Acetaldehyde Reference Exposure Levels

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

CAS: 75-07-0

H2C

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1. Summary

Acetaldehyde has been prioritized as a Toxic Air Contaminant (TAC) with the potential to differentially impact infants and children. Acetaldehyde was chosen for a focused literature review due to its effects as a respiratory irritant and possible exacerbation of asthma. In addition, acetaldehyde has high California Hot Spots and mobile source emissions, and secondary formation in the atmosphere (OEHHA 2001). Based on acute and chronic inhalation studies conducted mostly in experimental animals, the target tissue for acetaldehyde has consistently been at the portal of entry with effects occurring primarily in the upper respiratory tract at lowest concentrations.

The major noncancer health effects of acute exposure in humans to acetaldehyde vapors consist of irritation to the eyes, skin, and respiratory tract. Low to moderate air concentrations (25 ppm to 200 ppm) cause eye and upper respiratory tract irritation. OEHHA used the critical effect of eye irritation as the basis for determination of the acute Reference Exposure Level (REL). The 8-hour Reference Exposure Level (REL) also accounted for sensory irritation based on an early human exposure study. Signs of acute toxicity in animals at high concentrations (~10,000 ppm) include labored respiration, mouth breathing, weight loss, and liver damage.

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

Children, especially those with diagnosed asthma, may be more likely to show impaired pulmonary function and symptoms of asthma than are adults following exposure to acetaldehyde.
1.1 Acetaldehyde Acute REL

Reference Exposure Level: 750 μg/m$^3$ (420 ppb)
Critical effect(s): Sensory irritation, eye redness and swelling
Hazard index target(s): Eyes, nose, throat

1.2 Acetaldehyde 8-Hour REL

Reference Exposure Level: 568 μg/m$^3$ (316 ppb)
Critical effect(s): Degeneration of olfactory nasal epithelium
Hazard index target(s): Respiratory system

1.3 Acetaldehyde Chronic REL

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

2. Physical & Chemical Properties

Description: Colorless liquid or gas (above 21°C)
Molecular formula: C$_2$H$_4$O
Molecular weight: 44.05 g/mol
Density: 0.79 g/cm$^3$
Boiling point: 21 °C
Melting point: -123.5 °C
Vapor pressure: 755 mm Hg @ 20°C
Odor threshold: 0.09 mg/m$^3$
Solubility: Miscible in all proportions with water and the most common organic solvents.
Conversion factor: 1.8 mg/m$^3$ = 1 ppm @ 25°C
3. Occurrence and Major Uses

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

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

Indoor concentrations of acetaldehyde often greatly exceed outdoor levels and appear to dictate personal exposures, which is consistent with the more significant and widespread indoor sources of this aldehyde. In 2002, the annual average outdoor concentration of acetaldehyde in the South Coast Air Basin was 2.5 µg/m$^3$ (1.4 ppb). In Brazil, which has a high usage of ethanol as a transportation fuel, outdoor acetaldehyde concentrations have been measured as high as 63 µg/m$^3$ (35 ppb) while a highway tunnel had 3.43 times higher levels of acetaldehyde (430 µg/m$^3$ (240 ppb)). The mean acetaldehyde concentrations in U.S. homes range from 15 to 36 µg/m$^3$ (8.3 to 20 ppb) but reached as high as 103 µg/m$^3$ (57.2 ppb) in newly manufactured homes (Zweidinger et al., 1990; Lindstrom et al., 1995; Hodgson et al., 2002; Kinney et al., 2002). Acetaldehyde concentrations measured in Southern California portable classrooms ranged from 5.7 to 12.8 µg/m$^3$ (3.2 to 7.1 ppb) with a mean of 9.8 µg/m$^3$ (5.4 ppb) (Shendell et al., 2004). Similar concentrations were found in classrooms of the main buildings. Measured concentrations of acetaldehyde in public/office buildings range from 3 to 16 µg/m$^3$ (1.7 to 8.9 ppb).

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

The concentration of breath acetaldehyde (endogenous level) in non-alcoholic, non-smokers range from 0.7 to 11.0 µg/m$^3$ (0.4 to 6.1 ppb), but can be somewhat higher in smokers (16 ± 3 µg/m$^3$ = 8.9 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 µg/m$^3$ (111 to 1222 ppb) (Shaskan and Dolinsky, 1985; Jones, 1995).

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

In Asian subjects with genetically deficient 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. The ethanol inhalation test was negative, but the acetaldehyde inhalation test (5, 10, 20, or 40 mg/ml) was positive because FEV$_{1.0}$ was decreased by 33.5% at the 20 mg/ml concentration of acetaldehyde (Saito et al., 2001).

### 4. Disposition

Acetaldehyde is readily absorbed through the lungs into the blood following inhalation exposure. Acetaldehyde is rapidly exchanged and equilibrated between blood entering the lungs and alveolar air. Male Sprague-Dawley rats exposed to acetaldehyde vapor concentrations in air ranging from 9 to 1000 mg/l (0.009 to 1 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, 1970; Egle Jr., 1972a; 1972b). In humans, the total respiratory tract retention of acetaldehyde was 45-70% when inhaled either orally or nasally (Egle, 1970). Physiological respiratory total retention in multiple breath experiments was independent of tidal volume, and uptake was controlled by frequency and duration of ventilation. Total respiratory tract retention of acetaldehyde in
dogs was found to be very close to human retention values and inversely related to ventilatory rate in the same manner as humans (Egle Jr., 1972b). Uptake was also found to be higher in the upper than the lower respiratory tract and unrelated to changes in concentration inhaled or tidal volume (Egle Jr., 1972b; Morris, 1997b).

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

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

Acetaldehyde rapidly diffuses through cellular membranes and is distributed to various organs for metabolism. The half-life in rats was 10 minutes, and the time to total body clearance was 40 minutes (Shiohara et al., 1984). Inhaled acetaldehyde does not undergo a first pass effect and is distributed to all tissues including the liver. Inhaled acetaldehyde undergoes extrahepatic metabolism and is metabolized by aldehyde dehydrogenase in the lungs to acetate. Aldehyde dehydrogenase is found in both the cytosol and the mitochondria. Inhaled acetaldehyde undergoes extrahepatic metabolism by the respiratory-olfactory epithelium, kidneys, blood, brain, and spleen, and only small amounts reach the liver. Acetaldehyde also crosses the blood-brain barrier.

Various isoenzymes of alcohol dehydrogenase transform ethanol into acetaldehyde, which in turn is rapidly oxidized by aldehyde dehydrogenase (ALDH) into nontoxic acetate. Functional genetic polymorphisms and ethnic variation exist at various genes encoding these enzyme proteins which all act to alter the rate of synthesis of the toxic metabolite acetaldehyde, or decrease its further oxidation. About 50% of the Asian population are alcohol-sensitive and have a deficiency or low activity in ethanol-metabolizing enzymes that can result in high blood and breath acetaldehyde levels following alcohol consumption.

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

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

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

5. Acute Toxicity of Acetaldehyde

5.1 Acute Toxicity to Adult Humans

The major acute effects of human exposure to acetaldehyde vapors consist of irritation to the eyes, skin and respiratory tract, and bronchoconstriction in asthmatics. The key study used to determine the acute Reference Exposure Level (REL) was a study performed in human volunteers investigating sensory response to solvent vapors (Silverman et al., 1946). The purpose of the study was to determine the sensory response limit for solvent concentrations when estimating ventilation requirements for comfortable working conditions. The sensory limits were reported and compared to the maximum allowable concentration, which was stated as 200 ppm for acetaldehyde at the time of the study. Twelve volunteer human subjects of both sexes were used for each solvent exposure. The time of exposure was fifteen minutes. During the 15 minute exposure period, motion pictures were shown to occupy the subjects’ attention and divert their thoughts from the atmospheric exposure in the chamber.

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

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

Acetaldehyde provocation tests have been conducted with asthmatic and non-asthmatic human subjects using aerosolized acetaldehyde solutions. Nine male asthmatic patients with nine age- and sex-matched healthy volunteers inhaled ascending doses of 5, 10, 20, or 40 mg/ml of acetaldehyde and bronchial responsiveness was measured (Myou et al., 1993). The acetaldehyde solutions were in saline and were inhaled from a nebulizer for 2 minutes by mouth tidal breathing wearing a noseclips. Forced expiratory volume (FEV\textsubscript{1}) was measured. A greater than 20% decrease in FEV\textsubscript{1} was caused by inhalation of acetaldehyde in a dose-dependent manner in asthmatics that received a placebo and did not receive a histamine H\textsubscript{1} blocker (terfenadine). Percent decrease in FEV\textsubscript{1} in asthmatics receiving the placebo after inhalation of each concentration of acetaldehyde was larger than that in asthmatics treated with terfenadine and in healthy subjects (Myou et al., 1993). These findings support the hypothesis that bronchial hyper-responsiveness is a precondition of acetaldehyde induced bronchoconstriction, which is caused indirectly via histamine release in asthmatics.

In another acute human study, nine asthmatic subjects were used to determine whether bronchial responsiveness to inhaled methacholine (a standard test used to identify agents that potentially exacerbate asthma) was altered when asthmatic subjects inhaled a sub threshold concentration of aerosolized acetaldehyde which did not cause bronchoconstriction, and whether any increase in bronchial hyper-responsiveness after acetaldehyde was mediated by histamine release. For each subject, the concentration of acetaldehyde producing a 20% fall in FEV\textsubscript{1} was determined (PC\textsubscript{20}) using ascending doses (5, 10, 20, or 40 mg/ml) of acetaldehyde. The mean concentration of PC\textsubscript{20} for the nine subjects was 23.3 mg/ml of acetaldehyde. A sub threshold concentration of 0.8 mg/ml acetaldehyde was chosen and the nine subjects inhaled that or saline for four minutes (Myou et al., 1994). After each inhalation, a methacholine provocation test was performed. Acetaldehyde potentiated bronchial hyper-responsiveness to provocation by methacholine (Myou et al., 1994). In addition, the investigators determined that histamine was not responsible for acetaldehyde induced bronchial hyper-responsiveness at sub threshold concentrations (Myou et al., 1994).

The response to methacholine and acetaldehyde challenges have also been measured in 81 non-smoking adults (61 asthmatics and 20 normal controls) to determine differences in airway responsiveness between asthmatics and healthy subjects and to examine the relationship between acetaldehyde responsiveness and the variability of peak expiratory flow (PEF) (Prieto et al., 2000). The results of this study indicate that airway hyper-responsiveness to acetaldehyde is a sensitive and specific indicator for separating normal and asthmatic subjects. Five to 40 mg/mL acetaldehyde solutions were inhaled and FEV\textsubscript{1} was measured 60 to 90 seconds after inhalation of each concentration until FEV\textsubscript{1} dropped by more than 20% (Prieto et al., 2000). In this study, the PC\textsubscript{20} values for acetaldehyde ranged from 1.96 to 40 mg/mL with a geometric mean value of 17.55 mg/mL. However, in this study, inhaled acetaldehyde was much less poten as a bronchoconstrictor than
methacholine in asthmatic patients. Peak expiratory flow variation was significantly but weakly related to acetaldehyde responsiveness (p = 0.004) (Prieto et al., 2000).

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

Another acute human study showed increased sensitivity to acetaldehyde by alcohol-sensitive adults (Myou et al., 1995). Seven male and three female asthmatics with alcohol-induced bronchoconstriction and sixteen asthmatics without alcohol-induced sensitivity were exposed to 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, and 80 mg/mL acetaldehyde or methacholine solution dissolved in saline for two minutes, administered with a nebulizer, and FEV$_1$ was recorded (Myou et al., 1995). Bronchial responsiveness was greater in the alcohol-sensitive asthmatic group compared to the alcohol-non-sensitive asthmatic subjects.

As indicated above, the provocation tests involved acetaldehyde solutions that were aerosolized, and then inhaled by mouth. Aerosolized acetaldehyde solutions have been shown to be about 265 times less potent than methacholine in constricting the airways of asthmatic subjects, with aerosolized acetaldehyde solutions of 80 mg/ml resulting in cough, dyspnea, and throat irritation in the asthmatic subjects (Myou et al., 1993). Studies involving aerosolized solutions of acetaldehyde were not used by OEHHA as the basis for the REL derivation.

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

### 5.2 Acute Toxicity to Infants and Children

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

Findings also support the view that toxic air contaminants, such as acetaldehyde, in communities in proximity to major emission sources, including both industrial and traffic sources, have adverse effects on asthma in children (Delfino et al., 2003). The average daily residential exposure to acetaldehyde in high school students living in inner-city neighborhoods of New York City and Los Angeles and living with a smoker was evaluated. The exposure concentration range measured in juveniles living with smokers was 6.3 to 14 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 $RD_{50}$ was calculated (the concentration eliciting a 50% decrease in respiratory rate). $RD_{50}$ values were 2932 and 2845 ppm for B6C3F1 and Swiss-Webster mice, respectively (Steinhagen and Barrow, 1984).

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

Similarly, male F-344 rats were exposed in a head-only inhalation chamber to acetaldehyde (approximately 800 to 10,000 ppm though exact concentrations from the graph were not provided in the paper) for 10 minutes and experienced sensory irritation as measured by reduction in respiratory rate (Babiuk et al., 1985). The $RD_{50}$ (the level inducing a 50% reduction in respiratory breathing rate) was 2991 ppm (95% CI 2411-3825) for this study (Babiuk et al., 1985).
In addition to sensory irritation, histopathological effects have been observed after exposure to acetaldehyde. Albino, male Wistar rats, 8 weeks old, were exposed for 6 hours a day, either on one or three day exposures on consecutive days, in a nose-only inhalation chamber to acetaldehyde (750 or 1500 ppm) (Cassee et al., 1996b). Acetaldehyde exposure resulted in histopathological nasal changes with the three-day exposure group consisting of increased incidence and severity of “single-cell necrosis” in olfactory epithelium with increasing concentration. Biochemical changes consisted of concentration-dependent increases of nonprotein sulfhydryl groups in nasal respiratory epithelium with one- and three-day exposure, which was statistically significant with exposure to 1500 ppm. Activities of biotransformation enzymes (glutathione peroxidase, glutathione S-transferase, glutathione reductase, formaldehyde dehydrogenase, and nonspecific aldehyde dehydrogenase) were not affected by any of the exposures (Cassee et al., 1996b).

Acute lethality studies have also been performed with acetaldehyde. In an historical acute inhalation study in rats, groups of eight per dose were exposed to acetaldehyde vapors 14,000 to 17,600 ppm (30,600 to 31,680 mg/m$^3$) for thirty minutes (Skog, 1950). The acute LD$_{50}$ value for acetaldehyde inhalation was 20,600 ppm (37,080 mg/m$^3$) (Skog, 1950).

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

Syrian Golden hamsters were exposed acutely to acetaldehyde vapors for 4 hours at doses ranging from 14,450 to 17,600 ppm (26,010 to 31,680 mg/m$^3$) (Kruysse, 1970). After one to two hours of exposure at all concentrations, the animals showed severe lacrimation, salivation, and nasal discharges. The 4-hour LC$_{50}$ was determined to be 17,000 ppm (30,600 mg/m$^3$) for this study. In all exposure groups, the animals that died during exposure had convulsions. However at all concentrations, some animals survived, but only after a deep narcosis and apnea (Kruysse, 1970).

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

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

Table 5.3.1 summarizes the acute animal data for acetaldehyde inhalation. The data indicate that humans are more sensitive to the acute effects of acetaldehyde than animals. For the endpoint of sensory irritation, measured as reduction in respiratory rate, the lowest RD$_{50}$ for mice and rats were 2845 and 2991 ppm, respectively. With respect to histopathological changes, effects were observed at 1500 ppm. In the acute lethality studies, the lowest LC$_{50}$ was 13,300 ppm in rats. In contrast, the LOAEL for humans was reported to be 25 ppm in one historical study (Silverman et al., 1946). Thus, humans appear to be at least 30 times more sensitive than animals to the acute effects of acetaldehyde.
<table>
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<th><strong>Endpoint</strong></th>
<th><strong>Strain/Species</strong></th>
<th><strong>Exposure</strong></th>
<th><strong>Response</strong></th>
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<td>Sensory irritation</td>
<td>B6C3F1 mice</td>
<td>750 to 4200 ppm for 10 min</td>
<td>RD$_{50}$ = 2932 ppm</td>
<td>(Steinhagen and Barrow, 1984)</td>
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<td></td>
<td>Swiss Webster mice</td>
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<td></td>
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<td></td>
<td>Wistar rats</td>
<td>10,436 to 16,801 ppm for 4 hours</td>
<td>LC$_{50}$ = 13,300 ppm</td>
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<tr>
<td></td>
<td>Syrian Golden hamsters</td>
<td>14,450 to 17,600 ppm for 4 hours</td>
<td>LC$_{50}$ = 17,000 ppm</td>
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<td>14,450 for one to 2 hours</td>
<td>lacrimation, salivation, and nasal discharges</td>
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<td></td>
<td>Wistar rats</td>
<td>10,436 ppm within first 30 min</td>
<td>restlessness, closed eyes and labored breathing</td>
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<td>Wistar rats</td>
<td>5000 ppm for 30 min</td>
<td>severe dyspnoea and excitation</td>
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<td></td>
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<td>5000 ppm for 20 minutes</td>
<td>crouching, bradypnea, closed eyes, and piloerection</td>
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<td></td>
<td>CD1 mice</td>
<td>200 ppm for 3 hours</td>
<td>increased pulmonary bactericidal activity</td>
<td>Aranyi et al., 1986</td>
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</table>
6. Chronic Toxicity of Acetaldehyde

6.1 Chronic Toxicity to Adult Humans

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

6.2 Chronic Toxicity to Infants and Children

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

6.3 Chronic Toxicity to Experimental Animals

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

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

<table>
<thead>
<tr>
<th>Degeneration of nasal olfactory epithelium</th>
<th>Exposure Group (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Number examined</td>
<td>40</td>
</tr>
<tr>
<td>Number affected</td>
<td>2</td>
</tr>
</tbody>
</table>

(Appelman et al., 1982)

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

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

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

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

In a pulmonary immune response study, groups of non-sensitized and ovalbumin (OA)-sensitized guinea pigs were exposed to 0 or 200 ppb (360 µg/m$^3$) acetaldehyde for 6 hr/day, 5 days/week for four weeks (Lacroix et al., 2002). In sensitized, acetaldehyde-exposed animals, subsequent challenge with OA aerosol did not modify the inflammatory and allergic responses induced by sensitization alone. In nonsensitized guinea pigs, acetaldehyde exposure resulted in slight irritation (metaplasia/hyperplasia) of the lung, trachea and nasal respiratory epithelium, and induced a significant increase in the number of alveolar macrophages and total number of cells in bronchoalveolar lavage fluid (Lacroix et al., 2002). The respiratory lesions in guinea pigs at 200 ppb acetaldehyde occurred at roughly 2000-fold less than the NOAEL established for respiratory lesions in rats and hamsters under similar exposure duration protocols. These data suggest that guinea pigs are considerably less sensitive to acetaldehyde exposure than rats or hamsters.

Inhaled acetaldehyde is genotoxic and is a clastogen, and inducer of sister chromatid exchanges (Dellarco, 1988). In vivo and in vitro studies have shown that acetaldehyde can form DNA-DNA and DNA-protein crosslinks (Morris, 1997a). Acetaldehyde vapor causes chronic tissue injury and tumor formation in nasal tissues at exposure concentrations of 750 ppm or higher (Feron et al., 1982; Woutersen et al., 1986).

**7.0 Developmental and Reproductive Toxicity**

Both clinical and experimental studies have shown that ethyl alcohol causes developmental and reproductive toxicity. Acetaldehyde, the primary metabolite of ethyl alcohol, has been suggested as a possible etiologic agent in fetal alcohol syndrome (FAS) (Pratt, 1980; West, 1994; Eriksson, 2001). Current studies suggest that ethyl alcohol and acetaldehyde work through different mechanisms, but it is still unknown if one or both are the basis for FAS. Acetaldehyde has been shown to cross the placenta in mice and was distributed to embryos (Blakley and Scott Jr., 1984). Placental transfer occurred when acetaldehyde was administered via i.p. injection to pregnant CD-1 mice at 200 mg/kg on day 10 of gestation, and acetaldehyde was detected within the embryo within 5 minutes (Silverman et al., 1946). Maximal concentrations of acetaldehyde were also reached in the maternal blood, liver, and yolk sac in the first five minutes.

Acetaldehyde also freely crosses the placenta of Wistar rats (Zorzano and Herrera, 1989). Following i.v. injection of acetaldehyde (10 mg/kg) to pregnant rats on gestation day 21, acetaldehyde concentrations reached peak levels within five minutes in the maternal blood, fetal blood, and amniotic fluid. After just two minutes of maternal intravenous administration of acetaldehyde at high concentrations, it freely crosses the placenta.
Acetaldehyde is a small liposoluble molecule and is able to cross membranes by simple diffusion (Zorzano and Herrera, 1989).

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

_in vitro_ models have found that acetaldehyde was teratogenic to 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. 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 μM acetaldehyde was effective in suppressing testicular steroidogenesis; however cell viability was unaffected.

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

8.1 Acetaldehyde Acute Reference Exposure Level

<table>
<thead>
<tr>
<th>Study</th>
<th>Silverman et al., 1946</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study population</td>
<td>24 adult human volunteers</td>
</tr>
<tr>
<td>Exposure method</td>
<td>Whole body inhalation</td>
</tr>
<tr>
<td>Exposure continuity</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Exposure duration</td>
<td>Eye and upper respiratory tract irritation</td>
</tr>
<tr>
<td>Critical effects</td>
<td></td>
</tr>
<tr>
<td>LOAEL</td>
<td>45 mg/m$^3$ (25 ppm)</td>
</tr>
<tr>
<td>NOAEL</td>
<td>not observed</td>
</tr>
<tr>
<td>Benchmark concentration</td>
<td>not derived</td>
</tr>
<tr>
<td>Time-adjusted exposure</td>
<td>not applied (sensory irritation, no Haber’s Law adjustment)</td>
</tr>
<tr>
<td>Human Equivalent Concentration</td>
<td></td>
</tr>
<tr>
<td>LOAEL uncertainty factor (UF$_L$)</td>
<td>6 (default: mild effect, no NOAEL)</td>
</tr>
<tr>
<td>Subchronic uncertainty factor (UF$_S$)</td>
<td>not applied</td>
</tr>
<tr>
<td>Interspecies uncertainty factor</td>
<td></td>
</tr>
<tr>
<td>Toxicokinetic (UF$_A$)</td>
<td>1 (default, human study)</td>
</tr>
<tr>
<td>Toxicodynamic (UF$_A$)</td>
<td>1 (default, human study)</td>
</tr>
<tr>
<td>Intraspecies uncertainty factor</td>
<td></td>
</tr>
<tr>
<td>Toxicokinetic (UF$_H$)</td>
<td>1 (site of contact; no systemic effects)</td>
</tr>
<tr>
<td>Toxicodynamic (UF$_H$)</td>
<td>10 (asthma exacerbation in children)</td>
</tr>
<tr>
<td>Cumulative uncertainty factor</td>
<td>60</td>
</tr>
<tr>
<td>Reference Exposure Level</td>
<td>750 $\mu$g/m$^3$ (420 ppb)</td>
</tr>
</tbody>
</table>

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

The study by Silverman et al., (1946) was selected for development of the acute REL as it investigated short-term exposure of human volunteers to acetaldehyde. Eye irritation was the most noticeable endpoint for non-asthmatic adults. Upper respiratory tract, nose, throat, and bronchial irritation typically followed that effect closely. Exposure to 50 ppm for 15 minutes caused moderate eye irritation in all subjects, whereas 25 ppm caused complaints of slight eye irritation in an unspecified number of volunteers. Nose and throat irritation and transient conjunctivitis were seen at concentrations of 200 ppm or greater. From the key study it was possible to determine a LOAEL of 25 ppm for slight eye irritation. However, a NOAEL was not determined for this study.

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

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

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

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

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

A toxicodynamic uncertainty factor (UF_H-d) of ten was used to account for the potential greater susceptibility of children. While ocular irritation is not expected to be substantially different between children and adults, the respiratory irritant effect, with documented potential to exacerbate asthma, is clearly an effect with the potential to differentially impact infants and children. The toxicodynamic component of the intraspecies uncertainty factor UF_H-d is therefore assigned an increased value of ten to account for potential asthma exacerbation. As mentioned earlier, asthmatics are more
sensitive to the irritative properties of inhaled aerosolized acetaldehyde solutions, which significantly decreased forced expiratory volume in one second (FEV$_1$) by more than 20% in asthmatics. And, alcohol sensitive asthmatics had a selective hyper-responsiveness to acetaldehyde (Myou et al., 1993; Fujimura et al., 1999; Prieto et al., 2000). These considerations are applied equally to the acute, 8-hour and chronic REL.

Limitations with the Silverman et al. (1946) key study include: small sample size, subjective and non-quantitative measure of irritation, absence of a clear description of exposure method and experimental procedure, which was further unsubstantiated by lack of a clear experimental procedure that was referenced as Cook et al. (1945).

In conclusion, using the LOAEL of 45 mg/m$^3$ (25 ppm) from Silverman et al. (1946) divided by the cumulative uncertainty factor of 60, an acute reference exposure level (REL) for acetaldehyde, with the endpoint of eye irritation, was determined to be 750 ug/m$^3$ or 420 ppb, which is the level considered safe for infants and children during an acute exposure period.
### 8.2 Acetaldehyde 8-Hour Reference Exposure Level

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

Both eye irritation and nasal mucosal histopathology are legitimate concerns for the 8-hour REL and occur in a broadly similar concentration range over the relevant time scale. The human study by Silverman et al. (1946) provided limited information on the experimental procedure, used a small sample size, and used a subjective non-quantitative form of measure as the endpoint (self-reported irritancy). Further, the repeated nature of an 8-hour REL makes use of the acute study inappropriate. Therefore, the 8-hour REL was derived using the subchronic animal study (Appelman et al., 1982; 1986) in rats exposed to acetaldehyde six hours per day, five days per week for four weeks. Incidence of degeneration of nasal olfactory epithelium was the most sensitive end-point.

<table>
<thead>
<tr>
<th>Study</th>
<th>Appelman et al., 1982; 1986</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study population</td>
<td>Wistar rats (10-40 animals/group)</td>
</tr>
<tr>
<td>Discontinuous whole-body inhalation exposure to 0, 273, 728, 910, 1820, 4004, 9100 mg/m$^3$ (0, 150, 400, 500, 1000, 2200, or 5000 ppm)</td>
<td></td>
</tr>
<tr>
<td>Exposure continuity</td>
<td>6 hours per day, 5 days/week</td>
</tr>
<tr>
<td>Critical effects</td>
<td>Nasal degeneration of olfactory epithelium</td>
</tr>
<tr>
<td>LOAEL</td>
<td>720 mg/m$^3$ (400 ppm)</td>
</tr>
<tr>
<td>NOAEL</td>
<td>270 mg/m$^3$ (150 ppm)</td>
</tr>
<tr>
<td>Benchmark Concentration (BMC$_{0.05}$) (using continuous model)</td>
<td>178 mg/m$^3$ (99 ppm)</td>
</tr>
<tr>
<td>Time-adjusted exposure</td>
<td>133.5 mg/m$^3$ (74.25 ppm) = (178<em>6/8</em>5/5)</td>
</tr>
<tr>
<td>Human DAF concentration</td>
<td>113.5 mg/m$^3$ (63.11 ppm) = 133.5*0.85 (DAF)</td>
</tr>
<tr>
<td>LOAEL uncertainty factor (UF$_L$)</td>
<td>1</td>
</tr>
<tr>
<td>Subchronic uncertainty factor (UF$_s$)</td>
<td>1</td>
</tr>
<tr>
<td>Interspecies uncertainty factor</td>
<td>2 (with DAF adjustment)</td>
</tr>
<tr>
<td>Toxicokinetic (UF$_{A,k}$)</td>
<td>$\sqrt[10]{10}$ (default: no interspecies toxicodynamic data)</td>
</tr>
<tr>
<td>Toxicodynamic (UF$_{A,d}$)</td>
<td>10 (asthma exacerbation in children)</td>
</tr>
<tr>
<td>Intraspecies uncertainty factor</td>
<td>200</td>
</tr>
<tr>
<td>Toxicokinetic (UF$_{H,k}$)</td>
<td>$\sqrt[10]{10}$ (inter-individual variation)</td>
</tr>
<tr>
<td>Toxicodynamic (UF$_{H,d}$)</td>
<td>1</td>
</tr>
<tr>
<td>Cumulative uncertainty factor</td>
<td>568 µg/m$^3$ (316 ppb)</td>
</tr>
</tbody>
</table>

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 \times t) cumulation, at least over moderate timeframes. Therefore, the average experimental exposure was adjusted from six to eight hours per day.

The 8-hour REL was determined using the Benchmark Dose (BMC) program (Crump and Howe, 1983; Crump, 1984) and procedures developed by the U.S. EPA (2003). The BC_{05} is defined as the 95% lower confidence limit of the concentration expected to produce a response rate of 5%. The animal data from the Appelman et al. (1982; 1986) studies were used to develop a BC_{05} for acetaldehyde.

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

<table>
<thead>
<tr>
<th>Dose (ppm)</th>
<th>Males</th>
<th>Number</th>
<th>Mean</th>
<th>Stdev</th>
<th>Females²</th>
<th>Number</th>
<th>Mean</th>
<th>Stdev</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>30</td>
<td>0.07</td>
<td>0.25</td>
<td></td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>10</td>
<td>2.6</td>
<td>1.17</td>
<td>0.97</td>
<td>10</td>
<td>0.9</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>10</td>
<td>2.5</td>
<td>0.63</td>
<td>0.97</td>
<td>10</td>
<td>3.6</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>10</td>
<td>2.8</td>
<td>0.63</td>
<td>0.67</td>
<td>10</td>
<td>5.1</td>
<td>1.91</td>
<td></td>
</tr>
<tr>
<td>2200</td>
<td>10</td>
<td>5.3</td>
<td>2.21</td>
<td>10</td>
<td>6.9</td>
<td>0.32</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Method</th>
<th>BMCL*</th>
<th>BMC*</th>
<th>P-value</th>
<th>AIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hill Model</td>
<td>100</td>
<td>205</td>
<td>0.07</td>
<td>55.96</td>
</tr>
<tr>
<td>Polynomial (2°)</td>
<td>101</td>
<td>126</td>
<td>0.02</td>
<td>56.18</td>
</tr>
<tr>
<td>Polynomial (3°)</td>
<td>97</td>
<td>165</td>
<td>0.03</td>
<td>55.95</td>
</tr>
</tbody>
</table>

* BMCL and BMC are in units of ppm.

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

The dosimetric adjustment factor (DAF) is a factor derived by OEHHA based on the modeled comparative flux of formaldehyde in the upper respiratory tracts of rats, rhesus monkeys and humans (Kimbell et al., 2001) (see Section 4.4.7.2.2 of the TSD). In this model, a three-dimensional, anatomically realistic, computational flow dynamic model was used to estimate mass flux across 20 consecutive bins representing the nasal passages. The mean flux at each bin was weighted by the percent of non-squamous epithelium in that bin to derive a weighted average flux for each bin. Averaging across all 20 bins provides an overall estimate of the flux for comparison between species (rat 13.63 pmol/mm²; human 30.80 pmol/mm²). Peak flux values were also estimated for the rat (2620 pmol/mm²) and human (2082 pmol/mm²), and averaged with the mean flux values to estimate the DAF. The DAF is the ratio of this value for the rat to that for humans. The reactivity of acetaldehyde is similar to that of formaldehyde. Therefore, application of the DAF to acetaldehyde assumes that it deposits similarly to formaldehyde in the nasal passages. The uncertainty associated with this assumption is reflected in the use of the toxicokinetic component of the interspecies uncertainty factor $U_{Ax}$ equalling two. Sensitivity to acetaldehyde of the rat olfactory epithelium is a major factor for olfactory tissue damage, even though the specific activity of aldehyde dehydrogenase is greater in the respiratory epithelium (Bogdanffy et al., 1998; Stanek and Morris, 1999).
The LOAEL uncertainty factor (UF$_L$) of one was chosen, since both a LOAEL and NOAEL were determined in the key studies (Appelman et al., 1982; Appelman et al., 1986), and the benchmark approach was used to determine the chronic REL. In addition, the subchronic uncertainty factor (UF$_S$) was assigned a value of one since this was a subchronic study.

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

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

An uncertainty factor (UF$_{H,k}$) of 3.16 was used to account for intra-individual toxicokinetic variation. The intraspecies uncertainty factor was selected because acetaldehyde is a reactive substance that produces lesions at the point of contact with the tissue, therefore there would be less variability to take into account for children versus adults. The toxicodynamic uncertainty factor (UF$_{H,d}$) of 10 was used to account for the potentially greater susceptibility of children and asthmatics. The resulting cumulative uncertainty factor was calculated as 200 and used to determine the acute REL of the experimental animal study.

The BC$_{95}$ of 99 ± 1.20 ppm from the Benchmark Dose analysis was time-adjusted from six to eight hours for five days per week. Then, the resulting value was multiplied by the dosimetric adjustment factor (DAF) based on PBPK modeling. Finally, the resulting value was divided by the cumulative uncertainty factor of 200. This produced an 8-hour REL with the endpoint of degeneration of olfactory epithelium in rats of 568 μg/m$^3$ (316 ppb) (Appelman et al., 1982; Appelman et al., 1986)

Eye irritation and nasal mucosal histopathology are both legitimate concerns for the 8-hour REL for acetaldehyde and occur in a broadly similar concentration range over the relevant time scale. However, repeated 8-hour exposures could result in tissue damage, therefore the REL using the animal study with a histopathological endpoint of 568 μg/m$^3$ (316 ppb) was used. The experimental animal study used as the basis for the 8-hour REL with an endpoint of degeneration of nasal olfactory epithelium would also be protective of the human sensory response too, since the acute REL derived from the Silverman et al. (1946) human study is higher. The animal study was chosen because it was a well-conducted study with adequate dose groups and a time-period relevant for the 8-hour REL. In addition, using benchmark dose and PBPK modeling decreased the uncertainty associated with the REL derivation compared with using the traditional NOAEL/LOAEL and HEC procedures.
8.3 Acetaldehyde Chronic Reference Exposure Level

Study population
Wistar rats (10-40 animals/group)

Exposure method
Discontinuous whole-body inhalation exposure to 0, 273, 728, 910, 1820, 4004, 9100 mg/m³ (0, 150, 400, 500, 1000, 2200, or 5000 ppm)

Exposure continuity
6 hours per day, 5 days/week

Exposure duration
4 weeks

Critical effects
Nasal degeneration of olfactory epithelium

LOAEL
720 mg/m³ (400 ppm)

NOAEL
270 mg/m³ (150 ppm)

Benchmark Concentration (BMC₀.₅)
178 mg/m³ (99 ppm)

Time-adjusted exposure
31.79 mg/m³ (17.68 ppm) = (178*6/24*5/7)

Human DAF concentration
27.02 mg/m³ (15.03 ppm) = 31.79*0.85 (DAF)

LOAEL uncertainty factor (UFᵢ)
1

Subchronic uncertainty factor (UFs)
√10 (exposure 8-12% of lifetime)

Interspecies uncertainty factor

Toxicokinetic (UFₐ₋ₖ)
2 (with DAF adjustment)

Toxicodynamic (UFₐ₋₃ₜ)
√10 (default: no interspecies toxicodynamic data)

Intraspecies uncertainty factor

Toxicokinetic (UFₕ₋ₖ)
√10 (inter-individual variation)

Toxicodynamic (UFₕ₋₃ₜ)
10 (asthma exacerbation in children)

Cumulative uncertainty factor
632

Reference Exposure Level
43 μg/m³ (24 ppb)

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

The chronic REL was based on four-week exposure data in rats from Appelman et al., (1982, 1986), and supported by Saldiva et al., (1985); Woutersen et al., (1986, 1984); and (Woutersen and Feron, 1987), which included a 28-month chronic study in rats. Incidence of degeneration of nasal olfactory epithelium was the most sensitive end-point. The proposed chronic REL was estimated by a benchmark concentration (BMC) modeling approach using the continuous polynomial and Hill models of analysis (Crump and Howe, 1983; Crump, 1984) as previously described in detail in Section 8.2. The average experimental exposure data were adjusted to reflect chronic exposure. Table 8.2.1 shows the data expressed as the mean and standard deviation of the degeneration of nasal olfactory epithelium by severity for each dose group, which were the data used for the BMC model. As shown in Table 8.2.2, three models were selected that best fit the data and their mean and standard deviation was 99 ± 1.20 ppm and therefore used as the BC₀.₅.
As described in detail in Section 8.2, OEHHA derived a dosimetric adjustment factor (DAF) for acetaldehyde rather than using the traditional Human Equivalent Concentration (HEC) value. The application of the DAF was based on a model developed with formaldehyde and assumes that acetaldehyde deposits similarly to formaldehyde in the nasal passages. The uncertainty associated with this assumption is reflected in the use of the toxicokinetic component of the interspecies uncertainty factor $U_{A-k}$ equaling two.

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

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

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

Intraspecies variability can be as much as a factor of 1,000-fold for VOCs measured in human subjects (Fenske and Paulson, 1999). An uncertainty factor ($U_{H-k}$) of 3.16 was used to account for intra-individual toxicokinetic variation. The intraspecies uncertainty factor was selected because acetaldehyde is a reactive substance that produces lesions at the point of contact with the tissue, therefore there would be less kinetic variability to take into account for children versus adults. The toxicodynamic uncertainty factor ($U_{H-d}$) of 10 was used to account for the potentially greater susceptibility of children and asthmatics.

The current chronic RfC for acetaldehyde determined by the U.S. EPA and based on Appelman et al., (1982; 1986) is 9 µg/m$^3$ (5 ppb) and is within the range of normal human breath acetaldehyde concentrations of 0.7 to 11.0 µg/m$^3$ (0.4 to 6.1 ppb). OEHHA’s proposed chronic REL of 43 µg/m$^3$ (24 ppb) is above the range of human breath concentrations of acetaldehyde, but is mostly exceeded when humans consume significant amounts of alcohol, resulting in human breath concentrations ranging from

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200 to 2200 µg/m³. Thus, frequent alcohol use and abuse by humans is a major source of acetaldehyde exposure to the airway tissue that can exceed the chronic REL.

Standard BMC methodology was not in place when U.S. EPA developed its RfC for acetaldehyde in 1991. The U.S. EPA (1991) determined an acetaldehyde RfC of 9 µg/m³ (5 ppb) based on the Appelman et al., (1982, 1986) studies using NOAEL/LOAEL methodology. The principal study established a NOAEL of 273 mg/m³. Adjustments for duration of exposure and corrections for relative areas of human and animal extrathoracic region of the respiratory tract were made resulting in a human equivalent concentration of 8.7 mg/m³. A total UF of 1000 (10 for intraspecies, 10 for extrapolation to chronic exposure, and 10 for interspecies and incompleteness of the database) was then applied, resulting in the RfC of 9 µg/m³.

The LOAEL of 750 ppm from the chronic exposure data by Woutersen et al., (1984, 1986) and Woutersen and Feron (1987) produced similar injuries and was confined to the nasal olfactory epithelium as the LOAEL of 400 ppm from the 4-week Appelman studies. Thus, the subchronic UF was reduced from 10 to 3.16, to account for similar findings from the chronic studies. Analyses were also performed on the incidence of respiratory epithelial changes using the LOAEL from the chronic rat studies, although it was a less sensitive end-point (Woutersen et al., 1984, 1986; Woutersen and Feron 1987). The 100% response rate at the LOAEL combined with the lack of a NOAEL prevented the chronic studies from becoming the basis of the REL.

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

8.4 Acetaldehyde as a Toxic Air Contaminant

In view of the potential of acetaldehyde to exacerbate asthma (Section 5.1, 5.2), and the differential impacts of asthma on children including higher prevalence rates, coupled with widespread exposure (e.g., indoors from exposure to environmental tobacco smoke, and outdoors due to numerous emissions sources), OEHHA recommends that acetaldehyde be identified as a toxic air contaminant (TAC) that may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).
9. References


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


