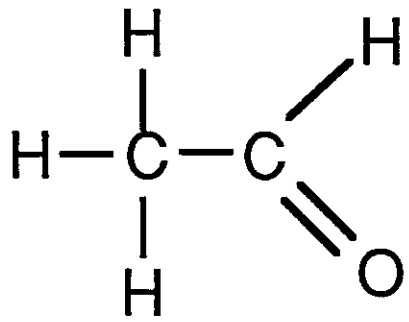
California Environmental Protection Agency

Air Resources Board

Acetaldehyde

as a Toxic Air Contaminant



Part B

Health Assessment

Stationary Source Division

November 1993

PART B

HEALTH EFFECTS OF ACETALDEHYDE

Prepared by

Air Toxicology and Epidemiology Section
Office of Environmental Health Hazard Assessment
2151 Berkeley Way, Annex 11
Berkeley, CA 94704-9980

James F. Collins, Ph.D.
Stanley V. Dawson, Sc.D.
George V. Alexeeff, Ph.D.

November 1993

TABLE OF CONTENTS

	Page
LIST OF FIGURES	v
LIST OF TABLES	vi
AUTHORS, CONTRIBUTORS, REVIEWERS	viii
1.0 SUMMARY	**1-1**
2.0 METABOLISM AND PHARMACOKINETICS	2-1
2.1 Introduction	2-1
2.2 Absorption	2-1
2.2.1 Inhalation Studies	2-1
2.2.2 Oral Studies	2-2
2.3 Distribution	2-5
2.4 Metabolism	2-7
2.4.1 Aldehyde Dehydrogenase Enzyme System	2-7
2.4.2 Extrahepatic Metabolism	2-9
2.4.3 Acetaldehyde Binding and Antibody Formation	2-12
2.4.4 Acetaldehyde Binding to DNA	2-13
2.5 Excretion	2-13
2.6 Conclusions	2-14
3.0 ACUTE TOXICITY	3-1
3.1 Human Health Effects	3-1
3.2 Animal Studies	3-1
3.2.1 Inhalation Exposure	3-1
3.2.2 Oral	3-4
3.2.2 Parenteral Administration	3-4
3.3 Toxicity of Peroxyacetyl Nitrate	3-4
3.4 Conclusions	3-5
4.0 SUBCHRONIC TOXICITY	4-1
4.1 Inhalation Studies	4-1
4.2 Oral Studies	4-2
4.3 Conclusions	4-3
5.0 CHRONIC TOXICITY	5-1
5.1 Introduction	5-1
5.2 Inhalation	5-1
5.3 Inhalation with Intratracheal Instillation of Benzo[a]pyrene	5-2
5.4 Intratracheal Instillation	5-3
5.5 Conclusions	5-3

TABLE OF CONTENTS (cont.)

	Page
6.0 REPRODUCTIVE AND DEVELOPMENTAL EFFECTS	6-1
6.1 Introduction	6-1
6.2 *In vivo* Studies	6-1
6.2.1 Developmental Toxicity	6-1
6.2.2 Reproductive Toxicity	6-2
6.2.3 Reporductive Toxicity	6-3
6.3 *In vitro* Studies	6-3
6.3.1 Developmental Toxicity	6-3
6.3.2 Reproductive Toxicity	6-4
6.4 Conclusions	6-4
7.0 GENOTOXICITY	7-1
7.1 Introduction	7-1
7.2 Gene Mutations	7-1
7.2.1 Bacteria	7-1
7.2.2 Yeast	7-1
7.2.3 Nematodes	7-3
7.2.4 Insects	7-3
7.2.5 Mammalian Cells	7-3
7.3 Chromosomal Aberrations	7-3
7.3.1 Plants	7-3
7.3.2 Insects	7-4
7.3.3 Mammalian Cell Cultures	7-4
7.3.4 Whole Mammals	7-5
7.4 Sister Chromatid Exchanges	7-5
7.4.1 CHO Cells	7-5
7.4.2 Human Lymphocytes	7-6
7.4.3 Whole-Mammal Bone Marrow Cells	7-7
7.5 DNA Damage	7-7
7.5.1 Bacteria	7-7
7.5.2 Mammalian Cell Culture	7-7
7.6 Cell Transformation	7-8
7.7 Conclusions	7-8
8.0 CARCINOGENICITY	8-1
8.1 Introduction	8-1
8.2 Epidemiological Study	8-1
8.3 Animal Studies	8-2
8.3.1 Inhalation	8-2
8.3.2 Inhalation + Intratracheal Instillation	8-8
8.3.3 Intratracheal Instillation	8-18
8.4 Carcinogenicity of Ethanol	8-18
8.5 Conclusions	8-22

9.0 QUANTITATIVE RISK ANALYSIS 9-1
9.1 Noncarcinogenic Risks 9-1
9.2 Carcinogenic Risks 9-1
9.2.1 Thresholds 9-2
9.2.2 Rat Nasal Carcinomas and the Multistage Model 9-2
9.2.3 Interspecies Scaling and Rat Nasal Carcinomas 9-6
9.2.4 Rat Nasal Carcinomas and Contact Scaling 9-8
9.2.5 Hamster Laryngeal Carcinomas and Multistage Model 9-9
9.2.6 Risk Estimate using the Gaylor-Kodell Approach 9-9
9.2.7 Population Risk from Acetaldehyde in Ambient Air 9-10
9.2.8 Selection of Best Value for Risk Assessment 9-10

APPENDIX A: Results of Reproductive and *in vivo* Developmental Toxicity Studies with Acetaldehyde A-1

APPENDIX B: Mutagenicity-Genotoxicity Testing of Acetaldehyde B-1

APPENDIX C: IARC and USEPA Classification Schemes for Carcinogens .. C-1

REFERENCES R-1

LIST OF FIGURES

Figure No.		Page
2-1	The retention of acetaldehyde with the open system design at three respiratory rates and three concentration ranges	2-3
2-2	The retention of acetaldehyde with the closed system design	2-4
2-3	The effect of increasing amounts of oral ethanol upon acetaldehyde and ethanol concentrations in alveolar air in one subject	2-8

LIST OF TABLES

Table No.		Page
2-1	Tissue Distribution of Acetaldehyde Following Acetaldehyde Inhalation and Intragastric Ethanol Administration	2-6
2-2	Estimated Kinetic Constants for the NAD^+-Dependent Oxidation of Acetaldehyde to Acetate Catalyzed by Homogenates of Respiratory and Olfactory Mucosa from Fischer-344 rats	2-10
3-1	Acute Toxicity Studies Conducted with Acetaldehyde	3-2
7-1	Genotoxicity of Acetaldehyde in Various Test Systems	7-2
8-1	Summary of Carcinogenicity Studies of Acetaldehyde in Rats and Hamsters	8-3
8-2	Summary of Respiratory Tract Hyperplastic and Preneoplastic Lesions in Rats Exposed to Acetaldehyde by Inhalation	8-5
8-3	Sites, Types, and Incidences of Respiratory Tract Tumors in Rats Exposed to Acetaldehyde by Inhalation	8-7
8-4	Incidence of Respiratory Tract Tumors in Hamsters Exposed to Either Air or Acetaldehyde Vapor	8-9
8-5	Types and Incidences of Respiratory Tract Tumors in Male Hamsters After 52 Weekly Intratracheal Instillations of B[a]P and Exposure to Air or Acetaldehyde Vapor	8-11
8-6	Treatment Protocol for Hamsters Exposed to Either Air or Acetaldehyde Vapor	8-13
8-7	Sites and Incidences of Respiratory Tract Tumors in Hamsters Exposed to Air or Acetaldehyde Vapor and Treated Intratracheally with B[a]P or Subcutaneously with DENA	8-14
8-8	Sites, Types, and Incidences of Respiratory Tract Tumors in Hamsters Exposed to Air or Acetaldehyde Vapor and Treated Intratracheally with B[a]P or Subcutaneously with DENA	8-16

LIST OF TABLES (continued)

Table No.		Page
8-9	Treatment of Hamsters in the Various Groups Used in the Intratracheal Instillation Study	8-19
8-10	Types and Incidences of Respiratory Tract Tumors in Hamsters Given Intratracheal Instillations of 0.9% NaCl Solution, Acetaldehyde, B[a]P, B[a]P + Acetaldehyde, DENA, or DENA + Acetaldehyde	8-20
8-11	Classification of Acetaldehyde's Carcinogenicity	8-24
9-1	Tumor Incidences in Male and Female Rats After 28 Months of Acetaldehyde Exposure	9-3
9-2	Carcinogenic Potency Determination by GLOBAL86: Inhalation Unit Risk/PPB Based on Rat Nasal Tumors	9-5

AUTHORS, CONTRIBUTORS, REVIEWERS

This document is based in part on a review of the literature on the toxicology of acetaldehyde prepared under contract with the Air Toxicology and Epidemiology Section by Dynamac Corporation. The Dynamac Corporation personnel involved in the preparation of the review were: Aisar Atrakchi, Ph.D. (Principal Author); Jess Rowland, M.S. and Dawn Webb, B.S. (Authors); William L. McLellan, Ph.D. and Allan Gray, Ph.D. (Reviewers); Karen Swetlow, B.S. (Technical Editor); and Nicolas P. Hajjar, Ph.D. (Department Manager). This document was prepared under contract with the California Department of Health Services, Hazard Evaluation Section, Berkeley, CA, Ms. Michelle Sabel, Project Officer. Melanie A. Marty, Ph.D. of the Air Toxicology and Epidemiology Section also reviewed the document.

Additionally, the authors gratefully acknowledge the assistance of Gloria Harrell and Laurie Bliss in preparation of this document.

1.0. SUMMARY

The health effects of acetaldehyde have been reviewed and evaluated to determine if acetaldehyde may be a toxic air contaminant, i.e., an air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

At ambient temperatures acetaldehyde is a gas. In vivo acetaldehyde is rapidly distributed throughout the body. Acute exposure leads to eye, skin, and respiratory tract irritation, as well as to other pathologic changes. At high doses acetaldehyde cause embryotoxicity and fetal malformations in rats. At current ambient levels of acetaldehyde, no acute or noncarcinogenic chronic effects are expected; however, hot spots have not been evaluated.

Acetaldehyde (CH_3CHO), a saturated, aliphatic aldehyde with a pungent, suffocating odor, is a colorless liquid that is volatile at room temperature. Since acetaldehyde is the immediate oxidation product of ethyl alcohol, its metabolic fate and toxic effects have been studied primarily in conjunction with the effects of alcohol.

Acetaldehyde is readily absorbed through the lungs into the blood following inhalation exposure and from the gastrointestinal tract following oral administration. Following inhalation exposure, it is transported by the blood to various organs for metabolism. Acetaldehyde undergoes transplacental transport when present at relatively high concentrations in the blood of pregnant rats. Following oral administration, it undergoes extensive first-pass metabolism in the liver; only 5% remains unchanged. Acetaldehyde is metabolized by aldehyde dehydrogenase to acetate and is eliminated in the urine, in expired air, and through the skin. When acetaldehyde is administered orally to rats at low concentrations, the elimination kinetics are first order but nonlinear when concentrations greater than 100 μM are reached. Because acetaldehyde is a reactive molecule, it is mostly bound to proteins and other blood components while in the bloodstream. Acetaldehyde causes lipid peroxidation and free radical formation resulting in cellular injury. It can also act as a hapten, and antibodies against acetaldehyde conjugates have been isolated from human serum.

The major effects of acetaldehyde vapor in humans consist of irritation to the eyes, skin, and respiratory tract. Information in the available literature is inadequate to assess the chronic effects of acetaldehyde exposure in humans. The American Conference of Governmental Industrial Hygienists (ACGIH) Time-Weighted Average Threshold Limit Value (TLV) for exposure 8 hours/day, 5 days/week, is 100 ppm. This is also the Occupational Safety and Health Administration's Permissible Exposure Level (PEL) for workers.

From the LD_{50} and LC_{50} values, acetaldehyde is considered to produce low acute toxicity in laboratory animals. Following single or repeated exposure, the entire respiratory tract is affected to varying degrees, depending on the concentration and length of exposure. Signs of toxicity include labored respiration, mouth breathing, weight loss, and liver damage.

Subchronic exposure to acetaldehyde caused varying degrees of inflammation and injury to the respiratory tract. Lesions included hyperplasia and metaplasia

of the olfactory mucosa. The NOAEL (No Observed Adverse Effect Level) and LOAEL (Lowest Observed Adverse Effect Level) from subchronic inhalation toxicity studies with rats are 150 ppm and 400 ppm, respectively.

In hamsters and rats, the chronic toxicity of acetaldehyde inhalation included increased mortality and growth retardation. Histopathological changes in the nose, larynx, and trachea included degeneration, hyperplasia, and metaplasia. A NOAEL for chronic toxicity could not be established. A LOAEL of 750 ppm was obtained for growth retardation.

The Office of Environmental Health Hazard Assessment (OEHHA) uses Reference Exposure Levels (RELs) to assess risk for noncancer health effects. The United States Environmental Protection Agency (USEPA) has developed a specific methodology to derive RELs and calls the resulting number the Reference Concentration (RfC) for the chemical. The RfC is an estimate, with an uncertainty spanning perhaps an order of magnitude, of a daily exposure to the human population, including sensitive subgroups, that is likely to be without appreciable risk of deleterious effects during a lifetime of exposure. The RfC is derived from a no (NOAEL) or lowest (LOAEL) observed adverse effect level in human or animal exposure, to which uncertainty or "safety" factors are applied. The USEPA has determined an RfC for acetaldehyde of 0.009 mg/m^3 (5 ppb) which is based on studies in which degeneration of the olfactory epithelium of rats was the most sensitive endpoint. OEHHA staff agree that below this RfC there will be no significant noncancer health effects due to acetaldehyde. Since the annual average ambient air level of acetaldehyde in California is estimated by the Air Resources Board in part A of this document to be 0.004 mg/m^3 (2 ppb), noncarcinogenic effects are not expected to occur since the air level is one-half of the RfC. However, "hot spots" of acetaldehyde exposure have not been evaluated.

Acetaldehyde has been shown to cross the placenta and enter the fetus in laboratory studies. Acetaldehyde caused developmental and reproductive toxicity in some rodents. Several intraperitoneal and intravenous developmental studies, *in vitro* studies, and a single reproductive study in male mice were found. The *in vivo* studies provided insufficient information upon which to base finite conclusions regarding developmental toxicity. Both the single male reproductive toxicity study and the *in vitro* testicular cell culture tests revealed effects on testosterone production. Various studies showed similarities between human and animal effects. No reproductive or developmental toxicity studies were found in which acetaldehyde was given via the inhalation or oral route.

Genotoxicity studies indicate that acetaldehyde is mutagenic in somatic cells. Acetaldehyde induced gene mutations in *Drosophila* but not in *Salmonella* assays. Conflicting results were obtained for mitochondrial mutations in *Saccharomyces cerevisiae*. Mammalian cell cultures indicated that acetaldehyde induces aneuploidy, micronuclei, chromosomal aberrations, and sister chromatid exchange. *In vivo*, acetaldehyde induced chromosome gaps and breaks in rat embryos after intra-amniotic injection, and sister chromatid exchange in mouse and hamster bone marrow cells after intraperitoneal injection. Acetaldehyde also has the potential to initiate cell transformation.

Inhalation studies in rats and hamsters provided positive evidence for acetaldehyde carcinogenicity. Rats and hamsters exposed to acetaldehyde via

inhalation of 750-3000 and 1500 ppm, respectively, developed nasal and respiratory tract tumors, and hamsters exposed via inhalation to 1650-2500 ppm also developed nasal and respiratory tract tumors. Nasal tumors found in rats were mainly squamous cell carcinomas and adenocarcinomas originating in the respiratory and olfactory epithelium, respectively. In hamsters, acetaldehyde-induced tumors were predominantly in the larynx. No useful epidemiologic data were available.

The International Agency for Research on Cancer has concluded that there is sufficient evidence for the carcinogenicity of acetaldehyde in animals; in humans, the evidence for carcinogenicity is inadequate. Overall, based on both the animal and human data, IARC considers that acetaldehyde is possibly carcinogenic to humans. The Environmental Protection Agency (U.S. EPA, 1991) has classified acetaldehyde in class B2, a probable human carcinogen, "based on increased incidence of nasal tumors in male and female rats and laryngeal tumors in male and female hamsters after inhalation exposure." Office of Environmental Health Hazard Assessment (OEHHA) staff concurs with these conclusions. In addition, OEHHA staff found no evidence for a carcinogenic threshold level for acetaldehyde.

OEHHA staff recommends that the range of risks for ambient exposures to acetaldehyde be based on the range of upper 95% confidence limits predicted from fitting a linearized multistage model to the male and female rat nasal tumor data after application of one of three plausible interspecies scaling factors: one which assumes that ppb concentrations are equivalent between species (i.e., a scaling factor of 1), a second which is a standard surface area correction factor, and a third which is a contact area correction factor. The range of estimated excess lifetime cancer risk is 0.97 to 27 x 10^{-6} per ppb (0.54 to 15 x $10^{-6}/\mu g/m^3$). The risk from continuous exposure for a 70 year lifetime to average ambient airborne concentrations in California, estimated by the Air Resources Board to be 2 ppb (4 $\mu g/m^3$), is 2 to 54 cases per million persons exposed. The lower and upper ends of the range are from female rat nasal tumors with no scaling factor and male rats with a contact area scaling factor, respectively. Within this range OEHHA staff believe that the best upper bound value is 4.8 x 10^{-6} per ppb, based on nasal tumors in male rats. Using the best value exposure to the ambient value of 2 ppb could result in up to 288 excess lifetime cancers (Upper 95% Confidence Limits) among the 30 million residents of the state. However, potential "hot spots" have not been evaluated.

The range of risk values represents several sources of uncertainty, including statistical uncertainty due to the relatively small number of animals used in the bioassay (less than the usual 50 per group). Other general sources of uncertainty, include the choice of the animal-to-human scaling factors, the choice of the extrapolation model, and the large range of extrapolation (five orders of magnitude) from the acetaldehyde concentrations used in the animal experiments to current ambient levels. In addition there is the possibility in light of the absence of an epidemiological connection between exposure to acetaldehyde and cancer that the risks in rats and hamsters may not be applicable to humans, i.e., acetaldehyde is only a potential human carcinogen. While a portion of the population is exposed to concentrations of acetaldehyde greater than 2 ppb, others will be exposed to less and thus have a lower risk.

Based on the findings of carcinogenicity and the results of the risk assessment, OEHHA staff finds that ambient acetaldehyde is an air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

2.0 METABOLISM AND PHARMACOKINETICS

2.1 Introduction

Acetaldehyde is the immediate oxidation product of ethyl alcohol. Most of the toxicity induced by alcohol consumption in humans has been related to the accumulation of acetaldehyde in various tissues. Consequently, the metabolic fate and effects of acetaldehyde have been generally studied in conjunction with the effects of alcohol. Acetaldehyde is also an intermediate byproduct of many metabolic cycles (Krebs and Perkins 1970). The small amount of acetaldehyde generated in the body is rapidly oxidized to acetate, primarily in the liver; therefore, a significant amount of acetaldehyde is seldom found in the body unless it is introduced exogenously or through ingestion of large amounts of alcohol (Krebs and Perkins 1970).

The following sections discuss acetaldehyde absorption, distribution, metabolism, and excretion. The absorption studies include both the inhalation and oral routes of exposure. The varied routes of exposure in the distribution and metabolism studies include the use of ethanol as a precursor. A search of the available literature revaled no data on dermal exposure.

2.2 Absorption

2.2.1 Inhalation Studies

Acetaldehyde is a volatile liquid with a boiling point of 20.6°C, a vapor pressure of 1.23 atm at 25°C, and a vapor density of 1.52 (air = 1). These properties allow rapid exchange and equilibration between blood entering the lungs and alveolar air (Ridge 1963). Watanabe et al. (1986) exposed male Sprague-Dawley rats to acetaldehyde vapor concentrations in air ranging from 9 to 1000 mg/l for 1 hour. Acetaldehyde inhalation resulted in blood concentrations considerably higher than liver levels; at a blood level greater than 10 ug/ml, the concentration of acetaldehyde in the liver was less than 2.5 ug/g. Moreover, following acetaldehyde inhalation, levels in arterial blood were higher than in peripheral venous blood (data not given). This latter finding led the study authors to suggest that acetaldehyde is rapidly absorbed from and metabolized by the lungs, which possess aldehyde dehydrogenase. Acetaldehyde also undergoes extrahepatic metabolism, and only minor amounts reach the liver.

Two inhalation studies were conducted with humans and dogs to determine the percent retention of inhaled acetaldehyde in the respiratory tract under given experimental conditions (Egle 1970, 1972a). Retention was defined as the percent difference between the amount of acetaldehyde inhaled in a given exposure period and the amount exhaled. In the first study, Egle (1970) measured the total respiratory tract retention in relation to respiratory frequency, duration of exposure, and concentration of acetaldehyde inhaled. Volunteers (males and females aged 23-60 years) inhaled acetaldehyde vapors through nose and mouth breathing. Acetaldehyde vapors (average concentration between 0.4 and 0.6 ug/ml) were inhaled from a respirometer. Two breathing systems, open and closed, were used. In the open system, acetaldehyde vapor was inhaled from a respirometer and exhaled into a collecting bag; in the closed system, acetaldehyde vapor was circulated through the system by a fan,

and exhaled air was returned to the respirometer. The closed system permitted a record of the entire ventilatory pattern, and from this, an estimate of the contact time. In a sine-wave breathing pattern, contact time was determined by measuring the base of the triangular pattern, dividing by two, and converting millimeters to seconds. The contact time of a square-wave breathing pattern was determined as the sum of parallel sides of a trapezoid divided by two and converted to seconds.

The results of both the open and closed systems indicated an inverse relationship between ventilatory rate and percent retention of acetaldehyde (Figures 2-1 and 2-2). Moreover, in the open system (Figure 2-1), percent uptake (the difference between the amount inhaled and the amount retained) declined as the concentration of acetaldehyde increased. No differences in uptake were noted among the volunteers with regard to the absolute uptake under given conditions or the pattern of change in uptake when conditions were varied. The uptake was defined as the difference between the amount in the system before and after the inhalation period (these values were derived from the initial and final concentrations and the volume of the system). Total acetaldehyde retention was the same for mouth and nose breathing. In humans, the total respiratory tract retention was 60% under the given experimental conditions; the physiological respiratory rate was 10 to 20 ml/min, and the tidal volume was 500-2000 ml. The results also showed that total retention in multiple breath experiments was independent of tidal volume, and that uptake was controlled by frequency and duration of ventilation. For example, the uptake at acetaldehyde concentrations ranging between 0.4 and 0.6 ug/ml was 78% at an average contact time of 10.1 seconds and only 59% at an average contact time of 1.4 seconds ($p < 0.02$).

In a followup study to compare the effects of acetaldehyde vapors on the total respiratory tract in dogs and humans and to test (in dogs only) the effects of high acetaldehyde vapor concentrations on different parts of the respiratory tract, Egle (1972a) exposed male and female mongrel dogs to acetaldehyde vapors ranging from 0.4 to 0.8 ug/ml. The authors concluded that the total respiratory tract retention of acetaldehyde in dogs was similar to human retention values and inversely related to the ventilatory rate. In the dog-only studies, the results showed higher uptake in the upper respiratory tract than in the lower tract; these results were independent of the inhaled concentrations or the tidal volume.

2.2.2 Oral Studies

Because of its small molecular size and lipophilic and water-miscibile nature, acetaldehyde is readily absorbed from the gastrointestinal tract following oral administration. Once absorbed, acetaldehyde enters the bloodstream; a large portion reaches the hepatic circulation and becomes metabolized by the liver, the major organ of acetaldehyde breakdown (Ridge 1963). Data describing the dose-absorption relationship are not available (Barry and Williams 1988).

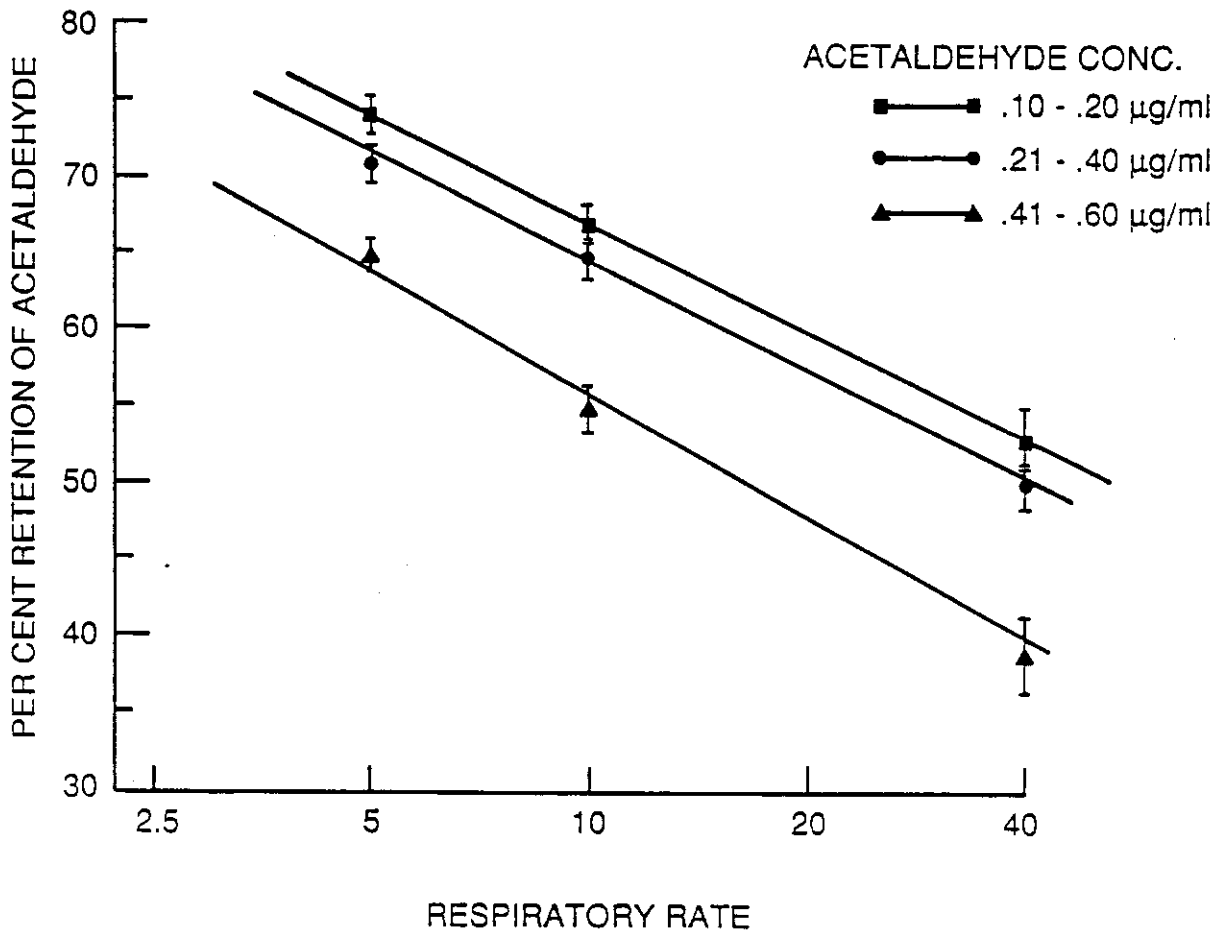


Figure 2-1. The retention of acetaldehyde with the open system design at three respiratory rates and three concentration ranges. Each point represents the average of 10 or more experiments.

SOURCE: Egle 1970.

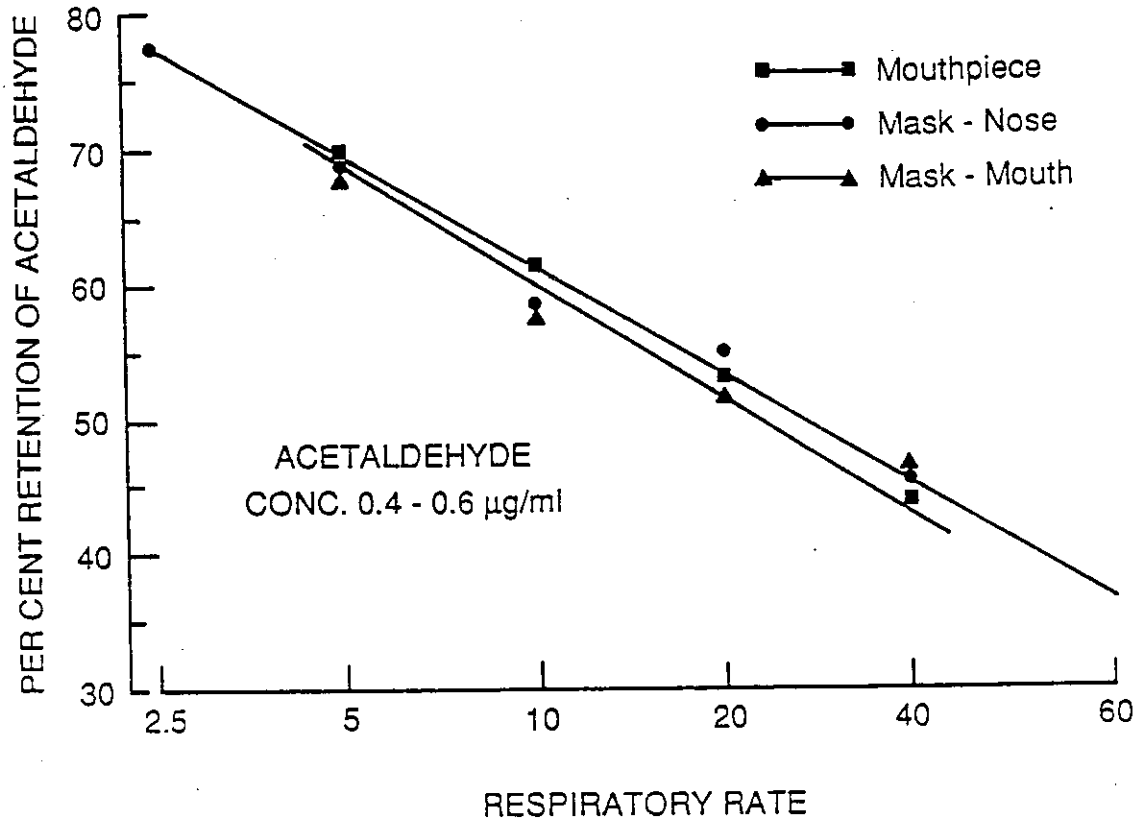


Figure 2-2. The retention of acetaldehyde with the closed system design. Three different types of breathing are compared. The overall average tidal volumes for each rate were 2.5/min, 2295 ml; 5/min, 1092 ml; 20/min, 762 ml; 40/min, 530 ml; and 80/min, 480 ml.

SOURCE: Egle 1970.

2.3 Distribution

Acetaldehyde readily diffuses through cellular membranes and is distributed to various organs for metabolism. It has been suggested that a normal endogenous level of acetaldehyde exists in blood from intestinal bacterial action and breakdown (Thurman and Pathman 1975). However, other studies have failed to detect such levels (Cohen and MacNamee 1976).

Hobara et al. (1985) exposed three male Sprague-Dawley rats to acetaldehyde air concentrations ranging from 1 to 20 mM for 1 hour. Within minutes postexposure, acetaldehyde was found in the blood, liver, kidneys, spleen, heart, and skeletal muscle, indicating rapid distribution. Immediately postexposure, the highest level of acetaldehyde was found in peripheral blood; at 15 minutes, arterial blood levels were 55% higher than peripheral venous blood levels. The lowest value was found in the liver (Table 2-1). These findings are in contrast to those reported by Watanabe et al. (1986), who reported that acetaldehyde levels were highest in arterial blood immediately following exposure. Hobara et al. (1985) reported that acetaldehyde disappeared rapidly from the blood; the half-life was 3.1 minutes. In contrast to findings related to inhalation of acetaldehyde, intragastric administration of ethanol at 3 g/kg produced blood and tissue acetaldehyde concentrations considerably lower than the values reported for inhalation exposure; the highest concentration was found in the liver (Table 2-1).

Shiohara et al. (1984) exposed rats by inhalation to 0.3 mmol acetaldehyde/l air (7000 ppm), 2 hours/day for 7 days, and measured blood levels at the end of the last exposure period. They found that acetaldehyde was rapidly removed from the blood; half-life was 10 minutes, and time to total body clearance was 40 minutes. Mean acetaldehyde blood concentration was 0.7, 0.2, and 0.1 mM at 5, 15, and 20 minutes, respectively, after inhalation was terminated. Following ingestion of 0.8 g/kg ethanol, acetaldehyde concentration in the hepatic blood of control nonalcoholics was 15.4 uM. Acetaldehyde could not be detected in the antecubital blood of controls (Nuutinen et al. 1984). A concentration-dependent increase was seen in acetaldehyde disappearance rate, which exhibited first-order Michaelis-Menten kinetics at acetaldehyde concentrations below 50 uM; the Michaelis constant (K_m) was 30-40 umol/l, and the V_{max} was 7 umol/l blood/minute. However, at blood acetaldehyde concentrations higher than 100 uM, the elimination kinetics followed a nonlinear pattern.

To demonstrate the rapid exchange of acetaldehyde between blood and exhaled air in humans, Freund and O'Hollaren (1965) used gas chromatography and detected acetaldehyde in alveolar air following ingestion of 0.5 ml ethanol/lb (859 mg/kg) body weight. Acetaldehyde could not be detected in alveolar air in a subject who consumed 1 ppm acetaldehyde in 500 ml orange juice (0.5 mg) containing no ethanol. However, it would seem highly unlikely that a dose this low (0.5 mg) would be detected in alveolar air, since acetaldehyde is extensively metabolized by the liver before it enters the blood circulation and the lung.

The results showed that acetaldehyde concentrations rose in proportion to ethanol intake when ethanol concentrations were less than 15-25 ug/100 ml. However, at higher ethanol concentrations, acetaldehyde values remained at a

TABLE 2-1

TISSUE DISTRIBUTION OF ACETALDEHYDE FOLLOWING ACETALDEHYDE INHALATION AND INTRAGASTRIC ETHANOL ADMINISTRATION

Tissue	Acetaldehyde Inhalation (nmol/g)	Ethanol Administration (nmol/g)
Blood[a]	1210	4.2
Liver	55	9.4
Kidney	213	2.1
Spleen	183	2.1
Heart muscle	277	2.3
Skeleton-muscle	345	1.7

[a]Blood levels were expressed as nmol/ml. Rats were exposed to 1-20 mM acetaldehyde vapor (in air) for 1 hour. The acetaldehyde levels were determined immediately after discontinuation of inhalation and 3 hours after the intragastric administration of ethanol at 3 g/kg body weight.

SOURCE: Hobara et al. 1985.

relatively constant level. As ethanol concentration dropped to 25 ug/100 ml or below, acetaldehyde concentration decreased rapidly (Figure 2-3).

Johannsson-Brittebo and Tjalve (1979) have used whole-body autoradiography to show the distribution of ^{14}C-acetaldehyde injected intravenously in mice. Within 1 minute, high radioactivity was detected in the heart muscle, diaphragm, kidney cortex, gastrointestinal mucosa, pancreas, salivary and lacrimal glands, bone marrow, nasal and bronchial mucosa, brown fat, plexus chorioidus, Harder's gland, and skeletal muscle. Low radioactivity was detected in the liver. After 5 minutes, the radioactivity in the heart, diaphragm, and skeletal muscle had decreased to low levels. Similar radioactivity distribution patterns were found in tissues 30 minutes to 24 hours postadministration. After 6 days of ^{14}C-acetaldehyde administration, radioactivity was high in the adrenal cortex, ovaries, bile, kidney cortex, nasal and bronchial mucosa, and skin; this was attributed to the incorporation of the 2-carbon moieties into other biosynthetic pathways, such as incorporation of acetyl-CoA into the citric acid cycle. Thus, acetaldehyde is rapidly distributed throughout the body and is incorporated into the body's metabolic cycles.

2.4 Metabolism

The liver is the main site of acetaldehyde metabolism. There is a strong "first pass" effect for ingested acetaldehyde and only 5% leaves the liver unchanged; this portion of acetaldehyde is transported by peripheral blood into other organs for its metabolism (Lubin and Westerfeld 1945; Westerfeld 1949). Inhaled acetaldehyde does not undergo a first pass effect and is distributed to all tissues including the liver. Acetaldehyde is oxidized by the enzyme aldehyde dehydrogenase to acetate. At least four additional enzymes can utilize acetaldehyde as a substrate. Xanthine oxidase, which occurs in the liver and in the blood and milk of some species, oxidizes acetaldehyde to acetate. Aldehyde mutase, found predominantly in the liver, preferentially reacts with one mole of acetaldehyde and one mole of pyruvate to produce acetate and lactate. Aldehyde aldolase and carboxylase, both of which are found in muscle, can metabolize acetaldehyde but apparently do not play a significant role in acetaldehyde breakdown *in vivo*.

Acetaldehyde blood concentrations have not been determined in persons exposed to the vapor, but have been determined following alcohol and paraldehyde administration; they were found not to exceed 0.2 mg/l.

The following sections discuss the aldehyde dehydrogenase enzyme system responsible for acetaldehyde metabolism, extrahepatic metabolism of acetaldehyde, and the compound's chemical reactivity.

2.4.1 Aldehyde Dehydrogenase Enzyme System

Aldehyde dehydrogenase is the main enzyme responsible for the breakdown of acetaldehyde. Its properties and subcellular locations have been extensively studied in the rat liver. This enzyme is found in both the cytosol and the mitochondria. The mitochondrial aldehyde dehydrogenase, which is the primary site of acetaldehyde oxidation in the rat, accounts for 80% of the

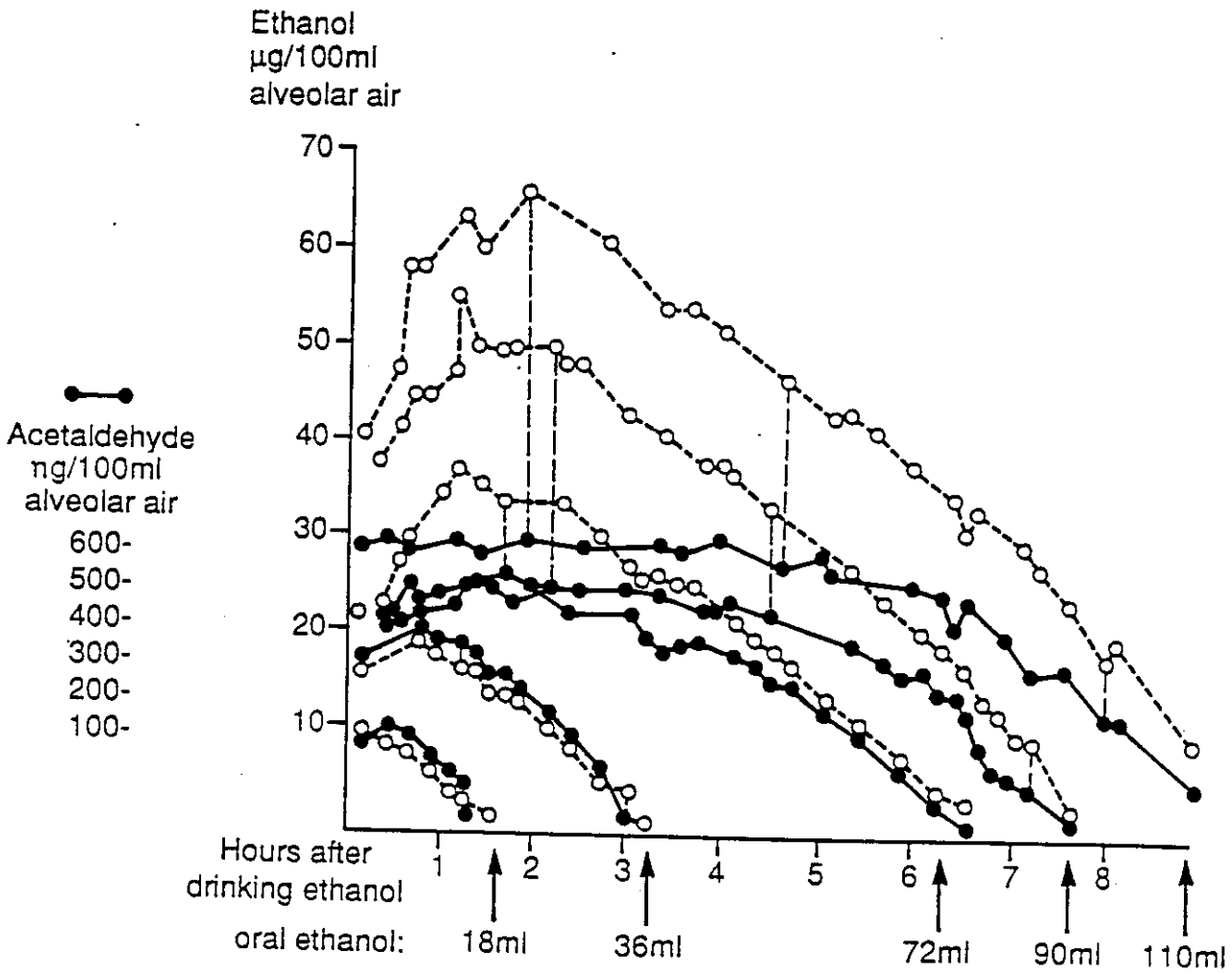


Figure 2-3. The effect of increasing amounts of oral ethanol upon acetaldehyde and ethanol concentrations in alveolar air in one subject. The circles represent ethanol, and the solid dots represent acetaldehyde concentrations. Each acetaldehyde curve is connected with its corresponding ethanol curve by three vertical lines.

SOURCE: Freund and O'Hollaren 1965.

acetaldehyde metabolizing activity in the mitochondria (Lindros et al. 1972; Horton and Barrett 1975, 1976). Two isozymes of aldehyde dehydrogenase are present in rat liver mitochondria. The first isozyme, designated as aldehyde dehydrogenase-I-NAD^+, has a K_m value for acetaldehyde oxidation of <5 uM (high affinity site); the second isozyme, designated as aldehyde dehydrogenase-II-NAD^+, has a K_m value of 1.5 mM (low affinity site). Acetaldehyde oxidation in the rat is linked to the mitochondrial electron transport chain at the NAD-aldehyde dehydrogenase site (Hasumura et al. 1976). Koivula et al. (1975) found two separable enzyme activities in both the cytosol and the microsomal fractions of the liver with properties similar to the mitochondrial enzymes with high K_m values.

Shiohara et al. (1984) exposed Sprague-Dawley rats to 0.3 mM acetaldehyde concentration in air for 2 hours/day for 7 or 14 successive days and killed them 24 hours after the last exposure. The authors found a significant ($p < 0.05$) decrease in liver mitochondrial low-K_m aldehyde dehydrogenase activity. They found similar results in the high-K_m aldehyde dehydrogenase of both mitochondrial and microsomal liver fractions. Acetaldehyde oxidation by intact liver mitochondria was decreased to 60% of that of controls. In contrast, brain mitochondrial aldehyde dehydrogenase activity and oxidation properties were unaffected by acetaldehyde inhalation. The authors suggested that only a small concentration of acetaldehyde had reached the brain.

The subcellular location of acetaldehyde oxidation in humans is debated. It has been suggested that the cytosol, not the mitochondria, is the primary site of acetaldehyde oxidation. However, more recently Cao et al. (1988), using beef and pig liver slices, showed that 60% of acetaldehyde oxidation was catalyzed by mitochondrial aldehyde dehydrogenase and only 20% by the high-K_m cytosolic enzyme. These findings are similar to those obtained in rats. Cao et al. concluded that the mitochondria appear to be the main site of acetaldehyde oxidation in all mammalian tissues including humans.

2.4.2 Extrahepatic Metabolism

In addition to hepatic mitochondrial metabolism, acetaldehyde is also metabolized by the respiratory-olfactory epithelium, kidneys, blood, brain, and spleen. Michoudet and Baverel (1987a) showed that in human and baboon renal cortex, acetaldehyde is oxidized to acetate by the kidney aldehyde dehydrogenase in a dose-dependent and linear manner with time. Acetaldehyde also crosses the blood-brain barrier and is metabolized by the brain aldehyde dehydrogenase (Shiohara et al. 1983). Forsander et al. (1969) showed a five- to sixfold higher level of acetaldehyde in peripheral blood as compared with that in the intact rat liver and hepatic vein. This was expected, since the former represents the total amount of acetaldehyde metabolized by all the extrahepatic organs. Oxidation rates of acetaldehyde were reported to be 2.0 umol/minute/g in the perfused rabbit liver and 1.1 umol/minute/g in the rat liver.

Casanova-Schmitz et al. (1984) exposed male F344 rats to 1500 ppm acetaldehyde for 6 hours/day for 5 days. At least two isozymes of aldehyde dehydrogenase were found in the nasal mucosa, differing with respect to their apparent V_{max} and K_m values (Table 2-2 shows these parameters in both the respiratory and olfactory mucosa). It is clear from the table that oxidation of acetaldehyde

occurred more rapidly in the homogenates of the respiratory than the olfactory mucosa. The nasal tissue is the first to contact acetaldehyde vapors upon inhalation. The presence of aldehyde dehydrogenase in the respiratory mucosa, as demonstrated in mice, rats, and hamsters, is important in the detoxification of acetaldehyde following inhalation. The aldehyde dehydrogenase acts as a defense mechanism, helping to minimize or prevent toxic injury to nasal tissues exposed to airborne compounds such as acetaldehyde (Kerns et al. 1983; Feron et al. 1982).

Michoudet and Baverel (1987b) prepared renal cortex tubules from the dog, rat, and guinea pig and incubated them with various concentrations of acetaldehyde. They found that acetaldehyde was metabolized at high rates in the tubules and in a dose-dependent fashion when high concentrations of acetaldehyde (5-10 mM) were tested. The rate of acetate accumulation was high in the dog and guinea pig but not in the rat, and exceeded the rate of conversion of acetate to acetyl-CoA by acetyl-CoA synthetase. These species differences were due to differences in the activities of these two enzymes. In rat renal cortex, acetyl-CoA synthetase was suggested to be the rate-limiting enzyme in acetaldehyde breakdown. The ratio of aldehyde dehydrogenase to acetyl-CoA synthetase in the dog, rat, and guinea pig was 1.13, 0.65, and 2.24, respectively. The acetaldehyde carbon removed but not accounted for by acetate was converted into carbon dioxide (CO_2). When concentrations of acetaldehyde (0.1-0.2 mM) closer to the physiological range were used, acetate did not accumulate. This study indicates that in vivo, the kidneys have high capacity to remove acetaldehyde from the circulation. Similarly, Marchner and Tottmar (1976) showed 50% lower acetaldehyde concentrations in renal venous blood of the rat after ethanol ingestion as compared with concentrations in the arterial blood. From all these findings, it is clear that the kidneys play a significant role in removing acetaldehyde from the circulation.

Results similar to those obtained in the above studies have been reported in the human and baboon renal cortex (Michoudet and Baverel 1987a). Acetaldehyde, 1-20 mM, was metabolized at high rates, in a dose-dependent fashion, and was completely oxidized to CO_2 and water in isolated human and baboon kidney cortex tubules. This indicated that the kidneys in vivo play a significant role in metabolism of acetaldehyde.

Pietruszko and Vallari (1978) were the first to demonstrate the presence of aldehyde dehydrogenase in human blood. Total catalytic enzyme activity was 0.017 umol/minute/ml blood incubated with 680 uM acetaldehyde. The enzyme was suggested to be located in the intracellular fraction of the blood.

In a recent study, Solomon (1988) investigated the effects of acetaldehyde on human red blood cell (RBC) enzyme activities in vivo. He tested 26 different enzymes and found that incubation of undiluted RBC lysates with 10 mM acetaldehyde at 37°C for 4 hours decreased aldolase activity by 42%, glutamic-pyruvic transaminase (GPT) by 52%, and glutamic-oxaloacetic transaminase (GOT) by 37%. In intact RBCs under identical incubation conditions, 10 mM acetaldehyde decreased GPT, GOT, and aldolase by 26%, 17%, and 53%, respectively. The acetaldehyde-mediated enzyme inhibition was both time- and temperature-dependent. There was no inhibition at 4°C, and in the first half hour there was no detectable inhibition of enzyme activity; however, within 3.5 hours, inhibition was significant. The findings were different with diluted hemolysate. Acetaldehyde had no effect on GPT, GOT, or aldolase

TABLE 2-2

ESTIMATED KINETIC CONSTANTS FOR THE NAD^+-DEPENDENT OXIDATION OF ACETALDEHYDE TO ACETATE CATALYZED BY HOMOGENATES OF RESPIRATORY AND OLFACTORY MUCOSA FROM FISCHER-344 RATS

AldDH isozyme	Tissue[a]	V_{max}[b] [nmol·min^{-1}·(mg protein)$^{-1}$]	K_m[b] (mM)
		Control	
I	Respiratory mucosa	128 ± 10	20 ± 3
	Olfactory mucosa	28 ± 4	22 ± 7
II	Respiratory mucosa	0.8 ± 0.9	$3 \times 10^{-4} \pm 9 \times 10^{-2}$
	Olfactory mucosa	2.2 ± 0.6	$1 \times 10^{-1} \pm 5 \times 10^{-2}$
		Exposed[c]	
I	Respiratory mucosa	140 ± 7	21 ± 2
	Olfactory mucosa	17.8 ± 0.6	14 ± 1
II	Respiratory mucosa	1.5 ± 1.1	$1 \times 10^{-1} \pm 2 \times 10^{-1}$
	Olfactory mucosa	1.3 ± 0.2	$7 \times 10^{-2} \pm 3 \times 10^{-2}$

[a]Mucosal samples from eight rats were combined for the measurement of the enzyme activity.
[b]Estimates shown are mean ± S.D., calculated by nonlinear regression analysis of the inital velocity on the substrate concentration; df = 13.
[c]Rats were exposed to 1500 ppm of acetaldehyde (6 hours/day, 5 days).

SOURCE: Casanova-Schmitz et al. 1984.

activities, suggesting the requirement of other metabolic conditions. The inhibition of aldolase was due to acetaldehyde oxidation, whereas transaminase inhibition seemed to require a nonoxidative pathway of acetaldehyde. It was suggested that acetaldehyde-mediated enzyme inhibition may play a role in the toxicity of alcoholics. Acetaldehyde-mediated transaminase inhibition was prevented by blocking glycolysis with sodium fluoride (Feig et al. 1971); therefore, some glycolytic intermediates may play a role in acetaldehyde-mediated transaminase inhibition. This was supported by Hoberman (1979a,b), where acetaldehyde was shown to react with glycolytic intermediates forming metabolites that bind to RBC proteins.

2.4.3 Acetaldehyde Binding and Antibody Formation

In addition to its metabolism by enyzmes, acetaldehyde is very reactive chemically. It has been reported to bind to proteins, the peptide glutathione, individual amino acids, and DNA. Schiff base formation appears to be the preferred initial reaction for acetaldehyde (Tuma and Sorrell, 1985) but other reactions are possible (USEPA, 1987).

Reaction of acetaldehyde with proteins was reported as early as 1949 (Mohammad et al. 1949). Acetaldehyde in the blood is found mostly in the bound form. It binds to albumin, hemoglobin, and other blood proteins (Donohue et al. 1983; Eriksson et al. 1977; Gaines et al. 1977; Mohammad et al. 1949). The maximum amount of acetaldehyde bound to rat erythrocytes is reported to be equivalent to 1.2 umol/ml of blood (Hagihara et al. 1981). Controversy exists concerning the presence of significant binding of acetaldehyde in human blood. Eriksson et al. (1977) reported 60% binding of acetaldehyde to whole blood in ethanol-dosed rats (different strains) that produced 20-300 uM acetaldehyde in the blood. Yet no significant binding was found in blood obtained from the antecubital vein of male and female human volunteers.

Gaines et al. (1977) reported that acetaldehyde irreversibly binds to erythrocyte membrane proteins such as hemoglobin in a manner similar to the binding of formaldehyde and glutaraldehyde (Stek 1972). They reacted isolated human erythrocyte ghosts with ^{14}C-acetaldehyde (1 mM containing 5 uCi of ^{14}C) and found that free acetaldehyde constituted 16% of the initial amount of label added; the remaining 84% was bound and remained in the suspension.

The binding of acetaldehyde to liver membrane proteins is known to alter the morphological and structural conformation of the membrane and perhaps the immunogenicity without having an effect on membrane function (Barry et al. 1984). Since the nonenzymatic browning of many foods involves, as a first step, an aldehyde-amine addition or condensation reaction, this phenomenon was used to demonstrate the interaction of acetaldehyde with various proteins (Mohammad et al. 1949). The reaction of acetaldehyde with bovine serum albumin (BSA) was temperature-, pH-, and substrate concentration-dependent and was evident by the appearance of the brown color and gelation of the reaction mixture. When proteins were isolated, they showed decreased solubility, lowered total nitrogen content, and a significant reduction in free amine content. This indicated the reaction of acetaldehyde with the amino groups of BSA. Acetaldehyde also reacted with guanidyl groups but to a lesser extent than with those of the amino groups of BSA.

Gelation of BSA by acetaldehyde was attributed to the formation of cross-links between the different reactive sites of protein molecules. Direct proof of BSA-acetaldehyde cross-linking was determined by osmotic pressure measurements, which revealed a large increase in the average molecular weight of soluble derivatives.

Acetaldehyde also reacted with some amino acids, such as alanine, serine, and threonine. The results were similar to those found with proteins. The reaction of acetaldehyde with proteins has been described as irreversible and covalent in nature. Such strong binding is due to the electrophilic properties of acetaldehyde, which can form both stable and unstable Schiff bases with the free amino groups of BSA and hepatic proteins (Donohue et al. 1983).

Acetaldehyde is well known to cause lipid peroxidation either directly through oxidation, or indirectly as a glutathione (GSH) conjugate (Shaw et al. 1981). Acetaldehyde binds to cysteine, one of the three amino acids constituting the GSH tripeptide. Depletion of GSH can reduce the scavenging of toxic free radicals by GSH. Although depletion of GSH per se is not enough to produce liver injury (Siegers et al. 1977), it should be accompanied by free radical formation. Williams and Barry (1986) showed that incubation of rat liver membranes with acetaldehyde led to the formation of products that activated neutrophils to degranulate and to produce cytotoxic superoxide ions. Tuma et al. (1984) incubated 200 uM ^{14}C-acetaldehyde with 6 mg/ml BSA with or without 5 mM ascorbate, a strong antioxidant. In the absence of ascorbate, acetaldehyde-BSA adduct formation reached a maximum after 24 hours of incubation and plateaued thereafter up to 240 hours. The percent acetaldehyde converted to stable adducts increased progressively with time and ranged from 35 to 70% after 240 hours. On the other hand, in the presence of ascorbate, there was a gradual and continuous increase in the formation of total acetaldehyde-BSA adducts throughout the reaction period. The total adducts formed were stable. Similar enhancement of stable adduct formation was seen in the reaction of acetaldehyde with cytochrome c, histones, and poly-L-lysine. Based on these findings, it was suggested that possible liver injury due to formation of stable hepatotoxic adducts may occur in individuals who consume megadoses of vitamin C and drink alcohol.

Fleisher et al. (1988) found that rabbits immunized with acetaldehyde-rabbit serum albumin (A-RSA) generated high titers of serum antibodies against A-RSA. The induced antibodies reacted negligibly against untreated RSA, indicating that A-RSA can serve as an antigen and immunize the host. In addition, A-RSA-immunized rabbits produced antibodies against acetaldehyde conjugates of human serum albumin, human gamma globulin, BSA, and bovine gamma globulin. These results suggest a role for immune mechanisms in the pathogenesis of alcohol liver disease. Indeed, Hoerner et al. (1988) found that the majority of alcoholic patients studied had elevated circulatory antibody titers against acetaldehyde-human hemoglobin and acetaldehyde-human serum albumin adducts.

2.4.4 Acetaldehyde Binding to DNA

Lam et al. (1986) reported the ability of acetaldehyde to crosslink DNA to protein in rat nasal respiratory mucosa. Crosslinking was demonstrated both in vitro, in homogenates of nasal respiratory mucosa incubated with acetaldehyde, and in vivo, after inhalational exposure of rats to

acetaldehyde. Such crosslinking indicates that acetaldehyde can react with DNA.

2.5 Excretion

The data on the disposition and elimination kinetics of acetaldehyde are scarce. Acetaldehyde can be excreted unchanged in urine, expired air, and skin (Baselt and Cravey 1989). Truitt and Walsh (1971) suggested that during periods of elevated blood levels, acetaldehyde metabolites would be excreted in the urine. Kallama and Hemminki (1983) injected Wistar rats with 120 uCi of ^{14}C-acetaldehyde and found that only 6% of the initial radioactivity was excreted in urine over a 7-day period. The main urinary products were acetate and two cysteine adducts constituting 2% of the radioactivity in urine collected 48 hours after ^{14}C-acetaldehyde administration. When a complex mixture of ^{14}C-cysteine and acetaldehyde was injected into the rats, 13.6% of radioactivity was detected in urine throughout 4 days in the form of 2-methylthiazolidine-4-carboxylic acid. This was in agreement with other studies, indicating that cysteine is a relatively poor trapping agent for acetaldehyde *in vivo*.

2.6 Conclusions

From the three inhalation studies investigating absorption, acetaldehyde was found to undergo rapid absorption and to reach equilibrium between the alveolar air and blood entering the lungs. Inhalation of 0.4 and 0.6 ug/ml acetaldehyde vapors in humans caused a decrease in tissue retention of vapors as the respiratory rate was increased. The total retention was 60% under physiological conditions of rate and tidal volume. No difference in total retention was found between nose and mouth breathing in humans. Total retention was independent of tidal volume, but uptake was dependent on frequency and duration of respiration. Similarity was found in total retention between the dog and human; in the dog, uptake was greater in the upper than in the lower tract, and it was independent of tidal volume and inhaled concentration.

Acetaldehyde is rapidly distributed to various organs when administered through inhalation, oral, or parental routes. At low concentrations, the acetaldehyde disappearance rate follows a first-order Michaelis-Menten kinetics with a K_m of 30-40 mM and a V_{max} of 7 umol/l blood/minute. At concentrations higher than 100 uM, the elimination kinetics are nonlinear.

The liver is the main site of acetaldehyde metabolism; extrahepatic metabolism also occurs in kidneys, lungs, blood, brain, and spleen. Aldehyde dehydrogenase is the enzyme system responsible for the metabolism of acetaldehyde into acetate. This enzyme is found in at least two isozyme forms in the cytosol and the mitochondia of various tissues. The mitochondrial aldehyde dehydrogenase is the main site of acetaldehyde oxidation both in rat liver and mammalian tissues including man. Oxidation occurs more rapidly in the respiratory mucosa than in the olfactory mucosa. Aldehyde dehydrogenase in the nasal tissue acts as a defense mechanism in detoxification of various airborne compounds, including acetaldehyde entering via inhalation. Acetaldehyde can be excreted unchanged in urine, expired air, and skin.

Acetaldehyde is a highly reactive molecule; it binds to amino acids and blood and membrane proteins. It causes lipid peroxidation leading to adduct formation and free radical-induced cell injury. Acetaldehyde can act as a hapten, and antibodies against acetaldehyde conjugates have been detected in human serum.

In conclusion, more complete studies are needed to examine the detailed pharmacokinetics of acetaldehyde metabolism, particularly via the inhalation route. The studies presented in this chapter have shown acetaldehyde to be rapidly absorbed, distributed, metabolized, and excreted. No accumulation in tissues occurs when administered via the various routes. At concentrations less than 100 uM, the elimination kinetics follow a first-order Michaelis-Menten linear relationship; the elimination kinetics are nonlinear at concentrations greater than 100 uM when acetaldehyde is given orally.

3.0 ACUTE TOXICITY

3.1 Human Health Effects

Inhalation of acetaldehyde vapors is the main route of occupational exposure in humans. The Occupational Safety and Health Administration's Time-Weighted Average (TWA) Permissible Exposure Limit (PEL) is 100 ppm (180 mg/m^3) for an exposure period of 8 hours/day, 5 days/week (OSHA 1989). The American Conference of Governmental Industrial Hygienists (ACGIH 1986) recommends a Threshold Limit Value (TLV) of 100 ppm (180 mg/m^3) for an exposure period of 8 hours/day, 5 days/week. OSHA has also promulgated a Short Term Exposure Limit (STEL) of 150 ppm.

The major effects of human exposure to acetaldehyde vapors consist of irritation to the eyes, skin, and respiratory tract. Low to moderate air concentrations (50-200 ppm) cause eye irritation and upper respiratory tract discomfort. Concentrations greater than 200 ppm may cause dyspnea and central nervous system depression (Baselt and Cravey 1989). Other clinical manifestations include erythema, coughing, pulmonary edema, and narcosis (Dreisbach 1980).

Human volunteers exposed to 50 ppm for 15 minutes experienced mild eye irritation (Silverman et al. 1946). Exposure to 134 ppm for 30 minutes produced mild upper respiratory tract irritation, and exposure to 200 ppm for 15 minutes produced transient conjunctivitis in all subjects. Sim and Pattle (1957) exposed 14 men, aged 18-45 years, to 133 ppm acetaldehyde for 30 minutes. Only mild irritation of the upper respiratory tract was evident.

When splashed in the eye, acetaldehyde liquid causes lacrimation and blurred vision (MCA, Inc. 1952). Prolonged exposure of the skin to liquid acetaldehyde causes erythema and burns; repeated contact may lead to dermatitis due to primary irritation or sensitization (ACGIH 1986).

3.2 Animal Studies

The acute animal toxicity studies discussed in this section include inhalation, oral, parenteral, and subcutaneous routes of administration with emphasis on the first three routes.

The acute LD_{50} values for acetaldehyde are summarized in Table 3-1. The acute inhalation LC_{50} is 20,000 ppm in rats exposed to acetaldehyde for 30 minutes (Skog 1950) and 13,300 ppm in rats exposed for 4 hours (Appleman et al. 1982). However, in another study inhalation of either 8,000 or 16,000 ppm acetaldehyde vapors for 8 hours caused no mortality in rats at 14 days postexposure (Smyth et al. 1951). The acute oral LD_{50} was reported to be 1232 mg/kg in mice and ranged from 1900 to 5300 mg/kg in rats. The LD_{50} for subcutaneous injection ranged from 560 to 640 mg/kg.

3.2.1 Inhalation Exposure

Following single or repeated exposure to acetaldehyde, the entire respiratory tract responds to varying degrees. The severity generally depends on the

TABLE 3-1

ACUTE TOXICITY STUDIES CONDUCTED WITH ACETALDEHYDE

Species	Number and Sex/Dose	Route of Administration	Dosage Eliciting Toxic Effects	Reference
Rat	5 males, 5 females	inhalation	LC_{50} 13,300 ppm/4 hr	Appleman et al. 1982
	--[a]	inhalation	LC_{50} 20,000 ppm 30 min	Skog 1950
	--	inhalation	LC_{Lo} 4000 ppm/4 hr	Lewis and Tatkin 1983
	--	inhalation	8000-16,000 ppm/8 hr; no deaths 14 days later	Smyth et al. 1951
Mouse	4 males	inhalation	RD_{50} 4946 ppm	Kane et al. 1980
	--	inhalation	RD_{50} 2845 ppm	Barrow 1982
Rat	--	oral	LD_{50} 1900 mg/kg	Windholz et al. 1983
	--	oral	LD_{50} 1930 mg/kg	Smyth et al. 1951
	--	oral	LD_{50} 5300 mg/kg	Omel'yanets et al. 1978
Mouse	--	oral	LD_{50} 1232 mg/kg	NRC 1977
	--	subcutaneous	LD_{50} 560 mg/kg	Skog 1950
Rat	--	subcutaneous	LD_{50} 640 mg/kg	Skog 1950

[a]Data were not reported in the literature.

concentration and exposure time. Appelman et al. (1982) exposed 20 SPF albino Wistar rats of each sex for 4 hours to acetaldehyde concentrations in air ranging from 10,436 to 16,801 ppm and observed the rats for 14 days. During the first 30 minutes of exposure, the animals were restless; they exhibited labored respiration with closed eyes. After 1 hour, the rats exhibited prostration with open eyes and severe mouth breathing. The 4-hour acute inhalation LC_{50} was estimated to be 13,300 ppm. Death occurred 2 to 6 days after exposure to the 15,683-ppm concentration.

Kane et al. (1980) studied the sensory irritant effect of acetaldehyde in male Swiss-Webster mice by recording the degree of respiratory rate depression. Groups of four animals were subjected to head-only exposures at varying concentrations for 10 minutes. Within a few seconds, the respiratory rate was reduced, and a pattern typical of sensory irritants was seen (Alarie 1966). Moreover, after 3 to 4 minutes, the animals exhibited a further reduction in rate as pronounced as the first. From the concentration-response relationship, the RD_{50} (the concentration that produced a 50% decrease in respiration rate) was calculated to be 4946 ppm. Concentrations of 1500 ppm and above resulted in respiratory rate depression. In another study, the RD_{50} of acetaldehyde in mice was calculated to be 2845 ppm (Barrow 1982). Neither of these studies reported histopathology but other studies have correlated RD_{50} concentrations with histopathology (Jiang et al. 1983, Buckley et al. 1984). The RD_{50} value indicates an irritation response to the chemical; it is useful in comparing relative potencies of chemicals as irritants and in establishing TLVs.

Unlike mice, F344 rats are not a good model for predicting acceptable occupational exposure concentrations to prevent sensory irritation in humans. Babiuk et al. (1985) exposed male Fischer 344 rats head only to various aldehydes. Both naive (no pretreatment) and formaldehyde-pretreated rats were used to determine whether pretreatment with formaldehyde could cause sensory irritation and cross-tolerance. All pretreated rats were exposed to 15 ppm formaldehyde 9 hours/day for 9 days; on the 10th day, the animals were exposed to acetaldehyde. The sensory irritant response was quantified by measuring respiratory rate depression using plethesmography. Acetaldehyde alone showed an RD_{50} value of 2991 ppm. After formaldehyde pretreatment, acetaldehyde showed cross-tolerance. The concentration response curve for acetaldehyde shifted to the right with a 3.5-fold increase in the RD_{50} from 2,991 to 10,601 ppm. They speculated that the mechanism of cross-tolerance is either a nonspecific injury to the nasal mucosa or modulation of a specific receptor in the trigeminal nerve endings.

Changes in arterial blood pressure and heart rate were measured in anesthetized rats exposed to 278-16,680 ppm (0.5-30 ug/ml) acetaldehyde (Egle 1972b). Blood pressure was significantly increased at concentrations of 1668 ppm (3 ug/ml) and greater, whereas heart rate was increased at 6672 and 13,900 ppm (12 and 25 ug/ml), respectively.

Liver damage is known to occur upon inhalation of large amounts of acetaldehyde. Tanaka et al. (1988) showed that acetaldehyde potentiates adenylate cyclase activity, which in turn stimulates cyclic adenosine 3',5'-monophosphate (cAMP) release in rats exposed to acetaldehyde for 2 hours (concentration not reported). This elevated cAMP increased the synthesis and release of biogenic amines that caused the liver damage.

3.2.2 Oral

Severe hypomotility (reduction of norman physical movements) was induced by intragastric administration of 100 mg/kg of acetaldehyde to male albino rats (Durlach et al. 1988). This almost immediate and severe hypomotility partially decreased after 5 minutes and plateaued for the remainder of the 15-minute test period. It could be reversed by calcium-N-acetylhomotaurine, the taurine analog having the greatest anti-acetaldehyde activity.

Mixed-breed male dogs dosed orally with 600 g/kg acetaldehyde showed frequent and severe bloody vomiting lasting for several hours (Booze and Oehme 1986). Only two of the acetaldehyde-dosed dogs exhibited slight tremors, and the remaining four dogs did not show any neurological or muscular symptoms. The two dogs exhibiting tremors had high acetaldehyde levels in their blood, whereas acetaldehyde either could not be detected or remained near the detection limit of 2 ng/ul in the other four dogs. All dogs appeared normal at 24 hours postdosing.

3.2.3 Parenteral Administration

Condouris and Havelin (1987) showed that intravenous injection of acetaldehyde at 1 to 10 mg/kg or infusion at 0.1 to 100 mg/kg/min to anesthetized cats resulted in increased mean arterial blood pressure and heart rate. Ventricular arrhythmias in the form of premature ventricular beats, bigeminy, and multifocal rhythms were seen at a threshold dose of 1-2.5 mg/kg. These effects of acetaldehyde were indirectly induced via the release of endogenous catecholamines from the heart. Similar results were found in guinea pigs (Mohan et al. 1981).

In mice, acetaldehyde injected intraperitoneally at 250 mg/kg three times, 10 minutes before 1-methyl-4-phenyl-1,2,3,4 tetrahydropyridine (MPTP), and 10 and 30 minutes after MPTP, potentiated the neurotoxicity of MPTP injected intraperitoneally at 36 mg/kg. This potentiation occurred through enhancement of striatal dopamine depletion and destruction of dopaminergic neurons in the substantia nigra (Corsini et al. 1987; Zuddas et al. 1987).

Acetaldehyde has been shown *in vitro* or following intravenous administration to cause cardiomyopathies by impairing protein synthesis (Lieber 1988) and by increasing the epinephrine content in the heart (Fujiwara et al. 1988). It stimulates the adrenal glands and hence increases the synthesis and release of biogenic amines (Lieber 1988). Acetaldehyde also impairs vitamin metabolism, particularly that of vitamin B (Veitch et al. 1975), and it affects the synthesis of vascular prostacyclin (Guivernau et al. 1987).

3.3 Toxicity of Peroxyacetyl Nitrate

Airborne acetaldehyde in the presence of nitrogen oxides can be converted to peroxyacetyl nitrate (PAN). PAN causes eye irritation in humans in the 1 ppm range (Stephens et al., 1961). In male A-strain mice, an LC_{50} (2 hours) of 106 ppm has been estimated (Campbell et al., 1967). Younger mice had a higher

LC_{50} (more resistant to PAN) than older mice. No lethality was seen at 97 ppm which indicates a very steep dose response curve. In rats the 4 hour LC_{50} was 95 ppm (Kruysse et al., 1977). Additional studies were done in rats for 4 and 13 weeks with exposures of 6 h/day, 5 days/week (Kruysse et al., 1977). In the 4 week study a NOEL of 4.1 ppm was obtained based on minimal behavioral disturbances, transient growth depression, slightly increased lung weights, and mild changes in the histopathology of the respiratory tract. The NOEL was 0.9 ppm. In the 13 week study the LOEL was 1 ppm, based on minimal irritation of the mucous membranes of the nasal cavity. The NOEL was 0.2 ppm. The toxic effects of PAN need to be considered as possible sequelae of acetaldehyde emissions.

3.4 Conclusions

In humans, the primary route of exposure to acetaldehyde is vapor inhalation. Acetaldehyde vapor is irritating to the skin, eyes, and respiratory tract. In animal studies, the 4-hour LC_{50} value in rats is 13,300 ppm, and the LD_{50} value ranges from 1900 to 5300 mg/kg. The RD_{50} is 2845 ppm in mice and the lowest observed effect level reported is 1500 ppm for a 10 minute exposure based on respiratory rate depression. It is hepatotoxic in rats when inhaled in large amounts. Acetaldehyde affects the cardiovascular system. It affects blood pressure and heart rate, causes cardiac arrhythmias through the release of catecholamines, and at high concentrations, acts as a depressant to the central nervous system and causes narcosis when administered via different routes. Acetaldehyde indirectly caused liver damage in rats by stimulating the synthesis and release of catecholamines. Oral administration of 100 mg/kg to rats caused severe hypomotility. Dogs orally dosed at 600 g/kg developed severe bloody vomiting. In mice, the neurotoxicity of MPTP was potentiated by acetaldehyde injected intraperitoneally at 250 mg/kg.

4.0 SUBCHRONIC TOXICITY

4.1 Inhalation Studies

Three subchronic inhalation studies in rats and one in hamsters were found in the literature. Appelman et al. (1982) exposed SPF rats to 0, 400, 1000, 2200, or 5000 ppm of acetaldehyde (0, 720, 1800, 3960, or 9000 mg/m^3, respectively) for 6 hours/day, 5 days/week for 4 weeks. Rats exposed to 5000 ppm exhibited severe dyspnea and marked excitation during the first 30 minutes of exposure. No significant clinical signs were observed at the lower exposure levels. Decreased body weight occurred in males dosed at 1000, 2200, and 5000 ppm and in females dosed at 5000 ppm. The percentage of lymphocytes was lower and that of neutrophils was higher in males and females exposed to 5000 ppm relative to the controls. No treatment-related changes were seen in clinical chemistry values. At the high dose, liver weights were significantly decreased in both sexes, and lung weights were increased only in males. Histopathological changes were seen at all doses tested, with the nose being more sensitive than the larynx, trachea, or lungs. The dorsal region of the nose exhibited the most severe changes, such as loss of microvilli, disarrangement of the epithelium, occasional loss of sensory cells at 400 ppm, and severe atrophy of the epithelium at 5000 ppm. At the two highest exposure concentrations, focal hyperplasia and stratified squamous metaplasia were seen. Damage to the larynx at 2200 and 5000 ppm consisted of hyperplastic and metaplastic stratified squamous epithelia. Metaplastic and hyperplastic changes in the tracheal epithelium were seen only at the highest exposure level.

The most severe effects appeared to occur in the posterior portion of the nose. The authors speculated that a combination of factors, including the impact of acetaldehyde due to the airstream through the nose, the polarity and solubility of acetaldehyde, and the higher sensitivity of the epithelium in this area, could explain the severity of the damage. The LOAEL was 400 ppm, but a NOAEL was not determined. The EPA has used this LOAEL to determine a Reference Concentration (RfC) for acetaldehyde (see Section 9.1).

In a later study, Appelman et al. (1986) tested intermittent and peak exposure effects of acetaldehyde vapor in groups of 10 male SPF Wistar rats. Two 4-week studies were performed using either (1) continuous exposure to 150 ppm or 500 ppm (270 or 900 mg/m^3, respectively) for 6 hours/day, 5 days/week; (2) two 3-hour exposures to 150 or 500 ppm for 5 days/week, interrupted by a nonexposure period of 1.5 hours between the first and second exposure; or (3) same as in (2) with four 5-minute peaks of 6 times the basic concentrations of 111 or 500 ppm per 3-hour exposure period. The peak exposure levels were 668 or 3043 ppm, respectively. A control group received fresh air only. Only rats exposed to 500 ppm with peak exposures of 3043 ppm showed irritation, displayed as eye blinking, excessive running, and nose twitching. In addition, the mean body weight of this group was significantly reduced compared to the controls. No effects on condition, behavior, or body weights were reported for animals exposed to 150 ppm with or without interruption or for those exposed to 111 ppm with or without peak exposures.

Histopathology revealed lesions of the epithelium from the dorsal part of the nose at 500 ppm (uninterrupted), but not at 150 or 111 ppm (interrupted). The effects noted in the respiratory tract at 500 ppm were comparable to those

noted at 404 ppm in the Appelman et al. (1982) study. In addition, a reduced phagocytic index was seen at the highest dose. Interruption of 500 ppm with peak concentrations of 3043 ppm caused a further reduction in the phagocytic index; nevertheless, there were no marked changes on the nasal epithelium. The NOAEL in this study was 150 ppm, and the LOAEL was 500 ppm.

Saldiva et al. (1985) exposed male Wistar rats via inhalation to 243 ppm acetaldehyde, 8 hours/day, 5 days/week for 5 weeks. Various pulmonary function tests were carried out in addition to gross and microscopic examinations of the respiratory system. The results revealed significant increases in functional residual capacity, residual volume, total lung capacity, and respiratory frequency. The authors excluded the possibility of emphysema or obstructive bronchial disease and suggested that the damage to the distal airways was unrelated to acetaldehyde. They suggested that these changes were the result of forced expiratory maneuvers such as applying negative pressure to simulate forced expiration, which can cause a premature collapse of the bronchioli.

The authors suggested that the increase in respiratory frequency was due to irritation of the airways. Gross examination did not reveal any differences between the control and acetaldehyde-treated groups. In contrast, histopathology showed an intense subacute inflammatory reaction in the nasal cavity characterized by hyperplasia of the olfactory epithelium and polymorphonuclear and mononuclear infiltration of the submucosa. The trachea and larynx were not examined.

Kruysse et al. (1975) exposed groups of Syrian golden hamsters to acetaldehyde vapor at concentrations of 0, 390, 1340, and 4560 ppm (0, 702, 2412, and 8208 mg/m^3, respectively), 6 hours/day, 5 days/week for 13 weeks. This study showed that more severe damage occurred in the upper rather than the lower respiratory tract, as was seen in rats (Saldiva et al. 1985). This suggested that the hamster is a less sensitive species than the rat. At 4560 ppm, a significant decrease in body weight and increase in relative heart, kidney, brain, testes, and lung weights were seen. Except for an increased relative kidney weight in males exposed to 1340 ppm, no other changes in organ or body weights were seen at the 390- and 1340-ppm doses. Lesions of the nasal cavity, larynx, trachea, bronchi, and lungs were seen at the highest dose, 4560 ppm. These lesions consisted of necrosis, inflammation, hyperplasia, and keratinizing squamous metaplasia. The NOAEL in this study was 1340 ppm, and the LOAEL was 4560 ppm.

4.2 Oral Studies

Both sexes of SPF Wistar rats received acetaldehyde in a test solution containing 25, 125, or 675 mg acetaldehyde/kg body weight/day for 4 weeks (Til et al. 1988). The only observed adverse effects were noted in rats receiving 675 mg/kg. These effects included hyperkeratosis of the forestomach, a significant decrease in food and liquid intake, and a slight increase in the relative weight of the kidneys in male rats compared to controls. No significant changes occurred in hematology values. The NOAEL was 125 mg/kg/day.

4.3 Conclusions

Few subchronic studies were found in the available literature. Acetaldehyde was found to be highly toxic to the entire respiratory tract. The effects included lesions such as hyperplasia and metaplasia of the olfactory mucosa and varying degrees of inflammation. The nose, trachea, and larynx were affected. Following oral administration, the NOAEL value in the rat was 125 mg/kg/day. Exposure of rats to acetaldehyde via inhalation gave a LOAEL value of 400 ppm and a NOAEL value of 150 ppm. The hamster appeared to be more resistant than the rat to the effects of acetaldehyde inhalation.

5.0 CHRONIC TOXICITY

5.1 Introduction

Limited information is available on the effects of acetaldehyde following chronic exposure. This chapter discusses chronic toxicity with relationship to two inhalation and two intratracheal instillation carcinogenicity studies in rats and hamsters. These studies are divided into three sections for clarity: inhalation, inhalation and intratracheal instillation, and intratracheal instillation. See Table 8-1 for a summary of these studies with regard to animal species, sex, number, dose, duration, and effects. The neoplastic changes observed in these studies are discussed in detail in Chapter 8 on carcinogenicity; only the nonneoplastic and/or preneoplastic lesions are presented in this chapter.

5.2 Inhalation

Woutersen et al. (1984, 1986) and Woutersen and Feron (1987) exposed male and female Wistar rats to acetaldehyde at concentrations of 0, 750, 1500, or 3000/1000 ppm (0, 1350, 2700, or 5400/1800 mg/m^3, respectively) 6 hours/day, 5 days/week for up to 28 months. The concentration in the high-dose group was gradually reduced from 3000 to 1000 ppm because of severe growth retardation, occasional loss of body weight, and early mortality. Animals in the high-dose group exhibited signs of severe respiratory distress, including salivation, labored breathing, and mouth breathing.

Mortality increased with dose; a clear dose-response relationship was evident. By day 715, all high-dose rats had died. Very few animals in the mid-dose group lived to study termination at day 844. Treatment caused dose-dependent growth retardation in males at all exposure concentrations and in females at the two highest concentrations. Consequently, the LOAEL was 750 ppm and a NOAEL could not be determined from this study.

The rats in the high-dose group showed signs of excitation, salivation, piloerection, and labored respiration; blood was seen around the nares of several animals. Despite a further reduction in the acetaldehyde concentration, a greater number of animals developed these symptoms after 12 months. In almost every high-dose rat that died early or was sacrificed moribund, the nose was partially or completely occluded by excessive amounts of keratin and inflammatory exudate. Several male and female rats also developed acute bronchopneumonia, occasionally accompanied by tracheitis.

Feron et al. (1982) exposed male and female Syrian golden hamsters to decreasing concentrations of acetaldehyde. The initial concentration was 2500 ppm (4500 mg/m^3); this was gradually decreased (between weeks 9 and 44) to 1650 ppm (2970 mg/m^3), 6 hours/day, 5 days/week for 52 weeks. The animals were sacrificed following a 29-week recovery period (at week 81). Mortality was slightly higher in hamsters exposed to acetaldehyde vapor than in controls. Body weights were recorded every 2 weeks during the first 6 weeks and monthly thereafter. Beginning at week 4, hamsters exposed to acetaldehyde had substantially lower body weights ($p < 0.05$) than those exposed to air. During the postexposure period

(53 to 81 weeks), differences in the body weights of exposed and control hamsters generally diminished but did not disappear. Except for a slight increase in alkaline phosphatase activity in females exposed to acetaldehyde, no significant differences in hematological or biochemical properties were observed between treated and control animals. The relative kidney and lung weights were higher in treated hamsters as compared with controls; the difference was statistically significant (p value not provided) only in treated females.

5.3 Inhalation with Intratracheal Instillation of Benzo[a]pyrene

Male Syrian golden hamsters exposed to air or 1500 ppm (2700 mg/m^3) acetaldehyde vapor, 7 hours/day, 5 days/week for 52 weeks, also received concurrent weekly intratracheal instillations of 0, 0.0625, 0.125, 0.25, 0.5, or 1.0 mg benzo[a]pyrene (B[a]P) for the same duration (Feron 1979). No differences in mortality rate were observed in hamsters exposed to acetaldehyde alone or air for up to 39 weeks; thereafter, the mortality of treated hamsters increased more rapidly than that of the control group (12 deaths as compared with 5 deaths at week 78, respectively). Hamsters exposed to acetaldehyde alone were generally more restless and had slightly lower body weights (maximum 10%) than controls. The hemoglobin, hematocrit, and red blood cell count values were significantly (p value not provided) lower in hamsters exposed to acetaldehyde than in controls exposed to air. In addition, in the acetaldehyde-exposed group, the urine contained more protein and the kidney weights were increased significantly as compared with the air-control groups (p value not provided). The mortality of hamsters exposed to acetaldehyde and receiving the high dose (52 mg) of B[a]P increased more rapidly than the B[a]P + air-exposed group when compared with respective controls ($p < 0.001$ in both groups).

In a similar experiment (Feron et al. 1982), male and female hamsters were exposed to air or to acetaldehyde vapor (initial concentration = 2500 ppm; final concentration = 1650 ppm; see Section 5-2 above) for 7 hours/day, 5 days/week for 52 weeks, and were given simultaneous weekly intratracheal instillations of 0.32 ml of 0.175% or 0.35% B[a]P (total dose, 18.2 or 36.4 mg/hamster, respectively) in saline, or subcutaneous injections once every 3 weeks of 0.2 ml of 0.0625% diethylnitrosamine (DENA) (total volume injected, 2.1 ul). The experiment was terminated after 81 weeks. Mortality was slightly higher in acetaldehyde-exposed hamsters relative to controls; also, these hamsters exhibited lower body weights than those exposed to air from week 4 onward. During the recovery period (53 to 81 weeks), the significant ($p < 0.05$) differences in body weights between exposed and control hamsters generally diminished but did not disappear.

There was a significant increase in mortality ($p < 0.05$) in animals treated with B[a]P and exposed to acetaldehyde or air over those exposed to acetaldehyde or air alone. In addition, mortality was considerably higher in animals, particularly in males, treated with the highest dose of B[a]P and exposed to acetaldehyde than in those given the same dose of B[a]P but exposed to air. There was low mortality in the DENA-treated group exposed to air.

5.4 Intratracheal Instillation

Feron (1979) gave male and female hamsters weekly or biweekly intratracheal instillations of 4 or 8 ul of acetaldehyde in 0.2 ml saline (approximately 32 to 73 mg/kg) for 52 weeks. Vehicle controls received 0.2 ml saline. The experiment was terminated at 104 weeks. Intratracheal instillation of acetaldehyde had no significant effect on mortality or body weight gain.

5.5 Conclusions

The major treatment-related chronic toxicity following inhalation exposure was increased mortality and growth retardation in hamsters and rats. Rats exposed to high concentrations (>1600 ppm) exhibited acute bronchopneumonia, occasionally accompanied by tracheitis and severe respiratory distress that included salivation, labored breathing, and mouth breathing. Inhalation of acetaldehyde resulted in histopathological alterations in the respiratory tract. NOAELs were not established for chronic toxicity. A LOAEL of 750 ppm can be reported for chronic toxicity. However, since the LOAEL for subchronic toxicity is lower (400 ppm), this putative chronic LOAEL does not reflect a likely LOAEL for acetaldehyde.

6.0 DEVELOPMENTAL AND REPRODUCTIVE EFFECTS

6.1 Introduction

Clinical and experimental studies have shown that ethyl alcohol causes developmental and reproductive toxicity. Ethyl alcohol (in alcoholic beverages) is a chemical known to the State of California to cause reproductive toxicity. Acetaldehyde, the primary metabolite of ethyl alcohol, has been suggested as a possible etiological agent in fetal alcohol syndrome. Its precise mode of action remains unknown. However, it has been shown to cross the placenta in mice (Blakely and Scott 1984b) as well as in rats (Zorzano and Herrera 1989) (see discussion above in Section 2.3, Distribution). A number of studies have been conducted to elucidate the mechanism of action of ethyl alcohol/acetaldehyde in causing developmental toxicity. This chapter summarizes studies in rodents that have evaluated *in vivo* and *in vitro* developmental and reproductive toxicity of direct exposure to acetaldehyde (as opposed to ethanol-mediated exposure). No studies were found in which acetaldehyde was administered via the inhalation or oral route; *in vivo* studies were limited to the intraperitoneal (ip) and intravenous (iv) routes of exposure. Only one reproductive toxicity study was found. *In vitro* studies include whole mouse and rat embryo culture systems for detecting developmental effects and various testicular cell culture systems for detecting effects on testosterone production.

6.2 In vivo Studies

6.2.1 Placental Transfer

Acetaldehyde is distributed to embryos via the placenta (Randall et al. 1978). Blakley and Scott (1984b) administered intraperitoneal injections of acetaldehyde to pregnant CD-1 mice at 200 mg/kg on day 10 of gestation. Within 5 minutes postadministration, acetaldehyde reached maximal concentrations in the maternal blood and liver, embryo, and yolk sac. The acetaldehyde rapidly disappeared; it was undetectable 2 hours after treatment.

Zorzano and Herrera (1989) found that acetaldehyde freely crossed the placenta in Wistar rats when present in maternal blood at high concentrations (100 uM or 4.4 mg/l). At all times following intravenous injection of acetaldehyde at 10 mg/kg to pregnant rats on gestation day 21, acetaldehyde concentrations in maternal blood were similar to those in fetal blood and amniotic fluid, and they reached equilibrium within 2 minutes of dosing. Peak concentrations were reached within 5 minutes of dosing; these were 1355, 1332, and 1913 uM in maternal blood, fetal blood, and amniotic fluid, respectively. However, at maternal blood concentrations below 80 uM, acetaldehyde was metabolized by fetal tissues or by the placenta and was not detected in fetal blood. The above studies indicate that acetaldehyde crosses the placenta and therefore can present an exposure to the fetus.

6.2.2 Developmental Toxicity

Studies in rodents that have examined the developmental toxicity of acetaldehyde following ip or iv administration of a single injection or multiple injections on different days during gestation are summarized in Appendix A (Table A-1).

In studies with rats, acetaldehyde has been shown to be teratogenic and embryolethal and has been shown to cause growth retardation. Sreenathan et al. (1982) evaluated the developing embryos and their membranes on gestation day (gd) 21 after a single ip injection of 50, 75, or 100 mg/kg acetaldehyde on gd 10, 11, or 12, or daily injections on gd 10-12 in Charles Foster (CF) rats. They found a significant ($p < 0.05$) increase in the number of resorptions. The incidence of litters with malformations was increased in the higher single-injected dose groups as well as in all triple-injected dose groups. Significant ($p < 0.05$) growth retardation was evidenced by reduced fetal body weights, crown-rump and tail length, transumbilical distance, umbilical cord length, and delayed skeletogenesis. In fetuses from dams dosed on gd 12 or 10-12, the placental weight was significantly ($p < 0.05$-0.001) reduced. No obvious dose-dependency was noted for these effects. In a time-course study, these same authors (Sreenathan et al. 1984) studied delayed ossification in gd 16-21 fetuses from CF rats that had received 50 mg/kg acetaldehyde ip on gd 8-15. Their results indicated that ossification of selected bones in the forelimbs, hindlimbs, and skull was significantly ($p < 0.05$-0.001) delayed by 1 to 2 days. An additional study (Padmanabhan et al. 1983) reported similar findings; CF rats were examined on gd 21 after ip administration of 50, 75, 100, or 150 mg/kg acetaldehyde on gd 8-15. Significant ($p < 0.01$-0.001) and dose-dependent increases were observed in the number of resorptions and the incidence of malformations. In addition, ossification was significantly ($p < 0.001$), but not dose-dependently, delayed in selected skull bones and in the axial skeleton.

Ali and Persaud (1988) investigated the effects of ip administration of 100 mg/kg acetaldehyde on gd 9-12 in Sprague-Dawley rats. Of the 16 recognizable morphologic endpoints evaluated on gd 12, only head length was significantly ($p < 0.05$) reduced in the treated animals when compared with the controls. Dreosti et al. (1981) examined the fetal development in Wistar rats on gd 20 after exposure to acetaldehyde (0.5 ml of a 3% saline solution, twice daily) throughout gestation. Slight but nonsignificant changes were noted in fetal body weight (decrease) and in the number of resorptions (increase). No increase in the incidence of malformations was observed.

In studies with CD-1 mice, acetaldehyde produced no effects on body weight and number of resorptions and malformations in gd 18 fetuses after five ip injections of 200 mg/kg each, administered during a 10-hour period on gd 10 (Blakley and Scott 1984a). No histopathological changes were observed in gd 10 fetuses from mice of the LACA strain after a single ip injection of approximately 60-480 mg/kg on gd 9 (Bannigan and Burke 1982). In C57Bl/6J mice, after one or two ip injections of 320 mg/kg acetaldehyde on gd 6, 7, 8, or 9, a nonsignificant decrease in fetal body weight and a slight increase in percent malformed fetuses (head and limb defects) were observed (Webster et al. 1983). However, in this latter study, control groups were not always used. Consequently, the slight increase in percent malformed fetuses that was observed cannot be compared with the spontaneous malformation rate normally

present in this mouse strain. Interpretation of these results is difficult.

In contrast, in CFLP mice given multiple iv injections of 40-80 mg/kg/day acetaldehyde on gd 7-9 (O'Shea and Kaufman 1979) or a single or multiple iv injections of 50 mg/kg on gd 6-9 (O'Shea and Kaufman 1981), a significant ($p < 0.05$-0.001) dose-related increase in the number of resorptions (days 10 and 19), decrease in the crown-rump length (days 10 and 19), and decrease in gd 19 fetal body weight were observed. In addition, slight increases (no statistical analysis reported) in the incidence of malformations (neural tube) and number of embryos failing to turn into a fetal position were noted on gd 10 only.

6.2.3 Reproductive Toxicity

In a male reproductive toxicity study (Lähdetie 1988), hybrid male mice (C57BL/6J x C_3H/He)F_1 were given daily ip injections of saline solution containing 62.5, 125, or 250 mg/kg acetaldehyde for 5 days. Animals were sacrificed 5 weeks after the beginning of treatment, and the testicular genotoxic effects were evaluated. No significant effects were observed on frequency of meiotic micronuclei, sperm count and morphology, and testicular and seminal vesicle weights. However, this study evaluated only selected end-points and, therefore, is not a representative study for male reproductive toxicity (see Conclusion for further discussion).

6.3 In vitro Studies

6.3.1 Developmental Toxicity

Several studies examined the embryotoxic properties of acetaldehyde utilizing whole mouse and rat embryos. These studies are summarized in Appendix A (Table A-2). Evaluations were conducted on cultures of postimplantation embryos recovered between gd 7 and 10.

In C_3H 8-day-old mouse embryos, acetaldehyde at doses of 7.4, 19.7, and 39.4 mg/l induced alterations in somite count, heartbeat, DNA synthesis, CNS development, and neural tube defects at all or some dose levels, while no consistent (and dose-related) response was noted in 9-day-old embryos (Thompson and Folb 1982). Exposure of ICR mouse embryos to acetaldehyde (range 17.6 ug/l to 1.7 g/l) resulted in increased embryolethality and incidence of malformations (cranial, facial, and limb effects) at all dose levels as well as dose-related growth retardation (Higuchi and Matsumoto 1984).

Albino rat embryos exposed to acetaldehyde on gd 9 at doses ranging from 0.20 to 1,980 mg/l caused significant ($p < 0.05$) growth retardation and an increased incidence of malformations. The lowest effective dose was 1.1 mg/l, and the no-effect level was 0.22 mg/l (Popov et al. 1982). Campbell and Fantel (1983) demonstrated similar results in 11-day-old Sprague-Dawley rat embryos after exposure to 5, 25, 50, 75, and 100 uM acetaldehyde on gd 10. The highest concentration was lethal; the no-effect level was 5 uM; and 25, 50, and 75 uM caused significant ($p < 0.01$) growth retardation and decreased total protein content.

In contrast, Priscott (1985) observed no significant effects in 11- and 12-day-old Wistar rat embryos previously exposed to 100 and 260 uM acetaldehyde on gd 10. At 800 uM, there was a rapid cessation of growth and development resulting in "an unrecognizable necrotic mass."

Preimplantation 2-cell stage embryos from CF-1 mice exposed to acetaldehyde at concentrations of 5, 10, 50, 100 and 500 mg/100 ml culture medium (Kalmus and Buckermaier 1989) resisted the lower concentrations of acetaldehyde, while doses of ≥50 mg/100 ml medium were lethal. These lethal doses are considered by some authors to be unusually high. Consequently, the 2-cell stage embryos are regarded as highly resistant to acetaldehyde.

6.3.2 Reproductive Toxicity

Reproductive in vitro toxicity studies have shown that acetaldehyde is a potent inhibitor of testicular steroidogenesis. Cicero et al (1980) exposed dispersed testicular cell cultures from 55- to 60-day-old Sprague-Dawley rats to acetaldehyde at concentrations of 0.02 to 1.0 mM. At acetaldehyde concentrations of 50 uM, testicular steroidogenesis was significantly inhibited. Johnson et al. (1981) examined the effects of acetaldehyde on three enzymes involved in the conversion of pregnenolone to testosterone. Testicular microsomal fractions were prepared from 60-day-old Wistar rats; 600 uM acetaldehyde, in the presence of androstenedione, significantly inhibited this conversion.

To assess potential acute impairment of testicular testosterone, Boyden et al. (1981) conducted a study in dogs with isolated blood-perfused testes. Acetaldehyde, at a dose level corresponding to blood levels of 0.2 mg/dl (approximately 50 uM, which is frequently found in humans), inhibited hCG-stimulated testosterone production.

6.4. Conclusions

Because of their study design, the in vivo studies do not permit specific conclusions about the developmental toxicity of acetaldehyde and its mode of action. Small sample numbers and evaluation of a limited number of endpoints are evident in most studies; nevertheless, certain general conclusions may be drawn. Different species and strains respond differently to acetaldehyde exposure. All rat strains examined, when given approximately the same ip dose of acetaldehyde, exhibited similar effects for number of resorptions, incidence, and type of malformations, and/or growth retardation. Some mouse strains exhibit resistance to acetaldehyde, and others show effects similar to those observed in rats. Mode and duration of administration (as observed in mice) influenced the degree (but not the type) of developmental toxicity. Some, but not all, studies show a dose-related response for the endpoints studied. As discussed by several authors, these studies demonstrate a great inter-litter variability in embryolethality; some litters were highly affected and others not at all. It has been suggested that this indication of great differences in susceptibility to acetaldehyde within a strain is due to genetic makeup; this has been observed in human offspring and their susceptibility to ethanol.

Only one male reproductive toxicity study for acetaldehyde exposure was found. Only a selected number of endpoints in one sex was examined, since the authors' intentions were to study the testicular genotoxic effects of acetaldehyde. Therefore, the negative results of the study should not be interpreted as evidence of acetaldehyde not being a male reproductive toxicant. For example, to cover the entire spermatogenic cycle in mice, sperm count/morphology, testicular weight/morphology, hormone levels, fertility, etc., should be examined periodically over a 35- to 42-day period. This study evaluated some endpoints at one time only (at the end of one complete spermatogenic cycle). In fact, in support of male reproductive toxicity are the results from studies with various testicular cell culture systems. Acetaldehyde seems to affect testosterone production; one author suggests that it does so by inhibiting one of four enzymes necessary for conversion of pregnenolone to testosterone.

The effects observed on embryos in culture are in agreement with those observed in in vivo studies. Both test systems have demonstrated similar types of malformations and growth retardation as well as embryolethality; species and strain differences exist in both; and some (but not all) results exhibit a dose-related response. Two-cell stage preimplantation embryos appear to be more resistant to acetaldehyde than are postimplantation embryos.

Acetaldehyde crosses the placenta and enters the fetal circulation. Acetaldehyde equilibrated within 2 minutes of injection of a high concentration into pregnant rats; it reached peak concentration within 5 minutes of dosing in maternal blood, fetal blood, and amniotic fluid.

Acetaldehyde has been shown to cause adverse developmental effects in some rodent species when administered at high doses via i.p. or i.v. injection. There is also evidence for toxicity to the embryo and to testicular function when there is direct exposure to acetaldehyde in vitro. In all but one of the in vivo studies discussed, maternal toxicity parameters were not reported and the authors did not discuss or determine the maternal versus the developmental no-effect and low-effect levels of acetaldehyde toxicity. However, several authors have emphasized the similarities between the types of effects observed in humans and those observed in rodents. The mechanisms by which acetaldehyde causes developmental and reproductive toxic effects in vitro and following high i.p. and i.v. doses in vivo are not known. There are also no data available to indicate the relevance of adverse effects under the conditions investigated to possible human exposures. It is, therefore, not possible at present to determine if acetaldehyde poses a reproductive or developmental hazard to humans. It is desirable that studies relevant to hazard identification for possible reproductive and developmental toxicity of acetaldehyde to humans be performed.

7.0 GENOTOXICITY

7.1 Introduction

Acetaldehyde has been tested for genotoxicity in a variety of *in vitro* and *in vivo* assays designed to detect gene mutations, chromosomal aberrations, sister chromatid exchanges, DNA damage, and cell transformation. Formation of Schiff bases and other adducts may be involved at the molecular level (see Section 2.4). Table 7-1 summarizes the findings for acetaldehyde genotoxicity as demonstrated in each test. The table in Appendix B summarizes the protocols and specific results for each individual study.

7.2 Gene Mutations

Acetaldehyde has been tested for the potential to induce gene mutations in bacteria, yeast, nematodes, insects, and mammalian cells.

7.2.1 Bacteria

Acetaldehyde was nonmutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538, TA102, or TA104 both in the presence and absence of metabolic activating systems (Laumbach et al. 1976; Rosenkranz 1977; Pool and Wiessler 1981; Marnett et al. 1985; Mortelmans et al. 1986). In one study, acetaldehyde (7793 ug/plate) caused a slight increase in mutant colonies of strain TA1535 without activation (16 revertants in the treated group versus 4 revertants in the water control); however, the very low background frequency made the results inconclusive (Rosenkranz 1977). It is unclear why acetaldehyde produces consistently negative results in the *Salmonella* assays while producing positive results in other test systems. It is possible that the standard Ames test is not suitable for detecting the mutagenicity of acetaldehyde because the compound is volatile, and precautions may not have been taken to prevent its evaporation (Dellarco 1988).

Conflicting results were obtained when acetaldehyde was tested for mutagenicity in liquid suspension assays with *Escherichia coli* WP2 uvrA. Nonactivated acetaldehyde was mutagenic at a concentration of 38.8 ug/ml (880 uM) when incubated in sealed tubes at 0°C for 30 minutes (Veghelyi et al. 1978), but was nonmutagenic in the same strain at concentrations ranging from 0.9 to 441 ug/ml without metabolic activation (20 to 10,000 uM) when incubated in capped tubes at 37°C for 18 hours (Hemminki et al. 1980). It has been suggested that the positive results obtained by Veghelyi et al. may be due to the lower incubation temperature, which would reduce the evaporation of acetaldehyde during treatment and the oxidation of acetaldehyde to acetic acid (Dellarco 1988).

7.2.2 Yeast

Bandas (1982, as cited in Dellarco 1988) reported that nonactivated acetaldehyde exhibited a weakly mutagenic effect on mitochondrial DNA (petite mutations) of the yeast *Saccharomyces cerevisiae*. A twofold increase in the spontaneous

TABLE 7-1

GENOTOXICITY OF ACETALDEHYDE IN VARIOUS TEST SYSTEMS

Endpoint	Test Systems	Results		
		Positive	Negative	Equivocal
Gene mutation	*Salmonella typhimurium*		X	
	Escherichia coli			X
	Caenorhabditis elegans			X
	Drosophila melanogaster sex-linked recessive lethal test	X		
	Human lymphocytes	X		
Chromosomal aberrations	*Vicia faba*	X		
	Drosophila melanogaster reciprocal translocation		X	
	Rat fibroblasts	X		
	Chinese hamster ovary cells	X		
	Human lymphocytes	X		
	In vivo bone marrow cells	X		
Sister chromatid exchange	Chinese hamster ovary cells	X		
	Human lymphocytes	X		
	In vivo bone marrow cells	X		
DNA damage	*Escherichia coli* pol A			X
	Mammalian cells (mouse lymphoma, rat hepatocytes, human lymphocytes, and human bronchial epithelial cells)		X	
Mammalian cell transformation	Kidney cells (HRPT[4])	X		

frequency of petite mutants was seen after treatment of cells with 23,490 ug/ml acetaldehyde for 90 minutes. However, this response was considered to be equivocal because it occurred at an extremely cytotoxic dose (96% of the cells were killed) and no dose-response was demonstrated. Dellarco (1988) considered the interpretation of the response (increase in mitochondrial mutations) to be uncertain because cytoplasmic mutations are less defined genetically than the nuclear mutations used in standard assays.

7.2.3 Nematodes

Acetaldehyde (783 or 7,830 ug/ml for 2 hours) was tested in the nematode *Caenorhabditis elegans* for its ability to induce mutations in the genes affecting the egg-laying system. The frequency of mutations (reduction in brood size) was increased at the lower (783 ug/ml) concentration ($1x10^{-4}$ in treated nematodes versus $6x10^{-6}$ in untreated controls); acetaldehyde was toxic at the higher concentration (Greenwald and Horvitz 1980).

7.2.4 Insects

Woodruff et al. (1985) tested acetaldehyde in the sex-linked recessive lethal (SLRL) test in *Drosophila melanogaster* by adult feeding and injection. Acetaldehyde was nonmutagenic after feeding (25,000 ppm), but 22,500 ppm given by injection produced 0.21% lethals versus 0.06% in the untreated control group.

7.2.5 Mammalian cells

He and Lambert (1990) used acetaldehyde to induce mutations at the hypoxanthine-guanine phosphoribosyl transferase (hprt) locus in freshly isolated human T-cell lymphocytes. Cells were incubated for 24 h with 1.2 to 2.4 mM acetaldehyde or for 48 h with 0.2 to 0.6 mM acetaldehyde. Cells showed decreasing survival with increasing acetaldehyde and a 3 to 16 fold increase in mutation frequency at the hprt locus relative to background (background mutation rate = 3.2 to $6.2x10^{-6}$). Several of the mutations involved partial deletions of the hprt gene. This study is the first demonstration of induction of mutations by acetaldehyde in human somatic cells.

7.3 Chromosomal Aberrations

Acetaldehyde has been tested for its ability to induce chromosomal aberrations in plant, insect, and mammalian cells.

7.3.1 Plants

Acetaldehyde produced a dose-dependent increase in the frequency of chromosomal aberrations (breaks and translocations) in cells of the root-tip meristem of *Vicia faba*. The clastogenic effect was seen following treatment with 220.5 to 2,205 ug/ml (5 to 50 mM) acetaldehyde for 24 hours at 12°C; higher temperatures

reduced the clastogenic effects of acetaldehyde (Rieger and Michaelis 1960).

7.3.2 Insects

As part of the full investigation of acetaldehyde in D. melanogaster, Woodruff et al. (1985) evaluated the brood-yielding results in the SLRL assay for the induction of reciprocal translocations. Acetaldehyde (22,500 ppm administered by injection) was negative.

7.3.3 Mammalian Cell Cultures

Bird et al. (1982) studied the production of micronuclei and chromosomal aberrations in primary rat skin fibroblasts exposed to concentrations of 4.4 to 441 ug/ml acetaldehyde for 12, 24, or 48 hours (micronucleus test) or 0.44 to 44.1 ug/ml acetaldehyde for 12 or 24 hours (chromosomal aberrations). Treatment resulted in a dose-dependent production of micronuclei; the lowest effective concentration was 22 ug/ml (2.4% cells with micronuclei versus 0.5% in control cultures after 12-hour treatment). In the chromosomal aberration assay, the 12-hour exposure to acetaldehyde induced an increase in aberrations (14% aberrant cells compared with 2% in control cultures) only at the 44.1 ug/ml concentration; chromatid breaks and gaps and acentric fragments were observed. Treatment for 24 hours increased the frequency of aberrations (12% at 4.4 ug/ml and 20% at 44.1 ug/ml as compared with 4% in control cultures), and the number of metaphases with specific aberrations (chromatid and chromosomal breaks, gaps, deletions, and fragmentation) was also increased. An increase in aneuploid cells was also seen at the 4.4- and 44.1-ug/ml dose levels; however, only the total incidences of aneuploidy were reported rather than the incidences of hyperploidy and hypoploidy.

Dulout and Furnus (1988) determined that the most notable cytogenetic effect of acetaldehyde in cultured Chinese hamster ovary (CHO) cells was aneuploidy and not chromosomal breakage. Acetaldehyde added for 24 hours to cultures at concentrations of 15.66, 31.32, or 46.98 ug/ml produced an increased frequency of aneuploidy compared to controls ($p < 0.001$). Aneuploidy was due mainly to an increase in the number of hypodiploid cells; the ratio of hypodiploid/hyperdiploid cells was 0.81 in the controls and 2.72, 2.73, and 3.57 in the 15.66-, 31.32-, and 46.98-ug/ml acetaldehyde-treated cells, respectively. In contrast to the induction of aneuploidy at all assayed levels, the frequency of structural aberrations (chromatid or isochromatid breaks, chromatid exchanges, and dicentric chromosomes) was significantly increased only at the two highest concentrations: 31.32 ug/ml (3.6% abnormal cells, $p < 0.025$) and 46.98 ug/ml (30.38% abnormal cells, $p < 0.001$). Acetaldehyde was not clastogenic at 15.66 ug/ml. These results suggested that acetaldehyde can elicit an aneuploidy effect at nonclastogenic concentrations. To a lesser degree, acetaldehyde also induced polyploidy as indicated by the significant increase in polyploidy cells at 15.66 ug/ml ($p < 0.001$) and 31.32 ug/ml ($p < 0.05$); concentrations were severely cytotoxic.

Obe et al. (1979) studied the chromosome-breaking activity of acetaldehyde in peripheral lymphocytes of a patient with Fanconi's anemia (deficiency in repairing DNA cross-links) and three normal individuals. After 24 hours of

treatment, acetaldehyde (7.83 or 15.7 ug/ml) caused a non-dose-related increase in the frequency of gaps, breaks, exchange-type aberrations, and acentric fragmentation in the cells from the subject with Fanconi's anemia. No clastogenicity was observed in the cells of the normal subjects.

Böhlke et al. (1983) examined the cytogenetic effects of acetaldehyde in cultured lymphocytes of German and Japanese subjects having different aldehyde dehydrogenase (ALDH) phenotypes. Cells, derived from the German subjects possessing both ALDH isozymes I and II and from the Japanese subjects possessing either isozyme II or isozymes I and II, were exposed for 72 hours to acetaldehyde concentrations ranging from 0.09 to 1.08 ug/ml. Cells from both populations showed dose-dependent increases in chromatid aberrations (especially achromatic lesions, chromatid breaks, and exchange-type aberrations) following exposure to acetaldehyde concentrations of 0.72 and 1.08 ug/ml; no significant differences were seen among the different ALDH phenotypes.

7.3.4 Whole Mammals

Barilyak and Kozachuk (1983) conducted a cytogenetic analysis of embryonic cells harvested 24 hours after a single intra-amniotic injection of 7830 ug/ml acetaldehyde was administered on gd 13 to female Wistar rats. A higher frequency of chromosome aberrations (mostly gaps and breaks) was seen in treated rat embryos (16 ± 1.5% metaphases with breaks) as compared with controls (3.8 ± 0.8%).

Lähdetie (1988) observed no significant increase in the frequency of meiotic micronuclei in early spermatids harvested from hybrid mice (C57Bl/6J x C3H/He)F1 given single intraperitoneal injections of 0, 125, 250, or 375 mg/kg acetaldehyde in saline and sacrificed 13 days posttreatment. No mice survived treatment with the highest dose (500 mg/kg).

7.4 Sister Chromatid Exchanges

A number of studies have shown acetaldehyde to be a strong inducer of sister chromatid exchanges (SCEs) in CHO cells and human peripheral blood lymphocytes *in vitro*.

7.4.1 CHO Cells

Obe and Ristow (1977) treated CHO cells once each day for 8 days with nonactivated acetaldehyde concentrations ranging from 3.92 to 31.32 ug/ml. Cells incubated with concentrations greater than 7.83 ug/ml were cytotoxic. Lower concentrations (3.92 and 7.83 ug/ml) caused dose-dependent increases in SCEs, which ranged from 13.56 SCEs/cell at 3.9 ug/ml to 28.35 SCEs/cell at 7.83 ug/ml. The background central frequency was 4.69 SCEs/cell.

Following a 24-hour exposure of CHO cells to nonactivated acetaldehyde concentrations ranging from 1.96 to 11.75 ug/ml, Obe and Beek (1979) found that the lowest effective concentration was 3.92 ug/ml, which produced 18.89 SCEs/cell

compared with 8.24 SCEs/cell in controls. The maximum response (22.08 SCEs/cell) was seen at the highest (11.75 ug/ml) concentration.

De Raat et al. (1983) reported that acetaldehyde (7.8 to 39.4 ug/ml for 24 hours) induced dose-related increases in SCEs both in the presence and absence of metabolic activation. The lowest concentration tested, 7.8 ug/ml, produced 17.25 SCEs/cell without activation and 15.40 SCEs/cell with S9 activation; corresponding values in control cultures were 9.20 and 13.45 SCEs/cell, respectively.

7.4.2 Human Lymphocytes

Ristow and Obe (1978) observed a dose-related increase in SCEs in cultured human whole-blood lymphocyte cultures exposed to 3.92 to 15.7 ug/ml acetaldehyde. The highest concentration (15.7 ug/ml) produced 14.18 SCEs/cell compared with 4.02 SCEs/cell in control cultures at 24 hours; when treated for 48 hours, the SCE frequency increased to 23.95 SCEs/cell.

Similarly, Norppa et al. (1985) observed statistically significant ($p < 0.001$) dose-related increases in SCEs following treatment of peripheral lymphocytes with 2.8 to 88.2 ug/ml acetaldehyde (-S9) for 48 hours.

In another study, exposure of lymphocytes to 3.92 or 7.83 ug/ml acetaldehyde (-S9) for 90 hours caused significant ($p < 0.01$), dose-dependent increases in SCEs; approximately 17 or 28 SCEs/cell were produced, respectively, in treated cells compared to 11 SCEs/cell in control cultures (Jansson 1982).

Böhlke et al. (1983) treated lymphocytes derived from German subjects possessing both ALDH isozymes and from Japanese subjects possessing either isozyme II or isozymes I and II with nonactivated acetaldehyde (3.97 to 47.6 ug/ml) for 72 hours. A dose-dependent increase in SCE frequencies was observed for both populations; no differences that could be related to the different ALDH phenotypes were observed.

Obe et al. (1986) showed that SCE induction was slightly reduced when ALDH was added directly to the culture medium. Exposure of human peripheral lymphocytes to 78.3 ug/ml acetaldehyde for 3 hours without metabolic activation produced approximately 28.2 SCEs/cell compared to 10 SCEs/cell in control cultures. When ALDH and NAD (required cofactor for ALDH) were added, the SCE frequency was reduced to 14.5 SCEs/cell. Treatment with acetaldehyde at 15.66 ug/ml for 3 hours (without added ALDH and NAD) produced approximately two- and threefold increases in SCE frequencies over the control values.

He and Lambert (1985) examined the SCE-inducing effect of acetaldehyde in human lymphocytes treated at different phases of the cell cycle. Exposure of cells to 4.4, 8.8, or 13.2 ug/ml resulted in a twofold increase of SCEs when acetaldehyde was added in the late G_1 phase of the cell cycle (23 hours post-mitogen stimulation) as compared to an earlier G_1-cell (at the time of mitogen stimulation) exposure. In addition, the authors demonstrated that the length of exposure affected SCE induction. For a 1-hour exposure, a 24-fold higher concentration (105.6 ug/ml) of acetaldehyde was required to elicit an SCE

response equivalent to that seen following a 70-hour exposure of the cells to 4.4 ug/ml.

7.4.3 Whole-Mammal Bone Marrow Cells

Obe et al. (1979) reported a significant ($p < 0.001$) increase in the SCE frequency of a single male mouse administered an intraperitoneal injection of acetaldehyde at either 0.01 (2.88 SCEs/cell) or 0.02 (6.40 SCEs/cell) mg/kg. However, the number of animals used was lower than the minimum of three animals/dose required for an *in vivo* study (Latt et al. 1981). Therefore, the positive results observed in this study are considered to be suggestive rather than definitive evidence of a genotoxic effect (Dellarco 1988).

Korte and Obe (1981) administered single intraperitoneal injections of acetaldehyde at 0.01, 0.1, or 0.5 mg/kg to groups of six or seven male and six or seven female Chinese hamsters. Doses equal to or greater than 0.6 mg/kg were lethal, and animals died within 30 minutes of injection. The SCE frequency was elevated only in the 0.5-mg/kg dose group (6.1 SCEs/cell) as compared with untreated controls (3.5 SCEs/cell, $p < 0.01$).

It is of note that the route of exposure in these two *in vivo* studies was intraperitoneal. It is uncertain, therefore, whether similar responses would be observed if a route relevant to human exposure (inhalation or oral) were used.

7.5 DNA Damage

Acetaldehyde-induced DNA damage was studied in DNA repair-deficient bacteria and in cultured mammalian cells.

7.5.1 Bacteria

Rosenkranz (1977) observed a weakly positive response in the *E. coli* pol A assay after exposure of the bacteria to 7938 ug/ml acetaldehyde in the absence of metabolic activation; the zone of inhibition for the DNA repair-deficient strain (pol A^-) was only slightly greater (12 mm) than that for the DNA repair-proficient (pol A^+) strain (8 mm). However, conditions may not have been ideal for the testing of a volatile material.

7.5.2 Mammalian Cell Culture

Results from *in vitro* alkaline elution assays showed that acetaldehyde did not produce detectable DNA damage in mouse lymphoma L5178Y/$TK^{+/-}$ cells exposed to 66 to 1936 ug/ml for 3 hours (Garberg et al. 1988); rat hepatocytes exposed to 1.3 to 132 ug/ml for 3 hours (Sina et al. 1983); human lymphocytes treated with 441 ug/ml for 4 hours (Lambert et al. 1985); or human bronchial epithelial cells receiving doses up to 44.1 ug/ml for 1 hour (Saladino et al. 1985). However, if acetaldehyde produces chromosomal aberrations and SCEs by DNA-DNA and/or DNA-protein cross-linking, it may not necessarily produce DNA strand breaks (Bradley

et al. 1979).

7.6 Cell Transformation

Eker and Sanner (1986) studied the potential of acetaldehyde to initiate transformation of the rat kidney cell line ($HRPT^4$) in a two-stage (initiation/promotion) cell transformation assay. The assay is based on anchorage-independent growth (i.e., transformed cells can grow in medium gelled with agar while "normal," anchorage-dependent cells cannot).

A 3-hour incubation with noncytotoxic concentrations of acetaldehyde up to 132 ug/ml was followed by continuous 6-day treatment with 0.1 ug/ml of the known tumor promoters: 12-O-tetradecanoyl-phorbol 13-acetate (TPA) or phorbol 12,13-didecanoate (PPD) or the non-tumor-promoting analogue, 4Á-phorbol 12,13-didecanoate (4Á-PPD). Cell viability was determined after 3 or 6 days; colonies growing in soft agar were scored after 14 days of incubation.

Exposure to acetaldehyde alone or TPA alone had no effect on cell survival; however, a significant increase (p value not provided) in viability was seen (10% above the control treated with solvent only) with the combined 4.4-ug/ml acetaldehyde/TPA-treatment. Viability was increased up to 70% above the control at the highest concentration (132 ug/ml acetaldehyde + TPA). Results further demonstrated that the promoter PPD was also effective in enhancing acetaldehyde-treated cell viability; however, the non-tumor-promoting phorbol analogue (4Á-PPD) had no effect. Transformation was confirmed by measuring colony growth in soft agar of the cells treated with 132 ug/ml acetaldehyde plus 0.1 ug/ml TPA. The number of colonies from acetaldehyde-treated cells (450 colonies) was approximately twofold higher than the control untreated cells (250 colonies). The results of this well-controlled but unconfirmed study indicate that acetaldehyde has the potential to act as an initiator of cell transformation.

7.7 Conclusions

The results of *in vitro* genotoxic assays indicate that acetaldehyde induces gene mutations in *Drosophila* and human cells but not in *Salmonella*. Positive gene mutation results were also reported in the nematode *Caenorhabditis*, but no dose-response was shown. Conflicting results were obtained for mitochondrial mutations in the yeast *Saccharomyces cerevisiae*. There is sufficient evidence indicating that acetaldehyde induces aneuploidy, micronuclei, chromosomal aberrations (breaks, gaps, and exchange-type aberrations), and sister chromatid exchanges in rat fibroblasts, CHO cells, and human lymphocytes. Chromosomal aberrations have also been detected in plants. Acetaldehyde has yielded negative results in tests for DNA strand breaks in cultured mammalian cells. *In vivo*, chromosome gaps and breaks were seen in rat embryos after a single intra-amniotic injection, and acetaldehyde-induced SCE have been detected in mice and hamster bone marrow cells after intraperitoneal injection. However, acetaldehyde did not increase the frequency of micronuclei in the early spermatids harvested from mice. Although unconfirmed, there is evidence from a well-controlled initiation/promoter cell transformation assay showing that acetaldehyde initiated transformation of rat kidney cells.

In summary, the available data indicate that acetaldehyde poses a mutagenic risk for somatic cells. Thus, acetaldehyde should be classified as genotoxic.

8.0 CARCINOGENICITY

8.1 Introduction

Only one epidemiologic study has investigated the carcinogenic potential of acetaldehyde. Major methodological limitations prevent the use of that study in determining acetaldehyde carcinogenicity. The determination of carcinogenicity rests on four studies using either rats or hamsters.

8.2 Epidemiological Study

Bittersohl (1974) conducted a morbidity survey to study the incidence of total cancer in an aldol and aliphatic aldehyde factory in the German Democratic Republic (GDR). The work force in this factory was potentially exposed to a product primarily consisting of acetaldol (70%) combined with smaller, but variable, amounts of acetaldehyde; butylaldehyde; crotonaldehyde; "large" condensed aldehydes such as hexatrial, hexatetral, and ethylhexal; traces of acrolein; and 20 to 22% water. The observation period extended from 1967 to 1972. The study cohort consisted of 220 people actively employed in the factory during the observation period; of these, approximately 150 were employed for more than 20 years. Air in the reduction process work site was sampled for various chemicals, including acetaldehyde. Acetaldehyde concentrations were found to range from 1 to 0.56 ppm; this level was far below the recommended GDR Maximum Allowable Concentration ("MAC value") of 55.6 ppm (100 mg/m^3) for this chemical.

Nine cases of cancer were identified in male workers during the 6-year study period. The distribution of cause-specific cancer was as follows: 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. An incidence rate of 6,000 per 100,000 population (9 cases/150 individuals employed for more than 20 years) for total cancer was calculated for this study cohort. In contrast, the incidence rate for cancer in the general population of the GDR during the same time period was 1,200 per 100,000. Analysis by latency showed that eight cases had an average latency period of 26 years (range $\pm$ 4 years), and one case (buccal cavity carcinoma) had a latency period of 13 years. Of the nine cases, five belonged to the 35- to 59-year age group, and the remaining four were over 65 years old. All had a history of smoking. One individual smoked 30 cigarettes per day and developed buccal cavity carcinoma with a latency period of 13 years; the remaining eight smoked between 5 and 10 cigarettes per day.

This study has the following major methodological limitations: the incidence rate was not age adjusted; concurrent exposure to other chemicals and cigarette smoke occurred; duration of exposure was short; a small number of subjects was studied; and information on subject selection, age, and sex distribution was lacking. Because of these limitations, IARC (1985) considered this study to be inadequate to evaluate the carcinogenicity of acetaldehyde.

8.3 Animal Studies

Four carcinogenicity studies with animals were found; acetaldehyde was administered by inhalation in all but part of one study, in which animals were dosed via intratracheal instillation. In the first two studies, one preliminary to the other and described together below, rats received acetaldehyde by inhalation alone. In the other two studies, hamsters received one or the other of two carcinogenic chemicals, either alone or simultaneously with acetaldehyde. In this test for the synergistic effects of acetaldehyde, the hamsters received each additional chemical via the noninhalation route previously found to produce malignancies. In part of one of these studies, hamsters received acetaldehyde by intratracheal instillation. Table 8-1 summarizes the experimental protocols and results of the animal studies.

8.3.1 Inhalation

Woutersen et al. (1984, 1986) exposed groups of 105 male and 105 female SPF-Wistar rats to atmospheres containing acetaldehyde concentrations of 0, 750, 1500, or 3000/1000 ppm (0, 1350, 2700, or 5400/1800 mg/m^3, respectively), 6 hours/day, 5 days/week for up to 28 months. The highest concentration was gradually decreased from 3000 ppm (days 0 to 141) to 1000 ppm (from day 313 forward) because of severe growth retardation, occasional loss of body weight, and early mortality.

Treatment-related nonneoplastic histopathological lesions observed in the nose, larynx, and lungs are summarized in Table 8-2. The most severe lesions were seen in the nose (degeneration, hyperplasia, and metaplasia) of animals in all test groups (except controls), and in the vocal cord region of the larynx (hyperplasia and squamous metaplasia) of several animals in the mid-dose (1500 ppm) and high-dose (3000 ppm) groups. Laryngeal lesions at the lower concentration (750 ppm) were comparable to controls.

The types and incidences of benign and malignant tumors of the respiratory tract are summarized in Table 8-3. Nasal tumors were mainly squamous cell carcinomas and adenocarcinomas originating from the respiratory and olfactory epithelium, respectively. The incidences of adenocarcinomas were significantly ($p < 0.01$) higher in both sexes of rats at all exposure concentrations when compared to controls; on the other hand, squamous cell carcinomas were significantly ($p < 0.01$) increased in males in the mid- and high-dose groups and in females in the high-dose group. No laryngeal or lung tumors were seen in male rats. In females, a carcinoma *in situ* was seen in the larynx in one animal in the mid-dose group, and a poorly differentiated adenocarcinoma of the lung was seen in one animal in the low-dose group. Tumors observed in the other organs of treated rats were comparable to those in the controls. The presence of nasal tumors at all exposure levels suggested that the latency period for nasal tumor induction was independent of the acetaldehyde concentration. The authors concluded that under the conditions of this study, acetaldehyde was carcinogenic to the nasal mucosa of rats.

In an extension of the above study, Woutersen and Feron (1987) examined the process of regeneration of damaged nasal mucosa. Rats exposed to acetaldehyde at concentrations described above for 52 weeks were sacrificed after a recovery

TABLE 8-1

SUMMARY OF CARCINOGENICITY STUDIES OF ACETALDEHYDE IN RATS AND HAMSTERS

Species/Strain	No./Sex/Group	Route of Exposure	Compound	Exposure Level	Duration of Exposure	Neoplastic and Nonneoplastic Lesions	Reference
Rat/Wistar	105/M 105/F	Inhalation	Acetaldehyde	0, 750, 1500, or 3000 decreasing to 1000 ppm	6 hr/day, 5 days/week for 28 months	Neoplasm: Squamous cell carcinomas and adenomas of the nose in males and females. Nonneoplastic lesions: Degeneration, hyperplasia, metaplasia, and squamous metaplasia in the nose and larynx in both sexes.	Woutersen et al. 1984, 1986
Hamster/Syrian golden	36/M 36/F	Inhalation	Acetaldehyde	0, 2500 ppm decreasing to 1650 ppm	7 hr/day, 5 days/week for 52 weeks	Neoplasm: Adenomas, adenocarcinomas, and anaplastic carcinomas of the nose and carcinomas in situ; squamous cell carcinomas and adeno-squamous carcinomas in the larynx of males and females. Nonneoplastic lesions: Rhinitis, hyperplasia, and metaplasia of the nasal, laryngeal, and tracheal epithelium in both sexes.	Feron et al. 1982
Hamster/Syrian golden	35/M 35/F	Intratracheal instillation	Saline Acetaldehyde B[a]P DENA Acetaldehyde + B[a]P	0.2 ml 4 or 8 ul 0.25% in 0.2 ml saline 0.25% in 0.2 ml saline 4 or 8 ul + 0.5% in 0.2 ml saline	1/week for 52 weeks	Neoplasm: Acetaldehyde alone did not induce respiratory tumors. Acetaldehyde neither enhanced nor inhibited the carcinogenicity of B[a]P or DENA. Noneoplastic lesions: Acetaldehyde alone induced peribronchiolar adenomatoid lesions.	Feron 1979

TABLE 8-1 (continued)

Species/Strain	Route of Exposure	No./Sex/Group	Compound	Level	Duration of Exposure	Neoplastic and Nonneoplastic Lesions	Reference
			Acetaldehyde + DENA	4 or 8 ul + 0.5% in 0.2 ml saline			
Hamster/Syrian golden	Inhalation + Intratracheal instillation	35/M + B[a]P	Acetaldehyde +	0 or 1500 ppm +week for 52 weeks	7 hr/day, 5 days/ respiratory tract tumors. A synergistic effect was	Neoplasm: Acetaldehyde alone did not induce seen in both benign and malignant tumor response in animals treated with acetaldehyde + the high dose (52 mg) of B[a]P. No synergistic effect of acetaldehyde was seen at lower doses. Nonneoplastic lesions: Hyper- and metaplastic lesions in the nasal, laryngeal, and tracheal epithelium.	Feron 1979
			B[a]P	0, 0.0625, 0.125, 0.25, 0.5, or 1.0 mg in 0.2 ml saline	1/week for 52 weeks		
Hamster/Syrian golden	Inhalation + Intratracheal instillation or Subcutaneous injection	30/M 30/F + B[a]P or DENA	Acetaldehyde +	0, 2,500-1,650 ppm week for 52 weeks	7 hr/day, 5 days/ laryngeal tumors. Combined treatment with high	Neoplasms: Acetaldehyde alone induced nasal and dose (36.4 mg) of B[a]P produced a synergistic effect in benign and malignant respiratory tract tumor response. No synergistic effects were seen with acetaldehyde + DENA treatment. Nonneoplastic lesions: Hyper- and metaplastic lesions in the nose and respiratory epithelium.	Feron et al. 1982
			B[a]P	0.35 or 0.70 mg in 0.2 ml saline	1/week for 52 weeks		
			DENA	0.625% in 0.2 ml saline	Once each week for a total of 17 injections		

TABLE 8-2

SUMMARY OF RESPIRATORY TRACT HYPERPLASTIC AND PRENEOPLASTIC LESIONS IN RATS EXPOSED TO ACETALDEHYDE BY INHALATION

Organ Examined and Site and Type of Lesion Observed	Incidence of Lesions in Rats Exposed to Acetaldehyde (ppm)							
	Males				Females			
	0	750	1500	3000	0	750	1500	3000
Nose	(49)[a]	(52)	(53)	(49)	(50)	(48)	(53)	(53)
Squamous metaplasia of respiratory epithelium								
Without keratinization	0	1	11[b]	1	0	3	14[b]	0
With keratinization	0	0	5	19[b]	0	1	16[b]	18[b]
Papillomatous hyperplasia with atypia and keratinization	0	0	0	2	0	0	0	6
Focal hyperplasia of respiratory epithelium	0	4	3	5	0	3	11[b]	2
Focal respiratory epithelial pseudoepitheliomatous hyperplasia	0	1	13[b]	3	0	0	20[b]	7
Focal olfactory epithelial squamous metaplasia								
Without hyperkeratosis	0	0	0	0	0	0	1	1
With hyperkeratosis	0	0	0	3	0	0	0	0
Focal basal cell hyperplasia of olfactory epithelium								
Without atypia	0	37[b]	9	0	0	42[b]	19[b]	0
With atypia	0	1	17[b]	0	0	0	5	0
Focal aggregates of (atypical) basal cells in the submucosa beneath the olfactory epithelium	0	0	23[b]	0	0	0	31[b]	2
Focal proliferation of glands in the loosely arranged submucosa beneath the olfactory epithelium	0	0	14[b]	5	0	4	18[b]	5[c]

TABLE 8-2 (continued)

Organ Examined and Site and Type of Lesion Observed	Incidence of Lesions in Rats Exposed to Acetaldehyde (ppm)							
	Males				Females			
	0	750	1500	3000	0	750	1500	3000
Larynx	(50)	(50)	(51)	(47)	(51)	(46)	(47)	(49)
Squamous metaplasia/hyperplasia								
Without hyperkeratosis	2	2	10[c]	9	1	0	6	9
With hyperkeratosis	1	4	13[b]	32[b]	0	3	17[b]	23[b]
Proliferation of dysplastic epithelium	0	0	1	0	0	1	4[c]	2
Lungs	(55)	(54)	(55)	(52)	(53)	(52)	(54)	(54)
Squamous metaplasia with hyperkeratosis of bronchial epithelium	0	0	0	1	0	0	0	0

[a]Numbers in parentheses represent numbers of animals examined.
[b]$p < 0.01$, according to Fisher's Exact test. All comparisons were made with the controls.
[c]$p < 0.05$, according to Fisher's Exact test. All comparisons were made with the controls.
All statistical analyses were done by the author.

SOURCE: Woutersen et al. 1986.

TABLE 8-3

SITES, TYPES, AND INCIDENCES OF RESPIRATORY TRACT TUMORS IN RATS EXPOSED TO ACETALDEHYDE BY INHALATION

Organ Examined and Site and Type of Lesion Observed	Incidence of Lesions in Rats Exposed to Acetaldehyde (ppm)							
	Males				Females			
	0	750	1500	3000	0	750	1500	3000
Nose	(49)[a]	(52)	(53)	(49)	(50)	(48)	(53)	(53)
Papilloma	0	0	0	0	0	1	0	0
Adenocarcinoma	0	16[b]	31[b]	21[b]	0	6[c]	26[b]	21[b]
Metastasizing adenocarcinoma	0	0	1	1	0	0	0	1
Carcinoma *in situ*	0	0	0	1	0	0	3	5
Squamous cell carcinoma	1	1	10[b]	15[b]	0	0	5	17[b]
Metastasizing squamous cell carcinoma	0	0	0	1	0	0	0	0
Larynx	(50)	(50)	(51)	(47)	(51)	(46)	(47)	(49)
Carcinoma *in situ*	0	0	0	0	0	0	1	0
Lungs	(55)	(54)	(55)	(52)	(53)	(52)	(54)	(54)
Poorly differentiated adenocarcinoma	0	0	0	0	0	1	0	0

[a]Numbers in parentheses represent numbers of animals examined.

[b]$p < 0.01$, according to Fisher's Exact test.

[c]$p < 0.05$, according to Fisher's Exact test.

SOURCE: Woutersen et al. 1986.

period of 26 weeks (10 males and 10 females) and 52 weeks (20 males and 20 females). During the recovery period, restoration of the olfactory epithelium was evident in the low-dose group (750 ppm), evident to a lesser degree in the mid-dose group (1500 ppm), and absent in rats in the high-dose group (3000/1000 ppm). During this period, the number of nasal tumors observed was almost the same as in the lifetime study, which indicated that proliferative epithelial lesions of the nose may develop into tumors even without continued acetaldehyde exposure. The authors concluded that the rat olfactory epithelium may regenerate after damage by acetaldehyde, provided that the mucosa is not completely devoid of basal cells and that Bowman's glands have not been totally destroyed.

Feron et al. (1982) exposed groups of 36 male and 36 female Syrian golden hamsters to room air (0 ppm) or to decreasing concentrations of acetaldehyde. The initial concentration was 2500 ppm (4500 mg/m^3), which was gradually decreased (between weeks 9 and 44) to 1650 ppm (2970 mg/m^3) 6 hours/day, 5 days/week for 52 weeks; the animals were sacrificed at 81 weeks. Acetaldehyde-induced nonneoplastic lesions were seen in the nose, larynx, and trachea. Nasal lesions consisted of rhinitis, thinning and degeneration of the layer of olfactory epithelium, hyperplasia and metaplasia of the respiratory epithelium, and thickening of the submucosa. Laryngeal and tracheal lesions characterized by slight to moderate focal hyperplasia and squamous metaplasia of the lining epithelium were seen in nearly all treated hamsters. Tumors were seen in both the nose (adenoma, adenocarcinoma, and anaplastic carcinoma) and the larynx (carcinoma in situ, squamous cell carcinoma, and adeno-squamous carcinoma, Table 8-4). The tumor incidences were 7 and 26% in males and 4 and 20% in females for the nose and larynx, respectively; only the increases in laryngeal tumors were statistically significant ($p < 0.05$) when compared with controls (see Table 8-4). In addition to tumors, hyperplasia and metaplasia with unequivocal nuclear cell atypia were seen in the larynx of males (17%) and females (15%); these lesions were not seen in control rats exposed to air. No nasal or respiratory tract tumors were seen in the control animals. Under the conditions of this study, acetaldehyde is considered to be carcinogenic in male and female hamsters.

These two studies (Woutersen et al. 1986; Feron et al. 1982) demonstrate that acetaldehyde is capable of inducing tumors in the nose and larynx of both hamsters and rats following chronic inhalation exposure. However, the localization of effects differs in these two species. In rats, the major tumor response occurred in the nose, and only one carcinoma was observed in the larynx. In contrast, the major tumor response in hamsters occurred in the larynx (10/43 developed laryngeal tumors), and only a few tumors (3/53) were found in the nose. This difference between the two species may be due to dissimilarities in their upper respiratory tract anatomy, breathing pattern (rats are obligatory nose-breathers, and hamsters may also breathe through the mouth), susceptibility of the epithelium to acetaldehyde, or a combination of these factors (Woutersen et al. 1984, 1986).

8.3.2 Inhalation + Intratracheal Instillation

In a second part of the above study (Feron 1979), groups of 35 male Syrian golden hamsters were exposed by inhalation to 0 or 1500 ppm (2700 mg/m^3) acetaldehyde vapor 7 hours/day, 5 days/week for 52 weeks. The animals also received a

TABLE 8-4

INCIDENCE OF RESPIRATORY TRACT TUMORS IN HAMSTERS EXPOSED TO EITHER AIR OR ACETALDEHYDE VAPOR

Site	Type of Tumor	Air		Acetaldehyde	
		Males	Females	Males	Female
Nose	Adenoma	0/24	0/23	1/27	0/26
	Adenocarcinoma	0/24	0/23	0/27	1/26
	Anaplastic carcinoma	0/24	0/23	1/27	0/26
Larynx	Polyp/papilloma	0/20	0/22	1/23	1/20
	Carcinoma *in situ*	0/20	0/22	3/23	0/20
	Squamous cell carcinoma	0/20	0/22	2/23	1/20
	Adeno-squamous carcinoma	0/20	0/22	0/23	2/20

SOURCE: Feron et al. 1982.

concurrent, weekly intratracheal instillation of 0, 0.0625, 0.125, 0.225, 0.5, or 1 mg benzo[a]pyrene (B[a]P) in saline for the same duration. Simultaneous exposure to acetaldehyde and B[a]P induced marked nonneoplastic lesions in the nasal cavity and trachea. However, after the 26-week recovery period, the lesions in the nasal cavity clearly diminished or completely disappeared. No treatment-related nonneoplastic lesions were seen in other areas of the respiratory tract or other organs. No respiratory tract tumors were seen in hamsters exposed to acetaldehyde alone. Various types of benign and malignant respiratory tract tumors were found in male hamsters treated with B[a]P or B[a]P plus acetaldehyde (Table 8-5). Combined treatment of acetaldehyde plus the high dose (52 mg) of B[a]P resulted in an increased tumor response; twice as many squamous cell carcinomas of the trachea (24/30 versus 11/28, $p = 0.002$) and bronchi (8/30 versus 4/28, $p = 0.20$) were seen as compared with those seen at the same dose of B[a]P without acetaldehyde. In addition, simultaneous exposure (at the high dose) also resulted in a distinct shortening of the latency period (28 weeks versus 50 weeks) for the induction of respiratory tract tumors as compared with those exposed to air plus B[a]P. This effect of acetaldehyde was not noticeable at any of the lower B[a]P levels. The results of this study indicate no evidence for carcinogenicity of acetaldehyde and limited evidence of co-carcinogenicity. This study had the following methodological limitations: only male hamsters were used, the duration of exposure was only 1 year, the study was terminated at 78 weeks, and only one exposure level of acetaldehyde was used. This single exposure level did exceed the maximum tolerated dose (MTD), since increased mortality and decreased body weight gain were noted (as described in Chapter 5.0, Chronic Toxicity). Moreover, cessation of treatment at 52 weeks might have caused regression in metaplastic lesions.

In an extension of the above study, Feron et al. (1982) exposed male and female hamsters to air or high concentrations (2500-1650 ppm, 4500-2970 mg/m^3) of acetaldehyde vapor 7 hours/day, 5 days/week for 52 weeks, and simultaneously treated the animals either with weekly intratracheal instillations of 0.35 or 0.70 mg B[a]P in saline for 2 weeks or with subcutaneous injections of 0.0625% DENA once every 3 weeks (total dose 2.1 ul/hamster) (Table 8-6). Following a 29-week recovery period, all hamsters were sacrificed (81 weeks). At the end of the exposure period, acetaldehyde-exposed animals displayed distinct nonneoplastic histopathological nasal changes similar to those observed in previous studies (e.g., thinning and degeneration of the layer of olfactory epithelium; hyperplasia and metaplasia of the respiratory epithelium). Table 8-7 shows the incidence of respiratory tract tumors in hamsters exposed to the various treatment regimens. Combined treatment of acetaldehyde and the high dose (36.4 mg) of B[a]P caused significant ($p < 0.05$) increases in the incidence of carcinomas in the trachea and bronchi of male hamsters compared with those treated with the same dose of B[a]P but exposed to air only (Table 8-8). The latency period of the tracheobronchial tumors was much shorter after combined exposure than after treatment with B[a]P alone. The enhancing effect of B[a]P-initiated respiratory tract tumor formation observed in this study was similar to that observed in the previous study (Feron 1979), in which inhalation at a lower concentration (1500 ppm) of acetaldehyde and intratracheal instillation of 52 mg of B[a]P produced a slight enhancing effect. There was no evidence that acetaldehyde exposure increased the incidence or affected the type of DENA-induced tumors in any part of the respiratory tract (see Tables 8-7 and 8-8). Based upon these findings, the authors concluded: "acetaldehyde is an irritant

TABLE 8-5

TYPES AND INCIDENCES OF RESPIRATORY TRACT TUMORS IN MALE HAMSTERS AFTER 52 WEEKLY INTRATRACHEAL INSTILLATIONS OF B[a]P AND EXPOSURE TO AIR OR ACETALDEHYDE VAPOR

	Incidence of Tumors											
	Air and B[a]P (mg)						1500 ppm Acetaldehyde and B[a]P (mg)					
Site and Type of Tumor	0	3.25	6.5	13	26	52	0	3.25	6.5	13	26	52
Animals killed after 52 weeks												
Number of animals examined	5	5	5	5	5	5	5	5	5	5	5	5
Number of animals with tumors	0	0	0	0	2	4	0	0	0	0	1	5
Total number of tumors	0	0	0	0	3	6	0	0	0	0	2	12
Trachea												
Papilloma	0	0	0	0	2	0	0	0	0	0	0	1
Squamous cell carcinoma	0	0	0	0	0	1	0	0	0	0	0	4
Bronchi												
Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	1	1
Squamous adenocarcinoma	0	0	0	0	0	0	0	0	0	0	0	1
Anaplastic carcinoma	0	0	0	0	0	1	0	0	0	0	0	0
Bronchioli and alveoli												
Adenoma	0	0	0	0	1	3	0	0	0	0	1	4
Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	0	1
Anaplastic carcinoma	0	0	0	0	0	1	0	0	0	0	0	0
Animals that died spontaneously or were killed after 78 weeks or when moribund												
Number of animals examined[a]	29	30	30	30	29	28	29	28	29	29	29	30
Number of animals with tumors	0	3	4	9	25	26	0	1	5	8	16	29
Total number of tumors	0	4	5	12	44	58	0	1	7	10	26	63
Larynx												
Papilloma	0	0	0	0	0	0	0	0	0	1	0	0
Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	0	0	1
Adenocarcinoma	0	0	0	0	0	0	0	0	0	1	0	0
Trachea												
Polyp	0	0	0	0	2	0	0	0	0	1	0	0
Papilloma	0	3	1	5	9	6	0	0	4	3	6	3
Squamous cell carcinoma	0	0	0	0	5	11	0	0	0	0	4	24[b]
Squamous adenocarcinoma	0	0	0	0	1	1	0	0	0	0	1	0
Adenocarcinoma	0	0	0	0	0	0	0	0	0	0	0	1
Fibrosarcoma	0	0	0	0	1	0	0	0	0	0	0	0
Bronchi												
Polyp	0	0	0	0	2	1	0	0	0	0	1	1
Papilloma	0	0	0	0	1	2	0	0	0	0	0	0
Squamous cell carcinoma	0	0	0	0	2	4	0	0	0	0	2	8
Squamous adenocarcinoma	0	0	0	0	1	2	0	0	0	0	1	3

TABLE 8-5 (continued)

Site and type of tumor	Incidence of Tumors											
	Air and B[a]P (mg)						1500 ppm Acetaldehyde and B[a]P (mg)					
	0	3.25	6.5	13	26	52	0	3.25	6.5	13	26	52
Adenocarcinoma	0	0	0	0	0	4	0	0	0	0	0	0
Anaplastic carcinoma	0	0	0	0	0	1	0	0	0	0	0	0
Bronchioli and alveoli												
Adenoma	0	1	4	7	17	16	0	1	3	4	9	16
Squamous cell carcinoma	0	0	0	0	2	4	0	0	0	0	0	2
Squamous adenocarcinoma	0	0	0	0	0	3	0	0	0	0	1	2
Adenocarcinoma	0	0	0	0	1	1	0	0	0	0	1	2
Anaplastic carcinoma	0	0	0	0	0	2	0	0	0	0	0	0

[a]A few animals were lost through cannibalism or autolysis.

[b]$p = 0.002$, according to Fisher's Exact test.

SOURCE: Feron 1979.

TABLE 8-6

TREATMENT PROTOCOL FOR HAMSTERS EXPOSED TO EITHER AIR OR ACETALDEHYDE VAPOR

	Number and Sex of Hamsters Exposed via Inhalation to Acetaldehyde or Air		Dosage and Route of Additional Treatments to Hamsters		
Group	Air (0 ppm)	Acetaldehyde (2500-1650 ppm)	Treatment	Dosage	Route
1	18 males 18 females	18 males 18 females	None	--[a]	--
2	18 males 18 females	18 males 18 females	Saline	0.2 ml/week	Intratracheal instillation
3	30 males 30 females	30 males 30 females	B[a]P	0.35 mg/week Total 18.2 mg	Intratracheal instillation
4	30 males 30 females	30 males 30 females	B[a]P	0.70 mg/week Total 36.4 mg	Intratracheal instillation
5	30 males 30 females	30 males 30 females	DENA (0.0625%)	0.2 ml every 3 weeks	Subcutaneous injection

[a]No information was provided.

SOURCE: Feron et al. (1982).

TABLE 8-7

SITES AND INCIDENCES OF RESPIRATORY TRACT TUMORS IN HAMSTERS EXPOSED TO AIR OR ACETALDEHYDE VAPOR AND TREATED INTRATRACHEALLY WITH B[a]P OR SUBCUTANEOUSLY WITH DENA[a]

Treatment					Number of animals with tumors of the						Total Number of Respiratory Tract Tumors
Inhalation	Intratracheal Instillation	Subcutaneous Injection		Number of Animals Examined[b]	Respiratory Tract (total)	Nose	Larynx	Trachea	Bronchi	Lungs	
				Males							
Air	--[c]	--	15								
Air	0.9% NaCl[d]	--	15†	30[e]	0(0%)	0	0	0	0	0	0
Air	B[a]P(18.2 mg)[f]	--		29	4(14%)	0	0	2	1	2	5
Air	B[a]P(36.4 mg)[g]	--		30	19(63%)	0	1	8	3	13[h]	27
Air	--	DENA[i]		29	12(41%)	2	7	3	3	0	15
Acetaldehyde	--	--	15								
Acetaldehyde	0.9% NaCl[d]	--	14*	29[e]	7(24%)[j]	2	6[j]	0	0	0	8
Acetaldehyde	B[a]P(18.2 mg)[f]	--		29	12(41%)[k]	2	8[j]	3	1	1	15
Acetaldehyde	B[a]P(36.4 mg)[g]	--		27	22(81%)	1	9[j]	14	5	3[j]	32
Acetaldehyde	--	DENA[i]		30	11(37%)	3	10	2	0	0	15
				Females							
Air	--	--	14								
Air	0.9% NaCl[d]	--	14*	28[e]	0(0%)	0	0	0	0	0	0
Air	B[a]P(18.2 mg)[f]			27	3(11%)	0	1	0	1	1	3
Air	B[a]P(36.4 mg)[g]	--		24	7(29%)	0	0	3	1	5	9
Air	--	DENA[i]		27	11(41%)	0	3	8	2	0	13

TABLE 8-7 (continued)

Treatment					Number of animals with tumors of the						Total Number of Respiratory Tract Tumors
Inhalation	Intratracheal Instillation	Subcutaneous Injection		Number of Animals Examined[b]	Respiratory Tract (total)	Nose	Larynx	Trachea	Bronchi	Lungs	
Acetaldehyde	--	--	15								
Acetaldehyde	0.9% NaCl[d]	--	14†	29[e]	5(17%)	1	4	0	0	0	5
Acetaldehyde	B[a]P(18.2 mg)[f]	--		29	11(38%)	1	7[k]	4[k]	0	1	13
Acetaldehyde	B[a]P(36.4 mg)[g]	--		29	16(55%)	0	4	10	2	3	19
Acetaldehyde	--	DENA[i]		28	8(29%)	2	7	0[k]	0	0	9

[a]See Table 8-10 for types of tumors.

[b]A few animals were lost through cannibalism or autolysis.

[c]No further treatment.

[d]0.2 ml weekly for 52 weeks.

[e]Animals killed at the end of the treatment period are not included in this table.

[f]0.35 mg weekly for 52 weeks.

[g]0.70 mg 52 weekly for 52 weeks.

[h]Two animals had more than one type of pulmonary tumor.

[i]0.125 ul given subcutaneously every 3 weeks for a total of 17 injections.

[j]p _0.01, according to the Chi-square test. All statistical analyses were done by the authors.

[k]p _0.05, according to the Chi-square test. The various groups of acetaldehyde-exposed animals were compared with the corresponding groups of air-exposed controls.

SOURCE: Feron et al. 1982.

TABLE 8-8

SITES, TYPES, AND INCIDENCES OF RESPIRATORY TRACT TUMORS IN HAMSTERS EXPOSED TO AIR OR ACETALDEHYDE VAPOR AND TREATED INTRATRACHEALLY WITH B[a]P OR SUBCUTANEOUSLY WITH DENA[a]

	Incidence of Tumors							
	Inhalation of Air				Inhalation of Acetaldehyde			
Site and Type of Tumor	0.9% NaCl[b,c]	B[a]P (18.2 mg)[d]	B[a]P (36.4 mg)[c]	DENA	0.9% NaCl[b,c]	B[a]P (18.2 mg)[d]	B[a]P (36.4 mg)[c]	DENA[e]
				Males				
Larynx	(20)[f]	(28)	(29)	(28)	(23)	(26)	(25)	(30)
Polyp/papilloma	0	0	1	7	1	1	1	6
Carcinoma *in situ*	0	0	0	0	3	3	1	3
Squamous cell carcinoma	0	0	0	0	2	6[g]	5[h]	1
Trachea	(30)	(29)	(29)	(29)	(28)	(28)	(27)	(30)
Polyp/papilloma	0	2	5	3	0	2	2	2
Squamous cell carcinoma	0	0	1	0	0	1	7[h]	0
Adenocarcinoma	0	0	0	0	0	0	3	0
Anaplastic carcinoma	0	0	1	0	0	0	0	0
Sarcoma	0	0	1	0	0	0	2	0
Bronchi	(30)	(29)	(30)	(29)	(28)	(29)	(27)	(30)
Polyp/papilloma	0	1	2	3	0	1	0	0
Squamous cell carcinoma	0	0	0	0	0	0	5[h]	0
Adenocarcinoma	0	0	1	0	0	0	0	0
				Females				
Larynx	(22)	(27)	(24)	(27)	(20)	(23)	(23)	(22)
Polyp/papilloma	0	1	0	3	1	2	1	1
Carcinoma *in situ*	0	0	0	0	0	0	2	3[h]
Squamous cell carcinoma	0	0	0	0	1	5[h]	1	3[h]
Adeno-squamous carcinoma	0	0	0	0	2	0	0	0

TABLE 8-8 (continued)

Site and Type of Tumor	Incidence of Tumors							
	Inhalation of Air				Inhalation of Acetaldehyde			
	0.9% NaCl[b,c]	B[a]P (18.2 mg)[d]	B[a]P (36.4 mg)[c]	DENA	0.9% NaCl[b,c]	B[a]P (18.2 mg)[d]	B[a]P (36.4 mg)[c]	DENA[e]
Trachea	(28)	(27)	(24)	(27)	(28)	(29)	(28)	(28)
Polyp/papilloma	0	0	1	8	0	3	1	0[g]
Squamous cell carcinoma	0	0	2	0	0	1	8	0
Anaplastic carcinoma	0	0	0	0	0	0	1	0
Bronchi	(28)	(27)	(24)	(27)	(29)	(29)	(29)	(28)
Papilloma	0	1	0	2	0	0	0	0
Adenocarcinoma	0	0	1	0	0	0	1	0
Adeno-squamous carcinoma	0	0	0	0	0	0	1	0

[a]Numbers of animals examined are given in parentheses. Animals killed at the end of the treatment period are not included in this table.

[b]No further treatment.

[c]Given intratracheally (0.2 mL), weekly during 52 weeks.

[d]Given intratracheally in 52 weekly doses of 0.35 mg.

[e]Given subcutaneously in 17 doses (once every 3 weeks) of 0.125 L.

[f]Given intratracheally in 52 weekly doses of 0.70 mg.

[g]$p < 0.01$, according to the chi-square test. All statistical analyses were done by the authors.

[h]$p < 0.05$, according to the chi-square test. The various groups of acetaldehyde-exposed animals were compared with the corresponding groups of air-exposed controls.

SOURCE: Feron et al. 1982.

as well as a carcinogen to the nose and larynx with a weak initiating and a strong 'promoting' (co-carcinogenic) activity."

8.3.3 Intratracheal Instillation

Feron (1979) gave 7 groups (35 males, 35 females) of Syrian golden hamsters weekly or biweekly intratracheal instillations of saline, acetaldehyde, B[a]P, B[a]P + acetaldehyde, diethylnitrosamine (DENA), or DENA + acetaldehyde for 52 weeks. The treatment schedule and doses are presented in Table 8-9, and the incidences of respiratory tract tumors are presented in Table 8-10. Intratracheal instillation of acetaldehyde alone did not induce any respiratory tract tumors but did produce extensive, irreversible, peribronchiolar adenomatoid lesions often accompanied by inflammatory changes. These lesions were not assumed to be preneoplastic because transition of the proliferated bronchioalveolar epithelium into a neoplastic growth was not observed. Acetaldehyde neither enhanced nor inhibited the carcinogenicity of B[a]P or DENA. Both B[a]P and DENA induced a variety of benign and malignant respiratory tract tumors. Under the conditions of the study (i.e., intratracheal instillation at the concentrations used), acetaldehyde did not act as either a primary carcinogen or a promoter.

8.4 Carcinogenicity of Ethanol

An indirect source of acetaldehyde in the body results following the ingestion of ethanol in alcoholic beverages, a voluntary exposure. Ethanol is rapidly metabolized to acetaldehyde and then to acetate. IARC has found that there is sufficient evidence for the carcinogenicity of alcoholic beverages in humans (IARC, 1988). The organs for which there is clear evidence of carcinogenicity from alcoholic beverages include the oral cavity and pharynx (excluding the nasopharynx), the larynx, the esophagus, and the liver. Whether the cancer is due to chronic irritation by ethanol, the action of the metabolic product acetaldehyde, or possibly even to another chemical present in all alcoholic beverages is not clear. After ingestion of ethanol the alveolar air of humans contains levels of acetaldehyde proportional to the amount of ethanol consumed, which would provide a periodic exposure of the respiratory tract to acetaldehyde. For example, consumption of 18 milliliters of ethanol, approximately the amount in one 12 ounce can of beer, resulted in an alveolar air concentration of 0.100 μg/100 ml (1 mg/m^3 or approximately 556 ppb acetaldehyde) (Freund and O'Halloren, 1965; Freund, 1967; USEPA, 1987). (In the USEPA document the alveolar concentrations of acetaldehyde are shown as μg/100 ml. In the original paper the concentrations are mμg/100 ml, a factor of 1000 lower. Our estimates are based on the figures in the original papers.) Higher intakes led to proportionally higher concentrations of acetaldehyde in alveolar air. At the highest intake tested, 110 ml ethanol, approximately the amount of ethanol in six 12 ounce cans of beer, an alveolar air concentration of 0.53-0.56 μg/100 ml alveolar air (approximately 3 ppm) persisted for 4 to 5 hours. The upper portions of the

TABLE 8-9

TREATMENT OF HAMSTERS IN THE VARIOUS GROUPS
USED IN THE INTRATRACHEAL INSTILLATION STUDY[a]

Group Number	Type of Intratracheal Instillation[b]
1	Weekly: 0.2 ml 0.9% NaCl solution
2	Weekly: 0.2 ml 2% acetaldehyde in 0.9% NaCl solution
3	Weekly: 0.2 ml 4% acetaldehyde in 0.9% NaCl solution
4	Biweekly: 0.2 ml 0.25% B[a]P in 0.9% NaCl solution
5	Weekly: one week 0.2 ml 2% acetaldehyde in 0.9% NaCl solution, and the other week 0.1 ml 4% acetaldehyde in 0.9% NaCl solution and 0.1 ml 0.5% B[a]P in 0.9% NaCl solution
6	Biweekly: 0.2 ml 0.25% diethylnitrosamine (DENA) in 0.9% NaCl solution
7	Weekly: one week 0.2 mL 2% acetaldehyde in 0.9% NaCl solution, and the other week 0.1 ml 4% acetaldehyde in 0.9% NaCl solution and 0.1 ml 0.5% DENA in 0.9% NaCl solution

[a]Each group initially consisted of 35 males and 35 females.
[b]The intratracheal instillations were carried out during a period of 52 weeks.

SOURCE: Feron 1979.

TABLE 8-10

TYPES AND INCIDENCES OF RESPIRATORY TRACT TUMORS IN HAMSTERS GIVEN INTRATRACHEAL INSTILLATIONS OF 0.9% NaCl SOLUTION, ACETALDEHYDE, B[a]P, B[a]P + ACETALDEHYDE, DENA, OR DENA + ACETALDEHYDE

Site and Type of Tumor	Incidence of Tumors													
	0.9% NaCl		4 ul Acet-aldehyde		8 ul Acet-aldehyde		0.25% B[a]P		0.5% B[a]P+ 4 ul Acet-aldehyde		0.25% DENA		0.5% DENA+ 4 ul Acet-aldehyde	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Animals killed after 13 weeks														
Number of animals examined	3	2	3	3	3	3	3	3	3	3	3	3	3	3
Trachea														
Papilloma	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Lungs														
Adenoma	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Animals killed after 26 weeks														
Number of animals examined	2	2	3	3	3	3	3	3	3	3	3	3	3	3
Trachea														
Papilloma	0	0	0	0	0	0	0	0	0	0	2	1	2	2
Bronchi														
Polyp	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Animals killed after 52 weeks														
Number of animals examined	3	2	3	3	3	3	3	3	3	3	3	3	3	3
Larynx														
Papilloma	0	0	0	0	0	0	0	0	0	0	1	1	0	3
Trachea														
Papilloma	0	0	0	0	0	0	0	0	1	0	3	3	3	3
Lungs														
Adenoma	0	0	0	0	0	0	0	0	0	0	2	2	3	3

TABLE 8-10 (continued)

Site and Type of Tumor	Incidence of Tumors													
	0.9% NaCl		4 ul Acet-aldehyde		8 ul Acet-aldehyde		0.25% B(a)P		0.5% B[a]P+ 4 ul Acet-aldehyde		0.25% DENA		0.5% DENA+ 4 ul Acet-aldehyde	
	M	F	M	F	M	F	M	F	M	F	M	F	M	F
Animals that died spontaneously or were killed at the end of the experimental period or when moribund														
Number of animals examined[a]	24	25	24	25	25	23	23	25	23	23	24	25	23	24
Larynx														
Papilloma	0	0	0	0	0	0	0	0	1	1	10	2	7	7
Carcinoma	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Trachea														
Polyp	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Papilloma	0	1	0	0	0	0	6	8	6	10	23	21	22	20
Squamous cell carcinoma	0	0	0	0	0	0	3	3	7	3	0	0	0	0
Anaplastic carcinoma	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Bronchi														
Polyp	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Papilloma	0	0	0	0	0	0	0	0	1	0	0	1	1	0
Squamous cell carcinoma	0	0	0	0	0	0	1	0	1	0	0	0	0	0
Lungs														
Adenoma	0	0	0	0	0	1	7	6	2	1	17	21	21	23
Adenocarcinoma	0	0	0	0	0	0	0	1	1	0	0	1	1	0
Squamous cell carcinoma	0	0	0	0	0	0	0	0	0	1	0	0	0	0

[a]A few animals were lost through autolysis or cannibalism.

SOURCE: Feron 1979.

respiratory tract would be exposed during exhalation. It is interesting that tumors of the larynx were observed in hamsters after acetaldehyde exposure and that tumors of the larynx are significantly increased in humans after ingestion of alcoholic beverages.

8.5 Conclusions

The only epidemiological study involving acetaldehyde exposure showed an increased incidence of total cancer among workers in an aldehyde factory in the German Democratic Republic between 1967 and 1972. However, this morbidity study had a number of limitations: concurrent exposure to other chemicals and cigarette smoke; short duration; small number of subjects; lack of information on subject selection, age, and sex distribution; and failure to age-adjust the incidence rate. Therefore, this study does not provide the evidence needed to evaluate the carcinogenicity of acetaldehyde.

Inhalation studies in rats and hamsters provide positive evidence for the carcinogenicity of acetaldehyde. The most severe nonneoplastic lesions induced by inhalation were in the nose (degeneration, hyperplasia, and metaplasia) and in the larynx (vocal cord region, predominantly hyperplasia and metaplasia). Following chronic inhalation exposure, these lesions evidently became neoplastic. Acetaldehyde induced predominantly nasal tumors in rats, mainly squamous cell carcinomas and adenocarcinomas originating in the respiratory and olfactory epithelium, respectively. In hamsters, acetaldehyde-induced tumors were found predominantly in the larynx. These effects indicate species differences in the sensitivity and response to acetaldehyde vapors.

In hamsters, inhalation of acetaldehyde potentiated the carcinogenicity of high doses of B[a]P administered by intratracheal instillation, causing a twofold increase in the number of squamous cell carcinomas of the trachea and bronchi as compared with the incidence in hamsters administered B[a]P alone. Moreover, the latency period for tumor induction decreased from 50 to 28 weeks as compared with that in hamsters exposed to air or B[a]P alone. There was no evidence that acetaldehyde exposure increased the incidence or affected the type of DENA-induced tumors in any part of the respiratory tract of hamsters.

Following intratracheal instillation, acetaldehyde did not produce respiratory tract tumors in hamsters. When given simultaneously with the respiratory carcinogens B[a]P or DENA, no potentiation of the carcinogenic effect of B[a]P or DENA in the respiratory tract of hamsters was found. In some combinations acetaldehyde did appear to exert a protective effect against some doses of other carcinogens in at least some parts of the hamster's respiratory tract, but the effects were not consistent enough to be interpretable.

In Tables 8-5, 8-8, and 8-10, acetaldehyde appears to exert a protective effect against cancer induction by known carcinogens at some anatomical sites. The strongest effect, which is statistically significant, is seen in Table 8-8 where 8 polyps/papillomas of the trachea are induced by diethylnitrosamine (DENA) in hamsters exposed to air, while none are induced by DENA in hamsters exposed to airborne acetaldehyde. Also in Table 8-10 acetaldehyde appears to protect against lung adenomas induced by benzo(a)pyrene. Yet, in the same table slightly

more adenomas are induced by DENA in the presence of acetaldehyde than in its absence. Some protective effects could be due to statistical variation while others could be real. For example, acetaldehyde is an irritant and increased mucus production caused by irritation might counteract the deleterious effect of a second chemical. However, establishment of such protective effects requires extensive experimentation, and, even if such protective effects are demonstrable, acetaldehyde alone causes respiratory tract tumors in both rats and hamsters (see above).

The International Agency for Research on Cancer (IARC) concluded that there is inadequate evidence in humans and sufficient evidence in experimental animals for the carcinogenicity of acetaldehyde (IARC 1985). Therefore IARC classified acetaldehyde as class 2B, a possible human carcinogen. The U.S. Environmental Protection Agency (U.S. EPA), using the guidelines for Carcinogen Risk Assessment, has classified acetaldehyde as a Group B2 probable human carcinogen, based on sufficient evidence of carcinogenicity in animals and inadequate evidence in humans (IRIS 1989, 1991) (Table 8-11). OEHHA staff concur that acetaldehyde is a potential human carcinogen. Details of the IARC and USEPA classification schemes, taken from IARC (1987) and USEPA (1986) respectively, are given in Appendix C.

TABLE 8-11

CLASSIFICATION OF ACETALDEHYDE'S CARCINOGENICITY

Organization	IARC	USEPA
Human evidence	inadequate	inadequate
Animal evidence	sufficient	sufficient
Class	2B	B2
Classification	possible	probable
Date	1985, 1987	1987

9.0 QUANTITATIVE RISK ANALYSIS

9.1. Noncarcinogenic risks

The United States Environmental Protection Agency (USEPA) has determined a Reference Concentration (RfC) for acetaldehyde of 0.009 mg/m^3 (5 ppb). The Reference Concentration of a chemical is an estimate, with an uncertainty spanning perhaps an order of magnitude, of a daily exposure to the human population, including sensitive subgroups, that is likely to be without appreciable risk of deleterious effects during a lifetime of exposure. The Reference Concentration is derived from a no (NOAEL) or lowest (LOAEL) observed adverse effect level in human or animal exposure, to which uncertainty or "safety" factors are applied. For acetaldehyde a NOAEL of 150 ppm (273 mg/m^3) for degeneration of the olfactory epithelium of rats was obtained from the studies of Appleman et al. (1982) and Appleman et al. (1986). 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/m^3, then an uncertainty factor of 1000 was applied. This factor was the product of factors of 10 to account for the varying sensitivity to chemicals in the human population, 10 to account for subchronic (26 weeks) to chronic extrapolation, and 10 to account both for uncertainty in the interspecies extrapolation using dosimetric adjustments and for the incompleteness in the database. At 0.004 mg/m^3 (2 ppb), the annual average ambient air level of acetaldehyde estimated in part A of this document by the Air Resources Board, noncarcinogenic effects are not expected to occur (see Chapters 3, 4 and 5) since it is one-half of the RfC. However, "hot spots" of acetaldehyde exposure have not been evaluated.

9.2. Carcinogenic risks

The USEPA draft Health Assessment Document for acetaldehyde (USEPA 1987) includes a quantitative risk assessment for cancer based on animal studies and a risk assessment for acetaldehyde is listed in its Integrated Risk Information System (IRIS) database (USEPA, 1991). Office of Environmental Health Hazard Assessment (OEHHA) staff have consulted the USEPA risk assessment in the preparation of this report.

OEHHA staff use the most sensitive sex, site and species for risk assessment (CDHS, 1985), unless other data appear to be more appropriate. In this case, OEHHA staff have used the rat nasal tumor data from the Woutersen et at. (1986) inhalation study (Table 8-3) and hamster laryngeal tumor data from the Feron et al. (1982) inhalation study (Table 8-4) to assess the cancer potency with the multistage model. Cancer risk at ambient levels was estimated by extrapolating downward 5 orders of magnitude from these data by means of the best fitting linearized multistage model. This model provides a reasonably health-protective risk estimate due in part to its property of furnishing a linear extrapolation of the 95% UCL on risk at low doses (CDHS, 1985; Howe et al., 1986).

Tumors in animals have occurred in 2 sites. There is no known site concurrence among species for carcinogens although some carcinogens do result in the same tumors in man and animals (e.g., induction of liver angiosarcomas by vinyl chloride). For acetaldehyde tumors occurred in the nasal area for

rats and in the larynx for hamsters. While it is assumed that the respiratory tract is the only organ affected by acetaldehyde, tumors in the nose in rats and in the larynx of hamsters do not directly mean that only tumors in the nose or larynx would occur in humans. In the case of formaldehyde (OEHHA, 1992), 98-99% of inhaled material is absorbed in the nasal passages in rodents, but much less is absorbed there in monkeys (Casanova et al., 1991) and also presumably in humans. By analogy much acetaldehyde should not be absorbed in the human nasal passages. In addition, humans are not obligate nose breathers. Thus, the entire human respiratory tract may be at risk for cancer induction by acetaldehyde.

9.2.1. Thresholds

A threshold dose of a toxic substance is one below which a specified outcome does not occur. While some threshold models for carcinogenesis have been proposed (based on, for example, saturation of detoxification enzymes, the existence of DNA repair mechanisms, or recurrent toxicity), none has been convincingly demonstrated.

An "epigenetic" mechanism that could result in a threshold has been invoked to explain the carcinogenic action of substances that do not directly produce genetic damage in short-term tests. At high concenntrations toxicants can kill cells. Indeed, in the case of acetaldehyde high concentrations lead to significant cell killing ("degeneration") in the respiratory epithelium (Wouterson et al., 1986) However, for acetaldehyde there is compelling evidence of genotoxicity because of binding to DNA and mutagenicity (Chapter 7). There is also experimental evidence for acetaldehyde acting as an initiator of tumorigenesis (Chapter 8). Therefore, OEHHA staff considers acetaldehyde-induced carcinogenesis as a genotoxic event and staff were unable to determine a threshold for the phenomenon.

9.2.2. Rat Nasal Carcinomas and the Multistage Model.

The data used to calculate cancer risk from the male and female rat nasal carcinomas observed in the Woutersen et al. (1986) inhalation study are given in Table 9.1 (USEPA, 1987). Three types of nasal tumors were observed: squamous cell carcinomas, adenocarcinomas, and carcinomas *in situ*. (Note that the tumors were determined after 28 months of exposure which is longer than the 24 months often used in lifetime experiments in rats.) The denominators are the same as those in Table 8-3 which are the numbers of animals examined for nasal changes. USEPA's risk assessment considered all 55 animals in the experimental groups to be at risk, whereas OEHHA staff used only the 49-53 animals of each group that were examined for nasal changes. Doses were converted to an equivalent continuous dose (USEPA 1987, 1991), because the animals were exposed for only 6 hours/day, 5 days/week. Because of the excessive morbidity (labored respiration, mouth breathing, excessive salivation, and blood around the external nostrils) encountered by Wouterson et al. with the animals exposed at the highest concentration of 3000 ppm, the concentration was eventually lowered to 1000 ppm, less than the middle concentration of 1500 ppm. Since the impact of this highly variable exposure is difficult to determine, it was not used in the running of the model.

TABLE 9-1

TUMOR INCIDENCE IN MALE AND FEMALE RATS
AFTER 28 MONTHS OF ACETALDEHYDE INHALATION

	Exposure (ppm)			Nasal Tumor
	Nominal	Measured	Continuous	Incidence
Males	0			1/49
	750	727	129.8	17/52
	1500	1438	256.8	41/53[a]
Females	0			0/50
	750	727	129.8	6/48[b]
	1500	1438	256.8	36/53

[a] The 1 metastasizing adenocarcinoma in Table 8-3 is considered to be one of the 31 total adenocarcinomas.
[b] Does not include the benign papilloma in Table 8-3.

Using the computer program GLOBAL86 (Howe et al., 1986), a linearized, time-independent multistage model was fit to the nasal carcinoma dose-response data. This multistage model may be expressed as:

$$P(d) = 1 - e^{-(q_0 + q_1 d + q_2 d^2 + .. + q_k d^k)}$$

where P(d) is the lifetime probability of cancer for a given dose d of carcinogen, q_0 is a constant that accounts for the background incidence of cancer occurring in the absence of the carcinogen, and q_1, q_2, ... q_k are coefficients that allow the data to be expressed to various powers of the dose of carcinogen to obtain the best fit of the model to the data.

The male rat nasal tumor data yielded a maximum likelihood estimate (MLE) for q_1 (the linear or slope term, which relates the probability of cancer to the first power of the dose of carcinogen administered in the equation for the multistage model) of 1.6×10^{-8} ppb^{-1}. "The multistage model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data" (USEPA 1985). In the present case, however, no higher order terms than 2, i.e., coefficients multiplied by dose raised to a power greater than 2, were obtained using the model. For the rat nasal cancer data, the equation therefore reduces to:

$$P(d) = 1 - e^{-(q_0 + q_1 d + q_2 d^2)}$$

The model generated an Upper 95% Confidence Limit (UCL) on q_1 (named q_1^* and referred to as the inhalation unit risk) of 3.2×10^{-6} ppb^{-1} (Table 9.2). The female rat data (Table 9.1) yielded a somewhat lower risk with a q_1^* equal to 9.3×10^{-7} ppb^{-1} (Table 9.2). Such UCLs are calculated because they: are more stable statistically, considering the uncertainty in the risk assessment, and are health protective, i.e., statistically there is only a 5% chance that the true value of q_1 is greater than the 95% UCL.

For acetaldehyde 1 ppb = 1.8 ug/m^3. Using the latter units, the MLE for q_1 equals 8.8×10^{-9} $(ug/m^3)^{-1}$ and the 95% UCL for q_1 (q_1^*) equals 1.8×10^{-6} $(ug/m^3)^{-1}$ for the male rat data.

In its risk assessment, the USEPA combined two experiments by Wouterson et al.: the lifetime exposure experiment and an experiment in which one year of exposure was followed by 1 year of recovery. OEHHA staff, however, used only the lifetime exposure experiment. Such experiments, when available, are preferable for risk assessment since environmental risk assessments usually assume lifetime exposures to low levels of carcinogens. USEPA also used two versions of the multistage model: the standard, non-time-dependent ("quantal") version and a time-dependent, variable-dose or time-to-tumor version which is preferable when the data are available for its use. However, in the case of the Wouterson et al. data, such data as: (1) the exact time of death of each animal, (2) whether a tumor was present at the time of its death, and (3) whether a tumor was incidental to or causal of the death, were not reported. Thus USEPA made several assumptions to apply the time-to-tumor version of the model. To minimize the use of additional assumptions, OEHHA staff decided to use the standard ("quantal") version of the model. Using the assumptions the

TABLE 9-2

CARCINOGENIC POTENCY DETERMINATION BY GLOBAL86:
INHALATION UNIT RISK/PPB BASED ON RAT NASAL TUMORS

	Interspecies scaling factor		
	(1.0)[a]	Metabolic[b]	Contact[c]
males	3.2×10^{-6}	4.8×10^{-6}	2.7×10^{-5}
females	9.7×10^{-7}	1.6×10^{-6}	6.3×10^{-6}

[a] Assumes ppb equivalent between species.
[b] Assumes metabolism throughout body.
[c] Assumes metabolism in respiratory tract only.

USEPA found that the time-to-tumor version gave risks that were about 20-25% higher than those obtained using the quantal version of the model (USEPA, 1987).

9.2.3 Interspecies Scaling and Rat Nasal Carcinomas

Cancer risk assessment usually requires a means of predicting human risk from the results of an animal bioassay. Without specific information to the contrary, OEHHA assumes that a surface area scaling factor is appropriate for scaling from animals to humans. Use of a scaling factor on applied exposure provides a pragmatic way of performing the extrapolation from rodents to humans.

In its risk assessment (USEPA 1987, 1991), the USEPA assumed that units of ppm (or ppb) are risk-equivalent across species, as it had done previously with other contact carcinogens including formaldehyde and epichlorohydrin. Such an approach leads to a risk range of 9.7×10^{-7} to 3.2×10^{-6} ppb^{-1} acetaldehyde, based on female and male rat nasal carcinomas, respectively (Table 9-2).

The present work makes the basic assumption that equal concentrations of carcinogen imply equal risk across species in order to develop scaling factors in two cases. Both cases derive from the same simplified sort of metabolic model, in which the intake rate of carcinogen just equals the overall rate of metabolism of the carcinogen. This equation, a first approximation in the absence of extensive data, leads to a mathematical expression for the concentration of the carcinogen in the target tissue as the measure of the carcinogenic effect. In the first case, a systemic scaling results from assuming a quasi-uniform distribution of the carcinogen throughout the body. For the particular assumption of metabolic rate proportional to body surface area, this scaling becomes identical to the default scaling option of the California Department of Health Services guidelines for carcinogen risk assessments (CDHS, 1985). (In the second case, discussed in Section 9.2.4, a contact scaling results from assuming that the carcinogenic effect takes place only in a thin layer of tissue at the point of entry of the carcinogen.)

The simple metabolic model used to obtain scaling factors in this assessment represents the affected tissue as a single-compartment in order to estimate the concentration of carcinogen in affected tissue. Among other simplifying assumptions, especially that the chemical processes are for practical purposes homogeneous and that the actual metabolic chain of reactions can be usefully approximated by a single rate limiting step, this analysis considers concentrations to be sufficiently small that linear kinetics govern in determining the scaling.

In a compartment which consists of a given volume with chemical processes occurring at a steady state, a mass balance requires that a single input rate equals the overall rate of disappearance by metabolism in that volume, if there is no outflow:

$$I = B\,M, \qquad (1)$$

where, for that volume, I = rate of input of carcinogen in ug/hr, B = tissue concentration of carcinogen in ug/l, and M = metabolic rate per concentration ((ug/hr)/(ug/l) = 1/h).

Thus, for that volume the tissue concentration is

$$B = I / M. \tag{2}$$

According to the assumption that equal values of tissue concentration imply equal risks of cancer across species, it follows from Equation 2 that humans, designated by subscript h, will have the same risk as the rodent test species, designated by r, for

$$(I / M)_h = (I / M)_r. \tag{3}$$

(In this work the subscript following the closing parenthesis applies to the entire expression within the parentheses.) For inhalation, Equation 3 becomes

$$(a V C / M)_h = (a V C / M)_r, \tag{4}$$

where a = proportion of carcinogen absorbed, V = inhalation rate in m^3/hr, and C = atmospheric concentration in ug/m^3.

The inhalation rate across mammalian species follows the allometric relation (Weibel, 1984):

$$V_h / V_r = (W_h / W_r)^{0.75}. \tag{5}$$

where W = body mass in kg.

Using this expression in Equation 4 gives:

$$(a W^{0.75} C / M)_h = (a W^{0.75} C / M)_r. \tag{6}$$

This equation is used in both the systemic case and the contact case below. The only difference is in the calculation of M.

The systemic case assumes that the concentration and metabolism are quasi-uniformly distributed throughout the body. Let n be the exponent in the allometric relation for M_b, which represents the average metabolic rate for the whole body.

$$(M_{br} / M_{bh}) = (W_r / W_h)^n, \tag{7}$$

Dividing this equation into Equation 6 yields

$$(a W^{0.75-n} C)_h = (a W^{0.75-n} C)_r, \tag{8}$$

Solving equation (8) for C_r provides an expression to use in the risk formula for test animals in order to convert it to one for the risk for humans exposed to C_h.

$$C_r = (a_h / a_r)(W_h / W_r)^{0.75-n} C_h. \tag{9}$$

Note that n = 2/3 gives OEHHA default scaling. Allometric data related to metabolic rate show that n may be somewhat greater than 2/3. Inhalation clearance measurements for vinyl chloride appear to give n = 0.69 from mouse to human (plot of data from Buchter et al. 1977, 1978, 1980; Filser and Bolt

1979). Oxygen metabolism *in vivo* gives n = 0.75 for an even greater range of body mass (Weibel 1984). The use of 0.75 corresponds to no net scaling based on body mass.

For the case of acetaldehyde, the lack of data requires the default assumption of n = 2/3. With the further assumption that the absorption coefficients are equal because of lack of data, Equation 9 gives the following scaling factors: 1.5 for the 400 g male rat and 1.6 for the 250 g female rat, assuming 70 kg body weight for both human sexes. Using these scaling factors risk values of 1.6×10^{-6} ppb^{-1} from female rat data and 4.8×10^{-6} ppb^{-1} from male rat data are obtained (Table 9.2).

9.2.4 Rat Nasal Carcinomas and Contact Scaling

The contact case assumes the concentration of carcinogen to be distributed only in the designated volume of a thin layer in the body and nowhere else. The metabolism per unit volume is assumed to occur in that layer at the same rate as in the rest of the body. The average rate of metabolism per concentration per unit volume in the body is $M_b/(W/D)$. Thus, the overall metabolism per concentration in the layer is:

$$M_l = M_b \; D \; A \; t \; / \; W \; , \qquad (10)$$

where D = average density of the body in kg/l, A = surface area of the layer in cm^2, and t = average thickness of the layer in cm.

Inserting this expression into Equation 6 insures that the concentration in that layer is constant across species.

$$(a \; C \; W^{1.75} \; / \; M_b A \; t \; D)_h = (a \; C \; W^{1.75} \; / \; M_b A \; t \; D)_r . \qquad (11)$$

Using Equation 7 and assuming that the surface area of the lung airways has an allometric exponent of 0.75, the exponent for "metabolic size" (Gross et al. 1982), this equation becomes:

$$(a \; W^{0.75+1-0.75-n} \; C \; / \; t)_h = (a \; W^{0.75+1-0.75-n} \; C \; / \; t)_r .$$

So the equivalent rat concentration is

$$C_r = (\; a_h \; / \; a_r \;)(\; W_h \; / \; W_r \;)^{1-n} \; (\; t_r \; / \; t_h \;) \; C_h . \qquad (12)$$

Assuming for lack of better information that the value of a/t is constant across species and again using n = 2/3, Equation 12 gives the following scaling factors: 5.6 for a 400 g male rat and 6.5 for a 250 g female rat, again assuming both human sexes have 70 kg body mass. The resultant risks of 6.3×10^{-6} ppb^{-1} for female rats and 2.7×10^{-5} ppb^{-1} for male rats (Table 9-2) would be used only to predict nasal or respiratory system cancers.

The experimental data of Lam et al. (1986) on the ability of acetaldehyde to crosslink DNA to protein and of Bogdanffy et al. (1986) on differential levels of aldehyde dehydrogenase in the rat respiratory tract are consistent with the use of a contact area scaling factor. Aldehyde dehydrogenase converts active acetaldehyde to acetic acid which is much less reactive than acetaldehyde.

Thus aldehyde dehydrogenation is a detoxifying reaction. The greatly reduced presence of aldehyde dehydrogenase in rat olfactory epithelium compared to other areas (Bogdanffy et al. 1986) means that acetaldehyde can accumulate in the olfactory epithelium and be available to react with DNA and to cause genetic damage. The olfactory epithelium is the area where nasal lesions and tumors occur in rats.

Using the different assumptions indicated above, a range of risk inhalation unit values is obtained, from 9.7×10^{-7} ppb^{-1} based on female rat data without a scaling factor applied to 2.7×10^{-5} ppb^{-1} based on male rat data with a contact area correction factor applied (Table 9.2).

9.2.5. Hamster Laryngeal Carcinomas and the Multistage Model

The multistage model was also fit to the tumor data in hamsters obtained by Feron et al. (1982). These authors observed both nasal and laryngeal tumors (Table 8-4) in a single group of hamsters exposed 6 hours/day, 5 days/week to an acetaldehyde level which was initially 2500 ppm but was gradually decreased to 1650 ppm. For the 52 weeks of exposure the average level was approximately 2075 ppm; the hamsters were terminated at 81 weeks. Since the tumorigenic effect was stronger for laryngeal tumors, these were used for risk assessment. For females there were no tumors in 20 control animals and 4 laryngeal tumors in 20 treated animals. The reported exposure concentration corresponds to 370.5 continuous ppm during the exposure period of 52 weeks and 238 continuous ppm over the 81 weeks of the experiment. Under the assumption that ppm (or ppb) exposures are equivalent in man and hamster (USEPA, 1987), the female hamster data result in a q_1^* of 1.9×10^{-6} ppb^{-1}. For males there were 0 tumors in 20 controls and 6 laryngeal tumors in 23 treated hamsters. A q_1^* of 2.3×10^{-6} ppb^{-1} was obtained with these data using the multistage model. These values are very close to the ones obtained for rats under the assumption of equality of ppb exposure (Table 9.2).

9.2.6. Risk Estimate using the Gaylor-Kodell Approach

The application of the linearized multistage model requires the use of a computer program to fit the model to the data and extrapolation into the range below the lowest dose tested. An alternative approach is the technique introduced by Gaylor and Kodell (Gaylor and Kodell 1979, Williams and Burson 1985). In this method the observed responses are fit to a model, then the 95% upper confidence limit (UCL) is determined on the predicted value from the model at the lowest tested dose of chemical for which cancer risk is increased over background. This UCL is then interpolated linearly from this point to the background incidence in order to determine an upper boundary line on risk. Under the assumption of strict linearity (not just at low doses), the true risk is predicted to be at or below this line with 95% probability. For nasal carcinoma in male rats this 95% UCL on the 750 ppm exposure level (129.8 continuous ppm) was determined to be 0.406 using the multistage model. Subtracting the background incidence of 0.020 (1/49) yielded an additional risk of 0.386. Dividing this net incidence by 129,800 ppb continuous exposure yielded an inhalation unit risk of 3×10^{-6} ppb^{-1}, which agrees with the results of the multistage model.

9.2.7 Population Risk from Acetaldehyde in Ambient Air

As stated above, air modeling of acetaldehyde in California has yielded an estimated mean ambient concentration of 2 ppb (4 $\mu g/m^3$). With an estimated California population of $30x10^6$ and upper 95% confidence limits on inhalation unit risk of $0.97\text{-}27x10^{-6}$ ppb^{-1} ($0.54\text{-}15x10^{-6}$ $(\mu g/m^3)^{-1}$) derived using the multistage model (Table 9.2), the upper 95% confidence limit estimate of excess cancers over a lifetime due to exposure to acetaldehyde would be:

$$30x10^6 \times 0.97\text{-}27x10^{-6}\ ppb^{-1} \times 2\ ppb = 58\text{-}1620 \text{ excess cancers}$$

Using the approach of Gaylor and Kodell, for an ambient level of 2 ppb, the individual risk is $3 \times 10^{-6} \times 2 = 6x10^{-6}$ which yields an estimate of 180 lifetime excess cancers. The predicted numbers would occur in a background of approximately 6 to 8 million cases in this population based on recent cancer data for Los Angeles County and for all of California (World Health Organization 1982, Silverberg and Lubera 1987, Boring et al. 1991).

The range of risk values represents several sources of uncertainty, including statistical uncertainty due to the relatively small number of animals used in the bioassay. Other general sources of uncertainty include the choice of the animal-to-human scaling factors, the choice of the extrapolation model, and the large range of extrapolation (five orders of magnitude) from the adjusted acetaldehyde concentrations used in the animal experiments to current ambient levels. In addition there is the possibility in light of the absence of an epidemiological connection between exposure to acetaldehyde and cancer that the risks in rats and hamsters may not be applicable to humans, i.e., acetaldehyde is only a potential human carcinogen. While a portion of the population is exposed to concentrations of acetaldehyde greater than 2 ppb, others will be exposed to less and thus have a lower risk.

Based on the findings of carcinogenicity and the results of the risk assessment, OEHHA staff find that ambient acetaldehyde is an air pollutant which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

9.2.8. Selection of Best Value for Risk Assessment

Using the multistage model, a range of inhalation unit risk values was obtained, from $9.7x10^{-7}$ ppb^{-1} for female rats without a scaling factor to $2.7x10^{-5}$ ppb^{-1} for male rats with a contact area correction (Table 9.2). For simplification of risk assessments a best value is often chosen from the range. In the case of acetaldehyde, a best value of $4.8x10^{-6}$ ppb^{-1} ($2.7x10^{-6}$ $(\mu g/m^3)^{-1}$) was selected. The male rat is more sensitive to tumor induction by acetaldehyde than the female rat and thus is the proper sex to select based on California procedures for cancer risk assessment. The value was obtained from the male rat data using the standard interspecies surface area correction factor. Use of the rat data set has additional health protection built in since it was obtained using tumor data obtained after 28 months of exposure to acetaldehyde which is longer than the normal exposure and observation period for most chemicals of 24 months. This best estimate predicts 288 excess lifetime cancer cases in the California population.

REFERENCES

Alarie Y (1966) Irritating properties of airborne materials to the upper respiratory tract. Arch Environ Health 13:433-449.

Ali F and Persaud T (1988) Mechanisms of fetal alcohol effects: role of acetaldehyde. Exp Pathol 33:17-21.

ACGIH (American Conference of Governmental Industrial Hygienists) (1986) Documentation of the Threshold Limit Values. 5th ed. Cincinnati, OH: ACGIH, p. 3.

ACGIH (American Conference of Governmental Industrial Hygienists) (1985) TLVs: Threshold Limit Values and Biological Exposure Indices for 1985-86. 5th ed. Cincinnati, OH: ACGIH, p. 9.

Anderson RA, Reddy JM, Joyce C, Willis BR, Van der Vend H and Zaneveld LJ (1982) Inhibition of mouse sperm capacitation by ethanol. Biol Reprod 27:833-840.

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

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

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

Badr FM, Bartke A, Dalterio S and Bulger W (1977) Suppression of testosterone production by ethyl alcohol. Possible mode of action. Steroids 30:647-655.

Bandas EL (1982) Study of the role of metabolites and impurities in the mutagenic action of ethanol on yeast mitochondria. Genetika 18:1056-1061.

Bannigan J and Burke P (1982) Ethanol teratogenicity in mice: a light microscope study. Teratology 26:247-254.

Barilyak IR and Kozachuk SY (1983) Embryotoxic and mutagenic activity of ethanol after intra-amniotic injection. Tsitologiya i Genetika 17:57-60.

Barrow CS (1982) Sensory irritation of inhaled aldehydes: structure activity studies. CIIT 2(9).

Barry RE, McGivan JD and Hayes M (1984) Acetaldehyde binds to liver cell membrane without affecting membranes function. Gut 25:412-416.

Barry RE and Williams AJK (1988) Metabolism of ethanol and its consequences for the liver and gastrointestinal tract. Dig Dis 6:194-202.

Baselt RC and Cravey RH (1989) Disposition of Toxic Drugs and Chemicals in Man. 3rd. ed. Year Book Medical Pub., Inc.

Bird RP, Draper HH and Basrur PK (1982) Effect of malonaldehyde and acetaldehyde on cultured mammalian cells; production of micronuclei and chromosomal aberrations. Mutat Res 101:237-246.

Bittersohl G (1975) Epidemiological research on cancer risk by aldol and aliphatic aldehydes. Environ Qual Safety 4:235-238.

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

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

Böhlke JU, Singh S and Goedde HW (1983) Cytogenetic effects of acetaldehyde in lymphocytes of Germans and Japanese: SCE, clastogenic activity, and cell cycle delay. Hum Genet 63:285-289.

Bogdanffy MS, Randall HW and Morgan KT (1986) Histochemical localization of aldehyde dehydrogenase in the respiratory tract of the Fischer-344 rat. Toxicol Appl Pharmacol 82:560-567.

Booze T and Oehme F (1986) An investigation of metaldehyde and acetaldehyde toxicities in dogs. Fund Appl Toxicol 6:440-446.

Boring CC, Squires TS, and Tong T (1991) Cancer statistics, 1991. CA- Cancer J Clinicians. 41, 19-36.

Boyden TW, Silvert MA and Pamenter RW (1981) Acetaldehyde acutely impairs canine testicular testosterone secretion. Eur J Pharmacol 70:571-576.

Bradley M, Hsu IC and Harris CC (1979) Relationships between sister chromatid exchange and mutagenicity, toxicity, and DNA damage. Nature 282:318-320.

Buchter A, Bolt HM, Kappus H and Bolt W (1977) Die gewebsverteilung von 1,2-^{14}C-vinylchlorod bei der ratte. Int Arch Occup Environ Health 39:27-32.

Buchter A, Bolt HM, Filser JG, Goergens HW, Laib RJ and Bolt W (1978) Pharmakokinetic and karzinogenese von vinyl chloride. Arbeitsmedizinische Risikobeurteilung. Verh Dtsch Ges Arbeitsmedizin (Gentner, Stuttgart) 18:111-124.

Buchter A, Filser JG, Peter H and Bolt HM (1980) Pharmacokinetics of vinyl chloride in the Rhesus monkey. Toxicol Lett 6:33-36.

Buckley LA, Jiang XZ, James RA, Morgan KT, and Barrow SC (1984) Respiratory tract lesions induced by sensory irritants at the RD50 concentration. Toxicol Appl Pharmacol 74:417-429.

California Department of Health Services (1985). Guidelines for Chemical Carcinogen Risk Assessments and their Scientific Rationale. State of California, Health and Welfare Agency. Sacramento, CA.

Campbell KI, Clarke GL, Emik LO and Plata RL (1967) The atmospheric contaminant peroxyacetyl nitrate. Acute inhalation toxicity in mice. Arch Environ Health 15:739-744.

Campbell MA and Fantel AG (1983) Teratogenicity of acetaldehyde in vitro: relevance to fetal alcohol syndrome. Life Sci 32:2641-2647.

Cao QN, Tu G-C and Weiner H (1988) Mitochondria as the primary site of acetaldehyde metabolism in beef and pig liver slices. Alcohol Clin Exp Res 12:720-724.

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

Casanova M, Morgan KT, Steinhagen WH, Everitt JI, Popp JA, Heck H D'A (1991) Covalent binding of inhaled formaldehyde to DNA in the respiratory tract of rhesus monkeys: Pharmacokinetics, rat-to-monkey interspecies scaling, and extrapolation to man. Fund Appl Toxicol 17:409-428.

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

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

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

Cobb CF, Ennis MF, Michael F, Van Thiel DH, Gavaler JS and Lester R (1978) Acetaldehyde and ethanol are direct testicular toxins. Surg Forum 29:641-644.

Cobb CF, Ennis MF, Van Thiel DH, Gavaler JS and Lester R (1980) Isolated testes perfusion: a method using a cell- and protein-free perfusate useful for the evaluation of potential drug injury. Metabolism 29:71-79.

Cohen G and MacNamee D (1976) On the absence of "endogenous" acetaldehyde in rat and mouse blood. Res Commun Chem Pathol Pharmacol 14:489-495.

Condouris GA and Havelin DM (1987) Acetaldehyde and cardiac arrhythmias. Arch Int Pharmacodyn 285:50-59.

Corsini GU, Zuddas A, Bonuccelli U, Schinelli S and Kopin IJ (1987) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) neurotoxicity in mice is enhanced by ethanol or acetaldehyde. Life Sci 40:827-832.

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

De Raat WK, Davis PB and Bakker GL (1983) Induction of sister-chromatid exchanges by alcohol and alcoholic beverages after metabolic activation by rat-liver homogenate. Mutat Res 124:85-90.

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

Dreisbach RH (1980) Acetaldehyde, metaldehyde, and paraldehyde. In: Handbook of Poisoning: Prevention, Diagnosis and Treatment. Los Angeles, CA: Lange Medical Publications, pp. 177-180.

Dreosti IE, Ballard FJ, Belling GB, Record IR, Manuel SJ and Hetzel BS (1981) The effect of ethanol and acetaldehyde on DNA synthesis in growing cells and on fetal development in the rat. Alcohol Clin Exp Res 5:357-362.

Dulout F and Furnus C (1988) Acetaldehyde induced aneuploidy in cultured Chinese hamster cells. Mutagenesis 3:207-211.

Durlach J, Rinjard P, Sprince H and Smith GG (1988) Similar antagonistic effects of a Ca-N-acetylhomotaurinate on depression of motor activity and lethality induced by acetaldehyde on ethanol. Meth Find Exper Clin Pharmacol 10:437-447.

Egle JL Jr (1970) Retention of inhaled acetaldehyde in man. J Pharmacol Exper Therap 174:14-19.

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

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

Eker P and Sanner T (1986) Initiation of in vitro cell transformation by formaldehyde and acetaldehyde as measured by attachment-independent survival of cells in aggregates. Eur J Cancer Clin Oncol 22:671-676.

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

Feig SA, Sohet SB and Nathan DG (1971) Energy metabolism in human erythrocytes: effect of sodium fluoride. J Clin Invest 50:1731-1737.

Feron VJ (1979) Effects of exposure to acetaldehyde in Syrian hamsters simultaneously treated with benzo[a]pyrene or diethylnitrosamine. Prog Exp Tumor Res 24:162-176.

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

Filser JG and Bolt HM (1979) Pharmacokinetics of halogenated ethylenes in rats. Arch Toxicol 42:123-136.

Fleisher JH, Lung CC, Meinke GC and Pinnas JL (1988) Acetaldehyde-albumin adduct formation: possible relevance to an immunologic mechanism in alcoholism. Alcohol 23:133-141.

Forsander OA, Hillbom ME and Lindros KO (1969) Influence of thyroid function on the acetaldehyde level of blood and liver of intact rats during ethanol metabolism. Acta Pharmacol Toxicol 27:410-419.

Freund G (1967) Serial determinations of acetaldehyde and acetone in alveolar air after standard amounts of ethanol. In: Biochemical Factors in Alcoholism. Maickel RP, ed. Oxford: Pergamon Press, pp. 89-95.

Freund G and O'Hollaren P (1965) Acetaldehyde concentrations in alveolar air following a standard dose of ethanol in man. J Lipid Res 6:471-477.

Fujiwara N, Shwa Y, Yoshizumi H, Yamatodani A and Wada H (1988) Preventive effects of L-alanine and L-glutamic acid on acute toxicity of acetaldehyde in mice. Jpn J Alc Drug Depend 23:58-69.

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

Garberg P, Akerblom E-L and Bolcsfoldi G (1988) Evaluation of a genotoxicity test measuring DNA strand breaks in mouse lymphoma cells by alkaline unwinding and hydroxyapatite elution. Mutat Res 203:155-176.

Gaylor DW and Kodell RL (1980) Linear interpretation algorithm for low dose risk assessment of toxic substances. J Environ Path Toxicol 4:305-312.

Greenwald IS and Horvitz HR (1980) Unc-93 (e1500): a behavioral mutant of Caenorhabditis elegans that defines a gene with a wild-type null phenotype. Genetics 96:147-164.

Gross EA, Swenberg JA, Fields, and Popp JA (1982) Comparative morphometry of the nasal cavity in rats and mice. J Anat 135:83-88.

Guivernau M, Baraona E and Lieber CS (1987) Acute and chronic effects of ethanol and its metabolites on vascular production of prostacyclin in rats. J Pharmacol Exp Therap 240:59-64.

Hagihara S, Sameshima Y, Kobayashi M and Obe F (1981) Behavior of acetaldehyde transported in blood. Biochem Pharmacol 30:651-661.

Hasumura Y, Teschke R and Lieber CS (1976) Characteristics of acetaldehyde oxidation in rat liver mitochondria. J Biol Chem 251:4908-4913.

He SM and Lambert B (1985) Induction and persistence of SCE-inducing damage in human lymphocytes exposed to vinyl acetate and acetaldehyde in vitro. Mutat Res 158:201-208.

He SM and Lambert B (1990) Acetaldehyde-induced mutation at the *hprt* locus in human lymphocytes in vitro. Environ Molec Mutag 16:57-63.

Hemminki K, Falck K and Vainio H (1980) Comparison of alkylation rates and mutagenicity of directly acting industrial and laboratory chemicals. Arch Toxicol 46:277-285.

Higuchi Y and Matsumoto N (1984) Embryotoxicity of ethanol and acetaldehyde: direct effects on mouse embryo in vitro. Congen Anom 24:9-28.

Hobara N, Watanabe A, Kobayashi M, Nakatsukasa H, Nagashima H, Fukuda T and Araki Y (1985) Tissue distribution of acetaldehyde in rats following acetaldehyde inhalation and intragastric ethanol administration. Bull Environ Contam Toxicol 35:393-396.

Hoberman HD (1979a) Synthesis of 5-deoxy-D-xylulose-1-phosphate by human erythrocytes. Biochem Biophys Res Comm 90:757-763.

Hoberman HD (1979b) Adduct formation between hemoglobin and 5-deoxy-L-xylulose-1-phosphate. Biochem Biophys Res Comm 90:764-768.

Hoerner M, Behrens UJ, Worner TM, Blacksberg I, Braly LF, Schaffner F and Lieber CS (1988) The role of alcoholism and liver disease in the appearance of serum antibodies against acetaldehyde adducts. Hematology 8:569-574.

Horton AA and Barrett MC (1975) The subcellular localization of aldehyde dehydrogenase in rat liver. Arch Biochem Biophys 167:426-436.

Horton AA and Barrett MC (1976) Rates of induction of mitochondrial aldehyde dehydrogenase in rat liver. Biochem J 156:177-183.

Howe, R.B., Crump, K., and van Landingham, C. (1986). GLOBAL86. Clement Associates. Ruston, LA.

IARC (International Agency for Research on Cancer) (1985) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Vol. 36: Allyl compounds, aldehydes, epoxides and peroxides. Lyon, France: IARC, pp. 101-132.

IARC (International Agency for Research on Cancer) (1987) IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Suppl. 7: Overall evaluations of carcinogenicity: an updating of IARC Monographs Volumes 1 to 42. Lyon, France: IARC.

IARC (1988) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 44. Alcohol drinking. Lyon, France: IARC.

Jansson T (1982) The frequency of sister chromatid exchanges in human lymphocytes treated with ethanol and acetaldehyde. Hereditas 97:301-303.

Jiang XZ, Buckley LA, and Morgan KT (1983) Pathology of toxic responses to the RD50 concentration of chlorine gas in the nasal passages of rats and mice. Toxicol Appl Pharmacol 71:225-236.

Johannsson-Brittebo E and Tjalve H (1979) Studies on the tissue-disposition and fate of nitrosourea in mice. Toxicology 13:275-285.

Johnston DE, Chiao Y-B, Gavaler JS and Van Thiel DH (1981) Inhibition of testosterone synthesis by ethanol and acetaldehyde. Biochem Pharmacol 36:1827-1831.

Kallama S and Hemminki K (1983) Urinary excretion products after the administration of ^{14}C-acetaldehyde to rats. J Appl Toxicol 3:313-316.

Kalmus G and Buckenmaier C (1989) Effects of ethanol and acetaldehyde on cultured pre-implantation mouse embryos. Experientia 45:484-487.

Kane LE, Dombroske R and Alarie Y (1980) Evaluation of sensory irritation from some common industrial solvents. Am Ind Hyg Assoc J 41:451-455.

Kerns WD, Pavkov KL, Donofrio DJ, Connell MM, Mitchell RI, Gralla EJ and Swenberg JA (1983) Carcinogenicity of formaldehyde in rats and mice after long term inhalation exposure. Cancer Res 43:4382-4392.

Koivula T, Koivusalo M and Lindros KO (1975) Liver aldehyde and alcohol dehydrogenase activities in rat strains genetically selected for their ethanol preferences. Biochem Pharmacol 24:1807-1813.

Korte A and Obe G (1981) Influence of chronic ethanol uptake and acute acetaldehyde treatment on the chromosomes of bone-marrow cells and peripheral lymphocytes of Chinese hamsters. Mutat Res 88:389-395.

Krebs HA and Perkins JR (1970) The physiological role of liver alcohol dehydrogenase. Biochem J 118:635-647.

Kruysse A, Feron VJ, Immel HR, Spit BJ and Van Esch GJ (1977) Short-term inhalation toxicity studies with peroxyacetyl nitrate in rats. Toxicology 8:231-249.

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

Lahdetie J (1988) Effects of vinyl acetate and acetaldehyde on sperm morphology and meiotic micronuclei in mice. Mutat Res 202:171-178.

Lam CW, Casanova M and Heck HD (1986). Decreased extractability of DNA from proteins in the rat nasal mucosa after acetaldehyde exposure. Fund Appl Toxicol 6:541-550.

Lambert B, Chen Y, He SM and Sten M (1985) DNA cross-links in human leucocytes treated with vinyl acetate and acetaldehyde in vitro. Mutat Res 146:301-303.

Latt SA, Allen J, Bloom SE, Carrano A, Falke E, Kram D, Schneider E, Schreck R, Tice R, Whitfield B and Wolff S (1981) Sister chromatid exchanges: a report of the Gene-Tox program. Mutat Res 87:17-62.

Laumbach AD, Lee S, Wong J and Streips UN (1976) Studies on the mutagenicity of vinyl chloride metabolites and related chemicals. Proceedings of the 3rd

International Symposium on the Prevention and Detection of Cancer, Vol. 1, pp. 155-169.

Lewis RJ and Tatkin RL (1983) Registry of Toxic Effects of Chemical Substances. 1981-1982 ed. Cincinnati, OH: U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health; p. 156; DHHS (NIOSH) publ. no. 83-107.

Lieber C (1988) Metabolic effects of acetaldehyde. Biochem Soc Trans 16:241-247.

Lindros KO, Vikma R and Forsander OA (1972) Utilization and metabolic effects of acetaldehyde and ethanol in the perfused rat liver. Biochem J 126:945-951.

Lubin M and Westerfeld WW (1945) The metabolism of acetaldehyde. J Biol Chem 161:503-512.

Marnett LJ, Hurd HK, Hollstein MC, Levin DE, Esterbauer H, Ames BN (1985) Naturally occurring carbonyl compounds are mutagenic in Salmonella tester strain TA104. Mutat Res 148:25-34.

Machner H and Tottmar O (1976) Inhibition of the acetaldehyde dehydrogenases in rat liver by a cyanamide derivative present in a commercial standard diet for small animals. Acta Pharmacol Toxicol 38:59.

MCA, Inc. (1952) Chemical Safety data sheet SD-43, Acetaldehyde, pp. 5-6, 12-15, Washington, DC.

Michoudet C and Baverel G (1987a) Metabolism of acetaldehyde in human and baboon renal cortex. FEBS Lett 216:113-117.

Michoudet C and Baverel G (1987b) Characteristics of acetaldehyde metabolism in isolated dog, rat, and guinea-pig kidney tubules. Biochem Pharmacol 36:3987-3991.

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

Mohan M, Rai UC, Reddy LP, Prasanna CV and Ramakrishnan S (1981) Cardiovascular effects of acetaldehyde in guinea pigs. Ind J Physiol Pharmacol 25:246-252.

Mortelmans K, Haworth S, Lawlor T, Speck W, Tainer B and Zeiger E (1986) Salmonella mutagenicity tests: II. Results from testing of 270 chemicals. Environ Mutagen 8:1-39.

NRC (National Research Council) (1977) Drinking Water and Health: Part II, Chapters 6 and 7. Washington, DC: National Academy of Sciences, pp. VI-219 - VI-220. Available from: NTIS, Springfield, VA; PB-270423.

Norppa H, Tursi F, Pfäffli P, Mäki-Paakkanen J and Järventaus H (1985) Chromosome damage induced by vinyl acetate through in vitro formation of acetaldehyde in human lymphocytes and Chinese hamster ovary cells. Cancer Res 45:4816-4821.

Nuutinen HU, Salaspuro MP Valle M, and Lindros KO (1984) Blood acetaldehyde gradient between hepatic and anticubital venous blood in ethanol-intoxicated alcoholics and controls. Eur J Clin Invest 14:306-311.

Obe G and Beek B (1979) Mutagenicity of aldehydes. Drug Alcohol Depend 4:91-94.

Obe G and Ristow H (1977) Acetaldehyde, but not ethanol, induces sister chromatid exchanges in Chinese hamster cells in vitro. Mutat Res 56:211-213.

Obe G, Natarajan AT, Meyers M and Den Hertog A (1979) Induction of chromosomal aberrations in peripheral lymphocytes of human blood in vitro, and of SCEs in bone-marrow cells of mice in vivo by ethanol and its metabolite acetaldehyde. Mutat Res 68:291-294.

Obe G, Jonas R and Schmidt S (1986) Metabolism of ethanol in vitro produces a compound which induces sister-chromatid exchanges in human peripheral lymphocytes in vitro: acetaldehyde, not ethanol, is mutagenic. Mutat Res 174:47-51.

Occupational Safety and Health Administration (OSHA) (1989) Air Contaminants - Permissible Exposure Limits. (Title 29 Code of Federal Regulations Part 1910.1000) U.S. Department of Labor, Washington, DC.

Office of Environmental Health Hazard Assessment (1992). Cancer risk assessment for airborne formaldehyde. State of California, California Environmental Protection Agency, Berkeley, CA.

Omel'yanets NI, Mironets NV, Martyshchenko NV, Gubareva IA, Piven LF and Starchenko SN (1978) Experimental substantiation of the maximum permissible concentrations of acetone and acetaldehyde in reclaimed potable water. Biol Aviakosm Med 1978:67-70.

O'Shea KS and Kaufman MH (1979) Teratogenic effects of acetaldehyde: implications for the study of fetal alcohol syndrome. J Anat 128:65-76.

O'Shea KS and Kaufman MH (1981) Effects of acetaldehyde on neuroepithelium of early mouse embryos. J Anat 132:107-118.

Padmanabhan R, Sreenathan RN and Singh S (1983) Studies of the lethal and teratogenic effects of acetaldehyde in the rat. Congen Anom 23:13-23.

Pietruszko R and Vallari RL (1978) Aldehyde dehydrogenase in human blood. FEBS Lett 92:89-91.

Pool BL and Wiessler M (1981) Investigations on the mutagenicity of primary and secondary Á-acetoxynitrosamines with Salmonella typhimurium: activation and deactivation of structurally related compounds by S-9. Carcinogenesis 2:991-997.

Popov VB, Vaisman BL, Puchkov VP and Ignat'eva TV (1982) Toxic effects of ethanol and its biotransformation products on post-implantation embryos in culture. Bull Exper Biol Med (Rus.) 92:1707-1710.

Priscott PK (1985) The effect of acetaldehyde and 2,3-butanediol on rat embryos developing in vitro. Biochem Pharmacol 34:529-532.

Randall C, Taylor W, Tabakoff B and Walker D (1978) Ethanol as a teratogen. In: Alcohol Acetaldehyde Metabolizing System. Thurman R, Williamson J, Drott H and Chance B, eds. New York: Academic Press, Vol. 3, pp. 659-670.

Ridge JW (1963) The metabolism of acetaldehyde by the brain in vivo. Biochem J 88:95.

Rieger R and Michaelis A (1960) Chromosomenaberrationen nach Einwirkung von Acetaldehyd auf Primawurzeln von Vicia faba. Biol Zbl 79:1-5.

Ristow H and Obe G (1978) Acetaldehyde induces cross-links in DNA and causes sister-chromatid exchanges in human cells. Mutat Res 58:115-119.

Rosenkranz HS (1977) Mutagenicity of halogenated alkanes and their derivatives. Environ Health Perspect 21:79-84.

Saladino AJ, Willey JC, Lechner JF, Grafstrom RC, LaVeck M and Harris CC (1985) Effects of formaldehyde, acetaldehyde, benzoyl peroxide, and hydrogen peroxide on cultured normal human bronchial epithelial cells. Cancer Res 45:2522-2526.

Saldiva P, Caldeira M, Massad E, Calheiros D, Cardoso L and Bohm G (1985) Effects of formaldehyde and acetaldehyde inhalation on rat pulmonary mechanics. J Appl Toxicol 5:288-292.

Santucci L, Graham TJ, Van Thiel DH (1983) Inhibition of testosterone production by rat Leydig cells with ethanol and acetaldehyde: prevention of ethanol toxicity with 4-methylpyrazole. Alcoholism 7:135-139.

Seigers CP, Schuett A and Strubelt O (1977) Influence of some hepatotoxic agents on hepatic glutathione levels in mice. Proc Europ Soc Toxicol 18:160-162.

Shaw S, Jayatilleke E, Ross WA, Gordon ER and Lieber CS (1981) Ethanol-induced lipid peroxidation: potentiation by long-term alcohol feeding and attenuation by methionine. J Lab Clin Med 98:417-424.

Shiohara E, Tsukada M, Yamazaki H, Nishiguchi K, Miyamoto R and Nakanishi S (1983) Effect of disulfiram and cyanamide treatments in vivo on the activity of aldehyde dehydrogenases of subcellular fractions of rat brain. Jpn J Alcohol Drug Dependence 18:117.

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

Silverberg E and Lubera JA (1988) Cancer statistics, 1988. CA: Cancer J Clinicians 38:5-22.

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

Sim VM, Pattle RE (1957) Effect of possible smog irritants on human subjects. JAMA J Am Med Assoc 165:1908-1913.

Sina JF, Bean CL, Dysart GR, Taylor VI and Bradley MO (1983) Evaluation of the alkaline elution/rat hepatocyte assay as a predictor of carcinogenic/mutagenic potential. Mutat Res 113:357-391.

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

Smyth HF Jr, Carpenter CP and Weil CS (1951) Range-finding toxicity data: list IV. Arch Ind Hyg 4:119-122.

Solomon LR (1988) Effects of acetaldehyde on human red cell metabolism: evidence for the formation of enzyme inhibitors. Clin Chim Acta 175:249-266.

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

Sreenathan RN, Singh S and Padmanabhan R (1984) Effects of acetaldehyde on skeletogenesis in the rat. Drug Alcohol Depend 14:165-174.

Stek TL (1972) Cross-linking the major proteins of the isolated erythrocyte membrane. J Mol Biol 66:295-305.

Stephens ER, Darley EF, Taylor OC and Scott WE (1961) Photochemical reaction products in air pollution. Int J Air Water Poll 4:79-100.

Tanaka T, Ogasamara H and Kanezaki A (1988) Alteration of cAMP in experimental liver damage induced by acetaldehyde inhalation. ISBRA.

Thompson PAC and Folb PI (1982) An in vitro model of alcohol and acetaldehyde teratogenicity. J Appl Toxicol 2(4):190-243.

Thurman RG and Pathman DE (1975) Withdrawal symptoms from ethanol: evidence against the involvement of acetaldehyde. In: The Role of Acetaldehyde in the Actions of Ethanol. Lindros DO and Ericksson CJP, eds. Satellite Symp Int Congr Pharmacol Helsinki, Finland: Foundation Alcohol Studies Publication Number 23, pp. 217.

Til HP, Woutersen RA and Feron VJ (1988) Evaluation of the oral toxicity of acetaldehyde and formaldehyde in a 4-week drinking water study in rats. Food Chem Toxicol 26:447-452.

Truitt EB and Walsh MJ (1971) The role of acetaldehyde in the actions of ethanol. In: The Biology of Alcoholism. Kissin B and Beglecter HB, eds. Vol. I, Biochemistry, New York, NY: Plenum Press.

Tuma DJ, Donohue TM, Medina VA and Sorrell (1984) Enhancement of acetaldehyde-protein adduct formation by L-ascorbate. Arch Biochem Biophys 234:377-381.

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

United States Environmental Protection Agency (USEPA) (1985) Health Assessment Document for Ethylene Oxide. Final Report. U.S. Environmental Protection Agency, Washington, D.C.

United States Environmental Protection Agency (USEPA) (1986) Guidelines for chemical carcinogen risk assessment. Federal Register 51:33992-34003.

United States Environmental Protection Agency (USEPA) (1987) Health Assessment Document for Acetaldehyde. External Review Draft. U.S. Environmental Protection Agency, Washington, D.C.

United States Environmental Protection Agency (USEPA) (1989) Integrated Risk Information Service (IRIS) (Database). U.S. Environmental Protection Agency, Research Triangle Park, NC.

United States Environmental Protection Agency (USEPA) (1991) Integrated Risk Information Service (IRIS)(Database). U.S. Environmental Protection Agency, Research Triangle Park, NC.

United States Environmental Protection Agency (USEPA) (1992) Integrated Risk Information Service (IRIS)(Database). U.S. Environmental Protection Agency, Research Triangle Park, NC.

Veghelyi PV, Osztovics M, Kardos G, Leisztner L, Szaszovszky E, Igali S and Imrei J (1978) The fetal alcohol syndrome: symptoms and pathogenesis. Acta Paed Acad Sci Hungar 19:171-189.

Veitch RL, Lumeng L, Li TK (1975) Clin Invest 55:1026-1032 (as cited in Gaines 1977).

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

Webster WS, Walsh DA, McEwen SE and Lipson AH (1983) Some teratogenic properties of ethanol and acetaldehyde in C57BL/6J mice; implications for the study of fetal alcohol syndrome. Teratology 27:231-243.

Weibel ER (1984) The Pathway for Oxygen. Harvard Univ. Press, Cambridge, MA.

Westerfeld WW (1949) Acetaldehyde disappearance and acetoin formation in vitro. Proc Soc Exp Biol Med 71:28-33.

Williams AJK and Barry RE (1986) Superoxide anion production and degranulation of rat neutrophils in response to acetaldehyde altered liver cell membranes. Clin Sci Mol Med 71:313-318.

Williams PL and Burson JL (1985) Industrial Toxicology. Van Nostrand Reinhold Co., New York, pp. 381-383, 459.

Woodruff RC, Mason JM, Valencia R and Zimmering S (1985) Chemical mutagenesis testing in Drosophila. V. Results of 53 coded compounds tested for the National Toxicology Program. Environ Mutagen 7:677-702.

World Health Organization (1982) Cancer Incidence in Five Continents. IARC Sci Publ Vol. IV. World Health Organization, Lyon, France.

Woutersen RA and Feron VJ (1987) Inhalation toxicity of acetaldehyde in rats. IV. Progression and regression of nasal lesions after discontinuation of exposure. Toxicology 47:295-304.

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

Woutersen RA, Appleman LM, Van Garderen-Hoetmer A and Feron VJ (1986) Inhalation toxicity of acetaldehyde in rats. III. Carcinogenicity study. Toxicology 41:213-232.

Yamaguchi T and Yamashita Y (1980) Mutagenicity of hydroperoxides of fatty acids and some hydrocarbons. Agric Biol Chem 44:1675-1678.

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

Zuddas A, Schinelli, Bonuccelli U, Corcini GU and Kopin IJ (1987) Combined treatment with ethanol or acetaldehyde and MPTP: a model of destruction of substantia nigra DA neurons in young mature mice. Proc Soc Neurosci 33:71.

APPENDIX A

RESULTS OF REPRODUCTIVE AND *IN VIVO* DEVELOPMENTAL TOXICITY STUDIES WITH ACETALDEHYDE

TABLE A-1

RESULTS OF *IN VIVO* REPRODUCTIVE AND DEVELOPMENTAL TOXICITY STUDIES WITH ACETALDEHYDE[a]

Species/Strain	Dose	Route of Administration	Duration of Exposure	Results	Comments	Reference
Rat/CF	50, 75, 100 mg/kg	ip	GD: 10, 11, or 12 or 10-12; Sac: D21	↑Resorp; variable in litters with malformations; ↓FW; ↓CRL; ↓TUD; ↓placental wt; ↓rate skeletogenesis	Small sample size, 5-13 litters/group; no dose-response trend. Multiple days of treatment did not increase severity of effect.	Sreenathan et al. 1982
Rat/CF	50, 75, 100, 150 mg/kg	ip	GD: 8-15; Sac: D21	↑Resorp; ↑No. litters with malformations; ↓FW; ↓CRL; ↓TUD; ↓placental wt; ↓in rate of ossification; lesions in placenta	Small sample size, 7-9 dose, ↓in growth parameters all show dose-response.	Padmanabhan et al. 1983
Rat/CF	50 mg/kg	ip	GD: 8-15 Sac: D16-21	Delayed ossification; ↑in litters with malformations	Small sample size, 5-9 litters/group.	Sreenathan et al. 1984
Rat/Sprague-Dawley	100 mg/kg	ip	GD: 9-12 Sac: D12	Reduction in head length	Sample dose size not reported.	Ali and Persaud 1988

TABLE A-1 (continued)

Species/Strain	Dose	Route of Administration	Duration of Exposure	Results	Comments	Reference
Rat/Wistar	0.5 mL of 3% (v/v) solution x2 daily injections (approximately 0-53 mg/kg/inj)	ip	Throughout gestation Sac: D20	↓FW; ↑resorp; ↓litter size	Days of treatment (gestation) not specified; sample size 5-7 litters/group.	Dreosti et al. 1981
Mouse/CD-1	200 mg/kg x5 in 10 hr (total dose 1000 mg/kg)	ip	GD: 10 Sac: D18	No effect on resorptions, FW, or malformations	Small sample size, 8-14 litters/group.	Blakley and Scott 1984a
Mouse/CD-1	200 mg/kg	ip	GD: 10 Sac: 5 min-24 hr postinjection	Maximal acetaldehyde levels within 5 min; undetectable by 2 hr	Acetaldehyde does gain access to fetus.	Blakley and Scott 1984b
Mouse/LACA	2, 4, 6% (approximately 60-480 mg/kg)	ip	GD: 9 Sac: 1-24 hr post-injection	No histopathological changes seen in fetus (light microscopy)	Study limited to light microscopic examination; sample size, 3 dams/group and 3 embryos/dam.	Bannigan and Burke 1982

TABLE A-1 (continued)

Species/Strain	Dose	Route of Administration	Duration of Exposure	Results	Comments	Reference
Mouse/C57BL/6J	320 mg/kg	ip	GD: 6,7, 8, or 9--single injection; 6, 7, 8, or 9--two injections Sac: D18	Nonsignificant ↓FW; slight ↑in % fetuses with head and limb defects	Small sample size, 5-14 litters/group, data set incomplete in that control groups absent for most treatments and FW not reported for each group; makes data essentially uninterpretable.	Webster et al. 1983
Mouse/CFLP	1 or 2% (approximately 40-80 mg/kg)	iv	GD: 7-9 Sac: D10 or D19	No effect on maternal wt gain; dose-related resorp; ↓CRL; ↓FW; ↓PC; ↑No. embryos failing to turn into fetal position (2%), ↑malformations	Small sample sizes/group (7-11) occurrence of terata difficult to evaluate due to small No. litters/treatment. In fact, No. of D10 litters reported in text disagrees with No. in tables.	O'Shea and Kaufman 1979
Mouse/CFLP	0.1 mL (% solution) (approximately 80 mg/kg)	iv	GD: 6, 7, or 8; 6-8; 7-8; or 7-9 Sac: D10 or D12	Day 10 ↑resorp; ↑No. embryos failing to turn to fetal position; ↓CRL and PC; ↑No. malformed	Small sample sizes, approx. 4-9 litters/group; no D12 control groups; no consistent trends seen with ↑No. days of dosing.	O'Shea and Kaufman 1981

TABLE A-1 (continued)

Species/Strain	Dose	Route of Administration	Duration of Exposure	Results	Comments	Reference
Mouse/C57BL/6J xC_3H/He) F_1	62.5, 125, 250 mg/kg	ip	Groups of 5 to 10 male mice were dosed 5 days Sac: 5 weeks after start of treatment	No adverse effects on frequency of micronuclei, count and morphology, and testicular and seminal vesicle weights	The only study available on the toxicity of the male reproductive system, limited number of endpoints specified.	Lahdetie 1988

Abbreviations:

ip = Intraperitoneal.
iv = Intravenous.
GD = Gestation day(s) acetaldehyde was administered.
Sac = Day of pregnancy that dam was sacrificed.
D = Day.
↑ = Increase.
↓ = Decrease.
Resorp = Resorptions.
FW = Fetal weight.
CRL = Crown-rump length.
TUD = Transumbilical distance.
PC = Protein content.

SOURCE: U.S. EPA 1988.

TABLE A-2

RESULTS OF IN VITRO DEVELOPMENTAL TOXICITY STUDIES OF ACETALDEHYDE

Embryo Culture (species/strain)	Dose	Age of Embryo	Results	Comments	Reference
Mouse/C_3H	7.4, 19.7, 39.4 mg/l	Explant: D8 or 9; Examine: 28 hr later	D8 ↓somite count; CNS abnormal; ↓embryonic DNA syntheses. D9: no consistent dose trends	↑rate of development for high-dose group on D9 makes significance of data unclear	Thompson and Folb 1982
Mouse/ICR	17.6 ug/l to 1.7 g/l	Explant: D8.5 Examine: 48 hr later	↑Embryolethality; ↑No. malformed; dose-response in growth retard. (↓DNA and PC; ↓CRL, ↓head length, ↓somite number)	Effects seen at all dose levels (17.6 ug/l)	Higuchi and Matsumoto 1984
Mouse/CF-1	5 to 500 mg/100 ml	Explant: D1.5 Examine: 105 hr	No effects up to 10 mg/100 ml; higher doses lethal	Pre-implantation 2-cell stage mouse embryos highly resistant	Kalmus and Buckenmaier 1989
Rat/Sprague Dawley	5 to 100 uM	Explant: D10 Examine: 25 hr later	Embryolethality at 100 uM growth retard. (↓DNA, ↓PC, ↓CRL, ↓head length)	Effects at all dose levels No dose-response trends for growth retardation	Campbell and Fantel 1983
Rat/Albino	0.20 to 1980 mg/l	Explant: D9.5 Examine: ?	Embryolethality; growth retard. (↓No. somites, ↓PC, ↓cranio-caudal length) ↑malformations	Sample size not provided; dose-response trends present	Popov et al. 1982

TABLE A-2 (continued)

Embryo Culture (species/strain)	Dose	Age of Embryo	Results	Comments	Reference
Rat/Albino	100, 260, and 800 uM	Explant: D10 Examine: Over 48-hr period	No effect at these doses on PC, DNA, somite no., or morphology; embryolethality at 800 uM	Author suggests that differences between his data and those of Campbell and Fantel may be result of using different strains of rats	Priscott 1985

Abbreviations:

D = Day.
= Increase.
= Decrease.
CNS = Central nervous system.
PC = Protein content.
CRL = Crown-rump length.
Retard = Retardation.

APPENDIX B

MUTAGENICITY-GENOTOXICITY TESTING OF ACETALDEHYDE

TABLE B-1

MUTAGENICITY-GENOTOXICITY TESTING OF ACETALDEHYDE

Organism	Metabolic Activation	Concentrations Tested[a]	Reported Result	Reference
GENE MUTATION				
Bacteria				
Salmonella typhimurium (reverse mutations)				
Plate test: Strains				
TA100	-S9	NR[b]	-	Laumbach et al. 1976
TA1535	±S9	0.44 to 110 ug/plate	-	Pool and Wiessler 1981
TA1535, TA1538	-S9	7,938 ug/plate	?[c]	Rosenkranz 1977
Preincubation method: Strains				
TA98, TA100, TA1535, TA1537	±S9	33 to 10,000 ug/plate	-	Mortelmans et al. 1986
TA104, TA102	-S9	1,000 ug/plate	-	Marnett et al. 1985

TABLE B-1 (continued)

Organism	Metabolic Activation	Concentrations Tested[a]	Reported Result	Reference
Escherichia coli (reverse mutation)				
Liquid incubation: Strains				
WP2*uvrA*	-S9	0.9-441 ug/ml	-	Hemminki et al. 1980
	-S9	38.8 ug/ml	+ (no dose-response relationship)	Veghelyi et al. 1978
Yeast				
Saccharomyces cerevisiae (mitochondrial mutations)	-S9	23,490 ug/ml for 30, 60, and 90 min	?[d]	Bandas 1982
Nematodes				
Caenorhabditis elegans (mutations affecting egg-laying)	-S9	783 and 7,830 ug/ml for 2 h	+ at 783 ug/ml (no dose-response relationship)	Greenwald and Horvitz 1980
Insects				
Drosophila melanogaster--Canton S (sex-linked recessive lethals)	--	22,500 ppm (injection of adult males; all germ cell stages treated)	+	Woodruff et al. 1985
		25,000 ppm (feeding)	-	

TABLE B-1 (continued)

Organism	Metabolic Activation	Concentrations Tested[a]	Reported Result	Reference
CHROMOSOMAL ABERRATIONS				
Plants				
Vicia faba (root-tips)		220.5 to 2,205 ug/ml for 24 h at 12 °C	+DR[e] (breaks and trans-locations)	Rieger and Michaelis 1960
Insects				
D. melanogaster--Canton S (heritable translocations)	--	22,500 ppm (injection of adult males; all germ cell stages treated)	-	Woodruff et al. 1985
Mammalian cell culture				
Rat skin fibroblasts	-S9	4.4 to 441 ug/ml for 12, 24, and 48 h	+DR (micro-nuclei)	Bird et al. 1982
		0.44 to 44.1 ug/ml for 12 and 24 h	+ (gaps, breaks, exchange-type aberrations, acentric fragments)	

TABLE B-1 (continued)

Organism	Metabolic Activation	Concentrations Tested[a]	Reported Result	Reference
			+?[f] (aneuploidy)	
Chinese hamster ovary (CHO) cells	-S9	15.66 to 46.98 ug/ml for 24 h	+DR (aneuploidy; chromatid or isochromatid breaks, chromatid exchanges and dicentric chromosomes; polyploidy)	Dulout and Furnus 1988
Human lymphocytes	-S9	7.83 and 15.7 ug/ml for 24 h	+ (gaps, breaks, exchange-type aberrations in Fanconi anemia cells, but was not clastogenic in normal lymphocytes at same dosages)	Obe et al. 1979
Human lymphocytes	-S9	3.97 to 47.6 ug/ml for 72 h	+DR (gaps, breaks, exchange-type aberrations)	Bhlke et al. 1983

TABLE B-1 (continued)

Organism	Metabolic Activation	Concentrations Tested[a]	Reported Result	Reference
Whole mammals				
Female rats/treated embryos (13th day of pregnancy)	--	7,830 ug/ml intra-amniotically	+ (gaps and breaks)	Barilyak and Kozachuk 1983
Hybrid male mice	--	0 to 500 mg/kg, single intraperitoneal (ip) injection	No effect on micronuclei frequency of early spermatids	Lahdetie 1988
SISTER CHROMATID EXCHANGE				
Mammalian cell culture				
CHO cells	-S9	3.92 and 7.83 ug/ml for 8 days	+DR	Obe and Ristow 1977
CHO cells	-S9	2 to 11.7 ug/ml for 24 h	+DR	Obe and Beek 1979
CHO cells	±S9	0.78 to 78 ug/ml--plus S9 0.78 to 39.4 ug/ml--minus S9 for 1 h	+DR (response similar +/- S9)	de Raat et al. 1983

TABLE B-1 (continued)

Organism	Metabolic Activation	Concentrations Tested[a]	Reported Result	Reference
Human lymphocytes	-S9	3.92 to 15.7 ug/ml for 24 h; 15.4 ug/ml for 48 h	+DR	Ristow and Obe 1978
Human lymphocytes	-S9	15.7 and 78 ug/ml for 3 h	+ (the presence of aldehyde dehydrogenase + NAD slightly reduced the response)	Obe et al. 1986
Human lymphocytes	-S9	3.97 to 7.83 ug/ml for 90 h	+DR	Jansson 1982
Human lymphocytes	-S9	3.97 to 47.6 ug/ml for 72 and 96 h	+DR	Bhlke et. al. 1983
Human whole-blood lymphocyte cultures	-S9	2.8 to 88.2 ug/ml for 48 h	+DR	Norrpa et al. 1985
Human lymphocytes	-S9	4.4 to 13.2 ug/ml for 47 and 70 h	+DR	He and Lambert 1985
		26.5 to 105.8 ug/ml for 1 h	+DR	

TABLE B-1 (continued)

Organism	Metabolic Activation	Concentrations Tested[a]	Reported Result	Reference
Whole Mammals				
Male CBA mice	--	0.01 and 0.02 mg/kg (ip injection)	+[g] (almost doubled background frequency at 0.02 mg/kg)	Obe et al. 1979
Male and female Chinese hamsters	--	0.01 to 0.5 mg/kg (ip injection)	+ (almost doubled background frequency at 0.5 mg/kg)	Korte and Obe 1981
OTHER ENDPOINTS INDICATIVE OF DNA DAMAGE				
Bacteria				
E. coli (*polA*$^+$/*polA*$^-$ assay)	-S9	7,830 ug/plate	weakly positive	Rosenkranz 1977

TABLE B-1 (continued)

Organism	Metabolic Activation	Concentrations Tested[a]	Reported Result	Reference
Mammalian cell culture (alkaline elution)				
Rat hepatocytes	-S9	1.3 to 132 ug/ml for 3 h	-	Sina et al. 1983
Human lymphocytes	-S9	441 ug/ml for 4 h	-	Lambert et al. 1985
Human bronchial epithelial cells	-S9	up to 44.1 ug/ml for 1 h	-	Saladino et al. 1985
Mouse lymphoma L5178Y/TK$^{+/-}$ cells	±S9	66 to 1936 ug/ml for 3 h	No increase in single-strand breaks	Garberg et al. 1988

TABLE B-1 (continued)

Organism	Metabolic Activation	Concentrations Tested[a]	Reported Result	Reference
(Cell transformation)				
Rat kidney cells, HRPT[4]	-S9	up to 132 ug/ml	Positive for initiation of cell transformation in the presence of tumor-promoting esters	Eker and Sanner 1986

[a]Source: Dellarco 1988.

[b]NR = Not reported.

[c]? = Although a slight increase in TA1535 revertants (16 revertants in treated versus 4 revertants in controls) was reported, the low background frequency renders the results inconclusive. Also, no dose-response relationship was demonstrated.

[d]? = marginal response that is regarded as equivocal because it occurred in the presence of high toxicity and no dose-response relationship was demonstrated.

[e]DR = Dose-related response.

[f]+? = Questionable positive because data for hyperploidy and hypoploidy were combined.

[g]+ = Positive results considered merely suggestive of an effect because only one mouse per treatment was tested and there was no sham-treated negative controls.

APPENDIX C

IARC AND USEPA CLASSIFICATION SCHEMES FOR CARCINOGENS

(*b*) *Experimental carcinogenicity data*

Data relevant to the evaluation of the carcinogenicity of the agent in animals are summarized. For each animal species and route of administration, it is stated whether an increased incidence of neoplasms was observed, and the tumour sites are indicated. If the agent produced tumours after prenatal exposure or in single-dose experiments, this is also indicated. Dose-response and other quantitative data may be given when available. Negative findings are also summarized.

(*c*) *Human carcinogenicity data*

Results of epidemiological studies that are considered to be pertinent to an assessment of human carcinogenicity are summarized. When relevant, case reports and correlation studies are also considered.

(*d*) *Other relevant data*

Structure-activity correlations are mentioned when relevant.

Toxicological information and data on kinetics and metabolism in experimental animals are given when considered relevant. The results of tests for genetic and related effects are summarized for whole mammals, cultured mammalian cells and nonmammalian systems.

Data on other biological effects in humans of particular relevance are summarized. These may include kinetic and metabolic considerations and evidence of DNA binding, persistence of DNA lesions or genetic damage in humans exposed to the agent.

When available, comparisons of such data for humans and for animals, and particularly animals that have developed cancer, are described.

13. EVALUATION

Evaluations of the strength of the evidence for carcinogenicity arising from human and experimental animal data are made, using standard terms.

It is recognized that the criteria for these evaluations, described below, cannot encompass all of the factors that may be relevant to an evaluation of the carcinogenicity of an agent. In considering all of the relevant data, the Working Group may assign the agent to a higher or lower category than a strict interpretation of these criteria would indicate.

(*a*) *Degrees of evidence for carcinogenicity to humans and to experimental animals and supporting evidence*

It should be noted that these categories refer only to the strength of the evidence that these agents are carcinogenic and not to the extent of their carcinogenic activity (potency) nor to the mechanism involved. The classification of some agents may change as new information becomes available.

(i) *Human carcinogenicity data*

The evidence relevant to carcinogenicity from studies in humans is classified into one of the following categories:

Sufficient evidence of carcinogenicity: The Working Group considers that a causal relationship has been established between exposure to the agent and human cancer. That is, a positive relationship has been observed between exposure to the agent and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence.

Limited evidence of carcinogenicity: A positive association has been observed between exposure to the agent and cancer for which a causal interpretation is considered by the Working Group to be credible, but chance, bias or confounding could not be ruled out with reasonable confidence.

Inadequate evidence of carcinogenicity: The available studies are of insufficient quality, consistency or statistical power to permit a conclusion regarding the presence or absence of a causal association.

Evidence suggesting lack of carcinogenicity: There are several adequate studies covering the full range of doses to which human beings are known to be exposed, which are mutually consistent in not showing a positive association between exposure to the agent and any studied cancer at any observed level of exposure. A conclusion of 'evidence suggesting lack of carcinogenicity' is inevitably limited to the cancer sites, circumstances and doses of exposure and length of observation covered by the available studies. In addition, the possibility of a very small risk at the levels of exposure studied can never be excluded.

In some instances, the above categories may be used to classify the degree of evidence for the carcinogenicity of the agent for specific organs or tissues.

(ii) *Experimental carcinogenicity data*

The evidence relevant to carcinogenicity in experimental animals is classified into one of the following categories:

Sufficient evidence of carcinogenicity: The Working Group considers that a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms (as described on p.23) in (a) two or more species of animals or (b) in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols.

Exceptionally, a single study in one species might be considered to provide sufficient evidence of carcinogenicity when malignant neoplasms occur to an unusual degree with regard to incidence, site, type of tumour or age at onset.

In the absence of adequate data on humans, it is biologically plausible and prudent to regard agents for which there is *sufficient evidence* of carcinogenicity in experimental animals as if they presented a carcinogenic risk to humans.

Limited evidence of carcinogenicity: The data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g., (a) the evidence of carcinogenicity is restricted to a single experiment; or (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the study; or (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential, or of certain neoplasms which may occur spontaneously in high incidences in certain strains.

Inadequate evidence of carcinogenicity: The studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative or quantitative limitations.

Evidence suggesting lack of carcinogenicity: Adequate studies involving at least two species are available which show that, within the limits of the tests used, the agent is not carcinogenic.. A conclusion of evidence suggesting lack of carcinogenicity is inevitably limited to the species, tumour sites and doses of exposure studied.

(iii) *Supporting evidence of carcinogenicity*

The other relevant data judged to be of sufficient importance as to affect the making of the overall evaluation are indicated.

(*b*) *Overall evaluation*

Finally, the total body of evidence is taken into account; the agent is described according to the wording of one of the following categories, and the designated group is given. The categorization of an agent is a matter of scientific judgement, reflecting the strength of the evidence derived from studies in humans and in experimental animals and from other relevant data.

Group 1 — The agent is carcinogenic to humans.

This category is used only when there is *sufficient evidence* of carcinogenicity in humans.

Group 2

This category includes agents for which, at one extreme, the degree of evidence of carcinogenicity in humans is almost sufficient, as well as agents for which, at the other extreme, there are no human data but for which there is experimental evidence of carcinogenicity. Agents are assigned to either 2A (probably carcinogenic) or 2B (possibly carcinogenic) on the basis of epidemiological, experimental and other relevant data.

Group 2A — The agent is probably carcinogenic to humans.

This category is used when there is *limited evidence* of carcinogenicity in humans and *sufficient evidence* of carcinogenicity in experimental animals. Exceptionally, an agent may be classified into this category solely on the basis of *limited evidence* of carcinogenicity in humans or of *sufficient evidence* of carcinogenicity in experimental animals strengthened by supporting evidence from other relevant data.

Group 2B — The agent is possibly carcinogenic to humans.

This category is generally used for agents for which there is *limited evidence* in humans in the absence of *sufficient evidence* in experimental animals. It may also be used when there is *inadequate evidence* of carcinogenicity in humans or when human data are nonexistent but there is *sufficient evidence* of carcinogenicity in experimental animals. In some instances, an agent for which there is inadequate evidence or no data in humans but *limited evidence* of carcinogenicity in experimental animals together with supporting evidence from other relevant data may be placed in this group.

Group 3 — The agent is not classifiable as to its carcinogenicity to humans.

Agents are placed in this category when they do not fall into any other group.

Group 4 — The agent is probably not carcinogenic to humans.

This category is used for agents for which there is *evidence suggesting lack of carcinogenicity* in humans together with *evidence suggesting lack of carcinogenicity* in experimental animals. In some circumstances, agents for which there is *inadequate evidence* of or no data on carcinogenicity in humans but *evidence suggesting lack of carcinogenicity* in experimental animals, consistently and strongly supported by a broad range of other relevant data, may be classified in this group.

References

1. IARC (1977) *IARC Monographs Programme on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Preamble (IARC intern. tech. Rep. No. 77/002)*, Lyon
2. IARC (1978) *Chemicals with* Sufficient Evidence *of Carcinogenicity in Experimental Animals* —. IARC Monographs *Volumes 1-17 (IARC intern. tech. Rep. No. 78/003)*, Lyon
3. IARC (1979) *Criteria to Select Chemicals for* IARC Monographs *(IARC intern. tech. Rep. No. 79/003)*, Lyon
4. IARC (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, Supplement 4, *Chemicals, Industrial Processes and Industries Associated with Cancer in Humans (IARC Monographs, Volumes 1 to 29)*, Lyon
5. IARC (1983) *Approaches to Classifying Chemical Carcinogens According to Mechanism of Action (IARC intern. tech. Rep. No. 83/001)*, Lyon
6. IARC (1973-1984) *Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity*, Numbers 1-12, Lyon

   Number 1 (1973) 52 pages
   Number 2 (1973) 77 pages
   Number 3 (1974) 67 pages
   Number 4 (1974) 97 pages
   Number 5 (1975) 88 pages
   Number 6 (1976) 360 pages

and well-conducted animal studies in different species.

The classifications "sufficient evidence" and "limited evidence" refer only to the weight of the experimental evidence that these agents are carcinogenic and not to the potency of their carcinogenic action.

C. Categorization of Overall Weight of Evidence for Human Carcinogenicity

The overall scheme for categorization of the weight of evidence of carcinogenicity of a chemical for humans uses a three-step process. (1) The weight of evidence in human studies or animal studies is summarized; (2) these lines of information are combined to yield a tentative assignment to a category (see Table 1); and (3) all relevant supportive information is evaluated to see if the designation of the overall weight of evidence needs to be modified. Relevant factors to be included along with the tumor information from human and animal studies include structure-activity relationships; short-term test findings; results of appropriate physiological, biochemical, and toxicological observations; and comparative metabolism and pharmacokinetic studies. The nature of these findings may cause one to adjust the overall categorization of the weight of evidence.

TABLE 1.—ILLUSTRATIVE CATEGORIZATION OF EVIDENCE BASED ON ANIMAL AND HUMAN DATA [1]

Human evidence	Animal evidence				
	Sufficient	Limited	Inadequate	No data	No Evidence
Sufficient	A	A	A	A	A
Limited	B1	B1	B1	B1	B1
Inadequate	B2	C	D	D	D
No data	B2	C	D	D	E
No evidence	B2	C	D	D	E

[1] The above assignments are presented for illustrative purposes. There may be nuances in the classification of both animal and human data indicating that different categorizations than those given in the table should be assigned. Furthermore, these assignments are tentative and may be modified by ancillary evidence. In this regard all relevant information should be evaluated to determine if the designation of the overall weight of evidence needs to be modified. Relevant factors to be included along with the tumor data from human and animal studies include structure-activity relationships, short-term test findings, results of appropriate physiological, biochemical, and toxicological observations, and comparative metabolism and pharmacokinetic studies. The nature of these findings may cause an adjustment of the overall categorization of the weight of evidence.

The agents are categorized into five groups as follows:

Group A—Human Carcinogen

This group is used only when there is sufficient evidence from epidemiologic studies to support a causal association between exposure to the agents and cancer.

Group B—Probable Human Carcinogen

This group includes agents for which the weight of evidence of human carcinogenicity is based on epidemiologic studies is "limited" and also includes agents for which the weight of evidence of carcinogenicity based on animal studies is "sufficient." The group is divided into two subgroups. Usually, Group B1 is reserved for agents for which there is limited evidence of carcinogenicity from epidemiologic studies. It is reasonable, for practical purposes, to regard an agent for which there is "sufficient" evidence of carcinogenicity in animals as if it presented a carcinogenic risk to humans. Therefore, agents for which there is "sufficient" evidence from animal studies and for which there is "inadequate evidence" or "no data" from epidemiologic studies would usually be categorized under Group B2.

Group C—Possible Human Carcinogen

This group is used for agents with limited evidence of carcinogenicity in animals in the absence of human data. It includes a wide variety of evidence, e.g., (a) a malignant tumor response in a single well-conducted experiment that does not meet conditions for sufficient evidence, (b) tumor responses of marginal statistical significance in studies having inadequate design or reporting, (c) benign but not malignant tumors with an agent showing no response in a variety of short-term tests for mutagenicity, and (d) responses of marginal statistical significance in a tissue known to have a high or variable background rate.

Group D—Not Classifiable as to Human Carcinogenicity

This group is generally used for agents with inadequate human and animal evidence of carcinogenicity or for which no data are available.

Group E—Evidence of Non-Carcinogenicity for Humans

This group is used for agents that show no evidence for carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

The designation of an agent as being in Group E is based on the available evidence and should not be interpreted as a definitive conclusion that the agent will not be a carcinogen under any circumstances.

V. References

Albert, R.E., Train, R.E., and Anderson, E. 1977. Rationale developed by the Environmental Protection Agency for the assessment of carcinogenic risks. J. Natl. Cancer Inst. 58:1537–1541.

Crump, K.S., Hoel, D.G., Langley, C.H., Peto, R. 1976. Fundamental carcinogenic processes and their implications for low dose risk assessment. Cancer Res. 16:2973–2979.

Dedrick, R.L. 1973. Animal Scale Up. J. Pharmacokinet. Biopharm. 1:435–461.

Feron, V.J., Gross, H.C., Griesemer, R., Peto, R., Agthe, C., Althoff, J., Arnold, D.L., Blumenthal, H., Cabral, J.R.P., Della Porta, G., Ito, N., Kimmerle, G., Kroes, R., Mohr, U., Napalkov, N.P., Odashima, S., Page, N.P., Schramm, T., Steinhoff, D., Sugar, J., Tomatis, L., Uehleke, H., and Vouk, V. 1980. Basic requirements for long-term assays for carcinogenicity. In: Long-term and short-term screening assays for carcinogens: a critical appraisal. IARC Monographs, Supplement 2. Lyon, France: International Agency for Research on Cancer. pp 21–83.

Freireich, E.J., Gehan, E.A., Rall, D.P., Schmidt, L.H., and Skipper, H.E. 1966. Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey and man. Cancer Chemother. Rep. 50:219–244.

Haseman, J.K. 1984. Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ. Health Perspect. 58:385–392.

Haseman, J.K., Huff, J., and Boorman, G.A. 1984. Use of historical control data in carcinogenicity studies in rodents. Toxicol. Pathol. 12:126–135.

Interagency Regulatory Liaison Group (IRLG). 1979. Scientific basis for identification of potential carcinogens and estimation of risks. J. Natl. Cancer Inst. 63:245–257.

Interdisciplinary Panel on Carcinogenicity. 1984. Criteria for evidence of chemical carcinogenicity. Science 225:682–687.

International Agency for Research on Cancer (IARC). 1982. IARC Monographs on the