



TOXICOLOGICAL REVIEW

OF

ACETALDEHYDE

(CAS No. 75-07-0)

**In Support of Summary Information on the
Integrated Risk Information System (IRIS)**

December 1999

NOTICE

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U.S. Environmental Protection Agency
Washington, DC

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LIST OF ABBREVIATIONS AND ACRONYMS

ALDH	aldehyde dehydrogenase
BaP	benzo(a)pyrene
BMC	benchmark concentration
DENA	diethylnitrosamine
FAS	fetal alcohol syndrome
HEC	human equivalent concentration
HPRT	hypoxanthine phosphoribosyltransferase
LEC ₁₀	
MTD	maximum tolerated dose
MF	modifying factor
NAD ⁺	
OR	odds ratio
PEL	permissible exposure limit
RfC	reference concentration
RfD	reference dose
RGDR	regional gas dose ratio
S _{ET}	surface areas for the extrathoracic portion of the respiratory tract
STEL-C	short-term exposure limit ceiling
TWA	time-weighted-average
UF	uncertainty factor
V _E	ventilation rates, in m ³ /day

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to acetaldehyde. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of acetaldehyde.

In Section 6, EPA has characterized its overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's Risk Information Hotline at 513-569-7254.

AUTHORS, CONTRIBUTORS, AND REVIEWERS

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1. INTRODUCTION

This document presents background and justification for the hazard and dose-response assessment summaries in EPA's Integrated Risk Information System (IRIS). IRIS summaries may include an oral reference dose (RfD), inhalation reference concentration (RfC) and a carcinogenicity assessment.

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis, but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrapulmonary or systemic effects). It is generally expressed in units of mg/m³.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per µg/m³ air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for acetaldehyde has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Proposed Guidelines for Carcinogen Risk Assessment* (1996a), and *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996b); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b); and memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

Literature search strategies employed for this compound were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, TSCATS, CCRIS, GENETOX, EMIC, EMICBACK, DART, ETICBACK, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Acetaldehyde is also known as acetic aldehyde, ethanal, or ethyl aldehyde. Some relevant physical and chemical properties of acetaldehyde are listed below (IARC, 1985; NTP, 1999):

CAS Registry number: 75-07-0

Structural formula: CH_3CHO

Molecular weight: 44.06

Vapor pressure: 755 mm Hg at 20°C

Water solubility: 0.1-1.0 mg/ml at 19°C

Conversion factor: 1 ppm = 1.80 mg/m³, 1 mg/m³ = 0.555 ppm (25°C, 760 mmHg)

At room temperature, acetaldehyde is a volatile, colorless liquid with a pungent, fruity odor. It is flammable, with a flash point of -40°C, and strongly reactive with a number of chemical classes. Acetaldehyde has a short-term exposure limit ceiling (STEL-C) of 25 ppm (45 mg/m³), recommended to protect against irritation (ACGIH, 1999). OSHA has promulgated an 8-hour PEL of 200 ppm, which corresponds to 360 mg/m³ (OSHA, 1993).

The primary use of acetaldehyde is as an ingredient in the chemical synthesis of a large number of compounds, including acetic acid, pyridine and pyridine bases, and peracetic acid. Small amounts of acetaldehyde are used as a food additive in such foods as milk products, baked goods, fruit juices, candies, and soft drinks, usually at levels up to 0.047%.

Acetaldehyde is ubiquitous in the environment, and is released from natural sources. It is a metabolic intermediate of higher plant respiration and alcohol fermentation. In humans, acetaldehyde is formed as a result of the metabolism of ethanol by alcohol dehydrogenase enzymes. Acetaldehyde has been detected in a wide variety of fruits, vegetables, and plant-derived oils. It has also been detected in oak leaves and tobacco plants. Acetaldehyde is found in high concentrations (up to 1200 ppm) in the smoke of tobacco and marijuana cigarettes.

Acetaldehyde may be present in the atmosphere as a result of natural sources or, more commonly, as a result of industrial emission or motor vehicle exhaust. In Brazil, where ethanol-fueled vehicles are slowly replacing traditional gasoline-powered vehicles, one study (Grosjean et al., 1990) reported that urban levels of atmospheric acetaldehyde range between 2.3 and 19.2 parts per billion (4.1-34.6 µg/m³) but may rise to as high as 240 ppb (430 µg/m³) in poorly ventilated areas, such as highway tunnels. The same study reported indoor levels ranging from 9.2 to 34 ppb (16.6-61.3 µg/m³). A recent study of airborne acetaldehyde levels in the United States reported ambient levels ranging from 2.4 to 3.1 µg/m³, but noted that in-vehicle exposure levels may reach as high as 66.7 µg/m³ (U.S. EPA, 1993). Atmospheric acetaldehyde is volatile

and prone to oxidation, with atmospheric residence times varying with climate and location, and ranging from 3 to 3000 hours, with average values ranging from 6 to 200 hours (U.S. EPA, 1993).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION/DEPOSITION

3.1.1. Oral Exposure

Booze and Oehme (1986) exposed 6 dogs by gavage to 600 mg/kg of acetaldehyde and examined acetaldehyde levels in blood. Plasma concentrations peaked at 15 minutes, indicating rapid absorption, and decreased rapidly thereafter. However, because of a high rate of vomiting (all six dogs vomited following exposure, several of them more than once), the rate and extent of absorption was not quantified.

3.1.2. Inhalation Exposure

Egle (1970) studied the retention of inhaled acetaldehyde in a group of 4 men and 4 women. Percent retention of acetaldehyde varied inversely with respiratory rate (in breaths per minute), with 5 breaths per minute resulting in approximately 70% retention, whereas 40 breaths per minute resulted in approximately 45% retention. Retention also varied with exposure concentration, with a higher concentration resulting in a lower percent retention. There was no difference between mouth breathing and nose breathing in total retention. Varying the tidal volume over a broad range did not affect uptake when other factors remained constant. The author concluded that the percent retention of acetaldehyde was dependent primarily upon the duration of the ventilatory cycle. In another study examining respiratory tract acetaldehyde uptake in humans, Dalhamn et al. (1968) reported that retention of the acetaldehyde from cigarette smoke in human subjects was nearly 100%.

Morris and Blanchard (1992) measured the deposition, calculated from the change in airborne concentration, of acetaldehyde in the upper respiratory tract of male Fischer 344 rats exposed to 0, 1, 10, 100, or 1000 ppm of acetaldehyde. Absolute deposition rates, in $\mu\text{g}/\text{minute}$, increased with increasing inspired concentration in a nonlinear fashion. However, fractional deposition decreased with increasing concentration, beginning at a deposition efficiency of 0.71 at 1 ppm, decreasing to 0.26 at 1000 ppm (values for cyclic air flow at 100 ml/minute). The trend of decreasing fractional deposition with increasing concentration was statistically significant. A trend toward decreased fractional deposition with increasing flow rate was also statistically significant. A significant association was also observed between flow rate and concentration with regards to their influence on fractional deposition. Later studies (Morris, 1997) demonstrated similar relationships between flow rate, concentration, and fractional deposition in mouse, hamster, and guinea pigs as well. Species differences in acetaldehyde deposition were found, which varied with exposure concentration. At high concentrations, uptake was significantly higher in the mouse, rat, and hamster than in the guinea pig. In contrast, uptake at 10 ppm was significantly lower in the rat than all other species examined. Similar results involving the correlation between respiratory rate and percent retention were reported by Egle (1972) in dogs.

Another animal study demonstrating high absorption levels following inhalation exposure was reported by Watanabe et al. (1986). Male Sprague-Dawley rats were exposed to airborne acetaldehyde at levels from 0.009 to 1.0 mg/m³ for 1 hour. Immediately following discontinuation of exposure, the rats were exsanguinated and analyzed for blood acetaldehyde levels. Acetaldehyde inhalation resulted in blood concentrations of ~1000 µM in blood 5 minutes after exposure. Acetaldehyde concentrations were higher in arterial blood than in venous blood.

3.1.3. Dermal Exposure

Acetaldehyde has been shown to cause dermal sensitization in guinea pigs (Bergh and Karlberg, 1999). However, no data on the dermal absorption of acetaldehyde in humans or animals are available.

3.2. DISTRIBUTION

Johansson-Brittebo and Tjälve (1979) examined the distribution of ¹⁴C-acetaldehyde in C57-B1 mice by whole-body autoradiography. Mice were injected intravenously with 5.0 µCi (~2 mg/kg) and monitored after 1, 5, and 30 minutes, 1, 4, and 24 hours, and 6 days. Immediately after injection, the highest activity was present in the heart muscle, diaphragm, and kidney cortex, with high levels also found in the gastrointestinal mucosa, exocrine pancreas, salivary and lacrimal glands, bone marrow, nasal and bronchial mucosa, brown fat, choroid plexus, Harder's gland, and skeletal muscles. Radioactivity in the liver was relatively low. By 5 minutes post-injection, the activity in the heart muscle, diaphragm, and skeletal muscles had decreased, but distribution to other areas of the body was relatively unchanged. Similar distribution pictures were also seen 30 minutes to 24 hours following injection, with the additional finding of radioactivity in tissues of steroid hormone synthesis.

Hobara et al. (1985) reported finding relatively low acetaldehyde levels in liver, and high levels of acetaldehyde in kidney, spleen, heart muscle, and skeletal muscle up to 25 minutes after a 1-hour inhalation exposure of Sprague-Dawley rats to airborne acetaldehyde levels between 1 and 20 mM in air (~25,000 ppm). Acetaldehyde levels in peripheral blood were highest, being 6- to 10-fold higher than any of the other tissues examined.

3.3. METABOLISM

The primary metabolic pathway for acetaldehyde metabolism involves a rapid metabolism by NAD⁺-dependant aldehyde dehydrogenase enzymes in the mitochondria. The resulting product, acetate, is then either further metabolized to carbon dioxide and water or incorporated into the body's 2-carbon pool and used in molecular synthesis reactions (Hardman et al., 1996). Aldehyde dehydrogenase (ALDH) genes have been located in myriad tissues throughout the body.

Nasal metabolism is believed to be of particular relevance to the toxicity and carcinogenicity of inhaled acetaldehyde. Homogenates from rat, mouse, and hamster nasal tissues showed aldehyde dehydrogenase activity with kinetics representative of a two-isoenzyme (high affinity and low affinity) model, while guinea pig homogenates showed kinetics representative of a

one-isoenzyme model (Morris, 1997). The V_{\max} for the guinea pig high-affinity-low-capacity enzyme was significantly greater than for all other species examined, and the K_m was significantly greater for the guinea pig enzyme than for all species examined except for the mouse, which had a nonsignificantly lower value. For the low-affinity enzyme, K_m values were similar for the rat and hamster, but significantly higher in mouse. In contrast, the V_{\max} value was greater for the rat than either the mouse or hamster. Bogdanffy et al. (1986) have demonstrated by histochemical localization that the olfactory epithelium is virtually devoid of ALDH activity though the respiratory epithelium is high in activity, and noted that this distribution correlates with the regional epithelial susceptibility to acetaldehyde.

Other animal studies (e.g. Shiohara et al., 1984) have demonstrated multiple ALDH enzyme activities in other organs as well, and at least 4 distinct ALDH isozymes have been identified. It appears that the bulk of acetaldehyde metabolism following inhalation exposure occurs in organs other than the liver (Watanabe et al., 1986), though the liver can readily metabolize acetaldehyde (Lubin and Westerfield, 1945; Hald et al., 1949), resulting in substantial first pass effects following oral exposure. Studies in humans (Greenfield and Pietruszko, 1977) have also demonstrated both high- and low-affinity ALDH isozymes.

3.4. ELIMINATION AND EXCRETION

The primary method of acetaldehyde elimination appears to be metabolism to acetate, as described above (Hardman et al., 1996). Following acute inhalation exposure, acetaldehyde is very rapidly removed from the blood, with a half-life in rats of ~3.1 minutes (Hobara et al., 1985). Booze and Oehme (1986) reported that blood acetaldehyde levels peaked at 15 minutes following a single gavage dose of 600 mg/kg and fell rapidly, though they were not able to determine a half time.

A small amount of the total acetaldehyde dose is excreted in the urine. Kallama and Hemminki (1983) reported approximately 6% recovery of administered activity in the urine seven days following intraperitoneal injection of [¹⁴C]acetaldehyde in rats. Booze and Oehme (1986) reported finding no acetaldehyde in the urine of six dogs exposed orally to a single dose of 600 mg/kg. However, all six dogs were observed to vomit often following exposure, making assessment of actual systemic dose questionable. Also, the authors measured unmetabolized acetaldehyde, which is unlikely to reach the urine due to high metabolic rates throughout the body.

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS - EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

In an early study, Silverman et al. (1946) reported on the sensory response to airborne acetaldehyde in groups of 12 volunteers of both genders. The majority of subjects experienced some degree of eye irritation at 50 ppm, and several experienced symptoms at 25 ppm of acetaldehyde. Those subjects who reported no eye irritation exhibited bloodshot eyes and

reddened eyelids after exposure to 200 ppm. Nose and throat irritation was not reported at airborne concentrations up to 200 ppm.

Only one cohort study regarding acetaldehyde exposure and incidence of cancer has been reported. Bittersohl (1974) examined a cohort of 220 individuals who were actively employed in an acetaldol and aliphatic aldehyde factory in the German Democratic Republic between 1967 and 1972, approximately 150 of whom were employed for more than 20 years in the factory. The numbers of male and female cohort members were not specified. Only workers actively employed during the study time were included in the study cohort. Acetaldehyde concentrations in workplace air were found to range from 1 to 7 mg/m³ (0.55-3.9 ppm). Nine cases of cancer (five squamous cell carcinomas of the bronchi, two squamous cell carcinomas of the mouth cavity, one adenocarcinoma of the stomach, and one adenocarcinoma of the cecum) were identified among males in the cohort. Of these, eight were reported to have smoked 5-10 cigarettes per day. The ninth smoked more than 30 cigarettes daily. Two female cancer cases were excluded from analysis because the author felt that the latency period for these cases was too short for their cancers to have been the result of industrial chemical exposure. A cancer incidence rate of 6000 per 100,000 population was calculated for this study cohort, compared to 1200 per 100,000 in the general German Democratic Republic population. Because the incidence rates were not age adjusted, and because this study has several other major methodological limitations (concurrent exposure to acetaldol, butyraldehyde, crotonaldehyde, “large” condensed aldehydes, and acrolein; smoking history [all patients with cancer reported having smoked]; and small number of subjects; lack of followup of workers who terminated employment before the study was begun; lack of information on subject selection, age and sex distribution), the data are considered inadequate to evaluate the carcinogenicity of acetaldehyde.

A cohort study of 29,139 men employed at any of three chemical manufacturing facilities in West Virginia over a 39-year period identified an increase in deaths caused by both cancers of the liver and lymphatic and hematopoietic tissue cancer (Rinsky et al. 1988). These findings led to the initiation of a case-control study by Ott et al. (1989a, 1989b), examining 129 cases of hematopoietic tissue malignancies taken from the larger cohort, with a detailed analysis of exposure classifications published in a separate report (Ott et al. 1989c). Co-exposure to other chemicals (e.g., acrylonitrile, alkyl sulfates, epichlorohydrin, metal salts, vinyl chloride) was common throughout exposure categories. In particular, job categories exposed to acetaldehyde were also co-exposed to acrylonitrile. The study revealed nonsignificant increases (95% lower confidence interval of the OR did not exceed 1.0) in odds ratios (OR) for acetaldehyde exposure when non-Hodgkins lymphoma (7 cases, OR 2.3), multiple myeloma (3 cases, OR 2.3), and non-lymphocytic leukemia (3 cases, OR 1.3) were examined. Therefore, a definitive association between acetaldehyde exposure and these cancers could not be made from these data.

4.2. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS - ORAL AND INHALATION

4.2.1. Oral Exposure

Til et al. (1988) exposed groups of 10 male and 10 female (20 male and 20 female for the unexposed group) Wistar rats to 0, 25, 125, or 625 mg/kg-day in drinking water for 4 weeks. To

account for potential effects of decreased water consumption in treated animals, an additional group of 10 male and 10 female rats was given drinking water in an amount equal to the amount of liquid consumed by the group given the highest dose. Endpoints examined included food and water intake, body weight, daily observations for condition and behavior, organ weights, hematologic and clinical chemistry parameters, urinalysis, and gross and histologic pathology. Acetaldehyde exposure resulted in no decrease in body weight or food consumption, but did lead to a decrease in liquid intake for both males and females at the highest dose only. There were no changes in hematology among the groups. In the 625 mg/kg-day male rats, the plasma level of urea was significantly increased and that of bilirubin was significantly decreased, while plasma alkaline phosphatase and alanine aminotransferase activities were significantly decreased in females exposed to 625 mg/kg-day. The relative weights of the kidneys were slightly, but significantly, increased in males, but not females, in the 625 mg/kg-day group; absolute weights were not reported. No change in the relative weights of the gonads, brain, heart, or liver were observed following acetaldehyde treatment. A slight (in 6/10 males and 6/10 females) or moderate (in 2/10 males and 2/10 females) focal hyperkeratosis of the stomach was observed in animals exposed to 625 mg/kg-day, but not in any of the other exposure levels. This study established a NOAEL of 125 mg/kg-day and a LOAEL of 625 mg/kg-day for histologic alterations of the gastrointestinal tract in male and female Wistar rats.

In another study reporting similar findings, Homann et al. (1997) exposed groups of 10 male Wistar rats to 0 or 120 mM acetaldehyde in drinking water for 8 months. The exposure level for the acetaldehyde-exposed rats was calculated by the researchers to be 324 ± 24 mg/kg-day. No significant changes in fluid intake or body weight were observed between groups, and no cancerous or dysplastic lesions were located in the upper gastrointestinal tract of rats from either group. The epithelial thickness of the tongue, epiglottis, and forestomach was increased in acetaldehyde-treated rats relative to controls, and was accompanied by a significant increase in the expression of Ki67, a marker of cellular proliferation. This study established a LOAEL of 324 mg/kg-day for histologic alterations of the gastrointestinal tract in male Wistar rats.

Evidence of gastrointestinal irritation has also been demonstrated in dogs. Following a single gavage exposure of 6 male dogs to 600 mg/kg of acetaldehyde in distilled water, Booze and Oehme (1986) reported vomiting in all six dogs, which continued for several hours and was often bloody. The two dogs with the highest plasma acetaldehyde levels developed slight tremors, which were transient. Upon gross examination, the stomachs of all acetaldehyde-treated dogs presented extensive hemorrhagic areas on the mucosa, suggestive of a severe irritation.

Other available oral studies have not examined gastrointestinal effects. HoLownia et al. (1992) exposed groups of 15 male Wistar rats daily by gavage to 0 or 800 mg/kg of acetaldehyde for 4 weeks. Following sacrifice, the livers were homogenized and the activity of superoxide dismutase (SOD), glutathione peroxidase, and catalase were measured. Acetaldehyde significantly reduced the level of SOD activity, but not of the other enzymes examined. Acetaldehyde treatment also significantly reduced the total level of sulfhydryl compounds in the liver, which the authors attributed to a decrease in glutathione levels.

The effect of long-term oral exposure to acetaldehyde on collagen biosynthesis in the liver of rats was examined (Pawlicka et al., 1991; Bankowski et al., 1993). Groups of 10 male Wistar rats were exposed to 0.05% acetaldehyde in the drinking water for up to 6 months (~75 mg/kg-

day, based on subchronic reference values for water intake and body weight from U.S. EPA, 1988). Acetaldehyde exposure resulted in a progressive increase of 5-³H-proline incorporation into collagen synthesized by the liver, but no change in noncollagen protein synthesis. Collagen content in the liver did not change significantly at early (up to 4 months) time points, but was significantly increased by 6 months of exposure. No differences in the types of collagen synthesized were noted.

4.2.2. Inhalation Exposure

A number of prechronic and chronic studies examining the effects of inhaled acetaldehyde have been reported by a team of Dutch researchers. Appelman et al. (1982) exposed groups of 10 male and 10 female Wistar rats to acetaldehyde for 6 hours/day, 5 days/week for 4 weeks at nominal concentrations of 0, 400, 1000, 2200, or 5000 ppm. Measured mean exposure concentrations were 0, 401, 941, 2217, or 4975 ppm (0, 730, 1713, 4035, or 9055 mg/m³, respectively). Growth retardation occurred in males of the 3 highest dose groups and in females of the highest dose group only. There were statistically significant differences in several blood chemistry parameters between the exposure groups and the control group, but none of them were concentration-related. Statistically significant changes in relative organ weight occurred only in the 5000 ppm group, and included decreased liver weights in both male and female rats and increased lung weights in male rats. Males exposed to 5000 ppm produced less urine, but it was of higher density. Compound-related histopathological changes were observed only in the respiratory system. The nasal cavity was most severely affected, with the anterior portions generally less affected than the posterior portions. Alterations in the nasal region demonstrated a concentration-response relationship. In the 400 ppm exposure group, a loss of microvilli accompanied by thinning and disarrangement of the epithelium in the dorsal part of the nose was observed in 9/10 of the males and 7/10 of the females. In animals exposed at the 1000 or 2200 ppm levels, more severe degenerative changes were observed, with hyperplastic and metaplastic changes in the olfactory and respiratory epithelium of the nasal cavities of all rats exposed to 2200 ppm. At 5000 ppm, the changes included severe degenerative hyperplastic and metaplastic changes, with a thickening of the submucosal region. The vocal cord region of the larynx of rats exposed to 2200 or 5000 ppm showed hyperplastic and metaplastic alterations, characterized by slightly keratinized stratified squamous epithelium. In the tracheal region, degeneration with hyperplasia and metaplasia occurred in the laryngeal and tracheal epithelium at the 2200 or 5000 ppm exposure levels; nearly all rats in the 5000 ppm group demonstrated a metaplasia of the respiratory epithelium, manifested by flat, elongated superficial cells and large basal cells containing huge, bizarre nuclei with large nucleoli. Tracheal changes were more severe in the upper trachea than in the lower trachea. Minor changes in the alveolar region of the lung were occasionally seen in animals of the high-dose group, but not in other groups. This study established a LOAEL of 400 ppm, based on histologic alterations of the nasal epithelium in Wistar rats; no NOAEL was established.

Appelman et al. (1986) exposed groups of 10 male Wistar rats for 6 hours/day, 5 days/week for 4 weeks to 0, 110/150, or 500 ppm (0, 200/273, or 910 mg/m³, respectively) of acetaldehyde. At each exposure concentration, three groups were examined. One group was exposed without interruption (150 or 500 ppm), a second group was interrupted for 1.5 hours between the first and second 3-hour period (150 or 500 ppm), and a third group (110 or 500 ppm) was interrupted as described with a superimposed peak exposure profile of 4 peaks at 6-fold

the basic concentration (660 or 3000 ppm) per 3-hour period. Urine samples were collected from all rats, and lung lavage was performed on 4-5 per group at the end of the experiment. Cell density, viability, number of phagocytosing cells, and phagocytic index were determined on the lavage fluid. Microscopic examination was performed on the nasal cavity, larynx, trachea with bifurcation and pulmonary lobes of all rats of all groups. Continuous and interrupted exposure to 500 ppm did not induce any visible effect on general condition or behavior, but animals exposed to 500 ppm with peak exposures of 3000 ppm showed signs of irritation. Mean body weights of the groups exposed to 500 ppm with interruption and with peak exposures were statistically significantly lower than those of the controls. Body weights were similar to controls in the other exposure groups. Mean lavage cell density and cell viability were significantly decreased in the groups exposed to 500 ppm with interruption, either with or without peak exposures. The mean percentage of phagocytosing cells and the phagocytic index were significantly lower than controls in all groups exposed to 500 ppm, particularly in the group with peak exposures. Histopathological changes attributable to exposure were found only in the nasal cavity, where degeneration of the olfactory epithelium was observed in all groups of rats exposed to 500 ppm. Interruption of the exposure or interruption combined with peak exposure did not appear to influence this adverse effect. No compound-related effects were observed in rats exposed to 150 ppm of acetaldehyde during the 4-week exposure period. This study established a NOAEL in rats of 150 ppm and a LOAEL of 500 ppm, based on histologic alterations of the nasal epithelium.

Kruyssen et al. (1975) conducted a 90-day inhalation study in Syrian golden hamsters (10/sex/concentration). The hamsters were exposed to acetaldehyde vapor at concentrations of 0, 390, 1340, or 4560 ppm (0, 702, 2412 or 8200 mg/m³, respectively), for 6 hours/day, 5 days/week for 90 days. At the highest concentration (4560 ppm), profound effects were observed. Body weights were significantly decreased (~25% reduction) relative to controls, and the relative weights of the heart, kidneys, brain, testicles, and lungs were significantly increased. In the nasal cavity, rhinitis, necrosis, rarefaction of turbinate bones, and metaplasia of respiratory and olfactory epithelium were noted, as well as a disappearance of subepithelial glands and thickening of the submucosa by an increased amount of fibrous tissue. In the larynx, the normal respiratory epithelium was replaced by a stratified squamous epithelium which was often keratinized, especially in the region caudad to the vocal cords. Likewise, large areas of the tracheas of high-dose hamsters were covered with a stratified squamous epithelium, often with heavy keratinization and inflammatory changes. Similar changes were evident to a lesser extent in the main stem bronchi. At the 1340 ppm exposure level, relative kidney weights in male hamsters were elevated, and some animals (4 male, 2 female) showed focal metaplastic responses of the tracheal epithelium, with small areas of stratified epithelium present. No treatment-related alterations were observed in animals exposed to 390 ppm of acetaldehyde. This study established a NOAEL of 390 ppm and a LOAEL of 1340 ppm, based on histopathologic alterations of the tracheal epithelium of hamsters.

Feron (1979) exposed groups of 35 male Syrian golden hamsters to airborne concentrations of 0 or 1500 ppm (2700 mg/m³) of acetaldehyde for 7 hours/day, 5 days/week for 52 weeks. Five animals were killed and autopsied immediately following cessation of exposure, and the remaining animals were observed for an additional 26 weeks. Immediately following 52 weeks of exposure, acetaldehyde-exposed animals showed marked lesions in the nasal cavity, including hyperplasia and metaplasia of the olfactory and respiratory epithelium and a slight to moderate rhinitis. In the trachea, slight focal hyper- and metaplastic changes of the lining

epithelium were observed in nearly all animals that were exposed to acetaldehyde. Following 26 weeks of recovery, these lesions had lessened in severity, but were still present. The larynx, trachea, bronchi, and bronchioli and alveoli were examined for increased tumor incidence. Acetaldehyde exposure did not result in increased incidence of tumors in these tissues at any time point examined. Thus, this study identified a LOAEL of 1500 ppm for histologic lesions in the nasal cavity.

In the second part of the study, groups of 35 male and 35 female Syrian golden hamsters were intratracheally instilled once weekly for 52 weeks with 0.2 ml of a 2 or 4% acetaldehyde solution (4 or 8 μ l, respectively), and observed for an additional 52 weeks. Extensive peribronchiolar adenomatoid lesions, often accompanied by inflammatory changes, were observed in many animals. Following 52 weeks of observation (study week 104), the inflammatory changes had reduced in severity, but the epithelial changes remained unaltered, though they had not progressed to a neoplastic state.

In a later study, Feron et al. (1982) exposed groups of 18 male and 18 female Syrian golden hamsters for 7 hours/day, 5 days/week for 52 weeks to a time-weighted average (TWA) concentration of 0 or 2028 ppm (3650mg/m³). The TWA concentration reflects an initial concentration of 2500 ppm that was lowered throughout the study to a final level of 1650 ppm to avoid early mortality) of acetaldehyde. After 52 weeks of exposure, animals were observed for an additional 29 weeks (81 weeks total study duration). Hamsters exposed to acetaldehyde had significantly lower body weights than those of controls from week 4 and onwards. Mortality was slightly higher in acetaldehyde-exposed hamsters than in control animals. There were no significant differences in hematological and biochemical findings between air-exposed and acetaldehyde-exposed hamsters, with the exception of an increase in alkaline phosphatase in exposed females. The relative weights of kidneys and lungs were higher in acetaldehyde-exposed hamsters than in controls, but were only statistically significantly elevated in females. Acetaldehyde-exposed animals killed at the end of exposure showed pathological changes in the nose, larynx, and trachea. Nasal alterations included rhinitis, thinning of the olfactory epithelium, and hyper- and metaplasia, often with heavy keratinization. In both the larynx and trachea, slight to moderate focal hyper- and metaplasia of the epithelium were found. Similar lesions were still found in the nose, larynx, and trachea at the end of the recovery period. Increased incidence of tumors of the larynx was noted following exposure to acetaldehyde, but was only statistically significant in males (6/29 in exposed compared to 0/30 for controls). For noncancer effects, this study identified a LOAEL of 2028 ppm.

A lifetime carcinogenicity study of acetaldehyde vapor in rats was conducted by Woutersen et al. (1985, 1986). Groups of 75 male and 75 female Wistar rats were exposed to 0, 750, 1500, or 3000 ppm (0, 1350, 2700, or 5400 mg/m³) acetaldehyde for 6 hours/day, 5 days/week for 28 months. Because of signs of severe toxicity in the 3000 ppm group, the concentration was gradually decreased to a final concentration of 1000 ppm in week 52 and onwards. The mean (TWA for high-dose) of the measured concentrations throughout the study were 0, 727, 1438, and 1592 ppm for the control, low-, mid-, and high-dose groups, respectively. Interim sacrifices occurred at weeks 13 (5 rats/sex), 26 (5 rats/sex), and 52 (10 rats/sex). Acetaldehyde exposure resulted in an increase in mortality in all exposed groups, compared to the controls, in a concentration-related manner (22, 11, 9, or 0 male rats survived to the end of the study in the 0, 750, 1500, and 3000 ppm groups, respectively; 28, 17, 11 or 0 female rats survived

to the end of the study in the 0, 750, 1500, and 3000 ppm groups, respectively). All of the male and female rats exposed to the highest exposure concentration died by day 715 of the study. Statistically significant retardations of growth occurred in males of each dose group (~8, 17, or 22% for 750, 1500, or 3000 ppm groups) and in the 1500 ppm (~14%) and 3000 ppm (~11%) females at study day 560. Significantly increased tumor incidences were limited to the nasal region, consisting primarily of adenocarcinomas and squamous cell carcinomas, with other respiratory tract tumors limited to a single laryngeal carcinoma and one pulmonary adenocarcinoma. In the nose, only one benign tumor was reported; all other nasal tumors were malignant. Total respiratory tract tumor incidences were 1/55, 17/54, 40/55, or 31/53 for control, 750, 1500, or 3000 ppm male rats, respectively, and 0/54, 8/55, 36/55, or 39/55 for control, 750, 1500, or 3000 ppm female rats, respectively. Nasal degeneration occurred in all dose groups, beginning as early as 52 weeks of exposure. It was characterized by basal cell hyperplasia in the 750 and 1500 ppm groups and squamous metaplasia, with slight to severe hyperkeratosis, in 1500 and 3000 ppm rats, beginning as early as week 13. The authors concluded that acetaldehyde vapor can cause severe nasal cytotoxicity, as well as carcinogenic effects in nasal tissues. These studies established a LOAEL for noncarcinogenic effects in the upper respiratory tract of 750 ppm.

In a concurrent study (Woutersen and Appelman, 1984; Woutersen and Feron, 1987), groups of 30 male and 30 female rats were exposed as described above for the carcinogenicity study, with exposure durations of 52 weeks followed by observation for up to 52 weeks of air exposure as a “recovery” period. Nonneoplastic nasal lesions observed in rats following 52 weeks of exposure had partially regressed following 52 weeks of recovery. Regenerative changes including small nerve bundles, groups of sensory cells, and groups of epithelial cells resembling acinar cells of the Bowman’s glands were evident after both 26 and 52 weeks of recovery. However, tumor incidence rates were similar to those in animals exposed throughout their lifetimes, indicating that after 52 weeks of exposure to acetaldehyde, proliferative epithelial lesions of the nose may develop into tumors even without continued exposure.

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

Imai and Omoto (1992) exposed 3- or 8-month-old pregnant Fisher rats (number per group not available) to 240 mg/kg-day of acetaldehyde, as a 2% aqueous solution via the oral route, daily throughout gestation, labor, and lactation. Because the manuscript was in Japanese, with an English translation of the abstract only, this study could not be completely evaluated. Maternal body weight gain was decreased between the 1st and 20th day of pregnancy in acetaldehyde-exposed rats. Eight-month-old rats, but not 3-month-old rats, showed a decreased placental weight relative to controls following acetaldehyde treatment. Slight (unspecified) histologic changes were observed in the brain, liver, and kidney of treated dams. No treatment-related differences in average pup number were observed, though acetaldehyde treatment resulted in a mild but statistically significant decrease in neonatal weight. Histologic examination of the brain, lung, liver, kidney, and thymus in offspring of treated rats revealed “visceral immaturity and hemorrhage,” compared to the controls, though incidence rates were not available. The significance of the reported findings cannot be evaluated without additional information that was not provided in the English abstract.

No developmental effects due to acetaldehyde exposure were found in an oral teratology study in rats performed by Hazleton Labs (1983). Twenty-two pregnant female CRL:COBS, CD[®](SD)BR rats were exposed by gavage on gestation days 6 through 15 to 0 or 400 mg/kg of acetaldehyde. Female rats were sacrificed at gestation day 20 and the fetuses were taken by cesarean section and examined. No statistically significant treatment-related changes in body weight, food consumption, or water consumption were reported. Gross pathological examination of the dams revealed no significant changes induced by acetaldehyde treatment. Gravid uterine weights were similar between control and acetaldehyde-treated rats. No changes in the number of corpora lutea, implantations, absorptions, or fetuses were observed in acetaldehyde-treated dams. Following visceral and skeletal examination of the fetuses, no treatment-related alterations were noted.

Whereas oral exposure of acetaldehyde appears to have slight, if any, effects on the developing organism, exposure by injection results in more pronounced changes. Sreenathan et al. (1982) evaluated the teratogenic effects of acetaldehyde in female CF rats. Pregnant animals were intraperitoneally injected with 0, 50, 75, or 100 mg/kg on days 10, 11, and/or 12 of gestation. Significant increases in fetal resorptions and malformations, including edema, microcephaly, micrognathia, micromelia, and others, were noted in treated animals. However, resorptions were confined to certain litters while others showed no effect, indicating extreme inter-litter variability. Acetaldehyde treatment resulted in a dose-related retardation of growth in rat fetuses, as well as retarded ossification of the squamous occipital, parietal, and frontal bones. The placentae of acetaldehyde-treated fetuses were reduced in size compared to controls and the umbilical cord length was significantly shorter in treated animals.

In another parenteral study, Fadel and Persaud (1991, 1993) exposed groups of 10 pregnant rats to 0 or 200 mg/kg of acetaldehyde by intraperitoneal injection throughout gestation and examined the fetuses for skeletal abnormalities. A significant reduction in the number of ossified bones, as well as the number of completely ossified bone centers, was found in the offspring of acetaldehyde-treated animals.

As a product of both maternal and fetal ethanol metabolism, acetaldehyde is believed to be involved in the development of the Fetal Alcohol Syndrome (FAS) (Hardman et al., 1996). Studies of injected acetaldehyde support this hypothesis, with similar morphologic alterations observed following maternal ethanol exposure and injection of acetaldehyde during gestation. However, the specific role of acetaldehyde in FAS has not been conclusively established.

4.4. OTHER

4.4.1. Genotoxicity

Acetaldehyde has tested negative for reverse mutation in *Salmonella typhimurium*, both with and without S-9 activating mixture (Litton Bionetics, Inc, 1979; Dellarco, 1988; Aeschbacher et al., 1989; Kato et al., 1989; Dillon et al., 1998), though its high volatility makes testing difficult. Evaluation of acetaldehyde in the SOS/*umu* test was also negative (Reifferscheid and Heil, 1996). Acetaldehyde has tested positive for mutations in the *Escherichia coli* *polA* system (Leifer et al., 1981), but negative in the *E. coli* K-12 *uvrB/recA* DNA repair host-mediated

assay (Kato et al., 1989; Hellmér and Bolcsfoldi, 1992). Exposure of *Saccharomyces cerevisiae* to acetaldehyde resulted in chromosomal damage, including both single-strand and double-strand breaks (Ristow et al., 1995).

Olin et al. (1996) reported a concentration-related increase in DNA-protein cross links in Chinese hamster ovary cells exposed to acetaldehyde, but not in human fibroblasts. Lower concentrations were not directly clastogenic in Chinese hamster ovary cells, but potentiated the clastogenic effects of several other DNA-damaging agents (Lin et al., 1989). Primary rat hepatocytes treated with 100 μ M acetaldehyde for 3 hours showed an increase in unscheduled DNA synthesis (Stevens et al., 1991). Acetaldehyde tested positive for induction of forward mutations in L5178Y mouse lymphoma cells (Wangenheim and Bolcsfoldi, 1988). Acetaldehyde exposure of up to 100 mM in freshly isolated human lymphocytes resulted in a dose-related increase in single-strand and double-strand DNA breaks, which the cells appeared to be unable to repair within 2 hours post-exposure (Singh and Khan, 1995). Exposure of human lymphocytes at acetaldehyde levels of up to 400 μ M resulted in an increased frequency of sister-chromatid exchanges, which was potentiated by the addition of an aldehyde dehydrogenase inhibitor (Helander and Lindahl-Kiessling, 1991). Acetaldehyde exposure resulted in a dose-related increase in micronucleus formation in human lymphocytes (Migliore and Nieri, 1991). Human lymphocytes exposed to acetaldehyde have shown an increased frequency of mutations at the HPRT locus (He and Lambert, 1990; Lambert et al., 1994). Acetaldehyde is capable of producing DNA-protein cross-links in human lymphoma cells (Costa et al., 1997). Vaca et al. (1998) demonstrated a dose-related formation of DNA adducts in human buccal epithelial cells exposed to up to 100 mM acetaldehyde *in vitro*.

Acetaldehyde induced dominant lethal effects in *Drosophila* following injection, but not following oral exposure (Woodruff et al., 1985). Chromosomal translocations in *Drosophila* have not been observed following injection of acetaldehyde (Woodruff et al., 1985), nor have acetaldehyde-related effects on X chromosome segregation been observed following oral exposure of *Drosophila* females (Rey et al., 1994). Acetaldehyde has tested positive in the *Drosophila* wing spot test (Graf et al., 1989). A concentration-dependent increase in DNA-protein crosslinks was found in the nasal mucosa of rats exposed by inhalation to 0, 100, 300, 1000, or 3000 ppm of acetaldehyde for 6 hours, which was increased in animals exposed to 1000 ppm for 5 consecutive days (Lam et al., 1986). Chinese hamsters exposed to 0.5 mg/kg by intraperitoneal injection, but not those exposed to 0.01 or 0.1 mg/kg, showed an increased frequency of sister-chromatid exchanges in bone marrow cells (Korte and Obe, 1981). CD-1 mice exposed by intraperitoneal injection to up to 380 mg/kg of acetaldehyde showed an increase in micronucleated polychromatic erythrocytes in a dose-related manner (Morita et al., 1997). Hybrid male mice exposed by intraperitoneal injection to up to 250 mg/kg of acetaldehyde daily for 5 days showed no alterations in sperm morphology or production (Lähdetie, 1988).

4.4.2. Co-carcinogenic Effects of Acetaldehyde

Hamsters exposed to acetaldehyde vapor (1500 ppm, 6 hours/day, 5 days/week) and intratracheally instilled with benzo(a)pyrene (BaP) weekly (1 mg/week for 52 weeks) produced twice the incidence of squamous cell carcinomas (24 with BaP-acetaldehyde co-exposure, compared to 11 with BaP alone), following a 52 week “recovery” period, compared with the same dose of BaP alone (Feron, 1979). This effect did not occur at lower doses of BaP. In

animals co-instilled with acetaldehyde (4 µl/instillation) and BaP (1 mg/week for 52 weeks), significantly fewer pulmonary tumors were observed (13 without acetaldehyde, 3 with acetaldehyde), despite morphologic alterations by acetaldehyde instillation alone. The authors postulated that the damaged pulmonary epithelium resulting from repeated acetaldehyde instillation is less sensitive to the carcinogenic effects of BaP.

In a followup study, Feron et al. (1982) exposed three groups each of control or acetaldehyde-exposed (TWA concentration: 2028 ppm, 7 hours/day, 5 day/week) Syrian golden hamsters to either weekly intratracheal instillations of benzo(a)pyrene (BaP) at one of two concentrations or a weekly subcutaneous injection of diethylnitrosoamine (DNA) for 52 weeks, followed by up to 28 weeks of observation (total duration: 80 weeks). Acetaldehyde co-exposure resulted in an approximately 4-fold increase in BaP-induced tumors at the highest BaP exposure level, with a much shorter latency period, but did not appear to affect the ability of DNA to induce respiratory tract tumors.

4.4.3. Dermal Sensitization

Bergh and Karlberg (1999) examined the dermal sensitizing properties of acetaldehyde in guinea pigs using a modified cumulative contact enhancement test. Animals were treated with 2.5, 5, or 10% acetaldehyde and challenged with 10% acetaldehyde solution. Acetaldehyde induced a dose-related inflammatory response on challenge at a peripheral site, indicating its potential as a dermal sensitizing agent.

4.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION – ORAL AND INHALATION

Data from human studies are not sufficient to evaluate the mode of action of acetaldehyde. Acetaldehyde is highly reactive, and has been regulated as an airborne irritant. Toxic responses seen in animals following inhalation of acetaldehyde generally occur in the upper respiratory tract, with the nasal region being the most affected. At low concentrations, respiratory tract lesions generally include a hyperplastic response and a thickening of the mucosal region. At higher levels, the response becomes metaplastic, with the normal respiratory or olfactory epithelium replaced by a stratified squamous epithelial layer, often with keratinization.

Following oral exposure to acetaldehyde in animals, the tongue, glottis, and forestomach exhibit similar histologic changes as are seen in the nose, larynx, and trachea following inhalation exposure. The basal epithelium thickens, accompanied by an increase in cellular proliferation, indicative of a hyperplastic response. At higher doses, keratinization can be observed. Effects on the liver of animals following oral exposure include alterations in enzyme levels and an increase in collagen synthesis.

Mechanistic data regarding the mode of action for noncancer effects of acetaldehyde are incomplete. However, the major effects seen following both oral and inhalation exposure are portal of entry effects; oral exposure results in proliferative effects in the tongue, glottis, and forestomach, while inhalation exposure results in similar effects in the nose, larynx, and trachea. This observation is suggestive of a direct cytotoxic action of acetaldehyde, perhaps due to its high

chemical reactivity, as the areas exposed to the greatest levels of acetaldehyde show the greatest effects. Acetaldehyde has been shown to bind to both proteins and DNA, and may exert its toxic effects by interacting with cellular macromolecules, resulting in cell death and subsequent regeneration. Chronic inhalation studies in rats have shown only partial reversal of acetaldehyde-induced lesions of the nose after up to 52 weeks of recovery, and then primarily at lower exposure levels.

4.6. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Under the 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a), acetaldehyde is classified as Group B2 (Probable Human Carcinogen), based on insufficient data on the carcinogenicity of acetaldehyde in humans and sufficient evidence of carcinogenicity in animals, as evidenced by increased incidence of upper respiratory tract tumors in male and female rats and male hamsters following inhalation exposure. Under the proposed cancer guidelines (U.S. EPA, 1996a, 1999), acetaldehyde is considered likely to be carcinogenic to humans.

While available human studies have weakly suggested a possible association between risk of various types of cancer and acetaldehyde exposure (Bittersohl, 1974; Ott et al., 1989a,b), limitations of the studies, including inability to control for concurrent exposures to other chemicals, make them inadequate for assessing the carcinogenic potential of acetaldehyde. Available chronic animal studies have demonstrated the carcinogenic potential of inhaled acetaldehyde in male and female rats, as well as in male hamsters (Feron et al., 1982; Woutersen and Appelman, 1984; Woutersen et al., 1985, 1986; Woutersen and Feron, 1987). Acetaldehyde-induced tumors in both rats and hamsters were only located in the upper respiratory tract, with the posterior nasal regions being the most prevalent site for increased tumor incidence. No studies suitable for evaluation of the oral carcinogenic potential of acetaldehyde were located.

There is evidence that acetaldehyde exposure is capable of eliciting genotoxic effects. While evaluations for mutagenic effects in bacteria have generally yielded negative results, tests for sister-chromatid exchanges, unscheduled DNA synthesis, and mutations in human and animal cells have demonstrated potentially genotoxic effects of acetaldehyde. *In vitro* treatment with acetaldehyde has also resulted in increased micronucleus formation in human lymphocytes and an increased incidence of DNA-protein cross-links and DNA adducts. *In vivo* exposure to acetaldehyde has resulted in increased incidence of DNA-protein crosslinks, sister-chromatid exchange, and micronucleus formation.

The exact mechanism of acetaldehyde-induced carcinogenic effects in animals is not known. Acetaldehyde is chemically reactive, and inhalation exposure to acetaldehyde results in substantial toxic effects in the nasal epithelium, including hyperplastic and metaplastic changes. It is possible that the carcinogenic effects seen following acetaldehyde inhalation are a result of this enhanced proliferation, a response to the substantial cytotoxic effects seen in chronic studies. However, acetaldehyde has also been shown to have mutagenic effects in mammalian cells, is capable of binding to protein, lipid, and DNA molecules, and causes increased levels of protein-DNA adducts in nasal tissues following inhalation exposure. Thus, a direct genotoxic effect of acetaldehyde cannot be ruled out. The carcinogenic effects are most likely the result of a

combination of the two mechanisms, whereby cytotoxicity-induced proliferative changes render the cells more susceptible to genotoxic effects, resulting in increased mutation frequency, which may lead to both additional cytotoxicity, enhancing the effect, or tumor formation. Animals exposed by inhalation for 52 weeks and allowed to recover for up to 52 weeks showed similar tumor incidence rates as animals exposed for their entire lifetime, suggesting that once the initial carcinogenic insult occurs, additional exposure is not necessary for progression of the lesion.

4.7. SUSCEPTIBLE POPULATIONS

4.7.1. Possible Childhood Susceptibility

No data are available regarding the effects of acetaldehyde in children. Several animal studies have demonstrated that acetaldehyde can have effects on the developing fetus (Sreenathan et al., 1982; Imai and Omoto, 1992; Fadel and Persaud, 1991, 1993). Data in animals also suggest that acetaldehyde may be the proximate chemical responsible for the fetal alcohol syndrome (Blakley and Scott, 1984; Hardman et al., 1996).

4.7.2. Possible Gender Differences

No data examining the differences in response between men and women are available. Available data from humans, including studies on irritation (Silverman et al., 1946), absorption (Egle, 1970), and racial susceptibility (Myou et al., 1993, 1994, 1995; Takada et al., 1994; Shimoda et al., 1996; Takao et al., 1998) have not directly compared responses of males and females, though both genders were represented in the study populations. Animal studies have not demonstrated any definitive differences in response to acetaldehyde between male and female animals.

4.7.3. Possible Racial Differences

A polymorphism of the ALDH₁ gene (mitochondrial, low K_m form of the enzyme) resulting in a nonfunctional enzyme is present in as much as 50% of the Asian population, as well as about 40% of the South American Indian population (Goedde and Agarwal, 1987). This polymorphism is not found in the Caucasian or Negroid populations. Individuals homozygous, and to a lesser extent those who are heterozygous, for the nonfunctional enzyme are less able to metabolize systemic acetaldehyde, and therefore show an elevated level of acetaldehyde in the blood after mild exposure to ethanol, which is metabolized to acetaldehyde by alcohol dehydrogenase enzymes. ALDH₁-deficient individuals are also more susceptible to topical alcohol and aldehyde application (Willkin and Fortner, 1985).

Within the ALDH₁-deficient population, asthmatics may show an even greater susceptibility to acetaldehyde. Studies have demonstrated that in Asians with asthma, but not in Caucasian asthmatics, an alcohol-induced asthmatic reaction can occur. This phenomenon has been correlated with blood acetaldehyde levels (Shimoda et al., 1996; Takao et al., 1998), as well as with an involvement of the aldehyde dehydrogenase polymorphism within the Asian population (Takada et al., 1994; Takao et al., 1998), suggesting that other sources that elevate blood acetaldehyde levels will also induce this reaction. Additionally, studies have demonstrated that

inhaled acetaldehyde can induce bronchoconstriction in Japanese asthmatics but not in healthy subjects (Myou et al., 1993). Acetaldehyde inhalation in Japanese asthmatics at concentrations below that needed to directly induce bronchoconstriction significantly increased bronchial responsiveness to methacholine (Myou et al., 1994). Tachyphylaxis to inhaled acetaldehyde develops within an hour of initial challenge (Myou et al., 1995). Thus, in Asian asthmatics, high concentrations of acetaldehyde in air can induce a bronchoconstrictive response, to which tolerance develops within an hour of initial exposure, while lower concentrations may potentiate the effects of other agents that induce bronchoconstriction. A guinea pig model for this phenomenon has been developed which may be of use in future studies (Fujimura et al., 1997).

5. DOSE RESPONSE ASSESSMENTS

5.1. ORAL RfD

5.1.1. Choice of Principal Study and Critical Effect

Chronic oral data on the non-cancer effects of acetaldehyde are not available. Available data from oral toxicity studies are suggestive of a direct action of acetaldehyde on the target tissues, likely irritant effects due to its very high chemical reactivity. Pharmacokinetic studies suggest that absorption will be high following oral exposure, but that significant levels of metabolism will occur in the liver, substantially reducing the systemic exposure to acetaldehyde. Acetaldehyde is rapidly removed from the blood, with a half-life in rats of approximately 3 minutes. Therefore, significant effects beyond the portal of entry following oral acetaldehyde exposure seem unlikely.

Til et al. (1988), tested several exposure levels of acetaldehyde in the drinking water of male and female rats exposed for 4 weeks. Histopathological changes (focal hyperkeratosis) were observed in the stomach of rats exposed to 625 mg/kg-day, but not at 25 or 125 mg/kg-day. The study by Homann et al. (1997) found similar changes in the stomach, as well as the tongue and epiglottis, following exposure of rats to 324 mg/kg-day in the drinking water for 8 months. Consistent with acetaldehyde-induced irritation of the gastrointestinal tract, Booze and Oehme (1986) reported severe gastrointestinal irritation resulting in emetic episodes in all 6 dogs exposed by single gavage to 600 mg/kg of acetaldehyde. A study of the developmental effects of acetaldehyde following gavage exposure to 400 mg/kg-day in rats produced negative results (Hazleton Labs, 1983). Another gavage study (Imai and Omoto, 1992) reported developmental effects in rats, including decreased pup weight and “visceral immaturity”, following exposure by gavage to 240 mg/kg-day. However, incomplete study details preclude an adequate evaluation of the Imai and Omoto (1992) study. Other oral studies (Pawlicka et al., 1991; HoLownia et al., 1992; Bankowski et al., 1993) were generally limited by the inclusion of only a single dose level and investigation of few endpoints. The Til et al. (1988) and Homann et al. (1997) were selected as co-critical studies.

5.1.2. Methods of Analysis

A benchmark dose (BMD) approach was considered, but was not utilized for this assessment. The Til et al. (1988) study tested several doses and provides empirical evidence that

a threshold exists for the gastrointestinal effects; the threshold falls between the mid- and high-dose levels. Because statistical curve fitting the incidence data (0, 0, 0, 80% in the control, low-, mid-, and high-dose groups, respectively) will not markedly decrease the uncertainty in estimating the no effect level, a NOAEL/LOAEL approach was used.

5.1.3. RfD Derivation - Including Application of Uncertainty Factors and Modifying Factors

The NOAEL and LOAEL identified in the Til et al. (1988) study was used for the operational derivation of the RfD. Although it is of relatively short duration, Til et al. (1988) is a more appropriate basis for RfD derivation than the Homann et al. (1997) study because it identified both a NOAEL (125 mg/kg-day) and a LOAEL (625 mg/kg-day), and the NOAEL is below the Homann et al. (1997) LOAEL (324 mg/kg-day).

Despite occurring in different target tissues, the basic effects seen following oral and inhalation exposures to acetaldehyde are similar, with hyper- and metaplastic changes, particularly stratification of the epithelium with keratinization, being the primary lesions. Four-week inhalation studies (Appelman et al., 1982, 1986) have demonstrated similar histologic alterations as those seen in chronic inhalation studies (Woutersen and Appelman, 1984; Woutersen et al., 1985, 1986; Woutersen and Feron, 1987). Though data are not available on the chronic effects of oral acetaldehyde exposure, the similarity of short-term effects following the oral and inhalation exposure, the putative mechanism of cytotoxicity based on chemical reactivity of the acetaldehyde molecule, and the similarity in lesions between short-term and chronic inhalation studies are suggestive of similar associations for noncancer effects between short and long-term oral studies. It is conceivable that repeated long-term exposure may lower the threshold of response, as represented by the NOAEL, but the uncertainty factor utilized for extrapolating from a less-than-chronic to a chronic study is likely to be sufficient to account for this possibility.

To the NOAEL of 125 mg/kg-day, an uncertainty factor of 3000 was applied (10 for interspecies variation, 10 for human variability, 10 for use of a subchronic study, and 3 for database insufficiencies). The database insufficiencies include a lack of a developmental toxicity study in a second species and a multi-generation reproductive toxicity study. A full factor of 10 was not considered necessary because (1) there is no evidence to suggest that acetaldehyde accumulates in the body, (2) a substantial first-pass effect is seen following oral acetaldehyde exposure, and (3) the high reactivity of the acetaldehyde molecule suggests that the portal of entry effects will be the most sensitive toxic endpoints. The modifying factor (MF) was set to 1. From these, the RfD for acetaldehyde is derived as follows:

$$\begin{aligned} \text{RfD} &= \text{NOAEL} \div (\text{UF} \times \text{MF}) \\ &= 125 \text{ mg/kg-day} \div (3000 \times 1) \\ &= 4\text{E-}2 \text{ mg/kg-day} \end{aligned}$$

Confidence in the co-principal studies is medium, as together the studies examined a sufficient number of endpoints in a sufficient number of animals, though only one examined multiple dose levels. Confidence in the database is low, owing to the lack of chronic oral data, lack of subchronic or chronic data in species outside the rat, and lack of adequate reproductive toxicity data. Low confidence in the RfD results.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect

A number of studies have examined the toxic effects of acetaldehyde following inhalation exposure. In both rats and hamsters, inhalation exposure to acetaldehyde results in damage to the upper respiratory tract, specifically the posterior region of the nose. Chronic studies in rats (Woutersen and Appelman, 1984; Woutersen et al., 1985, 1986; Woutersen and Feron, 1987) and hamsters (Feron et al., 1979, 1982) have demonstrated these effects, often in combination with tumor formation. Short-term studies have found similar effects at lower inhaled concentrations in both rats (Appelman et al., 1982, 1986) and hamsters (Kruyssen et al., 1975). While none of the chronic studies established a NOAEL, both the studies of Appelman et al. (1982, 1986) and Kruyssen et al. (1975) established NOAEL values for nasal lesions.

Based on the classifications given in the Methods for Derivation of Inhalation Reference Concentrations (U.S. EPA, 1994b), acetaldehyde is considered to be a category 1 gas because (1) it reacts rapidly with respiratory tract tissues, specifically in the nasal cavity, (2) it is highly water soluble, and (3) it does not significantly accumulate in the blood. A structurally similar compound, formaldehyde, is also categorized as a category 1 gas. The regional gas dose ratio (RGDR) for a category 1 gas with effects in the upper respiratory tract is defined as:

$$RGDR = \frac{(Dose_{ET})_A}{(Dose_{ET})_H} = \frac{\left(\frac{V_E}{S_{ET}}\right)_A}{\left(\frac{V_E}{S_{ET}}\right)_H}$$

where:

V_E = Ventilation rates, in m^3/day .

S_{ET} = Surface areas for the extrathoracic portion of the respiratory tract.

A and H are subscripts for animal and human values, respectively.

Using ventilation rate values taken from U.S. EPA (1988) and surface area values from U.S. EPA (1994b), RGDR values were calculated for both the rat and the hamster, and used to calculate human equivalent concentrations (HEC) for the NOAEL and LOAEL values from the available inhalation studies, as defined by U.S. EPA (1994b). The adjusted values are presented below:

Table 1. NOAEL_{HEC} and LOAEL_{HEC} Values From Inhalation Studies of Acetaldehyde

Study	Species	RGDR	NOAEL	LOAEL	NOAEL _[HEC]	LOAEL _[HEC]
Appelman et al., 1982	Rat	0.153	-----	401 ppm	-----	19.7 mg/m ³

Appelman et al., 1986	Rat	0.153	150 ppm	500 ppm	7.46 mg/m ³	24.6 mg/m ³
Woutersen et al., 1985*	Rat	0.153	-----	727 ppm	-----	35.8 mg/m ³
Kruyssen et al., 1975	Hamster	0.0436	390 ppm	1340 ppm	5.45 mg/m ³	18.8 mg/m ³
Feron et al., 1979	Hamster	0.0436	-----	1500 ppm	-----	21.0 mg/m ³
Feron et al., 1982	Hamster	0.0436	-----	2028 ppm	-----	28.4 mg/m ³

* Exposures for Woutersen et al., 1985, 1986, 1987 and Woutersen and Appelman, 1984 were identical

The Kruyssen et al. (1975) study identified the lowest NOAEL_{HEC} and LOAEL_{HEC} values, and was chosen as the critical study for the derivation of an RfC. This study examined several exposure concentrations in both male and female hamsters, and established both NOAEL and LOAEL values for histologic alterations of the nasal epithelium. The effects seen were similar to those reported following longer-term exposures in hamsters (Feron et al., 1979, 1982), as well as both short and long-term studies in rats (Appelman et al., 1982, 1986; Woutersen and Appelman, 1984; Woutersen et al., 1985, 1986; Woutersen and Feron, 1987).

5.2.2. Methods of Analysis

A benchmark concentration (BMC) approach was considered, but was not utilized because the Kruyssen et al. (1975) study does not provide incidence data. Thus, the relationship between exposure level and response could not be appropriately modeled. As described above, NOAEL and LOAEL values were adjusted for duration of exposure, then used to calculate NOAEL_[HEC] and LOAEL_[HEC] values, as outlined in the Methods for Derivation of Inhalation Reference Concentrations (U.S. EPA, 1994b).

5.2.3. RfC Derivation - Including Application of Uncertainty Factors and Modifying Factors

The following uncertainty factors (UF) were applied to the NOAEL_[HEC] of 5.45 mg/m³: 3 for interspecies extrapolation using dosimetric adjustments; 10 for human variability; 10 for the use of a less than chronic study; and 3 for database insufficiencies. The total uncertainty factor is 1000.

A partial UF of 3 was used for database insufficiencies because data on the developmental and reproductive effects of acetaldehyde following inhalation exposure are not available. A full factor of 10 was not considered necessary because pharmacokinetic data suggest that because of the rapid metabolism of acetaldehyde and a lack of substantial accumulation in the body following inhalation exposure, portal of entry effects, specifically nasal lesions, are likely to be the most sensitive endpoint. The modifying factor (MF) was set to 1. Using these, the RfC for acetaldehyde is derived as follows:

$$\text{RfC} = \text{NOAEL} \div (\text{UF} \times \text{MF})$$

$$= 5.45\text{mg}/\text{m}^3 \div (1000 \times 1)$$

$$= 5\text{E}-3 \text{ mg}/\text{m}^3$$

Confidence in the principal study is medium, as the Kruijsse et al. (1975) study is a well-designed study that used adequate numbers of male and female hamsters, examined sensitive endpoints, and utilized appropriate controls. However, the study was of subchronic duration, and the most sensitive effects examined were not quantified, and thus no statistical analysis of the critical effect was possible. Also, the study was performed only in one species, though later studies in other species and for longer durations have shown similar effects. Confidence in the database is low due to the lack of chronic data establishing NOAELs and due to the lack of reproductive and developmental toxicity data following inhalation exposure. Low confidence in the RfC results.

5.3. CANCER ASSESSMENT

5.3.1. Oral Slope Factor

No data on the long-term carcinogenic effects of oral exposure to acetaldehyde in humans or animals were located. Because the tumors seen following inhalation exposure to acetaldehyde are located in the respiratory tract, a route-to-route extrapolation from the available inhalation data was not attempted.

5.3.2. Inhalation Unit Risk

No human data suitable for the derivation of an inhalation unit risk for acetaldehyde were located. The lifetime carcinogenicity study of Woutersen et al (1985, 1986) was chosen as the principal study for the derivation of an inhalation unit risk. Though the highest exposure group began with an exposure level of 3000 ppm, it was reduced several times during the study. Therefore, a time-weighted average of 1592 ppm over the entire exposure (715 days, due to increased mortality) was used. The measured concentrations for each group were converted to human equivalent concentrations, using the RGDR for a category 1 gas as derived in section 5.2.1, as follows:

Table 2. Conversion to Human Equivalent Concentrations

	Control	Low Dose	Mid Dose	High Dose
Measured Concentration (ppm)	0	727	1439	1592
Convert to mg/m ³ from ppm	(ppm x MW)/24.45 = (ppm x 44.06)/24.45			
Concentration (mg/m ³)	0	1308	2590	2866
Adjust for partial exposure	ppm x 6 hours/24 hours x 5days/7 days			

Adjusted Concentration (mg/m ³)	0	234	463	512
Convert to HEC using RGDR _[ET]	ppm x 0.153 (RGDR _[ET] see above in 5.2.1.)			
HEC (mg/m ³)	0	36	71	78

Two mathematical approaches were utilized to calculate LEC₁₀ values from which to derive an inhalation unit risk. The first utilized the time-weighted average (TWA) of the highest exposure concentration, along with measured concentrations of the other exposure concentrations, to model the association of incidence rates and exposure concentration. The second applied a variant of the Armitage-Doll multistage model with a time-dependent dose pattern (Crump and Howe, 1984). This model utilizes the exact time-dependent dose pattern rather than a single measure, such as a time-weighted average. The LEC₁₀ values calculated by each model are presented below, along with the resulting unit risk values.

5.3.2.1. Polynomial Fit Utilizing the Time-Weighted Average (TWA)

LEC₁₀ values were calculated from the incidence data using a polynomial curve fitting program (Global86) as shown below. Because the highest exposure level appeared to exceed the MTD, the calculations were run both with and without the inclusion of that group. When the high-concentration group was included, the data were adjusted to correct for early mortality, which was greatest in the high-concentration animals, by excluding animals that died prior to 52 weeks of exposure, which was the first time point at which a nasal tumor was reported.

Table 3. LEC₁₀ Values Calculation from Incidence Data

	Incidence 0 mg/m ³	Incidence 36 mg/m ³	Incidence 71 mg/m ³	Incidence 78 mg/m ³	LEC ₁₀ (mg/m ³)	Goodness of Fit (P-Value) [†]
Males (all groups) *	1/55	17/52	40/54	31/41	7.43	0.63
Males (high excluded)	1/55	17/54	40/55	-----	8.08	1.00
Females (all groups) *	0/54	8/54	36/55	39/48	17.47	0.11
Females (high excluded)	0/54	8/55	36/55	-----	17.66	0.15

* Animals that died before 52 weeks of exposure were excluded from this analysis.

[†] A p-value greater than 0.05 is a statistically acceptable fit.

The most conservative (health-protective) value, that from male rats of all groups, was selected for further calculations. The inhalation unit risk was then derived as follows:

$$\begin{aligned}
 \text{Unit Risk} &= 0.1 \div \text{LEC}_{10} \\
 &= 0.1 \div 7.43 \text{ mg/m}^3 \\
 &= 0.1 \div 7430 \text{ } \mu\text{g/m}^3 \\
 &= 1.3\text{E-}5 \text{ (}\mu\text{g/m}^3\text{)}^{-1}
 \end{aligned}$$

From this value, the following risk levels are calculated:

<u>Risk Level</u>	<u>Concentration</u>
E-4 (1 in 10,000)	8E+0 $\mu\text{g/m}^3$
E-5 (1 in 100,000)	8E-1 $\mu\text{g/m}^3$
E-6 (1 in 1,000,000)	8E-2 $\mu\text{g/m}^3$

5.3.2.2. Multistage Model with a Time-dependent Dose Pattern

Because the exposure concentration of the highest exposure level was varied throughout the study, a variant of the Armitage-Doll multistage model with a time-dependent dose pattern was also used (Crump and Howe, 1984). This model utilizes the exact time-dependent dose pattern rather than a single measure, such as a time-weighted average. Because data correlating individual animal tumor incidence with time-to-tumor data, such as is required by this model, were not available, the reported mortality data, tumor incidences at monitored timepoints, and variations in exposure levels (converted to HEC in mg/m³, as described above) were used to generate a data set suitable for use in the model. Specific parameters used, as well as the model output, are found in Appendix A. The model returned an LEC₁₀ value of 5.08 mg/m³, from which an inhalation unit risk is calculated as follows:

$$\begin{aligned}
 \text{Unit Risk} &= 0.1 \div \text{LEC}_{10} \\
 &= 0.1 \div 5.08 \text{ mg/m}^3 \\
 &= 0.1 \div 5080 \text{ }\mu\text{g/m}^3 \\
 &= 2.0\text{E-}5 \text{ (}\mu\text{g/m}^3\text{)}^{-1}
 \end{aligned}$$

From this value, the following risk levels are calculated:

<u>Risk Level</u>	<u>Concentration</u>
E-4 (1 in 10,000)	5E+0 $\mu\text{g/m}^3$
E-5 (1 in 100,000)	5E-1 $\mu\text{g/m}^3$
E-6 (1 in 1,000,000)	5E-2 $\mu\text{g/m}^3$

5.3.2.3. *Conclusions and Recommendations*

The inhalation unit risk values derived using a standard polynomial fitting program (Global86) and the model utilizing a time-dependent dose pattern were similar, differing by less than a factor of two. However, the individual animal time-to-tumor data required for the time-dependent Armitage-Doll multistage model were not given in or easily estimated from the study, making the input parameters for calculation of the value using this model questionable. Due to uncertainties in the approximation of the input parameters for the time-dependent model, the unit risk value derived using the polynomial fit from the TWA, $1.3\text{E-}5 \text{ (}\mu\text{g/m}^3\text{)}^{-1}$, is recommended, even though the value derived from the time-dependent model is slightly more conservative (health-protective).

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1. HUMAN HAZARD POTENTIAL

Acetaldehyde (CAS no. 75-07-0) is a 2-carbon aldehyde with the chemical formula $\text{C}_2\text{H}_4\text{O}$ (structural formula CH_3CHO) and a molecular weight of 44.06. At room temperature, acetaldehyde is either a vapor or a clear, colorless liquid (boiling point 21°C). Industrially, acetaldehyde is used as an intermediate in a number of chemical synthesis processes. At very low levels, acetaldehyde is used as an additive in many food products. Acetaldehyde is derived from a number of natural and anthropogenic sources, and is ubiquitous in the environment.

Due to its high chemical reactivity, acetaldehyde is an irritant of the eyes, skin, mucous membranes, throat, and respiratory tract (Silverman et al., 1946; U.S. EPA, 1993). Absorption of acetaldehyde by the oral and inhalation routes is high, both in animals and in humans (Egle, 1970, 1972; Booze and Oehme, 1986; Watanabe et al., 1986; Morris and Blanchard, 1992; Morris, 1997). Following exposure, blood levels peak and fall very rapidly, with a measured half life in rats of 3 minutes (Hobara et al., 1985). Once absorbed, it is rapidly metabolized to acetate by aldehyde dehydrogenase enzymes, and enters the body's 2-carbon pool. However other metabolic pathways may also be active, particularly in the case of very high levels of acetaldehyde, and binding to DNA and proteins can occur (Lam et al., 1986; Costa et al., 1997; Vaca et al.,

1998). Urinary excretion of acetaldehyde following exposure is minimal (Kallama and Hemminik, 1983).

Following oral exposure of animals to acetaldehyde, observed effects include hyperplasia of the tongue, epiglottis, and forestomach (Til et al., 1988; Homann et al., 1997), as well as increased liver collagen synthesis and superoxide dismutase enzyme activity (Pawlicka et al., 1991; HoLownia et al., 1992; Bankowski et al., 1993). There are no data in humans following oral exposure to acetaldehyde, so the relevance of these findings to human exposures is not clear. There are no data on the carcinogenicity of acetaldehyde following oral exposure to humans or animals.

The primary health effects observed in humans following inhalation exposure to acetaldehyde appear to be irritation of the eyes, skin, and respiratory tract, with the eyes being the most sensitive of these targets (Silverman et al., 1946). Erythema, coughing, pulmonary edema, and necrosis may also occur and, at extremely high concentrations, so may respiratory paralysis and death (U.S. EPA, 1993; ACGIH 1999). Acetaldehyde exposure may also induce bronchoconstriction in susceptible asthmatics (Myou et al., 1993, 1994, 1995). The mechanism(s) of action for these effects have not been conclusively established, but are likely due to the high reactivity of the acetaldehyde molecule.

Inhalation studies in rodents have demonstrated profound effects on the nasal region of the respiratory tract, including hyperplasia, metaplasia, and the development of tumors. While absorption of acetaldehyde following inhalation exposure in both animals and humans is high, rodents are obligate nose-breathers, and have a different breathing pattern than humans. While this would be expected to reduce the risk of acetaldehyde-induced nasal lesions in humans, it may increase the risk of effects lower in the respiratory tract. Chronic inhalation studies have resulted in nasal tumors in both rats and hamsters (Feron et al., 1982; Woutersen and Appelman, 1984; Woutersen et al., 1985, 1986; Woutersen and Feron, 1987), though human data (Bittersohl et al., 1974; Ott et al., 1989a, 1989b) have been insufficient for evaluating the carcinogenic effects of acetaldehyde.

No data are available on the effects of acetaldehyde on developing humans. While acetaldehyde derived from ethanol metabolism is believed to cause developmental effects, animal studies examining the developmental toxicity of acetaldehyde following oral exposure have been inconclusive, with the two available oral studies reporting differing results. Following injection, acetaldehyde has been demonstrated to cause fetal malformations. No data are available on the reproductive effects of inhaled acetaldehyde.

Based on the weight of evidence, acetaldehyde is classified as group B2, a probable human carcinogen, based on the 1986 guidelines (U.S. EPA, 1986a). Under the proposed cancer guidelines (U.S. EPA, 1996b, 1999), acetaldehyde is likely to be carcinogenic to humans following inhalation exposure, although its carcinogenicity cannot be determined following oral exposure.

6.2. DOSE RESPONSE

6.2.1. Noncancer/Oral

There are no chronic oral dose-response data for acetaldehyde in humans or animals. One subchronic study (Til et al., 1988) established a NOAEL and LOAEL for acetaldehyde, and was selected as a critical study for the derivation of an RfD. An 8-month drinking water study, reported by Homann et al. (1997), reported no NOAEL and a LOAEL of 324 mg/kg-day for the same endpoint, and was selected as a co-critical study. The NOAEL of 125 mg/kg-day was divided by an uncertainty factor of 3000, based on four areas of uncertainty, resulting in a chronic RfD of 4E-2 mg/kg-day. Confidence in the principal studies is medium, as the studies examined a large number of endpoints in a sufficient number of animals of both sexes at several exposure concentrations, but did so only in one species and had differing durations. Confidence in the database is low, owing to the lack of chronic oral data, lack of data in species other than the rat, and insufficient data on developmental and reproductive effects. Low confidence in the RfD results.

6.2.2. Noncancer/Inhalation

No studies on the inhalation of acetaldehyde in humans suitable for the derivation of an RfC were located. Both chronic and subchronic inhalation exposure to acetaldehyde have pronounced effects on the respiratory tract of rodents, specifically on the nasal epithelium. A subchronic study in male and female hamsters (Kruyssen et al., 1975) established a NOAEL of 390 ppm and a LOAEL of 1340 ppm, based on histologic alterations of the upper respiratory tract. From these values, a chronic RfC was derived by dividing the NOAEL_[HEC] of 5.45 mg/m³ by an uncertainty factor of 1000 (3 for interspecies differences, 10 for human variability, 10 for a less than chronic study, and 3 for database insufficiencies, including the lack of reproductive and developmental studies following inhalation exposure), resulting in a chronic RfC of 5x10⁻³ mg/m³. Confidence in the principal study is medium, as the Kruyssen et al. (1975) study is a well-designed study that used adequate numbers of male and female hamsters, examined sensitive endpoints, and utilized appropriate controls. However, the study was of subchronic duration, and the most sensitive effects examined were not quantified, and thus no statistical analysis of the critical effect was possible. Confidence in the database is low due to the lack of chronic data establishing NOAELs and due to the lack of reproductive and developmental toxicity data following inhalation exposure. Low confidence in the RfC results.

6.2.3. Cancer/Oral and Inhalation

Data for humans are inadequate to evaluate an association between acetaldehyde and cancer. For the oral route of exposure, animal data are likewise lacking, and the derivation of an oral slope factor is not possible. Studies in rats and hamsters have shown a treatment-related increase in the incidence of respiratory tract tumors following acetaldehyde exposure, particularly in the nasal region. A lifetime carcinogenicity study (Woutersen et al., 1985, 1986) was chosen as the key study for the derivation of an inhalation unit risk. After conversion of the measured exposure levels to human equivalent concentrations (HEC), an LEC₁₀ of 7.43 mg/m³ was calculated using incidence rates for total respiratory tract tumors in male rats. From this value, an inhalation unit risk of 1.3x10⁻⁵ (µg/m³)⁻¹ was calculated.

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Appendix A - Output from Multistage Model with Time-Dependent Dose Pattern

DATE: 09-16-99

TIME: 14:37:58

ADOLL-1 84 (SEPT-1984)

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K.S. CRUMP & COMPANY, INC.
 1201 GAINES STREET
 RUSTON, LA 71270
 (318) 255-4800

Acetaldehyde MALE RATS

TIME INTERVALS AND THE ASSOCIATED DOSES

	TIME INTERVAL -----	DOSE ----
LEVEL 1	0 ---- 121.0	.0000
LEVEL 2	0 ---- 121.0	36.00
LEVEL 3	0 ---- 121.0	71.00
LEVEL 4	0 ---- 20.00	149.0
	---- 30.00	107.0
	---- 34.00	100.0
	---- 43.00	71.00
	---- 45.00	83.00
	---- 51.00	73.00
	---- 99.00	48.00
	---- 102.0	.0000

THE 4 OBSERVATIONS AT LEVEL 1

TIME ----	# OF ANIMALS -----	TUMOR INDICATOR -----	TIME ----	# OF ANIMALS -----	TUMOR INDICATOR -----
70.0	1	3	70.0	2	1
104.0	15	1	121.0	37	1

THE 8 OBSERVATIONS AT LEVEL 2

TIME ----	# OF ANIMALS -----	TUMOR INDICATOR -----	TIME ----	# OF ANIMALS -----	TUMOR INDICATOR -----
52.0	1	3	52.0	2	1
70.0	2	3	87.0	4	1
104.0	6	3	104.0	9	1
121.0	8	3	121.0	21	1

THE 8 OBSERVATIONS AT LEVEL 3

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
52.0	1	3	70.0	4	3
70.0	1	1	87.0	10	3
104.0	4	3	104.0	7	1
121.0	21	3	121.0	7	1

THE 7 OBSERVATIONS AT LEVEL 4

TIME	# OF ANIMALS	TUMOR INDICATOR	TIME	# OF ANIMALS	TUMOR INDICATOR
52.0	14	1	70.0	11	3
70.0	3	1	87.0	13	3
87.0	3	1	104.0	7	3
104.0	4	1			

THE LIKELIHOOD IS -59.8115944166 FOR THE 1 STAGE ACTIVE OUT OF 1 STAGES

THE LIKELIHOOD IS -17.4453798432 FOR THE 1 STAGE ACTIVE OUT OF 2 STAGES

THE LIKELIHOOD IS -28.1676681475 FOR THE 2 STAGE ACTIVE OUT OF 2 STAGES

THE LIKELIHOOD IS -10.0466279918 FOR THE 1 STAGE ACTIVE OUT OF 3 STAGES

THE LIKELIHOOD IS -13.6995645899 FOR THE 2 STAGE ACTIVE OUT OF 3 STAGES

THE LIKELIHOOD IS -8.70422326565 FOR THE 1 STAGE ACTIVE OUT OF 4 STAGES

THE LIKELIHOOD IS -11.8301996943 FOR THE 2 STAGE ACTIVE OUT OF 4 STAGES

THE LIKELIHOOD IS -8.37907150971 FOR THE 1 STAGE ACTIVE OUT OF 5 STAGES

THE LIKELIHOOD IS -11.6242786598 FOR THE 2 STAGE ACTIVE OUT OF 5 STAGES

THE LIKELIHOOD IS -8.28638058980 FOR THE 1 STAGE ACTIVE OUT OF 6 STAGES

THE LIKELIHOOD IS -11.5967448951 FOR THE 2 STAGE ACTIVE OUT OF 6 STAGES

A 6 STAGE MODEL WITH STAGE NUM. 1 ACTIVE

THE MAXIMUM LIKELIHOOD ESTIMATION OF:

PROBABILITY FUNCTION COEFFICIENTS

Q(0)= .674363479433E-14

Q(1)= .668660591996E-14

INDUCTION TIME

T(0)= 2.30568

THE MAXIMUM LIKELIHOOD IS -8.28638058980

MAXIMUM LIKELIHOOD ESTIMATES OF EXTRA RISK

TIME INTERVAL AND DOSE RATIOS FOR LOWER LIMITS

TIME INTERVAL	DOSE
-----	----
0 ---- 121.0	1.000

ADOLL-1 84 LOWER CONFIDENCE LIMITS ON DOSE FOR FIXED RISK

RISK	MLE DOSE	LOWER BOUND ON DOSE	UPPER BOUND ON RISK	CONFIDENCE LIMIT INTERVAL	TIME	COEFFICIENTS FOR CONFIDENCE LIMIT
----	-----	-----	-----	-----	-----	-----
.1000	5.6350	5.0815	.11027	95.0%	121.000	Q(0)= .11178863890E-13 Q(1)= .13088749669E-13 (T - 13.031396819)
1.0000E-06	5.34829E-05	4.82300E-05	1.10891E-06	95.0%	121.000	Q(0)= .11178863890E-13 Q(1)= .13088749669E-13 (T - 13.031396819)
1.0000E-07	5.34829E-06	4.82300E-06	1.10891E-07	95.0%	121.000	Q(0)= .11178863890E-13 Q(1)= .13088749670E-13 (T - 13.031396820)

TIME INTERVAL AND DOSE PATTERN FOR UPPER LIMITS ON RISK

TIME INTERVAL	DOSE
-----	----
0 ---- 121.0	1.000

ADOLL-1 84 UPPER CONFIDENCE LIMITS ON RISK FOR FIXED DOSE

DOSE	MLE RISK	UPPER BOUND ON RISK	CONFIDENCE LIMIT INTERVAL	TIME	COEFFICIENTS FOR CONFIDENCE LIMIT
----	-----	-----	-----	-----	-----
1.000	1.00699E-04	1.00699E-04	95.0%	52.000	Q(0)= .65833513068E-14 Q(1)= .75278190719E+26 (T - 51.999989503)
1.000	7.36853E-03	7.36882E-03	95.0%	104.00	Q(0)= .35641483061E-14 Q(1)= .96489669327E-14 (T - 8.3348726051)
1.000	1.85239E-02	2.05205E-02	95.0%	121.00	Q(0)= .11178863890E-13 Q(1)= .13088749669E-13 (T - 13.031396819)

NORMAL COMPLETION!