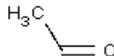


Acetaldehyde Reference Exposure Levels

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

CAS: 75-07-0



1. Summary

Based on acute and chronic inhalation studies conducted mostly in experimental animals, the target tissue for acetaldehyde has consistently been at the portal of entry with effects occurring primarily in the upper respiratory tract at lowest concentrations. The major noncancer health effects of acute exposure in humans to acetaldehyde vapors consist of irritation to the eyes, skin, and respiratory tract. Low to moderate air concentrations (25 ppm to 200 ppm) cause eye and upper respiratory tract irritation. Moderate concentrations (~ 300 ppm or greater) also cause bronchoconstriction in asthmatics as measured by a greater than 20% drop in forced expiratory volume (FEV₁). Signs of acute toxicity in animals at high concentrations (~10,000 ppm) include labored respiration, mouth breathing, weight loss, and liver damage. The studies described in this document include those published through the Spring of 2008.

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

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

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

1.1 Acetaldehyde Acute REL

<i>Reference Exposure Level</i>	470 µg/m³ (260 ppb)
<i>Critical effect(s)</i>	Sensory irritation, bronchoconstriction, eye redness and swelling
<i>Hazard index target(s)</i>	Bronchi, eyes, nose, throat

1.2 Acetaldehyde 8-Hour REL

<i>Reference Exposure Level</i>	300 µg/m³ (160 ppb)
<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>	140 µg/m³ (80 ppb)
<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 ₂ H ₄ O
<i>Molecular weight</i>	44.05 g/mol
<i>Density</i>	0.79 g/cm ³
<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 ³
<i>Solubility</i>	Miscible in all proportions with water and the most common organic solvents.
<i>Conversion factor</i>	1.8 mg/m ³ = 1 ppm @ 25°C

3. Occurrence and Major Uses

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

An emissions study of new building materials found that samples of carpet, fiberboard, particleboard, and non-rubber resilient flooring emitted acetaldehyde (Burt et al., 1996; IWMB, 2003). Air concentrations based on the acetaldehyde emission rates from these various building products, when modeled to standard State office and classroom dimensions, ranged from 4.6 to 26 $\mu\text{g}/\text{m}^3$ (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 measured levels of acetaldehyde of 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) (Hodgson et al., 2004). Similar concentrations were found in classrooms of the main buildings. Measured concentrations of acetaldehyde in public/office buildings range from 3 to 16 $\mu\text{g}/\text{m}^3$ (1.7 to 8.9 ppb).

Environmental tobacco smoke (ETS) has been found to be a source of environmental acetaldehyde. Although long-term acetaldehyde levels in smoking and non-smoking homes tend to be similar, acetaldehyde concentrations in homes as a result of exposure from ETS for nonsmoking Californians has been estimated at 11-15 $\mu\text{g}/\text{m}^3$ (6.1 to 8.3 ppb) (Miller et al., 1998). Concentrations of acetaldehyde measured over a 72-hour period in 57 homes ranged from 3 to 23 $\mu\text{g}/\text{m}^3$ (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 a genetic deficiency of the enzyme aldehyde dehydrogenase (ALDH), the concentration of acetaldehyde in the breath after drinking can reach 8.8-22 mg/m^3 (4.9 to 12.2 ppm). Higher concentrations of acetaldehyde have been shown to activate mast cells, which then induce histamine release. In one case study, a patient had a severe bronchial asthma attack after ingesting food containing small amounts of alcohol, and was found to be homozygous for the ALDH-2 mutant genotype. Both acetaldehyde and ethanol inhalation tests were performed on the patient. The ethanol inhalation test was negative, but acetaldehyde inhalation (5, 10, 20, or 40 mg/ml) decreased FEV_{1.0} by 33.5% at 20 mg/ml (Saito et al., 2001).

4. Disposition

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

Two studies were performed using humans and dogs to determine the percent retention of inhaled acetaldehyde in the respiratory tract (Egle Jr, 1970; Egle Jr., 1972a; 1972b). In humans, the total respiratory tract retention of acetaldehyde was 45-70% when inhaled either orally or nasally (Egle Jr, 1970). Physiological respiratory total retention in multiple breath experiments was independent of tidal volume, and uptake was controlled by frequency and duration of ventilation. Total respiratory tract retention of acetaldehyde in dogs was found to be very close to human retention values and inversely related to ventilatory rate in the same manner as humans (Egle Jr., 1972b). Uptake was also found to be higher in the upper than the lower respiratory tract and unrelated to changes in concentration inhaled or tidal volume (Egle Jr., 1972b).

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

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

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

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

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

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

At least two isozymes of aldehyde dehydrogenase were found in the rodent nasal mucosa, differing with respect to their apparent V_{max} and K_m values (Morris, 1997a). Male F344 rats were exposed to 1500 ppm acetaldehyde for 6 hours/day for 5 days. Oxidation of acetaldehyde occurred more rapidly in the homogenates of the respiratory than the olfactory mucosa (Morris, 1997a). The nasal tissue is the first to contact acetaldehyde vapors upon inhalation. The

aldehyde dehydrogenase acts as a defense mechanism helping to minimize or prevent toxic injury to nasal tissues exposed to airborne compounds. Pretreatment with an ALDH inhibitor reduced nasal acetaldehyde deposition rates (Morris, 1997a).

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

5. Acute Toxicity of Acetaldehyde

5.1 Acute Toxicity to Adult Humans

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

Silverman et al. (1946) exposed human volunteers to acetaldehyde to determine the sensory response limit for solvent concentrations when estimating ventilation requirements for comfortable working conditions (Silverman et al., 1946). The sensory limits were reported and compared to the maximum allowable concentration, which was stated as 200 ppm for acetaldehyde at the time of the study. Twelve volunteer human subjects of both sexes were used for each solvent exposure. During the 15 minute exposure period, motion pictures were shown to occupy the subjects' attention and divert their thoughts from the atmospheric exposure in the chamber. The results, though described in a limited way, are useful because the analysis was performed in human subjects and the concentrations tested were as low as 25 ppm. At 50 ppm, the majority of subjects experienced eye irritation (number not specified). The subjects that did not report eye irritation had reddened eyelids and bloodshot eyes after exposure at 200 ppm. Several subjects reported unspecified irritation at 25 ppm and "objected strenuously." Finally, nose and throat irritation were reported as occurring at concentrations greater than 200 ppm (Silverman et al., 1946).

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

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

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

In further experiments with the same group of volunteers, the influence of oral terfenadine, a histamine H1 blocker, was examined as was the bronchial responsiveness to methacholine (challenge with methacholine is a common asthma identification test). The response seen after inhalation of acetaldehyde was completely suppressed by pretreatment with terfenadine, which supports the hypothesis that bronchial hyper-responsiveness is a precondition of acetaldehyde induced bronchoconstriction, which is caused indirectly via histamine release in asthmatics (Myou et al., 1993). A rough estimate from the dose response curve as presented in the paper, suggests a PC_{20} for acetaldehyde (acetaldehyde concentration producing a 20% reduction in FEV_1) of about 20 mg/ml (Myou et al., 1993). The acetaldehyde aerosol concentration as mg/m^3 in this study can be estimated as follows. The nebulizer was operated at 5 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.14 ml/minute. When given at this rate a 20 mg

acetaldehyde/ml solution (the estimated PC₂₀) corresponds to a concentration in air of approximately 560 mg/m³ (about 314 ppm).

In a subsequent acute human study, nine asthmatic subjects of Japanese origin were used to determine whether bronchial responsiveness to inhaled methacholine (STET: a standard test used to identify agents that potentially exacerbate asthma) was altered when asthmatic subjects inhaled a sub threshold concentration of aerosolized acetaldehyde which did not cause bronchoconstriction, and whether any increase in bronchial hyper-responsiveness after acetaldehyde was mediated by histamine release (Myou et al., 1994). For each subject, the concentration of acetaldehyde producing a 20% fall in FEV₁ was determined (PC₂₀) using ascending doses (5, 10, 20, or 40 mg/ml) of acetaldehyde. The mean concentration of PC₂₀ for the nine subjects was 23.3 mg/ml of acetaldehyde (Myou et al., 1994). The nebulizer was operated at 5 liters air/minute for 4 minutes with an acetaldehyde solution output of 0.14 ml/minute. Therefore, a 23.3 mg acetaldehyde/ml solution corresponds to a concentration in air of approximately 652 mg/m³ (about 362 ppm).

In part two of this study, nine subjects inhaled a sub threshold concentration of 0.8 mg/ml acetaldehyde at 0.14 ml/minute for four minutes or saline followed by provocation with a range of increasing methacholine concentrations (Myou et al., 1994). FEV₁ was measured before and after treatment. Acetaldehyde potentiated bronchial hyper-responsiveness to provocation by methacholine (Myou et al., 1994) producing a marked reduction in PC₂₀-MCH (0.48 mg/ml versus 0.85 mg/ml after saline treatment) (Myou et al., 1994).

Myou et al. (1995) examined tachyphylaxis occurring in response to repeated inhalation of histamine or acetaldehyde in nine asthmatic subjects. The mean acetaldehyde concentration causing a 20% decrease in FEV₁ increased significantly from a geometric mean of 18.4 mg/ml (with a geometric standard error (GSEM) of 0.14) to 45.2 mg/ml (GSEM 0.14) over a period of one hour (p<0.002). The mean histamine concentrations causing a 20% decrease in FEV₁ were no different.

In a later study in asthmatics of Japanese origin, the hypothesis was tested that asthmatics that are sensitive to alcohol (showing bronchoconstriction after drinking alcohol) also have increased airway responsiveness to inhaled acetaldehyde when compared to asthmatics not sensitive to alcohol (Fujimura et al., 1999). Ten alcohol-sensitive asthmatics and 16 alcohol insensitive asthmatics (20-65 years) of Japanese origin inhaled acetaldehyde aerosol for 2 minutes by tidal mouth breathing and FEV₁ was measured. Increasing concentrations of acetaldehyde solutions in saline (0.04 to 80 mg acetaldehyde/ml) were inhaled until FEV₁ showed a fall of 20%. In the alcohol-sensitive group the geometric mean PC₂₀ was 21.0 mg/ml (range not reported), whereas in the alcohol-insensitive group this was 31.7 mg/ml (range not reported). The difference between the groups, however, was not statistically significant (Fujimura et al., 1999). The aerosol was produced using a DeVilbiss 646 nebulizer operated by compressed air at 5 liters/minute with a nebulizer output of 0.14 ml/minute. The nebulizer was operated at 5 liters air/minute for 2 minutes with a acetaldehyde solution output of 0.14 ml/minute. At this rate, inhalation of acetaldehyde solutions of 0.04 to 80 mg/ml corresponds to concentrations in air of approximately 1.12 to 2240 mg/m³. Similarly, the geometric mean PC₂₀ in the alcohol-sensitive

group corresponds to approximately 588 mg/m³ (about 330 ppm) and the geometric mean PC₂₀ in the alcohol-insensitive group to approximately 888 mg/m³ (about 500 ppm).

In the key study (Prieto et al., 2000) used for the acute REL determination, the responses to methacholine and acetaldehyde challenges were measured in 81 non-smoking adults to determine differences in airway responsiveness between asthmatics and healthy subjects and to examine the relationship between acetaldehyde responsiveness and the variability of peak expiratory flow (PEF). Prieto et al. (2000) examined whether the bronchoconstriction seen in Japanese asthmatics after inhalation of acetaldehyde also occurred in Caucasian subjects. They exposed 61 mildly asthmatic subjects and 20 healthy subjects (control group) to aerosolized acetaldehyde (5 to 40 mg acetaldehyde/ml) for two minutes using a two-minute tidal breathing-method and FEV₁ was measured 60 to 90 seconds after inhalation of each concentration until FEV₁ dropped by more than 20%. In this study, the PC₂₀ values for acetaldehyde ranged from 1.96 to 40 mg/mL with a geometric mean value of 17.55 mg/mL and the 95% confidence interval of the geometric mean (95% CI 4.72-38.3 mg/ml). Therefore, the lower limit of the geometric 95% CI was 4.72 mg/ml (Prieto, 2008). Effectively, the data are for the geometric mean (antilog of the mean PC₂₀ acetaldehyde) and the 95% CI is for the antilog of this value also. This is the traditional way these type of data are presented for acetaldehyde, methacholine or AMP (Prieto 2008). In the asthma group 56/61 subjects showed bronchoconstriction compared to 0/20 in the control group. Inhaled acetaldehyde was much less potent as a bronchoconstrictor than methacholine in asthmatic patients. Peak expiratory flow variation was significantly but weakly related to acetaldehyde responsiveness (p = 0.004). The results obtained by Prieto et al. (2000) indicate that airway hyper-responsiveness to acetaldehyde is a sensitive and specific indicator for separating normal and asthmatic subjects.

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In the Prieto et al. (2000) study, aqueous solutions containing acetaldehyde were nebulized in a Hudson 1720 nebulizer operated by compressed air at 6 liters/minute with a nebulizer output of 0.18 ml/minute. Flow rates were reported in a National Advisory Committee document from the U.S. EPA, based on a personal communication from the Prieto group (NAS, 2004). The nebulizer was operated at 6 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.18 ml/minute. At this rate, inhalation of acetaldehyde solutions of 5 to 40 mg/ml corresponds to concentrations in air of approximately 150 to 1200 mg/m³. The observed geometric mean PC₂₀ of 17.55 mg/ml corresponds to 527 mg/m³ (about 293 ppm) and the lower 95% confidence interval of 4.72 mg/ml corresponds to approximately 142 mg/m³ (about 79 ppm).

In a follow-up study, Prieto et al. (2002a) exposed mildly asthmatic subjects (age 18-58 years) to 2.5 to 80 mg acetaldehyde/ml using a Hudson 1720 nebulizer with an output of 5 liters/minute. In the first group, 16 subjects were measured for their response to acetaldehyde which was compared to that of methacholine and adenosine-5'-monophosphate (two bronchoconstrictive agents of known potency). In the second group of 14 subjects, repeatability and side effects of acetaldehyde inhalation were examined. For acetaldehyde the PC₂₀ ranged from 8.4 to 80 mg/ml with a geometric mean of 38.9 mg/ml (geometric mean values for methacholine and AMP were 0.6 and 17.4 mg/ml, respectively). The response to acetaldehyde was found to be moderately repeatable. For the group in which repeatability was examined, for acetaldehyde concentrations producing a >20% fall in FEV₁, most subjects had cough (64%), dyspnea (57%) or throat

irritation (43%) (Prieto et al., 2002a). The nebulizer was operated at 5 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.16 ml/minute. At this rate, inhalation of acetaldehyde solutions of 2.5 to 80 mg/ml corresponds to concentrations in air of approximately 80 to 2560 mg/m³. The observed geometric mean PC₂₀ of 38.9 mg/ml corresponds to approximately 1245 mg/m³ (about 692 ppm).

In a further volunteer study, Prieto et al. (2002b) studied comparative airway responsiveness to acetaldehyde (2.5 mg to 80 mg/ml) in subjects with allergic rhinitis (n=43), asthmatics (n=16), and healthy subjects (n=19). The number of subjects with a fall in FEV₁ >20% was 8/43 in the group with allergic rhinitis, 13/16 in the asthmatic group and 0/19 in the healthy subjects group. PC₂₀ values in the group with allergic rhinitis ranged from 15.5 to 80.0 mg/ml with a geometric mean of 67.7 mg/ml whereas in the asthmatic group PC₂₀ ranged from 8.4 to 80.0 mg/ml with a geometric mean of 35.5 mg/ml (p < 0.001) (Prieto et al., 2002b). The PC₂₀ values in the allergic rhinitis group were also significantly lower than in the healthy control group (p = 0.04) (Prieto et al., 2002b). The nebulizer was operated at 5 liters air/minute for 2 minutes with an acetaldehyde solution output of 0.16 ml/minute. Thus, inhalation of acetaldehyde solutions (2.5 to 80 mg/ml) corresponds to concentrations in air of 80 to 2560 mg/m³. The observed geometric mean of 67.7 mg/ml corresponds to approximately 2166 mg/m³ (about 1210 ppm) and the geometric mean of 35.5 mg/ml to approximately 1136 mg/m³ (about 631 ppm).

As indicated above, the provocation tests involved acetaldehyde solutions that were aerosolized, and then inhaled by mouth. Aerosolized acetaldehyde solutions have been shown to be about 265 times less potent than methacholine in constricting the airways of asthmatic subjects, with aerosolized acetaldehyde solutions of 80 mg/ml resulting in cough, dyspnea, and throat irritation in the asthmatic subjects (Myou et al., 1993). In addition, the exposure times were very short (several minutes) and the concentrations eliciting a response in FEV₁ were much higher. However, it is important to note that in the Myou et al. (1994) study, aerosolized acetaldehyde potentiated bronchial hyper-responsiveness to provocation by methacholine at concentration equivalents in the air of about 22.4 mg/m³ (or 12.5 ppm) similar to the concentration that produced eye irritation (25 ppm) in human volunteers as seen in the Silverman et al. (1946) study. This response is of concern and an experimental analog to asthma. This may be indicative that the same chemo-sensory response triggered both the reactivity in the airways and eye irritation. The potentiation of methacholine-induced bronchoconstriction shows the potential of acetaldehyde at concentrations of approximately 12.5 ppm or higher to exacerbate asthma. It should be noted that the model of nebulizer used was shown to have inconsistent delivery; thus the estimate of concentration of acetaldehyde that potentiated methacholine-induced bronchoconstriction is uncertain.

In summary, exposure to acetaldehyde, at concentrations as low as 25 ppm, resulted in sensory irritation in human volunteers (Silverman et al., 1946). Aerosolized acetaldehyde at concentrations equivalent to approximately 12.5 ppm potentiated bronchial hyper-responsiveness to provocation by methacholine (Myou et al., 1994). Adult asthmatics showed large inter-individual variation in PC₂₀ values (59 ppm to 1200 ppm) (Prieto et al., 2000). Finally, adult asthmatics that inhaled aerosolized solutions of acetaldehyde showed increased irritation and bronchoconstriction at 293 ppm (Prieto et al., 2000). Table 5.1.1 summarizes the aerosolized acetaldehyde provocation studies in adult human volunteers.

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

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

* Values [for PC₂₀, which are geometric means are](#) converted from mg/mL of aerosolized acetaldehyde to approximate concentration in air (ppm).

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(79 ppm ¶
lower 95% CI)

5.2 Acute Toxicity to Infants and Children

No studies on the effects of acute exposure to acetaldehyde in non-adult humans were located. However, as noted above for adults, there is some evidence that following acute exposure to acetaldehyde, asthmatics are more sensitive to acetaldehyde exposure and are likely to show symptoms such as wheezing, shortness of breath, bronchoconstriction, and/or decrements in pulmonary function consistent with immediate and/or delayed bronchoconstriction. Furthermore, some asthmatics may respond with significant reductions in lung function due to the irritant effects, sensitized or not. The potential association between acetaldehyde exposure and asthma is of special concern for children because they have higher prevalence rates of asthma than adults, and their asthma episodes can be more severe due to their smaller airways. Hospitalization rates of children for asthma, especially for the first four years of life, are higher than for other age groups (Mannino et al., 1998). In addition, infants and children may have qualitatively different responses due to different target tissue sensitivities during windows of susceptibility in the developmental process.

Findings also support the view that toxic air contaminants, such as acetaldehyde, in communities in proximity to major emission sources, including both industrial and traffic sources, have adverse effects on asthma in children (Delfino et al., 2003). The average daily residential exposure to acetaldehyde in high school students living in inner-city neighborhoods of New York City and Los Angeles and living with a smoker was evaluated. The exposure concentration range measured in juveniles living with smokers was 6.3 to 14 µg/m³ (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 RD₅₀ was calculated (the concentration eliciting a 50% decrease in respiratory rate). RD₅₀ 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 RD₅₀ 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 RD₅₀ (the level inducing a 50% reduction in respiratory breathing rate) was 2991 ppm (95% CI 2411-3825) for this study (Babiuk et al., 1985).

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

Acute lethality studies have also been performed with acetaldehyde. In an historical acute inhalation study in rats, groups of eight per dose were exposed to acetaldehyde vapors 7,778 to 31,667 mg/m³ (14,000 to 57,000 ppm) for thirty minutes (Skog, 1950). The acute LD₅₀ value (reported as LD₅₀) for acetaldehyde inhalation was 20,600 ppm (37,000 mg/m³) (Skog, 1950).

Appelman et al. (1982) determined the LC₅₀ for acetaldehyde using twenty male and twenty female albino Wistar rats. The animals were exposed for 4 hours in horizontally placed glass exposure cylinders with a total airflow through the cylinder of 8 l/min. Concentrations were given as the mean of 10 to 15 determinations and were as follows: 10,436, 12,673, 15,683, and 16,801 ppm. Within the first half-hour of the four-hour LC₅₀ 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 LC₅₀ and the 95% confidence limits were calculated to be 13,300 ppm (95% CL: 11,200, 15,400) (Appelman et al., 1982).

Syrian Golden hamsters were exposed to acetaldehyde vapors for 4 hours at doses ranging from 14,450 to 17,600 ppm (26,010 to 31,680 mg/m³) (Kruyssen et al., 1975). After one to two hours of exposure at all concentrations, the animals showed severe lacrimation, salivation, and nasal discharge. The 4-hour LC₅₀ was determined to be 17,000 ppm (30,600 mg/m³) for this study. In all exposure groups, the animals that died during exposure had convulsions. Some animals survived at all concentrations, but only after a deep narcosis and apnea (Kruyssen et al., 1975).

Aldehyde dehydrogenase 2 (ALDH2) is an important enzyme that oxidizes acetaldehyde. Isse et al. (2005) compared the acute acetaldehyde toxicity between wild-type (Aldh2^{+/+}) and Aldh2-inactive transgenic (Aldh2^{-/-}) mice after inhalation. The null aldehyde dehydrogenase 2 (ALDH2) transgenic mice (-/-) or 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 ALDH (-/-) mice were showing a staggering gait (Isse et al., 2005). This study concluded that acute acetaldehyde toxicity after inhalation is higher in aldehyde dehydrogenase 2 knockout than in wild-type mice (Isse et al., 2005).

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

Table 5.3.1 summarizes the acute animal data for acetaldehyde inhalation. The data indicate that humans may be more sensitive to the acute effects of acetaldehyde than animals. For the endpoint of sensory irritation, measured as reduction in respiratory rate, the lowest RD₅₀ 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 LC₅₀ was 13,300 ppm in rats. In contrast, the LOAEL for human sensory irritation was reported to be 25 ppm in

one historical study (Silverman et al., 1946). In addition, potentiation of methacholine-induced bronchoconstriction was shown in one study at approximately 12.5 ppm.

Table 5.3.1 Summary of Acute Studies in Experimental Animals

Endpoint	Strain/Species	Exposure	Response	Reference
Sensory irritation	B6C3F1 mice	750 to 4200 ppm for 10 min	RD ₅₀ = 2932 ppm	(Steinhagen and Barrow 1984)
	Swiss Webster mice	750 to 4200 ppm for 10 min	RD ₅₀ = 2845 ppm	(Steinhagen and Barrow 1984)
	F-344 rats	~800 to 10,000 ppm for 10 min	RD ₅₀ = 2991 ppm	Babiuk et al., 1985
	Wistar rats	2800, 4600, or 6500 ppm for 30 min	RD ₅₀ = 3046 ppm	Cassee et al., 1996a
Histopathological	Wistar rats	750 or 1500 ppm for 1 and 3 days	Olfactory epithelial lesions at 1500 ppm	Cassee et al., 1996b
Lethality	Rats	14,000 to 57,000 ppm for 30 min	LD ₅₀ = 20,600 ppm	Skog, 1950
	Wistar rats	10,436 to 16,801 ppm for 4 hours	LC ₅₀ = 13,300 ppm	Appelman et al., 1982
	Syrian Golden hamsters	14,450 to 17,600 ppm for 4 hours	LC ₅₀ = 17,000 ppm	Kruysse et al., 1975
Behavioral/Other effects	Syrian Golden hamsters	14,450 for one to 2 hours	lacrimation, salivation, and nasal discharges	Kruysse et al., 1975
	Wistar rats	10,436 ppm within first 30 min	restlessness, closed eyes and labored breathing	Appelman et al., 1982
	Wistar rats	5000 ppm for 30 min	severe dyspnoea and excitation	Appelman et al., 1982
		5000 ppm for 20 minutes	crouching, 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 males of the same rat species at 150 and 500 ppm, resulted in observed degeneration of olfactory nasal tissues at 500 ppm, but not in the 150 ppm exposure group (Appelman et al., 1986). Therefore, 150 ppm was designated the no observable adverse effect level (NOAEL).

Table 6.3.1: Incidence of Nasal Olfactory Tissue Effects in Rats

Degeneration of nasal olfactory epithelium	<u>Exposure Group (ppm)</u>						
	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/m³) acetaldehyde for 8 hr/day, 5 days/week for 5 weeks resulted in an “intense” nasal inflammatory reaction with olfactory epithelium hyperplasia and polymorphonuclear and mononuclear infiltration of the submucosa (Saldiva et al., 1985). Changes in pulmonary mechanics, including increased functional residual capacity, residual volume, total lung capacity, and respiratory frequency was observed, but may have been the result of mechanical damage during pulmonary function testing.

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

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

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

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

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

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

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

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

In a subchronic exposure of hamsters to 0, 390, 1340, or 4560 ppm acetaldehyde 6 hr/day, 5 days/week for 90 days resulted in growth retardation, and ocular and nasal irritation in the high dose group. Histopathological changes were observed only in the respiratory tract and consisted

of necrosis and inflammatory changes of the epithelium in the nasal cavity, larynx, bronchi and lungs in the high dose animals, and mild tracheal epithelial lesions in the mid-dose group. No adverse effects were observed at 390 ppm (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 (n = 8) of non-sensitized and ovalbumin (OA)-sensitized guinea pigs were exposed to 0 or 200 ppb (360 µg/m³) acetaldehyde or 0 or 600 ppb benzaldehyde for 6 hr/day, 5 days/week for four weeks (Lacroix et al., 2002). Two animals from each group were examined histologically and 6 animals from each group underwent bronchoalveolar lavage. Analyses of protein, PGE₂ and leukotriene content, and cellularity of the BALF were reported. In sensitized animals, acetaldehyde exposure did not modify the inflammatory and allergic response to subsequent challenge with ovalbumin (OA) aerosol relative to that induced by sensitization alone. Interestingly, benzaldehyde exposure suppressed the response of sensitized guinea pigs to OA challenge. In nonsensitized guinea pigs, acetaldehyde exposure resulted in "slight irritation" (n = 2) of the lung, trachea and nasal respiratory epithelium, and induced a significant increase in the number of alveolar macrophages, but not eosinophils or neutrophils, in bronchoalveolar lavage fluid (n = 6) (Lacroix et al., 2002). There was no increase in total protein, PGE₂, or leukotriene content in the BALF. Acetaldehyde exposure did not change any of these parameters in OA-sensitized animals. Limitations of the Lacroix et al. (2000) study include a lack of quantitative data for irritation and reported large variability in the concentration of acetaldehyde in the chamber atmosphere. In addition, there was no acetaldehyde-induced exacerbation of response to OA challenge in the sensitized animals. If the slight irritation seen in

non-sensitized animals was exposure related, the effect would be expected to be greater in the sensitized animals than the non-sensitized animals, but there was no increase in response in acetaldehyde-exposed sensitized animals beyond sensitization alone. Finally, human data indicate exacerbation of methacholine induced bronchoconstriction after acetaldehyde exposure, yet acetaldehyde exposure did not exacerbate OA challenge in this study. Given the lack of quantitative data on irritation, lack of exacerbation of response by acetaldehyde in OA-sensitized animals, and the inconsistency of this study with other rodent studies vis-à-vis irritation NOAELs, we decided against using this study for the 8-hour or chronic REL.

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

7.0 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. As a small lipid soluble molecule, acetaldehyde is able to cross membranes by simple diffusion (Zorzano and Herrera, 1989a). Acetaldehyde has been shown to cross the placenta in mice and distribute to embryos (Blakley and Scott Jr., 1984b). Placental transfer occurred when acetaldehyde was administered via i.p. injection to pregnant CD-1 mice at 200 mg/kg on day 10 of gestation, and acetaldehyde was detected within the embryo within 5 minutes (Blakley and Scott Jr., 1984a; b). Maximal concentrations of acetaldehyde were also reached in the maternal blood, liver, and yolk sac in the first five minutes.

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

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

of 50, 75, or 100 mg/kg, teratogenicity, embryoletality, and growth retardation were observed (Blakley and Scott Jr., 1984a).

In vitro models have found that acetaldehyde was teratogenic to C₃H mouse embryos between 8 and 10 days of gestation after 28 hours of exposure (Thompson and Folb, 1982). Morphological parameters and DNA synthesis were measured and correlated. Eight and nine-day embryos were exposed to doses of 7.4, 19.7, or 39.4 mg/l acetaldehyde in incubation medium. The 39.4 mg/l dose group at eight days showed a significant effect on somite count, neural tube fusion, CNS development (size and symmetry), and significant reduction in DNA synthesis. The nine-day embryos at 39.4 mg/l had increased somite count, absent heart beat, and a significant increase in limb development, while the 19.7 mg/l group had significant abnormalities in development of visceral arches, CNS development, and reduction in DNA synthesis.

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

Both acetaldehyde and ethanol significantly inhibited the gonadotropin-stimulated biosynthesis of testosterone, and acetaldehyde 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 μ M acetaldehyde was effective in suppressing testicular steroidogenesis; however, cell viability was unaffected.

8.0 Derivation of Reference Exposure Levels

8.1 Acetaldehyde Acute Reference Exposure Level

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

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

<i>Study</i>	Prieto et al., 2000
<i>Study population</i>	61 adult asthmatic human volunteers
<i>Exposure method</i>	Inhalation by nebulizer
<i>Exposure continuity</i>	
<i>Exposure duration</i>	2-4 minutes
<i>Critical effects</i>	Bronchoconstriction, PC ₂₀ >20% drop in FEV ₁
<i>LOAEL</i>	142 mg/m ³ (79 ppm)
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	not derived
<i>Time-adjusted exposure</i>	not applied
<i>Human Equivalent Concentration</i>	not applied
<i>LOAEL uncertainty factor (UF_L)</i>	10 (severe effect, no NOAEL)
<i>Subchronic uncertainty factor (UF_s)</i>	not applied
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	1 (default, human study)
<i>Toxicodynamic (UF_{A-d})</i>	1 (default, human study)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	1 (inter-individual variation)
<i>Toxicodynamic (UF_{H-d})</i>	30 (asthma exacerbation in children, hyper-responsiveness to methacholine)
<i>Cumulative uncertainty factor</i>	300
<i>Reference Exposure Level</i>	470 µg/m³ (260 ppb)

Sixty-one asthmatic subjects were used to determine the concentration of acetaldehyde producing a 20% fall (PC₂₀) in Forced Expiratory Volume in one second (FEV₁) using ascending doses (5 to 40 mg/ml) of aerosolized acetaldehyde solutions. The [geometric](#) mean concentration of PC₂₀ for the sixty-one subjects was 17.55 mg/ml of acetaldehyde [and the values ranged from 1.96 to 40 mg/mL \(Prieto et al., 2000\). The 95% confidence interval of the geometric mean was \(4.72-38.3 mg/ml\) \(Prieto, 2008\). Therefore, the lower bound of the geometric 95% CI was 4.72 mg/ml, which was converted to ppm, and was the value used as the point of departure for the acute REL derivation \(Prieto, 2008\)](#). The Hudson 1720 nebulizer was operated at 6 liters air/minute for 2 to 4 minutes with an acetaldehyde solution output of 0.18 ml/minute. Therefore,

Deleted: The lower bound of the 95% confidence interval was 4.72 mg/ml

Deleted: (Prieto et al., 2000), which was converted to ppm and was the value used for the acute REL derivation.

the lower 95% confidence interval of 4.72 mg/ml corresponds to approximately 142 mg/m³ (about 79 ppm), which was used as the LOAEL for the acute REL determination.

Deleted: a 17.55 mg acetaldehyde/ml solution corresponds to a concentration in air of approximately 527 mg/m³ (about 293 ppm). T

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

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

The toxicodynamic component of the intraspecies uncertainty factor UF_{H-d} was assigned an increased value of 30 for the acute REL determination due to multiple lines of evidence. A portion of the UF_{H-d} is applied to account for the potential greater susceptibility of children. The respiratory irritant effect of acetaldehyde, with documented potential to exacerbate asthma, is an effect with the potential to differentially impact infants and children. Myou et al., 1994 demonstrated hyper-responsiveness to methacholine after provocation with a sub-threshold dose of aerosolized acetaldehyde at concentrations equivalent to approximately 12.5 ppm. Additional studies have also shown that adult asthmatics are more sensitive to the irritative properties of inhaled aerosolized acetaldehyde solutions, which significantly decreased their forced expiratory volume in one second (FEV₁) by more than 20% (Myou et al., 1993; Myou et al., 1994; Fujimura et al., 1999; Prieto et al., 2002a; Prieto et al., 2002b). Finally, alcohol sensitive asthmatics had a selective hyper-responsiveness to acetaldehyde (Myou et al., 1993; Myou et al., 1994; Fujimura et al., 1999).

Myou et al. (1994) also observed that aerosolized acetaldehyde potentiated bronchial hyper-responsiveness to provocation by methacholine at concentration equivalents in the air (22.4 mg/m³ or 12.5 ppm) similar to the concentration that produced eye irritation (25 ppm) in human volunteers as seen in the Silverman et al. (1946) study. This response is of concern and an experimental analog to asthma. This may be indicative that the same chemo-sensory response triggered both the reactivity in the airways and eye irritation. The potentiation of methacholine-induced bronchoconstriction shows the potential of acetaldehyde at concentrations of approximately 12.5 ppm or higher to exacerbate asthma. Of note however, some uncertainty is associated with the use of a DeVillbis nebulizer, which has been shown to have considerable variability in aerosol output and delivered dose (Hollie et al., 1991).

In conclusion, using the LOAEL of 142 mg/m³ (79 ppm) for bronchoconstriction from Prieto et al. (2000), divided by the cumulative uncertainty factor of 300, an acute reference exposure level (REL) for acetaldehyde was determined to be 470 µg/m³ (260 ppb). This level is considered safe for infants and children during an acute exposure period.

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

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

<i>Study</i>	Silverman et al., 1946
<i>Study population</i>	24 adult human volunteers
<i>Exposure method</i>	Whole body
<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 ³ (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_L)</i>	6 (default: mild effect, no NOAEL)
<i>Subchronic uncertainty factor (UF_s)</i>	not applied
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	1 (default, human study)
<i>Toxicodynamic (UF_{A-d})</i>	1 (default, human study)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	1 (site of contact; no systemic effects)
<i>Toxicodynamic (UF_{H-d})</i>	10 (asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	60
<i>Reference Exposure Level</i>	750 µg/m³ (420 ppb)

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

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

A default uncertainty factor of six is associated with the use of a LOAEL for mild effects in the absence of a NOAEL (see Section 4.4.5 of the TSD). The study was performed in humans, therefore the interspecies uncertainty factor, toxicokinetic (UF_{A-k}) and toxicodynamic (UF_{A-d}) components were each assigned the default value of one. Eye irritancy appears to be more a

function of concentration rather than duration of exposure (Yang et al., 2001), so no time correction factor was applied.

For the toxicokinetic component of the intraspecies uncertainty factor (UF_{H-k}) a value of one was used since sensory irritation is not expected to involve large toxicokinetic differences among individuals, and the effects are largely confined to the site of contact, in this case, the eyes, nose, and upper respiratory tract, with negligible or no systemic effects. The deposition kinetics of reactive gases is generally thought not to be greatly different between adults and children. Because of this, a value of one is used for the kinetic component of the intraspecies uncertainty factor (UF_{H-k}), rather than a more extended values of $\sqrt{10}$ 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 their forced expiratory volume in one second (FEV_1) by more than 20% (Prieto et al., 2000; Prieto et al., 2002b). And, alcohol sensitive asthmatics had a selective hyper-responsiveness to acetaldehyde (Myou et al., 1993; Myou et al., 1994; Fujimura et al., 1999). These considerations are applied equally to the acute, 8-hour and chronic REL.

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

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

8.2 Acetaldehyde 8-Hour Reference Exposure Level

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

Bronchoconstriction, eye irritation and nasal mucosal histopathology are all legitimate concerns for the 8-hour REL and occur in a broadly similar concentration range over the relevant time scale. The repeated nature of an 8-hour REL makes use of the acute studies inappropriate. Therefore, the 8-hour REL was derived using the subchronic animal study (Appelman et al.,

1982; 1986) in rats exposed to acetaldehyde six hours per day, five days per week for four weeks. Incidence of degeneration of nasal olfactory epithelium was the most sensitive end-point. These data are supported by Dorman et al. (2008) who reported endpoints of degeneration of the nasal olfactory and respiratory epithelia.

<i>Study</i>	Appelman et al., 1982; 1986
<i>Study population</i>	Wistar rats (10-40 animals/group)
<i>Exposure method</i>	Inhalation exposure to 0, 273, 728, 910, 1820, 4004, 9100 mg/m ³ (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>	Degeneration of olfactory epithelium
<i>LOAEL</i>	720 mg/m ³ (400 ppm)
<i>NOAEL</i>	270 mg/m ³ (150 ppm)
<i>Benchmark Concentration (BMC₀₅) (using continuous model)</i>	178 mg/m ³ (99 ppm)
<i>Human equivalent concentration</i>	242.1 mg/ m ³ (134.6 ppm)(99 ppm* 1.36 (DAF) Teegarden et al. (2008)
<i>Time-adjusted exposure</i>	86.5 mg/m ³ (48.1 ppm) = (134.6*6/24*20/10*5/7)
<i>LOAEL uncertainty factor (UF_L)</i>	1
<i>Subchronic uncertainty factor (UFs)</i>	√10 (exposure 8-12% of lifetime)
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	1 (interspecies PBPK model for acetaldehyde)
<i>Toxicodynamic (UF_{A-d})</i>	√10 (default: no interspecies toxicodynamic data)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	√10 (inter-individual variation)
<i>Toxicodynamic (UF_{H-d})</i>	10 (potential asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	300
<i>Reference Exposure Level</i>	300 µg/m³ (160 ppb)

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

The 8-hour REL was determined using the Benchmark Dose (BMDS) program developed by the U.S. EPA (2003). The BMC₀₅ 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 BMC₀₅ for acetaldehyde.

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

Table 8.2.1. Incidence of Degeneration of Olfactory Epithelium using Weighted Means by Severity¹.

Dose (ppm)	<u>Males</u>			<u>Females²</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

¹Severity 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.

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

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

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

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

The standard Human Equivalent Concentration (HEC) adjustment using an RGDR was not used for the dosimetric interspecies extrapolation. Instead, species information based on pharmacokinetic modeling for toxicants that result in specific nasal olfactory tissue damage was applied for interspecies extrapolation of acetaldehyde toxicity (Teeguarden et al., 2008).

Dosimetry data for the nasal olfactory epithelium shows that the rat is more efficient in scrubbing organic vapors in this region of the nasal cavity than humans (Frederick et al., 1998; Frederick et al., 2001). Consequently, rats receive a similar, or greater, tissue dose of inhaled organic vapors than humans in the olfactory epithelium. Sensitivity to acetaldehyde of the rat olfactory epithelium is a major factor for olfactory tissue damage, even though the specific

activity of aldehyde dehydrogenase is greater in the respiratory epithelium (Bogdanffy et al., 1998; Stanek and Morris, 1999). The interspecies adjustment also takes into account differences in the deposition of inhaled vapors and breathing rates. While rodents are obligate nose breathers, humans are not, which has implications for exposure of nasal tissues. Other factors when extrapolating toxicity findings from rodents to humans include dosimetry, nasal anatomy and airflow dynamics, target tissue metabolism, species differences in gross anatomy, distribution of nasal airway epithelia, and distribution and composition of mucous secretory products (Feron et al., 2001).

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

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

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

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

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

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

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

Dorman et al. (2008) conducted a 13-week study in male F344 rats (n=12 per group) with acetaldehyde exposures of 0, 50, 150, 500, or 1500 ppm. They reported degeneration of olfactory and respiratory epithelium (Dorman et al., 2008). The LOAEL and NOAEL for the endpoint of degeneration of olfactory nasal epithelium were 150 and 50 ppm, respectively for the observations at 65 days (Table 6.3.2). Benchmark concentration analysis was performed on the data and several models provided a BMC_{05} in close agreement with the NOAEL (quantal linear $BMC_{05} = 45.3$ ppm and probit $BMC_{05} = 48.3$ ppm), but statistically were not as reliable due to the small sample size and dose spacing. Adjusting the NOAEL using the dosimetric adjustment factor (DAF) of 1.36, as described previously, based on the PBPK model for acetaldehyde (Teeguarden et al., 2008), yielded a NOAEL of 68 ppm. Thus the BMC_{05} value from the Dorman study and also the LOAEL and NOAEL values from the same study are supportive of the 8-hour REL determined from the data of Appelman et al. (1982; 1986).

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

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

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

8.3 Acetaldehyde Chronic Reference Exposure Level

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

<i>Study</i>	Appelman et al., 1982; 1986
<i>Study population</i>	Wistar rats (10-40 animals/group)
<i>Exposure method</i>	Inhalation exposure to 0, 273, 728, 910, 1820, 4004, 9100 mg/m^3 (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>	Degeneration of olfactory epithelium
<i>LOAEL</i>	720 mg/m^3 (400 ppm)
<i>NOAEL</i>	270 mg/m^3 (150 ppm)
<i>Benchmark Concentration (BMC₀₅) (using continuous model)</i>	178 mg/m^3 (99 ppm)
<i>Human equivalent concentration</i>	242.1 mg/m^3 (134.6 ppm) (= 99 * 1.36 (DAF) Teeguarden et al. (2008)
<i>Time-adjusted exposure</i>	43.2 mg/m^3 (24 ppm) = (134.6*6/24*5/7)
<i>LOAEL uncertainty factor (UF_L)</i>	1
<i>Subchronic uncertainty factor (UF_S)</i>	$\sqrt{10}$ (exposure 8-12% of lifetime)
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	1 (intraspecies PBPK model for acetaldehyde)
<i>Toxicodynamic (UF_{A-d})</i>	$\sqrt{10}$ (default: no interspecies toxicodynamic data)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	$\sqrt{10}$ (inter-individual variation)
<i>Toxicodynamic (UF_{H-d})</i>	10 (potential asthma exacerbation in children)
<i>Cumulative uncertainty factor</i>	300
<i>Reference Exposure Level</i>	140 $\mu\text{g}/\text{m}^3$ (80 ppb)

The chronic REL was based on four-week exposure data in rats from Appelman et al., (1982, 1986), and supported by Saldiva et al., (1985); Woutersen et al., (1986, 1984); and (Woutersen

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

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

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

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

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

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

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

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

The current chronic RfC for acetaldehyde determined by the U.S. EPA and based on Appelman et al. (1982; 1986) is $9 \mu\text{g}/\text{m}^3$ (5 ppb) and is within the range of normal human breath acetaldehyde concentrations of 0.7 to $11.0 \mu\text{g}/\text{m}^3$ (0.4 to 6.1 ppb). OEHHA's proposed chronic REL of $140 \mu\text{g}/\text{m}^3$ (76 ppb) is above the range of human breath concentrations of acetaldehyde, but is mostly exceeded when humans consume significant amounts of alcohol, resulting in human breath concentrations ranging from 200 to $2200 \mu\text{g}/\text{m}^3$. 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.

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

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

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

8.4 Acetaldehyde as a Toxic Air Contaminant

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

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