/LOAEL approach is used. Both approaches employ uncertainty factors to address shortcomings in available toxicity data when deriving the RELs.

9.2 Selection of Critical Studies

The available studies of acute lung toxicity in humans and animals were unsuitable for the derivation of an acute REL. Human data were limited to case reports and small occupational clinical or epidemiological studies with limited reporting and inadequate exposure data. Animal studies in many cases are complicated by less relevant exposure routes (e.g. subcutaneous injection, intratracheal installation), or the endpoints examined were not the most sensitive. Instead, it was found that acute or short-term studies of immunotoxicity provided

a better basis for this derivation. In the derivations for the acute REL we have selected critical studies based on two related toxic endpoints, namely immunotoxicity and pneumotoxicity. The acute REL critical study (Graham et al., 1978) and its supporting study (Adkins et al., 1979) are both based on immunotoxicity and give values of 0.2 and 0.7 µg Ni/m^3, respectively. Another study we considered was that of Ishihara et al. (2002) on bronchial inflammatory responses and mucus secretion in rats but the exposure of 5 hr/day x 5 days/week was too extensive for the 1 hour aREL.

The 8-Hour REL uses the NTP (1994c) $NiSO_4$ inhalation study in rats as the critical study and the Graham et al. (1978) as a supporting study. In this case we used a NOAEL approach but for a very large study with several time intervals up to 2 years. We also considered two other studies for the 8-hour REL (see Table 21). The chronic RELs for nickel compounds and for NiO also use NTP studies for $NiSO_4$ in rats, and NiO in mice. Both studies show similar effects of lung toxicity (e.g., alveolar proteinosis) but the derivation of the cRELs differ somewhat in that the rat data could be analyzed using a computer program for particle deposition in rats and humans (MPPD2) whereas the mouse used published data on deposition calculations since the MPPD2 model does not analyze deposition in the mouse lung. Both data sets were analyzed for dose response (i.e. $BMDL_{05}$). For the oral REL we adopted a study previously used in our drinking water program to set the public health goal (PHG). In all cases we apply uncertainty factors according to our published guidance (OEHHA, 2008) and the sufficiency of the data available in deriving the final REL proposals.

9.3 Acute Reference Exposure Level (aREL)

Study	Graham et al., 1978; supported by Adkins et al. (1979)
Study population	Immunotoxicity in mice,
Exposure method	Inhalation of 100 to 490 µg/m^3 $NiCl_2$
Critical effects	Depressed antibody response
BMDL	165 µg Ni/m^3 (-100 plaques/10^6 cells)
Exposure duration	2 hours
Extrapolated 1 hour concentration	233 µg Ni/m^3 (usingC^n x T = K, with n =2)
BMR uncertainty factor	√10 (clear response at BMR)
Interspecies uncertainty factor	10(default)
Intraspecies uncertainty factor	(√10PD * 10PK)
Cumulative uncertainty factor	1000
Reference Exposure Level	0.2 µg Ni/m^3

An acute REL of 0.2 µg Ni/$m^3$for mild effects following a 1-hour exposure was derived using the study of Graham *et al*. (1978) as the basis. This study is discussed above in section 8.4 and a dose response analysis is shown in Figure 6. The study gives a clear linear dose response. It involved an adequate number of animals per dose group (14-29) and each group was compared with its own controls. An extrapolation from the BMDL of 165 µg Ni/m^3 to that of a 1-

hour exposure was made using the time adjustment formula $C^n * T = K$, where n = 2. This yielded a 1-hour value of 233 µg/m^3. An overall uncertainty factor of 1000 was applied. This included a factor of √10 to allow for the fact that the BMDL was calculated for a benchmark response rate (BMR) which was considered to be a clearly measurable and biologically significant response. Interspecies and individual variabilities were represented by the usual defaults of 10 and 30, respectively. This results in a 1-hour REL of 0.2 µg Ni/m³.

The data of Graham et al. are supported by Adkins et al. (1979), who demonstrated increased mortality in mice exposed to $NiCl_2$ aerosol followed by streptococcal infection. In this case a BMDL of 365 µg Ni/m^3 for a doubling of mortality (from 3.74 to 7.5%) was obtained with the continuous power model. Other acute studies, particularly Ishihara et al. (2002) on lung toxicity, are less suitable to deriving a one-hour value. This aREL value should be reevaluated if human immunotoxicity or other human data become available. The aREL specifically does not apply to nickel carbonyl, which releases both nickel and carbon monoxide.

9.4 8-Hour Reference Exposure Level (8-hour REL):

Study	NTP, 1994c (supported by Graham *et al*., 1978)
Study population	*Female and male rats*
Exposure method	inhalation of 0.12 to 0.5 mg $NiSO_4$/m³ 6.2hr/d x 5d/wk, 16 days to 24 months
Critical effects	alveolar macrophage hyperplasia, alveolar proteinosis, chronic active inflammation
NOAEL	*0.03 mg/Ni/m3*
Exposure duration	*6.2 hours/day x 5/7 days/week) for 13 weeks*
Extrapolated 8 hour concentration	*5.7 µg Ni/m³ (30 µg/m3 x 0.264 DAF)*
Interspecies uncertainty factor	*√10 (default)*
Intraspecies uncertainty factor	*30*
Cumulative uncertainty factor	*100*
Reference Exposure Level	*0.06 µg Ni/m³*

The studies and endpoints considered in deriving the 8-hour REL are summarized in Table 21. The 8-hour REL proposed is based on the NTP (1994c) bioassay results on non-neoplastic lung lesions. This study provides daily exposures of 6.2 hours for five days/week for durations of 16 days to 24 months(Table 22). The data were unsuitable for benchmark dose analysis. The most consistent value presented was a NOAEL of 0.03 mg Ni/m^3 for alveolar macrophage hyperplasia in female rats (Table 22). This would give a daily value of 5.7 µg Ni/m^3 (30 µg Ni/m^3 x 0.264DAF x 5/7 days/wk). A value of 1 for UF_L was used since an acceptable NOAEL was identified. Determination of the DAF

in this study is described below in the section on derivation of the chronic REL. A model was used to account for rat to human differences in upper and lower airway deposition of nickel particles and it seems likely that deposition is the key event leading to subsequent lung toxicity. Therefore, a UF_{A-k} subfactor of 1 was applied to pharmacokinetic differences and $UF_{A-d} = \sqrt{10}$ for pharmacodynamic differences, for a total $UF_A = \sqrt{10}$. An intraspecies uncertainty factor (UF_H) of 30 was used incorporating a subfactor of 10 for pharmacodynamic differences and $\sqrt{10}$ for pharmacokinetic differences. The value of UF_{H-d} of 10 addresses potential increased sensitivity of infants and children vs. adults to continuous exposures to airborne nickel particles. There is also pharmacokinetic uncertainty, but this is somewhat lessened by the deposition model which was also appled to several child lung structures. With a cumulative uncertainty factor of 100 ($\sqrt{10}$ x 30) the calculated 8-hour REL would be 0.06 µg Ni/m^3. The experimental exposures were 6.2 hours and repeated daily exposures were made over a period of 13 weeks.

A suitable supporting study for the 8 hour REL is the Graham et al. (1978) study, the immunotoxicity endpoint and the 2 hr BMDL of 165 µg Ni/m^3. Where the 1-hour extrapolation yielded a value of 233 µg/m^3 the 8-hour value was 82 µg/m^3. In this derivation we used an uncertainty factor (UF_L) of $\sqrt{10}$ for the BMDL, which replaces the LOAEL. The BMDL has the advantage over the LOAEL of using all the dose-response data, although in this case the benchmark response was considered to represent a measurable non-zero response rate. However, since a dose-response model was used, a smaller UF than would be applied for a LOAEL is adequate. There was insufficient confidence in the reported NOAEL to base a REL on that value. For interspecies uncertainty (UF_A) we adopted the usual value of 10 which can be considered to account equally for pharmacokinetic and pharmacodynamic differences between mice and humans. For intraspecies uncertainty (UF_H) we used a value of 30, which includes a subfactor of 10 for pharmacodynamic differences (i.e., child sensitivity) and $\sqrt{10}$ for pharmacokinetic differences. Using the cumulative uncertainty factor of 1000 yields an 8-hour REL of 0.08 µg/m^3. Repeated exposures to airborne nickel may have a greater impact on infants and children than on adults due to its targeting of the immune system and lung function, and its asthma inducing capability. Thus, following our approved guidelines, we have used a full UF_H of 30.

The advantage of the NTP study is multiple doses in two species and both sexes with extended durations of exposure. Daily exposures are close to eight hours and approximate the type of repeated exposures the 8-hour REL is intended to address. However, the Graham et al. (1978) study addresses an alternate toxic endpoint albeit with greater uncertainty due to study design limitations. The Ishihara et al. (2002) data on lung inflammation and mucus secretion endpoints generally fall in between the Graham et al. and the NTP studies in severity and duration of exposure, however the derived REL values appear to be consistent with the more severe lung and immunotoxicity effects evaluated.

Table 21. Studies and Toxic Endpoints Considered for the 8-Hour REL

Study	Endpoint	Criterion	Duration	8 hr Adjusted Value	Cumulative uncertainty factor	Proposed REL µg/m^3
Graham et al., 1978	Immunotoxicity	BMDL = 165 µg/m^3 (BMD_{100} = 284 µg/m^3)	2hr (single inhalation exposure)	82.3 µg/m^3	1000	0.082
NTP 1994c	Lung toxicity in rats	NOAEL = 0.03 mg/m^3	6.2 hr/d x 5 d/wk, 16 d, ≤ 24 mo	5.7 µg/m^3	100	0.06
Ishihara et al. 2002	Lung inflammation, total cells/µL in BALF	$BMDL_{1SD}$ = 5.5 µg (BMD_{1SD} = 9.8 µg)	5 hr/d x 5 d/wk x 1wk	19.6 µg/m^3	300	0.065
"	Lung inflammation, total protein in BALF, mg/mL	$BMDL_{1SD}$ = 18.6 µg (BMD_{1SD} = 26.9 µg)	5 hr/d x 5 d/wk x 1 wk	66.4 µg/m^3	300	0.22
"	Lung inflammation, total elastolytic activity in BALF	$BMDL_{1SD}$ = 50.0 µg (BMD_{1SD} = 53.0 µg)	5 hr/d x 5 d/wk x 1 wk	178 µg/m^3	300	0.60
"	Mucus secretion, sialic acid in BALF, µg/mL	$BMDL_{1SD}$ = 13.5 µg (BMD_{1SD} = 23.0 µg)	5 hr/d x 5 d/wk x 1wk	48.2 µg/m^3	300	0.16
Pandey & Srivastava, 2000	Decreased sperm motility percent	$BMDL_{1SD}$ = 2.91 mg $NiSO_4$/kg	1 oral dose/d x 5 d/wk x 5 wk	0.47 mg Ni/kg-d	1000	3.3
"	Increased Sperm Abnormalities percent	$BMDL_{1SD}$ = 0.46 mg $NiSO_4$/kg	1 oral dose/d x 5 d/wk x 5 wk	0.074 mg Ni/kg	1000	0.52
"	Increased Sperm Abnormalities percent	$BMDL_{1SD}$ = 0.34mg $NiCl_2$/kg	1 oral dose/d x 5 d/wk x 5 wk	0.060 mg Ni/kg	1000	0.42

Note: BALF = bronchoalveolar lavage fluid; for spermatotoxicity it was assumed that the hexahydrate salts were used, for the inhalation equivalent level it was assumed that only 50% of nickel would be absorbed via the inhalation route in addition to a 70 kg body weight and a 20 m^3/d inhalation rate (i.e. mouse µg/kg/d x 70 kg/20 m^3/d/0.5 = human µg/m^3.

Table 22. Non-neoplastic Lung Toxicity Observed with Inhalation of Nickel Sulfate (NTP, 1994c).

Effect	16 days (animals/dose group)*	13 weeks	7 months	15 months	24 months
		(N)OAEL or (L)OAEL mg Ni/m^3			
Male Mice					
Lung Inflammation	0.77L (5)	0.44N(10)	0.22N(5)	0.11N(5)	0.056N(61)
Alveolar Macrophage Hyperplasia		0.056N(10)	0.11N(5)	0.056N(5)	0.056N(61)
Fibrosis		0.22N(10)			
Female Mice					
Lung Inflammation	0.77L(5)	0.22N(10)	0.22N(5)	0.11N(5)	0.056L(60)
Alveolar Macrophage Hyperplasia		0.056N(10)	0.11N(5)	0.11N(5)	0.056L(60)
Fibrosis		0.22N(10)			
Male Rats					
Lung Inflammation	0.7L(5)	0.11N(10)	0.03L(5)	0.06N(5)	0.03N(53)
Alveolar Macrophage Hyperplasia		0.03L(10)	0.03N(5)	0.06N(5)	0.03N(53)
Fibrosis				0.11N(5)	0.03N(53
Female Rats					
Lung Inflammation	0.7L(5)	0.06N(10)	0.03N(5)	0.06N(5)	0.03N(53)
Alveolar Macrophage Hyperplasia		0.03L(10)	0.03N(5)	0.06N(5)	0.03N(53)
Fibrosis				0.11N(5)	0.03N(53)

*Note: animals exposed to $NiSO_4$ aerosol for 6.2 hr/day, 5days/week.

9.5 Derivation of Chronic Reference Exposure Levels (cRELS)

The studies conducted by NTP (1994a & c) were used as the bases for the chronic RELs. These studies all showed similar non-carcinogenic effects in rats and mice, regardless of the form of nickel administered. It therefore appears that soluble and insoluble forms of nickel cause similar effects in rodents. For nickel sulfate the NOAELs for alveolar proteinosis are virtually identical for male or female rats (Table 22). The data set for exposures of 24 months duration was used in the development of the cREL for nickel and nickel compounds other than nickel oxide. Benchmark dose analysis was undertaken with the results shown in Table 23. A benchmark concentration of 0.0305 mg Ni/m^3, which is the average of the values obtained for alveolar proteinosis in male and female rats, was selected.

Table 23. Benchmark Dose Analysis of Lung Effects Induced by $NiSO_4$ in Two-Year Studies (NTP, 1994c)

Species, Sex, Endpoint, Quantal Response	**Model**	**Goodness of Fit, X^2, p**	**BMD_{05} mg Ni/m^3**	**$BMDL_{05}$ mg Ni/m^3**
Rats, Male				
Macrophage Hyperplasia, 7/54,9/53,35/53,48/53	Log logistic	1.30, 0.25	0.024	0.016
Alveolar proteinosis 0/54,0/53,12/53,41/53	**Multistage**	**1.68, 0.64**	**0.036**	**0.029**
Rats, Female				
Macrophage Hyperplasia, 9/53,10/53,32/53,45/54	Multistage	3.94, 0.14	0.018	0.007
Alveolar proteinosis 1/52,0/53,22/53,49/54	**Log probit**	**2.02, 0.16**	**0.038**	**0.032**

For extrapolation to humans the multiple-path particle dosimetry model (MPPD) version two was used to derive a dosimetric adjustment factor (DAF) to calculate a human equivalent concentration (HEC), see Table 24.

Table 24. Lung Deposition of $NiSO_4 \cdot 6H_2O$ and NiO Particles Predicted by the Hsieh et al. (1999a, c) and the Age-Specific MPPD Model (Version 2)*

Age Distribu-tion	$NiSO_4$ Hsieh et al. 1999a		NiO Hseih et al. 1999c		$NiSO_4$ MPPD2		NiO MPPD2	
MMAD, µm	2.33		2.80		2.50		2.46	
gsd	2.20		1.87		2.38		1.87	
Density, g/cm^3	2.07		7.45		2.07		6.67	
Concn. mg/m^3	0.12, 0.2, 0.50		1.25, 2.5, 5.0		0.12		1.25	
Species	Rat		Mouse		Rat		Rat	
TB + ALV	**ADF**	**DAF**	**ADF**	**DAF**	**ADF**	**DAF**	**ADF**	**DAF**
Rat, adult	0.0769	1.00	0.0354	1.00	0.089	1.00	0.1289	1.00
Human 3 months	0.3982	0.193	0.4491	0.0788	0.4008	0.2225	0.4329	0.30
Human 3 years	0.3246	0.237	0.3674	0.0964	0.3245	0.274	0.3552	0.36
Human 9+ years	0.4086	0.188	0.4631	0.0764	0.4047	0.2199	0.4502	0.29
Human 14 years	0.3653	0.21	0.3209	0.1102	0.3600	0.2472	0.4039	0.32
Human 21 years	0.2643	0.291	0.2957	0.1197	0.2479	0.3597	0.3026	0.43
Human mean		0.224		0.096		0.264		0.338

*Note: MPPD = Multi-Pathway Particle Dosimetry model run with particle concentration of 1 $\mu g/m^3$, rat nasal breathing and human oronasal normal augmenter, ADF = airway deposition fraction (tracheobronchial plus alveolar), DAF = dosimetric adjustment factor (Human Equivalent Concentration = DAF x Animal Concentration);The MPPD model was developed by the CIIT Center for Health Research, The National Institute of Public Health and the Environment, The Netherlands (RIVM), the Ministry of Housing Spatial Planning and the Environment, The Netherlands, and the National Institute for Occupational Safety and Health (NIOSH). See Brown et al. (2005) for model comparisons.

In using the ratio of animal to human deposition fractions $(Fr)_A/(Fr)_H$ as the DAF, our approach differs from that of U.S.EPA (1994). In their regional deposited dose rate ratio (RDDRr) approach they would multiply the deposition ratio by the ratios of adult minute volumes $(V_E)_A/(V_E)_H$ and regional surface areas $(SA)_H/(SA)_A$ to estimate a deposited dose. In our case this adjustment would approximately double the DAF to 0.554 from 0.264. We have chosen not to apply this adjustment since our human fractional deposition in the above ratio is the average of several age-specific MPPD2 model predictions. We believe that this ratio would be significantly discounted by the RDDRr approach, which does not include deposition predictions for children. Note that in Table 17 all of the child models show higher airway deposition fractions than adult (0.32 to 0.4 vs. 0.25 for adult).

We have investigated the use of the MPPD2 model in deposition and clearance simulations to estimate alveolar dosimetry in units of µg Ni retained/day/m^2 alveolar surface area (TB clearance is very rapid and doesn't figure in the retention rates) for the various age-specific models. The results indicate an average retention ratio $(R)_A/(R)_H$ of 0.61 leading to a DAF of about 2/3 the value we are currently using. For the present time we propose to continue using the simple deposition fraction ratio as providing the most direct and unmanaged value without additional assumptions about clearance rates and adult values etc.

With a DAF of 0.26 the HEC was calculated as 1.4 µg/m^3. The uncertainty factors applied to this value were UF_L = 1 since a NOAEL was identified. The interspecies uncertainty factor UF_A = √10 was used since the MPPD2 model accounted for rat to human differences in upper and lower airway deposition of nickel particles and it seems likely that deposition is the key event leading to subsequent lung toxicity (e.g., alveolar proteinosis). Therefore, a UF_A subfactor of 1 would then apply to pharmacokinetic differences and √10 for pharmacodynamic differences. The default intraspecies uncertainty factor (UF_H) of 30 was used, incorporating a subfactor of 10 for pharmacodynamic differences and √10 for pharmacokinetic differences. The value of 10 addresses potential increased sensitivity of infants and children vs. adults to continuous exposures to airborne nickel particles. There is also pharmacokinetic uncertainty but this is somewhat lessened by the MPPD2 model which was also applied to several child lung structures. A cumulative uncertainty factor of 100 was then used to derive a chronic REL of 0.014 µg/m^3.

9.6 cREL for Nickel and Nickel Compounds (except nickel oxide)

Study	National Toxicology Program, 1994c
Study population	Male and female F344/N rats (52-53 per group)
Exposure method	Discontinuous inhalation
Critical effects	Pathological changes in lung, lymph nodes, and nasal epithelium: (1) active pulmonary inflammation, (2) macrophage hyperplasia, (3) alveolar proteinosis, (4) fibrosis, (5) lymph node hyperplasia, (6) olfactory epithelial atrophy
$BMDL_{05}$	30.5 µg/m^3 (alveolar proteinosis, male and female mean)
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Average experimental exposure	5.4 µg Ni/m^3 for NOAEL group (30 x 6/24 x 5/7)
Human equivalent concentration	1.4 µg Ni/m^3 for NOAEL group males (particulate with respiratory effects, DAF = 0.26 based on MMAD = 2.50 µm, gsd = 2.38 µm, density = 2.07 g/cm^3 by MPPD2 model)
LOAEL uncertainty factor	1(default)
Subchronic uncertainty factor	1(default)
Interspecies uncertainty factor	√10 (√10 PD * 1 PK)
Intraspecies uncertainty factor	30 (10 PD * √10 PK)
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.014 µg Ni/m^3

A supporting study is that of Berge and Skyberg (2003) measuring pulmonary fibrosis in nickel refinery workers over a 22 year period. The authors found a weak but positive dose response for pulmonary fibrosis and cumulative nickel exposure expressed as (mg Ni/m^3)-yr. The best model fit to the data was obtained with the unadjusted data on soluble nickel of 0.35 (mg/m^3)-yr for the $BMDL_{01}$ (1% excess risk, multistage model) (Table 25). Converting this value to a lifetime continuous value (8/24 hr x 5/7 days x 1/70 yr) gives 1.2 µg/m^3 equivalent and applying a 30-fold UF_H would give a supporting value for the cREL of 0.04 µg/m^3. The respiratory lesions observed in the Oller et al. (2008) chronic rat study with nickel metal powder give lower cREL values, particularly for alveolar proteinosis (0.004 µg Ni/m^3 female and 0.007 µg Ni/m^3 male), but the material is probably atypical of ambient air exposures.

Table 25. Benchmark Dose Analysis of Pulmonary Fibrosis in Nickel Refinery Workers (data from Berge & Skyberg, 2003)

Nickel type, cumulative dose	Quantal response	Adjustment, goodness of fit χ^2, P Multistage Model	BMD_{01} (mg/m^3)-yr	$BMDL_{01}$ (mg/m^3)-yr
Soluble Ni: 0.03, 0.27, 1.03, and 4.32 (mg/m^3)-yr	6/254, 3/246, 13/283, 25/263	None, 2.21, 0.33	0.51	0.35
	6/254, 4/246, 12/283, 13/263	Age, smoking, asbestos, sulfidic Ni, 2.21, 0.33	1.38	0.69
	6/254, 4/246, 12/283, 16/263	Age, smoking, asbestos, 1.72, 0.42	0.98	0.56
Sulfidic Ni: 0.01, 0.08, 0.33, 1.73 (mg/m^3)-yr	4/264, 9/237, 15/282, 19/263	None, 3.91, 0.14	0.33	0.19
	4/264, 9/237, 11/282, (8/263)	Age, smoking, asbestos, soluble Ni, 3.27, 0.20; (1.87, 0.17)	No Value for full data set; (0.15 without top dose)	No Value for full data set; (0.063 without top dose)
	4/267, 10/237, 13/282, 12/263	Age, smoking, asbestos, 4.16, 0.125	0.95	0.34

9.7 Nickel Oxide

For nickel oxide the benchmark dose analysis of the lung lesion data from NTP (1994a) gives an improved value of 117 µg Ni/m^3 for the $BMDL_{05}$. The results of the analysis are summarized in Table 26. The derivation of the chronic REL for NiO is similar to that for other nickel compounds shown above with only a slightly different DAF resulting in a proposed cREL for NiO of 0.06 $µg/m^3$ based on pulmonary inflammation in male and female mice.

Table 26. Benchmark Dose Analysis of Lung Effects Induced by NiO in Two-Year Studies (NTP, 1994a)*

Species, Sex, Endpoint, Quantal Response	Model	Goodness of Fit, X^2, p	BMD_{05} mg Ni/m^3 (see note)	$BMDL_{05}$ mg Ni/m^3 (see note)
Rats, Male				
Bronchiolar hyperplasia 0/52,7/51,10/53,18/52	Quantal Linear	0.22, 0.89	0.15	0.004
Mice, Male				
Lung inflammation 0/57,21/67,34/66,55/69	Quantal Linear	0.09, 0.95	0.16	0.052
Alveolar proteinosis 0/57,12/67,22/66,43/69	Quantal Linear	0.09, 0.96	0.33	0.13
Mice, Female				
Lung inflammation 7/64,43/66,53/63,52/64	Multistage Cubic	0, 1.0	0.056	0.028
Alveolar proteinosis 0/64,8/66,17/63,29/64	Quantal Linear	0.14, 0.93	0.40	0.12

*Note: BMD and BMDL values are in mg Ni/m^3 continuous

Note that since the MPPD2 model does not calculate airway deposition fractions for the mouse we have included airway deposition fractions from Hsieh et al. (1999c) in Table 24. These authors used the following values for NiO: MMAD = 2.8 µm; gsd = 1.87; density = 7.45 g/cm^3; and concentrations from 1.25 to 5.0 mg NiO/m^3. Predicted mouse deposition fraction for the tracheobronchial region was 0.0096 and for the alveoli was 0.0258 with a total (TB + Alv) of 0.0354. This is much lower than the MPPD2 rat deposition fraction of 0.1289 (OEHHA) or 0.0801 in Hsieh et al. (1999a). Applying this mouse deposition from Hsieh gives a lower DAF of 0.096 and consequently lower HEC of 2.0 µg Ni/m^3. We applied the following uncertainty factors in the derivation of the cREL summarized below. Since an adequate chronic BMDL was available, the UF_L is 1. For interspecies uncertainty we used the same UF_A and rationale as for nickel (above). We assumed that alveolar deposition was the key event leading to subsequent lung toxic effects (e.g., alveolar proteinosis) and that the dosimetric adjustment factor (DAF) would adequately account for the interspecies differences. We applied a factor of √10 for pharmacodynamic differences between mice and humans. For intraspecies differences we applied a UF_H of 30 using the same rationale as with the values derived above. A subfactor of 10 was used to account for the anticipated greater sentitivity of infants and children to continuous exposure to airborne nickel oxide particles. A subfactor of √10 was applied for pharmacokinetic differences between children and adults. The cumulative UF of

100 (√10 x 30) was applied to the HEC of 2.0 µg Ni/m^3 to derive the cREL of 0.02 µg Ni/m^3. This derivation is summarized below.

Study	National Toxicology Program, 1994a
Study population	Male and female B6C3F$_1$ mice (57-69 per group)
Exposure method	Discontinuous inhalation
Critical effects	Pathological changes in lung: (1) active pulmonary inflammation, (2) alveolar proteinosis
BMDL$_{05}$	117 µg Ni/m^3 (alveolar proteinosis)
Exposure continuity	6 hours/day, 5 days/week
Exposure duration	104 weeks
Average experimental exposure	20.9 µg Ni/m^3 for LOAEL group (117 x 6/24 x 5/7)
Human equivalent concentration	2.0 µg Ni/m^3 for BMDL$_{05}$ for female mice (particulate with respiratory effects, DAF = 0.096 based on MMAD = 2.80 µm, gsd = 1.87, density = 7.45 g/cm^3, from Hsieh et al. 1999c)
LOAEL uncertainty factor	1(default)
Subchronic uncertainty factor	1(default)
Interspecies uncertainty factor	√10 (√10PD * 1PK)
Intraspecies uncertainty factor	30 (10 PD * √10 PK)
Cumulative uncertainty factor	100
Inhalation reference exposure level	0.02 µg Ni/m^3 as NiO

The human epidemiological literature predominantly describes cancer mortality rates from occupational exposures to nickel compounds, but does not specifically examine non-cancer effects. However, it is clear from many case reports that allergies and dermatitis can occur in exposed workers. Hypersensitive reactions to nickel have not been quantitatively studied in humans or animals; therefore it is not possible to develop an REL based on immunological hypersensitivity at the present time. A host of subacute and subchronic animal studies have shown nickel to affect certain immunological responses unrelated to hypersensitivity, but the applicability of these results to chronic human exposures and responses involves considerable uncertainty. Furthermore, data show that nickel may precipitate onset of asthma in occupational settings.

The results of the NTP studies and these dose response analyses support the speciation of nickel oxide for noncancer effects. The health effects data for nickel oxide indicate that its adverse pulmonary effects were less severe (absence of fibrosis, lower chronic lung inflammation severity scores) at higher doses than the pulmonary effects observed for nickel sulfate and nickel subsulfide. The higher chronic REL value for nickel oxide of 0.06 µg/m^3 reflects these dose response differences. OEHHA therefore concludes that 0.06 µg/m^3 is

an appropriate REL for nickel oxide. However, in setting inhalation exposure RELs for groups of compounds, OEHHA uses the most sensitive strain, species, sex, chronic endpoint, and agent for each group of substances. Therefore, as the pulmonary toxicity of the relatively insoluble nickel subsulfide is greater than that of nickel oxide and closer to that of nickel sulfate, OEHHA proposes to use the chronic REL derived from nickel sulfate for all other nickel compounds.

It should be noted tha although the non-neoplastic lung effects seen in the animal studies discussed above were relatively mild, similar effects in humans may be serious or even fatal.

9.8 Data Strengths and Limitations for Development of the Chronic RELs

The strengths of the inhalation REL include the availability of controlled lifetime exposure inhalation studies in multiple species at multiple exposure concentrations and with adequate histopathological analysis and the observation of a NOAEL. The major areas of uncertainty are the lack of adequate human exposure data and the lack of lifetime toxicity studies in any non-rodent species. The toxicological response to various inhaled nickel compounds in children compared to adults is also an area of uncertainty addressed by a larger uncertainty factor for intra-individual variation (UF_H). Nickel targets the immune system and the lung, which are likely a more susceptible system and organ in exposed infants and children.

9.9 Oral Chronic Reference Level

Study	NiPERA (2000a,b) supported by Smith *et al*., 1993
Study population	Rats (Sprague-Dawley)
Exposure method	Aqueous gavage
Critical effects	Perinatal mortality in two generation study
LOAEL	2.23 mg Ni/kg-d
NOAEL	1.12 mg Ni/kg-d
Exposure continuity	Continuous
Exposure duration	Chronic (70 weeks)
Average exposure	1.12 mg/kg-day
Human equivalent concentration	1.12 mg/kg-day
LOAEL uncertainty factor	1(default)
Subchronic uncertainty factor	1(default)
Interspecies uncertainty factor	10(default)
Intraspecies uncertainty factor	10(default)
Cumulative uncertainty factor	100
Oral reference exposure level	0.0112 mg/kg-day

In addition to being inhaled, airborne nickel can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic REL for nickel is also required.

The proposed oral REL for nickel uses the same three studies used to support OEHHA's Public Health Goal for nickel in drinking water. OEHHA (2000) identified the oral dose of 1.12 mg/kg-d from the lower dose-range of (NiPERA, 2000b) as the appropriate NOAEL value. This NOAEL is lower than the doses at which early pup mortality was observed (LOAEL of 2.23 mg/kg-d) in the preliminary study (NiPERA. 2000a) and the LOAEL of 1.3 mg Ni/kg-d reported by Smith et al. (1993). The oral REL derivation summarized above used uncertainty factors of 10 each for interspecies, and intraspecies extrapolations. The final value is 0.0112 mg Ni/kg-d or 11.0 µg Ni/kg-d. Haber et al. (2000) have proposed an oral reference dose of 8 µg Ni/kg-d based on albuminuria seen in female Wistar rats exposed to $NiSO_4$ for six months (Vsykocil et al., 1994). In our view the limitations of the Vsykocil et al. study, particularly the lack of a clear dose response, render it less acceptable than the NiPERA studies as the basis for a chronic oral REL. All of the inhalation-based RELs derived above give much lower intake values than the oral chronic REL and are considered sufficiently protective of nickel-mediated developmental or reproductive toxicity.

10 Nickel as a Toxic Air Contaminant that Disproportionately Impacts Children

There is a potential for exposure to nickel and nickel compounds in view of its widespread occurrence and numerous uses (see section 3). Nickel is a minor component of airborne particulate matter (PM) and may play a role in the toxicity of PM. It also occurs in tobacco smoke. The adverse impacts of nickel compounds on the respiratory and immune systems (including asthma), and also the increased perinatal mortality and reduced birth weight observed in animal studies of reproductive toxicity (see Section 6), are among the types of effect leading to the potential for differential impacts on infants and children. OEHHA therefore recommends that nickel be identified as a toxic air contaminant, which may disproportionately impact children, pursuant to Health and Safety Code, Section 39669.5(c).

11 References

Abbracchio M, Simmons-Hansen J and Costa M. (1982). Cytoplasmic dissolution of phagocytized crystalline nickel sulfide particles: A prerequisite for nuclear uptake of nickel. J Toxicol Environ Health 9:663-676.

Adkins B, Richards JH and Gardner DE. (1979). Enhancement of experimental respiratory infection following nickel inhalation. Environ Res: 20:33-42.

Afridi HI, Kazi TG, Jamali MK, Kazi GHG, Arain MB, Jalbani N, Shar GQ and Sarfaraz RA. (2006). Evaluation of toxic metals in biological samples (scalp hair, blood and urine) of steel mill workers by electrothermal atomic absorption spectroscopy. Toxicol Ind Health 22:381-393.

Afridi HI, Kazi TG, Kazi NG, Jamali MK, Arain MB, Sirajuddin, Baig JA, Kandhro GA, Wadhwa SK and Shah AQ. (2010). Evaluation of cadmium, lead, nickel and zinc status in biological samples of smokers and nonsmokers hypertensive patients. J Hum Hyperten 24:34-43.

Agrawal H, Eden R, Zhang X, Fine PM, Katzenstein A, Miller JW, Ospital J, Teffera S and Cocker DR III. (2009). Primary particulate matter from ocean-going engines in the southern California air basin. Environ Sci Technol 43:5398-5402.

Ahamed M. (2011). Toxic response of nickel nanoparticles in human lung epithelial A549 cells. Toxicol in Vitro 25:930-936.

Ahamed M, Akhtar MJ, Siddiqui MA, Ahmad J, Musarrat J, Al-Khedhairy AA, Alsalhi MS and Alrokayan SA. (2011). Oxidative stress mediated apoptosis induced by nickel ferrite nanoparticles in cultured A549 cells. Toxicology 283: 101-108.

Akesson B and Skerfving S. (1985). Exposure in welding of high nickel alloy. Int Arch Occup Environ Health 56:111-117.

Amacher DE and Paillet SC. (1980). Induction of trifluorothymidine resistant mutants by metal ions in L5178Y/TK$^{+/-}$ cells. Mutat Res 78:279-288.

Ambrose AM, Larson PS, Borzelleca JF and Hennigar GR, Jr. (1976). Long term toxicologic assessment of nickel in rats and dogs. J Food Sci Technol 13:181-187.

Andersen I and Svenes KB. (1989). Determination of nickel in lung specimens of thirty-nine autopsied nickel workers. Int Arch Occup Environ Health 61:289-295.

Andersen O. (1983). Effects of coal combustion products and metal compounds on sister chromatid exchange (SCE) in a macrophage cell line. Environ Health Perspect 47:239-253.

Andrew AS and Barchowsky A. (2000). Nickel-induced plasminogen activator inhibitor-1 expression inhibits the fibrinolytic activity of human airway epithelial cells. Toxicol Appl Pharmacol 168:50-57.

Andrew As, Klei LR and Barchowsky A. (2001). AP-1-dependent induction of plasminogen activator inhibitor-1 by nickel does not require reactive oxygen. Am J Physiol Lung Cell Mol Physiol 281:L616-L623.

Andrews RK, Blakeley RL and Zerner B. (1988). Nickel in proteins and enzymes. In: *Metal Ions in Biological Systems. Vol 23 Nickel and Its Role in Biology*. Sigel H and Sigel A eds. Marcel Dekker, New York, pp. 165-284.

Angerer J and Lehnert G. (1990). Occupational chronic exposure to metals. II: Nickel exposure of stainless steel welders—biological monitoring. Int Arch Occup Environ Health 62:7-10.

ATSDR (2005). Toxicological Profile for Nickel. Public Health Service, U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry. August, 2005.

Amlacher E and Rudolph C (1981). The thymidine incorporation inhibiting screening system (TSS) to test carcinogenic substances: a nuclear DNA synthesis suppressive short-term test. Arch Geschwultstforsch 51:605-610.

Arhami M, Sillanpaa M, Hu S, Olson MR, Schauer JJ and Sioutas C. (2009). Size-segregated inorganic and organic components of PM in the communities of the Los Angeles harbor. Aerosol Sci Technol 43:145-160.

Arita A and Costa M (2009). Epigenetics in metal carcinogenesis: nickel, arsenic, chromium, and cadmium. Metallomics 1:222-228.

Arlauskas A, Baker RS, Bonin AM, Tandon RK, Crisp PT and Ellis J. (1985). Mutagenicity of metal ions in bacteria. Environ Res 36:379-388.

Au A, Ha J, Hernandez M, Polotsky A, Hungerford DS and Frondoza CG. (2006). Nickel and vanadium metal ions induce apoptosis of T-lymphocyte Jurkat cells. J Biomed Mater Res 79A:512-521.

Barchowsky A, Soucy NV, O'Hara KA, Hwa J, Noreault TL and Andrew AS. (2002). A novel pathway for nickel-induced Interleukin-8 expression. J Biol Chem 277:24225-24231.

Basrur PK and Gilman JPW. (1067). Morphologic and synthetic response of normal and tumor muscle cultures to nickel sulfide. Cancer Res 27:1168-1177.

Bell ML, Ebisu K, Peng RD, Samet JM and Dominici F. (2009). Hospital admissions and chemical composition of fine particle pollution. Am J Respir Crit Care Med 179(12):1115-1120.

Bell ML, Belanger K, Ebisu K, Gent JF, Lee HJ, Koutrakis P and Leaderer BP. (2010). Prenatal exposure to fine particulate matter and birth weight. Epidemiology 21(6):884-891.

Benoff S, Cooper GW, Centola GM, Jacob A, Hershlag A and Hurley IR. (2000). Metal ions and human sperm mannose receptors. Andrologia 32:317-239.

Benson JM, Carpenter RL, Hahn FF, Haley PJ, Hanson RL, Hobbs CH, Pickrell JA and Dunnick JK. (1987). Comparative inhalation toxicity of nickel subsulfide to F344/N rats and B6C3F1 mice exposed for 12 days. Fundam Appl Toxicol 9:251-265.

Benson JM, Carpenter RL, Hahn FF, Haley PJ, Hanson RL, Hobbs CH, Pickrell CH and Dunnick JK. (1987). Comparative inhalation toxicity of nickel subsulfide to F344/N rats and B6C3F1 mice exposed for twelve days. Fundam Appl Toxicol 9:251-265.

Benson JM, Burt DG, Carpenter RL, Eidson AF, Hahn FF, Haley PJ, Hanson RL, Hobbs CH, Pickrell CH and Dunnick JK. (1988). Comparative inhalation toxicity of nickel sulfate to F344/N rats and B6C3F1 mice exposed for twelve days. Fundam Appl Toxicol 10:164-178.

Benson JM, Barr EB, Bechtold WE, Cheng YS, Dunnick JK, Eastin WE, Hobbs CH, Kennedy CH and Maples KR. (1994). Fate of inhaled nickel oxide and nickel subsulfide in F344/N rats. Inhal Toxicol 6:167-183.

Berge SR and Skyberg K. (2003). Radiographic evidence of pulmonary fibrosis and possible etiologic factors at a nickel refinery in Norway. J Environ Monit 5:681-688.

Beyersmann D and Hartwig A. (2008). Carcinogenic metal compounds: recent insight into molecular and cellular mechanisms. Arch Toxicol 82:493-512.

Biggart NW and Costa M. (1986). Assessment of the uptake and mutagenicity of nickel chloride in *Salmonella* tester strains. Mutat Res 175:209-215.

Biggart NW and Murphy E Jr. (1988). Analysis of metal-induced mutations altering the expression or structure of a retroviral gene in a mammalian cell line. Mutat Res 198:115-130.

Biggart NW, Gallick GE and Murphy EC Jr. (1987). Nickel-induced heritable alterations in retroviral transforming gene expression. J Virol 61:2378-2388.

Boisleve F, Kerdine-Romer S and Pallardy M. (2005). Implication of the MAPK pathways in the maturation of human dendritic cells induced by nickel and TNF-α. Toxicology 206:233-244.

Borg K and Tjalve H. (1988). Effect of thiram and dithiocarbamate pesticides on the gastrointestinal absorption and distribution of nickel in mice. Toxicol Lett 42:87-98.

Borg K and Tjalve H. (1989). Uptake of $^{63}Ni^{2+}$ in the central and peripheral nervous system of mice after oral administration: Effects of treatment with halogenated 8-hydroxyquinolines. Toxicology 54:59-68.

Boscolo P, Andreassi M, Sabbioni E, Reale M, Conti P, Amerio P and Di Gioacchino M. (1999). Systemic effects of ingested nickel on the immune system of nickel sensitized women. Life Sci 64(17):1485-1491.

Broday L, Peng W, Kuo MH, Salnikow K, Zoroddu M and Costa M. (2000). Nickel compounds are novel inhibitors of histone H4 acetylation. Cancer Res 60:238-241.

Broder I, Smith JW, Corey P and Holness L. (1989). Health status and sulfur dioxide exposure of nickel smelter workers and civic laborers. J Occup Med 31(4):347-353.

Brown JS, Wilson WE and Grant LD. (2005). Dosimetric comparisons of particle deposition and retention in rats and humans. Inhal Toxicol 17:355-385.

Buchvald D and Lundeberg L. (2004). Impaired responses of peripheral blood mononuclear cells to nickel in patients with nickel-allergic contact dermatitis and concomitant atopic dermatitis. Br J Dermatol 150:484-492.

Burnet FM. (1974). *Intrinsic Mutagenesis, a Genetic Approach to Ageing*. J. Wiley, New York.

Burnett RT, Brook J, Dann T, Delocla C, Philips O, Cakmak S, Vincent R, Goldberg MS and Krewski D. (2000). Association between particulate- and gas-phase components of urban air pollution and daily mortality in eight Canadian cities. Inhal Toxicol 12(Suppl 4):15-39.

Caicedo M, Jacobs JJ, Reddy, A and Hallab NJ. (2008). Analysis of metal ion-induced DNA damage, apoptosis, and necrosis in human (Jurkat) T-cells demonstrates Ni^{2+} and V^{3+} are more toxic than other metals: Al^{3+}, Be^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Mo^{5+}, Nb^{5+}, Zr^{2+}. J Biomed Mater Res 86A:905-913.

CARB (1991). Technical Support Document: Proposed Identification of Nickel as a Toxic Air Contaminant. Parts A and B. California Air Resources Board, California Environmental Protection Agency, Sacramento, CA.

CARB. (2008). Annual Statewide Toxics Summary: Nickel (http://www.arb.ca.gov/adam/toxics/statepages/nistate.html).

CARB (2009). The California Almanac of Emissions and Air Quality – 2009 Edition. (http://www.arb.ca.gov/aqd/almanac/almanac09/toc09.htm)p 4-10, 4-11.

CDTSC. (1985). Review: Nickel and compounds. California Department of Toxic Substances Control. November 15, 1985.

Carroll S and Wood EJ. (2000). Exposure of human keratinocytes and fibroblasts in vitro to nickel sulfate ions induces synthesis of stress proteins Hsp72 and Hsp90. Acta Derm Venerol 80(2):94-97.

Carter JD, Ghio AJ, Samet JM and Devlin RB. (1997). Cytokine production by human airway epithelial cells after exposure to an air pollution particle is metal-dependent. Toxicol Appl Pharmacol 146:180-188.

Carvalho SM and Ziemer PL. (1982). Distribution and clearance of ^{63}Ni administered as $^{63}NiCl_2$ in the rat: Intratracheal study. Arch Environ Contam Toxicol 11:245-248.

Casey CE and Neville MC. (1987). Studies in human lactation 3: molybdenum and nickel in human milk during the first month of lactation. Am J Clin Nutr 45(5):921-926.

Chakrabarti SK and Bai C. (1999). Role of oxidative stress in nickel chloride-induced cell injury in rat cortical slices. Biochem Pharmacol 58:1501-1510.

Chashschin VP, Artunia GP and Norseth T. (1994). Congenital defects, abortion and other health effects in nickel refinery workers. Sci Total Environ 148:287-291.

Chen CY, Wang YF, Huang WR and Huang YT. (2003). Nickel induces oxidative stress and genotoxicity in human lymphocytes. Toxicol Appl Pharmacol 189:153-159.

Chen H, Ke Q, Kluz T, Yan Y and Costa M. (2006). Nickel ions increase histone H3 lysine 9 dimethylation and induce transgene silencing. Mol Cell Biol 26(10):3728-3737.

Chen LC and Lippmann M. (2009). Effects of metals within ambient air particulate matter (PM) on human health. Inhal Toxicol 21:1-31.

Cheng RYS, Zaho A, Alvord WG, Powell DA, Bare RM, Masuda A, Takahashi T, Anderson LM and Kasprzak KS. (2003). Gene expression dose-response changes in microarrays after exposure of human peripheral lung epithelial cells to nickel(II). Toxicol Appl Pharmacol 191:22-19.

Christensen OB and Moller H. (1975). External and internal exposure to the antigen in the hand eczema of nickel allergy. Contact Dermatitis 1(3):136-141.

Christensen OB and Lagesson V. (1981). Nickel concentration of blood and urine after oral administration. Ann Clin Lab Sci 11:119-125.

Ciccarelli RB and Wetterhahn KE. (1984). Molecular basis for the activity of nickel. In: Sunderman FW, *et al*., eds. *Nickel in the Human Environment*. Proceedings from joint symposium held at IARC, Lyon, France. Lyon: IARC, p. 201-213.

Cirla AM, Bernabeo F, Ottoboni F and Ratti R. (1985). Nickel induced occupational asthma: Immunological and clinical aspects. In: Brown SS, Sunderman FW, eds. *Progress in Nickel Toxicology*. Boston (MA): Blackwell Scientific Publications. p. 165-168.

Clemens F and Landolph JR. (2003). Genotoxicity of samples of nickel refinery dust. Toxicol Sci 73:114-123.

Clemons GK and Garcia JF. (1981). Neuroendocrine effects of acute nickel chloride administration in rats. Toxicol Appl Pharmacol 61:343-348.

Condevaux F, Guichard J, Forichon A, Aujoulat M and Descotes J. (2001). Compared effects of morphine and nickel chloride on NK cell activity in vitro in rats and monkeys. J Appl Toxicol 21:431-434.

Conway K and Costa M. (1989). Nonrandom chromosomal alterations in nickel-transformed Chinese hamster embryo cells. Cancer Res 49:6032-6038.

Coogan TP, Latta DM, Snow ET and Costa M. (1989). Toxicity and carcinogenicity of nickel compounds. CRC Crit Rev Toxicol 19(4):341-384.

Cooke MS, Olinski R and Evans MD. (2006). Does measurement of oxidative damage to DNA have clinical significance? Clin Chim Acta 365:30-49.

Costa M. (1991). Molecular mechanisms of nickel carcinogenesis. Annu Rev Pharmacol Toxicol 31:321-337.

Costa M and Heck JD. (1982). Specific nickel compounds as carcinogens. Trends Pharmacol Sci 3:408-410.

Costa M, Simmons-Hansen J, Bedrossian C, Bonura J and Caprioli RM. (1981). Phagocytosis, cellular distribution and carcinogenic activity of particulate nickel compounds in tissue culture. Cancer Res 41:2868-2876.

Costa M, Cantoni O, de Mars M and Swartzendruber DE. (1982). Toxic metals produce an S-phase-specific cell cycle block. Res Commun Chem Pathol Pharmacol 38:405-419.

Costa M, Zhuang Z, Huang X, et al. (1994). Molecular mechanisms of nickel carcinogenesis. Sci Total Environ 148:191-199.

Costa M, Davidson TL, Chen H, Ke Q, Zhang P, Yan Y. Huang C and Kluz T (2005). Nickel carcinogenesis: Epigenetics and hypoxia signaling. Mutat Res 592:79-88.

Cronin E, Di Michiel AD and Brown SS. (1980). Oral challenge in nickel-sensitive women with hand eczema. In: *Nickel Toxicology*, eds. Brown SS and Sunderman FW. Academic Press Inc. (London) Ltd., London, UK.

Daldrup T, Haarhoff K and Szathmary SC. (1983). [Fetal nickel sulfate intoxication]. Beitr Gerichtl Med 41:141-144.

Danadevi K, Rozati R, Reddy PP and Grover P. (2003). Semen quality of Indian welders occupationally exposed to nickel and chromium. Reprod Toxicol 17(4):451-456.

Danadevi K, Rozati R, Banu BS and Grover P. (2004). In vivo genotoxic effect of nickel chloride in mice leukocytes using comet assay. Food Chem Toxicol 42:751-757.

Das KK and Dasgupta S. (1997). Alteration of testicular biochemistry during protein restriction in nickel treated rats. Biol Trace Elem Res 60:243-249.

Das KK and Dasgupta S. (2000). Effect of nickel on testicular nucleic acid concentrations of rats on protein restriction. Biol Trace Elem Res 73:175-180.

Das KK and Dasgupta S. (2002). Effect of nickel sulfate on testicular steroidogenesis in rats during protein restriction. Environ Health Perspect 110(9):923-926.

Davidson T, Salnikow K and Costa M. (2003). Hypoxia Inducible Factor-1α-independent suppression of aryl hydrocarbon receptor-regulated genes by nickel. Mol Pharmacol 634(6):1485-1493.

Davidson T, Chen H, Garrick MD, D'Angelo GD and Costa M. (2005). Soluble nickel interferes with cellular iron homeostasis. Mol Cell Biochem 270:157-162.

Davies JE. (1986). Occupational asthma caused by nickel salts. J Soc Occup Med 36:29-31.

Deknudt GH and Leonard A. (1982). Mutagenicity tests with nickel salts in the male mouse. Toxicology 25:289-292.

Deng C, Lee HH, Xian H, Yao M and Ou B. (1988). Chromosomal aberrations and sister chromatid exchanges of peripheral blood lymphocytes in Chinese electroplating workers: Effect of nickel and chromium. J Trace Elem Exp Med 1:57-62.

Deng CZ, Fons MP, Rosenblatt J, El-Zein RA, Abdel-Rahman SZ and Albrecht T. (2006). Nickel potentiates the genotoxic effect of benzo[*a*]pyrene in Chinese hamster lung V79 cells. Environ Mol Mutagen 47:150-161.

DFG (2005). *Maximum concentrations and biological tolerance values at the workplace. XIII Carcinogenic substances*. Deutsche Forschungsgemeinschaft, Wiley-VCH, Weinheim, pp.193-199.

DHS (1998). Statistics for Chemicals Detected in Public Drinking Water Sources (µg/L) 1984-1997. Drinking Water Technical Programs Branch, Department of Health Services, State of California, Sacramento, CA.

Dhir H, Agarwal K, Sharma A and Talukder G. (1991). Modifying role of *Phyllanthus emblica* and ascorbic acid against nickel clastogenicity in mice. Cancer Lett 59:9-18.

Diamond GL, Goodrum PE, Felter SP and Ruoff WL. (1998). Gastrointestinal absorption of metals. Drug Chem Toxicol 21(2):223-251.

Dieter MP, Jameson CW, Tucker AN, Luster MI, Hong HL and Boorman GA. (1988). Evaluation of tissue disposition, myelopoietic, and immunologic responses in mice after long-term exposure to nickel sulfate in the drinking water. J Toxicol Environ Health 24(3):356-372.

Diwan BA, Kasprzak KS and Rice JM. (1992). Transplacental carcinogenic effects of Nickel(II) acetate in the renal cortex, renal pelvis and adenohypophysis in F344/NCr rats. Carcinogenesis 13:1351-1357.

Dominici F, Peng RD, Ebisu K, Zeger SL, Samet JM and Bell ML. (2007). Does the effect of PM_{10} on mortality depend on PM nickel and vanadium content? A reanalysis of the NMMAPS Data. Environ Health Perspect 115(12):1701-1703.

Donskoy E, Donskoy M, Forouhar F, Gillies CG, Marzouk A, Reid MC, Zaharia O and Sunderman FW Jr. (1986). Hepatic toxicity of nickel chloride in rats. Ann Clin Lab Sci 16(2):108-117.

Doreswamy K, Shrilatha B, Rajeshkumar T and Muralidhara. (2004). Nickel-induced oxidative stress in testis of mice: evidence of DNA damage and genotoxic effects. J Androl 25(6):996-1003.

Dostal LA, Hopfer SM, Lin SM and Sunderman FW Jr. (1989). Effects of nickel chloride on lactating rats and their sucking pups, and the transfer of nickel through rat milk. Toxicol Appl Pharmacol 101:220-231.

Duke JM. (1980). Nickel in rocks and ores. In: Nriagu JO ed. *Nickel in the Environment*. New York, NY: John Wiley and Sons, Inc., p 51-65.

Dunnick JK, Benson JM, Hobbs CH, Hahn FF, Cheng YS and Eidson AF. (1988). Comparative toxicity of nickel oxide, nickel sulfate, and nickel subsulfide after 12 days of inhalation exposure to F344/N rats and B6C3F1 mice. Toxicology 50:145-156.

Dunnick JK, Elwell MR, Benson JM, Hobbs CH, Hahn FF, Haly PJ, Cheng YS, and Eidson AF. (1989). Lung toxicity after 13-week inhalation exposure to nickel oxide, nickel subsulfide, or nickel sulfate hexahydrate in F344/N rats and B6C3F1 mice. Fundam Appl Toxicol 12:584-594.

Edwards MJ. (1986). Hyperthermia as a teratogen: A review of experimental studies and their clinical significance. Terat Carcin Mutagen 6:563-582.

Egedahl R, Carpenter M and Lundell D. (2001). Mortality experience among employees at a hydrometallurgical nickel refinery and fertilizer complex in Fort Saskatchewan, Alberta (1954-95). Occup Environ Med 58:711-715.

Elias Z, Muir JM, Pierre F, Gilgenkrantz S, Schneider O, Baruthio F, Daniere MC and Fontana JM. (1989). Chromosome aberrations in peripheral blood lymphocytes of welders and characterization of their exposure by biological samples analysis. J Occup Med 31:477-483.

Ellen TP, Kluz T, Harder ME, Xiong J and Costa M. (2009). Heterochromatinization as a potential mechanism of nickel-induced carcinogenesis. Biochemistry 48:4326-4632.

Englert N. (2004). Fine particles and human health—a review of epidemiological studies. Toxicol Lett 149:235-242.

English JC, Parker RDR, Sharma RP and Oberg SG. (1981). Toxicokinetics of nickel in rats after intratracheal administration of a soluble and insoluble form. Am Ind Hyg Assoc J 42:486-492.

Fernandez-Nieto M, Quirce S, Carnes J and Sastre J (2006). Occupational asthma due to chromium and nickel salts. Int Arch Occup Environ Health 79:483-486.

Finch GL, Fisher GL and Hayes TL. (1987). The pulmonary effects and clearance of intratracheally instilled Ni_3S_2 and TiO_2 in mice. Environ Res 42:83-93.

Fletcher GG, Rossetto FE, Turnbull JD and Nieboer E. (1994). Toxicity, uptake, and mutagenicity of particulate and soluble nickel compounds. Environ Health Perspect 102(suppl 3):69-79.

FDA (2000). Total Diet Study Statistics and Element Results. Revision 1, 1991-1998 Food and Drug Administration, Washington, DC.

Forgacs Z, Paksy K, Lazar P and Tatrai E. (1998). Effect of Ni^{2+} on the testosterone production of mouse primary Leydig cell culture. J Toxicol Environ Health A 55:213-224.

Forti E, Salovaara S, Cetin Y, Bulgheroni A, Tessadri R, Jennings P, Pfaller W and Prieto P. (2011). In vitro evaluation of the toxicity induced by nickel soluble and particulate forms in human airway epithelial cells. Toxicol in Vitro 25:454-461.

Franklin M, Koutrakis P and Schwartz J. (2008). The role of particle composition on the association between $PM_{2.5}$ and mortality. Epidemiology 19(5):680-689.

Franks SJ, Ward JP, Tindall MJ, King JR, Curtis A and Evans GS. (2008). A mathematical model of the in vitro keratinocyte response to chromium and nickel exposure. Toxicol in Vitro 22:1088-1093.

Freitas M, Gomes A, Porto G and Fernandes E. (2010). Nickel induces oxidative burst, NF-κB activation and interleukin-8 production in human neutrophils. J Biol Inorg Chem 15:1275-1283.

Gao F, Brant KA, Ward RM, Cattley RT, Barchowsky A and Fabisiak JP. (2010). Multiple protein kinase pathways mediate amplified IL-6 release by human lung fibroblasts co-exposed to nickel and TLR-2 agonist, MALP-2. Toxicol Appl Pharmacol 247:146-157.

Gawkrodger DJ, Cook SW, Fell GS and Hunter JAA.. (1986). Nickel dermatitis: The reaction to oral nickel challenge. Br J Dermatol 115:33-38.

Gazel A, Rosdy M, Tornier C, De Brugerolle De Fraissinette A and Blumenberg M. (2008). Transcriptional profiling defines the effects of nickel in human epidermal keratinocytes. J Cell Physiol 217:686-692.

Ghobadi S, Nemat-Gorgani M, Golabi SM, Zare HR and Mooavi-Movahedi AA. (2000). Nickel-induced substrate inhibition of bovine liver glutamate dehydrogenase. J Enzyme Inhib Med Chem 15(5):497-508.

Glaser U, Hochrainer D, Oldiges H and Takenaka S. (1986). Long-term inhalation studies with NiO and As_2O_3 aerosols in Wistar rats. Int Congr Ser Excerpta Med 676: 325-328.

Goebeler M, Meinardus-Hager G, Roth J, Goerdt S and Sorg C. (1993). Nickel chloride and cobalt chloride, two common contact sensitizers, directly induce expression of intercellular adhesion molecule-1(ICAM-1), vascular cell adhesion molecule-1(VCAM-1), and endothelial leukocyte adhesion molecule (ELAM-1) by endothelial cells. J Invest Dermatol 100:759-765.

Golokov VA, Bojtsov IV and Kotov LN. (2003). Single and multiple early afterdepolarization caused by nickel in rat atrial muscle. Gen Physiol Biophys 22:275-278.

Graham JA, Gardner DE, Miller FJ, Daniels MJ and Coffin DL. (1975). Effect of nickel chloride on primary antibody production in the spleen. Environ Health Perspect 12:109-113.

Graham JA, Miller FJ, Daniels MJ, Payne EA and Gardner DE. (1978). Influence of cadmium, nickel and chromium on primary immunity in mice. Environ Res 16:77-87.

Grandjean P. (1984). Human exposure to nickel. In: *Nickel in the Human Environment*. Proceedings of a joint symposium held at IARC. Lyon, France, 8-11 March 1983. International Agency for Research on Cancer (IARC) Scientific Publication No. 53, IARC, Lyon pp.469-485.

Grandjean P, Andersen O and Nielsen GD. (1988). Carcinogenicity of occupational nickel exposures: An evaluation of the epidemiologic evidence. Am J Ind Med 13:193-209.

Green MHL, Muriel WJ, Bridges BA. (1976). Use of simplified fluctuation test to detect low levels of mutagens. Mutat Res 38:33-42.

Guan F, Zhang D, Wang X and Chen J. (2007). Nitric oxide and bcl-2 mediated the apoptosis induced by nickel(II) in human T hybridoma cells. Toxicol Appl Pharmacol 221:86-94.

Gurley LR, Tobey RA, Valdez JG, Halleck MS and Barham SS. (1983). Biological availability of nickel arsenides: Toxic effects of particulate Ni_5As_2. Sci Tot Environ 28:415-432.

Haal ML, Hodrejarv H and Rouk H. (2004). Heavy metals in roadside soils. Proc Est Acad Sci Chem 53(4):182-200.

Haber LT, Diamond GL, Zhao Q, Erdreich L and Dourson ML. (2000). Hazard identification and dose response of ingested nickel-soluble salts. Regul Toxicol Pharmacol 31:231-241.

Hack CE, Covington TR, Lawrence G, Shipp AM, Gentry R, Yager J and Clewell HJ III. (2007). A pharmacokinetic model of the intracellular dosimetry of inhaled nickel. J Toxicol Environ Health A 70:445-464.

Haley PJ, Bice DE, Muggenburg BA, Hahn FF and Benjamin SA. (1987). Immunopathologic effects of nickel subsulfide on the primate pulmonary immune system. Toxicol Appl Pharmacol 88:1-12.

Haley PJ, Shopp GM, Benson JM, Cheng YS, Bice DE and Luster MI. (1994). The immunotoxicity of three nickel compounds following 13-week inhalation exposure in the mouse. Fundam Appl Toxicol 15:476-487.

Hamilton-Koch W, Snyder RD and Lavelle JM. (1986). Metal-induced DNA damage and repair in human diploid fibroblasts and Chinese hamster ovary cells. Chem Biol Interact 59:17-28.

Harkin A, Hynes MJ, Masterson E, Kelly JP, O'Donnell JM and Connor TJ. (2003). A toxicokinetic study of nickel-induced immunosuppression in rats. Immunopharmacol Immunotoxicol 25(4):655-670.

Haro RT, Furst A and Falk H. (1968). Studies on the acute toxicity of nickelocene. Proc West Pharmacol Soc 11:39-42.

Harris RM, Williams TD, Hodges NJ and Waring RH. (2011). Reactive oxygen species and oxidative DNA damage mediate the cytotoxicity of tungsten-nickel-cobalt alloys in vitro. Toxicol Appl Pharmacol 250:19-28.

Hartmann M and Hartwig A. (1998). Disturbance of DNA damage recognition after UV-irradiation by nickel(II) and cadmium(II) in mammalian cells. Carcinogenesis 19(4):617-621.

Hartwig A and Beyersmann D. (1989). Enhancement of UV-induced mutagenesis and sister chromatid exchanges by nickel ions in V79 cells: Evidence for inhibition of DNA repair. Mutat Res 217:65-73.

Hartwig A, Kruger I and Beyersmann D. (1994). Mechanisms in nickel genotoxicity: The significance of interactions with DNA repair. Toxicol Lett 72:353-358.

Hays MD, Cho SH, Baldauf R, Schauer JJ and Shafer M. (2011). Particle size distributions of metal and non-metal elements in an urban near-highway environment. Atmos Environ 45:925-934.

Hfaiedh N, Allagui MS, El Feki A, Gaubin Y Murat JC Soleilhavoup JP and Croute F. (2005). Effects of nickel poisoning on expression pattern of the 72/73 and 94 kDa stress proteins in rat organs and in the COS-7, HepG2, and A549 cell lines. J Biochem Mol Toxicol 19:12-18.

Hogetveit AC, Barton RT and Kostol CO. (1978). Plasma nickel as a primary index on exposure in nickel refining. Ann Occup Hyg 21:113-120.

Hohnadel DC, Sunderman FW Jr, Nechay MW and McNeely MD. (1973). Atomic absorption spectrometry of nickel, copper, zinc, and lead in sweat collected from healthy subjects during sauna bathing. Clin Chem 19(11):1288-1292.

HSDB (1994). Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM version). Denver, CO: Micromedex, Inc.

HSDB (1995). Hazardous Substances Data Bank. National Library of Medicine, Bethesda, MD (CD-ROM version). Denver, CO: Micromedex, Inc.

Ho W and Furst A. (1973). Nickel excretion by rats following a single treatment. Proc West Pharmac Soc 16:245-248.

Hoey MJ. (1966). The effects of metallic salts on the histology and functioning of the rat testis. J Reprod Fert 12:461-471.

Hopfer SM, Linden JV, Rezuke WN, O'Brien JE, Smith L, Watters F and Sunderman FW. (1987). Increased nickel concentrations in body fluids of patients with chronic alcoholism during disulfiram therapy. Res Commun Chem Pathol Pharmacol 55:101-109.

Horak E and Sunderman FW. (1973). Fecal nickel excretion by healthy adults. Clin Chem 19:429-430.

Horie M, Nishio K, Fujita K, Kato H, Nakamura A, Kinugasa S, Endoh S, Miyauchi A, Yamamoto K, Murayama H, Niki E, Iwahashi H, Yoshida Y and Nakanishi J. (2009). Ultrafine NiO particles induce cytotoxicity in vitro by cellular uptake and subsequent Ni(II) release. Chem Res Toxicol 22:1415-1426.

Horie M, Fukui H, Nishio K, Endoh S, Kato H, Fujita K, Miyauchi A, Nakamura A, Shichiri M, Ishida N, Kinugasa S, Morimoto Y, Niki E, Yoshida Y and Iwahashi H. (2011). Evaluation of acute oxidative stress induced by NiO nanoparticles *in vitro* and *in vivo.* J Occup Health 53:64-74.

Hsie AW, Johnson NP, Couch DB, San Sebastian JR, O'Neill JP and Forbes NL. (1979). Quantitative mammalian cell mutagenesis and a preliminary study of the mutagenic potential of metallic compounds. In: Kharasch N, ed. *Trace Metals in Health and Disease*. New York, NY: Raven Press, pp. 55-69.

Hsieh TH, Yu CP and Oberdörster G. (1999a). A dosimetry model of nickel compounds in the rat lung. Inhal Toxicol 11:229-248.

Hsieh TH, Yu CP and Oberdörster G. (1999b). Modeling of deposition and clearance of inhaled Ni compounds in the human lung. Regul Toxicol Pharmacol 30(1):18-28.

Hsieh TH, Yu CP and Oberdörster G. (1999c). Deposition and clearance models of Ni compounds in the mouse lung and comparisons with the rat models. Aerosol Sci Technol 31:358-372.

Hueper WC. (1958). Experimental studies in metal cancerigenesis. Arch Pathol 65:600-607.

Hui G and Sunderman FW Jr. (1980). Effects of nickel compounds on incorporation of [^{3}H]-thymidine into DNA in rat liver and kidney. Carcinogenesis 1:297-304.

Huvinen M, Uitti P, Oksa P, Palmroos P and Laippala P. (2002). Respiratory health effects of long-term exposure to different chromium species in stainless steel production. Occup Med 52:203-212.

IOM (2001). Nickel. In: *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc.* Institute of Medicine, National Academy Press, Washington, DC, pp 521-529.

IARC (1986). *Tobacco Smoking. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 38*. International Agency for Research on Cancer, Lyon, France.

IARC (1990). *Chromium, Nickel, and Welding. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 49*. International Agency for Research on Cancer, Lyon, France.

ICRP (1994). Human Respiratory Tract Model for Radiological Protection. International Commission on Radiological Protection. Publication 66 Annals of the ICRP, 24(1-3). Elsevier Science Inc., Tarrytown (NY).

IPCS (1991). Environmental Health Criteria 108: Nickel. International Programme on Chemical Safety, World Health Organization, Geneva.

Ishihara Y, Kyono H, Serita F, Toya T, Kawashima H and Miyasaka M. (2002). Inflammatory responses and mucus secretion in rats with acute bronchiolitis induced by nickel chloride. Inhal Toxicol 14:417-430.

Ishimatsu S, Kawamoto T, Matsuno K and Kodama Y. (1995). Distribution of various nickel compounds in rat organs after oral administration. Biol Trace Elem Res 49(1):43-52.

Iwitzki F, Schlepegrell R, Eichhorn U, Kaina B, Beyersmann D and Hartwig A. (1998). Nickel(II) inhibits the repair of O^6-methylguanine in mammalian cells. Arch Toxicol 72:681-689.

Jacobsen N, Alfheim I and Jonsen J. (1978). Nickel and strontium distribution in some mouse tissues. Passage through the placenta and mammary glands. Res Commun Chem Pathol Pharmacol 20:571-584.

Jarabek AM, Asgharian B and Miller FJ. (2005). Dosimetric adjustments for interspecies extrapolation of inhaled poorly soluble particles (PSP). Inhal Toxicol 17:317-334.

Jasim S and Tjalve H. (1984). Effect of thiuram sulphides on the uptake and distribution of nickel in pregnant and non-pregnant mice. Toxicology 32:297-313.

Jasim S and Tjalve H. (1986a). Mobilization of nickel by potassium ethylxanthate in mice: comparison with sodium diethyldithiocarbamate and effect of intravenous versus oral administration. Toxicol Lett 31:249-255.

Jasim S and Tjalve H. (1986b). Effect of thiuram sulphides on the uptake and distribution of nickel in pregnant and non-pregnant mice. Toxicology 32:297-313.

Jensen CS, Lisby S, Larsen JK, Veien NK and Menne T. (2004). Characterization of lymphocyte subpopulations and cytokine profiles in peripheral blood of nickel-sensitive individuals with systemic contact dermatitis after oral nickel exposure. Contact Dermatitis 50:31-38.

Ji W, Yang L, Yu L, Yuan J, Hu D, Zhang W, Yang J, Pang Y, Li W, Lu J, Fu J, Chen J, Lin Z, Chen W and Zhuang Z. (2008). Epigenetic silencing of O^6-methylguanine DNA methyltransferase gene in NiS-transformed cells. Carcinogenesis 29(6):1267-1275.

Jia C, Roman C and Hegg CC. (2010). Nickel sulfate induces location-dependent atrophy of mouse olfactory epithelium:Protective and proliferative role of purinergic receptor activation. Toxicol Sci 115(2):547-556.

Kang GS, Gillespie PA, Gunnison A, Moreira AL, Tchou-Wong KM and Chen LC. (2011). Long-term inhalation exposure to nickel nanoparticles exacerbated atherocslerosis in a susceptible mouse model. Environ Health Perspect 119(2):176-181.

Kakela R, Kakela A and Hyvarinen H. (1999). Effects of nickel chloride on reproduction of the rat and possible antagonistic role of selenium. Comp Biochem Physiol C 123:27-37.

Kanematsu M, Hara M and Kada T. (1980). Rec-assay and mutagenicity studies on metal compounds. Mutat Res 77:109-116.

Karaczyn AA, Golebiowski F and Kasprzak KS. (2006). Ni(II) affects ubiquitination of core histones H2B and H2A. Exp Cell Res 312:3252-3259.

Kasprzak KS, Waalkes MP and Porier LA. (1986). Antagonism by essential divalent metals and amino acids of nickel(II)-DNA binding in vitro. Toxicol Appl Pharmacol 82:336-343.

Kasprzak KS, Diwan BA, Konishi N, Misra M and Rice JM. (1990). Initiation by nickel acetate and promotion by sodium barbital of renal cortical epithelial tumors in male F344 rats. Carcinogenesis 11(4):647-652.

Kasprzak KS. (1991). The role of oxidative damage in metal carcinogenicity. Chem Res Toxicol 4:604-615.

Kasprzak KS, Diwan BA, Rice JM, Misra M, Riggs CW, Olinski R and Dizdaroglu M. (1992). Nickel (II)-mediated oxidative DNA base damage in renal and hepatic chromatin of pregnant rats and their fetuses. Possible relevance to carcinogenesis. Chem Res Toxicol 5:809-815.

Kaur P and Dani HM. (2003). Carcinogenicity of nickel is the result of its binding to RNA and not to DNA. J Environ Pathol Toxicol Oncol 22(1):29-39.

Kawanishi S, Inoue S, Oikawa S, Yamashita N, Toyokuni S. Kawanishi M and Nishino K. (2001). Oxidative DNA damage in cultured cells and rat lungs by carcinogenic nickel compounds. Free Radic Biol Med 31(1):108-116.

Kawanishi S, Oikawa S, Inoue S and Nishino K. (2002). Distinct mechanisms of oxidative DNA damage induced by carcinogenic nickel subsulfide and nickel oxides. Environ Health Perspect 110:789-791.

Ke Q, Davidson T, Chen H, Kluz T and Costa M. (2006). Alterations of histone modifications and transgene silencing by nickel chloride. Carcinogenesis 27(7):1481-1488.

Ke Q, Davidson T, Kluz T, Oller A and Costa M. (2007). Fluorescent tracking of nickel ions in human cultured cells. Toxicol Appl Pharmacol 219(1):18-23.

Ke Q, Ellen TP and Costa M. (2008). Nickel compounds induce histone ubiquitination by inhibiting histone deubiquitinating enzyme activity. Toxicol Appl Pharmacol 228(2):190-199.

Kelly MC, Whitaker G, White B and Smyth MR. (2007). Nickel(II)-catalyzed oxidative guanine and DNA damage beyond 8-oxoguanine. Free Radic Biol Med 42:1680-1689.

Kiilunen M. (1997). Occupational exposure to chromium and nickel in the 1980s in Finland. Sci Tot Environ 199(1-2):91-101.

Kitamura T, Tanaka T, Watanabe J, Uchida K, Kanegasaki S, Yamada Y and Nakata K. (1999). Idiopathic pulmonary alveolar proteinosis as an autoimmune disease with neutralizing antibody against granulocyte/macrophage colony-stimulating factor. J Exp Med 190(6):875-880.

Kleeman MJ and Cass GR. (1999). Effect of emissions control strategies on the size and composition distribution of urban particulate air pollution. Environ Sci Technol 33:177-189.

Knight JA, Plowman MR, Hopfer SM and Sunderman FW. (1991). Pathological reactions in lung, liver, thymus, and spleen of rats after subacute parenteral administration of nickel sulfate. Am Clin Lab Sci 21:275-283.

Kodama Y, Tanaka Z, Matsuno K, Tanaka I and Tsuchiya K. (1985). Pulmonary deposition and clearance of a nickel oxide aerosol by inhalation. Biol Trace Elem Res 7:1-8.

Koizumi C, Usada K, Hayashi S, Dote T and Kono K. (2004). Urinary nickel: measurement of exposure by inductively coupled plasma argon emission spectroscopy. Toxicol Ind Health 20:103-108.

Koller A, Rubanyi G, Ligeti L and Kovach AG. (1982). Effect of verapramil and phenoxybenzamine on nickel-induced coronary vasoconstriction in anaesthetized dog. Acta Physiol Acad Sci Hung 59(3):287-290.

Kollmeier H, Seemann JW, Muller KM, Rothe G, Wittig P and Schejbal VB. (1987). Increased chromium and nickel content in lung tissue and bronchial carcinoma. Am J Ind Med 11:659-669.

Koniaris LG, Zimmers-Koniaris T, Hsiao EC, Chavin K, Sitzmann JV and Farber JM. (2001). Cytokine-responsive gene-2/IFN-inducible protein-10 expression in multiple models of liver and bile duct injury suggests a role in tissue regeneration. J Immunol 167(1):399-406.

Krachler M, Prohaska T, Koellensperger G, Rossipal E and Stingeder G. (2000). Concentrations of selected trace elements in human milk and in infant formulas determined by magnetic sector field inductively coupled plasma-mass spectrometry. Biol Trace Elem Res 76(2):97-112.

Krudysz MA, Froines JR, Fine PM and Sioutas C. (2008). Intra-community spatial variation of size-fractionated PM mass, OC, EC, and trace elements in the Long Beach, CA area. Atmos Environ 42:5374-5389.

LaBella FS, Dular R, Lemon P, Vivian S and Queen G. (1973). Prolactin secretion is specifically inhibited by nickel. Nature 245:330-332.

Laden F, Neas LM, Dockery DW and Schwartz J. (2000). Association of fine particulate matter from different sources with daily mortality in six U.S. cities. Environ Health Perspect 108(10):941-947.

Larramendy ML, Popescu NC and DiPaolo JA. (1981). Induction by inorganic metal salts of sister chromatid exchanges and chromosome aberrations in human and Syrian hamster cell strands. Environ Mutagen 3:597-606.

Lee SH. (2006). Differential gene expression in nickel (II)-treated normal rat kidney cells. Res Commun Mol Pathol Pharmacol 119(1-6):77-87.

Lei YX, Chen JK and Wu ZL. (2001). Detection of DNA strand breaks, DNA-protein crosslinks and telomerase activity in nickel-transformed BALB/c-3T3 cells. Teratogenesis Carcinog Mutagen 21:463-471.

Li Q, Suen TC, Sun H, Arita A and Costa M. (2009). Nickel compounds induce apoptosis in human bronchial epithelial Beas-2B cells by activation of c-Myc through ERK pathway. Toxicol Appl Pharmacol 235:191-198.

Lim JH, Sabin LD, Schiff KC and Stolzenbach KD. (2006). Concentration, size distribution, and dry deposition rate of particle-associated metals in the Los Angeles region. Atmos Environ 40:7810-7823.

Linak WP, Miller CA and Wendt JOL. (2000). Comparison of particle size distributions and elemental partitioning from the combustion of pulverized coal and residual fuel oil. J Air Waste Manage Assoc 50:1532-1544.

Lippmann M, Ito K, Hwang JS, Maciejczyk P and Chen LC. (2006). Cardiovascular effects of Ni in ambient air. Environ Health Perspect 114(11):1662-1669.

Lisby S, Hansen LH, Skov L, Menne T and Baadsgaard O. (1999a). Nickel-induced activation of T cells in individuals with negative patch test to nickel sulphate. Arch Dermatol 291:247-252.

Lisby S, Hansen LH, Menne T and Baadsgaard O. (1999b). Nickel-induced proliferation of both memory and naïve T cells in patch test-negative individuals. Clin Exp Immunol 117:217-222.

Lu S, Duffin R, Poland C, Daly P, Murphy F, Drost E, MacNee W, Stone V and Donaldson K. (2009a). Efficacy of simple short-term *in vitro* assays for predicting the potential of metal oxide nanoparticles to cause pulmonary inflammation. Environ Health Perspect 117(2):241-247.

Lu X, Bao X, Huang Y, Qu Y, Lu H and Lu Z. (2009b). Mechanisms of cytotoxicity of nickel ions based on gene expression profiles. Biomaterials 30:141-148.

Lundborg M and Camner P. (1984). Lysozyme levels in rabbit lung after inhalation of nickel, cadmium, cobalt, and copper chlorides. Environ Res 34:335-342.

Lynn S, Yew FH, Chen KS and Jan KY. (1997). Reactive oxygen species are involved in nickel inhibition of DNA repair. Environ Mol Mutagen 29:208-216.

McConnell LH, Fink JN, Schlueter DP and Schmidt MG. (1973). Asthma caused by nickel sensitivity. Ann Int Med 78:888-890.

McDowell SA, Gammon K, Bachurski CJ, Wiest JS, Leikauf JE, Prows DR and Leikauf GD. (2000). Differential gene expression in the initiation and progression of nickel-induced acute lung injury. Am J Respir Cell Mol Biol 23:466-474.

McGregor DB, Brown A, Cattanach P and Edwards D. (1988). Responses of the L5178Y TK+/TK- mouse lymphoma cell forward mutation assay. III. 72 coded chemicals. Environ Mol Mutagen 12:85-154.

McNeeley MD, Sunderman FW, Nechay MW and Levine H. (1971). Abnormal concentrations of nickel in serum in cases of myocardial infarction, stroke, burns, hepatic cirrhosis, and uremia. Clin Chem 17:1123-1128.

McNeeley MD, Nechay MW and Sunderman FW. (1972). Measurements of nickel in serum and urine as indices of environmental exposure to nickel. Clin Chem 18:992-995.

Mann E, Ranft U, Eberwein G, Gladtke D, Sugiri D, Behrendt H, Ring J, Schafer T, Begerow J, Wittsiepe J, Kramer U and Wilhelm M. (2010). Does airborne nickel exposure induce nickel sensitization? Contact Dermatitis 62:355-362.

Marzin DR and Phi HV. (1985). Study of the mutagenicity of metal derivatives with *Salmonella typhimurium* TA102. Mutat Res 155:49-51.

Mas A, Holt D and Webb M. (1985). The acute toxicity and teratogenicity of nickel in pregnant rats. Toxicology 35:47-57.

Mas A, Peligero MJ, Arola L and Alemany M. (1986). Distribution and kinetics of injected nickel in the pregnant rat. Clin Exp Pharmacol Physiol 13:91-96.

Mastromatteo E. (1986). Yant memorial lecture: Nickel. Am Ind Hyg Assoc J 47:589-601.

Mathur AK, Dikshith TSS, Lal MM and Tandon SK. (1978). Distribution of nickel and cytogenetic changes in poisoned rats. Toxicology 10:105-113.

Mayer C, Klein RG, Wesch H, et al. (1998). Nickel subsulfide is genotoxic *in vitro* but shows no mutagenic potential in respiratory tract tissues of BigBlue(TM) rats and Muta(TM) Mouse mice *in vivo* after inhalation. Mutat Res 420(1-3):85-98.

M'Bemba-Meka P, Lemieux N and Chakrabarti SK. (2006). Role of oxidative stress, mitochondrial membrane potential, and calcium homeostasis in nickel subsulfide-induced human lymphocyte death in vitro. Sci Total Environ 369:21-34.

M'Bemba-Meka P, Lemieux N and Chakrabarti SK. (2007). Role of oxidative stress and intracellular calcium in nickel carbonate hydroxide-induced sister-chromatid exchange, and alterations in replication index and mitotic index in cultured human peripheral blood lymphocytes. Arch Toxicol 81:89-99.

Mehta M, Chen LC, Gordon T, Rom W and Tang MS. (2008). Particulate matter inhibits DNA repair and enhances mutagenesis. Mutat Res 657:116-121.

Meijer C, Bredberg M, Fischer T, et al. (1995). Ear piercing and nickel and cobalt sensitization in 520 young Swedish men doing compulsory military service. Contact Dermat 32:147-149.

Menden EE, Elia VJ, Michael LW and Petering HG. (1972). Distribution of cadmium and nickel of tobacco during cigarette smoking. Environ Sci Technol 6:830-832.

Menne T and Maibach HI. (1989). Nickel allergic contact dermatitis: A review. J Am Coll Toxicol 8(7):1271-1273.

Menzel DB, Deal DL, Tayyeb MI, Wolpert RL, Roger JR III, Shoaf CR, Sandy J, Wilkinson K, and Francovitch RJ. (1987). Pharmacokinetic modeling of the lung burden from repeated inhalation of nickel aerosols. Toxicol Lett 38:33-43.

Menzel DB. (1988). Planning and using PB-PK models:an integrated inhalation and distribution model for nickel. Toxicol Lett 43:67-83.

Minang JT, Arestrom I, Zuber B, Jonsson G, Troye-Blomberg M and Ahlborg N. (2006a). Nickel-induced IL-10 down-regulates Th1- but not Th2-type cytokine responses to the contact allergen nickel. Clin Exp Immunol 143:494-502.

Minang JT, Arestrom I, Zuber B, Jonsson G, Troye-Blomberg, Lundeberg L and Ahlborg N. (2006b). Nickel, cobalt, chromium, palladium and gold induce a mixed Th1- and Th2-type cytokine response in vitro in subjects with contact allergy to the respective metals. Clin Exp Immunol 146:417-426.

Miyaki M, Akamatsu N, Ono T and Koyama H. (1979). Mutagenicity of metal cations in cultured cells from Chinese hamster. Mutat Res 68:259-263.

Miyazawa M, Ito Y, Kosaka N, Nukada Y, Sakaguchi H, Suzuki H and Nishiyama N. (2008). Role of MAPK signaling pathway in the activation of dendritic-type cell line, THP-1, induced by DNCB and $NiSO_4$. J Toxicol Sci 33(1):51-59.

Moed H, Boorsma DM, Stoof TJ, Von Blomberg BME, Bruynzeel DP, Scheper RJ, Gibbs S and Rustemeyer T. (2004). Nickel-responding T cells are CD4+ CLA+ CD45RO+ and express chemokine receptors CXCR3, CCR4 and CCR10. Br J Dermatol 151:32-41.

Mohanty PK. (1987). Cytotoxic effect of nickel chloride on the somatic chromosomes of Swiss albino mice *Mus musculus*. Curr Sci 56:1154-1157.

Mongan M, Tan Z, Chen L, Peng Z, Dietsch M, Su B, Leikauf G and Xia Y. (2008). Mitogen-activated protein kinase kinase kinase 1 protects against nickel-induced acute lung injury. Toxicol Sci 104(2):405-411.

Morimoto Y, Nambu Z, Tanaka I, Higashi T, Yamoto H, Hori H, Cho S and Kido M. (1995). Effects of nickel oxide on the production of tumor necrosis factor by alveolar macrophages of rats. Biol Trace Elem Res 48:287-296.

Muir DC, Julian J, Jadon N, Roberts R, Roos J, Chan J, Maehle W and Morgan WK. (1993). Prevalence of small opacities in chest radiographs of nickel sinter plant workers. Br J Ind Med 50(5):428-431.

Myron DR, Zimmerman TJ, Shuler TR, Klevay LM, Lee DE and Nielsen FH. (1978). Intake of nickel and vanadium by humans. A survey of selected diets. Am J Clin Nutr 31:527-531.

Munch D. (1993). Concentration profiles of arsenic, cadmium, chromium, copper, lead, mercury, nickel, zinc, vanadium and polynuclear aromatic hydrocarbons (PAH) in forest soil beside an urban road. Sci Tot Environ 138(1-3):47-55.

NAS (1975). *Nickel*. National Academy of Sciences. National Academy Press, Washington, DC

NIOSH (1995). National Institute for Occupational Safety and Health. Chemical listing and documentation of revised IDLH values. Available at http://www.cdc.gov/niosh/intridl4.html.

NRC (1975). Medical and Biologic Effects of Environmental Pollutants. Nickel. Division of Medical Sciences, National Research Council, National Academy of Sciences, Washington, DC.

NTP (1994a). Technical Report on the Toxicology and Carcinogenesis Studies of Nickel Oxide in F344/N Rats and B6C3F1 Mice. NTP TR 451, NIH Publication No. 94-3363. National Toxicology Program. U.S. Department of Health and Human Services.

NTP (1994b). Technical Report on the Toxicology and Carcinogenesis Studies of Nickel Subsulfide in F344/N Rats and B6C3F1 Mice. NTP TR 453, NIH Publication No. 94-3369. National Toxicology Program. NTP. U.S. Department of Health and Human Services.

NTP (1994c). Technical Report on the Toxicology and Carcinogenesis Studies of Nickel Sulfate Hexahydrate in F344/N Rats and B6C3F1 Mice. NTP TR 454, NIH Publication No. 94-3370. National Toxicology Program. U.S. Department of Health and Human Services.

NTP (1998). Draft RoC Background Document for Nickel Compounds. National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

NiPERA (2000a). A One-Generation Reproduction Range-Finding Study in Rats with Nickel Sulfate Hexahydrate. Final Report. 12/28/00 Springborn Laboratories, Inc. Spencerville, OH 45887, sponsored by the Nickel Producers Environmental Research Association (NiPERA), Durham, NC.

NiPERA (2000b). An Oral (Gavage) Two-Generation Reproduction Toxicity Study in Sprague-Dawley Rats with Nickel Sulfate Hexahydrate. Final Report. 12/22/00. Springborn Laboratories, Inc. Spencerville, OH, sponsored by the Nickel Producers Environmental Research Association (NiPERA), Durham, NC.

Nierboer E, Stafford AR, Evans SL and Dolovich J. (1984). Cellular binding and/or uptake of nickel(II)ions. In: *Nickel in the Human Environment*. IARC Scientific Publication No. 53. International Agency for Research on Cancer, Lyon, pp.321-331.

Nieboer E, Tom RT and Sanford WE. (1988). Nickel metabolism in man and animals. In: Sigel H and Sigel A (eds.) *Metal Ions in Biological Systems. Vol 23 Nickel and Its Role in Biology*. Marcel Dekker, Inc., New York, pp. 91-121.

Nielsen GD, Andersen O and Jensen M. (1993). Toxicokinetics of nickel in mice studied with the gamma-emitting isotope ^{57}Ni. Fundam Appl Toxicol 21:236-243.

Nielsen FH. (1996). Other trace elements: Nickel. In: Ziegler EE, Filer LJ Jr (eds.) *Present Knowledge in Nutrition, 7th Edition*, ILSI. Washington, DC, pp360-364.

Nielsen GD, Soderberg U, Jorgensen PJ, Templeton DM, Rasmussen SN, Andersen KE and Grandjean P. (1999). Absorption and retention of nickel from drinking water in relation to food intake and nickel sensitivity. Toxicol Appl Pharmacol 154(1):67-75.

Nielsen NH and Menne T. (1993). Nickel sensitization and ear piercing in an unselected Danish population. Golstrup Allergy Study. Contact Dermatitis 29:16-21.

Nishi K, Morimoto Y, Ogami A, Murakami M, Myojo T, Oyabu T, Kadoya C, Yamamoto M, Todoroki M, Hirohashi M, Ymansaki S, Fujita K, Endo S, Uchida K, Yamamoto K, Nakanishi J and Tanaka I. (2009). Expression of cytokine-induced neutrophil chemoattractant in rat lungs by intratracheal instillation of nickel oxide nanoparticles. Inhal Toxicol 21(12):1030-1039.

Novey HS, Habib M and Wells ID. (1983). Asthma and IgE antibodies induced by chromium and nickel salts. J Allergy 72(4):407-412.

O'Rourke MK, Van De Water PK, Jin S, et al. (1999). Evaluations of primary metals from NHEXAS Arizona: distributions and preliminary exposures. J Expo Anal Environ Epidemiol 9:435-445.

Obone E, Chakrabarti SK, Bai C, Malick MA, Lamontagne L and Subramanian KS. (1999). Toxicity and bioaccumulation of nickel sulfate in Sprague-Dawley rats following 13 weeks of subchronic exposure. J Toxicol Environ Health A 57:379-401.

OEHHA. (2000a). Public Health Goal for Nickel in Drinking Water. Pesticide and Environmental Toxicology Branch, Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, CA.

OEHHA. (2000b). Air Toxics Hot Spots Program Risk Assessment Guidelines Part IV. Technical Support Document for Exposure Assessment and Stochastic Analysis. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency, Oakland, CA, p 10-4.

Ogami A, Morimoto Y, Myojo T, Oyabu T, Murakami M, Todoroki M, Nishi K, Kadoya C, Yamamoto M and Tanaka I. (2009). Pathological features of different sizes of nickel oxide following intratracheal instillation in rats. Inhal Toxicol 21:812-8.

Ohashi F, Fukui Y, Takada S, Moriguchi J, Ezaki T and Ikeda M. (2006). Reference values for cobalt, copper, manganese, and nickel in urine among women of the general population in Japan. Int Arch Occup Environ Health 80:117-126.

Ohshima S. (2003). Induction of genetic instability and chromosomal instability by nickel sulfate in V79 Chinese hamster cells. Mutagenesis 18(2):133-137.

Ohno H, Hanaoka F and Yamada M. (1982). Inducibility of sister chromatid exchanges by heavy metal ions. Mutat Res 104:141-145.

Oliveira JP, Pereira ME, de Siqueira PB and da Silva CS. (2000). Urinary nickel as bioindicator of workers' Ni exposure in a galvanizing plant in Brazil. Int Arch Occup Environ Health 73:65-68.

Oller AR and Erexson G. (2007). Lack of micronuclei formation in bone marrow of rats after repeated oral exposure to nickel sulfate hexahydrate.Mutat Res 626:102-110.

Oller AR, Kirkpatrick DT, Radovsky A and Bates HK. (2008). Inhalation carcinogenicity study with nickel metal powder in Wistar rats. Toxicol Appl Pharmacol 233:262-275.

Onkelinx C, Becker J and Sunderman FW Jr. (1973). Compartmental analysis of the metabolism of ^{63}Ni (II) in rats and rabbits. Chem Pathol Pharmacol 6(2):663-676.

Ostro B, Feng WY, Broadwin R, Green S and Lipsett M. (2007). The effects of components of fine particulate air pollution on mortality in California: Results from CALFINE. Environ Health Perspect 115(1):13-19.

Ottolenghi AD, Haseman JK, Payne WW, Falk HL, and MacFarland HN. (1974). Inhalation studies of nickel sulfide in pulmonary carcinogenesis of rats. J Natl Cancer Inst 54(5):1165-1172.

Ouyang W, Zhang D, Li J, Verma UN, Costa M and Huang C. (2009). Soluble and insoluble nickel compounds exert a differential inhibitory effect on cell growth through IKKα-dependent cyclin D1 down-regulation. J Cell Physiol 218:205-214.

Oyabu T, Ogami A, Morimoto Y, Shumada M, Lenggoro W, Okuyama K and Tanaka I. (2007). Biopersistence of inhaled nickel oxide nanoparticles in rat lung. Inhal Toxicol 19(Suppl 1):55-58.

Pan J, Chang Q, Wang X, Son Y, Zhang Z, Chen G, Luo J, Bi Y, Chen F and Shi X. (2010). Reactive oxygen species-activated Akt/ASK1/p38 signaling pathway in nickel compound-induced apoptosis in BEAS 2B cells. Chem Res Toxicol 23:568-577.

Pandey R, Kumar R, Singh SP, Saxena DK and Srivastava SP.(1999). Male reproductive effect of nickel sulphate in mice. BioMetals 12:339-346.

Pandey R and Srivastava SP. (2000). Spermatotoxic effects of nickel in mice. Bull Environ Contam Toxicol 64:161-167.

Pang D, Burges DCL and Sorohan T. (1996). Mortality study of nickel platers with special reference to cancers of the stomach and lung. Occup Environ Med 53:714-717.

Patel MM, Hoepner L, Garfinkel R, Chillrud S, Reyes A, Quinn JW, Perera F and Miller RL. (2009). Ambient metals, elemental carbon, and wheeze and cough in New York City children through 24 months of age. Am J Resp Crit Care Med 180(11):1107-1113.

Patierno SR and Costa M. (1985). DNA-protein cross-links induced by nickel compounds in intact cultured mammalian cells. Chem Biol Interact 55:75-91.

Patriarca M, Lyon TD and Fell GS. (1997). Nickel metabolism in humans investigated with an oral stable isotope. Am J Clin Nutr 66:616-621.

Peskin AV and Shiyahova L. (1986). Cell nuclei generate DNA-nicking superoxide radicals. FEBS Lett 194(2):317-321.

Phillips JI, Green FY, Davies JCA and Murray J. (2010). Pulmonary and systemic toxicity following exposure to nickel nanoparticles. Am J Ind Med 53:763-767.

Pikalek P and Necasek J. (1983). The mutagenic activity of nickel in *Corynebacterium* sp. Folia Microbiol 26:17-21.

Polidori A, Cheung KL, Arhami M, Delfino RJ, Schauer JJ and Sioutas C. (2009). Relationships between size-fractionated indoor and outdoor trace elements at four retirement communities in southern California. Atmos Chem Phys 9:4521-4536.

Prasad CM, Nair KC and Sheth UK. (1980). Reversal of digoxin induced cardiac arrhythmias by nickel chloride. Res Commun Chem Pathol Pharmacol 27:405-408.

Prows DR and Leikauf GD. (2001). Quantitative trait analysis of nickel-induced acute lung injury in mice. Am J Respir Cell Mol Biol 24:740-746.

Prows DR, McDowell SA, Aronow BJ and Leikauf GD. (2003). Genetic susceptibility to nickel-induced acute lung injury. Chemosphere 51:1139-1148.

Raithel HJ, Schaller KH, Akslen LA, et al. (1989). Analyses of chromium and nickel in human pulmonary tissue. Investigations in lung cancer patients and a control population under special consideration of medical expertise aspects. Int Arch Occup Environ Health 61:507-512.

Rana SVS. (2008). Metals and apoptosis: Recent developments. J Trace Elem Med Biol 22:262-284.

Reprotext® System. Dabney B, (ed.) Denver (CO): Micromedex, Inc.; 1999. (Edition expires 1/31/1999).

RTI (1988). Fertility and Reproductive Performance of the F_1 Generation. Final Study Report (III of III). Two-Generation Reproduction and Fertility Study of Nickel Chloride Administered to CD Rats in the Drinking Water. 9/23/88. Research Triangle Institute, Research Triangle Park, NC. Prepared for the Office of Solid Waste Management, U.S. Environmental Protection Agency, Washington, DC.

Rezuke WN, Knight JA and Sunderman FW Jr. (1987). Reference values for nickel concentrations in human tissues and bile. Am J Ind Med 11:419-426.

Rietschel RL, Fowler JF, Warshaw EM, Belsito D, DeLeo VA, Maibach HI, Marks JG, Mathias CGT, Pratt M, Sasseville D, Storrs FJ, Taylor JS and Zug KA. (2008). Detection of nickel sensitivity has increased in North American patch-test patients. Dermatitis 19(1):16-19.

Rivedal E and Sanner T (1980). Synergistic effect on morphological transformation of hamster embryo cells by nickel sulphate and benz[a]pyrene. Cancer Lett 8:203-208.

Roberts JR, Young SH, Castranova V and Antonini JM. (2009). The soluble nickel component of residual oil fly ash alters pulmonary host defenses in rats. J Immunotoxicol 6(1):49-61.

Rosen SH, Castleman B, Liebow AA, Enzinger FM and Hunt RTN. (1958). Pulmonary alveolar proteinosis. N Engl J Med 258:1123-1142.

Rossman TG. (2009). Inappropriate cytotoxicity measurements. Environ Mol Mutagen 50(2):81.

Rostami AA. (2009). Computational modeling of aerosol deposition in respiratory tract: A review. Inhal Toxicol 21:262-290.

Rubanyi G and Kovach AG. (1980). Cardiovascular actions of nickel ions. Acta Physiol Acad Sci Hung 55(4):345-353.

Ruth J. (1986). Odor thresholds and irritation levels of several chemical substances: A review. Am Ind Hyg Assoc J 47:142-151.

Sabin LD, Lim JH, Venezia MT, Winer AM, Schiff KC, and Stolzenbach KD. (2006). Dry deposition and resuspension of particle-associated metals near a freeway in Los Angeles. Atmos Environ 40:7528-7538.

Sabin LD and Schiff KC. (2008). Dry atmospheric deposition rates of metals along a costal transect in soutnern California. Atmos Environ 42:6606-6613.

Sahu RK, Katsifis SP, Kinney PL and Christie NT. (1995). Ni(II)-induced changes in cell cycle duration and sister-chromatid exchanges in cultured human lymphocytes. Mutat Res 327:217-225.

Saito K and Menzel D. (1986). Accumulation and efflux of nickel from cultured pneumocytes. Tohoku J Exp Med 148:295-302.

Sakar B (1984). Nickel metabolism. In: *Nickel in the Human Environment*. IARC Scientific Publication No. 53. International Agency for Research on Cancer, Lyon, France, pp. 367-384.

Salnikow K, Davidson T and Costa M. (2002). The role of hypoxia-inducible signaling pathway in nickel carcinogenesis. Environ Health Perspect 110(Suppl 5):831-834.

Salnikow K, Davidson T, Zhang Q, Chen LC, Su W and Costa M. (2003). The involvement of hypoxia-inducible transcription factor-1-dependent pathway in nickel carcinogenesis. Cancer Res 63:3524-3530.

Salnikow K, Davidson T, Kluz T, Chen H, Zhou D and Costa M. (2003). GeneChip analysis of signaling pathways effected by nickel. J Environ Monit 5:206-209.

Saplakoglu U, Iscan M and Iscan M. (1997). DNA single-strand breakage in rat lung, liver and kidney after single and combined treatments of nickel and cadmium. Mutat Res 394(1-3):133-140.

Salnikow K, Cosentino S, Klein C and Costa M. (1994). Loss of thromospondin transcriptional activity in nickel-transformed cells. Mol Cell Biol 14:851-858.

Salnikow K, Su W, Blagosklonny MV and Costa M. (2000). Carcinogenic metals induce hypoxia-inducible factor-stimulated transcription by reactive oxygen species- independent mechanism. Cancer Res 60(13):3375-3378.

Saxholm HJK, Reith A and Brogger A. (1981). Oncogenic transformation and cell lysis in C3H/10T1/2 cells and increased sister chromatid exchange in human lymphocytes by nickel subsulfide. Cancer Res 41:4136-4139.

Schmidt M, Raghavan B, Muller V, Vogl T, Fejer G, Tchaptchet S, Keck S, Kalis C, Nielsen Pj, Galanos C, Roth J, Skerra A, Martin SF, Freudenberg MA and Goebeler M. (2010). Crucial role for human Toll-like receptor 4 in the development of contact allergy to nickel. Nature Immunol 11:814-9

Schroeder HA, Balassa JJ and Tipton IH. (1962). Abnormal trace metals in man: Nickel. J Chron Dis 15:51

Schroeder HA and Mitchener M. (1971). Toxic effects of trace elements on the reproduction of mice and rats. Arch Environ Health 23:102-106.

Schwerdtle T and Hartwig A. (2006). Bioavailability and genotoxicity of soluble and particulate nickel compounds in cultured human lung cells. Mat-Wiss u Werkstofftech 37(6):521-525.

Sen P and Costa M. (1986a). Incidence and localization of sister chromatid exchanges induced by nickel and chromium compounds. Carcinogenesis 7:1527-1533.

Sen P and Costa M. (1986b). Pathway of nickel uptake influences its interaction with heterochromatic DNA. Toxicol Appl Pharmacol 84:278-285.

Sen P, Conway K and Costa M. (1987). Comparison of the localization of chromosome damage induced by calcium chromate and nickel compounds. Cancer Res 47:2142-2147.

Serita F, Kyono H and Seki Y. (1999). Pulmonary clearance and lesions in rats after a single inhalation of ultrafine metallic nickel at dose levels comparable to the Threshold Limit Value. Ind Health 37:353-363.

Seymour JF and Presneill JJ. (2002). Pulmonary alveolar proteinosis. Progress in the first 44 years. Am J Crit Care Med 166:215-235.

Shacklette HT and Boerngen JG. (1984). Element concentration in soils and other surficial materials of the conterminous United States. U.S. Geological Survey professional paper 1270. U.S. Geological Survey, Alexandria, VA.

Shiao YH, Lee SH and Kasprzak KS. (1998). Cell cycle arrest, apoptosis and p53 expression in nickel(II) acetate-treated Chinese hamster ovary cells. Carcinogenesis 19(7):1203-1207.

Singla A, Kaur J and Mahmood A. (2006). Alterations in the expression of intestinal enzymes in rats exposed to nickel. J Appl Toxicol 26:397-401.

Sivulka DJ, Conard BR, Hall GW and Vincent JH. (2007). Species-specific inhalable exposures in the nickel industry: A new approach for deriving inhalation occupational exposure limits. Regul Toxicol Pharmacol 48:19-34.

Slotkin TA and Seidler FJ. (2008). Developmental neurotoxicants target neurodifferentiation into serotonin phenotype: Chlorpyrifos, diazinon, dieldrin and divalent nickel. Toxicol Appl Pharmacol 233:211-219.

Smialowicz RJ, Rogers RR, Riddle MM, Garner RJ, Rowe DG and Luebke RW. (1985). Immunologic effects of nickel: II. Suppression of natural killer cell activity. Environ Res 36:56-66.

Smith CJ, Livingston SD and Doolittle DJ. (1997). An international literature survey of “IARC Group I Carcinogens” reported in mainstream cigarette smoke. Fd Chem Toxicol 35:1107-1130.

Smith MK, George EL, Stober JA, Feng HA, Kimmel GL. (1993). Perinatal toxicity associated with nickel chloride exposure. Environ Res 61:200-211.

Snow ET. (1992). Metal carcinogenesis: Mechanistic implications. Pharmacol Ther 53:31-65.

Sobti RC and Gill RK. (1989). Incidence of micronuclei and abnormalities in the head of spermatozoa caused by the salts of a heavy metal nickel. Cytologia 54:249-254.

SCAQMD. (2008). MATES III. Multiple Air Toxics Exposure Study in the South Coast Air Basin. Draft Final Report. South Coast Air Quality Management District. Diamond Bar ,CA. pp.2-7, 2-15.

Spiegelberg T, Kordel W and Hochrainer D (1984). Effects of NiO inhalation on alveolar macrophages and the humoral immune systems of rats. EcoToxicol Environ Safety 8:516-525.

Solomons NW, Viteri F, Shuler TR and Nielsen FH. (1982). Bioavailability of nickel in man: effects of foods and chemically-defined dietary constituents on the absorption of inorganic nickel. J Nutr 112:39-50.

Spruit D and Bongaarts PJM. (1977). Nickel content of plasma, urine and hair in contact dermatitis. Dermatologica 154:291-300.

Sunderman FW, Shen SK, Mitchell JM, Allpass PR and Damjanov I. (1978). Embryotoxicity and fetal toxicity of nickel in rats. Toxicol Appl Pharmacol 43:381-390.

Sunderman FW (1986). Kinetics and biotransformation of nickel and chromium. In: Stern RM, Berlin A, Fletcher AC, Jarvisalo J, (eds.) *Health Hazards and Biological Effects of Welding Fumes and Gases.* Amsterdam: Excerpta Medica, pp. 229-247.

Sunderman F, Dingle B, Hopfer S and Swift T. (1988). Acute nickel toxicity in electroplating workers who accidentally ingested a solution of nickel sulfate and nickel chloride. Am J Ind Med 14:257-266.

Sunderman FW. (1989). Mechanisms of metal carcinogenesis. Scand J Work Environ Health 15:1-12.

Sunderman FW, Reid MC, Shen SK and Kevorkian CB (1983). Embryotoxicity and teratogenicity of nickel compounds. In: Clarkson TW, Nordberg GF and Sager PR (eds.) *Reproductive and Developmental Toxicity of Metals*. Plenum Press, New York, pp. 399-416.

Sunderman FW, Hopfer SM, Knight JA, McCully KS, Cecutti AG, Thornhill PG, Conway K, Miller C, Patierno SR and Costa M. (1987). Physicochemical characteristics and biological effects of nickel oxides. Carcinogenesis 8:305-313.

Sunderman FW, Hopfer SM, Sweeney KR, Marcus AH, Most BM and Creason J. (1989). Nickel absorption and kinetics in human volunteers. Proc Soc Exp Biol Med 191:5-11.

Sunderman FW and Oskarsson A. (1991). Nickel. In: Merian E (ed.) *Metals and Their Compounds in the Environment*. VCH Verlagsgesellschaft, New York, NY pp. 1101-1126.

Svenes KB and Andersen I. (1998). Distribution of nickel in lungs from former nickel workers. Int Arch Occup Environ Health 71(6):424-428.

Swierenga SHH and McLean JR. (1985). Further insights into mechanisms of nickel-induced DNA damage: studies with cultured rat liver cells. In: Brown SS and Sunderman FW Jr (eds.), *Progress in Nickel Toxicology*, Blackwell Scientific Publications, Oxford, pp. 101-104.

Szakmary E, Movaio V, Naray M and Ungvary G. (1995). Haemodynamic effect of nickel chloride in pregnant rats. Acta Physiol Hungar. 83(1):3-12.

Takahashi S, Oishi M, Takeda E, Kubota Y, Kikuchi T and Furuya K. (1999). Physicochemical characteristics and toxicity of nickel oxide particles calcined at different temperatures. Biol Trace Elem Res 69:161-174.

Tanaka I, Ishimatsu S, Matsuno K, Kodama Y and Tsuchiya K. (1985). Biological half time of deposited nickel oxide aerosol in rat lung by inhalation. Biol Trace Elem Res 8:203-210.

Tanaka I, Ishimatsu S, Haratake J, Horie A and Kodama Y. (1988a). Biological half time in rats exposed to nickel monosulfide (amorphous) aerosol by inhalation. Biol Trace Elem Res 17:237-246.

Tanaka I, Horie A, Haratake J, Kodama Y and Tsuchiya K. (1988b). Lung burden of green nickel oxide aerosol and histopathological findings in rats after continuous inhalation. Biol Trace Elem Res 16:19-26.

Teeguarden JG, Gearhart J, Clewell HJ III, Covington TR, Nong A and Andersen ME. (2007). Pharmacokinetic modeling of manganese. III. Physiological approaches accounting for background and tracer kinetics. J Toxicol Environ Health A 70:1515-1526.

Thomas KW, Pellizzari ED and Berry MR. (1999). Population-based intakes and tap water concentrations for selected elements in the EPA Region V National Human Exposure Assessment Survey (NHEXAS). Expo Anal Environ Epidemiol 9:402-413.

Tkeshalashvilli LK, Reid TM, McBride TJ and Loeb LA. (1993). Nickel induces a signature mutation for oxygen free radical damage. Cancer Res 53(18):4172-4174.

Torjussen W and Andersen I. (1979). Nickel concentrations in nasal mucosa, plasma and urine in active and retired nickel workers. Ann Clin Lab Sci 9:289-298.

Torjussen W, Zachariasen H and Andersen I. (2003). Cigarette smoking and nickel exposure. J Environ Monit 5:198-201.

Toya T, Serita F, Sawatari K and Fukuda K. (1997). Lung lesions induced by intratracheal instillation of nickel fumes and nickel oxide powder in rats. Ind Health 35:69-77.

Trombetta D, Mondello MR, Cimino F, Cristani M, Pergolizzi S and Saija A. (2005). Toxic effect of nickel in an in vitro model of human oral epithelium. Toxicol Lett 159:219-225.

U.S. EPA (1994). Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. EPA/600/8-90/066F, United States Environmental Protection Agency. Washington, DC p 4-37.

U.S.EPA (1986). Health Assessment Document for Nickel and Nickel Compounds. EPA/600/8-83/012F, United States Environmental Protection Agency. Washington, DC: p. 3-3.

U.S.EPA (1988). Recommendations for and Determination of Biological Values for use in Risk Assessment. PB88-179874. United States Environmental Protection Agency. Washington, DC.

U.S.EPA (2007). Integrated Risk Information System, IRIS Glossary. Available at http:\\www.epa.gov\iris\help_gloss.htm

Uthus EO. (1999). Compartmental model of nickel metabolism in rats based on orally administered ^{63}Ni. Proc NY Acad Sci 53:92-96.

Vaktskjold AL, Talykova LV, Chashchin VP, Nieboer E, Thomassen Y and Odland JO. (2006). Genital malformations in newborns of female nickel-refinery workers. Scand J Work Environ Health 32(1):41-50.

Vaktskjold A, Talykova LV, Chashchin VP, Odland JO, and Nieboer E. (2007). Small-for gestational-age newborns of female refinery workers exposed to nickel. Intl J Occup Environ Health 20(4):327-338.

Vaktskjold A, Talykova LV, Chashchin VP, Odland JO and Nieboer E. (2008a). Maternal nickel exposure and congenital musculoskeletal defects. Am J Ind Med 51(11):825-33.

Vaktskjold AL, Talykova LV, Chashchin VP, Odland JO and Nieboer E. (2008b). Spontaneous abortions among nickel-exposed female refinery workers. Int J Environ Health Res 18(2):99-115.

Valentine R and Fisher GL (1984). Pulmonary clearance of intratracheally administered $^{63}Ni_3S_2$ in strain A/J mice. Environ Res 34:328-334.

Van Soestbergen M and Sunderman FW. (1972). ^{63}Ni complexes in rabbit serum and urine after injection of $^{63}NiCl_2$. Clin Chem 18(12):1478-1484.

Von Burg, R (1997). Toxicology update. Nickel and some nickel compounds. J Appl Toxicol 17(6):425-431.

Vyskocil A, Viau C and Cizkova M. (1994). Chronic nephrotoxicity of soluble nickel in rats. Hum Exp Toxicol 13:689-693.

Waksvik H and Boysen M. (1982). Cytogenic analysis of lymphocytes from workers in a nickel refinery. Mutat Res 103:185-190.

Wehner AP and Craig DK. (1972). Toxicology of inhaled NiO and CoO in Syrian golden hamsters. Am Ind Hyg Assoc J 33:147-155.

Woollam DHM (1972). *Advances in Teratology. Vol. 5*. Academic Press, New York. p. 57.

Wong PK. (1988). Mutagenicity of heavy metals. Bull Environ Contam Toxicol 40:597-603.

Xie J, Funakoshi T, Shimada H and Kojima S. (1995). Effects of chelating agents on testicular toxicity in mice by acute exposure to nickel. Toxicology 103:147-155.

Yan Y, Kluz T, Zhang P, Chen H and Costa M. (2003). Analysis of specific lysine histone H3 and H4 acetylation and methylation status in clones of cells with a gene silenced by nickel exposure. Toxicol Appl Pharmacol 190:272-277.

Yokota K, Johyama Y, Kunitani Y, Michitsuji H and Yamada S. (2007). Urinary elimination of nickel and cobalt in relation to airborne nickel and cobalt exposures in a battery plant. Int Arch Occup Environ Health 80:527-531.

Yoshioka N, Nakashima H and Hosoda K, Eitaki Y, Shimada N and Omae K. (2008). Urinary excretion of an oxidative stress marker, 8-hydroxyguanine (8-OH-Gua), among nickel-cadmium battery workers. J Occup Health 50(3):229-235.

Yu CP and Xu GB. (1986). Predictive models for deposition of diesel exhaust particulates in human and rat lungs. Aerosol Sci Technol 5:337-347.

Yu CP, Hsieh TH, Oller AR and Oberdörster G. (2001). Evaluation of the human nickel retention model with workplace data. Regul Toxicol Pharmacol 33:165-172.

Zeromski J, Jezewska E, Sikora J and Kasprzak KS. (1995). The effect of nickel compounds on immunophenotype and natural killer cell function of normal human lymphocytes. Toxicology 97:39-48.

Zhang Z, Salnikow K, Kluz T, Chen LC, Su WC and Costa M. (2003). Inhibition and reversal of nickel-induced transformation by the histone deacetylase inhibitor trichostatin A. Toxicol Appl Pharmacol 192:201-211.

Zhou X, Li Q, Arita A, Sun H and Costa M. (2009). Effects of nickel, chromate, and arsenite on histone 3 lysine methylation. Toxicol Appl Pharmacol 236:78-84.

Zhuang ZX, Shen Y, Shen HM, Ng V and Ong CN. (1996). DNA breaks and poly (ADP-ribose) polymerase activation induced by crystalline nickel subsulfide in MRC-5 lung fibroblast cells. Hum Exp Toxicol 15:891-897.

Zienolddiny S, Svendrud DH, Ryberg D, Mikalsen AB and Haugen A. (2000). Nickel(II) induces microsatellite mutations in human lung cancer cell lines. Mutat Res 452:91-100.

Appendix A: Additional Toxicological Data on Nickel and its Compounds.

A1 Air Pollution Studies: Nickel as a Component of Particulate Matter

Inhalation exposure to airborne particulate matter (PM) has been linked to multiple adverse respiratory and cardiovascular effects including premature deaths (Englert, 2004). PM of 2.5 µm or less is considered more hazardous since a larger percentage of fine particles are retained in the lung compared with larger particles. $PM_{2.5}$ contains a variety of heavy metals such as iron (Fe), vanadium (V) and nickel (Ni). Several studies in the past several years have found associations between nickel as a metal constituent of $PM_{2.5}$ or PM_{10} and both mortality and morbidity. In a study of daily mortality in 60 National Mortality and Morbidity Air Pollution Study (NMMAPS) cities in the United States Lippmann et al. (2006) found that the association between PM_{10} and mortality was significantly higher in cities where the nickel component level was high (95^{th} percentile) versus when it was low (5^{th} percentile). The difference was 0.6 percent per 10 µg/m^3 increase in PM_{10}. A subsequent reanalysis of the NMMAPS data found that when counties included in the New York community were excluded the effect modification by nickel was much weaker and no longer statistically significant (Dominici et al., 2007). Another study of mortality in 25 U.S. cities found that the effect of $PM_{2.5}$ on mortality increased significantly (0.37%) when $PM_{2.5}$ mass contained a higher proportion of nickel (Franklin et al., 2008). In a study of mortality and sources of $PM_{2.5}$ in six U.S. cities, Laden et al. (2000) found that an increase in nickel from the 5^{th} to 95^{th} percentile of exposure (10.3 ng/m^3) was associated with a significant 1.5% increase in daily mortality. Burnett et al. (2000) studied mortality and fine particulate matter components in 8 Canadian cities. Nickel was significantly associated with mortality in both single pollutant models and multi-pollutant models, which included ozone. A study of mortality and fine particulate components in nine California counties failed to find any association for nickel (Ostro et al., 2007).

Patel et al. (2009) investigated associations between respiratory symptoms in the first 24 months of age and specific components of $PM_{2.5}$ including elemental carbon (EC), Ni, V, and Zn. The study included 653 children. Twenty-four-hour average ambient concentrations of $PM_{2.5}$ and $PM_{2.5}$ fractions of Ni, V, Zn, and EC were measured every third day by the New York State Department of Environmental Conservation. Data on subject characteristics, residence, ETS exposure, and respiratory symptoms were collected by questionnaires administered to mothers every three months. Associations between metals, EC and $PM_{2.5}$ and the presence of wheeze and cough were analyzed using generalized additive mixed effects models. In single pollutant models each pollutant was analyzed as a parametric continuous variable. For each subject, 3-month moving average concentrations of Ni, V, Zn, EC, and $PM_{2.5}$ were

calculated for each symptom-reporting period. Significant positive associations were observed between metals and wheeze but not cough. The analysis was conducted using general additive mixed models adjusted for sex, ethnicity, postnatal ETS exposure and calendar time. The authors found that an increase in interquartile range concentration of ambient nickel (0.014 $\mu g/m^3$) was associated with a 28% increased probability of wheeze ($p = 0.0006$). The findings were robust to the inclusion of co-pollutants EC, NO_2, copper and iron.

The largest effect estimates were seen with nickel. In models that adjusted for sex, ethnicity, postnatal ETS exposure, and calendar time, an increase of 0.014 μg Ni/m^3 was associated with a 28% increased probability of wheeze ($P = 0.0006$). The authors conclude that exposure to $PM_{2.5}$ associated metals (particularly Ni) and EC may be associated with asthma morbidity in urban children as young as 2 years of age. Perhaps the biggest limitation of the study is that exposure estimates were based on two monitoring stations in the general residential area, which exhibited significant differences in Ni and EC between them. These may not represent true exposures as accurately as personal or residential measurements. Alternatively the exposed population is one of specific concern to OEHHA and the study involves realistic exposure conditions.

In a recent study of birth weight and constituents of $PM_{2.5}$ in three Connecticut counties and one Massachusetts county from 2000 to 2004, Bell et al (2010) found that an interquartile range increase in nickel resulted in an 11% increase in term low birth weight. The analysis was adjusted for tobacco use, alcohol use, marital status, age, race and education of the mother. Looking at change in birth weight, the authors found a significant decrease in birth weight associated with third trimester exposure to nickel. A study of 106 U.S. counties estimated county and season specific relative risks of cardiovascular and respiratory hospital admissions associated with $PM_{2.5}$ chemical components (Bell et al 2009). The authors found that the effect of $PM_{2.5}$ on both respiratory and cardiovascular admissions was significantly modified by the fraction of nickel in the $PM_{2.5}$ mass. An interquartile range increase in nickel resulted in 19% increase in the association between $PM_{2.5}$ and cardiovascular admissions and a 223% increase for respiratory admissions. These increases were robust to adjustment for elemental carbon or vanadium for the cardiovascular but not for the respiratory hospital admissions. Lippmann et al. (2006) and Chen and Lippmann (2009) analyzed and reviewed the data on health-related effects caused by inhalation of airborne particulate matter (PM) and metals within PM in the National Morbidity, Mortality, and Air Pollution Study (NMMAPS). Based on human and laboratory animal studies of concentrated PM and human population studies for which health effects and PM composition data were available, they reached the following conclusions: (1) residual oil fly ash (ROFA) was the most toxic source-related mixture, and (2) Ni and V, which are characteristic of ROFA, were the most influential components for acute cardiac function changes and excess short-term mortality. The difference in PM_{10} mortality risk estimates (in percent/10-$\mu g/m^3$ increase in PM_{10}) per 5^{th} to 95^{th} percentile difference in the the PM component across 60 metropolitan areas for which speciation data were

available showed Ni and V with high risk coefficients of 0.6 (their Fig. 1). Dominici et al. (2007) analyzed the same data and more or less came to the same conclusion.

Franklin et al. (2008) investigated the role of particle composition on the association between $PM_{2.5}$ and mortality in 25 communities including six in California. The study sites included $PM_{2.5}$ mass concentration and daily mortality data for at least two years between 2000 and 2005. The data were obtained from the U.S. EPA's Technology Transfer Network Air Quality System and the National Center for Health Statistics. Meteorologic data were obtained form the National Climatic Data Center. 1,313,983 nonaccidental deaths were examined. Thirty-one percent of deaths were due to cardiovascular, 10% were due to respiratory disease, and 7% were due to stroke. The average number of PM2.5 days examined per community was 1451 and the number of speciation days was 321. Seasonally averaged PM2.5 concentrations ranged from well below the National Ambient Air Quality Standard of 15 $\mu g/m^3$ in Sacramento, CA in spring (6.7 $\mu g/m^3$) to over twice the standard in Bakersfield and Fresno, CA in winter (34.4 and 33.4 $\mu g/m^3$, respectively). There was a 0.74% (95% CI = 0.41-1.07%) increase in nonaccidental deaths associated with a 10 $\mu g/m^3$ increase in 2-day averaged $PM_{2.5}$ mass concentration. The association was smaller in the west than in the east and was highest in spring. It was increased when $PM_{2.5}$ mass contained a higher proportion of aluminum, arsenic, sulfate, silicon, and nickel. The combination of aluminum, sulfate and nickel also modified the effect. The results support the concept that mass alone is an insufficient metric when evaluating the health effects of PM exposure and that metal ions, specifically nickel may play a role in the toxic mechanism.

In summary it appears that nickel is a component of ambient PM, which contributes to the overall toxicity, but the available data are not consistent as to the extent of this effect. This and the fact that the studies all involve mixed exposures where the overall effects are dominated by other components and properties of PM make these data unsuitable for consideration as the basis for an REL.

A2 Genetic Toxicity

While genetic toxicology generally provides key supporting documentation for cancer risk assessment rather than the present noncancer assessment, we believe that mutagenicity and other genetox effects, particularly oxidative DNA damage, may contribute to chronic diseases such as heart disease, neurodegenerative diseases, diabetes mellitus, rheumatoid arthritis and aging, irrespective of their role in initiation and promotion of tumors (Burnet, 1974; Cooke et al., 2006; Kelly et al., 2007). In particular nickel's effects on the epigenome and gene expression indicate the probability that nickel's genetic toxicity is relevant to its noncancer effects.

The International Agency for Cancer Research (IARC, 1990), the International Program on Chemical Safety (IPCS, 1991), and NTP (1998) have reviewed the genotoxicity of nickel and nickel compounds in humans. Waksvik and Boysen (1982) studied groups of nickel refinery workers (9-11 workers in each group) and observed increases in chromosomal aberrations compared to controls. Deng et al. (1988) found elevated levels of both sister chromatid exchanges and chromosome aberrations (gaps, breaks, fragments) in seven electroplating workers exposed to nickel and chromium. Kiilunen et al. (1997) found that the frequency of micronucleated epithelial cells in the buccal mucosa of nickel refinery workers in the Helsinki area was not significantly elevated versus controls. The significance of these study results is somewhat limited due to the small sample sizes and the possibility that some workers were exposed to genotoxic compounds other than nickel. We summarize genetic toxicity findings in human test systems in Table 27.

Chen et al. (2003) evaluated the effects of nickel chloride on genotoxicity in human lymphocytes in vitro. Peripheral blood mononuclear cells (PBMC, primarily lymphocytes) were collected from five randomly selected healthy individuals (aged 18 to 23). Isolated lymphocytes (2 x 10^6 cells/µL) were incubated in saline solution with 0, 0.5, 1.0, 2.5, 5.0, or 10.0 mM $NiCl_2$ for one hour at 37ºC with continuous shaking in the dark. The levels of intracellular reactive oxygen species (ROS), lipid peroxidation, hydroxyl radical (•OH), and DNA damage via the Comet assay were evaluated.

The viability of the lymphocytes based on either trypan blue or neutral red exclusion decreased in a dose-dependent manner (neutral red control 92.3 % vs. 69.7% at 10 mM $NiCl_2$). Intracellular oxidants measured by dichlorofluorescin (DFC) increased in a dose-dependent manner (control 4.8% vs. 59.9% fluorescence intensity at 10 mM $NiCl_2$) with all dose levels significantly greater than the control. 2-Thiobarbituric acid reactant substances (TBARS) were also significantly increased compared to control at all $NiCl_2$ levels (control 156.5 vs. 553.7 nmol/10^6 cells at 10 mM $NiCl_2$). Lipid peroxidation in lymphocytes was significantly increased by three-fold with 10 mM $NiCl_2$.

Hydroxy radical production was measured by the hydroxylation of salicylate to 2,3-dihydroxybenzoate (2,3-DHB) and 2,5-dihydroxybenzoate (2, 5-DHB) byproducts. Both byproducts were significantly increased by $NiCl_2$ in a dose-dependent manner. The greater increase was seen with 2, 3-DHB (control 33.3 vs. 80.5 nM/10^6 cells at 10 mM $NiCl_2$). DNA damage as assessed by the extent of cell tailing in the Comet assay was increased in a dose-dependent manner (control 60 vs. 260 arbitrary units at 10 mM $NiCl_2$). The authors conclude that the generation of •OH radical was responsible for the $NiCl_2$-induced DNA strand breakage as evidenced by the dose-dependent association with •OH radical generation and comet tailing. The high correlation of DNA damage and DHB byproducts (r^2 = 0.9519) indicates that ROS in Ni-treated lymphocytes are

responsible for Ni-induced oxidative stress. The generation of Ni-induced •OH radical may play an important role in genotoxicity in human cells.

Table 27. Genotoxicity of Nickel Compounds in Human Test Systems

(adapted from ATSDR, 2005)

Compound	Test System	End point	Result	Reference
Nickel chloride	Human diploid fibroblasts	DNA damage	-	Hamilton-Koch et al., 1986
Nickel sulfate	Human gastric mucosal cells	DNA damage	-	Pool-Zobel et al., 1994
Nickel chloride	Human HeLa cells	DNA replication	+	Chin et al., 1994
Nickel sulfate, Nickel sulfide	Human lymphocytes	Sister chromatid exchange	+	Andersen, 1983; Larremendy et al., 1981; Ohno et al., 1982; Saxholm et al., 1981
Nickel sulfate	Human lymphocytes	Chromosome aberration	+	Larremendy et al., 1981
Nickel subsulfide	Human lymphocytes	Sister chromatid exchange	+	Arrouijal et al., 1982
		metaphase analysis	+	
		micronuclei formation	+	
Nickel sulfate	Human bronchial epithelial cells	Chromosome aberration	+	Lechner et al., 1984
Nickel subsulfide, Nickel oxide, Nickel sulfate, Nickel acetate	Human foreskin cells	Cell transformation	+	Bidermann and Landolph,1987
Nickel oxide, Nickel subsulfide, Nickel carbonate hydroxide, Nickel sulfate	Human lymphocytes	Sister chromatid exchange	-	Waksvik and Boysen, 1982
			+	M'Bemba-Meka et al. 2007
Nickel chloride	Human lymphocytes	DNA strand breakage	+	Chen et al., 2003
		Comet assay	+	
Nickel containing particles	Human A549 lung cells	Cytotoxicity	+	Mehta et al., 2008
		DNA repair capacity	+	
		mutation frequency	+	

Broday et al. (2000) observed nickel-induced inhibition of histone H4 acetylation in yeast and human cells in vitro. *Saccharomyces cerevisiae* cells were grown in medium with 0, 0.2 or 0.5 mM $NiCl_2$ for 1, 3, or 6 cell generations. Histones were isolated and analyzed with antibodies specific for H4 acetyl-lysine 5, 8, 12, or 16. The addition of 0.5 mM $NiCl_2$ suppressed the growth-related accumulation of lysine acetylation at all four lysine residues compared with the control cells. The effect of nickel on the levels of histone acetylation was also examined in human lung carcinoma A549 cells treated with soluble $NiCl_2$ and insoluble Ni_3S_2. The soluble nickel treatment of 0 or 3 mM $NiCl_2$ did not change the level of H4 acetylation. Nickel subsulfide treatment at 0, 0.5, 1.0 μg/cm^2 for two days (40 to 80% confluent growth) resulted in a concentration-dependent decrease in H4 acetylation at Lys-12. The concentrations used were reported as nontoxic. What toxic effects may result from altered histone acetylation patterns in vivo, particularly when coupled with Ni-induced DNA methylation, are unknown.

Jia and Chen (2008) studied nickel-induced DNA damage and cell death in human leukemia HL-60 cells and the protecting role of antioxidants. Cells were treated for up to 96 hr with 0, 0.5, 1.0, or 10.0 mM Ni^{2+}. Ten mM Ni^{2+} was rapidly fatal to cells along with a concomitant increase in DNA fragmentation as measured by flow cytometry with propidium iodide. Lower concentrations of Ni^{2+} also resulted in DNA fragmentation and death but at lower levels and after much longer exposures, i.e. no less than 48 or 72 hr at 1.0 or 0.5 mM, respectively. Nickel treatment of HL-60 cells also resulted in a release of malondialdehyde (MDA) in a dose- and time-dependent manner. The antioxidants ascorbic acid and *N*-acetyl-cysteine significantly reduced the Ni-induced generation of MDA and DNA fragmentation in a dose-dependent manner. Alternatively, H_2O_2 increased both Ni-induced MDA generation and DNA fragmentation also in a dose-dependent manner. Similar results were obtained for the cell death endpoint.

Mehta et al. (2008) evaluated the effects of particulate matter containing nickel and chromium on nucleotide excision repair capacity (NER) in human lung cells in vitro. They observed that human A549 cells exposed to 100 μg/mL of urban particulate matter (collected in the Washington DC area) for 24 hr had only a 10% reduction in viability, but a 35% reduction in repair capacity, and a five-fold increase in mutation frequency. The authors interpret their results with a view to three potential mechanisms: (1) particle components such as heavy metals and aldehydes directly modify repair proteins and DNA; (2) ROS and secondary products of ROS modify repair proteins and DNA; and (3) direct modification of DNA replication proteins by heavy metals and aldehydes reduce the fidelity of DNA replication. Specifically “Ni and cadmium can induce repair protein-DNA damage complex formation. Aldehydes, Cr, and Ni are known to have a high affinity towards thiol groups and histones and, therefore, their potential targets could be zinc finger structures in DNA binding motifs.”

The Agency for Toxic Substances and Disease Registry (ATSDR, 2005), NTP (1998), Snow (1992), Kasprzak (1991), IPCS (1991), Costa (1991), IARC (1990), the California Air Resources Board (CARB, 1991), and Sunderman (1989) have reviewed the genotoxicity data and mode of action of nickel and nickel compounds. In Table 28 are summarized the *in vitro* and *in vivo* genotoxicity data of nickel compounds in microbial and mammalian test systems. In general the data suggest that nickel does not alter the frequency of gene mutations in non-mammalian systems although some studies have found gene mutations (ATSDR, 2005). The results in mammalian systems are stronger with increased gene mutations found at the HGPRT locus in Chinese hamster V79 cells (Hartwig and Beyermann, 1989; Miyaki et al., 1979) but not in Chinese hamster ovary (CHO) cells (Hsie et al., 1979). Increased gene mutations were also seen in CHO AS52 cells (*grp* locus) (Fletcher et al., 1994), mouse lymphoma cells (Amacher and Paillet, 1980; McGregor et al., 1988), and virus-infected mouse sarcoma cells (Biggart and Murphy, 1988; Biggart et al., 1987).

Table 28. Genotoxicity of Nickel in Microbial and Mammalian Test Systems (updated from ATSDR, 2005)

Compound	Test System	End point	Result	Reference
Microbial systems				
Nickel chloride, Nickel nitrate, Nickel sulfate	*Salmonella typhimurium*	Gene mutation	-	Arlauskas et al., 1985; Biggart and Costa, 1986; Marzin and Phi, 1985;Wong, 1988
Nickel chloride	*Escherichia coli*	Gene mutation	-	Green et al., 1976
Nickel chloride	*Escherichia coli*	DNA replication	+	Chin et al., 1976
Nickel chloride	*Corynebacter-ium* sp.	Gene mutation	+	Pikalek and Necasek, 1983
Nickel oxide, Nickel trioxide	*Bacillus subtilis*	DNA damage	-	Kanematsu et al., 1980
Nickel chloride	*Saccharomyces cerevisiae*	Histone H4 acetylation decreases at Lys5,8,12,16	+	Broday et al., 2000

Table 28. Genotoxicity of Nickel in Microbial and Mammalian Test Systems (updated from ATSDR, 2005)

Compound	Test System	End point	Result	Reference
Mammalian systems				
Nickel chloride	CHO cells	Gene mutation at the HGPRT locus	-	Hsie et al., 1979
Nickel chloride	Virus-infected mouse cells	Gene mutation	+	Biggart and Murphy, 1988; Biggart et al., 1987
Nickel chloride Nickel sulfate	Mouse lymphoma cells	Gene mutation	+	Amacher and Paillet, 1980; McGregor et al., 1988
Nickel chloride	Chinese hamster V79 cells	Gene mutation	+	Hartwig and Beyersmann, 1989; Miyaki et al., 1979
Nickel chloride Crystalline NiS	CHO cells	DNA damage	+	Hamilton-Koch et al., 1986; Patierno and Costa, 1985
NiO (black and green, <10 µm)), NiS (amorphous, <10 µm), Nickel subsulfide (< 10µm), Nickel chloride, Nickel sulfate, Nickel acetate	CHO AS52 cells	Gene mutation (*grp* locus)	+	Fletcher et al., 1994
Nickel chloride, Nickel sulfate, NiS (crystalline)	Hamster cells	SCE	+	Andersen, 1983; Larremendy et al., 1981; Ohno et al., 1982; Saxholm et al., 1981
Nickel sulfate, Nickel chloride, NiS	Hamster cells	Chromosome aberration	+	Conway and Costa, 1989; Larremendy et al., 1981; Sen and Costa, 1986b; Sen et al., 1987

Table 28. Genotoxicity of Nickel in Microbial and Mammalian Test Systems (updated from ATSDR, 2005)

Compound	Test System	End point	Result	Reference
Nickel sulfate	Rat bone marrow and spermatogonia cells	Chromosome aberration	-	Mathur et al., 1978
Nickel chloride, Nickel sulfate, Nickel nitrate	Mouse bone marrow cells	Micronucleus test (oral)	+	Sobti and Gill, 1989
Nickel chloride	Mouse bone marrow cells	Chromosome aberrations (i.p.)	-	Dhir et al., 1991
Nickel chloride	Mouse bone marrow cells	Micronucleus test (i.p.)	-	Deknudt and Leonard, 1982
Nickel acetate	Mouse	Dominant lethal test (i.p.)	-	Deknudt and Leonard, 1982
Nickel subsulfide (< 10 µm)	Human lung fibroblast MRC-5 cells	DNA strand breaks	+	Zhuang et al., 1996
		PADPRP activation	+	
Nickel chloride	CHO Cells	DNA repair inhibition	+	Lynn et al. 1997; Iwitzki et al. 1998
Nickel subsulfide (97% < 10 µm, 70% < 5 µm)	Transgenic mouse	Gene mutation (inhalation)	-	Mayer et al., 1998
Nickel subsulfide (97% < 10 µm, 70% < 5 µm)	Rat	Gene mutation respiratory tissue (inhalation)	-	Mayer et al., 1998
Nickel sulfide, (0.5 µg/cm^2), Nickel chloride (50 µmol/L), Nickel sulfate, (100 µmol/L)	BALB/c-3T3 Ni-transformed cells in vitro	DNA strand breaks (comet)	+	Lei et al. 2001
		DNA-protein crosslinks	+	
		Telomerase	+	
Nickel sulfate	Chinese hamster V79 cells	Gene mutation	+	Ohshima, 2003
		Chromosome aberrations	+	
		Aneuploidy	+	
		Polyploidy	+	

Table 28. Genotoxicity of Nickel in Microbial and Mammalian Test Systems (updated from ATSDR, 2005)

Compound	Test System	End point	Result	Reference
Nickel sulfate	Human lung tumor cell line HCC15	Induction of microsatellite mutations	+	Zienolddiny et al., 2000
	Human lung tumor cell line NCI-H2009		+	
	Human lung tumor cell line A427		+	
Nickel subsulfide (particle size not stated)	Human lung carcinoma A549 cells	Histone H4 acetylation decrease at Lys 12	+	Broday et al., 2000
Nickel chloride	Male Mice	Dominant lethal mutation	+	Doreswamy et al., 2004
Nickel chloride	Male Mice	DNA fragmentation	+	Danadevi et al., 2004
Nickel chloride	Human lung carcinoma A549 cells	Histone H4 acetylation	-	Broday et al., 2000
Nickel sulfate	Male Rats	Micronuclei formation oral	-	Oller and Erexson, 2007
Nickel chloride	Human leukemia HL-60 cells	DNA fragmentation	+	Jia and Chen, 2008
		cell death	+	
Nickel arsenide	Mouse embryo C3H/10T1/2 Cl 8 cells	Cell transformation	+	Clemens and Landolph, 2003
		Chromosome aberrations	+	
Tungsten-nickel-cobalt alloy 91-6-3 particles	Cultured L6-C11 rat muscle cells	DNA damage	+	Harris et al. (2011)
		Caspase-3 inhibition	+	
		hypoxia	+	
		cyto-toxicity	+	
$Ni(OH)_2$ nanoparticles (size not specified)	Hyperlipidemic(ApoE -/-) Mice, 79 µg Ni/m^3	Mitochondrial DNA damage in the aorta	+	Kang et al. (2011)

A2.2.1 Studies *in vitro*

Examination of the genotoxicity database for soluble nickel compounds indicated that they generally did not cause mutation in bacterial test systems. Positive results have been observed (1) in tests for single and double DNA strand breaks and/or crosslinks in both human and animal cells, (2) in tests for cell transformation, (3) in tests for sister chromatid exchanges and chromosomal aberrations in hamster and human cells, and (4) in tests for mutation at the HGPRT locus in animal cells (IARC, 1990).

Several studies reported that nickel compounds have the ability to enhance the cytotoxicity and mutagenicity of other DNA damaging agents such as ultra-violet light, benzo(a)pyrene, cis-platinum, and mitomycin C (Hartwig and Beyersmann, 1989; Christie, 1989; Rivedal and Sanner, 1980). Hartwig et al. (1994) showed that Ni^{2+} inhibited the removal of pyrimidine dimers and repair of DNA strand break in HeLa cells after exposure to ultra-violet light or X-rays. Hartmann and Hartwig (1998) demonstrated that the inhibition of DNA repair was effective at a relatively low concentration, 50 µM Ni^{2+}, and partly reversible by the addition of Mg^{2+}. Based on these observations, they suggested that Ni^{2+} disturbed DNA protein interactions essential for the DNA repair process by the displacement of essential metal ions.

Soluble nickel compounds can inhibit the normal DNA synthesis, impair or reduce the fidelity of DNA repair, and transform initiated cells in vitro. Basrur and Gilman (1967) and Swierenga and McLean (1985) showed that nickel chloride inhibited DNA synthesis in primary rat embryo cells and in rat liver epithelial cells. Costa et al. (1982) found that nickel chloride at 40-120 µM selectively blocked cell cycle progression in the S phase in Chinese hamster ovary cells.

Abbracchio et al. (1982) demonstrated that Chinese hamster ovary cells maintained in a minimal salts/glucose medium accumulated 10-fold more ^{63}Ni than did cells maintained in a minimal salts/glucose medium with 5 mM cysteine. The results were obtained after the removal of surface-associated radioactivity by treating the cells with trypsin. They also showed that supplementation of the salts/glucose medium with fetal bovine serum decreased in a concentration dependent fashion both the Ni^{2+} uptake and cytotoxicity.

Nieborer et al. (1984) demonstrated that chelation of Ni^{2+} by amino acids and proteins has a significant effect on the cellular uptake of Ni^{2+} in human B-lymphoblasts, human erythrocytes, and rabbit alveolar macrophages. They observed that addition of L-histidine or human serum albumin at physiological concentrations to the cell cultures reduced Ni^{2+} uptake by 70% -90%. The concentration of nickel used in the study was $7x10^{-8}$ M (or 4.1 µg/L); it was comparable to serum nickel levels observed in workers occupationally exposed to nickel.

Findings of Nieborer et al. (1984) and Abbracchio et al. (1982) indicate the important role of specific amino acids and proteins in regulating the uptake and cytotoxicity of Ni^{2+}. For this reason, when in vitro genotoxicity test results are compared, it is important to standardize the concentration of these chelating agents.

Zhuang et al. (1996) treated MRC-5 human lung fibroblast cells with crystalline Ni_3S_2 (0, 2.5, 5.0, 10.0, or 20 μg/cm^2) for four hours and DNA strand breaks measured by single cell electrophoresis (comet assay). All Ni-treated cells gave significantly increased tail lengths compared to the control ($P < 0.01$). A linear dose-response was observed up to 10 μg Ni/cm^2 (their Fig 2a). Significant leakage of lactate dehydrogenase was seen at 10 and 20 μg/cm^2 and increased activity of poly (ADP-ribose) polymerase (PADPRP) at 5.0 μg/cm^2 and above ($P < 0.01$). PADPRP is a nuclear enzyme associated with DNA damage and repair. PADPRP activity (pmol/μg DNA) was directly correlated with tail length (μm) in the comet assay ($R = 0.971$).

Lynn et al. (1997) studied the role of Ni^{2+} and ROS on enzymes of DNA repair in CHO cells in vitro. Nickel chloride exposure increased cellular oxidant levels in CHO cells in a dose-dependent manner between two and eight mM. When inhibitors of glutathione (BSO, buthione sulfoximine) or catalase (3ATA, 3-aminotriazole) were included with nickel chloride the cytotoxicity of Ni^{2+} was significantly enhanced. The effect was more pronounced in UV-irradiated cultures indicating that ROS were involved in the cytotoxic effect of nickel as well as the enhancing effect of nickel on UV cytotoxicity. The authors tested the effect of H_2O_2 on Ni inhibition of DNA polymerase and ligation. In the presence of 0.1 mM $NiCl_2$ or 1.0 mM H_2O_2, the activities of DNA ligation were about 85% and 50% of control, respectively. The activity of DNA ligation decreased to 9.3% when cell extracts were treated with 0.1 mM $NiCl_2$ and then with 1.0 mM H_2O_2. This level was significantly lower than expected by simple additivity (χ^2 analysis).

This synergistic inhibition induced by Ni plus H_2O_2 was also observed in DNA polymerization in which activity fell to 46.5% after treatment with 0.1 mM $NiCl_2$ and 2.0 mM H_2O_2. The results indicate that DNA ligation is more sensitive to oxidant enhanced Ni inhibition than DNA polymerase. A 30-minute incubation with glutathione could completely remove the inhibition of Ni or recover ligation activity to 80% of control following H_2O_2 treatment or only 45% of control following Ni plus H_2O_2. Ni has a high binding affinity with cellular proteins ($K = 10^9$ M^{-1}). The redox potential of Ni^{2+} is very high but can be lowered by binding to suitable ligands, such as the imidazole nitrogen of histidine. In the presence of oxidants such as H_2O_2, Ni^{2+}/Ni^{3+} redox cycling can occur leading to the formation of free radicals such as •OH. Radical formation can lead to irreversible damage to proteins involved in DNA repair, replication, recombination and transcription and contribute to the toxic effects of nickel.

Mayer et al. (1998) tested Ni_3S_2 in a *lacI* transgenic BigBlue Rat 2 embryonic fibroblast cell line exposed for two hours to 0, 0.01, 0.04, or 0.17 mM Ni_3S_2. The

mutation frequencies were 4(control), 7.2, 10.4, and 34.1 (x 10^{-5}), respectively. However, molecular analysis in one-third of the mutants did not show DNA sequence change in the *lacI* gene despite loss of function. DNA damage as indicated by fragmentation in the comet assay was also seen in lung and nasal mucosa cells at 0.04 and 0.3 mM Ni_3S_2. Transgenic mice and rats were also exposed by inhalation for two hours (nose only) to 24-352 mg Ni_3S_2/m^3. Control animals were exposed to 8-126 mg $CaCO_3/m^3$and sacrificed immediately after exposure. Transgenic test animals were sacrificed after an expression time of 14 days. Nasal mucosa and lung tissues were removed and frozen until analysis. The spontaneous mutation frequencies of the *lacZ* in mice or the *lacI* in rats was not significantly increased compared to controls in these tissues by exposure to 10 mg Ni_3S_2/kg bw and 6 mg Ni_3S_2/kg bw, respectively.

Iwitzki et al. (1998) studied the effect of nickel chloride on the induction and repair of O^6-methylguanine and N^7-methylguanine after treatment with N-methyl-N-nitrosourea (MNU) in Chinese hamster ovary cells. The CHO cells were transfected with human O^6-methylguanine-DNA methyltransferase (MGMT) cDNA, and compared with MGMT-deficient parental cells. For N^7-methylguanine repair, there was no marked difference in the kinetics of lesion removal with or without nickel. However, nickel (II) led to a significant decrease in repair of O^6-methylguanine lesions. Seventy-eight percent of O^6-methylguanine was repaired in 24 hours in the absence of nickel, while this was reduced to 48% with 250 µ*M* Ni^{2+}. Nickel-induced inhibition of repair exhibited a dose-dependence in the 50-250 µM range. Repair inhibition was accompanied by an increase in MNU-induced cytotoxicity in nickel-treated cells but not in MGMT-deficient controls.

Kawanishi et al. (2001, 2002) described two separate mechanisms of oxidative DNA damage induced by 20 µM $NiSO_4$ in studies with calf thymus DNA, 10 µg/mL of various Ni compounds in cultured HeLa cells, or rats exposed intratracheally. With calf thymus DNA treated with Ni(II) and H_2O_2 they observed a time- and peroxide-dependent increase in 8-hydroxydeoxyguanosine (8-OH-dG). Ni(II) or H_2O_2 alone gave little or no increase in 8-OH-dG. With HeLa cells, incubation with Ni_3S_2 (10 µg/mL) for 24 hr significantly increased 8-OH-dG in extracted cellular DNA. Similar incubations (10 µg/mL) with Ni_2O_3 (black), NiO (green), or $NiSO_4$ did not induce 8-OH-dG formation. A significant increase in of 8-OH-dG was found in DNA extracted from lungs of 3 to 5 rats treated with 1.0 mg each of the nickel compounds intratracheally. The mean 8-OH-dG formation was Ni_3S_2 (2.57±0.87), Ni_2O_3 (black, 2.33±0.55), NiO (green, 2.33±0.61), $NiSO_4$ (1.65±0.97), and control (0.78±0.51) in units of 8-OH-dG/dG x 10^5. All mean increases were significantly greater than the control mean ($P < 0.05$). The results were interpreted by the authors as supporting a direct mode of DNA damage whereby Ni(II) enters the cells and then interacts with endogenous and/or Ni_3S_2-produced H_2O_2 to give reactive oxygen species that cause DNA damage. Additionally an indirect mode of oxidative DNA damage via inflammation is also supported. In this mode the sources of endogenous oxygen radicals are phagocytic cells such as neutrophils and macrophages. All of the

nickel compounds can operate via the indirect mode while nickel subsulfide can also act directly.

Lei et al. (2001) measured DNA strand breaks, DNA-protein crosslinks, and telomerase activity in nickel-transformed BALB/c-3T3 cells in vitro. The transformed loci were induced by insoluble crystalline NiS (particle size not specified, 0.5 μg/cm^2), soluble $NiCl_2$ (50 μM) and $NiSO_4$ (100 μM). All three compounds showed statistically significant DNA strand breaks by the comet assay (single cell electrophoresis). The mean tail lengths of 100 comets were control 13.4, NiS 51.9, $NiCl_2$ 48.3, and $NiSO_4$ 42.2 μm, (all $P < 0.01$ vs. control). DNA-protein crosslinks were measured by ^{125}I-postlabelling techniques. Again all three nickel compounds gave significantly increased crosslinks compared to the control non-transformed cells 618, NiS 2414, $NiCl_2$ 1127, and $NiSO_4$ 988 cpm/μgDNA (all $P < 0.05$). In this case NiS was clearly much more active than the soluble nickel compounds. Telomerase activities were detected in all three nickel-transformed cells but the activity was much higher with NiS and $NiCl_2$ than with $NiSO_4$.

Ohshima (2003) studied genetic instability induced by nickel sulfate in V79 Chinese hamster cells. The cells were treated with 320 μM $NiSO_4$ for 24 hr at low cell density of 100 cells/100 mm diameter dish and clones selected from single surviving cells. When post-treatment cells were grown to 23-25 population doublings, the mutation frequency at the hypoxanthine phosphoribosyltransferase (HPRT) locus and chromosome aberration frequency of each clone were measured. Five out of 37 clones from Ni-treated cells showed increased frequencies of HPRT mutations ($\geq 1 \times 10^{-4}$), while only 1/37 control clones showed a mutation rate this high. Also, 17/37 clones from treated cells showed structural chromosomal aberrations vs. 3/37 for the controls. These included chromatid gaps and breaks, chromosome gaps and breaks, exchange, ring, and dicentric aberrations. The frequencies of chromosome gaps, ring, and dicentric aberrations were statistically significantly increased compared to controls, as was mean frequency of all aberrations ($P < 0.05$, *t*-test). Numerical aberrations were also observed in clones from Ni-treated cells: 8/37 for aneuploidy and 11/37 for polyploidy. Only a few control clones showed such numerical aberrations. The authors conclude that nickel sulfate can induce genetic and chromosomal instability in V79 cells.

Oxidative DNA damage has been implicated as a contributing factor in neurodegeneration and heart disease as well as cancer and may figure in many degenerative diseases. Several studies to date have focused on the formation of the primary products of DNA oxidation: 7, 8-dihydro-8-oxoguanine (8-oxoG) and 8-hydroxy-2'-deoxyguanosine (8-OH-dG). Kelly et al. (2007) studied the oxidation of guanine, 8-oxoG and DNA by a Ni(II)/H_2O_2 system in vitro. They observed erratic oscillatory-like formation of 8-oxoG from free guanine and from DNA. Oxidation of 8-oxoG by Ni(II)/H_2O_2 led to guanidinohydantoin (GH) or its oxidized analog (oxGH). The authors conclude that the instability of 8-oxoG (and presumably 8-OH-dG) in this system and its further oxidation products indicate a

complex oxidative mechanism for guanine and unsuitability as a biomarker of DNA damage. However, it's not yet clear how quantitatively significant these "further" oxidative steps are under usual exposure scenarios.

Another problem with interpreting DNA adduct data is revealed by the study of Kaur and Dani (2003) on the relative nickel binding to RNA versus DNA. Female Sprague-Dawley rats (3 x 0.15 kg) were administered i.p. injections of $^{63}NiCl_2$. After 24 hr the animals were sacrificed and selected tissues removed for analysis. The subcellular distribution of ^{63}Ni in the liver, kidney, spleen and lungs was highest in the nucleus. About 10% to 50% of the nuclear radioactivity level was seen in the mitochondria, lysosomes, and microsomes. Further analysis of the nuclear fraction showed that in each tissue the large majority of ^{63}Ni label was associated with RNA rather than with DNA or nucleoproteins. The highest association observed was with kidney RNA. In vitro binding of $^{63}NiCl_2$ to DNA, denatured DNA, highly polymerized (HP) DNA, and RNA showed the maximum binding to RNA and HP DNA. Binding to DNA and denatured DNA was less than 25% of these values. Significant differences were observed between the infrared (IR) spectra of RNA and DNA incubated in vitro with $NiCl_2$, which also support the radiolabel findings. The authors postulate that Ni(II) may act by controlling gene expression post-transcriptionally via interaction with mRNA. Loss of mRNA has been reported in nickel-transformed cells (Salnikow et al., 1994).

Deng et al. (2006) observed that treatment of V79 cells with $NiCl_2$ after, but not before, exposure to benzo[a]pyrene (BaP) or its diol-epoxide (BPDE) metabolite led to significant enhancements of chromosome damage compared to control cells. Treatment of V79 cell for two hours with 0, 1, 5, 10, or 20 µg/mL of $NiCl_2$ resulted in proportions of aberrant cells of 0.75%, 0.75%, 1.0%, 1.3%, and 1.8 %, respectively. A similar value, 1.3% was obtained with 0.5 µg/mL BaP. Treatment of $NiCl_2$ at 5, 10, or 20 µg/mL after BaP exposure gave 9.3%, 12%, or 13% aberrant cells (all $P < 0.05$). The large majority of aberrations were chromosome breaks. The authors interpret the Ni-mediated potentiation of BaP genetic toxicity as a result of nickel inhibition of nucleotide excision repair (NER).

A2.2.2 Studies *in vivo*

The clastogenic potential of soluble nickel compounds has been shown in many in vivo studies. Sobti and Gill (1989) reported that oral administration of nickel sulfate (28 mg Ni/kg bw), nickel nitrate (23 mg Ni/kg bw), or nickel chloride (43 mg Ni/kg bw) to mice increased the frequency of micronuclei in the bone marrow at 6 and 30 hours after treatment. Details of the study were not reported and it was not clear how many animals were used in each experiment. Mohanty (1987) reported that intraperitoneal injections of nickel chloride at 6, 12, or 24 mg/kg bw increased the frequency of chromosomal aberrations in bone-marrow cells of Chinese hamsters. However, Mathur et al. (1978) observed that intraperitoneal injections of nickel sulfate at 3 and 6 mg/kg bw did not induce chromosomal aberrations in bone-marrow cells and spermatogonia of male albino rats. Saplakoglu et al. (1997) administered 44.4 mg nickel chloride/kg bw to rats via

subcutaneous injections and did not observe increased levels of single-strand breaks in cultured lung, liver, or kidney cells.

Similarly, Deknudt and Leonard (1982) administered 25 mg/kg bw nickel chloride and 56 mg/kg nickel nitrate (about 50% of the LD_{50} in both cases) to mice by intraperitoneal injection and did not detect a significant increase of micronuclei in the bone marrow of the animals after 30 hours. Inhibition of DNA synthesis has been observed in vivo. Amlacher and Rudolph (1981) observed that intraperitoneal injections of nickel sulfate at 15 - 30% of the LD_{50} to CBA mice suppressed DNA synthesis in hepatic epithelial cells and in the kidney. Hui and Sunderman (1980) also reported that intramuscular injections of nickel chloride to rats at 20 mg Ni/kg bw inhibited DNA synthesis in the kidney.

Danadevi et al. (2004) administered $NiCl_2$ to 4-week old male Swiss mice. Eight groups of five animals each were given 0, 3.4, 6.8, 13.6, 27.2, 54.4, or 108.8 mg $NiCl_2$/kg bw by gavage. One group was given 25 mg cyclophosphamide/kg bw i.p. as a positive control. Blood was collected from each animal at 24, 48, and 72 hr, one week and two weeks post-treatment. DNA damage was assessed by single cell electrophoresis of leucocytes (comet assay). All doses produced significant dose-dependent DNA damage ($P < 0.05$) when compared to controls at 24, 48, 72 hr and one week. Clinical signs included loss in weight and feed intake at doses ≥ 13.6 mg $NiCl_2$/kg bw. From 72 hr post-treatment the mean comet lengths of all doses gradually decreased and after two weeks the lower doses (≤13.6 mg/kg) were not significantly different from the negative controls.

Oller and Erexson (2007) found a lack of micronuclei formation in 6 male Sprague-Dawley rats/dose group exposed to 0, 125, 250, or 500 mg $NiSO_4 \cdot 6H_2O$/kg-d for 3 days. At least 2000 polychromatic erythrocytes (PCEs) per animal were analyzed for micronuclei. Average micronuclei (2000/animal) found were 0.07, 0.01, 0.07, 0.06%, respectively. Nickel concentrations found in plasma and bone marrow were significantly higher in all dose groups than in the control animals.

Jia and Chen (2008) extended their study of antioxidant protection against nickel-induced DNA fragmentation to 40 male C57 mice and ascorbic acid (ASA) as antioxidant. Five groups of eight mice each were treated with a single daily i.p. injection for two weeks with 0, 2.0, 20.0 mg/kg-d $NiCl_2$, 2.0 + 5.0 mg/kg-d ASA, or 20.0 + 5.0 mg/kg-d ASA. DNA fragmentation and malondialdehyde (MDA) generation were measured in peripheral blood mononuclear cells (PBMC) and serum, respectively. Without ASA significant dose-dependent DNA fragmentation and MDA generation was observed. For DNA fragmentation the mean (± SD, N = 8) for 0, 2, and 20 mg Ni/kg-d were 4.68 ± 0.89%, 9.83 ± 1.16%* and 15.25(1.91) %*, respectively (*$P < 0.01$). MDA in serum also showed a significant but shallower increase. Treatment of Ni + ASA showed slight, non-statistically significant, increases of MDA and DNA fragmentation. For the latter the values were 4.68(0.89), 6.16(0.88), and 7.85(1.1), respectively. MDA values gave a shallower response. No trend tests were provided. The

authors suggest the use of ascorbic acid to ameliorate the chronic toxic effects in individuals occupationally exposed to nickel compounds.

A number of hypotheses have been proposed about the mechanisms that can explain the observed genotoxicity and transformation potential of soluble nickel compounds. Costa et al. (1982) and Sahu et al. (1995) showed that soluble nickel compounds affected cell growth by selectively blocking the S-phase of the cell cycle. Kasprzak (1991) and Sunderman (1989) suggested that most of the genotoxic characteristics of Ni^{2+} including DNA strand breaks, DNA-protein crosslinks, and chromosomal damage could be explained by the ability of Ni^{2+} to generate oxygen free radicals. While Ni^{2+} in the presence of inorganic ligands is resistant to oxidation, Ni^{2+} chelated with peptides has been shown to be able to catalyze reduction-oxidation reactions. Andrews et al. (1988) observed that certain peptides and proteins (especially those containing a histidine residue) form coordination complexes with Ni^{2+}. Many of these complexes have been shown to react with O_2 and/or H_2O_2 and generate oxygen free radicals (such as •OH) *in vitro* (Bossu et al., 1978; Inoue and Kawanishi, 1989; Torreilles and Guerin, 1990; Nieboer et al., 1984 and 1988). It is important to note that the major substrates for nickel mediated oxygen activation, O_2 and H_2O_2, are found in mammalian cells, including the nucleus (Peskin and Shlyahova, 1986).

Tkeshelashvili et al. (1993) showed that mutagenesis of Ni^{2+}in a bacterial test system could not only be enhanced by the addition of both hydrogen peroxide and a tripeptide glycyl-glycyl-L-histidine but also could be reduced by the addition of oxygen radical scavengers. Huang et al. (1993) treated Chinese hamster ovary cells with 0 to 5 mM nickel chloride and the precursor of fluorescence dye, 2, 7-dichlorofluorescin diacetate, and observed a significant increase of fluorescence in intact cells around the nuclear membranes. The effect was related to the concentration of the nickel chloride and was detectable at or below 1 mM. Since only strong oxidants, such as hydrogen peroxide and other organic hydroperoxides, can oxidize the nonfluorescent precursor to a fluorescent product, Huang et al. (1993) suggested that Ni^{2+} increased the level of such oxidants in intact cells.

Evidence of oxidative damage to cellular and genetic materials as a result of nickel administration has also been obtained from a number of *in vivo* studies. There are data indicating lipid peroxidation participates in the pathogenesis of acute nickel poisoning (Sunderman et al., 1985; Donskoy et al., 1986; Knight et al., 1986; Kasprzak et al., 1986 and Sunderman et al., 1987). Stinson et al. (1992) subcutaneously dosed rats with nickel chloride and observed increased DNA strand breaks and lipid peroxidation in the liver 4-13 hours after the treatment. Kasprzak et al. (1992) administered nickel acetate (5.3 mg Ni/kg bw) to pregnant rats by a single or two intraperitoneal injections and identified eleven oxidized purine and pyrimidine bases from the maternal and fetal liver and kidney tissues. Most of the products identified were typical hydroxyl radical-produced derivatives of DNA bases, suggesting a role for hydroxyl radical in the induction of their formation by Ni^{2+}. In two other animal studies, Kasprzak et al. (1990 and

1992) also observed elevated levels of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) in the kidneys of rodents administered a single intraperitoneal injection of nickel acetate. Formation of 8-OH-dG is often recognized as one of the many characteristics of •OH attack on DNA.

Besides generating oxygen free radicals, Ni^{2+} can also weaken cellular defense against oxidative stresses. Donskoy et al. (1986) demonstrated that administration of soluble nickel compounds depleted free-radical scavengers (e.g., glutathione) or catalase, superoxide dismutase, glutathione peroxidase, or other enzymes that protect against free-radical injury in the treated animals.

Insoluble crystalline nickel compounds are generally found to be more potent in genetic toxicity assays than the soluble or amorphous forms of nickel. To find out the reason for this phenomenon, Harnett et al. (1982) compared the binding of ^{63}Ni to DNA, RNA, and protein isolated from cultured Chinese hamster ovary cells treated with either crystalline nickel sulfide (^{63}NiS) or a soluble nickel compound, $^{63}NiCl_2$ (both at 10 µg/mL). They reported that in the case of $^{63}NiCl_2$ treatment, cellular proteins contained about 100 times more bound ^{63}Ni than the respective RNA or DNA fractions; whereas in cells treated with crystalline ^{63}NiS, equivalent levels of nickel were associated with RNA, DNA, and protein. In absolute terms, RNA or DNA had 300 to 2,000 times more bound nickel following crystalline ^{63}NiS treatment compared to cells treated with $^{63}NiCl_2$. Fletcher et al. (1994) reported similar findings. Chinese hamster ovary cells were exposed to either water-soluble or slightly water-soluble salts. They observed relatively high nickel concentrations in the cytosol and very low concentrations in the nuclei of the cells exposed to the water-soluble salts. In contrast, they found relatively high concentrations of nickel in both the cytosol and the nuclei of the cells exposed to the slightly water-soluble salts.

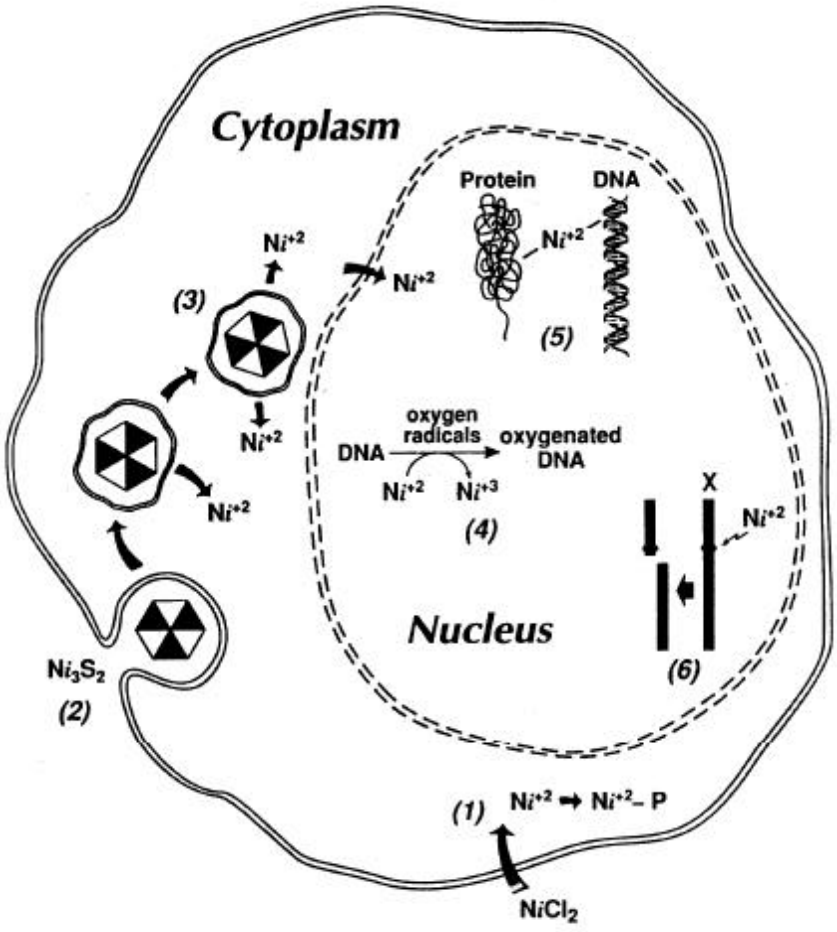
Sen and Costa (1986) and Costa et al. (1994) theorized that this is because NiS and $NiCl_2$ are taken up by cells through different mechanisms. Ni^{2+} has a high affinity for protein relative to DNA; treatment of cells with soluble nickel compounds resulted in substantial binding of the metal ion to cytoplasmic proteins, with a small portion of the metal ion eventually reaching the nucleus. When cells are treated with crystalline nickel sulfide, the nickel containing particles were phagocytosed and delivered to sites near the nucleus. This mode of intracellular transport reduces the interaction of Ni^{2+} with cytoplasmic proteins and peptides.

To support their theory, Sen and Costa (1986) exposed Chinese hamster ovary cells to nickel chloride alone, nickel chloride-albumin complexes, nickel chloride-liposomes, and nickel chloride-albumin complexes encapsulated in liposomes. They found that at a given concentration (between 100 and 1,000 µM), cellular uptakes of nickel were 2-4 fold higher when the ovary cells were exposed to nickel chloride-liposomes or nickel chloride-albumin complexes encapsulated in liposomes than to nickel chloride alone or nickel chloride-albumin complexes. Even at comparable levels of cellular nickel (approximately 300 pmole Ni/10^6

cells), fragmentation of the heterochromatic long arm of the X chromosome was only observed in cells treated with nickel encapsulated in liposomes and not in those exposed to nickel or nickel-albumin. Based on these data, they suggested that the higher genotoxic potency of crystalline nickel sulfide and nickel encapsulated in liposomes was not primarily due to the higher cellular nickel concentration, but rather to the way nickel ion was delivered into cells.

IARC (1980) suggested that cellular binding and uptake of nickel depend on the hydro- and lipophilic properties of the nickel complexes to which the cells are exposed. Nickel-complexing ligands, L-histidine, human serum albumin, D-penicillamine, and ethylenediaminetetraacetic acid, which form hydrophilic nickel complexes, inhibited the uptake of nickel by rabbit alveolar macrophages, human B-lymphoblasts, and human erythrocytes. Diethyldithiocarbamate and sodium pyridinethione, however, which form lipophilic nickel complexes, enhanced the cellular uptake of nickel. Several ideas and findings bearing on the mode of action of nickel genotoxicity have been integrated into a scheme proposed by NTP (1996a) and reproduced in Figure 7.

Figure 7. Possible Mechanisms of Nickel-induced Genotoxicity (from NTP, 1996a).



1) Soluble nickel compounds such as nickel chloride diffuse into the cell; Ni^{2+} ions are rapidly bound to cytoplasmic proteins (P) (Lee et al., 1993).
2) Insoluble nickel compounds such as nickel subsulfide are phagocytized into the cell and move toward the nucleus (Costa et al., 1982).
3) Lysosomal breakdown of insoluble nickel compounds releases large quantities of Ni^{2+} ions that concentrate adjacent to the nuclear membrane (Costa and Heck, 1983).
4) Oxidative damage is induced in DNA by nickel ions bound to nuclear proteins ($Ni^{2+} \rightarrow Ni^{3+}$), releasing active oxygen species (Tkeshelashvili et al., 1993; Sugiyama, 1994).
5) DNA-protein crosslinks are produced by Ni^{2+} ions binding to heterochromatin (Lee et al., 1982; Patierno and Costa, 1985; Sen and Costa, 1986).
6) Binding of nickel ions to the heterochromatic regions of the long arm of the X chromosome, which may contain a senescence gene and a tumor suppressor gene, can cause deletion of all or part of this region, leading to an immortalization of the cell and clonal expansion (Conway and Costa, 1989; Klein et al., 1991).

In general nickel genotoxicity is the result of indirect mechanisms. Three mechanisms predominate: (1) interference with cellular redox regulation and induction of oxidative stress and possible oxidative DNA damage; (2) inhibition of major DNA repair systems resulting in genomic instability and accumulation of mutations; and (3) deregulation of cell proliferation by induction of signaling pathways or inactivation of growth controls including tumor suppressor genes (Beyersmann and Hartwig, 2008).

A3 Effects on Gene Expression and the Epigenome

The effects of nickel on the epigenome are summarized in Table 29. Effects on DNA methylation and/or histone methylation, acetylation, or ubiquitination may influence the initiation and/or progression of chronic diseases in addition to cancer. In their review of metal epigenetics Arita and Costa (2009) conclude :

> "Taken together, numerous data suggest that epigenetic changes contribute more to nickel-induced toxic and carcinogenic effects than mutagenic effects."

Yan et al. (2003) studied histone modifications and gene silencing in nickel-treated *gpt* (guanine phophoribosyl transferase gene) transgenic G12 Chinese hamster cells. Four nickel-induced *gpt*-silenced G12 clones (N24, N37, N96, N97) obtained by treatment with NiS or Ni_3S_2 were used (particle sizes not specified). These clones were readily reverted to wild type (*gpt*$^+$) by treatment with 5-azacytidine. Analysis of chromatin proteins associated with Ni-silenced *gpt* gene was by chromosome immunoprecipitation assay (ChIP). The results showed hypoacetylation of both histones H3 and H4 in all four silenced G12 cell clones. Histone H4 acetylation of N24 was higher than the other clones but much lower than G12 control cells. The ChIP assay also showed hypoacetylation of histone H3-K9 in all four silenced clones. Alternatively, methylation was higher than controls in three of four silenced clones. Overall the results indicate that gene silencing induced by nickel involved the loss of histone acetylation and the activation of histone methylation. Silenced clones exhibited an increase in the methylation of the lysine 9 in histone H3.

Zhang et al. (2003) observed inhibition and reversal of nickel-induced transformation by the histone deacetylase inhibitor trichostatin A (TSA). Human T85 osteoblastic cells (HOS) were exposed to 0, 0.15, or 0.30 μg/cm^2 Ni_3S_2 or 0, 1, 2 mM $NiCl_2$ for 24 hr. The cells were rinsed, allowed to grow for 48 hr and the Ni treatment repeated. This procedure was repeated nine times. Either 5.0 ng/mL or 25 ng/mL TSA were added to the cells four hr before each exposure. Ni treated HOS cells exhibited dose-dependent increases in anchorage-independent colonies with both nickel compounds (ca. 500-750/10^5 cells vs. 250/10^5 cells in controls). Similar exposure to mouse PW cells showed 150 - 250/10^5 cells for $NiCl_2$ and 1500-2200/10^5 cells for Ni_3S_2 vs. 0 for controls. TSA treatment caused a dose-dependent suppression of Ni-induced transformation of HOS and PW cells. For HOS cells treated with 2 mM $NiCl_2$ the extent of

transformation at 0, 5.0, and 25.0 ng/mL TSA was 100%, 59.5% ($P < 0.05$), and 51.0% ($P < 0.01$), respectively. For HOS cells treated with 0.30 μg/cm^2 Ni_3S_2 the extent of transformation was 100%, 93.3% and 78.9% ($P<0.05$), respectively. Suppression was greater in the mouse PW cells (range 67 to 39%). Isolated Ni-transformed clones of mouse PW cells were reverted to normal by treatment with 5.0 ng/mL or 25.0 ng/mL TSA. Transformed cells ranged from 33 to 65% at 5 ng TSA/mL, 16 to 36% at 25.0 ng TSA/mL vs. 100% in untreated Ni-transformed clones.

Costa et al. (2005) found that exposure of human lung A540 cells to NiS particles for 48 to 72 hours resulted in most of the nickel bound in the cell nuclei. In contrast cells exposed to soluble $NiCl_2$ resulted in Ni ions localized in the cytoplasm. This result is consistent with reports that short-term (1-3 days) exposure to crystalline nickel particles can epigenetically silence target genes near heterochromatin, while similar short-term exposure to soluble nickel does not silence the genes. However, longer term (3 weeks) exposure to soluble nickel is also able to induce gene silencing. Nickel compounds were also found to activate hypoxia-signaling pathways. This probably results from nickel compounds blocking iron uptake leading to cellular iron depletion, affecting iron-containing enzymes. The inhibition of iron-dependent enzymes, such as aconitase and HIF proline hydroxylases may stabilize HIF protein and activate hypoxic signaling. Nickel and hypoxia decrease histone acetylation and increase methylation of H3 lysine 9. The loss of histone acetylation and methylation of lysine 9 in H3 results in global silencing of gene expression. Costa et al. also observed increases in the ubiquitination of histones of H2A and H2B in A549 cells after only 8 hours exposure to 1 mM $NiCl_2$. No changes were seen in ubiqitinated H4 as a result of similar exposures for up to 72hr.

Ke et al. (2006) studied alterations of histone modifications and transgene silencing by $NiCl_2$. Human lung bronchoepithelial A549 cells in culture were exposed to 0, 0.25, 0.50, or 1.0 mM $NiCl_2$ for 24 hr. Using pan-acetylated histone antibodies, the global levels of histone acetylation on histones H2A, H2B, H3 and H4 were measured following nickel exposure. The nickel doses had no effect on cell viability whereas histone acetylation was decreased in all four-core histones. A similar loss of histone acetylation was also observed in human hepatoma Hep3B cells, mouse epidermal C141 cells and *gpt* transgenic Chinese hamster G12 cells. Nickel treatment also resulted in increases of ubiquitination of H2A and H2B in a dose-dependent manner. The G12 *gpt* transgenic cell line was used to measure Ni-induced gene silencing in cells treated for 7 to 21 days with 50 or 100 μM $NiCl_2$ or 1 μg/cm^2 NiS. Treatments of three days or longer, resulted in increased frequency of 6-thioguanine (6-TG) resistant colonies, suggesting silencing of the *gpt* transgene in a time-dependent manner. After Ni-treatment the cells were placed in normal medium for either one or five weeks. The mRNA levels of the *gpt* transgene, which were very low after Ni treatment, returned to basal level after five weeks recovery. The data suggest that the nickel-induced effects were epigenetic.

Chen et al. (2006) reported that $NiCl_2$ treatment of human lung carcinoma A549 cells induced increases in histone H3 lysine 9 dimethylation and transgene silencing. Nickel(II) ions were found to increase global histone H3K9 mono- and dimethylation but not trimethylation. Increases in dimethylation occurred at ≥ 250 µM Ni(II) in a time-dependent manner. Nickel exposure decreased the activity of histone H3K9 methyltransferase G9a thus interfering with the histone dimethylation process. Cultured transgenic *gpt*$^+$ *hprt*$^-$ G12 cells were used to study Ni-induced gene silencing. Both acute and chronic nickel exposures decreased the expression of the *gpt* transgene in G12 cells. The cells were exposed to Ni(II) for 3 to 25 days to 50 or 100 µM $NiCl_2$ then selected for the *gpt*$^-$ phenotype by growing cells in the presence of 6-thioguanine (6-TG). Nickel exposure increased the frequency of 6-TG^r variants in a dose- and time-dependent manner. The variants were treated with 5-aza-2'-deoxycytidine resulting in a very high percentage reversion from *gpt*$^-$ to *gpt*$^+$ phenotype. Such a high frequency of reversion indicates that Ni(II) silenced the *gpt* transgene via an epigenetic rather than a genetic mechanism involving mutations or deletions. Overall the results indicated that the increase in H3K3 dimethylation played a key role in the *gpt* transgene silencing due to Ni(II) exposure.

Karaczyn et al. (2006) observed that human lung cells treated with Ni(II) resulted in a stimulation of mono-ubiquitination of H2A and H2B histones. Cultured 1HAEo and HPL1D human diploid lung cells were treated for 1 to 5 days with 0.05 to 0.5 mM Ni(II) acetate. Cell viability, assessed by Trypan blue exclusion, ranged from 90% at the low nickel concentration to 55-65% at the high concentration. Maximum stimulation of ubiquitination of H2B histone was reached in 24 hr at 0.25 mM Ni(II) in both cell lines. The authors note: "covalent modifications of core histones in chromatin, such as acetylation, methylation, phosphorylation, ribosylation, ubiquitination, sumoylation, and possibly others (e.g. deimination and biotinylation) serve as regulatory mechanisms of gene transcription." Usually increased ubiquitination of histone H2B is associated with gene silencing and decreased ubiquitination with gene activation, although this may depend on gene location. The authors interpret their results on Ni-induced histone ubiquitination as part of nickel's adverse effects on gene expression and DNA repair.

Ji et al. (2007) investigated epigenetic alterations in a set of DNA repair genes in NiS-treated 16HBE human bronchial epithelial cells (0, 0.25, 0.5, 1.0, or 2.0 µg Ni/cm^2 for 24 hr). The silencing of the O^6-methylguanine DNA methyltransferase (MGMT) gene locus and upregulation of DNA methyltransferase 1 (DNMT1) expression was observed in treated cells. Other epigenetic alterations included DNA hypermethylation, reduced histone H4 acetylation and a decrease in the ratio of Lys-9 acetylated/methylated histone H3 at the MGMT CpG island in NiS-transformed 16HBE cells. It is likely that Ni-induced alterations in DNA and histones contribute to altered gene expression, cytotoxicity and tumorigenicity.

Ke et al. (2008) demonstrated the both water-soluble and insoluble nickel compounds induce histone ubiquitination (uH2A and uH2B) in a variety of cell lines. Human A529 lung cells were treated with $NiCl_2$ (0.25, 0.5, and 1.0 mM) or Ni_3S_2 (0.5 and 1.0 μg/cm^2) for 24 hr. After exposures histones were isolated and Western blots performed using antibody against uH2A. $NiCl_2$ and Ni_3S_2 exposures resulted in increased levels of uH2A in a dose-dependent manner. Other mouse and human cell lines tested were C141, Beas-2B, HeLa, and Hep3B. In each case $NiCl_2$ treatment resulted in increased levels of uH2A. In vitro assays indicated that the presence of nickel did not affect the levels of ubiquinated histones through increased synthesis; instead nickel significantly prevented loss of uH2A and uH2B presumably inhibiting putative deubiquitinating enzyme(s). The study indicates that nickel ions may alter epigenetic homeostasis in cells.

Li et al. (2009) studied signaling pathways induced by nickel in non-tumorigenic human bronchial epithelial Beas-2B cells. Both 0.25 mM and 1.0 mM $NiSO_4$ exposures for 24 hr significantly up-regulated *c-Myc* protein in Beas-2B cells in a time-dependent manner. Because of the central role of *c-Myc* in cell growth regulation, cell apoptosis was also studied. Beas-2B cells were treated with $NiSO_4$ and whole cell lysates to determine poly (ADP-ribose) polymerase (PARP) cleavage, a marker for cell apoptosis. Nickel ions at 0.5 and 1.0 mM significantly induced PARP cleavage, indicating $NiSO_4$- induced apoptosis in the Beas-2B cells. Knockout of *c-Myc* and its restoration in a rat cell system confirmed the role of *c-Myc* in Ni(II)-induced apoptosis. Ni(II) ions increased the *c-Myc* mRNA concentration and *c-Myc* promoter activity but not *c-Myc* mRNA and protein stability. By the use of pathway specific inhibitors the investigators concluded that Ni(II) induced *c-Myc* in Beas-2B cells via the *Ras/ERK* signaling pathway. The study suggests possible roles for *c-Myc* in Ni-induced toxicity.

Ellen et al. (2009) observed that nickel ion Ni^{2+} condenses chromatin to a greater extent than the natural divalent cation in the cell, the magnesium ion Mg^{2+}. The authors found a significant difference in circular dichroism spectropolarimetry (CD) of oliginucleosomes exposed to the divalent cations. The maximum molar ellipticity at 272 nm decreased from ~6000 in the absence of cations to ~5000 with 0.6mM Mg^{2+}. In the presence of 0.6mM Ni^{2+} the molar ellipticity was reduced to ~3000. The authors note that this condensation or heterochromatinization of chromatin within a region containing a target gene would inhibit further molecular interactions essentially silencing the gene. In addition they used a model system that incorporated a transgene, the bacterial xanthine guanine phophoribosyl transferase gene (*gpt*) near and far from a heterochromatic region of the genome in two cell lines of Chinese hamster V79-derived cells. The model demonstrated by a Dnase I protection assay that nickel treatment protected the *gpt* gene sequence from Dnase I exonuclease degradation. The authors propose Ni-induced condensation of chromatin as a mechanism of nickel-mediated gene regulation.

The effects of nickel, chromate, and arsenite on histone 3 lysine 4 (H3K4) methylation in human A549 cells was evaluated by Zhou et al. (2009). Treatment of human lung carcinoma A549 cells with $NiCl_2$ (1.0 mM), Cr(VI) (10 μM), or As(III) (1.0 μM) significantly increased tri-methyl H3K4 after 24 hr exposure. Seven days exposure to lower levels (e.g., 50 μM Ni(II)) also increased tri-methyl H3K4. The results indicate that the metals studied alter various histone tail modifications, which can affect the expression of genes that may cause cell transformation or other cytotoxic effects. The specific genes that may be affected by these alterations are unknown. Other relevant DNA methylation and mapping of post-translational modifications of histones in the promoter regions of target genes warrant further investigation.

Table 29. Effects of Nickel on the Epigenome*

Study	Compound	Gene or factor affected	Effect	Cell Type	Comments or other effects
Li et al., 2009	$NiSO_4$	c-Myc	↑	BEAS-2B	Apoptosis induced.
		c-Myc	↑	HaCaT	
Guan et al., 2007	$NiCl_2$	bcl-2	↓	T cells Jurkat	Apoptosis induced, NO ↑.
Andrew & Barchowsky, 2000	Ni_3S_2	PAI-1	↑	BEAS-2B	Fibrinolysis inhibited. Particle sizes < 2.5μm
Andrew et al., 2001	Ni_3S_2	PAI-1	↑	BEAS-2B	Fibrinolysis inhibited. Particle sizes < 2.5μm
		c-Jun	↑		
		c-Fos	↑		
Salnikow et al., 2002	$NiCl_2$	HIF-1α	↑	Mouse fibroblasts HIF-1α knockout	Hypoxia, *Nip3* and prolyl-4-hydroxylase are HIF-1 dependent; *HSP70*, *GADD45*, *p21* and *p53* are HIF-1 independent; *ATM, GADD153*, *Jun B* and *MDR-1* are mixed.
		Cap43	↑		
		Nip3	↑		
		Prolyl-4-hydroxylase	↑		
		HSP70	↑		
		GADD45	↑		
		p21	↑		
		p53	↑		
	$NiCl_2$	HIF-1α	↑	Mouse fibroblasts	

*Note: BEAS, human bronchial epithelial cells; HaCaT, human keratinocyte cells; NO, nitric oxide generation; PAI-1, plasminogen activator inhibitor-1; HIF-1α, hypoxia-inducible transcription factor-1; AhR, aryl hydrocarbon receptor; A549, human lung bronchoepithelial cells; H3B, human hepatoma cells; H3K9, histone H3 lysine 9; HOS, human osteoblastic cell line; PW, mouse embryo fibroblasts; *MGMT*, O^6-methylguanine DNA methyltransferase gene locus; *DNMT1*, DNA methyltransferase 1 gene; 16HBE, Ni-transformed human bronchial epithelial cells; H3K9ac, histone H3 lys-9 acetylation; H4ac, histone H4 acetylation; H3K9me2, histone H3 Lys-9 methylation; NHBE, normal human bronchial epithelial cells; ↑, enhanced activity; ↓, reduced activity.

Table 29. Effects of Nickel on the Epigenome*

Study	Compound	Gene or factor affected	Effect	Cell Type	Comments or other effects
Salnikow et al., 2003		*Cap43*	↑	HIF-1α knockout	HIF-1 independent genes up-regulated *GADD45, p21, ATM, p53, Jun B;* genes up-regulated in HiF-1α deficient cells *HSP70, NGFb, IP-10, CD44 antigen, melanocortin 1 receptor, heparin-binding EGF-like, SGK kinase, BCL-2-like, E-MAP-115.*
		Bcl-2	↑		
		Nip3	↑		
		EGLN1	↑		
		HIG1	↑		
		Prolyl-4-hydroxylase	↑		
		Focal adhesion kinase	↑		
Davidson et al., 2003	$NiCl_2$	HIF-1α	↑	Mouse fibroblasts HIF-1α knockout	All genes suppressed were HIF-independent including *prostaglandin endoperoxide synthase I* and *glutathione S –transferases μ, α3,* and *α Ya*
		AhR	↓		
		CYP1B1	↓		
		NQO1	↓		
		UDP glucuronyl-transferase 1A6	↓		
Li et al., 2004	$NiCl_2$ Ni_3S_2	HIF-1α *Cap43* protein expression	↑	Mouse C141 epidermal cells and PI-3K and Akt deficient mutants	Activation of phosphatidylinositol 3-kinase (PI-3L), Akt, and p70S6 kinase ($p70^{S6k}$). Particle sizes of Ni_3S_2 not specified.
Broday et al., 2000	$NiCl_2$ Ni_3S_2	Histone H4 acetylation	↓	A549 lung carcinoma cells, yeast cells	Lysine 12 acetylation in H4 inhibited in A549 cells and at Lys 12, 16, 5, and 8 in yeast. Particle sizes of Ni_3S_2 not specified.

*Note: BEAS, human bronchial epithelial cells; HaCaT, human keratinocyte cells; NO, nitric oxide generation; PAI-1, plasminogen activator inhibitor-1; HIF-1α, hypoxia-inducible transcription factor-1; AhR, aryl hydrocarbon receptor; A549, human lung bronchoepithelial cells; H3B, human hepatoma cells; H3K9, histone H3 lysine 9; HOS, human osteoblastic cell line; PW, mouse embryo fibroblasts; *MGMT*, O^6-methylguanine DNA methyltransferase gene locus; *DNMT1*, DNA methyltransferase 1 gene; 16HBE, Ni-transformed human bronchial epithelial cells; H3K9ac, histone H3 lys-9 acetylation; H4ac, histone H4 acetylation; H3K9me2, histone H3 Lys-9 methylation; NHBE, normal human bronchial epithelial cells; ↑, enhanced activity; ↓, reduced activity.

Table 29. Effects of Nickel on the Epigenome*

Study	Compound	Gene or factor affected	Effect	Cell Type	Comments or other effects
Yan et al., 2003	Ni_3S_2 NiS	*gpt*+ gene silencing	↓	G12 Chinese hamster transgenic *gpt*+ cells	Histones H3 and H4 hypoacetylated,H3K9 methylated, H3K9 deacetylated. Particle sizes of Ni_3S_2 not specified.
Ke et al., 2006; 2008	$NiCl_2$ NiS	*gpt*+ *gpt*-	↓	A549 cells, Hep3B cells, G12 Chinese hamster transgenic *gpt*+ cells, and *gpt*- clones N24, N37, N96	Histones H2A, H2B, H3 and H4 deacetylated, increases of H3K9 dimethylation, increases of H2A and H2B ubiquitination, minimal cytotoxicity. Ni acts by inhibiting deubiquitination. Particle sizes of NiS not specified.
Chen et al., 2006	$NiCl_2$	*gpt*+ gene silencing	↓	G12 Chinese hamster transgenic *gpt*+ cells, A549 cells	Increased mono- and dimethylation of histone H3K9, decreased H3K9 methyltransferase G9a. gpt silencing reversed by dimethylation of H3K9 with 5-aza-2'-deoxycytidine.
Zhang et al., 2003	Ni_3S_2 $NiCl_2$	Reversion of Ni-induced cell transformation	↑	Human HOS TE85 cells, mouse PW cells	Cells treated with histone deacetylase inhibitor trichostatin A (TSA) had increased frequency of revertants in transformed cells. Particle sizes of Ni_3S_2 not specified.
Ji et al., 2008	NiS	*MGMT* *DNMT1*	↓ ↑	NiS-transformed human 16HBE cells	Silencing of *MGMT* associated with DNA hypermethylation, altered histones H3K9me2, H4ac and H3K9ac, and *DNMT1* upregulation. Particle sizes of NiS not specified.
Karaczyn et al., 2006	Ni(II) counter ion unspecified	Dysregulation of H2B ubiquitination	↑	1HAEo- and HPL1D human lung cells	Histone H2B and H2A ubiquitination stimulated by Ni(II) exposure. H2B was monoubiquinated and H2A mono- and diubiquinated.

*Note: BEAS, human bronchial epithelial cells; HaCaT, human keratinocyte cells; NO, nitric oxide generation; PAI-1, plasminogen activator inhibitor-1; HIF-1α, hypoxia-inducible transcription factor-1; AhR, aryl hydrocarbon receptor; A549, human lung bronchoepithelial cells; H3B, human hepatoma cells; H3K9, histone H3 lysine 9; HOS, human osteoblastic cell line; PW, mouse embryo fibroblasts; *MGMT*, O^6-methylguanine DNA methyltransferase gene locus; *DNMT1*, DNA methyltransferase 1 gene; 16HBE, Ni-transformed human bronchial epithelial cells; H3K9ac, histone H3 lys-9 acetylation; H4ac, histone H4 acetylation; H3K9me2, histone H3 Lys-9 methylation; NHBE, normal human bronchial epithelial cells; ↑, enhanced activity; ↓, reduced activity.

Table 29. Effects of Nickel on the Epigenome*

Study	Compound	Gene or factor affected	Effect	Cell Type	Comments or other effects
Kang et al., 2003	$NiCl_2$	Histone acetylation Reactive oxygen species (ROS)	↓	Human Hep3B hepatoma cells	Dose- and time-dependent decrease in H4 acetylation. Ni(II) inhibited histone acetyltransferase (HAT) but not histone deacetylase (HDAC). ROS involved in MOA.
Zhou et al., 2009	$NiCl_2$	Histone methylation	↓	A549 cells	H3K4 increased di- and tri-methylation but not mono-methylation.
			↓	NHBE cells	

*Note: BEAS, human bronchial epithelial cells; HaCaT, human keratinocyte cells; NO, nitric oxide generation; PAI-1, plasminogen activator inhibitor-1; HIF-1α, hypoxia-inducible transcription factor-1; AhR, aryl hydrocarbon receptor; A549, human lung bronchoepithelial cells; H3B, human hepatoma cells; H3K9, histone H3 lysine 9; HOS, human osteoblastic cell line; PW, mouse embryo fibroblasts; *MGMT*, O^6-methylguanine DNA methyltransferase gene locus; *DNMT1*, DNA methyltransferase 1 gene; 16HBE, Ni-transformed human bronchial epithelial cells; H3K9ac, histone H3 lys-9 acetylation; H4ac, histone H4 acetylation; H3K9me2, histone H3 Lys-9 methylation; NHBE, normal human bronchial epithelial cells; ↑, enhanced activity; ↓, reduced activity.

Salnikow et al. (2002) studied gene expression in nickel(II) treated mouse embryo fibroblasts with and without the hypoxia-inducible transcription factor-1 (HIF -1α $^{+/+}$, HIF-1α $^{-/-}$). HIF-1α strongly induces hypoxia-inducible genes, including the tumor marker gene *Cap43*. The wild type and knockout cells were exposed to 1.0 mM $NiCl_2$ for 20 hr and gene expression assessed by cRNA hybridization and GeneChip microarray analysis. Nickel exposure induced genes involved in glucose metabolism in HIF-1α-proficient cells. Of 12 glycolytic enzyme genes studied by microarray 10 were induced by Ni(II) exposure in proficient but not in HIF-1α deficient cells. Glucose-6 phosphate dehydrogenase and hexokinase I were the only unaffected genes. Nickel(II) was also found to induce some genes in HIF-1α proficient and deficient cells (*HSP70, GADD45, p21, p53, ATM, GADD, JunB,* and *MDR-1).*

In a subsequent study, Salnikow et al. (2003) found a number of genes induced by Ni(II) in HIF-1α deficient but not in proficient cells. Among these genes are *NGF-β*, *SGK*, *IP10*, *CD44*, heparin binding EGF-like, melanocortin 1 receptor, *Grg1*, *BCL-2*-like, and tubulin-binding protein *E-Map-115*. IFN-inducible protein 10 (IP10) is a chemokine that targets T cells and NK cells. The elevation of *IP10* expression has been demonstrated in human diseases including chronic cirrhosis and biliary atresia (Koniaris et al., 2001). Most of the nickel-induced genes appear to be related to stress response. A number of genes were significantly suppressed by nickel exposure in an HIF-1-dependent manner (i.e. suppression was greater in HIF-1α proficient cells compared with HIF-1α deficient cells) including monocytes chemoattractant protein 1 (*MCP-1*) and the tumor suppressor gene *Zac1*. *Zac1* induces apoptosis and cell cycle arrest and was not suppressed in HIF-1α deficient cells. Neuropilin-1 (*Npn-1*) was also suppressed by nickel in an HIF-1α-dependent manner. Neuropilin is a transmembrane receptor in endothelial and other cells. The effects of nickel on gene expression after 20 hr exposure were transient and disappeared after nickel removal, although chronic nickel exposure can lead to selection of cells in which these changes persist.

Salnikow et al. (2003) evaluated the modulation of gene expression by $NiCl_2$ and Ni_3S_2 in two mouse and one human cell lines. Mouse embryo fibroblast cell lines MEF-HIF1α and PW were exposed to 0, 0.03, 0.1, 0.3, 1.0, or 2.0 μg Ni_3S_2/cm^2 or 0, 0.125, 0.25, 0.5, 1.0, or 2.0 mM $NiCl_2$ for 20 hr. Total RNA was isolated from Ni-exposed and control cells and cDNA prepared for GeneChip analysis. Both soluble and insoluble nickel compounds induced similar signaling pathways in the mouse cell lines. The microarray data indicated increases in expression of genes involved in glucose metabolism including glucose transporter I and glycolytic enzymes such as hexokinase II, phosphofructokinase, pyruvate kinase, and triosephosphate and glucose phosphate isomerases and lactate dehydrogenase. All of these genes are induced by hypoxia, suggesting that nickel similarly induces the HIF-1 transcription factor, which regulates these genes. Other HIF-1 genes induced included Tdd5, Egln I, Nip3, Est and Gly96. The results indicate that the form of nickel has little effect on the Ni-induced alterations of gene expression and is therefore expected to have little effect on carcinogenic or other toxic potential *in vivo*.

Davidson et al. (2003) studied the interaction of the aryl hydrocarbon receptor (AhR) pathway and the hypoxia inducible factor-1α (HIF-1α) pathway in nickel-exposed cells. HIF-1α knockout and wild type cells were derived from C57B mice. Mouse cells exposed to1.0 mM $NiCl_2$ for 24 hr exhibited the suppression of several AhR-regulated genes including CYP1B1, NQO1, UDP-glucuronyltransferase 1A6, and glutathione *S*-transferase Ya. All of the observed AhR-dependent genes except glutathione *S*-transferase θ1 were down regulated in the HIF-1α knockout cells. The most suppressed gene was CYP1B1, which was reduced 22.9-fold in wild type cells and 29.7-fold in knockout cells. Desferrioxamine and hypoxia were also able to suppress basal and inducible expression levels of AhR genes. Dimethyloxalylglycine, an inhibitor of Fe(II)- and 2-oxoglutarate (2-OG)-dependent dioxygenases also inhibited AhR-dependent gene expression in an HIF-1α-dependent manner. The authors conclude that an Fe(II)-, 2-OG- or oxygen-dependent enzyme may be involved in the regulation of AhR-dependent transcriptional activity by nickel(II).

Lee (2006) studied differential gene expression in nickel(II)-treated normal rat kidney cells. NRK-52E cells were exposed for two months to 0, 160 and 240 μM Ni^{2+} (acetate). cDNAs corresponding to mRNAs for which expression levels were altered by nickel were isolated, sequenced and followed by GenBank Blast homology search. Specificity of differential expression of cDNAs was determined by reverse transcriptase-polymerase chain reaction. Two of the nickel(II) responsive differential display clones were down regulated: SH3 glutamic acid-rich protein (SH3BGRL3) and fragile histidine triad (FHIT). One clone was up-regulated, metallothionein. The expression of these mRNAs was nickel concentration-dependent. The author notes that SH3BGRL3 probably belongs to the thioredoxin-like superfamily. These small disulfide-reducing enzymes act as hydrogen donors and are thought to be involved in regenerating glutathionated proteins. Down-regulation of SH3BGRL3 may be related to apoptotic death of NRK-52E cells induced by nickel (e.g., as noted by Shiao et al., 1998). Metallothionein is involved in the regulation of physiologically important trace metals such as copper and the detoxification of toxic metals. Since the kidney is a target organ of nickel toxicity the observed up-regulation of metallothionein is not surprising.

Prows et al. (2003) used cDNA microarray analysis in nickel sensitive (A/J) and resistant (C57BL/6J) mouse strains. The mice were exposed continuously to $NiSO_4$ 150 μg Ni/m^3 (MMAD = 0.22 μm, gsd = 1.85) for 3, 8, 24, or 48 hr. Significant expression changes were identified in one or both strains for more than 100 known genes. The results indicated a temporal pattern of increased cell proliferation, extracellular matrix repair, hypoxia, and oxidative stress, followed by reduced surfactant proteins. Fifteen functional candidate genes were associated with expression ratio differences of two-fold or greater between strains for at least one exposure time. Of these two genes—metallothionein-1 (*Mt1*) on chromosome 8 and SP-B (*Sftpb*) on chromosome 6—map to QTL intervals linked to nickel-induced acute lung injury survival.

A4 Mechanisms of Toxicity

It is possible that the effects of nickel on the various elements of the immune system and its ability to induce lung injury are related on a mechanistic level. This may involve increased levels of oxidative stress, both directly via Ni-induced formation of reactive oxygen species (ROS) and by modulation of signaling pathways promoting inflammatory processes. This section is not meant to be a comprehensice review of mechanistic studies. Rather, we provide a synopsis of several mechanistic studies examining potential mechanisms of action of nickel compounds.

Inhalation of nickel dust has been associated with increased incidence of pulmonary fibrosis. A potential mechanism is via inactivation of the pulmonary fibrinolytic cascade (Andrew and Barchowsky, 2000). Andrew et al. (2001) studied the effect of nickel subsulfide on activator protein-1 (AP-1) induction of plasminogen activator inhibitor-1 (PAI-1). Addition of 2.34 µg Ni/cm^2 Ni_3S_2 (<2.5µm) to a layer of cultured BEAS-2B human airway epithelial cells stimulated intracellular oxidation, induced c-Jun and c-Fos mRNA levels, increased phospho- and total c-Jun levels, and increased PAI-1 mRNA levels over a 24-hr treatment period. No cytotoxicity was observed with nickel treatment. Pretreatment with the antioxidants N-acetyl-L-cysteine and ascorbic acid blocked the nickel-induced increases in reactive oxygen species (ROS) but did not affect the nickel induction of PAI-1. The results indicate that the potential effect of nickel on fibrinolytic activity is independent of its participation in redox cycling.

Barchowsky et al. (2002) exposed BEAS-2B human airway epithelial cells in culture to non-cytotoxic levels (based on cell survival assays) of Ni_3S_2 (< 2.5 µm diameter) and observed increased expression of the inflammatory cytokine interleukin-8 (IL-8). Confluent layers of cultured cells were treated with 2.34 µg Ni/cm^2 nickel subsulfide for 24 or 48 hr. After 48 hr there was a statistically significant increase in IL-8 protein in the culture medium compared to the control (ca. 2.3 vs. 0.9 ng/mL, $P < 0.001$, their Fig. 1). No increase was seen after 24 hr. IL-8 mRNA levels preceded the increase in IL-8 protein. Transient exposure to soluble nickel sulfate failed to increase IL-8 mRNA. Further study revealed that nickel induced IL-8 transcription through a novel pathway that requires both AP-1 and non-traditional transcription factors, Fos and cJun. The authors note that the protracted course of particulate nickel-stimulated IL-8 production observed in the study contrasts with the immediate IL-8 induction in response to cytokines, hypoxia, and many inhaled toxicants. Thus the study indicates "particulate Ni_3S_2 activates specific signaling cascades following uptake by pulmonary epithelial cells. These activated cascades stimulate parallel pathways for inducing transcription of both inflammatory and profibrotic genes."

Mongan et al. (2008) studied the role of mitogen activated protein kinase kinase kinase 1 (MAK3K1) in nickel-induced acute lung injury in mice. Wild type mice and MAK3K1 deficient mutants were exposed to $NiSO_4 \cdot 6H_2O$ aerosol (MMAD = 0.2 µm) at 150 µg/m^3 continuously and survival times recorded. Inactivation of

one functional allele in *Map3k1*$^{+/\Delta KD}$ heterozygous mutants did not alter survival; however, *Map3k1* homozygous mutants died significantly sooner than wild type control mice. Wild type and heterozygous mutants showed 20% survival at 110 hr compared to 20% survival at 80 hr for the homozygous mutants (N = 6 mice/group, $P < 0.01$ by *t*-test). During exposure, the mice developed severe dyspnea, with gross lung pathology showing air trapping and extensive hemorrhagic edema indicative of acute lung injury. Other experiments carried out in vitro with mouse embryo fibroblast cells indicate that MAK3K1 protects against lung injury by inhibiting the Ni-induced activation of c-jun N-terminal kinases (JNKs).

Carter et al. (1997) noted that the induction of inflammatory cytokines in human airway epithelial cells by airborne particulate pollution was dependent on particle metal content, particularly vanadium and nickel. Miyazawa et al. (2008) concluded that $NiSO_4$ could activate p38 MAPK and ERK and stimulate the release of TNF-α in THP-1 cells.

Li et al. (2009) concluded that nickel sulfate induced *c-Myc* in human bronchial epithelial cells via the *Ras/ERK* signaling pathway. Freitas et al. (2010) found that Ni(II) as nickel nitrate induced oxidative burst in human neutrophils where significant increases in chemiluminescence were seen at ≥250 µM Ni(II) and a clear dose response extended down to 7.8 µM Ni(II). Forti et al. (2011) evaluated the effects of $NiCl_2$ and Ni metal particles (0.5-1.0 µm diameter) on Calu-3 human bronchial epithelial cells in vitro. Exposure to $NiCl_2$ or Ni metal particles resulted in disruption of epithelial cell barrier function as demonstrated by transepithelial electrical resistance and increased oxidative stress as indicated by Ni-induced ROS and upregulation of stress-inducible genes (i.e., *MT1X, HSP70*, *HMOX-1*, and *γGCS*). The effects were partially attributed to an increase in intracellular levels of Ni^{2+} ions.

Horie et al. (2009) evaluated uptake and subsequent Ni^{2+} release in A549 human lung cells exposed to ultrafine NiO particles (black NiO = 20 nm; green NiO = 100 nm). Ultrafine NiO particles showed higher cytotoxicity than fine NiO particles (600-2000 nm) and up to 150-fold higher degree of dissolution in the cell culture medium than fine particles. The authors conclude that intracellular Ni^{2+} release may be a key factor determining the cytotoxicity of NiO and that ultrafine particles release more Ni^{2+} than fine particles.

Nickel metal nano particles (Ni NP, <100 nm in diameter) induce a number of toxic responses in human lung epithelial A549 cells (Ahamed, 2011). The cells were exposed to Ni NP (0, 1, 2, 5, 10, 25 µg/mL) for 24 hr or 48 hr. Cell viability decreased linearly with dose for both 24 and 48 hr Ni metal NP exposures, by up to 80 and 90% , respectively. Significant increases ($P < 0.05$) were seen in LDH leakage, ROS generation, and lipid peroxidation at ≥2 µg/mL. Significant decreases in cellular GSH at ≥2 µg/mL were also seen. The authors concluded that Ni metal NP toxicity to human lung cells in vitro was mediated by oxidative stress. Horie et al. (2011) evaluated the acute oxidative stress induced by NiO

nanoparticles in vivo and in vitro. Black NiO nanoparticles (20 nm) were evaluated with human A549 cells in vitro, and responses in vivo were examined by intratracheal instillation of nanoparticles in rats. The levels of intracellular ROS and lipid peroxidation in A549 cell increased with increasing exposure to NiO nanoparticles. Increased gene expression of lipid peroxide heme oxygenase-1 (HO-1) and surfactant protein-D (SP-D) were also seen in A549 cells. The lipid peroxide level in BALF significantly increased after 24 hr instillation. LDH leakage was also observed in BALF of exposed rats. The authors concluded that NiO nanoparticles induced oxidative stress-related lung injury.

Ahamed et al. (2011) studied the toxicity of nickel ferrite nanoparticles (26 nm) in A549 human lung cells. The $NiFe_2O_4$ particles at doses of 1 to 100 µg/mL induced dose-dependent cytotoxicity as demonstrated by MTT, NRU and LDH assays. Nickel ferrite nanoparticles were also seen to induce oxidative stress by ROS generation and GSH depletion. Quantitative real-time PCR analysis showed that following exposure, the level of mRNA expression of cell cycle checkpoint protein p53 and apoptotic proteins (bax, caspase-3 and caspase-9) were significantly up-regulated, whereas expression of anti-apoptotic proteins (survivin and bcl-2) were down-regulated. The authors concluded that nickel ferrite nanoparticles induced apoptosis in A549 cells through ROS generation and oxidative stress via p53, survivin, bax/bcl-2, and caspase pathways.

Long-term exposure of hyperlipidemic apoprotein E-deficient mice to $Ni(OH)_2$ nanoparticles (5 nm diameter, count median diameter of agglomerates = 40 nm, gsd = 1.50) resulted in significant oxidative stress and inflammation in the lung and extrapulmonary organs (Kang et al., 2011). The $ApoE^{-/-}$ mice were exposed to 0 or 79 µg Ni/m^3 for 5 hr/day, 5 days/week for 1 week (6 mice/group) or 5 months (16 mice/group). Pulmonary responses included significant increases in the number of cells, number of neutrophils and total protein in BALF of Ni exposed mice compared to controls at either exposure duration ($P < 0.01$). Relative increases in proinflammatory genes (mRNA) *Ccl-2* and *Il-6* were seen in the lung at 1 week ($P < 0.01$) and *Ccl-2*, *Il-6*, and *Tnf-α* at 5 months ($P < 0.05$). Significantly, increases in expression of *Ccl-2* and *Il-6* ($P < 0.05$) were also seen after 5 months Ni exposure in the heart and of *Ccl-2*, *Il-6*, and *Tnf-α* ($P<0.01$) in the spleen. Also relative mRNA levels of *Ccl-2*, *Vcam-1* and *Cd68* were all increased in aortas from 5 months Ni-exposed mice ($P <0.01$). After 5 months exposure to Ni nanoparticles relative *Ho-1* mRNA levels, indicative of oxidative stress, were significantly increased in lung > heart > spleen > aorta (all $P < 0.05$). Mitochondrial DNA damage in the aorta was also observed after 5 months exposure ($P < 0.01$) as were relative increases in plaque area in four regions of the aorta (all $P < 0.01$). This paper demonstrates that inhaled $Ni(OH)_2$ nanoparticles can induce oxidative stress and inflammation, not only in the lung but systemically in the cardiovascular system and can ultimately contribute to the progression of atherosclerosis in an $ApoE^{-/-}$ mouse system.

A possible mechanism leading to nickel pneumotoxicity may involve Ni-induced reactive oxygen species (ROS) and electrophiles initiating prooxidant activity, which in turn activates signaling pathways, including MAPK and multiple proteins involved in the pathway (p38, JNK, ERK). This leads to activation of transcription factors that initiate inflammatory processes and subsequent immunological effects leading to respiratory effects such as alveolar proteinosis. That is, the mechanism of respiratory effects derives from activation/inactivation of signaling pathways. A similar scheme was described by Pan et al. (2010) for Ni-induced apoptosis in human Beas-2B cells via the Akt/ASK1/p38 signaling pathway.

Appendix B

B.1 Berkeley Madonna Code for Sunderman et al. Human Oral Nickel Model.

METHOD RK4 {integration routine}

STARTTIME = 0

STOPTIME=24 {hours}

DT = 0.02 {step time or integration interval, i.e. 1200 steps total}

{Nickel biokinetic model of Sunderman et al. 1989; model units µg, hr}

{Nickel compartments, µg Ni initial values}

init Agi = 50*BW {Ni dose given in water 50 µg/kg body weight}

init Aserum = 0

init Aurine = 0

init Atissues = 0

{Model parameters, /hr unless otherwise specified}

Kf = 0.092 {zero-order rate constant for dietary absorption of nickel}

K01 = 0.28 {first-order rate constant for intestinal absorption of oral NiSO4 in water}

K10 = 0.21 {first-order rate constant for nickel excretion in urine}

K12 = 0.38 {first-order rate constant for nickel transfer from serum to tissues}

K21 = 0.08 {first-order rate constant for nickel transfer from tissues to serum}

BW = 70 {kg}

{Model differential equations calculate masses of nickel in respective compartments over 24 hours}

d/dt(Agi) = -Kf - K01*Agi

d/dt(Aserum) = Kf + K01*Agi - K10*Aserum - K12*Aserum + K21*Atissues

d/dt(Atissues) = K12*Aserum - K21*Atissues

d/dt(Aurine) = K10*Aserum

Massbal = Agi + Aurine+ Aserum + Atissues {sum of model compartments equals dose input}

B.2 Berkeley Madonna Code for Nickel Keratinocyte Model of Franks et al.

METHOD RK4 {integration routine}

STARTTIME = 0

STOPTIME=24

DT = 0.02

{Model parameters}

dni = 2.62E-5 {rate of cell death due to nickel ions, /µM/hr}

bci = 0 {/hr, rate of cytokine release by nickel affected cells}

kn = 13.3 {/hr, rate of ion exchange}

un = 2.2 {unitless, partition coefficient}

dn = 0.00875 {/hr, rate of natural wastage of cells}

dc = 0.133 {/hr, rate of natural decay of cytokines within the media}

bcn = 6.25E-5 {µM/hr, rate of cytokine release by nickel affected cells}

n0 = 0.0165 {volume of cells, mL}

A0 = 100 {µM}

cpg = c*1.77E4*1000 {IL-1ά, pg/mL}

init c = 0 {initial concentration IL-1ά}

init Ai = 0 {initial intracellular Ni concentration}

init n = n0 {initial volume of keratinocytes}

init Ac = A0 {initial extracellular concentration of Ni}

{model differential equations}

d/dt(n) = -kd*n {volume fraction of keratinocytes}

kd = dni*Ai + dn

d/dt(Ac) = -kn*n*(un*Ac - Ai) + kd*n*Ai {extracellular nickel}

d/dt(Ai) = kn*n*(un*Ac - Ai) - Kd*n*Ai {intracellular nickel}

d/dt(c) = bcn*n + bci*n*Ai - dc*(1-n)*c {IL-1ά cytokine release}

B.3 Intracellular Dosimetry Model of Inhaled Nickel Subsulfide.

METHOD RK4 {integration routine}

STARTTIME = 0

STOPTIME= 168 {hours}

DT = 0.01

DTOUT = 0.5

{Nickel mass μmoles Ni_3S_2}

init Agi = 0

init Asurf = Concn*Vmuc*0.23/MW

init Aionic = Concn*Vmuc*0.10/MW

init Aven = 0

init Avacuol = 0

init Acyto = 0

init Acytprot = 0

init Aperinuc = 0

init Aperinucytprot = 0

init Anucl = 0

init Anucprot = 0

{Concentrations, µmol/mL}

Csurf = Asurf/Vsurf

Cionic = Aionic/Vmuc

Ccyto = Acyto/Vcyto

Cperinuc = Aperinuc/Vperinuc

Cven = Aven/Vven

Cnucl = Anucl/Vnucl

Cnuni = 3*Cnucl

{Volumes, mL}

Vcyto = 0.54*Vtb

Vnucl = 0.06*Vtb

Vperinuc = 0.1*Vtb

Vtb =0.07*Vlu

Vlu = 0.014*BW

Vsurf = Vtb

Vionic = Vtb

Vven = 0.04*BW

Vmuc = 100 {mL}

{Model parameters}

VmiC = 10 {µmol/hr/μm^2}

Vmi = VmiC*2.4E11

Kmi = 1E9 {µmol/mL}

VmeC = 0.001 {µmol/hr/μm^2}

Vme = VmeC*2.4E11

Kme = 1E9{µmol/mL}

Kdm = 0.0001{/h}

Kdv = 0.0106 {/h}

PAcpC = 0.011{μm^2/hr}

PAcp = PAcpC

PApnC = 1.5

PApn = PApnC

ClpC = 1E-8 {mL/hr/cell}

Clp = ClpC*1E9 {/1E9 cells}

ClmC = 1.0E-11{mL/hr/cell}

Clm = ClmC*1E9 {/1E9 cells}

AbcC = 1E3{µmol/mL}

Abc = AbcC

AbpC = 1E3

Abp = AbpC

AbnC = 1E4

Abn = AbnC

Kbc = 1E9 {µmol/mL}

Kbn = 1E9

Kbp = 1E9

Frac = 0.08

Md = 0.1*MMAD

MMAD = 3.75 {µm}

Concn = 10 {µg/mL}

MW = 234.19

BW = 7E4

Protmg = 161{mg/1E9 cells}

Acytomg = Acyto/Protmg {µmol Ni/mg cytosol protein}

{Differential equations, µmol/hr}

d/dt(Agi) = Asurf*Clmc + Aionic*Clmc

d/dt(Asurf) = - Asurf*Kdm - Csurf*Clmc - Csurf*Clp

d/dt(Aionic) = Asurf*Kdm - Cionic*Vmi/(Kmi + Cionic) - Cionic*Clm

d/dt(Aven) = Ccyto*Vme/(Kme + Ccyto)

d/dt(Acyto) = Cionic*Vmi/(Kmi + Cionic) - Ccyto*Vme/(Kme + Ccyto) + Avacuol*Kdv*Frac - Ccyto*Abc/(Kbc + Ccyto) - Acyto*PAcp

d/dt(Avacuol) = Csurf*Clp - Avacuol*Kdv*Frac - Avacuol*Kdv*(1-Frac)

d/dt(Acytprot) = Ccyto*Abc/(Kbc + Ccyto)

d/dt(Aperinuc) = Avacuol*Kdv*(1-Frac) + Acyto*PAcp - Cperinuc*Abp/(Kbp + Cperinuc) - Aperinuc*PApn

d/dt(Aperinucytprot) = Cperinuc*Abp/(Kbc + Cperinuc)

d/dt(Anucl) = Aperinuc*PApn - Cnucl*Abn/(Kbn + Cnucl)

d/dt(Anucprot) = Cnucl*Abn/(Kbn + Cnucl)

B.4 PBPK Rat Model for NiO Inhalation Based on Teeguarden et al.

METHOD Stiff {integration routine}

STARTTIME = 0

STOPTIME= 8640 (12 months}

DT = 0.001

DTOUT = 0.25

{Draft PBPK model for nickel inhaled as nickel oxide; model loosely based on Teeguarden et al. 2007 Mn model w/ Pi's based on Ishimatsu et al.1995 and lung clearance based on Benson et al. 1994 and Tanaka et al. 1985}

{NiO in tissues, µg}

init Aart =0 {arterial blood}

init Aven = 0 {venous blood}

init Amusc = 0 {muscle shallow}

init Amuscdeep = 0 {muscle deep}

init Abone = 0

init Abonedeep =0

init Akid = 0 {kidney shallow}

init Akiddeep = 0 {kidney deep}

init Aliv = 0 {liver shallow}

init Alivdeep = 0 {liver deep}

init Alu = 0 {lung shallow}

init Alungdeep = 0 {lung deep}

init Alungdep = 0 {lung surface deposition}

init Anpdeep = 0 {nasopharynx deep}

init Anpdep = 0 {nasopharynx surface deposition}

init Anp = 0 {nasopharynx shallow}

init Agi = 0 {gastro-intestinal tract}

init Afeces = 0

init Aurine = 0

{Cardiac output, alveolar ventilation, body weight L/hr, kg}

BW = 0.325 {body weight}

Qtot = 14.6*BW^0.74 {cardiac output}

Qalv = 1.2*Qtot {alveolar ventilation}

{Blood flows, L/hr}

Qmusc = 0.534*Qtot

Qbone = 0.122*Qtot

Qkid = 0.141*Qtot

Qliv = 0.183*Qtot

Qnp = 0.01*Qtot

{Tissue volumes, L}

Vart = 0.0224*BW

Vblood = 0.0676*BW

Vmusc = 0.738*BW

Vbone = 0.021*BW

Vbonedeep = 0.052*BW

Vkid = 0.007*BW

Vliv = 0.034*BW

Vlu = 0.007*BW

Vnp = 0.0038*BW

Vtb = 0.01107*BW

Vpu = 0.01107*BW

Vven = 0.0452*BW

Vdeplu = Vtb + Vpu

{Concentrations µg Ni/L}

Cart = Cvlung {arterial concentration}

Cvmusc = Amusc/(Vmusc * Pmusc) {concentration leaving the muscle shallow compartment}

Cmusc = (Amuscdeep+Amusc)/Vmusc {total concentration in muscle}

Cvbone = Abone/(Vbone*Pbone)

Cbone = (Abonedeep + Abone)/Vbone

Cvkid = Akid/(Vkid*Pkid)

Ckid = (Akiddeep + Akid)/Vkid

Cvliv = Aliv/(Vliv*Pliv)

Cliv = (Alivdeep + Aliv)/Vliv

Cvnp = Anp/(Vnp*Pnp)

Cnp = (Anpdeep + Anp)/Vnp

Cvlung = Alu/(Vlu*Plung)

Clung = (Alungdeep + Alu)/Vlu

Cven = Aven/Vven {venous concentration}

Cvtot = (Qmusc*Cvmusc + Qkid*Cvkid + Qliv*Cvliv + Qbone*Cvbone + Qnp*Cvnp)/Qtot {mixed venous concentration}

Cair = IF TIME <= 140 THEN 600 ELSE 0 {140 hr exposure to 600 µg/m3}

Tvol = Qalv/0.6 {tidal volume}

{tissue/blood partition coefficients, unitless}

Pmusc = 0.8

Pbone = 1.0

Pkid = 16.0

Pliv = 2.0

Plung = 4.0

Pnp = 0.3

{Clearance rates, /hr}

Kf = 0.0001*BW^-0.25

Kinmusc = 0.017*BW^-0.25 {rate constants for nickel moving into and out of deep tissue compartments}

Kinbone = 0.105*BW^-0.25

Kinkid = 0.146*BW^-0.25

Kinliv = 0.621*BW^-0.25

Kinnp = 0.035*BW^-0.25

Kinlung = 0.035*BW^-0.25

Koutmusc = 0.0035*BW^-0.25

Koutbone = 0.085*BW^-0.25

Koutkid = 0.007*BW^-0.25

Koutliv = 0.015*BW^-0.25

Koutnp = 0.035*BW^-0.25

Koutlung = 0.0002*BW^-0.25

Kurine = 0.15 {kidney shallow to urine}

Kfeces = 0.5 {GI tract to feces}

Kai = 0.25 {GI tract to liver shallow}

Kbile = 0.05 {Liver to GI tract}

Kgi = 0.1 {respiratory tract to GI tract, i.e. swallowed particles mechanically removed from lung}

{rate constants for uptake from respiratory tract surface into shallow and deep compartments for lung and nasopharynx}

KdepSL = 2.0*BW^-0.25

KdepDL = 0.0*BW^-0.25

KdepSN = 0.2*BW^-0.25

KdepDN = 0.0*BW^-0.25

{fractional coeffs for deposited particles}

fdepNP = 0.2 {nasopharnyx}

fdepTB = 0.08 {tracheobroncheal}

fdepPu = 0.05 {pulmonary}

fdepLu = fdepTB + fdepPu

{differential equations}

d/dt(Abone) = Qbone*(Cart - Cvbone) - Kinbone*Cvbone*Vbone + Koutbone*Abonedeep

d/dt(Abonedeep) = Kinbone*Cvbone*Vbone - Koutbone*Abonedeep

d/dt(Amusc) = Qmusc*(Cart - Cvmusc) - Kinmusc*Cvmusc*Vmusc + Koutmusc*Amuscdeep

d/dt(Amuscdeep) = Kinmusc*Cvmusc*Vmusc - Koutmusc*Amuscdeep

d/dt(Akid) = Qkid*(Cart - Cvkid) - Kinkid*Cvkid*Vkid + Koutkid*Akiddeep

d/dt(Akiddeep) = Kinkid*Cvkid*Vkid - Koutkid*Akiddeep

d/dt(Alu) = Qtot*(Cvtot - Cvlung) - Kinlung*Cvlung*Vlu + Koutlung*Alungdeep + kdepSL*Alungdep

d/dt(Alungdeep) = Kinlung*Cvlung*Vlu - Koutlung*Alungdeep + kdepDL*Alungdep

d/dt(Alungdep) = fdepLu*Cair*Tvol - kdepDL*Alungdep - kdepSL*Alungdep - Alungdep*Kgi

d/dt(Aven) = Qmusc*Cvmusc + Qbone*Cvbone + Qkid*Cvkid + Qliv*Cvliv + Qnp*Cvnp - Qtot*Cven

d/dt(Aart) = Qtot*(Cvlung - Cart)

d/dt(Aliv) = Qliv*(Cart - Cvliv) - Kbile*Cvliv*Vliv - Kinliv*Cvliv*Vliv + Koutliv*Alivdeep - Aliv*Kbile

d/dt(Alivdeep) = Kinliv*Cvliv*Vliv - Koutliv*Alivdeep

d/dt(Anp) = Qnp*(Cart - Cvnp) - Kinnp*Cvnp*Vnp + Koutnp*Anpdeep + kdepSN*Anpdep

d/dt(Anpdeep) = kdepDN*Anpdep - Koutnp*Anpdeep + Kinnp*Cvnp*Vnp

d/dt(Anpdep) = fdepNP*Cair*Tvol - kdepDN*Anpdep - kdepSN*Anpdep - Anpdep*Kgi

d/dt(Agi) = Anpdep*Kgi + Alungdep*Kgi - Kai*Agi - Kfeces*Agi + Aliv*Kbile

d/dt(Afeces) = Kfeces*Agi

d/dt(Aurine) = Akid*Kurine

MASSBAL1 = Abone + Akid + Aliv + Anp + Amusc + Alu

MASSBAL2 = Abonedeep + Akiddeep + Alivdeep + Anpdeep + Amuscdeep + Alungdeep

MASSBAL3 = Anpdep + Alungdep

MASSBAL4 = Aurine + Afeces + Agi

MASSTOT = MASSBAL1 + MASSBAL2 + MASSBAL3 + MASSBAL4

Table 30. Comparison of Predicted and Observed Nickel Tissue Concentrations Twelve Months after a 140 Hours Exposure to NiO Aerosol.*

Tissue µg/L	8.0 mg/m^3 Model	Observed	O/P	0.6 mg/m^3 Model	Observed	O/P
Bone	5.95	ND		0.45	ND	
Kidneys	99.62	100 ± 90	1.00	7.47	80 ± 30	10.7
Liver	116.72	110 ± 70	0.94	8.75	50 ± 20	5.7
Nasopharynx	3.47	ND		0.26	ND	
Muscle	15.82	ND		1.19	ND	
Lung	285826	277000 ± 98000	0.97	21437	17000 ± 4000	0.79

*Note: NiO aerosol MADD = 1.2 µm, gsd = 2.2. Model exposure was continuous for 140 hr, actual exposure was discontinuous over a one month period (not specified but probably about 6 hr/day x 5 days/week x 30days).

B.5 Biokinetic Model of Uthus (1999) for Oral $NiCl_2$ in the Rat.

METHOD Stiff

STARTTIME = 0

STOPTIME= 10000 {minutes}

DT = 0.02

DTOUT = 10

{Uthus biokinetic model for 63Ni in the rat, Proc ND Acad Sci, 53:92-96(1999)}

{model compartments, ug Ni}

init GI_1 = 0.84 {ug at 12.7 uCi/ug Ni}

init GI_2 = 0

init GI_11 = 0

init Feces_3 = 0

init Blood_16 = 0

init Blood_15 = 0

init Blood_10 = 0

init Blood_4 = 0

init Liver_5 = 0

init Liver_6 = 0

init Liver_12 = 0

init Urine_9 = 0

init Urine_13 = 0

init Body_7 = 0

init Body_8 = 0

init Body_14 = 0

{mass transfer rate constants, /min}

K2_1 = 0.975

K3_11 = 0.000543

K4_1 = 0.025

K4_5 = 0.14

K4_7 = 0.3

K4_15 = 0.02

K5_4 = 0.155

K5_6 = 0.055

K6_5 = 0.05

K6_12 = 0.00003

K7_4 = 1.0

K7_8 = 0.005

K8_7 = 0.05

K8_14 = 0.0004

K9_13 = 0.0007

K10_4 = 0.0525

K11_2 = 0.001

K12_6 = 0.00175

K13_4 = 1.05

K14_8 = 0.0075

K15_10 = 0.066667

K15_16 = 0.0015

K16_15 = 0.01

{model differential equations, ug/min}

d/dt(GI_1) = -GI_1*K2_1 - GI_1*K4_1

d/dt(GI_2) = GI_1*K2_1 - GI_2*K11_2

d/dt(GI_11) = GI_2*K11_2 - GI_11*K3_11

d/dt(Feces_3) = GI_11*K3_11

d/dt(Blood_4) = GI_1*K4_1 - Blood_4*K5_4 + Liver_5*K4_5 - Blood_4*K10_4 + Blood_15*K4_15 - Blood_4*K13_4 - Blood_4*K7_4 + Body_7*K4_7

d/dt(Blood_10) = Blood_4*K10_4 - Blood_10*K15_10

d/dt(Blood_15) = Blood_10*K15_10 - Blood_15*K4_15 + Blood_16*K15_16

d/dt(Blood_16) = -Blood_16*K15_16 + Blood_15*K16_15

d/dt(Liver_5) = Blood_4*K5_4 - Liver_5*K4_5 -Liver_5*K6_5 + Liver_6*K5_6

d/dt(Liver_6) = Liver_5*K6_5 - Liver_6*K5_6 - Liver_6*K12_6 + Liver_12*K6_12

d/dt(Liver_12) = Liver_6*K12_6 - Liver_12*K6_12

d/dt(Urine_13) = Blood_4*K13_4 - Urine_13*K9_13

d/dt(Urine_9) = Urine_13*K9_13

d/dt(Body_7) = Blood_4*K7_4 - Body_7*K4_7 - Body_7*K8_7 + Body_8*K7_8

d/dt(Body_8) = Body_7*K8_7 - Body_8*K7_8 + Body_14*K8_14 - Body_8*K14_8

d/dt(Body_14) = Body_8*K14_8 - Body_14*K8_14

{Mass balance}

Mass_1 = GI_1 + GI_2 + GI_11 + Feces_3

Mass_2 = Blood_4 + Blood_10 + Blood_15 + Blood_16

Mass_3 = Liver_5 + Liver_6 + Liver_12

Mass_4 = Body_7 + Body_8 + Body_14

Mass_5 = Urine_13 + Urine_9

Mass_total = Mass_1 + Mass_2 + Mass_3 + Mass_4 + Mass_5

PCRECOV = Mass_total*100/0.84 {percent recovery of administered Ni}