

## **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**

**Acetaldehyde**

**Acrolein**

**Arsenic**

**Formaldehyde**

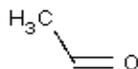
**Manganese**

**Mercury**

## Acetaldehyde Reference Exposure Levels

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

CAS: 75-07-0



### 1. Summary

Acetaldehyde has been prioritized as a Toxic Air Contaminant (TAC) with the potential to differentially impact infants and children. Acetaldehyde was chosen for a focused literature review 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). 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. OEHHA used the critical effect of eye irritation as the basis for determination of the acute Reference Exposure Level (REL). The 8-hour Reference Exposure Level (REL) also accounted for sensory irritation based on an early human exposure study. Signs of acute toxicity in animals at high concentrations (~10,000 ppm) include labored respiration, mouth breathing, weight loss, and liver damage.

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.

**1.1 Acetaldehyde Acute REL**

<i>Reference Exposure Level</i>	<b>750 µg/m<sup>3</sup> (420 ppb)</b>
<i>Critical effect(s)</i>	Sensory irritation, eye redness and swelling
<i>Hazard index target(s)</i>	Eyes, nose, throat

**1.2 Acetaldehyde 8-Hour REL**

<i>Reference Exposure Level</i>	<b>568 µg/m<sup>3</sup> (316 ppb)</b>
<i>Critical effect(s)</i>	Degeneration of olfactory nasal epithelium
<i>Hazard index target(s)</i>	Respiratory system

**1.3 Acetaldehyde Chronic REL**

<i>Reference Exposure Level</i>	<b>43 µg/m<sup>3</sup> (24 ppb)</b>
<i>Critical effect(s)</i>	Degenerative, inflammatory and hyperplastic changes of the nasal mucosa in animals
<i>Hazard index target(s)</i>	Respiratory system

**2. Physical & Chemical Properties**

<i>Description</i>	Colorless liquid or gas (above 21°C)
<i>Molecular formula</i>	C <sub>2</sub> H <sub>4</sub> O
<i>Molecular weight</i>	44.05 g/mol
<i>Density</i>	0.79 g/cm <sup>3</sup>
<i>Boiling point</i>	21 °C
<i>Melting point</i>	-123.5 °C
<i>Vapor pressure</i>	755 mm Hg @ 20°C
<i>Odor threshold</i>	0.09 mg/m <sup>3</sup>
<i>Solubility</i>	Miscible in all proportions with water and the most common organic solvents.
<i>Conversion factor</i>	1.8 mg/m <sup>3</sup> = 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, glyococal, 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 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  $\mu\text{g}/\text{m}^3$  (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  $\mu\text{g}/\text{m}^3$  (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  $\mu\text{g}/\text{m}^3$  (35 ppb) while a highway tunnel had 3.43 times higher levels of acetaldehyde (430  $\mu\text{g}/\text{m}^3$  (240 ppb)). The mean acetaldehyde concentrations in U.S. homes range from 15 to 36  $\mu\text{g}/\text{m}^3$  (8.3 to 20 ppb) but reached as high as 103  $\mu\text{g}/\text{m}^3$  (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  $\mu\text{g}/\text{m}^3$  (3.2 to 7.1 ppb) with a mean of 9.8  $\mu\text{g}/\text{m}^3$  (5.4 ppb) (Shendell *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  $\mu\text{g}/\text{m}^3$  (1.7 to 8.9 ppb).

Environmental tobacco smoke (ETS) has been found to be a dominant 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  $\mu\text{g}/\text{m}^3$  (6.1 to 8.3 ppb) (Miller *et al.*, 1998). Fifty-seven homes using a 72-hour exposure period were studied to assess the concentrations of acetaldehyde in the air, which ranged from 3 to 23  $\mu\text{g}/\text{m}^3$  (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  $\mu\text{g}/\text{m}^3$  (0.4 to 6.1 ppb), but can be somewhat higher in smokers ( $16 \pm 3 \mu\text{g}/\text{m}^3 = 8.9\text{ppb}$ ). 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  $\mu\text{g}/\text{m}^3$  (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 genetically deficient ALDH the concentration of acetaldehyde in the breath after drinking can reach 8.8-22  $\text{mg}/\text{m}^3$  (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. The ethanol inhalation test was negative, but the acetaldehyde inhalation test (5, 10, 20, or 40 mg/ml) was positive because FEV<sub>1.0</sub> was decreased by 33.5% at the 20 mg/ml concentration of acetaldehyde (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  $\text{mg}/\text{m}^3$  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, 1970; Egle Jr., 1972a; 1972b). In humans, the total respiratory tract retention of acetaldehyde was 45-70% when inhaled either orally or nasally (Egle, 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; Morris, 1997b).

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, 1997b).

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 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.

Various isoenzymes of alcohol dehydrogenase transform ethanol into acetaldehyde, which in turn is rapidly oxidized by aldehyde dehydrogenase (ALDH) into nontoxic acetate. 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 and have a deficiency or low activity in ethanol-metabolizing enzymes that can result in high blood and breath acetaldehyde levels 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  $V_{max}$  and  $K_m$  values (Morris, 1997b). 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, 1997b). 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, 1997b).

Acetaldehyde can be eliminated unchanged in urine, expired air, and skin (Baselt and Cravey, 1989). Acetaldehyde is highly reactive and can bind to amino acids and blood and membrane proteins (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). Acetaldehyde causes lipid peroxidation, which can lead to adduct formation and free radical-induced cell injury. Acetaldehyde that is metabolized by aldehyde dehydrogenase to acetate is readily excreted in the urine. Acetaldehyde can act as a hapten; and antibodies against acetaldehyde conjugates have been detected in human and rabbit serum (Gaines et al., 1977).

## **5. Acute Toxicity of Acetaldehyde**

### **5.1 Acute Toxicity to Adult Humans**

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 sensory response to solvent vapors (Silverman et al., 1946). The purpose of the study was to determine the sensory response limit for solvent concentrations when estimating ventilation requirements for comfortable working conditions. 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. The time of exposure was fifteen minutes. 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. 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. Nine male asthmatic patients with nine age- and sex-matched healthy volunteers inhaled ascending doses of 5, 10, 20, or 40 mg/ml of acetaldehyde and bronchial responsiveness was measured (Myou et al., 1993). The acetaldehyde solutions were in saline and were inhaled from a nebulizer for 2 minutes by mouth tidal breathing wearing a noseclip. Forced expiratory volume (FEV<sub>1</sub>) was measured. A greater than 20% decrease in FEV<sub>1</sub> was caused by inhalation of acetaldehyde in a dose-dependent manner in asthmatics that received a placebo and did not receive a histamine H<sub>1</sub> blocker (terfenadine). Percent decrease in FEV<sub>1</sub> in asthmatics receiving the placebo after inhalation of each concentration of acetaldehyde was larger than that in asthmatics treated with terfenadine and in healthy subjects (Myou et al., 1993). These findings support the hypothesis that bronchial hyper-responsiveness is a precondition of acetaldehyde induced bronchoconstriction, which is caused indirectly via histamine release in asthmatics.

In another acute human study, nine asthmatic subjects were used to determine whether bronchial responsiveness to inhaled methacholine (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. For each subject, the concentration of acetaldehyde producing a 20% fall in FEV<sub>1</sub> was determined (PC<sub>20</sub>) using ascending doses (5, 10, 20, or 40 mg/ml) of acetaldehyde. The mean concentration of PC<sub>20</sub> for the nine subjects was 23.3 mg/ml of acetaldehyde. A sub threshold concentration of 0.8 mg/ml acetaldehyde was chosen and the nine subjects inhaled that or saline for four minutes (Myou et al., 1994). After each inhalation, a methacholine provocation test was performed. Acetaldehyde potentiated bronchial hyper-responsiveness to provocation by methacholine (Myou et al., 1994). In addition, the investigators determined that histamine was not responsible for acetaldehyde induced bronchial hyper-responsiveness at sub threshold concentrations (Myou et al., 1994).

The response to methacholine and acetaldehyde challenges have also been measured in 81 non-smoking adults (61 asthmatics and 20 normal controls) 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). The results of this study indicate that airway hyper-responsiveness to acetaldehyde is a sensitive and specific indicator for separating normal and asthmatic subjects. Five to 40 mg/mL acetaldehyde solutions were inhaled and FEV<sub>1</sub> was measured 60 to 90 seconds after inhalation of each concentration until FEV<sub>1</sub> dropped by more than 20% (Prieto et al., 2000). In this study, the PC<sub>20</sub> values for acetaldehyde ranged from 1.96 to 40 mg/mL with a geometric mean value of 17.55 mg/mL. However, in this study, 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 ( $p = 0.004$ ) (Prieto et al., 2000).

Bronchial responsiveness to acetaldehyde has been studied in sixteen asthmatics subjects without alcohol sensitivity and ten subjects with alcohol-induced bronchoconstriction (Fujimura et al., 1999). In this acute human study, subjects inhaled acetaldehyde or methacholine (0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, and 20 mg/ml) in saline administered by nebulizer. The mean provocative concentration of acetaldehyde ( $PC_{20}$  in the alcohol and non-alcohol-sensitive groups were 21.0 mg/mL and 31.7 mg/mL acetaldehyde, respectively. Bronchial responsiveness of acetaldehyde relative to methacholine was augmented in asthmatic subjects with alcohol-induced bronchoconstriction when compared to asthmatic subjects who were tolerant to alcohol (Myou et al., 1993; Fujimura et al., 1999; Prieto et al., 2000)

Another acute human study showed increased sensitivity to acetaldehyde by alcohol-sensitive adults (Myou et al., 1995). Seven male and three female asthmatics with alcohol-induced bronchoconstriction and sixteen asthmatics without alcohol-induced sensitivity were exposed to 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, and 80 mg/mL acetaldehyde or methacholine solution dissolved in saline for two minutes, administered with a nebulizer, and  $FEV_1$  was recorded (Myou et al., 1995). Bronchial responsiveness was greater in the alcohol-sensitive asthmatic group compared to the alcohol-non-sensitive asthmatic subjects.

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). Studies involving aerosolized solutions of acetaldehyde were not used by OEHHA as the basis for the REL derivation.

In summary, exposure to acetaldehyde at concentrations as low as 25 ppm result in sensory irritation in human volunteers. Adult asthmatics that inhaled aerosolized solutions of acetaldehyde showed increased irritation and bronchoconstriction.

## **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 asthma-like 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 on asthma, 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, which may result in more hospitalizations of children, especially for the first four years of life (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  $\mu\text{g}/\text{m}^3$  (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  $\text{RD}_{50}$  was calculated (the concentration eliciting a 50% decrease in respiratory rate).  $\text{RD}_{50}$  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  $\text{RD}_{50}$  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  $\text{RD}_{50}$  (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, either on 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 14,000 to 17,600 ppm (30,600 to 31,680 mg/m<sup>3</sup>) for thirty minutes (Skog, 1950). The acute LD<sub>50</sub> value for acetaldehyde inhalation was 20,600 ppm (37,080 mg/m<sup>3</sup>) (Skog, 1950).

Appelman et al. (1982) determined the LC<sub>50</sub> for acetaldehyde after acute exposure in rats. Twenty male and twenty female albino Wistar rats were used for the acute study. The animals were exposed 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. Rats were exposed to acetaldehyde once for four hours. Within the first half-hour of the four-hour LC<sub>50</sub> 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<sub>50</sub> 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 acutely to acetaldehyde vapors for 4 hours at doses ranging from 14,450 to 17,600 ppm (26,010 to 31,680 mg/m<sup>3</sup>) (Kruyssen, 1970). After one to two hours of exposure at all concentrations, the animals showed severe lacrimation, salivation, and nasal discharges. The 4-hour LC<sub>50</sub> was determined to be 17,000 ppm (30,600 mg/m<sup>3</sup>) for this study. In all exposure groups, the animals that died during exposure had convulsions. However at all concentrations, some animals survived, but only after a deep narcosis and apnea (Kruyssen, 1970).

Aldehyde dehydrogenase 2 (ALDH2) is an important enzyme that oxidizes acetaldehyde. Isse et al. (2005) compared the acute acetaldehyde toxicity between wild-type (Aldh2<sup>+/+</sup>) and Aldh2-inactive transgenic (Aldh2<sup>-/-</sup>) mice after inhalation. The null aldehyde dehydrogenase 2 (ALDH2) transgenic mice (-/-) or wildtype (+/+) 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 wildtype 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  $180 \pm 35$  ppm), twice the threshold limit value, for single and multiple three-hour exposures, which were 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 are 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<sub>50</sub> 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<sub>50</sub> was 13,300 ppm in rats. In contrast, the LOAEL for humans was reported to be 25 ppm in one historical study (Silverman et al., 1946). Thus, humans appear to be at least 30 times more sensitive than animals to the acute effects of acetaldehyde.

**Table 5.3.1 Summary of Acute Studies in Experimental Animals**

<b>Endpoint</b>	<b>Strain/Species</b>	<b>Exposure</b>	<b>Response</b>	<b>Reference</b>
Sensory irritation	B6C3F1 mice	750 to 4200 ppm for 10 min	RD <sub>50</sub> = 2932 ppm	(Steinhagen and Barrow, 1984)
	Swiss Webster mice	750 to 4200 ppm for 10 min	RD <sub>50</sub> = 2845 ppm	(Steinhagen and Barrow, 1984)
	F-344 rats	~800 to 10,000 ppm for 10 min	RD <sub>50</sub> = 2991 ppm	Babiuk et al., 1985
	Wistar rats	2800, 4600, or 6500 ppm for 30 min	RD <sub>50</sub> = 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 17,600 ppm for 30 min	LD <sub>50</sub> = 20,600 ppm	Skog, 1950
	Wistar rats	10,436 to 16,801 ppm for 4 hours	LC <sub>50</sub> = 13,300 ppm	Appelman et al., 1982
	Syrian Golden hamsters	14,450 to 17,600 ppm for 4 hours	LC <sub>50</sub> = 17,000 ppm	Kruyssen, 1970
Behavioral/Other effects	Syrian Golden hamsters	14,450 for one to 2 hours	lacrimation, salivation, and nasal discharges	Kruyssen, 1970
	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, bradypnea, 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 the same rat species, but males only, 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<sup>3</sup>) 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 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 trachea epithelial lesions in the mid-dose group. No adverse effects were observed at 390 ppm (Kruyssen 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 Kruyssen 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 of non-sensitized and ovalbumin (OA)-sensitized guinea pigs were exposed to 0 or 200 ppb ( $360 \mu\text{g}/\text{m}^3$ ) acetaldehyde for 6 hr/day, 5 days/week for four weeks (Lacroix et al., 2002). In sensitized, acetaldehyde-exposed animals, subsequent challenge with OA aerosol did not modify the inflammatory and allergic responses induced by sensitization alone. In nonsensitized guinea pigs, acetaldehyde exposure resulted in slight irritation (metaplasia/hyperplasia) of the lung, trachea and nasal respiratory epithelium, and induced a significant increase in the number of alveolar macrophages and total number of cells in bronchoalveolar lavage fluid (Lacroix et al., 2002). The respiratory lesions in guinea pigs at 200 ppb acetaldehyde occurred at roughly 2000-fold less than the NOAEL established for respiratory lesions in rats and hamsters under similar exposure duration protocols. These data suggest that guinea pigs are considerably less sensitive to acetaldehyde exposure than rats or hamsters.

Inhaled acetaldehyde is genotoxic and is a clastogen, and inducer of sister chromatid exchanges (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).

## 7.0 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. Acetaldehyde has been shown to cross the placenta in mice and was distributed to embryos (Blakley and Scott Jr., 1984). 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 (Silverman et al., 1946). 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, 1989). 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. After just two minutes of maternal intravenous administration of acetaldehyde at high concentrations, it freely crosses the placenta.

Acetaldehyde is a small liposoluble molecule and is able to cross membranes by simple diffusion (Zorzano and Herrera, 1989).

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., 1984).

*In vitro* models have found that acetaldehyde was teratogenic to C<sub>3</sub>H 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. 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 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  $\mu$ M acetaldehyde was effective in suppressing testicular steroidogenesis; however cell viability was unaffected.

Currently, acetaldehyde is on the Proposition 65 list of carcinogens (listed April 1, 1988); however, it is not currently under consideration as a developmental and reproductive toxicant (DART).(OEHHA, 2007).

## 8.0 Derivation of Reference Exposure Levels

### 8.1 Acetaldehyde Acute Reference Exposure Level

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

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 study by Silverman *et al.*, (1946) was selected for development of the acute REL as it investigated short-term exposure of human volunteers to acetaldehyde. Eye irritation was the most noticeable endpoint for non-asthmatic adults. 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. From the key study it was possible to determine a LOAEL of 25 ppm for slight eye irritation. However, a NOAEL was not determined for this study.

OEHHA was unable to use pulmonary response as an endpoint because the studies done to date in humans have all used aerosolized acetaldehyde vs. inhalation of acetaldehyde vapor. However, in numerous studies on adult humans with and without asthma,

provocation with acetaldehyde resulted in significant pulmonary decrements and more so in asthmatics.

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.

In this key 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., 1999; 2001; 2002; Doty et al., 2004).

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 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. 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  $\sqrt{10}$  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 forced expiratory volume in one second (FEV<sub>1</sub>) by more than 20% in asthmatics. And, alcohol sensitive asthmatics had a selective hyper-responsiveness to acetaldehyde (Myou et al., 1993; Fujimura et al., 1999; Prieto et al., 2000). These considerations are applied equally to the acute, 8-hour and chronic REL.

Limitations with the Silverman et al. (1946) key 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 that was referenced as Cook et al. (1945).

In conclusion, using the LOAEL of 45 mg/m<sup>3</sup> (25 ppm) from Silverman et al. (1946) divided by the cumulative uncertainty factor of 60, an acute reference exposure level (REL) for acetaldehyde, with the endpoint of eye irritation, was determined to be 750 ug/m<sup>3</sup> or 420 ppb, which is the level considered safe for infants and children during an acute exposure period.

## 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).

Both eye irritation and nasal mucosal histopathology are legitimate concerns for the 8-hour REL and occur in a broadly similar concentration range over the relevant time scale. The human study by Silverman et al. (1946) provided limited information on the experimental procedure, used a small sample size, and used a subjective non-quantitative form of measure as the endpoint (self-reported irritancy). Further, the repeated nature of an 8-hour REL makes use of the acute study 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.

<i>Study</i>	Appelman <i>et al.</i> , 1982; 1986
<i>Study population</i>	Wistar rats (10-40 animals/group)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposure to 0, 273, 728, 910, 1820, 4004, 9100 mg/m <sup>3</sup> (0, 150, 400, 500, 1000, 2200, or 5000 ppm)
<i>Exposure continuity</i>	6 hours per day, 5 days/week
<i>Exposure duration</i>	4 weeks
<i>Critical effects</i>	Nasal degeneration of olfactory epithelium
<i>LOAEL</i>	720 mg/m <sup>3</sup> (400 ppm)
<i>NOAEL</i>	270 mg/m <sup>3</sup> (150 ppm)
<i>Benchmark Concentration (BMC<sub>05</sub>) (using continuous model)</i>	178 mg/m <sup>3</sup> (99 ppm)
<i>Time-adjusted exposure</i>	133.5 mg/m <sup>3</sup> (74.25 ppm) = (178*6/8*5/5)
<i>Human DAF concentration</i>	113.5 mg/m <sup>3</sup> (63.11 ppm) = 133.5*0.85 (DAF)
<i>LOAEL uncertainty factor (UF<sub>L</sub>)</i>	1
<i>Subchronic uncertainty factor (UF<sub>s</sub>)</i>	1
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>A-k</sub>)</i>	2 (with DAF adjustment)
<i>Toxicodynamic (UF<sub>A-d</sub>)</i>	√10 (default: no interspecies toxicodynamic data)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>H-k</sub>)</i>	√10 (inter-individual variation)
<i>Toxicodynamic (UF<sub>H-d</sub>)</i>	10 (asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	200
<i>Reference Exposure Level</i>	<b>568 µg/m<sup>3</sup> (316 ppb)</b>

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. Therefore, the average experimental exposure was adjusted from six to eight hours per day.

The 8-hour REL was determined using the Benchmark Dose (BMC) program (Crump and Howe, 1983; Crump, 1984) and procedures developed by the U.S. EPA (2003). The BC<sub>05</sub> 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 BC<sub>05</sub> 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 BMC program using continuous modeling. The Hill and Polynomial models in the BMC 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  $99 \pm 1.20$  ppm and used as the BC<sub>05</sub>.

**Table 8.2.1. Incidence of Degeneration of Olfactory Epithelium using Weighted Means by Severity<sup>1</sup>.**

Dose (ppm)	<u>Males</u>			<u>Females<sup>2</sup></u>		
	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

<sup>1</sup>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.

<sup>2</sup>In the 150 and 500 ppm dose groups, only male animals were used.

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

Method	BMCL*	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

\* BMCL and BMC are in units of ppm.

The standard Human Equivalent Concentration (HEC) adjustment 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. 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. This 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) 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 (Kimbell et al., 2001) (see Section 4.4.7.2.2 of the TSD). In this model, a three-dimensional, anatomically realistic, computational flow dynamic model was used 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<sup>2</sup>; human 30.80 pmol/mm<sup>2</sup>). Peak flux values were also estimated for the rat (2620 pmol/mm<sup>2</sup>) and human (2082 pmol/mm<sup>2</sup>), and averaged with the mean flux values to estimate the DAF. The DAF is the ratio of this value for the rat to that for humans. The reactivity of acetaldehyde is similar to that of formaldehyde. Therefore, application of the DAF to acetaldehyde assumes that it deposits similarly to formaldehyde in the nasal passages. The uncertainty associated with this assumption is reflected in the use of the toxicokinetic component of the interspecies uncertainty factor UF<sub>A-k</sub> equaling two. 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 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. In addition, the subchronic uncertainty factor (UFs) was assigned a value of one since this was a subchronic study.

The value of two was chosen for the toxicokinetic component of the interspecies uncertainty factor ( $UF_{A-k}$ ) to account for additional differences between humans and rodents. For example, it is unknown if humans have nasal ALDH2. In addition, previous studies have shown marked differences between animal species (hamster, rat, mice, and guinea pig) in the uptake of acetaldehyde vapor (Morris, 1997b). However, since acetaldehyde exerts mainly a localized effect on nasal olfactory epithelium, toxicokinetics including distribution and metabolism play less of a key role.

The toxicodynamic portion of the interspecies uncertainty factor ( $UF_{A-d}$ ) is 3.16 because the key studies are in non-primates and data on toxicodynamic interspecies differences are not available.

An uncertainty factor ( $UF_{H-k}$ ) of 3.16 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 variability to take into account for children versus adults. 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 200 and used to determine the acute REL of the experimental animal study.

The  $BC_{05}$  of  $99 \pm 1.20$  ppm from the Benchmark Dose analysis was time-adjusted from six to eight hours for five days per week. Then, the resulting value was multiplied by the dosimetric adjustment factor (DAF) based on PBPK modeling. Finally, the resulting value was divided by the cumulative uncertainty factor of 200. This produced an 8-hour REL with the endpoint of degeneration of olfactory epithelium in rats of  $568 \mu\text{g}/\text{m}^3$  (316 ppb) (Appelman et al., 1982; Appelman et al., 1986)

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 using the animal study with a histopathological endpoint of  $568 \mu\text{g}/\text{m}^3$  (316 ppb) 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 too, 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 procedures.

### 8.3 Acetaldehyde Chronic Reference Exposure Level

<i>Study</i>	Appelman <i>et al.</i> , 1982; 1986
<i>Study population</i>	Wistar rats (10-40 animals/group)
<i>Exposure method</i>	Discontinuous whole-body inhalation exposure to 0, 273, 728, 910, 1820, 4004, 9100 mg/m <sup>3</sup> (0, 150, 400, 500, 1000, 2200, or 5000 ppm)
<i>Exposure continuity</i>	6 hours per day, 5 days/week
<i>Exposure duration</i>	4 weeks
<i>Critical effects</i>	Nasal degeneration of olfactory epithelium
<i>LOAEL</i>	720 mg/m <sup>3</sup> (400 ppm)
<i>NOAEL</i>	270 mg/m <sup>3</sup> (150 ppm)
<i>Benchmark Concentration (BMC<sub>05</sub>) (using continuous model)</i>	178 mg/m <sup>3</sup> (99 ppm)
<i>Time-adjusted exposure</i>	31.79 mg/m <sup>3</sup> (17.68 ppm) = (178*6/24*5/7)
<i>Human DAF concentration</i>	27.02 mg/m <sup>3</sup> (15.03 ppm) = 31.79*0.85 (DAF)
<i>LOAEL uncertainty factor (UF<sub>L</sub>)</i>	1
<i>Subchronic uncertainty factor (UF<sub>s</sub>)</i>	√10 (exposure 8-12% of lifetime)
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>A-k</sub>)</i>	2 (with DAF adjustment)
<i>Toxicodynamic (UF<sub>A-d</sub>)</i>	√10 (default: no interspecies toxicodynamic data)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>H-k</sub>)</i>	√10 (inter-individual variation)
<i>Toxicodynamic (UF<sub>H-d</sub>)</i>	10 (asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	632
<i>Reference Exposure Level</i>	<b>43 µg/m<sup>3</sup> (24 ppb)</b>

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 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 (BMC) modeling approach using the continuous polynomial and Hill models of analysis (Crump and Howe, 1983; Crump, 1984) 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 BMC 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 BC<sub>05</sub>.

As described in detail in Section 8.2, OEHHA derived a dosimetric adjustment factor (DAF) for acetaldehyde rather than using the traditional Human Equivalent Concentration (HEC) value. The application of the DAF was based on a model developed with formaldehyde and assumes that acetaldehyde deposits similarly to formaldehyde in the nasal passages. The uncertainty associated with this assumption is reflected in the use of the toxicokinetic component of the interspecies uncertainty factor  $UF_{A-k}$  equaling two.

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 three 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.*, (1985) observed "intense" nasal lesions in rats exposed to 442 mg/m<sup>3</sup> (243 ppm) for slightly longer exposure durations than that used by Appelman *et al.*, (1982; 1986).

The value of two was also chosen for the toxicokinetic component of the interspecies uncertainty factor ( $UF_{A-k}$ ) to account for additional differences between humans and rodents. For example, it is unknown if humans have nasal ALDH2. In addition, previous studies have shown marked differences between animal species (hamster, rat, mice, and guinea pig) in the uptake of acetaldehyde vapor (Morris, 1997b). However, since acetaldehyde exerts mainly a localized effect on nasal olfactory epithelium, toxicokinetics including distribution and metabolism play less of a key role.

The toxicodynamic portion of the interspecies uncertainty factor ( $UF_{A-d}$ ) is 3.16 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 ( $UF_{H-k}$ ) of 3.16 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 ( $UF_{H-d}$ ) of 10 was used to account for the potentially greater susceptibility of children and asthmatics.

The current chronic RfC for acetaldehyde determined by the U.S. EPA and based on Appelman *et al.*, (1982; 1986) is 9 µg/m<sup>3</sup> (5 ppb) and is within the range of normal human breath acetaldehyde concentrations of 0.7 to 11.0 µg/m<sup>3</sup> (0.4 to 6.1 ppb). OEHHA's proposed chronic REL of 43 µg/m<sup>3</sup> (24 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  $\mu\text{g}/\text{m}^3$ . 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.

Standard BMC methodology was not in place when U.S. EPA developed its RfC for acetaldehyde in 1991. The U.S. EPA (1991) determined an acetaldehyde RfC of 9  $\mu\text{g}/\text{m}^3$  (5 ppb) based on the Appelman *et al.*, (1982, 1986) studies using NOAEL/LOAEL methodology. The principal study established a NOAEL of 273  $\text{mg}/\text{m}^3$ . Adjustments for duration of exposure and corrections for relative areas of human and animal extrathoracic region of the respiratory tract were made resulting in a human equivalent concentration of 8.7  $\text{mg}/\text{m}^3$ . A total UF of 1000 (10 for intraspecies, 10 for extrapolation to chronic exposure, and 10 for interspecies and incompleteness of the database) was then applied, resulting in the RfC of 9  $\mu\text{g}/\text{m}^3$ .

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 reduced from 10 to 3.16, 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**

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), OEHHHA 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): 978-96.

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

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.

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 V, Crump D, Gardiner D and Gavin M (1994). Assessment of a passive sampler for the determination of aldehydes and ketones in indoor air. *Environmental Technology* 15: 679-685.

Burt J, Nelson N and Kaufman J (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.

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.

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, 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.

Crump KS (1984). A new method for determining allowable daily intakes. *Fundam Appl Toxicol* 4(5): 854-871.

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

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 [<sup>14</sup>C]acetaldehyde to serum albumin. *Arch Biochem Biophys* 220(1): 239-246.

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 JL, Jr. (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, Kruijse 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): 235-42.

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.

HSDB. (2004). Hazardous Substances Data Bank. from <http://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. from [www.ciwmb.ca.gov/Publications](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.

Kimbell JS, Subramaniam RP, Gross EA, Schlosser PM and Morgan KT (2001). Dosimetry modeling of inhaled formaldehyde: Comparisons of local flux predictions in the rat, monkey, and human nasal passages. *Toxicol Sci* 64(1): 100-110.

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: 539-46.

Kruysse A. (1970). Acute inhalation toxicity of acetaldehyde in hamsters. *Centraal Instituut Voor Voedingsonderzoek*.

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 A, Proffitt D and Fortune C (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 Environ Epidemiol* 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, 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.

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.

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

OEHHA. (2007). *Safe Drinking Water and Toxic Enforcement Act of 1986. Chemicals Known to the State to Cause Cancer or Reproductive Toxicity. June 1, 2007.* Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. [http://www.oehha.ca.gov/prop65/prop65\\_list/files/060107LST.pdf](http://www.oehha.ca.gov/prop65/prop65_list/files/060107LST.pdf).

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, 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.

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.

Shendell DG, Winer AM, Stock TH, Zhang L, Zhang JJ, Maberti S and Colome SD (2004). Air concentrations of VOCs in portable and traditional classrooms: results of a pilot study in Los Angeles County. *J Expo Anal Environ Epidemiol* 14(1): 44-59.

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.

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.

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): 513-6.

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 <sup>14</sup>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.

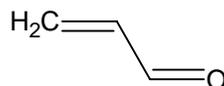
Zorzano A and Herrera E (1989). Disposition of ethanol and acetaldehyde in late pregnant rats and their fetuses. *Pediatr Res* 25(1): 102-106.

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. U.S. Environmental Protection Agency. 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 ( $\leq 1$  ppm) causes irritation of the eyes, nose and throat. 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.

### 1.1 Acrolein Acute REL

*Reference Exposure Level*

**2.3  $\mu\text{g}/\text{m}^3$  (1.0 ppb)**

*Critical effect(s)*

Subjective ocular irritation

*Hazard Index target(s)*

Eyes

### 1.2 Acrolein 8-Hour REL

*Reference Exposure Level*

**1.6  $\mu\text{g}/\text{m}^3$  (0.68 ppb)**

*Critical effect(s)*

Lesions in respiratory epithelium

*Hazard Index target(s)*

Respiratory

### 1.3 Acrolein Chronic REL

*Reference Exposure Level*

**0.1  $\mu\text{g}/\text{m}^3$  (0.05 ppb)**

*Critical effect(s)*

Lesions in respiratory epithelium

*Hazard Index target(s)*

Respiratory

## 2. Physical & Chemical Properties

<i>Description</i>	Colorless or yellow liquid with piercing disagreeable odor
<i>Molecular formula</i>	C <sub>3</sub> H <sub>4</sub> O
<i>Molecular weight</i>	56.1 g/mol
<i>Density</i>	0.843 g/cm <sup>3</sup> @ 20° C
<i>Boiling point</i>	53° C
<i>Melting point</i>	-87° C
<i>Vapor pressure</i>	220 mm Hg @ 20° C
<i>Flashpoint</i>	-26° C
<i>Explosive limits</i>	2.8% - 31% by volume
<i>Solubility</i>	soluble in ethanol, diethyl ether, and up to 20% w/v in water
<i>Odor threshold</i>	0.5 ppm
<i>Metabolites</i>	glycidaldehyde, acrylic acid
<i>Conversion factor</i>	1 ppm in air = 2.3 mg/m <sup>3</sup> @ 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 preliminarily 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 ( $\leq 0.25$  ppm) and rapidly become more pronounced with increasing concentration, while brief exposure (1.5 min) to 0.3 ppm ( $0.7 \text{ mg/m}^3$ ) 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  $7 \text{ mg/m}^3$ , 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 ( $0.14 \text{ mg/m}^3$ ) 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  $\mu\text{M}$  acrolein for 10 or 20 minutes significantly increased the maximal contractile response to a specific antigen (*Dermatophagoides pteronyssinus*) by  $20.5 \pm 6.5\%$  and  $34.9 \pm 7.4\%$ , respectively. In addition, in sensitized tissue pre-exposed to 0.3  $\mu\text{M}$  acrolein for 10 minutes, contractile response was increased by  $33.5 \pm 6.2\%$  and  $32.5 \pm 5.1\%$  for carbachol and histamine, respectively. Thus acrolein exposure appears to exacerbate 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.

#### *Predisposing Conditions for Acrolein Toxicity*

**Medical:** 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), and mucus hyper-secretion (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.

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

## 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**

<b>Study</b>	<b>Model</b>	<b>Exposure</b>	<b>Outcome</b>
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 <sub>2</sub> O/ml. Acetylcholine to double airway resistance dropped from 114 to 44.7 µg/kg/min
Buckley et al. 1984	Nasal histopathology at (RD <sub>50</sub> ) in mice;	1.7 ppm, 6 h/d, 5d	Exfoliation and squamous metaplasia of epithelium
Morris et al. 2003	Decrease in respiratory rate (RD <sub>50</sub> ) in mice	0.3, 1.6, 3.9 ppm, 10 min	Control RD <sub>50</sub> at 1.50 ppm vs 0.82 ppm in allergic mice
Kane et al. 1979	Decrease in respiratory rate (RD <sub>50</sub> ) in mice	15 min	RD <sub>50</sub> 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 h 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<sup>3</sup>) 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 h/d, 5 d/wk 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<sup>3</sup>) 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 h and measured the induction of bronchial hyperresponsiveness by the amount of infused acetylcholine necessary to double specific airway resistance 1, 2, 6, and 24 h after exposure compared to baseline. The dose of acetylcholine required to double airway resistance decreased from  $114.0 \pm 6.6$  to  $44.7 \pm 4.2$   $\mu\text{g}/\text{kg}/\text{min}$  ( $p < 0.001$ ) at 2 h following acrolein exposure and remained low for at least 24 h. Acrolein exposure was found to increase levels of the bronchoconstrictor leukotriene C<sub>4</sub> (LTC<sub>4</sub>) in bronchoalveolar lavage fluids prior to the observation of bronchial hyperresponsiveness. This hyperresponsiveness was prevented by treatment with an inhibitor of LTC<sub>4</sub> synthesis or an LTC<sub>4</sub> receptor antagonist. Acrolein was thus shown to induce bronchial hyperresponsiveness, an effect apparently mediated by LTC<sub>4</sub>.

Buckley et al. (1984) investigated whether lesions occur in the respiratory tract of Swiss-Webster mice after exposure to the RD<sub>50</sub> 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<sub>50</sub>, 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<sub>50</sub> as a measure of sensory irritation and estimated an RD<sub>50</sub> 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 h per day on one or three successive days. Immediately following the last exposure, the

rats were killed. Mucosa 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/m<sup>3</sup>) 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/m<sup>3</sup>) for 3 hours, McNulty et al. (1984) reported a 63% decrease in glutathione in nasal mucosal but not in liver. In addition, <sup>14</sup>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<sup>3</sup>) for 8 hours (Astry and Jakab, 1983). In this study, the control exposure was not described.

## **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 (IARC, 1985). However, a metabolite of acrolein, the reactive epoxide glycidaldehyde, has been shown to be mutagenic and carcinogenic in mice and rats. Therefore, acrolein has been designated a Group C substance, with possible human carcinogenic potential (U.S.EPA, 1987). In addition, acrolein-DNA adducts have been found in aortic tissue following 6 hr 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  $\mu\text{M}$ ) 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  $\mu\text{M}$ , 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.) at relatively high concentrations in smokers' homes (1.6-3.6  $\mu\text{g}/\text{m}^3$ ; 0.70-1.57 ppm (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. These effects were 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<sup>3</sup>) (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<sup>3</sup>). 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<sup>3</sup>) 6 h/d, 5 d/wk 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<sup>3</sup>) 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 or of 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<sup>3</sup>) 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<sup>3</sup>) 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<sup>3</sup>) 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 interstitia 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 bronchiopneumonia. 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. Kutzman (1981) studied reproductive fitness in male and female rats following acrolein inhalation for 6 h/d, 5 d/wk 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<sup>3</sup>) 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

<i>Study</i>	Darley et al., 1960
<i>Study population</i>	36 healthy human volunteers
<i>Exposure method</i>	5 min exposure: carbon-filter respirators worn
<i>Exposure continuity</i>	
<i>Exposure duration</i>	5 min
<i>Critical effects</i>	subjective ocular irritation
<i>LOAEL</i>	0.06 ppm
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	not applied
<i>Human Equivalent Concentration</i>	n/a
<i>LOAEL uncertainty factor (UF<sub>L</sub>)</i>	6 (default: mild effect, no NOAEL)
<i>Subchronic uncertainty factor (UF<sub>s</sub>)</i>	not applied
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>A-k</sub>)</i>	1 (default: human study)
<i>Toxicodynamic (UF<sub>A-d</sub>)</i>	1 (default: human study)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>H-k</sub>)</i>	1 (site of contact; no systemic effects)
<i>Toxicodynamic (UF<sub>H-d</sub>)</i>	10 (greater susceptibility of children to asthma exacerbation)
<i>Cumulative uncertainty factor</i>	60
<i>Reference Exposure Level</i>	<b>2.3 µg/m<sup>3</sup> (1.0 ppb)</b>

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 (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 values 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. The acute REL for acrolein exposure is calculated to be  $2.3 \mu\text{g}/\text{m}^3$  (1.0 ppb).

The acute REL above is supported by a study in humans by Weber-Tschopp et al. (1977). During a 40 min 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  $2.7 \mu\text{g}/\text{m}^3$  (1.2 ppb).

<i>Study</i>	Weber-Tschopp et al. (1977)
<i>Study population</i>	54 healthy human volunteers
<i>Exposure method</i>	Exposure chamber
<i>Exposure continuity</i>	Increasing concentration (0-0.6 ppm)
<i>Exposure duration</i>	40 min
<i>Critical effects</i>	subjective ocular irritation
<i>LOAEL</i>	0.07 ppm
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	not applied
<i>Human Equivalent Concentration</i>	n/a
<i>LOAEL uncertainty factor (UF<sub>L</sub>)</i>	6 (default: mild effect, no NOAEL)
<i>Subchronic uncertainty factor (UF<sub>s</sub>)</i>	not applied
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>A-k</sub>)</i>	1 (default: human study)
<i>Toxicodynamic (UF<sub>A-d</sub>)</i>	1 (default: human study)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>H-k</sub>)</i>	1 (site of contact; no systemic effects)
<i>Toxicodynamic (UF<sub>H-d</sub>)</i>	10 (asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	60
<i>Reference Exposure Level</i>	<b>2.7 µg/m<sup>3</sup> (1.2 ppb)</b>

A similar acute REL was calculated as shown below based on lesions in nasal epithelium in rats exposed to acrolein for 6 h/d for 3 d (Casseo 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 BMC<sub>0.5</sub> of 56 µg/m<sup>3</sup> 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<sup>n</sup>\*T=K (n=3) to adjust the 18 hours of exposure to 1 hour that gave 147 µg/m<sup>3</sup> (see Section 5.7.1 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.

<i>Study</i>	Cassee et al., 1996b
<i>Study population</i>	11 rats
<i>Exposure method</i>	
<i>Exposure continuity</i>	6 hr/day
<i>Exposure duration</i>	3 days
<i>Critical effects</i>	lesions of the respiratory epithelium
<i>LOAEL</i>	1.73 ppm
<i>NOAEL</i>	not observed
<i>Benchmark concentration (BMC<sub>05</sub>)</i>	56 µg/m <sup>3</sup>
<i>Time-adjusted exposure</i>	C <sup>n</sup> *T n = 3
<i>Extrapolated concentration</i>	147 µg/m <sup>3</sup> (56 <sup>3</sup> *6/1*3/1) <sup>1/3</sup>
<i>Human concentration adjustment</i>	125 µg/m <sup>3</sup> = 147*0.85 (DAF)
<i>LOAEL uncertainty factor (UF<sub>L</sub>)</i>	not applied
<i>Subchronic uncertainty factor (UF<sub>s</sub>)</i>	not applied
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>A-k</sub>)</i>	2 (with DAF adjustment)
<i>Toxicodynamic (UF<sub>A-d</sub>)</i>	√10 (default: no interspecies toxicodynamic data)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>H-k</sub>)</i>	1
<i>Toxicodynamic (UF<sub>H-d</sub>)</i>	10 (asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	60
<i>Reference Exposure Level</i>	<b>2.1 µg/m<sup>3</sup> (0.91 ppb)</b>

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). Kimbel 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<sup>2</sup>; human 30.80 pmol/mm<sup>2</sup>). Peak flux values were also estimated for the rat (2620 pmol/mm<sup>2</sup>) and human (2082 pmol/mm<sup>2</sup>), 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<sub>A-k</sub> of 2.

## 8.2 Acrolein 8-Hour Reference Exposure Level

<i>Study</i>	Kutzman et al., 1985
<i>Study population</i>	96 adult Fisher-344 rats
<i>Exposure method</i>	Discontinuous whole body 0.4 – 4.0 ppm
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	62 days
<i>Critical effects</i>	Lesions in the respiratory epithelium
<i>LOAEL</i>	0.4 ppm
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	$C^n * T = K$ , where $n = 1.2$
<i>Extrapolated 8 hour concentration</i>	310 ppb = $(0.4^{1.2} * 6 / 8)^{1/1.2}$
<i>Human concentration adjustment</i>	260 ppb = $310 * 0.85$ (DAF)
<i>LOAEL uncertainty factor (UF<sub>L</sub>)</i>	6 (default: mild effect, no NOAEL)
<i>Subchronic uncertainty factor (UFs)</i>	not applied
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>A-k</sub>)</i>	2 (default with DAF adjustment)
<i>Toxicodynamic (UF<sub>A-d</sub>)</i>	$\sqrt{10}$ (default: no interspecies toxicodynamic data)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>H-k</sub>)</i>	1
<i>Toxicodynamic (UF<sub>H-d</sub>)</i>	10 (asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	380
<i>Reference Exposure Level</i>	<b>1.6 <math>\mu\text{g}/\text{m}^3</math> (0.68ppb)</b>

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 Kutzman et al. (1985) following exposure to acrolein for 6 h/d, 5 d/wk for 62 d. The critical effect of lesion formation is not a sensory irritancy effect so a time (T) adjustment was applied using  $C^n * T = K$ , where  $n = 1.2$  (Table G-1, Appendix G of the TSD) to extrapolate to an 8 hour concentration of 310 ppb. Use of  $C^n$  (where C is concentration) is a modification of Haber's Law as described in Section 5.7.1 of the TSD. K is a constant. A UF of 6 was applied for the use of a LOAEL. An adjusted human concentration of 260 ppb was estimated using a DAF of 0.85 (see previous section for a discussion of the DAF). Use of the DAF is expected to correct for pharmacokinetic differences between species so an interspecies kinetic UF of 2 was used instead of  $\sqrt{10}$ . The default interspecies UF<sub>A-d</sub> of  $\sqrt{10}$  was applied to compensate for the absence of data on pharmacodynamic differences between species. An intraspecies UF<sub>H-k</sub> 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 380 and an 8-hour REL of  $1.6 \mu\text{g}/\text{m}^3$  (0.68 ppb).

### 8.3 Acrolein Chronic Reference Exposure Level

<i>Study</i>	Kutzman et al., 1985
<i>Study population</i>	96 adult Fischer-344 rats
<i>Exposure method</i>	Discontinuous whole body to 0 – 4.0 ppm
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	62 days
<i>Critical effects</i>	Lesions in the respiratory epithelium
<i>LOAEL</i>	0.4 ppm
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time adjusted exposure</i>	$0.071 \text{ ppm} = 0.4 * 6/24 * 5/7$
<i>Human concentration adjustment</i>	$60 \text{ ppb} = 0.071 * 0.85 \text{ (DAF)}$
<i>LOAEL uncertainty factor (<math>UF_L</math>)</i>	6 (default: mild effect, no NOAEL)
<i>Subchronic uncertainty factor (<math>UF_S</math>)</i>	$\sqrt{10}$ (exposure 8-12% of lifetime)
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (<math>UF_{A-k}</math>)</i>	2 (with DAF adjustment)
<i>Toxicodynamic (<math>UF_{A-d}</math>)</i>	$\sqrt{10}$ (default: no interspecies toxicodynamic data)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (<math>UF_{H-k}</math>)</i>	1
<i>Toxicodynamic (<math>UF_{H-d}</math>)</i>	10 (asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	1200
<i>Reference Exposure Level</i>	<b><math>0.12 \mu\text{g}/\text{m}^3</math> (0.05 ppb)</b>

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 chronic REL was developed based on 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). In addition to the UFs applied to the 8-hr REL, a UF of  $\sqrt{10}$  was applied since this was a subchronic exposure study representing 8-12% of the rat's lifetime. The cumulative UF of 1200 gives a chronic REL of  $0.12 \mu\text{g}/\text{m}^3$  (0.05 ppb). For comparison with the proposed chronic REL, a chronic REL was estimated from the data of Feron et al. (1978) in rats, which found a LOAEL of 0.4 ppm after exposure for 13 weeks. Using time extrapolation and a DAF of 0.85, a human equivalent

concentration of 60 ppb ( $138 \mu\text{g}/\text{m}^3$ ) was calculated. A UF of 6 was applied for LOAEL to NOAEL conversion,  $\sqrt{10}$  for use of subchronic exposure, and  $\sqrt{10}$  for interspecies toxicodynamic variability. A factor of 2 was used for interspecies toxicokinetic uncertainty associated with the use of the DAF. For intraspecies variability, while these data are from adult animals, a  $\text{UF}_{\text{H-k}}$  of 1 was used since the pharmacokinetic differences between adult and young animals are not expected to be as great as the potential pharmacodynamic differences, for which an intraspecies  $\text{UF}_{\text{H-d}}$  of 10 was employed. This gave an estimated chronic REL of  $0.12 \mu\text{g}/\text{m}^3$  (0.05 ppb). This study was considered supportive of the Kutzman results.

<i>Study</i>	Feron et al. (1978)
<i>Study population</i>	48 adult Wistar rats
<i>Exposure method</i>	Discontinuous whole body to 0 – 4.9 ppm
<i>Exposure continuity</i>	6 hr/day, 5 day/week
<i>Exposure duration</i>	13 weeks
<i>Critical effects</i>	Lesions in respiratory epithelium
<i>LOAEL</i>	0.4 ppm
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	$0.071 \text{ ppm} = 0.4 * 6 / 24 * 5 / 7$
<i>Human concentration adjustment</i>	$60 \text{ ppb} = 0.071 * 0.85 \text{ (DAF)}$
<i>LOAEL uncertainty factor (<math>\text{UF}_L</math>)</i>	6 (default: mild effect, no NOAEL)
<i>Subchronic uncertainty factor (<math>\text{UF}_s</math>)</i>	$\sqrt{10}$ (exposure 8-12% of lifetime)
<i>Interspecies Uncertainty Factor</i>	
<i>Toxicokinetic (<math>\text{UF}_{\text{A-k}}</math>)</i>	2 (with DAF adjustment)
<i>Toxicodynamic (<math>\text{UF}_{\text{A-d}}</math>)</i>	$\sqrt{10}$ (default: no interspecies toxicodynamic data)
<i>Intraspecies Uncertainty Factor</i>	
<i>Toxicokinetic (<math>\text{UF}_{\text{H-k}}</math>)</i>	1
<i>Toxicodynamic (<math>\text{UF}_{\text{H-d}}</math>)</i>	10 (asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	1200
<i>Reference Exposure Level</i>	<b><math>0.12 \mu\text{g}/\text{m}^3</math> (0.05 ppb)</b>

The U.S. EPA (2003) based its RfC of  $0.02 \mu\text{g}/\text{m}^3$  on the study by Feron et al. (1978) from which a HEC of  $0.02 \text{ mg}/\text{m}^3$  was derived based on a regional gas dosimetric ratio (RGDR) of 0.14 and an adjusted LOAEL of  $0.16 \text{ mg}/\text{m}^3$  ( $0.14 * 0.16 = 0.02$ ). 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 instead of a RGDR. This, combined with UFs of 6 for interspecies uncertainty (2 for use of the DAF,  $\sqrt{10}$  for toxicodynamic differences),  $\sqrt{10}$  for the use of a subchronic study, and 6 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  $0.2 \mu\text{g}/\text{m}^3$ , 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 designated 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 identified 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.

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 Kruyssen 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, Kruyssen 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, 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 Agreement 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, Douppnik 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 Douppnik 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 Casanova-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](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-<sup>14</sup>C]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.

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

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.

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](http://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 key values are summarized below.

#### 1.1 Inorganic Arsenic Acute REL

<i>Reference Exposure Level</i>	<b>0.2 µg As/m<sup>3</sup></b>
<i>Critical effect(s)</i>	Decreased fetal weight in mice
<i>Hazard Index target(s)</i>	Development (teratogenicity); cardiovascular system; nervous system

#### 1.2 Inorganic Arsenic 8-Hour REL

<i>Reference Exposure Level</i>	<b>0.015 µg/ As/m<sup>3</sup></b>
<i>Oral Reference Exposure Level</i>	0.0035 µg/kg bw-day
<i>Critical effect(s)</i>	Decreased intellectual function in 10 year old children
<i>Hazard Index target(s)</i>	Development (teratogenicity); cardiovascular system; nervous system; lung; skin

#### 1.3 Inorganic Arsenic Chronic REL

<i>Reference Exposure Level</i>	<b>0.015 µg As/m<sup>3</sup></b>
<i>Oral Reference Exposure Level</i>	0.0035 µg/kg bw-day
<i>Critical effect(s)</i>	Decreased intellectual function in 10 year old children
<i>Hazard Index target(s)</i>	Hematologic

## 2. Physical & Chemical Properties

**Table 2.1 Arsenic and Arsenic Species\***

<b>Molecular formula</b>	<b>Molecular weight</b>	<b>Percent As by weight</b>	<b>Synonyms</b>	<b>CAS Registry Number</b>
As	74.92	100%	Arsenic black, metallic arsenic	7440-38-2
As <sub>2</sub> O <sub>3</sub> As <sub>4</sub> O <sub>6</sub>	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 <sub>3</sub>	181.28	41.3%	Arsenic butter, trichloroarsine, arsenious chloride	7784-34-1
As <sub>2</sub> O <sub>5</sub>	229.82	65.2%	Arsenic pentoxide, arsenic anhydride, arsenic oxide, arsenic acid anhydride	1303-28-2
AsHNa <sub>2</sub> O <sub>4</sub>	185.91	40.3%	Arsenic acid disodium salt, disodium arsenate, sodium arsenate dibasic	7778-43-0
AsHNa <sub>2</sub> O <sub>3</sub>	130.92	57.2%	Arsenous acid disodium salt, arsenious acid sodium salt	7784-46-5
AsH <sub>3</sub>	77.94	96.12	Arsine, arsane, arsenic hydride, arsenous hydride, hydrogen arsenide, arsenic trihydride	7784-42-1
As(OH) <sub>3</sub>			Arsenous acid	13464-58-9
AsO(OH) <sub>3</sub>	141.93	52.78	Arsenic acid, orthoarsenic acid	7778-39-4
As <sub>4</sub> S <sub>4</sub>	427.92	70.03	Arsenic disulfide, realgar, red arsenic sulfide	
CH <sub>3</sub> AsO(OH) <sub>2</sub>	139.97	53.51	Monomethylarsonic acid	124-58-3
CH <sub>3</sub> As(OH) <sub>2</sub>	123.77	60.41	Monomethylarsonous acid	25400-23-1
(CH <sub>3</sub> ) <sub>2</sub> AsO(OH)	137.99	54.28	Dimethylarsinic acid, cacodylic acid	75-60-5
(CH <sub>3</sub> ) <sub>2</sub> AsOH	121.99	61.40	Dimethylarsinous acid	55094-22-9
(CH <sub>3</sub> ) <sub>3</sub> AsO	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)**

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

**2.2 Arsenic Trioxide (ATSDR, 2000)**

<i>Description</i>	As <sub>2</sub> O <sub>3</sub> : White solid, glassy, amorphous lumps or crystal
<i>Molecular formula</i>	See Table 2.1
<i>Molecular weight</i>	197.84
<i>Density</i>	As <sub>2</sub> O <sub>3</sub> : 3.865 g/cm <sup>3</sup>
<i>Boiling point</i>	As <sub>2</sub> O <sub>3</sub> : 460°C
<i>Melting point</i>	As <sub>2</sub> O <sub>3</sub> : 274°C
<i>Solubility</i>	Oxides: slightly soluble in water 17g/L, insoluble in alcohol, chloroform, ether.
<i>Metabolites</i>	Dimethylarsinic acid, methylarsonic acid

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

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	AsH <sub>3</sub>
<i>Molecular weight</i>	77.93
<i>Specific gravity (Water = 1)</i>	1.689 @ 84.9°C
<i>Boiling point</i>	-62.55°C
<i>Melting point</i>	-117°C
<i>Vapor pressure</i>	Greater than 1 atm
<i>Vapor density (Air = 1)</i>	2.695
<i>Solubility</i>	soluble in chloroform and benzene, slightly soluble in water (20 mL/100 mL at 20 C), ethyl alcohol and in alkalis
<i>Odor threshold</i>	0.5 ppm
<i>Odor description</i>	garlic-like or fishy odor
<i>Metabolites</i>	oxidation to arsenite, arsenate, other unidentified (Landrigan et al., 1982; Carter et al., 2003)
<i>Conversion factor</i>	1 ppm = 3.19 mg/m <sup>3</sup> @ 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, 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 a dopant in the semiconductor industry. 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) arsenic species (e.g., arsenite) have often exhibited greater acute toxicity than pentavalent (+5) species (e.g., arsenate). 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 (-3), while less studied, appears to progress similarly after its oxidation to arsenite (+3) 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 ( $\text{As}^{\text{III}}$ ) and arsenate ( $\text{As}^{\text{V}}$ ), as well as the organic methyl arsonate (MMA), dimethylarsinic acid (DMA), trimethylarsine (TMA), and or arsenobetaine (in fish).

## 4.1 Inorganic Arsenic Oxides

### 4.1.1 Absorption and Distribution

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  $\mu\text{m}$  activity median thermodynamic diameter particles at 0.31 to 2.03  $\text{m}^3/\text{hr}$  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  $\mu\text{g}/\text{m}^3$  of arsenic aerosol of MMAD of 5.0  $\mu\text{m}$ , 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  $\mu\text{g}$  As/g creatinine. 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  $\mu\text{g}$  As/ $\text{m}^3$ . After daily inhalation exposure of 100  $\mu\text{g}$  As (III)/ $\text{m}^3$  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/ $\text{cm}^2\text{-hr}$ .

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 [<sup>74</sup>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<sup>2</sup> 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 [<sup>73</sup>As] arsenate from soil or water *in vivo* in Rhesus monkeys and *in vitro* in human cadaver skin. Water solutions of [<sup>73</sup>As] arsenate at low (0.024 ng/cm<sup>2</sup>) or high (2.1 µg/cm<sup>2</sup>) 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<sup>2</sup>) 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<sup>III</sup>) reacts and binds to sulfhydryl groups while arsenate (As<sup>V</sup>) has

chemical properties similar to those of phosphate.  $\text{As}^{\text{V}}$  also has affinity for sulfhydryl groups; however, its affinity is approximately 10-fold less than  $\text{As}^{\text{III}}$  (Jacobson-Kram and Montalbano, 1985). The distribution and retention patterns of  $\text{As}^{\text{III}}$  and  $\text{As}^{\text{V}}$  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  $^{74}\text{As}$ -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. 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.

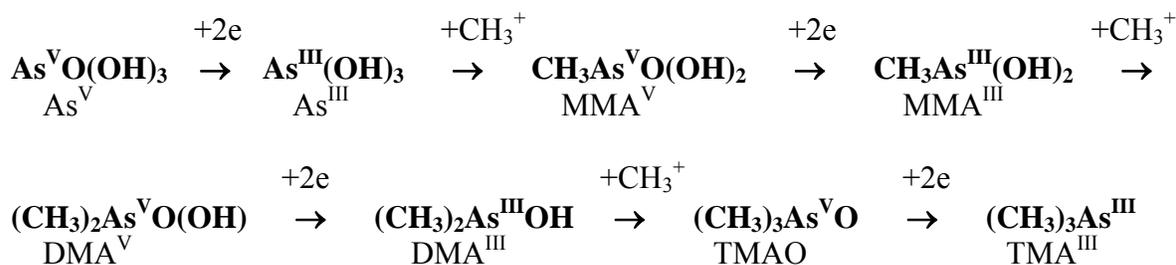
The effect of dose on arsenate disposition was evaluated in adult female B6C3F<sub>1</sub> mice dosed orally with 0.5 to 5000  $\mu\text{g}/\text{kg}$  [ $^{73}\text{As}$ ]-arsenate in water (Hughes et al., 1994). Urine was collected at several time points over a 48-hr period, and feces at 24 and 48 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.

#### 4.1.2 Metabolism

Most studies of arsenic metabolism have involved administration of inorganic arsenic (Asi) as arsenate ( $\text{As}^{\text{V}}$ ) or arsenite ( $\text{As}^{\text{III}}$ ) to an experimental animal or a human, and detection of Asi and the methylated metabolites methylarsonic acid ( $\text{MMA}^{\text{V}}$ ) and dimethylarsinic acid ( $\text{DMA}^{\text{V}}$ ) 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  $\text{As}^{\text{III}}$ ,  $\text{MMA}^{\text{V}}$ ,  $\text{MMA}^{\text{III}}$ ,  $\text{DMA}^{\text{V}}$ ,  $\text{DMA}^{\text{III}}$ ,  $\text{TMAO}^{\text{V}}$ , 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 ( $\text{As}(\text{SG})_3$ ) is converted to monomethyl-(MADG) and dimethyl-(DMAG) conjugates which are hydrolyzed to  $\text{MMA}^{\text{III}}$  and  $\text{DMA}^{\text{III}}$ , 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<sup>III</sup> and DMA<sup>III</sup> 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<sup>III</sup> 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<sup>III</sup> declined and MMA<sup>III</sup> output gradually increased. From 40 min after i.v. As<sup>III</sup> administration, MMA<sup>III</sup> was the dominant form of biliary arsenic. Within two hr 9.2 percent of the dose was excreted in the bile as MMA<sup>III</sup>. Injection of arsenate produced a mixture of As<sup>V</sup>, As<sup>III</sup> and MMA<sup>III</sup> in the bile. Curiously, rats injected with MMA<sup>V</sup> did not excrete MMA<sup>III</sup>.

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

Styblo et al. (1996) observed 50 μM arsenite inhibition of DMA<sup>V</sup> 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-<sup>3</sup>H]-adenosyl-L-methionine by measuring the formation of [methyl-<sup>3</sup>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<sup>-4</sup> M for hamster DMTase to 1.8x10<sup>-6</sup> 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  $\mu\text{M}$  arsenite was  $99.3 \pm 1.87$  pmol  $\text{CH}_3/\text{hr}/10^6$  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  $\text{CH}_3/\text{hr}/10^6$  cells, over a 50-fold difference in apparent methylation rate.

While the reduction of arsenate and  $\text{MMA}^{\text{V}}$  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  $[^{14}\text{C}] \text{MMA}^{\text{V}}$ ) and exhibited substrate saturation at about 300  $\mu\text{M}$ . 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 ( $\text{MMA}^{\text{V}}$ ) 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  $\text{MMA}^{\text{V}}$  reductase activity was 91.4 nmol  $\text{MMA}^{\text{III}}/\text{mg}$  protein/hr in brain and 61.8 nmol  $\text{MMA}^{\text{III}}/\text{mg}$  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  $\text{MMA}^{\text{V}}$  reductase in brain is curious and may help explain some of the neurotoxic effects of arsenic. Due to relatively low affinity of the  $\text{MMA}^{\text{V}}$  reductase ( $K_{\text{M}} = 2.2 \times 10^{-3}$  M) compared to the methyl transferases ( $K_{\text{M}} = 5-9 \times 10^{-6}$  M), the  $\text{MMA}^{\text{V}}$  reduction is thought to be the rate-limiting step in arsenic metabolism (Zakharyan and Aposhian, 1999). The partially purified human liver  $\text{MMA}^{\text{V}}$  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  $\mu\text{g As/L}$ ). 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  $\pm$  SE) were: Asi,  $11.8 \pm 1.0$ ; MMA,  $26.9 \pm 1.2$ ; and DMA,  $61.3 \pm 1.4$ . 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<sup>V</sup> 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 ( $\mu\text{g/g creatinine}$ ) 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<sup>V</sup> reductase genes. For the arsenate reductase gene (hPNP) 48 polymorphic sites were identified while 33 were found in the MMA<sup>V</sup> reductase gene (hGSTO1-1). For the EA individuals the MMA<sup>V</sup> 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<sup>V</sup> 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<sup>V</sup>/MMA<sup>V</sup> (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.

### 4.1.3 Excretion

Several authors have studied the kinetics of As excretion in humans. Tam et al. (1979) administered <sup>74</sup>As arsenic acid (0.01  $\mu\text{g}$ , ca. 6  $\mu\text{Ci}$ ) 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 <sup>74</sup>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).

#### 4.1.4 PBPK Models

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.

## 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 ( $\text{As}^{-\text{III}}\text{H}_3$ ) 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,  $\text{As}^{\text{III}}$ , arsenobetaine (AsB), and to a lesser extent  $\text{As}^{\text{V}}$ . The data indicate a capability to oxidize  $\text{As}^{-\text{III}}$  to  $\text{As}^{\text{V}}$  species probably via arsenite  $\text{As}(\text{OH})_3$ . 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  $\text{mg}/\text{m}^3$  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  $\text{As}^0$  and hydrogen; (2) reaction of  $\text{AsH}_3$  with oxidized thiols to form diarsine  $\text{AsH}_2\text{-AsH}_2$  (proposed) and reduced thiol  $\text{RSH}$ ; and (3) possible reaction between arsine and oxygen species, producing arsine hydroperoxide  $\text{H}_2\text{AsOOH}$  (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:



**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 (-III) > organo-arsine derivatives > arsenites (III) > arsenoxides (III) > arsenates (V) > pentavalent organic compounds (V) > arsonium metals (I) > metallic arsenic (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

#### 5.3.1 Inhalation Exposure

The lethal concentration low ( $LC_{Lo}$ ) for  $AsCl_3$  in the cat for a 20-minute inhalation exposure is 100 ppm (740 mg/m<sup>3</sup>) (Flury, 1921). In the mouse, the  $LC_{Lo}$  of  $AsCl_3$  for a 10-minute exposure is 338 ppm (2500 mg/m<sup>3</sup>) (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.

#### 5.3.2 Immunotoxicity

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<sub>2</sub>O<sub>3</sub> 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  $\mu\text{g As/m}^3$ ; (2) 5 times to 519  $\mu\text{g As/m}^3$ ; and (3) 20 times to 505  $\mu\text{g As/m}^3$ . 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 <sup>35</sup>S-labeled *Klebsiella pneumoniae*. A single exposure to 271, 496, and 940  $\mu\text{g As/m}^3$ , but not 123  $\mu\text{g As/m}^3$ , resulted in significantly decreased bactericidal activity. Five exposures to 519  $\mu\text{g As/m}^3$  and twenty exposures to both 245 and 505  $\mu\text{g As/m}^3$  resulted in decreased bactericidal activity. The studies indicate a NOAEL for immunotoxicity of 123  $\mu\text{g As/m}^3$ . This study provides a partial mode of action of arsenic-induced increase in mortality due to experimental lung infections with the mouse pathogen *S.zoopidemicus*. The second bactericidal assay with radiolabelled *K.pneumoniae* provides a plausible explanation, namely that arsenic exposure above 123  $\mu\text{g/m}^3$  inhibits normal immune bactericidal response in the lung.

### 5.3.3 Organ and Enzyme Effects

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<sub>2</sub>O<sub>4</sub>) on gestation day 8 was lethal to 44% of all embryos (Willhite and Ferm, 1984). A smaller dose (10 mg/kg) of sodium arsenite (III) (AsHNaO<sub>2</sub>) administered in the same manner resulted in 90% embryonic lethality.

Fetal malformations, including exencephaly, resulted from an intravenous injection of AsH<sub>3</sub>Na<sub>2</sub>O<sub>4</sub> (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  $\text{AsH}_2\text{NaO}_3$  (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  $\text{As}_2\text{O}_3$  at 1 mg/m<sup>3</sup> for 5 months; no maternal toxicity was observed (Kamkin, 1982). At the LOAEL, 0.3 mg/m<sup>3</sup>, 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  $\text{As}_2\text{O}_3$  at a concentration of 40 mg/m<sup>3</sup> for 48 hours (Kamil'dzhanov, 1982). 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 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  $\text{As}_2\text{O}_3$  aerosol for 4 hours/day on gestational days 9-12 at concentrations of 0, 0.26, 2.9, or 28.5 mg  $\text{As}_2\text{O}_3/\text{m}^3$  (~0.2, 2.2, and 21.6 mg As/m<sup>3</sup>). The aerosol was generated by spraying an aqueous solution of  $\text{As}_2\text{O}_3$ . On the 18<sup>th</sup> 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 <sub>2</sub> O <sub>3</sub> (mg/m <sup>3</sup> )	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<sup>3</sup> 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<sub>2</sub>O<sub>3</sub>/m<sup>3</sup> (0.76 µg As/m<sup>3</sup>) 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<sub>2</sub>O<sub>3</sub>/m<sup>3</sup> for 48 hours (Kamil'dzhanov, 1982). Similarly, motility was decreased after: (1) a 120-hour exposure to 7.95 mg/m<sup>3</sup>; (2) a 252-hour exposure to 1.45 mg/m<sup>3</sup>; and (3) an 800-hour exposure to 0.36 mg/m<sup>3</sup>.

Holson et al. (1999) administered arsenic trioxide (As<sub>2</sub>O<sub>3</sub>) 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<sub>2</sub>O<sub>3</sub>/m<sup>3</sup> (measured means: 0.24, 2.6, 8.3 mg As/m<sup>3</sup>). Maternal toxicity evidenced by the occurrence of resorptions, 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<sup>3</sup> (3.4 mg As<sub>2</sub>O<sub>3</sub>/m<sup>3</sup>). No treatment-related malformations or developmental variations were observed at any exposure level. The NOAEL for developmental toxicity was 8.3 mg As/m<sup>3</sup> (11 mg As<sub>2</sub>O<sub>3</sub>/m<sup>3</sup>). 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<sub>3</sub>AsO<sub>4</sub>) 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 ( $\text{As}^{\text{V}}$ ) i.p. or arsenic trioxide ( $\text{As}^{\text{III}}$ ) i.p. or by gavage on GD 9 to 25 CrI: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](http://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)).

#### 6.1.1 Inhalation Exposure

Smelter workers, exposed to concentrations of arsenic up to 7 mg As/m<sup>3</sup>, 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<sup>3</sup>, with As<sub>2</sub>O<sub>3</sub> 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<sup>3</sup> inorganic arsenic for two or more years (Perry et al., 1948).

### 6.1.2 Oral Exposure

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 ( $\geq 300$  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<sub>2</sub>O<sub>3</sub> (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  $\geq$  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  $\mu$ g/L. For cumulative arsenic exposures of  $<$ 0.1, 0.1-4.9, and  $\geq$  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  $\mu\text{g As/mg}$  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

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  $\mu\text{g/L}$ , 9, 36; 100-399  $\mu\text{g/L}$ , 66, 34;  $\geq 400$   $\mu\text{g/L}$ , 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 ( $\text{FEV}_1$ ) 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  $\mu\text{g/L}$  increase in arsenic level was associated with a 45.0 mL decrease (95% C.I.; 6.2, 83.9) in  $\text{FEV}_1$  ( $P = 0.02$ ) and a 41.4 mL decrease (95% C.I.; -0.7, 83.5) in FVC ( $P = 0.054$ ). 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).

### 6.2.1 Skin Effects

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 ( $\leq 9$  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 ( $\mu\text{g/L}$ ) 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.

### 6.2.2 Neurodevelopmental Effects

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 higher risk to children than adults. 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  $\mu\text{g/g}$ ) but similar Pb exposures (8.9 vs. 9.7  $\mu\text{g Pb/dL}$  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  $\mu\text{g/g}$  (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  $\leq$  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  $\leq 1$  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.,  $184.99 \text{ ppb} \times 1008.6 \text{ cm}^3/\text{d} \times 11.28 \text{ yr} \times 365 \text{ d/yr} \times 10^{-3}$ ). 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 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  $> 50 \mu\text{g/L}$  had significantly lower Performance and Full-Scale scores than did children with water As levels  $< 5.5 \mu\text{g/L}$ . Using the Full-Scale raw score, As water concentrations of 10 and  $50 \mu\text{g/L}$  were associated with decrements of 3.8 and 6.4 points, respectively. The relationships between urinary arsenic concentration ( $\mu\text{g As/g creatinine}$ ) 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.

### 6.2.3 Lung Effects

Smith et al. (1998) studied lung and urinary bladder cancer mortality in a region of northern Chile (Antofagasta) where the residents were exposed to arsenic in their drinking water. Arsenic levels ranged from a population weighted average of  $570 \mu\text{g/L}$  between 1955 and 1969 to  $100 \mu\text{g/L}$  by 1980. The mortality ratios (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,  $P < 0.001$ ). 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.

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 from 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).

#### **6.2.4 Reproductive and Other Effects**

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<sup>3</sup>; 10-100 ng/m<sup>3</sup>; and > 100 ng/m<sup>3</sup>). 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  $\geq 200$   $\mu\text{g/L}$  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 in 12 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  $\mu\text{g As}_2\text{O}_3/\text{m}^3$  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  $\text{As}_2\text{O}_3/\text{kg}/\text{day}$  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  $\text{As}_2\text{O}_3$  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  $\text{As}_2\text{O}_3$ . 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  $\text{As}_2\text{O}_3/\text{kg}/\text{day}$  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 following 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 ( $0.3 \text{ mg}/\text{m}^3$ ), 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  $\mu\text{g}/\text{m}^3$  (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 blank-corrected air concentration of arsine ranged from 0.010 to 0.067  $\text{mg}/\text{m}^3$ . While these concentrations are below the OSHA PEL 8-hour TWA of 0.2  $\text{mg}/\text{m}^3$ , 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<sub>50</sub> values reported by Gates (1946) are as follows: 120-210 ppm (380-670  $\text{mg}/\text{m}^3$ ) for 10 minutes in rats, 110 ppm (350  $\text{mg}/\text{m}^3$ ) for 30 minutes in dogs (equivalent to 190 ppm (610  $\text{mg}/\text{m}^3$ ) for 10 minutes), and 200-300 ppm (640-960  $\text{mg}/\text{m}^3$ ) for 10 minutes in rabbits. An LC<sub>50</sub> in mice was reported as 31 ppm (99  $\text{mg}/\text{m}^3$ ) for a 50-minute

exposure (Levy, 1947). The survival time of the fatalities (4 days) was reported to be more or less independent of exposure concentration (2500 mg/m<sup>3</sup> to 25 mg/m<sup>3</sup>) and exposure duration.

The study by Levy (1947) varied exposure durations for each given concentration of arsine. Because the mortality data were not presented in conventional form by the standard LC<sub>50</sub> 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 ( $\geq 5$  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 than simply concentration times time.

**Table 7.3.1 Arsine Mortality in Mice: Results from Levy (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<sub>05</sub> and BD<sub>05</sub>

Craig and Frye (1988) reported a 4-hour LC<sub>50</sub> 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<sub>50</sub> for females vs. 46.8 ppm LC<sub>50</sub> 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<sub>50</sub>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 <sup>1</sup>	$ED_{05}$ 60 min <sup>1</sup>	$BD_{05}$ 60 min <sup>1</sup>	$BD_{01}$ 60 min <sup>1</sup>
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 <sup>2</sup>	29	20	16	13

<sup>1</sup> Exposure time was extrapolated to 60 minutes, if needed, using a modification of Haber’s equation ( $C^n \times T = K$ ). 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,  $n = 1.8$ .

<sup>2</sup> 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/m<sup>3</sup>) and a NOAEL of 5 ppm (16 mg/m<sup>3</sup>) 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 demarcation 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 ( $\delta$ -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/m<sup>3</sup>) 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

<i>Study</i>	Nagymajtenyi et al., 1985
<i>Study population</i>	pregnant mice
<i>Exposure method</i>	maternal inhalation exposure
<i>Exposure continuity</i>	
<i>Exposure duration</i>	4 hours per day on gestation days 9, 10, 11, and 12
<i>Critical effects</i>	decreased fetal weight
<i>LOAEL</i>	0.26 mg/m <sup>3</sup> As <sub>2</sub> O <sub>3</sub> (0.197 mg As/m <sup>3</sup> )
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	n/a
<i>Human Equivalent Concentration</i>	n/a
<i>LOAEL uncertainty factor (UF<sub>L</sub>)</i>	10 (no NOAEL)
<i>Subchronic uncertainty factor (UFs)</i>	n/a
<i>Interspecies Uncertainty Factor</i>	
<i>Toxicokinetic (UF<sub>A-k</sub>)</i>	√10 (animal study)
<i>Toxicodynamic (UF<sub>A-d</sub>)</i>	√10 (animal study)
<i>Intraspecies Uncertainty Factor</i>	
<i>Toxicokinetic (UF<sub>H-k</sub>)</i>	√10 (remaining interindividual variation: study considered effects on fetus or infant)
<i>Toxicodynamic (UF<sub>H-d</sub>)</i>	√10 (interindividual variation)
<i>Cumulative uncertainty factor</i>	1,000
<i>Reference Exposure Level</i>	<b>0.0002 mg As/m<sup>3</sup> (0.20 µg As/m<sup>3</sup>)</b>

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  $\mu\text{g As/m}^3$  (260  $\mu\text{g As}_2\text{O}_3/\text{m}^3$ ) 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  $\mu\text{g As/m}^3$ , so 200  $\mu\text{g As/m}^3$  was taken as a LOAEL. Maternal toxicity data were not reported. This study is used as the basis of the acute REL:

$$0.2 \text{ mg/m}^3/1000 = 0.0002 \text{ mg/m}^3 = 0.2 \text{ } \mu\text{g As/m}^3 \text{ (equivalent to 0.065 ppb arsine gas)}$$

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 ( $\text{UF}_L$ ) 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 ( $\text{UF}_{A-k}$ ) and pharmacodynamic ( $\text{UF}_{A-d}$ ) differences, each assumed to be the  $\sqrt{10}$ . A final uncertainty factor of 10 was applied to address human interindividual differences in pharmacokinetics ( $\text{UF}_{H-k}$ ) and pharmacodynamics ( $\text{UF}_{H-d}$ ) 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

<i>Study</i>	Wasserman et al. (2004); Tsai et al. (2003)
<i>Study population</i>	201 children 10 years of age
<i>Exposure method</i>	drinking water
<i>Exposure continuity</i>	continuous
<i>Exposure duration</i>	9.5 to 10.5 years
<i>Critical effects</i>	Decrease in intellectual function, adverse effects on neurobehavioral development
<i>LOAEL</i>	0.23 $\mu\text{g As/m}^3$ based on est. LOAEL of 2.27 $\mu\text{g/L}$ (Wasserman et al., 2004; see Section 8.3.1.1)
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	117.8 $\mu\text{g As/L}$ (0.094 to 790 $\mu\text{g As/L}$ )
<i>Human DAF concentration</i>	
<i>LOAEL uncertainty factor (<math>UF_L</math>)</i>	3 (LOAEL estimated by quantitative analysis of study data)
<i>Subchronic uncertainty factor (<math>UF_s</math>)</i>	1 (default: duration >8% of lifetime)
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (<math>UF_{A-k}</math>)</i>	1 (default: human study)
<i>Toxicodynamic (<math>UF_{A-d}</math>)</i>	1 (default: human study)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (<math>UF_{H-k}</math>)</i>	$\sqrt{10}$ (remaining interindividual variation: study considered effects on 10 year-old but not infant)
<i>Toxicodynamic (<math>UF_{H-d}</math>)</i>	$\sqrt{10}$ (default, interindividual variation)
<i>Cumulative uncertainty factor</i>	30
<i>Inhalation Reference Exposure Level</i>	<b>0.015 <math>\mu\text{g As/m}^3</math></b>
<i>Oral Reference Exposure Level</i>	0.0035 $\mu\text{g/kg-d}$

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 Review and Analysis of Studies Supporting a Chronic REL

#### 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/ $\mu\text{g}/\text{L}$ . Assuming an adverse effect level of one point loss, then the corresponding arsenic concentration can be calculated as:

$$-1\text{point}/-0.44\text{ point}/\mu\text{g}/\text{L} = 2.27\ \mu\text{g}/\text{L}.$$

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  $2.3\ \mu\text{g}/\text{d}$ . 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  $1564\ \text{mL}/\text{day}$  the intake would be somewhat higher at  $3.6\ \mu\text{g}/\text{d}$  (OEHHA, 2000; Table 8.3). Since 10-year old males would inhale about  $9.9\ \text{m}^3/\text{d}$  (OEHHA, 2000), if airborne arsenic were 100% absorbed, this oral effect level would be equivalent to an inhalation level of  $2.3\ \mu\text{g}/\text{day}/9.9\ \text{m}^3/\text{day} = 0.23\ \mu\text{g}/\text{m}^3$ . Assuming a more realistic inhalation absorption of 50% would give a value of  $0.46\ \mu\text{g}/\text{m}^3$ . 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  $0.015\ \mu\text{g}/\text{m}^3$  can be calculated. An oral value based on the average study body weight of  $21.9\ \text{kg}$  and 100% oral absorption would be  $2.3\ \mu\text{g}/\text{d}/21.9\ \text{kg} = 0.105\ \mu\text{g}/\text{kg}\text{-day}$ . Applying the same overall uncertainty factor of 30 the oral health protective value would be  $0.105\ \mu\text{g}/\text{kg}\text{-day}/30 = 0.0035\ \mu\text{g}/\text{kg}\text{-day}$ .

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 ( $BMD_{05} = 49.75$ ;  $BMDL_{05} = 31.2$  ppb) and SA ( $BMD_{05} = 28.81$ ;  $BMDL_{05} = 19.73$  ppb) both using a linear model. For cumulative As intake the PM endpoint data were similarly fit by a linear model ( $BMD_{05} = 194.1$ ;  $BMDL_{05} = 122.7$  mg) and the SA data by a polynomial (quadratic) model ( $BMD_{05} = 39.1$ ;  $BMDL_{05} = 25.4$  mg; see Fig. 1). The SA endpoint appears to be the most sensitive. Based on the SA  $BMDL_{05}$  of 19.7 ppb and 1 L/d drinking water intake a minimum effect level of 19.7  $\mu\text{g}/\text{d}$  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  $\mu\text{g}/\text{day}$  (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  $19.7 \mu\text{g}/\text{d}/30 = 0.658 \mu\text{g}/\text{d}$  is calculated. Assuming inhalation of 10  $\text{m}^3/\text{d}$  and 50 % absorption (default) this value can be converted to an inhalation value of  $0.658 \mu\text{g}/\text{day}/(0.50 \times 10 \text{m}^3/\text{day}) = 1.32 \mu\text{g}/\text{m}^3$ . Using the SA cumulative  $BMDL_{05}$  of 25.4 mg As and 10 years exposure, an effect level of  $25.4 \text{mg}/(10 \text{yr} \times 365 \text{days}/\text{yr}) = 6.96 \mu\text{g}/\text{day}$  is calculated. Using the same assumptions and UFs as above, an inhalation value of  $0.044 \mu\text{g}/\text{m}^3$  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  $0.046 \mu\text{g}/\text{m}^3$  or  $0.05 \mu\text{g}/\text{m}^3$  (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  $6.96 \mu\text{g}/\text{day}/50 \text{kg} = 0.139 \mu\text{g}/\text{kg}\cdot\text{d}$ . Applying the same overall uncertainty factor of 30 would give  $0.139 \mu\text{g}/\text{kg}\cdot\text{day}/30 = 0.0046 \mu\text{g}/\text{kg}\cdot\text{day}$ .

The quantal responses for skin lesions in young children ( $\leq 9$  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  $BMD_{01} = 54.4 \mu\text{g}/\text{L}$  and a  $BMDL_{01} = 39.3 \mu\text{g}/\text{L}$ . For adolescents, the best fitting model was the log probit ( $X^2 = 0.77$ ,  $P = 0.68$ ) with a  $BMD_{01} = 77.3 \mu\text{g}/\text{L}$  and a  $BMDL_{01} = 47.4 \mu\text{g}/\text{L}$ . 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  $\mu\text{g}/\text{d}$  for one Liter/day water intake. For conversion to inhalation equivalent, young children are assumed to inhale 9.9  $\text{m}^3/\text{day}$  and adolescents 14  $\text{m}^3/\text{day}$  (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  $\mu\text{g}/\text{m}^3$ .

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  $BMD_{05} = 0.035 \mu\text{g}$  As/g hair and  $BMDL_{05} = 0.0155 \mu\text{g}$  As/g (polynomial model). A slope of  $-3.2$  IQ points/ $\mu\text{g}/\text{g}$  was derived from the  $BMDL_{05}$ . Using the conversion factor of  $0.01 \mu\text{g}$  As/g hair/ $\mu\text{g}$  As/Liter of water (Kurtio et al. 1998), a decrease of 1 IQ point would be equivalent to chronic consumption of 30  $\mu\text{g}$  As/L water (OEHHA, 2004). At one liter/day water consumption the 30  $\mu\text{g}/\text{d}$  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:  $30 \mu\text{g}/\text{day}/(10 \text{ m}^3/\text{day} \times 0.50 \times 30\text{UF}) = 0.20 \mu\text{g}/\text{m}^3$ .

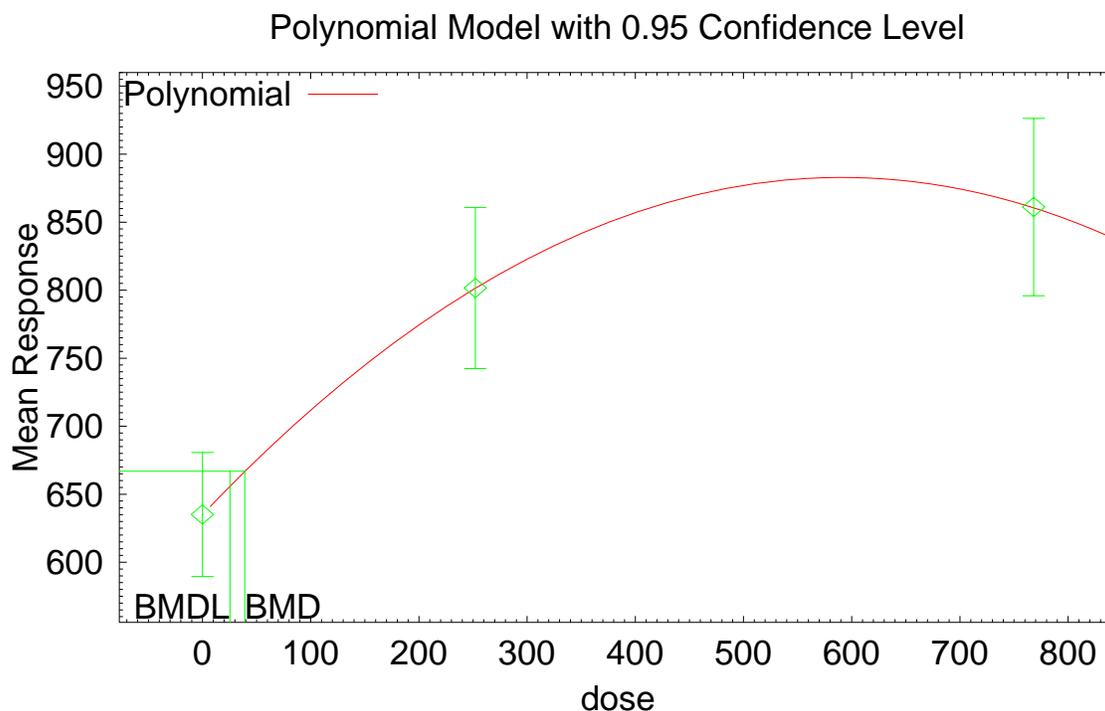
The visual perception data from Siripitayakunkit et al. (2001) was subjected to continuous benchmark dose analysis. The BMDL<sub>035</sub> of 2.40  $\mu\text{g}/\text{g}$  hair (polynomial model) was near the low level mean minus one SD score (20.5), presumably an adverse effect level on visual perception as defined by the authors. The linear model gave a higher value (3.69  $\mu\text{g}/\text{g}$ ) 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  $\mu\text{g}/\text{d}$  for this endpoint ( $2.40 \mu\text{g}/\text{g} \div 0.01 \mu\text{g}/\text{g}/\mu\text{g}/\text{Liter} \times 1 \text{ Liter}/\text{day} \div 30\text{UF} = 8.00 \mu\text{g}/\text{d}$ ). An inhalation value was derived as above:  $8.0 \mu\text{g}/\text{day}/(10 \text{ m}^3/\text{day} \times 0.50) = 1.6 \mu\text{g}/\text{m}^3$ .

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 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 to 1.6  $\mu\text{g}/\text{m}^3$ . The geometric mean of the three cognitive endpoint values is 0.053  $\mu\text{g}/\text{m}^3$ .

**Table 8.3.1. Inhalation Values Derived from Human Child Studies**

Study	Toxic Endpoint	Criterion	Value	Derived cREL, $\mu\text{g}/\text{m}^3$
Wasserman et al. (2004)	Intellectual function	One point loss	2.27 $\mu\text{g}/\text{d}$	0.015
Siripitayakunkit et al. (1999)	IQ	One point loss	-3.2 IQ/ $\mu\text{g}/\text{g}$ hair As	0.20
Siripitayakunkit et al. (2001)	Visual perception loss	LOAEL	240 $\mu\text{g}/\text{d}$	1.6
Mazumder et al. (1998)	Skin Lesions	LED <sub>01</sub>	39-47 $\mu\text{g}/\text{d}$	0.68-0.79
Tsai et al. (2003)	Neurobehavioral effects	LED <sub>05</sub>	7 $\mu\text{g}/\text{d}$	0.05

**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  $\mu\text{g}/\text{day}$  in Wasserman *et al* (2004) and 7  $\mu\text{g}/\text{day}$  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).

#### 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  $\mu\text{g As}/\text{m}^3$  for decreased bactericidal activity in mice (Aranyi et al., 1985);
- 200  $\mu\text{g As}/\text{m}^3$  for decreased fetal weight in mice (Nagymajtenyi et al., 1985); and
- 270  $\mu\text{g As}/\text{m}^3$  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  $\mu\text{g As}/\text{m}^3$ . 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  $\mu\text{g As}_2\text{O}_3/\text{m}^3$  (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 ( $\text{ED}_{01}$ ) and its 95% lower confidence limit ( $\text{LED}_{01}$ ), 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  $\text{ED}_{01}$  and  $\text{LED}_{01}$  values with goodness of fit P value meeting the acceptable fit criterion of  $P \geq 0.1$  were 359 and 189  $\mu\text{g}/\text{L}$  for CVD and 268 and 166  $\mu\text{g}/\text{L}$  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  $\text{LED}_{01}$  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  $\mu\text{g}/\text{L}$  based on the two dose metrics. For CVD with a 30 UF the corresponding values were 0.28 to 1.3  $\mu\text{g}/\text{L}$  (for details of analysis see OEHHA, 2004). Assuming 20  $\text{m}^3/\text{day}$  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  $\mu\text{g}/\text{m}^3$  and for CVD 0.28 to 1.26  $\mu\text{g}/\text{m}^3$ .

BMD analysis of the ISHD data from Chen et al. (1996) showed that these data were well fit by the QL dose-response equation ( $\text{ED}_{01} = 8.27$  (mg/L)-yr,  $X^2 = 0.26$ ,  $P = 0.88$ ). The  $\text{LED}_{01}$  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  $\mu\text{g}/\text{L}$  can be derived (OEHHA, 2004). Assuming 20  $\text{m}^3/\text{day}$  inhalation, 2 Liters/day

water consumption and 50 percent inhalation absorption the corresponding health protective inhalation value for ISHD would be  $0.16 \mu\text{g}/\text{m}^3$ .

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. For HT the  $\text{LED}_{10}$  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  $\text{LED}_{10}$  of  $7.4 (\text{mg}/\text{L})\text{-yr}$ . 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  $\text{LED}_{10}$  value of  $6.3 (\text{mg}/\text{L})\text{-yr}$  from Bangladesh is quite similar to comparable value of  $7.2 (\text{mg}/\text{L})\text{-yr}$  from the Taiwan study (OEHHA, 2004). Health protective drinking water concentrations with a cumulative uncertainty factor of 30 ranged from  $0.55$  to  $0.68 \mu\text{g}/\text{Liter}$ . Assuming  $20 \text{ m}^3/\text{day}$  inhalation,  $2 \text{ Liters}/\text{day}$  water consumption and 50 percent inhalation absorption the corresponding health protective inhalation value for HT would be  $0.55$  to  $0.70 \mu\text{g}/\text{m}^3$ .

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  $(\text{mg}/\text{L})\text{-yr}$ . 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  $\text{BMDL}_1$  values (1% response) of  $71.5 \mu\text{g}/\text{L}$  and  $0.66 (\text{mg}/\text{L})\text{-yr}$  were obtained ( $X^2 = 3.8$ ,  $P = 0.15$ ,  $\text{d.f.} = 2$ ). 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  $\text{BMDL}_{10}$ 's of  $0.49 \mu\text{g}/\text{L}$  and  $0.004 (\text{mg}/\text{L})\text{-yr}$  ( $X^2 = 4.45$ ,  $P = 0.11$ ,  $\text{d.f.} = 2$ ). TWA  $\text{BMDL}$ s for systolic and pulse hypertension in arsenic exposed subpopulations with lower intakes of B vitamins were also evaluated. The  $\text{BMDL}_{10}$  values for populations with low dietary folate ranged from  $62$  to  $405 \mu\text{g}/\text{L}$  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).

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  $\text{LED}_{05}$  for the adjusted values appear the best choice for a chronic criterion for arsenic-induced diabetes mellitus, i.e.,  $8.8 (\text{mg}/\text{L})\text{-yr}$  from Lai *et al.* and  $0.21 \text{ mg}/\text{L}$  from Rahman *et al.* The health protective drinking water derived from these values with a cumulative UF of 30 were  $0.84$  and  $1.4 \mu\text{g}/\text{L}$ , respectively (OEHHA,

2004). Assuming 20 m<sup>3</sup>/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<sup>3</sup>.

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<sup>3</sup>/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<sup>3</sup>. 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<sub>01</sub>) 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<sub>01</sub>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<sup>3</sup>/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<sup>3</sup>.

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<sub>10</sub> of 6.28 µg/L with mid-point based exposure estimates, and the former giving a BMDL<sub>10</sub> 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<sub>10</sub> 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<sub>10</sub> = 96.0 µg/L (X<sup>2</sup> = 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 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 one order of magnitude from 0.16 to 1.7  $\mu\text{g}/\text{m}^3$ . These adult values exceed the child-based values (range 0.015 to 1.6  $\mu\text{g}/\text{m}^3$ ). Therefore the proposed chronic REL value of 0.015  $\mu\text{g}/\text{m}^3$  is derived from the child arsenic exposure studies evaluated above and the adult studies provide supporting information.

**Table 8.3.2 Inhalation Values Derived from Adult Human Drinking Water Studies**

Study	Toxic Endpoint	Criterion	Value	Derived chronic REL, ( $\mu\text{g}/\text{m}^3$ )
Chiou et al. (1997b)	Cerebrovascular disease	LED <sub>01</sub>	378 $\mu\text{g}/\text{d}$	1.26
Chiou et al. (1997b)	Cerebrovascular infarct	LED <sub>01</sub>	332 $\mu\text{g}/\text{d}$	0.33
Chen et al. (1996a)	Ischemic Heart Disease Mortality	LED <sub>01</sub>	5.53 (mg/L)-yr	0.16
Chen et al. (1995)	Hypertension	LED <sub>10</sub>	5.8 (mg/L)-yr	0.55
Chen et al. (2006)	Systolic and pulse hypertension	SHT LED <sub>01</sub> PHT LED <sub>10</sub>	71.5 $\mu\text{g}/\text{L}$ 0.49 $\mu\text{g}/\text{L}$	1.43 0.0033
Lai et al. (1994)	Diabetes mellitus	LED <sub>05</sub>	8.8 (mg/L)-yr	0.85
Rahman et al. (1998)	Diabetes mellitus	LED <sub>05</sub>	0.21 mg/L	1.4
Mazumder et al. (1998)	Skin keratosis	LED <sub>01</sub>	50 $\mu\text{g}/\text{L}$	0.33
Rahman et al. (2006)	Skin keratosis or altered pigmentation	LED <sub>10</sub>	65.4 $\mu\text{g}/\text{L}$	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

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  $\mu\text{g}/\text{kg}\text{-day}$  is also proposed.

## 9. Arsinic 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 (AEG-2, disabling) of 0.17 ppm ( $500 \mu\text{g}/\text{m}^3$ ) 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 AEG-1 (Non-disabling) was considered inappropriate. Also the reliance on animal data was considered more “scientifically valid than AEGs 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  $\text{BMDL}_{1SD}$  of 2.17 ppm ( $6.9 \text{ mg}/\text{m}^3$ ) 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 aREL for a one hour exposure was  $2.17 \text{ ppm}/300 = 0.0072 \text{ ppm}$  ( $23 \mu\text{g}/\text{m}^3$ ).

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 ( $0.2 \mu\text{g}/\text{m}^3$  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 ( $4.98 \text{ mg}/\text{m}^3$ )/300UF = 0.053 ppm ( $17 \mu\text{g}/\text{m}^3$ ). 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  $\text{mg}/\text{m}^3$ . 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  $0.04 \text{ mg/m}^3/30 \text{ UF} = 0.001 \text{ mg/m}^3$  or  $1 \mu\text{g/m}^3$ . 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  $0.26 \mu\text{g/m}^3$ . 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 $\mu\text{g/m}^3$
Peterson and Bhattacharyya, 1985	Reticulocytosis in mice 1 hour exposure	BMDL <sub>1SD</sub> 2.17 ppm 6.9 $\text{mg/m}^3$	Acute 23
Peterson and Bhattacharyya, 1985	As above with 8-hour adjustment	1.6 ppm 4.98 $\text{mg/m}^3$	8-hour 17
Williams <i>et al.</i> , 1981	Headache, nausea, weakness, and vomiting in exposed workers	0.01 to 0.07 $\text{mg/m}^3$ , average 0.04 $\text{mg/m}^3$ LOAEL.	8-hour 1.0
Blair <i>et al.</i> , 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.,  $0.015 \mu\text{g/m}^3$  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. Battelle 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 effects 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.
- Levy GA (1947). A study of arsine poisoning. *Quart J Exp Physiol* 34: 47-67.
- Lewis DR, Southwick JW, Ouellet-Hellstrom R, Rensch 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 Cothorn 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.

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 Cothorn 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, Selyes 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](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 poisoning 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 extrapolation. 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 of 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 Massachusetts School 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, Nemecek 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 (<sup>74</sup>As) 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: *Biological 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 <sup>74</sup>As-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, Lolocono NJ, Cheng Z, Hussain I, Momotaj H and Graziano JH (2004). Water arsenic exposure and children's intellectual function in Araihaazar, 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 Toxicological 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 administered 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

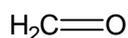
methyltransferases that methylate inorganic arsenic. *Toxicol Appl Pharmacol* 140(1): 77-84.

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.

# 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, asthma-like symptoms, 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 of asthma than are adults following chronic exposure to formaldehyde.

### 1.1 Formaldehyde Acute REL

<i>Reference Exposure Level</i>	<b>55 µg/m<sup>3</sup> (44 ppb)</b>
<i>Critical effect(s)</i>	Mild and moderate eye irritation
<i>Hazard Index target(s)</i>	Eye irritation

### 1.2 Formaldehyde 8-Hour REL

<i>Reference Exposure Level</i>	<b>9 µg/m<sup>3</sup> (7 ppb)</b>
<i>Critical effect(s)</i>	Asthma-like respiratory symptoms
<i>Hazard Index target(s)</i>	Respiratory

### 1.3 Formaldehyde Chronic REL

<i>Reference Exposure Level</i>	<b>9 µg/m<sup>3</sup> (7 ppb)</b>
<i>Critical effect(s)</i>	Asthma-like respiratory symptoms
<i>Hazard Index target(s)</i>	Respiratory

## 2. Physical & Chemical Properties

<i>Description</i>	Colorless gas
<i>Molecular formula</i>	CH <sub>2</sub> O
<i>Molecular weight</i>	30.03 g/mol
<i>Density</i>	0.815 g/L @ -20° C
<i>Boiling point</i>	-19.5° C
<i>Melting point</i>	-92° C
<i>Vapor pressure</i>	3883 mm Hg @ 25° C
<i>Flashpoint</i>	300° C
<i>Explosive limits</i>	7% - 73%
<i>Solubility</i>	soluble in water, alcohol, ether and other polar solvents
<i>Odor threshold</i>	0.05-0.5 ppm
<i>Metabolites</i>	formic acid
<i>Conversion factor</i>	1 ppm in air = 1.24 mg/m <sup>3</sup> @ 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. 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 mucous layer of the respiratory tract is reversibly hydrated to methylene glycol. Both the hydrated and free formaldehyde may be absorbed into the epithelial layer where formaldehyde may bind reversibly to glutathione to form S-hydroxymethylglutathione. This 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<sub>2</sub> 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).

## **5. Acute Toxicity of Formaldehyde**

### **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.

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<sup>3</sup>) for 35 minutes. Thresholds were 1.2 ppm (1.5 mg/m<sup>3</sup>) for eye and nose irritation, 1.7 ppm (2.1 mg/m<sup>3</sup>) for eye blinking, and 2.1 ppm (2.6 mg/m<sup>3</sup>) for throat irritation.

Kulle et al. (1987) exposed nonasthmatic humans to up to 3.0 ppm (3.7 mg/m<sup>3</sup>) 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 hours after exposure.

**Table 5.1 Mean Symptom Difference ( $t_{180}-t_0$ )  $\pm$  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 $\pm$ 0.00	0.22 $\pm$ 0.15	0.44 $\pm$ 0.18	1.00 $\pm$ 0.29	<0.0001
Nose/throat irritation	0.00 $\pm$ 0.00	0.11 $\pm$ 0.11	0.33 $\pm$ 0.17	0.22 $\pm$ 0.15	0.054
Eye irritation	0.00 $\pm$ 0.00	0.44 $\pm$ 0.24	0.89 $\pm$ 0.26	1.44 $\pm$ 0.18	<0.0001
Chest discomfort	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.11 $\pm$ 0.11	0.00 $\pm$ 0.00	0.62
Cough	0.00 $\pm$ 0.00	0.11 $\pm$ 0.11	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.11
Headache	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.11 $\pm$ 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). n=9

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<sup>3</sup>). 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<sup>3</sup>) 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/m<sup>3</sup> (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<sub>1</sub>) (defined as > 20% or more) and FEV<sub>1</sub>/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<sub>1</sub> of 20% or more during exposure. One of 22 asthmatics had a greater than 20% reduction in FEV<sub>1</sub> (-25.8%) at 17 minutes into exposure following a 15 minute moderate exercise session (minute ventilation [V<sub>E</sub>] = 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<sub>1</sub> (-24.4%) and in FEV<sub>1</sub>/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<sub>1</sub> (-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<sub>1</sub> of greater than 20% following exposure also exhibited FEV<sub>1</sub>/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<sub>aw</sub>) 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  $0.70 \pm 2.13$  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 asthma-like conditions 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 asthma-like 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.

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<sub>1</sub>) were observed in 12 occupationally-exposed workers challenged with 1.67 ppm (2.5 mg/m<sup>3</sup>) 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<sup>3</sup> 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. The significance of this result is unclear but may have repercussions for toxicological effects mediated by active oxygen species.

#### *Predisposing Conditions for Formaldehyde Toxicity*

**Medical:** Persons with eye, skin, respiratory, or allergic conditions (especially asthma) may be more sensitive to the effects of formaldehyde (ATSDR, 1999). Asthmatics sensitized to formaldehyde may be more sensitive to formaldehyde at low concentrations than non-sensitized individuals.

## 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 asthma-like 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. 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<sup>3</sup> (500-1,400 ppm) formaldehyde vapor for 30 minutes, the LC<sub>50</sub> was found to be 1,000 mg/m<sup>3</sup> (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<sup>3</sup>) formaldehyde vapor killed 3/5 rabbits, 8/20 guinea pigs, and 17/50 mice. The report stated that formaldehyde exposure resulted in delayed lethality.

Alarie (1981) determined the 10 minute LC<sub>50</sub> 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<sub>05</sub> and BC<sub>05</sub> 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, Nagorny et al. (1979) determined a 4-hour formaldehyde LC<sub>50</sub> in rats and mice to be 588 mg/m<sup>3</sup> (474 ppm) and 505 mg/m<sup>3</sup> (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<sup>3</sup>) formaldehyde resulted in deaths from massive pulmonary hemorrhage and edema, but a 2 hour exposure to 0.14 mg/l (140 mg/m<sup>3</sup>) 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<sup>3</sup>) 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<sup>3</sup>) formaldehyde for 8 hours. An 8-hour exposure to ≥0.3 ppm (≥ 0.4 mg/m<sup>3</sup>) formaldehyde was sufficient to produce a significant increase in airway reactivity. Similar effects occurred after > 9 ppm (> 11 mg/m<sup>3</sup>) 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  $10 \text{ mg/m}^3$  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-IgG1 (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-IgG1 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.

## 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.

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<sup>3</sup>) 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 0.06 ppm (0.09 mg/m<sup>3</sup>) 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)

	<b>Formaldehyde</b>	<b>Reference</b>	<b>Rate difference</b>	
	% (n=96)	% (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<sup>3</sup> (0.08- 0.89 ppm) with peaks of up to 5 mg/m<sup>3</sup> (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<sup>3</sup> range.

**Table 6.1.2 Distribution of Histological Characteristics Associated with Formaldehyde Exposure** (from Edling et al., 1988)

<b>Histological characteristic</b>	<b>Grading score</b>	<b>Point score</b>	<b>Workers</b>	<b>%</b>
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	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 was 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**

	No	Histological score						Mean
		0	1	2	3	4	5	
Exposed	37	3	16	5	9	1	3	1.9
Controls	37	5	17	10	5	0	0	1.4

Rhinoscopic 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<sup>3</sup>) 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<sup>3</sup>) (range = 0.24 - 0.55 mg/m<sup>3</sup>). 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<sup>3</sup>) 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<sub>1</sub>, FEF<sub>25-75</sub>, FEF<sub>50</sub>), 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<sub>1</sub>, and FEF<sub>25-75</sub>. 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<sub>1</sub>, FEF<sub>25-75</sub>, and FEF<sub>75-85</sub>, 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<sub>1</sub>, FEV<sub>1</sub>/FVC, and forced expiratory flow rate at 25% through 75% of FVC (FEF<sub>25-75</sub>), 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<sup>3</sup>) 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<sup>3</sup>) 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<sub>1</sub>, FEV<sub>1</sub>/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<sup>3</sup>) and 0.05 ppm (0.08 mg/m<sup>3</sup>), 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<sup>3</sup>). 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<sub>1</sub>, FEV<sub>1</sub>/FVC ratio, FEF<sub>50</sub>, FEF<sub>25</sub>, and FEF<sub>75</sub> 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<sub>1</sub>, 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<sup>3</sup>).

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<sub>1</sub> ( $p < 0.05$ ) 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<sub>1</sub>) were reported in 12 workers challenged with 1.67 ppm (2.5 mg/m<sup>3</sup>) 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 neurologically, 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 6.1). 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)

Bronchitis	Formaldehyde (ppb)			P value $X^2$ trend
	≤ 40 (N)	41-60 (N)	>60 (N)	
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
<b>Asthma</b>				
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  $\pm$  SE:  $-1.28 \pm 0.46$  vs  $0.09 \pm 0.27$ ). Measurements of PEFR in the morning suggested that children with asthma ( $n = 4$ ) were more severely affected than healthy children (coefficient  $\pm$  SE:  $-1.45 \pm 0.53$  vs  $0.09 \pm 0.15$ ) (Table 6.2). 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)

<b>Factor</b>	<b>Child coefficient <math>\pm</math> SE</b>	<b>Adult coefficient <math>\pm</math> SE</b>
HCHO house mean	$-1.28 \pm 0.46$	$0.09 \pm 0.27$
Morning vs bedtime	$-6.10 \pm 3.0$	$-5.90 \pm 1.10$
HCHO bdrm mean/morning	$0.09 \pm 0.15$	$-0.07 \pm 0.04$
HCHO bdrm mean/morning/asthma	$-1.45 \pm 0.53$	

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 asthma symptoms. Estimates of the relative risk for 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  $\geq 50$  ppb compared with those from homes with levels  $\leq 50$  ppb ( $15.5$  ppb eNO vs  $8.7$ ;  $p = 0.02$ ).

Garrett et al. (1999) examined the association between formaldehyde levels at home (median  $15.8 \mu\text{g}/\text{m}^3$ ; maximum  $139 \mu\text{g}/\text{m}^3$ ) and atopy and allergic sensitization in 148 children, 7-14 years of age. The risk of atopy increased by 40% with each  $10 \mu\text{g}/\text{m}^3$  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  $32 \mu\text{g}/\text{m}^3$  (26 ppb) and  $92 \mu\text{g}/\text{m}^3$  (75 ppb), respectively, after adjustment for exposure continuity. These values are based on data on nasal and eye irritation observed in Wilhelmsson and Holstrom (1992), and histological lesions in the nasal cavity 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.

### **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 sensitization (Sorg et al., 2001b) and altered expression of stress hormones (Sorg et al., 2001a).

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 of increased incidences 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 18<sup>th</sup> 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<sup>3</sup>) 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<sup>3</sup>) 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., 2001a) and on neurobehavioral changes in rats (Sorg et al., 2001b). To study formaldehyde's effects on the HPA, Sorg et al. (2001a) 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 mRNA 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<sup>3</sup>, but not at 0.5 mg/m<sup>3</sup>, 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.

## 8. Derivation of Reference Exposure Levels

### 8.1 Formaldehyde Acute Reference Exposure Level

<i>Study</i>	Kulle et al., 1987
<i>Study population</i>	19 nonasthmatic, nonsmoking humans
<i>Exposure method</i>	Whole body to 0.5-3.0 ppm
<i>Exposure continuity</i>	
<i>Exposure duration</i>	3 hr
<i>Critical effects</i>	mild and moderate eye irritation
<i>LOAEL</i>	1 ppm
<i>NOAEL</i>	0.5 ppm
<i>Benchmark concentration</i>	0.44 ppm
<i>Time-adjusted exposure</i>	not applied
<i>Human Equivalent Concentration</i>	not applied
<i>LOAEL uncertainty factor (UF<sub>L</sub>)</i>	not applied
<i>Subchronic uncertainty factor (UF<sub>s</sub>)</i>	not applied
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>A-k</sub>)</i>	1 (default, human study)
<i>Toxicodynamic (UF<sub>A-d</sub>)</i>	1 (default, human study)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>H-k</sub>)</i>	1 (site of contact; no systemic effects)
<i>Toxicodynamic (UF<sub>H-d</sub>)</i>	10 (asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	10
<i>Reference Exposure Level</i>	<b>55 µg/m<sup>3</sup> (44 ppb)</b>

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, and it featured human subjects with short-term exposures to a range of formaldehyde concentrations that 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 BMC<sub>05</sub> for eye irritation, estimated using log-probit analysis (Crump, 1984). The BMC<sub>05</sub> is defined as the 95% lower confidence limit of the concentration expected to produce a response rate of 5%. The resulting BMC<sub>05</sub> from this analysis was 0.44 ppm (0.53 mg/m<sup>3</sup>) 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<sub>H-k</sub>) 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. 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.

## 8.2 Formaldehyde 8-Hour Reference Exposure Level

<i>Study</i>	Wilhelmsson and Holmstrom, 1992
<i>Study population</i>	66 chemical plant workers
<i>Exposure method</i>	Discontinuous occupational exposure
<i>Exposure continuity</i>	8 hr/day, 5 days/week (assumed)
<i>Exposure duration</i>	10 years (average); range 1-36 years
<i>Critical effects</i>	Nasal obstruction and discomfort, lower airway discomfort, and eye irritation.
<i>LOAEL</i>	Mean 0.26 mg/m <sup>3</sup> (range 0.05 – 0.6 mg/m <sup>3</sup> ) (described as exposed group)
<i>NOAEL</i>	Mean of 0.09 mg/m <sup>3</sup> (described as control group of office workers)
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	0.09 mg/m <sup>3</sup> (time adjustment not applied)
<i>Human Equivalent Concentration</i>	not applied
<i>LOAEL uncertainty factor (<math>UF_L</math>)</i>	1 (NOAEL observed)
<i>Subchronic uncertainty factor (<math>UF_s</math>)</i>	not applied
<i>Interspecies Uncertainty Factor</i>	
<i>Toxicokinetic (<math>UF_{A-k}</math>)</i>	1 (default, human study)
<i>Toxicodynamic (<math>UF_{A-d}</math>)</i>	1 (default, human study)
<i>Intraspecies Uncertainty Factor</i>	
<i>Toxicokinetic (<math>UF_{H-k}</math>)</i>	1 (site of contact; no systemic effects)
<i>Toxicodynamic (<math>UF_{H-d}</math>)</i>	10 (asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	10
<i>Reference Exposure Level</i>	<b>9 µg/m<sup>3</sup> (7 ppb)</b>

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<sup>3</sup> during the work day compared with a referent group exposed to 0.09 mg/m<sup>3</sup>. 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 was applied.

For comparison, the 8-hour REL of  $9 \mu\text{g}/\text{m}^3$  is similar to the value of  $10 \mu\text{g}/\text{m}^3$  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.

<i>Study</i>	Swiecichowski et al., 1993
<i>Study population</i>	25-35 adult male guinea pigs
<i>Exposure method</i>	Whole body exposure
<i>Exposure continuity</i>	
<i>Exposure duration</i>	8 hr
<i>Critical effects</i>	Increased specific pulmonary resistance
<i>LOAEL</i>	1.0 ppm
<i>NOAEL</i>	0.59 ppm
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	not applied
<i>Human Equivalent Concentration</i>	0.49 ppm ( $610 \mu\text{g}/\text{m}^3$ ) ( $0.59 * \text{RGDR } 0.826$ for pulmonary effects)
<i>LOAEL uncertainty factor (<math>UF_L</math>)</i>	1 (default: NOAEL observed)
<i>Subchronic uncertainty factor (<math>UF_s</math>)</i>	not applied
<i>Interspecies Uncertainty Factor</i>	
<i>Toxicokinetic (<math>UF_{A-k}</math>)</i>	6 (with HEC adjustment)
<i>Toxicodynamic (<math>UF_{A-d}</math>)</i>	1 (with HEC adjustment)
<i>Intraspecies Uncertainty Factor</i>	
<i>Toxicokinetic (<math>UF_{H-k}</math>)</i>	1 (no systemic effect)
<i>Toxicodynamic (<math>UF_{H-d}</math>)</i>	10 (asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	60
<i>Reference Exposure Level</i>	<b><math>10 \mu\text{g}/\text{m}^3</math> (8 ppb)</b>

**Formaldehyde Chronic Reference Exposure Level**

<i>Study</i>	Wilhelmsson and Holmstrom, 1992 supported by Edling et al., 1988
<i>Study population</i>	66 human chemical plant workers
<i>Exposure method</i>	Discontinuous occupational exposure
<i>Exposure continuity</i>	8 hr/day, 5 days/week (assumed)
<i>Exposure duration</i>	10 years (average); range 1-36 years
<i>Critical effects</i>	Nasal obstruction and discomfort, lower airway discomfort; histopathological nasal lesions including rhinitis, squamous metaplasia, and dysplasia
<i>LOAEL</i>	Mean 0.26 mg/m <sup>3</sup> (range 0.05 – 0.6 mg/m <sup>3</sup> ) (described as exposed group)
<i>NOAEL</i>	Mean of 0.09 mg/m <sup>3</sup> (described as control group of office workers)
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	0.09 mg/m <sup>3</sup> for NOAEL group
<i>Human Equivalent Concentration</i>	not applied
<i>LOAEL uncertainty factor (UF<sub>L</sub>)</i>	not applied
<i>Subchronic uncertainty factor (UF<sub>s</sub>)</i>	not applied
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>A-k</sub>)</i>	1 (default, human study)
<i>Toxicodynamic (UF<sub>A-d</sub>)</i>	1 (default, human study)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>H-k</sub>)</i>	1 (no systemic effects)
<i>Toxicodynamic (UF<sub>H-d</sub>)</i>	10 (asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	10
<i>Reference Exposure Level</i>	<b>9 µg/m<sup>3</sup> (7 ppb)</b>

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<sup>3</sup>) 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<sup>3</sup>, 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  $10 \mu\text{g}/\text{m}^3$  (8 ppb), similar to the chronic REL calculated from the critical study.

<i>Study</i>	Rumchev et al., 2002
<i>Study population</i>	88 asthmatic children (mean age 25 mo); 104 nonasthmatic controls (mean age 20 mo)
<i>Exposure method</i>	Ambient in home
<i>Exposure continuity</i>	Continuous assumed
<i>Exposure duration</i>	range 0.5-3 years
<i>Critical effects</i>	Parent-reported asthma-related respiratory symptoms
<i>LOAEL</i>	$60 \mu\text{g}/\text{m}^3$
<i>NOAEL</i>	$30 \mu\text{g}/\text{m}^3$ (lower limit of NOAEL range)
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	not applied
<i>Human Equivalent Concentration</i>	$30 \mu\text{g}/\text{m}^3$
<i>LOAEL uncertainty factor (<math>UF_L</math>)</i>	1
<i>Subchronic uncertainty factor (<math>UF_s</math>)</i>	not applied
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (<math>UF_{A-k}</math>)</i>	1 (default, human study)
<i>Toxicodynamic (<math>UF_{A-d}</math>)</i>	1 (default, human study)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (<math>UF_{H-k}</math>)</i>	1 (study performed in children)
<i>Toxicodynamic (<math>UF_{H-d}</math>)</i>	$\sqrt{10}$ (inter-individual variation)
<i>Cumulative uncertainty factor</i>	$\sqrt{10}$
<i>Reference Exposure Level</i>	<b><math>10 \mu\text{g}/\text{m}^3</math> (8 ppb)</b>

The Rumchev study supports an association with exposure to formaldehyde and the observation of asthma-like symptom 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  $9 \mu\text{g}/\text{m}^3$  (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 dysplasia 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” ( $\leq 14$  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 ( $\geq 365$  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.

#### **8.4 Formaldehyde as a Toxic Air Contaminant**

In view of the differential impacts on infants and children identified in Section 6.2, OEHHA recommends that formaldehyde be identified 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	NOAEL	Time	LOAEL						Cum	REL	REL	
				ppm	ppm	adj	DAF	UF	UFak	UFad	UFhk	UFhd	UFsc	UF	ppb	µg/m <sup>3</sup>
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

## 9.0 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>.

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.

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.

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.

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.

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]. *Tsitologiya* 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.
- 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.

Nagorny 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.

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*. Available online at [http://www.oehha.ca.gov/air/chronic\\_rels/pdf/relsP32k.pdf](http://www.oehha.ca.gov/air/chronic_rels/pdf/relsP32k.pdf).  
[http://www.oehha.ca.gov/air/chronic\\_rels/pdf/relsP32k.pdf](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.

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.

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 adrenocorticotrophic 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 (2001a). 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 (2001b). Repeated formaldehyde effects in an animal model for multiple chemical sensitivity. *Annals of the New York Academy of Sciences* 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.

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.

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.

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.

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.

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.

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.

#### 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*  
*Critical effect(s)*

**0.05  $\mu\text{g}/\text{m}^3$**   
Impairment of neurobehavioral function in  
humans

*Hazard index target(s)*

Nervous system

#### 1.3 Manganese Chronic REL

*Reference Exposure Level*  
*Critical effect(s)*

**0.03  $\mu\text{g}/\text{m}^3$**   
Impairment of neurobehavioral function in  
humans

*Hazard index target(s)*

Nervous system

## 2. Physical and Chemical Properties

**Table 2.1 Manganese and Manganese Species\***

<i>Molecular Formula</i>	<i>Synonyms</i>	<i>Molecular Weight</i>	<i>CAS Reg. No.</i>
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 <sub>2</sub>	manganese dioxide; black manganese oxide	86.94 g/mol	1313-13-9
Mn <sub>3</sub> O <sub>4</sub>	manganese tetroxide; trimanganese tetraoxide; manganomanganic oxide	228.82 g/mol	1317-35-7
MnCl <sub>2</sub>	manganese chloride; manganese dichloride; manganous chloride	125.84 g/mol	7773-01-5
<i>Description</i>	Lustrous, gray-pink metal (Mn); green (MnO), black (MnO <sub>2</sub> ) or pink (MnCl <sub>2</sub> ) crystals; brownish-black powder (Mn <sub>3</sub> O <sub>4</sub> )		
<i>Molecular formula</i>	see Table 2.1		
<i>Molecular weight</i>	see Table 2.1		
<i>Density (in g/cm<sup>3</sup>)</i>	7.21-7.4 (Mn – depending on allotropic form); 5.43-5.46 (MnO); 4.88 (Mn <sub>3</sub> O <sub>4</sub> ); 2.977 @ 25°C (MnCl <sub>2</sub> )		
<i>Boiling point</i>	2095°C (Mn); not available (MnO); unknown (Mn <sub>3</sub> O <sub>4</sub> ); 1190°C (MnCl <sub>2</sub> )		
<i>Melting point</i>	1246°C (Mn); 1839°C (MnO); 1567°C (Mn <sub>3</sub> O <sub>4</sub> ); 650°C (MnCl <sub>2</sub> ) (CRC, 2005)		
<i>Vapor pressure</i>	1 torr @ 1292°C (Mn); non-volatile at room temperature (Mn <sub>3</sub> O <sub>4</sub> ); not available (MnO; MnCl <sub>2</sub> )		
<i>Solubility</i>	Sol. in dil. acids and aq. solns. of Na- or K-bicarbonate (Mn); sol. in NH <sub>4</sub> Cl, insol. in H <sub>2</sub> O (MnO); insol. in H <sub>2</sub> O, HNO <sub>3</sub> , or cold H <sub>2</sub> SO <sub>4</sub> (MnO <sub>2</sub> -(Merck, 1976); insol. in H <sub>2</sub> O, sol. in HCl (Mn <sub>3</sub> O <sub>4</sub> ); 72.3 g/100 ml H <sub>2</sub> O @ 25°C (MnCl <sub>2</sub> )		
<i>Conversion factor</i>	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. Manganese dioxide is used in batteries and may also be generated from the welding of manganese alloys. Manganese tetroxide may be generated in situations where other oxides of manganese are heated in air (NIOSH, 2005). 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<sup>3</sup>.

### 4. Metabolism / Toxicokinetics

Manganese can enter the body by both the oral and inhalation routes. Dermal absorption of manganese is insignificant. Essential manganese is 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 it subsequently bound in plasma to  $\beta_1$ -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 accumbans and the caudate putamen. Other mechanisms also appear to contribute to brain uptake of manganese including a divalent metal transporter (DMT-I), 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).

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 rats, Roels *et al.* (1997) used

intratracheal instillation as a surrogate for inhalation for comparison with the oral route (gavage). Intratracheal instillation of MnCl<sub>2</sub> (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<sub>2</sub> (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<sub>2</sub> (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<sub>2</sub>/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 result 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 <sub>2</sub> Intratracheal (1.2 mg/kg)	68	205	48	27
MnCl <sub>2</sub> Gavage (24.3 mg/kg)	68	0	22	0
MnO <sub>2</sub> Intratracheal (1.2 mg/kg)	41	48	34	31
MnO <sub>2</sub> Gavage (24.3 mg/kg)	0	0	0	0

Using the same exposure protocol with the less soluble MnO<sub>2</sub>, 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<sub>2</sub>, Mn blood levels following intratracheal MnO<sub>2</sub> 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<sub>2</sub>, 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 rats to 0, 2, or 6 mg/kg Mn(III)-pyrophosphate or Mn(II)Cl<sub>2</sub> 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 (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. 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  $^{54}\text{MnCl}_2$  and insoluble  $^{54}\text{Mn}_3\text{O}_4$  after instillation into the 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 ( $^{54}\text{Mn}$ ) 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.

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 it has been demonstrated that 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). 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. It is not clear how significant this route of exposure is in humans.

The major route of excretion of manganese is via bile, although a lesser amount is excreted via urine (Davis et al., 1993; ATSDR, 2000). 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).

## 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). However, a pulmonary inflammatory response is also associated with the inhalation of particulates in general and does not appear to be dependent on the manganese content. 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 female CD-1 mice to manganese oxide ( $Mn_3O_4$ ) aerosols (Adkins et al., 1980) that resulted in a NOAEL of  $2.9 \text{ mg/m}^3$  based on respiratory effects (edema). The other is a 24 hr exposure of guinea pigs to  $22 \text{ mg/m}^3$   $MnO_2$  (Bergstrom, 1977) that resulted in a NOAEL of  $0.9 \text{ mg/m}^3$ , corrected for one hour, again for respiratory effects (inflammatory reaction). However, no LOAEL was observed in either study, and no manganese acute inhalation studies 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 (ATSDR, 2000). 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 (PET) (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 (ATSDR, 2000), 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 exposure levels to manganese that are too low to cause frank manganism. Lucchini and his co-workers studied a group of 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 0.054 mg Mn per m<sup>3</sup> air at the time of the study. Earlier exposures were higher. In order to obtain a measure of cumulative exposure the investigators calculated a “cumulative exposure index” for each worker based on their exposure history in the factory. The cumulative exposure indices 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 cumulative exposure index 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.

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<sup>3</sup> \* year, with a geometric mean of 0.793 mg Mn/m<sup>3</sup> \* 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 LOAEL for this study, based on neurobehavioral effects, corresponds to 0.15 mg respirable manganese dust/m<sup>3</sup> (geometric mean of the LIRD divided by the average exposure time = 0.793 mg Mn/m<sup>3</sup> \* year / 5.3 year = 0.15 mg Mn/m<sup>3</sup>). This LOAEL was used to calculate the 8-hr and chronic RELs.

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 value concentrations of  $0.18 \text{ mg/m}^3$  at one smelter, and  $0.41 \text{ mg/m}^3$  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.

## 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. Infants and children are expected to be more susceptible than adults to the toxic effects of elevated manganese because they absorb greater amounts of manganese in their gastrointestinal tract, the infant liver has not yet developed the homeostatic mechanisms to maintain safe levels of manganese in the blood and target tissues (especially the brain), and the blood-brain barrier is incompletely formed. Indeed, this susceptibility is evident in a study by Wasserman *et al.* (2006) of 10-year old children ( $n = 142$ ) in Bangladesh exposed to manganese in drinking water ( $< 200$ ,  $200-499$ ,  $500-999$ ,  $>1,000 \text{ } \mu\text{g/l}$ ). Comparing the lowest and highest dose groups ( $< 200$  vs  $> 1,000 \text{ } \mu\text{g/l}$ ), 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  $<10 \mu\text{g As/l}$ . 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  $\beta = -4.56$ ,  $p < 0.01$ ; Performance  $\beta = -3.82$ ,  $p < 0.01$ ).

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, Collip *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 children who were hyperkinetic and exhibited learning disabilities. In comparison with normal children of the same age, significantly elevated levels of hair manganese ( $0.434 \mu\text{g/g}$ ; measured at 8 years of age) were reported in children with learning disabilities and hyperactivity compared with normal children ( $0.268 \mu\text{g/g}$ ) ( $p < 0.05$ ). An association between poorer performance in school and elevated hair manganese ( $1.242 \mu\text{g/g}$ ) has also been observed among children in China compared with children with more normal manganese levels (Zhang *et al.*, 1995).

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 20<sup>th</sup> 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 1<sup>st</sup> ( $R = 0.40 - 0.47$ ,  $p < 0.05$ ) and 3<sup>rd</sup> grades ( $R = 0.38 - 0.48$ ,  $p < 0.05$ ), and in the 3<sup>rd</sup> 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 62-64<sup>th</sup> week of gestation (i.e. postnatally) were correlated only with teachers' reports of externalizing behaviors in the 1<sup>st</sup> ( $R = 0.40$ ,  $p < 0.05$ ) and 3<sup>rd</sup> 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., 2006).

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 (<20<sup>th</sup> percentile in the Wide Range Assessment of Visual-motor Abilities). No obvious neurobehavioral problems were noted for either the parents or the older sibling.

### **6.2.1 Potential for Differential Effects in Children**

Infants and children may be more susceptible than adults to manganese toxicity for the following reasons:

1. Newborns absorb more manganese from the gastrointestinal tract than do adults.
2. 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.
3. Some infant formulas and foods are high in manganese.
4. The newborn's brain is still developing and the blood-brain barrier is not completely formed.
5. Modeling of the inhalation dosimetry of particles (0.001-10  $\mu\text{m}$ ), comparing neonates (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). In addition, infants and young children experience overall higher deposition of particles than adults.

## **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.

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.

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<sup>3</sup> respirable manganese dust for five h/d, five d/wk (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% 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.

The distribution of manganese in primate brain, and its neurobehavioral and cognitive effects in *Cynomolgus* macaques following weekly intravenous injection of MgSO<sub>4</sub> (10-15 or 3.26-4.89 mg/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). 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.

#### **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 (ATSDR, 2000). 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

## 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  $\mu\text{g}/\text{day}$ ) beginning at birth show decreased dopamine in the striatum and poorer performance on behavioral tests (Tran et al., 2002b). 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  $\text{mg}/\text{m}^3$ , while those exposed to 0.07-8.61  $\text{mg}/\text{m}^3$  (geometric mean 0.94  $\text{mg}/\text{m}^3$ ) 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  $\mu\text{g}/\text{g}$  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  $\mu\text{g}/\text{g}$  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  $\mu\text{g MnCl}_2$ ) introduced into the third ventricle of the brain significantly and dose-dependently stimulated the release of leutinizing hormone (LH). This effect was apparently at the level of the hypothalamus as pretreatment with the LH releasing hormone (LHRH) receptor antagonist, acycline, 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  $\text{MnCl}_2$  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.

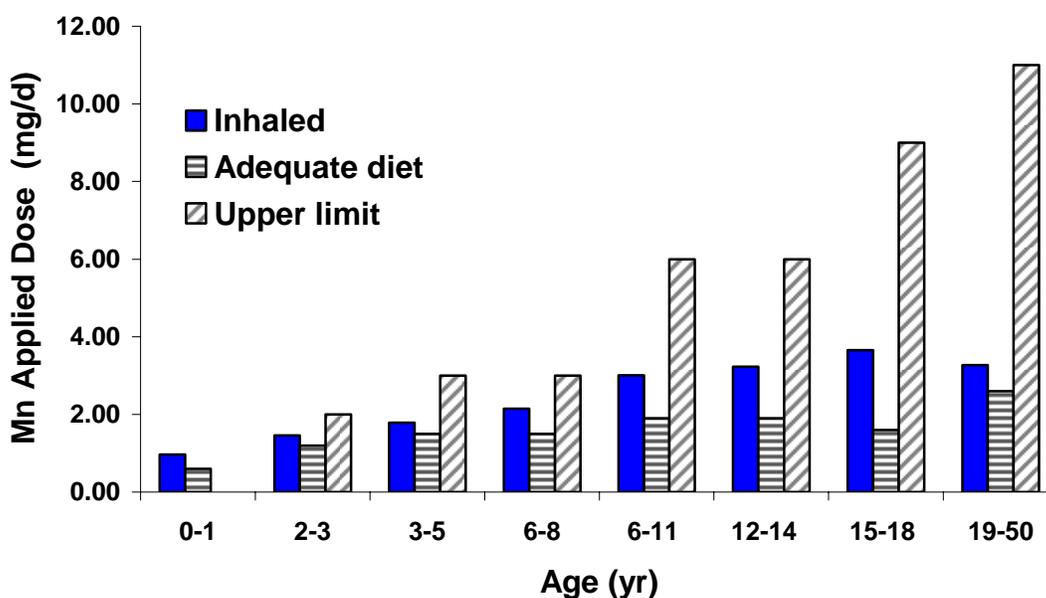
In adult male rats, exposure to 1,000 ppm manganese sulfate in drinking water for 12 weeks significantly suppressed sexual performance compared with controls as measured by prolonged ejaculatory latencies ( $p < 0.001$ ), and increased post-ejaculatory intervals ( $p < 0.05$ ). Displays of aggressive 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) (Ponnappakkam 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., 2001; Dorman et al., 2002b). To provide perspective on potential manganese exposure from inhalation relative to the suggested upper limits for age-dependent dietary intake, Figure 8.1 below shows the amount of manganese children of various ages and lactating mothers (19-50 yr old) would inhale if they were exposed to the manganese levels reported in the Roels *et al* (1992) occupational study ( $0.215 \text{ mg/m}^3$ ) compared with the recommended adequate intakes and upper limits listed in Table 6.5.1. The study by Roels is used in the development of the RELs below, and the value of  $0.215 \text{ mg/m}^3$  was selected from that study as it represents a real-world mean level of exposure that is associated with neurotoxicity in adults. The figure presents applied doses, not the actual amount absorbed since it is not known if humans absorb manganese more efficiently from the lungs than from the gut, as was shown to be the case for rats (Roels *et al.*, 1997). However, the graph suggests that for infants and children receiving adequate dietary manganese, additional exposure to manganese by inhalation presents a greater risk for manganese overdose than it does for adults at a given air concentration. Further, evidence of neurodevelopmental toxicity in animals and humans underscores increased sensitivity in children relative to adults. This enhanced risk among infants and children will be addressed in the development of the RELs below.

**Figure 8.1 Manganese Dose from Inhalation and Diet by Age**



## 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

<i>Study</i>	Roels <i>et al.</i> , 1992
<i>Study population</i>	102 workers in a battery plant
<i>Exposure method</i>	Inhalation of workplace air
<i>Exposure continuity</i>	
<i>Exposure duration</i>	8 hr/day, 0.2-17.7 yr (mean 5.3 yr)
<i>Critical effects</i>	Impaired neurobehavior: visual reaction time, eye-hand coordination, hand steadiness
<i>LOAEL</i>	0.15 mg/m <sup>3</sup>
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	0.107 mg/m <sup>3</sup> (0.15 mg/m <sup>3</sup> x 5/7)
<i>LOAEL uncertainty factor (UF<sub>L</sub>)</i>	6 (default, no NOAEL, mild effect)
<i>Subchronic uncertainty factor (UF<sub>s</sub>)</i>	√10 (default 8-12% of lifetime)
<i>Interspecies Uncertainty Factor</i>	
<i>Toxicokinetic (UF<sub>A-k</sub>)</i>	1 (default: human study)
<i>Toxicodynamic (UF<sub>A-d</sub>)</i>	1 (default: human study)
<i>Intraspecies Uncertainty Factor</i>	
<i>Toxicokinetic (UF<sub>H-k</sub>)</i>	10 (greater lung deposition in children)
<i>Toxicodynamic (UF<sub>H-d</sub>)</i>	10 (greater susceptibility of children to neurotoxicity)
<i>Cumulative uncertainty factor</i>	2000
<i>Reference Exposure Level</i>	<b>0.05 µg/m<sup>3</sup></b>

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 0.05 µg/m<sup>3</sup> based on impairment of neurobehavioral function in humans in the occupational study of Roels *et al.* (1992) described in Section 6.1. This study yielded an average occupational exposure for the exposed group (LOAEL, no NOAEL observed) of 0.15 mg Mn/m<sup>3</sup> corresponding to a time-adjusted concentration of 0.107 mg/m<sup>3</sup> (based on an 8 hour TWA occupational exposure for 5 days/week).

A cumulative UF of 2,000 was used, comprising a 6 for the LOAEL uncertainty factor associated with mild effects (see Section 4.4.5 of the TSD),  $\sqrt{10}$  for subchronic to chronic conversion (average exposure duration = 5.3 yr; Section 4.4.6 of the TSD), and 10 each for intraspecies toxicokinetic ( $UF_{H-k}$ ) 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  $\mu\text{m}$  size range in the lungs of neonates relative to adults exposed to similar particulate levels in ambient air (Ginsberg et al., 2005). In addition, based on studies with neonatal and adult rats (Dorman et al., 2005), neonates accumulate higher levels of manganese in the brain more quickly than do adults with similar exposures.

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.

### 8.3 Manganese Chronic Reference Exposure Level

<i>Study</i>	Roels et al., 1992
<i>Study population</i>	102 workers in a battery plant
<i>Exposure method</i>	Inhalation of workplace air
<i>Exposure continuity</i>	
<i>Exposure duration</i>	8 hr/day, 0.2-17.7 yr (mean 5.3 yr)
<i>Critical effects</i>	Impaired neurobehavior: visual reaction time, eye-hand coordination, hand steadiness
<i>LOAEL</i>	0.15 mg/m <sup>3</sup>
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	0.054 mg/m <sup>3</sup> (0.15 mg/m <sup>3</sup> x 10/20 x 5/7)
<i>LOAEL uncertainty factor (<math>UF_L</math>)</i>	6 (default, no NOAEL, mild effect)
<i>Subchronic uncertainty factor (<math>UF_s</math>)</i>	$\sqrt{10}$ (default 8-12% of lifetime)
<i>Interspecies Uncertainty Factor</i>	
<i>Toxicokinetic (<math>UF_{A-k}</math>)</i>	1 (default: human study)
<i>Toxicodynamic (<math>UF_{A-d}</math>)</i>	1 (default: human study)
<i>Intraspecies Uncertainty Factor</i>	
<i>Toxicokinetic (<math>UF_{H-k}</math>)</i>	10 (greater lung deposition in children)
<i>Toxicodynamic (<math>UF_{H-d}</math>)</i>	10 (greater susceptibility of children to neurotoxicity)
<i>Cumulative uncertainty factor</i>	2000
<i>Reference Exposure Level</i>	<b>0.03 <math>\mu\text{g}/\text{m}^3</math></b>

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 proposed chronic REL for manganese is  $0.03 \mu\text{g}/\text{m}^3$ , based on impairment of neurobehavioral function in humans in the occupational study of Roels et al. (1992). This study yielded an average occupational exposure for the exposed group (LOAEL, no NOAEL observed) of  $0.15 \text{ mg Mn}/\text{m}^3$  corresponding to a time-adjusted concentration of  $0.054 \text{ mg}/\text{m}^3$  (based on an 8 hour TWA occupational exposure to  $10 \text{ m}^3$  manganese contaminated air per day out of  $20 \text{ m}^3$  total air inhaled per day over 5 days/week).

A cumulative UF of 2,000 was used, comprising a 6 for the LOAEL uncertainty factor associated with mild effects (see Section 4.4.5 of the TSD), 3 for subchronic to chronic conversion (average exposure duration = 5.3 yr; Section 4.4.6 of the TSD), and 10 each for intraspecies toxicokinetic ( $\text{UF}_{\text{H-k}}$ ) and toxicodynamic ( $\text{UF}_{\text{H-d}}$ ) uncertainty. This REL is based on healthy adult male workers with adjustments for the potentially greater susceptibility of children. The intraspecies  $\text{UF}_{\text{H-k}}$  of 10 was chosen in part to reflect the 3-4-fold greater deposition of inhaled particulates in the 1-10  $\mu\text{m}$  range in the lungs of neonates relative to adults exposed to similar particulate levels in ambient air (Ginsberg et al., 2005). In addition, based on studies with neonatal and adult rats (Dorman et al., 2005), neonates accumulate higher levels of manganese in the brain more quickly than do adults with similar exposures.

A  $\text{UF}_{\text{H-d}}$  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  $0.05 \mu\text{g}/\text{m}^3$  (U.S.EPA, 1993) also based on Roels et al. (1992).

#### **8.4 Manganese as a Toxic Air Contaminant**

In view of the potential for high exposure 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.

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.

ATSDR. (2000). *Toxicological profile for manganese*. U.S. Department of Health and Human Services.

Barbeau A (1984). Manganese and extrapyramidal disorders (a critical review and tribute to Dr. George C. Cotzias). *Neurotoxicology* 5(1): 13-35.

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.

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.

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.

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.

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 (2005). 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, Struve MF, James RA, McManus BE, Marshall MW and Wong BA (2001). Influence of dietary manganese on the pharmacokinetics of inhaled manganese sulfate in male CD rats. *Toxicol Sci* 60(2): 242-51.

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.

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 (2006). Prenatal manganese levels linked to childhood behavioral disinhibition. *Neurotoxicol Teratol*.

Fechter LD (1999). Distribution of manganese in development. *Neurotoxicology* 20(2-3): 197-201.

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.

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.

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, McGlothlan 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.

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.

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 manganese: ten years of follow-up. *Neurology* 50(3): 698-700.

Kilburn CJ (1987). Manganese, malformations and motor disorders: findings in a manganese-exposed population. *Neurotoxicology* 8(3): 421-9.

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.

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.

Merck (1976). *The Merck Index*. Rahway, NJ: Merck and Co.

NIOSH. (2005). *NIOSH Pocket Guide to Chemical Hazards; Manganese tetroxide (as Mn)*. Centers for Disease Control. <http://www.cdc.gov/niosh/npg/npgd0381.html>

NWU. (2006). Nutrition Fact Sheet: Manganese. Retrieved 8-17-07, from <http://www.feinberg.northwestern.edu/nutrition/factsheets/manganese.html>.

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.

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 (MnCl<sub>2</sub>, MnO<sub>2</sub>) on the absorption and cerebral distribution of manganese in rats. *Arch Toxicol* 71(4): 223-30.

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.

Schneider JS, Decamp E, Fritz S, Schultz A, Emberger H and Guilarte TR (2006). Effects of chronic manganese exposure on cognitive and motor functioning in nonhuman primates. *Journal of Neurochemistry* 96(Suppl. 1): 89.

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

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.

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>.

Wennberg A, Hagman M and Johansson L (1992). Preclinical neurophysiological signs of parkinsonism in occupational manganese exposure. *Neurotoxicology* 13(1): 271-4.

Woolf A, Wright R, Amarasiriwardena C and Bellinger D (2002). A child with chronic manganese exposure from drinking water. *Environmental Health Perspectives* 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<sup>0</sup> Elemental; Quicksilver)

CAS 7439-97-6

## 1. Summary

Elemental mercury exposures adversely affect several organ systems, with the effects of acute, high level inhalation exposures first appearing 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 gate, 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*  
*Critical effect(s)*  
*Hazard Index target(s)*

**0.6 µg Hg/m<sup>3</sup> (0.07 ppb Hg<sup>0</sup>)**  
CNS disturbances in offspring  
Nervous system

### 1.2 Mercury 8-Hour REL

*Reference Exposure Level*  
*Critical effect(s)*  
*Hazard Index target(s)*

**0.06 µg Hg/m<sup>3</sup> (0.007 ppb Hg<sup>0</sup>)**  
Impairment of neurobehavioral functions  
in humans  
Nervous system

### 1.3 Mercury Chronic REL

*Reference Exposure Level*  
*Critical effect(s)*  
*Hazard Index target(s)*

**0.03 µg Hg/m<sup>3</sup> (0.004 ppb Hg<sup>0</sup>)**  
Impairment of neurobehavioral functions  
in humans  
Nervous system

## 2. Physical & Chemical Properties - Elemental Mercury

<i>Description</i>	Silver-white, heavy, mobile, odorless, liquid metal
<i>Molecular formula</i>	Hg <sup>0</sup>
<i>Molecular weight</i>	200.59 g/mol
<i>Density</i>	13.53 g/cm <sup>3</sup> @ 25° C
<i>Boiling point</i>	356.73° C
<i>Melting point</i>	-38.7° C
<i>Vapor pressure</i>	2 x 10 <sup>-3</sup> mm Hg @ 25° C
<i>Solubility</i>	soluble in nitric acid, to some extent in lipids, and up to 0.28 μmol in water @ 25° C
<i>Odor threshold</i>	odorless
<i>Conversion factor</i>	1 ppm in air = 8.34 mg/m <sup>3</sup> @ 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<sup>3</sup> (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 be protective of public health. 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 ( $\text{Hg}^0$ ) may be oxidized by catalase and peroxidase to the more toxic inorganic forms. Cellular membranes and the blood-brain barrier are readily permeable to  $\text{Hg}^0$ , but much less so to the inorganic forms. Residual  $\text{Hg}^0$  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  $\text{H}_2\text{O}_2$ . 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 mercuric 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 poisonings (Levin et al., 1988). Acute exposure to  $\text{Hg}^0$  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  $\text{Hg}^0$ . 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  $\text{Hg}^0$  were reported in 6 male workers exposed to an estimated concentration of 44 mg  $\text{Hg}/\text{m}^3$  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  $\text{Hg}^0$  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/ $\text{m}^3$  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.

#### *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 susceptibility 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  $\text{Hg}^0$  are largely limited to case reports with little or no information on actual exposure levels. In children who inhale high levels of toxic  $\text{Hg}^0$  vapors, pulmonary dysfunction is the primary cause of mortality. For example, autopsy of a 4-month-old child who died following acute exposure to  $\text{Hg}^0$  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  $\text{Hg}^0$  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)  $\text{Hg}/\text{m}^3$  for 1 hr, or 27 mg (3.2 ppm) for 2 hr. A dose-dependence of lung pathology and mortality was reported. No mortality was observed during the subsequent 15 d following the 1 hr exposure, while 50% mortality and more severe lesions were seen during the first 5 d following the 2 hr 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  $\text{mg}/\text{m}^3$  (0.79 - 0.90 ppm) mercury vapor for 4 hr 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/m<sup>3</sup> (0.22 ppm) of Hg<sup>0</sup> 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 5.3.1 Effects of Prenatal Metallic Mercury on Motor Activity**

Activity	Day	3 months			14 months		
		Control (SEM)	0.07 mg/kg/d	0.2 mg/kg/d	Control (SEM)	0.07 mg/kg/d	0.2 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 5.3.2 Prenatal Metallic Mercury and Learning Deficits**

Morris maze	Day	7 months			15 months		
		Control	0.07 mg/kg/d	0.2 mg/kg/d	Control	0.07 mg/kg/d	0.2 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  $25 \mu\text{g}/\text{m}^3$  (0.003 ppm) for 2-20 hr, or to  $500 \mu\text{g}/\text{m}^3$  (0.06 ppm) for 5-240 min. At  $25 \mu\text{g}/\text{m}^3$ , 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  $500 \mu\text{g}/\text{m}^3$ , 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  $29.7 \text{ mg Hg}/\text{m}^3$  (3.6 ppm) (Ashe et al., 1953). Exposure of rabbits to  $31.3 \text{ mg Hg}/\text{m}^3$  (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  $25 \mu\text{g}/\text{m}^3$  (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- $\beta$ -glucosaminidase have been seen in workers exposed to approximately 25-60  $\mu\text{g}/\text{m}^3$  (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  $\text{mg}/\text{m}^3$  (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  $\text{mg}/\text{m}^3$  (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  $\mu\text{g}/\text{L}$ , 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  $\mu\text{g}/\text{L}$  to an air concentration of 25  $\mu\text{g}/\text{m}^3$  (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  $\mu\text{g}/\text{L}$  corresponding to a TWA exposure of 25  $\mu\text{g Hg}/\text{m}^3$  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  $\mu\text{g Hg}/\text{m}^3$  (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  $\mu\text{g}/\text{m}^3$  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  $\mu\text{g Hg}/\text{m}^3$  (range 0.7 to 42  $\mu\text{g}/\text{m}^3$ ) 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  $\mu\text{g}/\text{L}$ ). The average concentration in air was estimated at 23  $\mu\text{g Hg}/\text{m}^3$  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  $\mu\text{g Hg}/\text{m}^3$  across worksites. The average level of exposure was estimated at 33  $\mu\text{g Hg}/\text{m}^3$  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  $\text{Hg}^0$  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 principle target organs for  $\text{Hg}^0$ . 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  $\text{Hg}^0$  and MeHg easily cross cell membranes, the blood brain barrier, and the placenta (Ask et al., 2002). Intracellular oxidation of  $\text{Hg}^0$  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  $\text{Hg}^0$  avidly bind to protein sulfhydryls and may inactivate enzymes. Disruption of protein synthesis has been reported after exposure to either  $\text{Hg}^0$  or MeHg, although the former is the more powerful inhibitor (NAS, 2000). The neurotoxic effects observed in adult rats following *in utero* exposure to  $\text{Hg}^0$ , MeHg, or both, are reportedly similar with MeHg potentiating the effects of  $\text{Hg}^0$  (Fredriksson et al., 1996). Given the high susceptibility of children to MeHg and the apparent similarities in mechanisms with  $\text{Hg}^0$ , children are expected to be more susceptible to  $\text{Hg}^0$  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 ( $\text{Hg}^{2+}$ ) 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  $0.06 \text{ mg/m}^3$  (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  $0.97 \text{ } \mu\text{g/g}$  tissue for MT-null and wild type, respectively. For comparison, the authors cite human brain mercury levels ranging from  $0.3 \text{ } \mu\text{g/g}$  in dental personnel to  $33 \text{ } \mu\text{g/g}$  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 ( $2 \text{ mg/kg/d}$  during days 6-9 of gestation), and metallic mercury ( $\text{Hg}^0$ ) vapor by inhalation ( $1.8 \text{ mg/m}^3$  (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  $0.1 \text{ mg Hg}^0/\text{kg/day}$ . 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  $\text{Hg}^0$  showed hyperactivity over all three measures of motor activity: locomotion, rearing and total activity. This effect was enhanced in the animals of the MeHg +  $\text{Hg}^0$  group. Compared to either the control or MeHg groups in the swim maze test, rats in the MeHg +  $\text{Hg}^0$  and  $\text{Hg}^0$  groups took longer to reach a submerged platform whose location they had learned the previous day. Similarly, both the MeHg +  $\text{Hg}^0$  and  $\text{Hg}^0$  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  $\text{Hg}^0$  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  $\text{Hg}^0$ .

The similarities in the effects of MeHg and  $\text{Hg}^0$  imply similar targets in the brain, which appears to be the case. Pregnant squirrel monkeys were exposed to mercury vapor (0.5 or 1  $\text{mg}/\text{m}^3$  (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  $\mu\text{g}/\text{g}$  tissue) than in offspring (0.20-0.70  $\mu\text{g}/\text{g}$ ) 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  $\sim 1 \text{ mg}/\text{m}^3$  (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 was 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  $\mu\text{g}/\text{g}$  tissue) from low to high doses, while that of the brain increased 608% (0.71 to 5.03  $\mu\text{g}/\text{g}$ ). 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  $\text{mg}/\text{m}^3$ ) 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  $\text{mg}/\text{m}^3$  (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  $\text{mg}/\text{m}^3$  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<sup>3</sup> 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

<i>Study</i>	Danielsson et al., 1993
<i>Study population</i>	groups of 12 pregnant rats
<i>Exposure method</i>	inhalation of metallic mercury vapors
<i>Exposure continuity</i>	
<i>Exposure duration</i>	1 hour per day
<i>Critical effects</i>	CNS disturbances in offspring
<i>LOAEL</i>	1.8 mg/m <sup>3</sup>
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	
<i>Human Equivalent Concentration</i>	n/a
<i>LOAEL uncertainty factor (UF<sub>L</sub>)</i>	10 (default; severe effect, no NOAEL)
<i>Subchronic uncertainty factor (UFs)</i>	
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>A-k</sub>)</i>	√10 (default, animal study)
<i>Toxicodynamic (UF<sub>A-d</sub>)</i>	10 (greater human vs rat susceptibility)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>H-k</sub>)</i>	√10 (default: critical study in young)
<i>Toxicodynamic (UF<sub>H-d</sub>)</i>	√10 (default: critical study in young)
<i>Cumulative uncertainty factor</i>	3000
<i>Reference Exposure Level</i>	<b>0.6 µg Hg/m<sup>3</sup> (0.07 ppb Hg<sup>0</sup>)</b>

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/m<sup>3</sup> 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<sub>A-k</sub>) variability was used, while a larger interspecies UF<sub>A-d</sub> 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 parallelogram approach to analyze in vivo and in vitro data on the responses 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 (BLL) of 1  $\mu\text{g}/\text{dL}$  compared with BLLs of less than 15  $\mu\text{g}/\text{dL}$  in primates (Gilbert and Rice, 1987) and less than 20  $\mu\text{g}/\text{dL}$  in rats (Cory-Slechta et al., 1985).

Since the critical study involved early life exposures, the default intraspecies toxicodynamic uncertainty factor ( $\text{UF}_{\text{H-d}}$ ) 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  $\mu\text{g}/\text{m}^3$  (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

<i>Study</i>	Piikivi and Hanninen (1989); Fawer et al. (1983); Piikivi and Tolonen (1989); Piikivi (1989); Ngim et al. (1992)
<i>Study population</i>	Humans
<i>Exposure method</i>	Inhalation of workplace air
<i>Exposure continuity</i>	8 hours per day, 5 days/week
<i>Exposure duration</i>	13.7 to 15.6 year
<i>Critical effects</i>	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
<i>LOAEL</i>	25 $\mu\text{g}/\text{m}^3$ (3 ppb)
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	18 $\mu\text{g}/\text{m}^3$ for LOAEL group
<i>LOAEL uncertainty factor (<math>UF_L</math>)</i>	10 (default, severe effect, no NOAEL)
<i>Subchronic uncertainty factor (<math>UF_s</math>)</i>	1
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (<math>UF_{A-k}</math>)</i>	1 (default: human study)
<i>Toxicodynamic (<math>UF_{A-d}</math>)</i>	1 (default: human study)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (<math>UF_{H-k}</math>)</i>	$\sqrt{10}$ (default for inter-individual variability)
<i>Toxicodynamic (<math>UF_{H-d}</math>)</i>	10 (greater susceptibility of children and their developing nervous systems)
<i>Cumulative uncertainty factor</i>	300
<i>Reference Exposure Level</i>	<b>0.06 <math>\mu\text{g Hg}/\text{m}^3</math> (0.007 ppb <math>\text{Hg}^0</math>)</b>

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 interval between daily 8-hr exposures is 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 24-hour uptake) 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  $\text{m}^3$  per day (i.e. half that assumed for a 24-hour period 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.

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  $25 \mu\text{g}/\text{m}^3$  (3 ppb) with a time-adjusted value of  $18 \mu\text{g}/\text{m}^3$  ( $25 \times 5/7$ ). 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  $\sqrt{10}$  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  $0.06 \mu\text{g}/\text{m}^3$  (0.007 ppb Hg °).

### 8.3 Mercury Chronic Reference Exposure Level

<i>Study</i>	Piikivi and Hanninen (1989); Fawer et al. (1983); Piikivi and Tolonen (1989); Piikivi (1989); Ngim et al. (1992)
<i>Study population</i>	Humans
<i>Exposure method</i>	Inhalation of workplace air
<i>Exposure continuity</i>	8 hours per day (10 m <sup>3</sup> /workday), 5 days/week
<i>Exposure duration</i>	13.7 to 15.6 year
<i>Critical effects</i>	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
<i>LOAEL</i>	25 µg/m <sup>3</sup> (3 ppb)
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	8.9 µg/m <sup>3</sup> for LOAEL group
<i>LOAEL uncertainty factor (UF<sub>L</sub>)</i>	10 (default, severe effect, no NOAEL)
<i>Subchronic uncertainty factor (UFs)</i>	1
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>A-k</sub>)</i>	1 (default: human study)
<i>Toxicodynamic (UF<sub>A-d</sub>)</i>	1 (default: human study)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>H-k</sub>)</i>	√10 (default for inter-individual variability)
<i>Toxicodynamic (UF<sub>H-d</sub>)</i>	10 (greater susceptibility of children and their developing nervous systems)
<i>Cumulative uncertainty factor</i>	300
<i>Reference Exposure Level</i>	<b>0.03 µg Hg/m<sup>3</sup> (0.004 ppb Hg<sup>0</sup>)</b>

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<sup>3</sup> (3 ppb). When adjusted for worker ventilation and workweek exposure, the LOAEL becomes 0.009 mg/m<sup>3</sup>. 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<sup>3</sup> (0.004 ppb Hg °).

The U.S.EPA (1995) based its RfC of  $0.3 \mu\text{g}/\text{m}^3$  (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  $\text{UF}_{\text{H-d}}$  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  $0.0012 \text{ mg Hg}/\text{L}$  (1.2 ppb) as a level of exposure expected to be protective of public health. This value was based on data from a 1993 study by the National Toxicology Program that supported a NOAEL of  $0.16 \text{ mg Hg}/\text{kg}\text{-day}$  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  $0.16 \mu\text{g Hg}/\text{kg}\text{-day}$ . 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.

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.

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.

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 H<sub>2</sub>O<sub>2</sub> 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. Sacramento, 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.

Taug 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.