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External Review Draft

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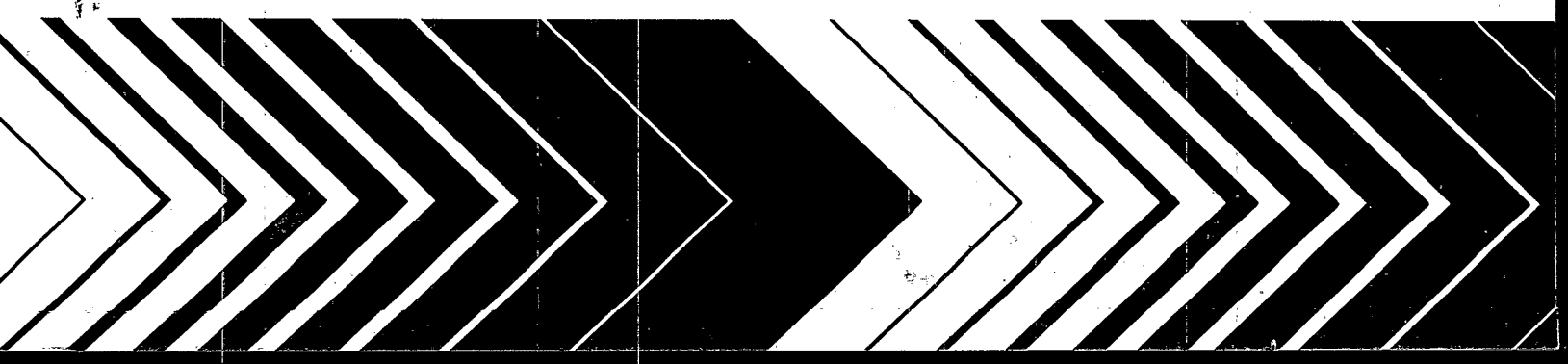
Health Assessment Document for Acetaldehyde

Review Draft

(Do Not Cite or Quote)

NOTICE

This document is a preliminary draft. It has not been formally released by EPA and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy and policy implications.



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U.S. ENVIRONMENTAL PROTECTION AGENCY
Office of Research and Development
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This document is an external draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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PREFACE

The Office of Health and Environmental Assessment has prepared this health assessment to serve as a source document for EPA use. The health assessment was developed for use by the Office of Air Quality Planning and Standards to support decision making regarding possible regulation of acetaldehyde as a hazardous air pollutant.

In the development of the assessment document, the scientific literature has been inventoried, key studies have been evaluated, and summary/conclusions have been prepared so that the chemical's toxicity and related characteristics are qualitatively identified. Observed effect levels and other measures of dose-response relationships are discussed, where appropriate, so that the nature of the adverse health responses is placed in perspective with observed environmental levels. The relevant literature for this document has been reviewed through July 1986.

Any information regarding sources, emissions, ambient air concentrations, and public exposure has been included only to give the reader a preliminary indication of the potential presence of this substance in the ambient air. While the available information is presented as accurately as possible, it is acknowledged to be limited and dependent in many instances on assumption rather than specific data. This information is not intended, nor should it be used, to support any conclusions regarding risk to public health.

If a review of the health information indicates that the Agency should consider regulatory action for this substance, a considerable effort will be undertaken to obtain appropriate information regarding sources, emissions, and ambient air concentrations. Such data will provide additional information for drawing regulatory conclusions regarding the extent and significance of public exposure to this substance.

ABSTRACT

Acetaldehyde, a chemical intermediate in the synthesis of several organic compounds, has an estimated production volume of 200 million pounds per year. Acetaldehyde is highly reactive and oxidized in air, and is ubiquitous in the environment, deriving from natural and anthropogenic sources.

It should be noted that a population exposed to environmental sources of acetaldehyde may be adding to a body burden of this compound produced by normal metabolism and by such life-style habits as cigarette smoking and ethanol consumption. No comparison of the relative magnitude of exposure from these various sources is deemed possible with the available data and, so, is not attempted in this document.

Acetaldehyde is rapidly and completely absorbed and is extensively metabolized to acetate, carbon dioxide, and water in mammalian systems. It readily forms adducts with membranal and intracellular macromolecules; such formation may be associated with its toxicity.

Acute inhalation of acetaldehyde resulted in depressed respiratory rate and elevated blood pressure in experimental animals. Acetaldehyde vapors produced systemic effects and growth retardation in the hamster in a chronic study. No LEL or NOEL has been established. The primary acute effect on humans is irritation of eyes, skin, and respiratory tract.

Acetaldehyde is mutagenic and may pose a risk for somatic cells, but evidence is inadequate with regard to germ cell mutagenicity. Data suggest that acetaldehyde may be a potential developmental toxin; however the majority of studies used parenteral routes of administration. The male and female reproductive toxicity of acetaldehyde has not been characterized. Based on positive carcinogenic responses in rats and hamsters and inadequate epidemiologic evidence, acetaldehyde is considered to be a probable human carcinogen. Using EPA's guidelines for Carcinogen Risk Assessment, acetaldehyde is classified in Group B2. An upper-limit incremental unit risk estimate for continuous lifetime exposure has been derived.

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1. SUMMARY OF HEALTH EFFECTS AND CONCLUSIONS

1.1 BACKGROUND INFORMATION

Acetaldehyde is a saturated aliphatic aldehyde with the chemical formula CH_3CHO. It is a colorless liquid, volatile at room temperature. In liquid form it is lighter than water; the vapors are heavier than air. Acetaldehyde has a pungent and suffocating odor, but at more dilute concentrations the odor is fruity and pleasant. It is used as a flavoring agent.

The vapor pressure of acetaldehyde is very high and it is soluble in water; it would be expected to vaporize from soil into the air, and leach from soil into water. It is readily metabolized by microorganisms. Significant bioaccumulation is unlikely.

Acetaldehyde is highly reactive and is readily oxidized in air. In the presence of a suitable catalyst, acetaldehyde will polymerize to paraldehyde, which is less reactive and volatile.

Chemical analysis in air is by high pressure liquid chromatography of a reaction product with Girard T reagent. The detection limit is 0.325 $\mu g/m^3$.

The current (1985) production of acetaldehyde in the United States is estimated to be 200 million pounds. The predominant use of acetaldehyde is as an intermediate in the synthesis of peracetic acid, pentaerythritol, pyridine, 1,3-butylene glycol, crotonaldehyde, terephthalate, lactic acid, and chloral.

Other uses include production of perfumes, polyester resins, dyes, metaldehyde, and as a food preservative and flavoring agent.

Acetaldehyde is produced from photooxidation reactions, and is an intermediate product of higher plant respiration; it is also formed as a product of incomplete wood combustion in residential fireplaces and woodstoves, burning of tobacco, coffee roasting, and coal refining and waste processing. Acetaldehyde is ubiquitous in the environment; levels up to 32 ppb have been measured in Los Angeles, California. In remote areas levels of 0.3 ppb have been measured.

Acetaldehyde is a component of photochemical smog; the atmospheric half-life is estimated to be 2 to 3 hours. The main product of photooxidation is peroxyacetyl nitrate. Degradation in water or soil would lead to acetic acid.

1.2 MAMMALIAN METABOLISM AND KINETICS OF DISPOSITION

The principal routes of entry of acetaldehyde into the body are by gastrointestinal and inhalation absorption. Acetaldehyde, whether from exogenous sources or generated from ethanol metabolism, is known to be very rapidly and extensively metabolized oxidatively in mammalian systems to a normal endogenous metabolite, acetate, primarily by aldehyde dehydrogenases and is widely distributed in body tissues. Acetate enters the metabolic pool of intermediary metabolism and is used in cellular energy production (end products CO_2 and water) or in synthesis of cell constituents. There are few studies of the kinetics of acetaldehyde of exogenous origin, i.e., from environmental exposure or experimental dosing. It is known, however, that all mammalian species have a high capacity to rapidly and virtually completely metabolize acetaldehyde by most tissues in the body, including the gastrointestinal mucosa and respiratory mucosa and lungs, although hepatic capacity is the highest. After oral or inhalation administration, experimental evidence indicates that a substantial first-pass metabolism in the liver or respiratory organs occurs, effectively limiting acetaldehyde access to the systemic circulation. However, adequate studies have not been conducted to establish dose-metabolism relationships or dose-blood concentration relationships.

Acetaldehyde readily crosses body compartmental membranes into virtually all body tissues, including the fetus, after administration or endogenous generation. Animal experiments have demonstrated a rapid exponential disappearance from circulating blood, consistent with first-order kinetics, with a short half-time of elimination of less than 15 min. Since less than 5 percent escapes unchanged in exhaled breath, and acetaldehyde is not known to be excreted into the urine, the elimination from the body is essentially by metabolism. While these observations suggest that the kinetics of acetaldehyde might best be described by nonlinear Michaelis-Menten kinetics, the high capacity of mammals to metabolize acetaldehyde indicates that even with very large assimilated doses, "saturation" kinetics will not be apparent.

Acetaldehyde is a highly reactive compound; for example, at high inhalation exposure concentrations it readily forms adducts nonenzymatically with membranal and intracellular macromolecules in the respiratory mucosa. Stable and reversible adduct formation including cross-linking have been demonstrated with proteins, nucleic acids (including DNA), and phospholipids. Moreover, even at "physiological levels" (10-150 μmol/L blood), acetaldehyde has been found to form adducts with cellular macromolecules. From these observations, it has been considered that acetaldehyde-adduct formation may play a role in the organ and cellular injury associated with acetaldehyde toxicities, and in the potential promotor or carcinogenic effect assigned to this compound. Acetaldehyde also readily reacts nonenzymatically with cysteine and glutathione to form stable and reversible adducts, respectively. Hence acetaldehyde is an effective depletor of these important cellular nonprotein thiols, which represent a "thiol defense" against the attack of toxic aldehydes and other mutagens and carcinogens.

1.3 MAMMALIAN TOXICITY

Studies with rats and mice showed acetaldehyde to be moderately toxic by the inhalation, oral, and intravenous routes. Acetaldehyde is a sensory irritant that causes a depressed respiration rate in mice. In rats, acetaldehyde increased blood pressure and heart rate after exposure by inhalation and i.v. injection. Acetaldehyde injected intraperitoneally to rats at 200 mg/kg significantly reduced the phospholipid concentration of pulmonary surfactant.

Acetaldehyde vapor at 1500 ppm for 52 weeks produced systemic effects in the hamster: growth retardation, slight anemia, increased UGOT activity, increased urine protein content, increased kidney weights, and histopathological changes in the nasal mucosa and trachea.

Intratracheal instillation of acetaldehyde (2 to 4 percent) to hamsters weekly or biweekly for up to 52 weeks caused severe hyperplastic and inflammatory changes in the bronchioalveolar region of the respiratory tract.

Hamsters exposed to levels of acetaldehyde vapor decreasing from 2500 ppm to 1650 ppm over 52 weeks had lower body weights than controls and distinct histopathological changes in the nose, trachea, and larynx.

Humans are frequently exposed to acetaldehyde from cigarette smoke, vehicle exhaust fumes, or other sources. Metabolism of ethanol would be the major source of acetaldehyde among consumers of alcoholic beverages.

The primary acute effect of exposure to acetaldehyde vapors is irritation of the eyes, skin, and respiratory tract. At high concentrations irritation and ciliastatic effects can occur, which could facilitate the uptake of other contaminants. Clinical effects include erythema, coughing, pulmonary edema, and necrosis. Respiratory paralysis and death has occurred at extremely high concentrations.

1.4 MUTAGENICITY

Acetaldehyde has been shown in studies by several different laboratories to induce sister chromatid exchanges in cultured mammalian cells (Chinese hamster cells and human peripheral lymphocytes) in a dose-related manner. The induction of SCEs by acetaldehyde has also been detected in the bone marrow cells of whole mammals, namely mice and Chinese hamsters. In addition to acetaldehyde's ability to induce SCEs, it has been shown to be a clastogen in mammalian cell cultures and plants. Acetaldehyde produced chromosomal aberrations (micronuclei, breaks, gaps, and exchange-type aberrations) in a dose-related manner. In Drosophila, chromosomal effects (i.e., reciprocal translocations) were not found after acetaldehyde treatment. The clastogenicity of acetaldehyde in whole mammals has not been sufficiently evaluated. In the one study that was available, female rats were intra-amniotically injected on the 13th day of gestation, and the treated embryos had high frequencies of chromosomal gaps and breaks.

Although acetaldehyde did not produce chromosomal translocations in Drosophila, it was found to induce gene mutations (sex-linked recessive lethals) at the same concentration when administered by injection. Positive results for gene mutations were reported in the nematode, Caenorhabditis, and an equivocal result was obtained for mitochrondrial mutations in yeast. Salmonella testing has been reported as negative. There were no available data on the ability of acetaldehyde to produce gene mutations in cultured mammalian cells.

Acetaldehyde has not been shown to cause DNA strand breaks in mammalian cells *in vitro*. However, if acetaldehyde produces SCEs and chromosomal aberrations by DNA-DNA or DNA-protein cross-linking, it may not necessarily produce DNA strand breaks.

In conclusion, there is sufficient evidence that acetaldehyde produces cytogenetic damage (chromosomal aberrations, micronuclei, and sister chromatid exchanges) in mammalian cells in culture. Although there are only three studies in whole mammals, they suggest that acetaldehyde produces similar effects *in vivo*. Acetaldehyde produced gene mutations in Drosophila and Caenorhabditis.

Thus, the available data, taken collectively, indicate that acetaldehyde is mutagenic and may pose a risk for somatic cells. Current knowledge, however, is inadequate with regard to germ cell mutagenicity because of the lack of information on the effects of acetaldehyde in mammalian gonads.

1.5 CARCINOGENICITY

Acetaldehyde has been tested for carcinogenicity in hamsters by intratracheal instillation and inhalation and in rats by subcutaneous injection and inhalation. In the inhalation studies of hamsters, exposure to acetaldehyde induced inflammatory changes, hyperplasia and metaplasia of the nasal, laryngeal, and tracheal epithelium, and tumors of the nose and larynx. In the intratracheal studies of hamsters, acetaldehyde enhanced the development of benzo(a)pyrene-initiated tracheobronchial carcinoma, but there was no evidence of acetaldehyde enhancing the development of diethylnitrosamine-initiated respiratory tract tumors. In one rat injection study, spindle cell carcinomas were produced at the injection site by repeated subcutaneous injections, but the experiment was considered inadequate for evaluation because of the small number of animals and the lack of a control group. One lifetime rat inhalation study showed that acetaldehyde exposure increased the number of animals with nasal tumors, both adenocarcinomas and squamous cell carcinomas, in a exposure-related manner. Adenocarcinomas were increased significantly in both male and female rats at all exposure levels, whereas squamous cell carcinomas were increased significantly in male rats at the middle and high exposure levels and in female rats at the high exposure level only. In addition, exposure-related increases in the incidence of multiple respiratory tract tumors were noted. In the same study in which groups of rats were exposed to acetaldehyde for 52 weeks followed by a recovery period of 52 weeks, the nasal tumor response was similar to that in the lifetime exposure group.

The only epidemiologic study involving acetaldehyde exposure, showed an increased crude incidence rate of total cancer in the workers as compared to the general population. This apparent increase cannot be validated as a real incidence increase because this crude rate was not age adjusted. The study has several other major methodological limitations. Hence, the study is considered inadequate to draw any positive or negative conclusions about the association of acetaldehyde with human cancer.

The repeated positive carcinogenic responses in rats and hamsters, together with supporting evidence of mutagenic activity, alkylating properties, and binding of DNA constitutes a sufficient level of evidence for animal carcinogenicity using EPA's Guidelines for Carcinogen Risk Assessment. Noting that the available epidemiologic data are inadequate for assessment of carcinogenic potential, the totality of the data is classified in EPA's weight of evidence category B2. Category B2 means that acetaldehyde should be considered a probable human carcinogen.

In order to provide a measure of possible impact upon public health, an upper-limit incremental unit cancer risk for acetaldehyde has been quantitatively estimated from nasal cancers observed in the rat inhalation study. The upper-limit incremental unit risk estimate is $q_1^* = 4.0 \times 10^{-3}$ $(ppm)^{-1}$ or $q_1^* = 2.2 \times 10^{-6}$ $(\mu g/m^3)^{-1}$ for a lifetime of continuous inhalation exposure. Because the rat study contains both lifetime and first-half lifetime exposure groups, with similar cancer experience in both groups, these incremental risk estimates can be applied to both types of human inhalation exposure. In terms of relative potency, on a per mole basis, acetaldehyde is the second weakest of 58 chemicals that the CAG has evaluated as suspect carcinogens, and is only about 1/25 to 1/250 as potent as formaldehyde. From a public health perspective, it should be noted that a population exposed to acetaldehyde from environmental sources may be at some additional level of risk due to concurrent cigarette smoking and ethanol consumption which also produce an incremental body burden to acetaldehyde.

1.6 REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

The data, in general, would support labeling acetaldehyde as a developmental toxicant; however, major issues must be resolved in order to do so. Questions remain as to differences that apparently exist between the rat and

mouse and the relevance of data that have been derived from studies employing intraperitoneal or intravenous routes of administration.

While all of the rat data are positive, the mouse data are equivocal. The reason for this species difference is not readily apparent. In the negative mouse studies, animals were injected with acetaldehyde on a single day of organogenesis, whereas the positive mouse studies included multiple days of injection. The rapid clearance of acetaldehyde by the mouse fetus may necessitate more prolonged exposure to produce developmental effects. In the rat studies, positive effects were seen with both single and multiple days of treatment. However, in at least one case, dose-response effects were seen only with more prolonged exposure. Clarification of species differences in pharmacokinetics of acetaldehyde is essential to resolving this conflict. Special attention should be paid to the maternal-fetal unit and the placental in such investigations.

Studies that employ intraperitoneal injections of acetaldehyde provide the opportunity for local uptake of the agent at concentrations that may well exceed those attained and maintained with occupational or environmental routes of exposure. Moreover, given the ubiquitous nature of acetaldehyde dehydrogenase (including placenta and fetus), it is quite likely that acetaldehyde would be rapidly eliminated following such exposures. The extrapolation of risk to the developing human cannot be based upon the current data. However, the data are suggestive enough to support the conduct of appropriate studies to ascertain the developmental toxicity of acetaldehyde before final risk estimations are derived.

There are no data on the effects of direct administration of acetaldehyde, in vivo, on the male reproductive system. Thus, definitive conclusions cannot be drawn at this time as to the potential male reproductive toxicity that might result from such exposure. However, the in vitro data strongly suggest the possibility for such toxicity and support the need for such data to be generated in in vivo systems.

2. INTRODUCTION

This health assessment document for acetaldehyde has been prepared by the Office of Health and Environmental Assessment (OHEA) as a basis for its evaluation of this chemical as a hazardous pollutant. It is intended by the Office to be one of several information sources to guide regulatory strategies of the EPA program offices. The preparation of this document involved the participation of the following groups: the Environmental Criteria and Assessment Office (ECAO/RTP), the Carcinogen Assessment Group (CAG), and the Reproductive Effects Assessment Group (REAG), of the U.S. Environmental Protection Agency. CAG prepared the carcinogenicity section of the document, and REAG prepared the reproductive, teratogenic, and genetic toxicology sections.

The basis of this document was a literature search using the health and environmental effects files in the following data base systems: National Library of Medicine (MEDLARS), Lockheed Information System (DIALOG), and System Development Corporation (ORBIT). The literature that was identified in the search was inventoried, and relevant studies were retrieved, evaluated, and summarized. Each chapter was written to include a summary of the significant aspects of acetaldehyde production, presence in the environment, and/or toxicity.

The major topics included in the document are physical and chemical properties, sampling and analytical methods, production and use, levels and sources in the environment, transport and fate, and biological effects. The discussion of biological effects includes the areas of metabolism and pharmacokinetics as well as mammalian toxicity to organ and tissue systems, carcinogenicity, mutagenicity, teratogenicity, and reproductive effects. Data on the effects of acetaldehyde in humans are also presented.

In the sections on animal toxicity, key studies are presented in a descriptive manner that includes information on the test species, dose or exposure regimen, route of exposure, types of effects seen with each dosage, number of animals in each test and control group, sex and age of the animals, and statistical significance. Information on the purity of the test material is specified

when the data were available. Emphasis is placed on observed effect levels and other measures of dose-response relationships.

This document is intended to serve as a basis for decision-making in the various regulatory offices within EPA as well as to inform the general public of the nature and extent of information available for assessment of health hazards resulting from exposure to acetaldehyde.

3. BACKGROUND INFORMATION

3.1 PHYSICAL AND CHEMICAL PROPERTIES

Acetaldehyde, also known as acetic aldehyde, ethanal, ethyl aldehyde, and methyl formaldehyde, is a saturated aldehyde with the chemical formula CH_3CHO. It is a colorless liquid, volatile at room temperature, and both the liquid and the vapors are highly flammable. Acetaldehyde as a liquid is lighter than water, and the vapors are heavier than air. It is soluble in water, alcohol, ether, acetone, and benzene. Though acetaldehyde has a pungent and suffocating odor, at dilute concentrations it has a fruity and pleasant odor. The conversion factor is 1 ppm = 1.8 mg/m^3 at 25°C and 760 mm Hg. The threshold odor concentration in air is 0.014 mg/m^3 - 0.06 mg/m^3. The physical and chemical properties of acetaldehyde are listed in Table 3-1.

3.1.1 Identification Numbers

The Chemical Abstracts Service (CAS) number is 75-07-0.

3.1.2 Significance of Physical and Chemical Properties with Respect to Environmental Behavior

Water solubility, vapor pressure, octanol/water partition coefficient, and degradation rates are the parameters which most influence the environmental behavior of acetaldehyde. As the vapor pressure of acetaldehyde is very high and it is soluble in water, the most important aspects of environmental behavior will be within the air and water compartments. This is due to vaporization from the soil into the air and leaching from soil into water. Acetaldehyde may, however, remain bound to organic constituents within the soil compartment, because of its high reactivity. Acetaldehyde is also readily metabolized by microorganisms (Versar, 1975). Significant bioaccumulation is unlikely, as acetaldehyde has a low octanol/water partition coefficient (Leo et al., 1971).

TABLE 3-1. PHYSICAL AND CHEMICAL PROPERTIES OF ACETALDEHYDE

Parameter	Value
Molecular weight	44.06
Melting point, °C	-123.50
Boiling point, °C	20.16
Dissociation constant (at 0°C, K_a)	0.7×10^{-14}
Partition coefficient (Log $P_{octanol/water}$)	0.43
Density (specific gravity at 18°C/4°C)	0.783
Volatility (vapor pressure at 20°C)	740 mm Hg
Vapor pressure (at 25°C)	1.23 atm
Refraction index (n_D^{20})	1.33113
Flash point (closed cup, °C)	-38
Flash point (open cup, °F)	-40
Vapor density (air = 1)	1.52
Autoignition temperature, °F	365

Source: American Conference of Governmental Industrial Hygienists (1980); Hagemeyer (1978); Windholz et al. (1983).

3.1.3 Chemical Reactions in the Environment

Acetaldehyde is highly reactive, as the oxygen or hydrogen ions can be easily replaced under the correct conditions (Hagemeyer, 1978). Acetaldehyde is readily oxidized with oxygen or air to form peracetic acid, acetic anhydride, or acetic acid (Hagemeyer, 1978). In the presence of a suitable catalyst, acetaldehyde may undergo rapid polymerization to form paraldehyde, which is much less reactive and volatile (Fairhall, 1957). The polymerization is exothermic and could result in combustion or explosion (Cooke, 1971).

3.2 ANALYTICAL METHODOLOGY

3.2.1 Chemical Analysis in Air

The National Institute of Occupational Safety and Health manual of analytical methods (NIOSH, 1979) describes method number S345 for the analysis

of acetaldehyde levels in air. A known volume of air is drawn through a midget bubbler which contains a solution of Girard T reagent. High pressure liquid chromatography is then used to analyze the acetaldehyde Girard T reagent derivative (NIOSH, 1979).

3.2.1.1 Range. Using a 60 liter sample, a range of 170-670 mg/m^3 was validated at an atmospheric temperature of 21°C and pressure of 756 mm Hg.

3.2.1.2 Detection Limit. The detection limit of this method is approximately 0.325 micrograms acetaldehyde, corresponding to a 50-microliter aliquot of 6.5 mg/mL standard (NIOSH, 1979). The detection limit may be extended by not diluting the sample prior to analysis.

3.2.1.3 Interference. Interference may be significant if the chromatographic conditions are not adjusted to separate other volatile aldehydes or ketones such as formaldehyde, acrolein, and acetone.

3.2.1.4 Precision and Accuracy. The coefficient of variation ($\overline{CV}_T$) for this method is 0.053 for the range of 170-670 mg/m^3. This value corresponds to a 19 mg/m^3 standard deviation at the OSHA standard level (NIOSH, 1979). Collection efficiency of the midget bubbler was found to be at least 0.998 with the range tested, so no collection correction is necessary.

3.2.1.5 Advantages. The acetaldehyde-Girard T reagent derivative has adequate storage stability if protected from light, and the collected samples can be analyzed by a quick instrumental method (NIOSH, 1979).

3.2.1.6 Disadvantages. The Girard T reagent solution must be stored in the dark, and the midget bubbler used in this method is awkward for collecting samples, and difficult to ship.

3.3 PRODUCTION, USE, AND ENVIRONMENTAL RELEASES

3.3.1 Production

The production of acetaldehyde has steadily decreased in the past decade due to a decrease in demand. Although there are three plants capable of producing acetaldehyde in the United States, two owned by Celanese Corporation and one owned by Texas Eastman Company, one or both of the Celanese plants have been on standby since 1981 (SRI, 1984; Mannsville Chemical Products Corp., 1983, 1984). Acetaldehyde can be produced by the oxidation of ethylene by a two-stage method using air or a one-stage method using oxygen. The Hoechst-Wacker two-stage process is the sole production method in the United States.

In this process, ethylene is oxidized by the palladium ion, and the palladium metal is reoxidized by cupric ion. The cuprous ion is then reoxidized to cupric ion with air, and the overall reaction yields CH_3CHO (Mannsville, 1983). Mannsville Chemical Products Corp. (1983) estimates the 1985 acetaldehyde production level in the United States to be 200 million pounds.

3.3.2 Use

The predominant use of acetaldehyde is as an intermediate in the synthesis of other chemicals. The production of acetic acid by the liquid-phase catalytic air oxidation of acetaldehyde has declined because other processes have been found to be more economical (Mannsville Chemical Products Corp., 1983, 1984). In addition to acetic acid, acetaldehyde has been used as a raw material for butyraldehyde, gloxal, glycerin, and vinyl acetate monomer. All now use other raw materials in the United States (Mannsville Chemical Products Corp., 1983). In Mexico, the La Cangrejera Celanese plant still uses acetaldehyde in significant amounts in the production of butyraldehyde and vinyl acetate (Mannsville Chemical Products Corp., 1983).

Acetaldehyde is now used as a raw material in the synthesis and production of peracetic acid, pentaerythritol, pyridine, 1,3-butylene glycol, crotonaldehyde, terephthalate, lactic acid and chloral. Approximately one-fourth of all U.S. acetaldehyde is converted to pentaerythritol, and one-fourth is used as a raw material for pyridine and substituted pyridines. Acetaldehyde is also used as a chemical intermediate in the production of perfumes, polyester resins, basic dyes, and metaldehyde (Windholz et al., 1983; U.S. EPA, 1982). Other uses of acetaldehyde include a fruit and fish preservative, a denaturant for alcohol, in fuel compositions, for hardening gelatin, and as a solvent in the rubber, tanning, and paper industries (Fishbein, 1979; Windholz et al., 1983). Acetaldehyde has also been used as an inhalant in catarrh and ozena (Fairhall, 1957) and in a wide variety of flavor compositions.

3.3.3 Substitute Chemicals/Processes

The production of acetic acid by methanol carbonylation is more economical than the use of the liquid-phase catalytic air oxidation of acetaldehyde. In addition to acetic acid, butyraldehyde, gloxal, glycerin and vinyl acetate monomer are no longer made from acetaldehyde in the United States (Mannsville Chemical Products Corp., 1983).

3.3.4 Environmental Release

3.3.4.1 Natural Releases. Acetaldehyde is produced from aliphatic and aromatic hydrocarbon photooxidation reactions, and peroxyacetyl nitrate (PAN) is a product of the photooxidation (Grosjean, 1982). It is an intermediate product of higher plant respiration. Trace amounts of acetaldehyde may be found in ripe fruit, and acetaldehyde may also be formed when alcoholic beverages are exposed to air (Fishbein, 1979).

3.3.4.2 Combustion. Acetaldehyde is formed as a product of incomplete wood combustion in residential fireplaces and woodstoves (Table 3-2). Ramdahl et al. (1982) estimate acetaldehyde levels of 0.5-992 mg/kg of fuel burned. In 1978, wood combustion accounted for 42 percent of the total national acetaldehyde emissions, and the coffee roasting process accounted for 36 percent of the total (Eimitus et al., 1978). Acetaldehyde is also released through the burning of tobacco, and Braven et al. (1967) estimated releases of 0.77 mg/cigarette smoked. The combustion of organic fuels, coal refining, and coal waste processing (Versar, 1975), release acetaldehyde into the environment. Acetaldehyde levels released through gasoline and diesel exhaust have been estimated at 0.8-4.9 ppm for gas exhaust (Seizinger and Dimitriades, 1972) and 3.2 ppm for diesel exhaust (Vogh, 1969). Acetaldehyde is also a combustion product of plastics (Boettner et al., 1973).

TABLE 3-2. ESTIMATED EMISSIONS OF ACETALDEHYDE TO THE AIR

Source	Emissions (1,000 kg/yr)
Residential wood combustion	5,056.4
Coffee roasting	4,411.5
Acetic acid	1,460.9
Vinyl acetate - from ethylene	1,094.6
Ethanol	57.8
Acrylonitrile	51.6
Acetic acid - from butane	20.8
Crotonaldehyde	4.5
Acetone and phenol from cumene	1.9
Acetaldehyde - hydration of ethylene	0.5
Polyvinyl chloride	0.2
Acetaldehyde-oxidation of ethanol	0.1

Source: Eimutis et al. (1978).

3.3.4.3 Production Processes. Plants which produce acetaldehyde, emit acetaldehyde, as do plants which produce ethanol, phenol, acrylonitrile, and acetone (Eimutis et al., 1978; Mannsville Chemical Products Corp., 1984; Delaney and Hughes, 1979). Chemical processes which involve acetaldehyde as an intermediate also emit acetaldehyde. This includes the production of peracetic acid, pentaerythritol, pyridine, terephtalic acid, 1,3-butylene glycol, and crotonaldehyde.

3.3.5 Environmental Occurrence

Acetaldehyde is ubiquitous in the environment. Environmental levels ranging from 0 to 32 ppb have been measured in the Los Angeles, California vicinity (Grosjean, 1982). Other urban levels are given in Table 3-3. Levels of 0 to 0.3 ppb have been measured in the remote, pristine area of Point Barrow, Alaska (Cavanagh et al., 1969).

TABLE 3-3. URBAN ATMOSPHERE LEVELS OF ACETALDEHYDE IN PARTS PER BILLION

Area	Range	Mean	Reference
Los Angeles, CA	0 - 32[a]	9.1	Grosjean, 1982
Claremont, CA	2.9 - 34.8[b]	14.0	Grosjean, 1982
Tulsa, OK	7 - 8.3	-	Arnts and Meeks, 1980
Nagoya, Japan	1.5 - 9.6	4.7	Hoshika, 1977

[a]30 day collection of 33 samples.
[b]27 day collection of 66 samples.

3.4 ENVIRONMENTAL TRANSPORT AND FATE

3.4.1 Transport

Acetaldehyde is a component of photochemical smog, and as such its movement within the atmosphere corresponds to that of the smog front. The high solubility of acetaldehyde in water increases the likelihood of its being leached into the soil compartment.

3.4.2 Fate

In the atmosphere, acetaldehyde would be degraded through photooxidation and oxidation by the HO radical, with a half-life of 2 to 3 hours (Hendry et

al., 1974; Calvert and Pitts, 1966). The main product of photooxidation is peroxyacetyl nitrate (PAN) (Grosjean, 1982).

Acetaldehyde is believed to be readily degraded in soils, sewage, and natural water systems. The degraded oxidation product is acetic acid. Acetaldehyde within the soil compartment is readily metabolized by microorganisms (Versar, 1975). Bioaccumulation is unlikely because acetaldehyde is readily metabolized (Browning, 1965) and has a low log $P_{octanol/water}$ value of 0.43 (Leo et al., 1971).

Acetaldehyde has been identified by Grosjean and Wright (1983) as a component of several samples of fog, ice fog, mist, cloudwater and rainwater.

3.5 REGULATIONS AND STANDARDS

3.5.1 Occupational Standards

The Occupational Safety and Health Adminstration's time-weighted average (TWA) for acetaldehyde is 200 ppm (360 mg/m^3) (Lewis and Tatkin, 1983). The American Conference of Governmental Industrial Hygienists (1985) have recommended a threshold limit value (TLV) of 100 ppm (180 mg/m^3). This is a time-weighted average for an 8 hour workday and a 40 hour work week. The ACGIH has also recommended a short-term exposure level (STEL) of 150 ppm (270 mg/m^3). This exposure level is based on 15 minute exposures for no more than 4 times/day, provided the time-weighted average is not exceeded.

In the United States, the maximum workplace concentration is 200 ppm (360 mg/m^3), while in West Germany, USSR, and East Germany the maximum workplace concentrations are 180 ppm (100 mg/m^3), 9 ppm (5 mg/m^3), and 200 ppm (360 mg/m^3), respectively (Bittersohl, 1974).

3.5.2 Food Tolerance

The FAO/WHO acceptable daily intake (ADI) of acetaldehyde is 0.0-2.5 mg/kg body weight. As a food additive, the level of use is 1-300 ppm (Doull et al., 1980).

3.5.3 Solid Waste Regulations

Acetaldehyde is listed as a hazardous waste constituent. Generation, treatment, transportation and storage of acetaldehyde must meet the requirements found in the Code of Federal Regulations (1985).

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4. MAMMALIAN METABOLISM AND KINETICS OF DISPOSITION

4.1 INTRODUCTION

Acetaldehyde (ethanal, ethylaldehyde, CH_3CHO) is a common member of the saturated aliphatic aldehydes which, with their related compounds, comprise one of the most important classes of industrial chemicals. In addition to acute and chronic industrial exposure, the multiplicity of human exposure to acetaldehyde (as reviewed in prior chapters) includes such mundane sources as peroral ingestion of fermented foods and beverages (Ribereau-Gayon and Peynaud, 1970) and inhaled tobacco smoke, in which acetaldehyde occurs in a high concentration (Newsome et al., 1965). Acetaldehyde is also an intermediate in several processes of intermediary metabolism (Krebs and Perkins, 1970); indeed, it has been suggested that a normal endogenous level of acetaldehyde exists in blood from intestinal bacterial action and metabolism (Thurman and Pathman, 1975). However, other investigators have failed to detect any such endogenous levels (Cohen and MacNamee, 1976). The small amounts of acetaldehyde that may be introduced or occur in the gut from normal nutrition are known to be rapidly oxidized to acetate in the liver, and consequently it is unlikely that significant amounts of acetaldehyde occur in the body in the absence of exposure to large amounts of exogenous aldehyde or ingestion of alcohol (Krebs and Perkins, 1970). With respect to exposure from tobacco smoking, Lindros (1978) has estimated that normal cigarette smoking (1000 ppm acetaldehyde) might result in 20 µg acetaldehyde per min reaching the lung blood, but he expressed doubt that this small amount could have any systemic detrimental effects (given the rapid endogenous conversion of aldehyde to acetate), except within the lungs themselves. The possibility of local tissue damage at the nasal and lung portal of entry is illustrated by recent findings that long-term daily inhaled acetaldehyde (750 to 3000 ppm) induced squamous cell carcinomas in the nasal respiratory mucosa and adenocarcinomas in the olfactory mucosa of the rat (Woutersen et al., 1984), and that it induced squamous cell carcinomas and other neoplasms in the nasal cavity and larynx of hamsters (Feron et al., 1982). The acute

toxicity of acetaldehyde appears also to be greater when the compound is given by inhalation versus the oral route (Table 4-1).

TABLE 4-1. ACUTE TOXICITY OF ACETALDEHYDE

Species	Route		Dosage (mg/kg)	Time of death (hr)	Reference
Frog	s.c.	LD	800		Supniewski (1927)
Mouse	s.c.	LD_{50}	560	0-24	Skog (1950)
Mouse	i.v.	LD_{50}	244	10 min	Akabane (1960)
Rat	p.o.	LD_{50}	1930 (1620-2240)	0-14	Smyth et al. (1951)
Rat	s.c.	LD_{50}	640	0-1	Skog (1950)
Rat	i.p.	LD_{50}	500	10 min	Skog (1950)
Rat	i.p.	LD_{100}	500	10 min	Stotz et al. (1944)
Rat	i.p.	LD_{100}	280	10 min	Akabane (1960)
Rat	Inhal.	LD_{50}	37 mg/l* of air	0-1	Skog (1950)
Rabbit	s.c.	LD	1200	0-24	Supniewski (1927)
Rabbit	i.v.	LD	300	instant	Supniewski (1927)

Source: Akabane (1960).

*About 18,000 ppm.

Acetaldehyde is also encountered in mammalian systems as the immediate metabolite of ethanol oxidation, and this source certainly represents one of the most prevalent forms of exposure to acetaldehyde. In this instance acetaldehyde is produced endogenously, in an ethanol dose-related manner, primarily by the liver; however, the circulating peripheral blood levels of acetaldehyde are far lower than in the liver and hepatic vein and range from physiological concentrations of 10 to 150 µmol/l after various doses of ethanol (Forsander et al., 1969; Eriksson and Sippel, 1977; Nuutinen et al., 1984). According to Lundquist (1981), the maximum total acetaldehyde content in blood of normal

persons metabolizing ethanol is even lower, about 2 to 3 μmol/l when methodological problems in determining acetaldehyde in blood are considered. Therefore, the concentration of ethanol-generated acetaldehyde in blood and tissues, as for example the lungs, may differ markedly from that occurring from exposure to exogenous acetaldehyde. The emphasis of this review therefore is on the metabolism and disposition of exogenous acetaldehyde, and only where appropriate is reference made to the voluminous literature ethanol-generated acetaldehyde.

Acetaldehyde is a highly reactive compound with a propensity to form Schiff bases with amine groups (O'Donnell, 1982). Its reactivity is primarily due to the difference in polarity of the bond between carbon and oxygen, the oxygen being negative and carbon being positive. Reactions of the carbonyl group usually involve additions to the carbon-oxygen double bond, and, as with $-NH_2$ groups, condensation results in stable and unstable azomethine (C = N-) compounds. In biological systems, these and other reactions of this aldehyde result in covalent binding with adduct formation with cellular macromolecules (nucleic acids, proteins, lipids) leading to impairment of function and cellular damage (Section 4.4.3). Hence, the high chemical reactivity of acetaldehyde and the adducts formed therefrom have importance as a probable mechanism for the toxicity associated with acetaldehyde exposure, including the toxicities associated with acute and chronic alcoholism (Collins, 1985). Other mechanisms, however, of alcohol-induced organ injury have been recently postulated that do not involve acetaldehyde but rather the nonoxidative metabolism of ethanol to fatty acid ethyl esters (Laposata and Lange, 1986).

Acetaldehyde is readily soluble in water and in organic solvents and is more lipid-soluble than ethanol. In water solution 60 percent of acetaldehyde occurs in the hydrated form ($CH_3CH(OH)_2$) (Bell et al., 1956). The equilibrium of hydration affects the nonenzymatic and enzymatic reactions of acetaldehyde since the very polar free carbonyl group is the more reactive species. These physicochemical properties, however, are also the basis for its ready diffusion across membranes at portals of entry into the body -- i.e., gastrointestinal tract and lung -- as well as across membranes of cellular and body compartments. In ambient environmental conditions, acetaldehyde, a volatile liquid, has a high vapor pressure of 760 torr at 20C, and hence inhalation is an important route of entry into the body.

4.2 ABSORPTION

4.2.1 Oral

When given orally, acetaldehyde is readily absorbed from the gastrointestinal tract; however, the dose-absorption relationships have not been fully determined by experiment. The acetaldehyde molecule is small, very little dissociated, and hydrated in aqueous solution. For these reasons, acetaldehyde is freely miscible with water and also relatively lipophilic, and it penetrates easily through biological membranes. Because the liver is a principal site for its oxidative metabolism, acetaldehyde is extracted by the liver from portal blood during absorption and metabolized by the liver, resulting in a marked first-pass effect. It is estimated that the liver is able to metabolize about 70 to 80 percent of the amount metabolized by the whole animal (Hald et al., 1949; Lubin and Westerfeld, 1945). Hence the first-pass effect results in only a small percentage of the dose reaching the systemic circulation. Oxidation rates for acetaldehyde have been estimated for the perfused rabbit liver as 2.0 µmol/min/g liver and 1.1 µmol/min/g rat liver (Hald et al., 1949; Lundquist et al., 1962). Forsander et al. (1969) have shown that the levels of acetaldehyde in the intact rat liver and in the hepatic vein are roughly similar, but the levels of acetaldehyde in peripheral blood are 5 to 6-fold lower. A similar concentration difference has also been observed for humans (Nuutinen et al., 1984). Table 4-1 indicates that the acute oral dose LD_{50} for the rat is large (2 g/kg) and 3- to 4-fold higher than after parenteral administration, i.e., acetaldehyde appears to be considerably less toxic when given by the oral route, presumably because of hepatic metabolism.

4.2.2 Dermal

Acetaldehyde vapor is irritating to the skin and mucous membranes (Babiuk et al., 1985). Applied topically to skin, acetaldehyde is cooling but irritating with a local anesthetic effect which seems to depend on its cooling and irritant properties. However, no studies appear to have been made of dermal absorption.

4.2.3 Pulmonary

The uptake of acetaldehyde into the body from inhalation exposure, or the exchange of acetaldehyde across the lung from alveolar air content to blood, (or from blood to alveolar air), has not been systematically studied. Because

acetaldehyde is a highly volatile liquid easily soluble in water and lipid, ready exchange across the lung is expected. According to one study (Forsander and Tuominen, 1975), the distribution of acetaldehyde between liquid and gas phases varies with the aqueous concentration, but for dilute solutions the ratio of acetaldehyde in solution:vapor in air is about 130; this value provides a rough approximation of the blood:gas solubility coefficient. However, with a half-life of only a few minutes, acetaldehyde is very rapidly metabolized in the body, and it is metabolized actively by the respiratory mucosa as well as by blood itself (Hagihara et al., 1981). Casanova-Schmitz et al. (1984) observed that rats exposed to 0.3 mmol acetaldehyde per liter of air (~7000 ppm) for 2 hr demonstrated only 0.7 mM in circulating blood 5 min after termination of inhalation. This observation suggests that, because of metabolism, the blood concentration during exposure steady-state is some fraction of 130.

Acetaldehyde also exchanges across the lung from blood into expired air. Freund and O'Hollaren (1965), Freund (1967), and Fukui (1969) detected by gas chromatography significant concentrations of acetaldehyde in alveolar air after human ingestion of ethanol, as shown in Figure 4-1. Breath acetaldehyde, which reflects acetaldehyde in alveolar capillaries, changed in parallel with breath and blood ethanol, and presumably with levels of ethanol-generated blood acetaldehyde. Similar experiments have demonstrated acetaldehyde in the breath of rats and mice after ethanol ingestion (Forsander and Sekki, 1974; Redmond and Cohen, 1972).

Dalhamn et al. (1968a,b) and Egle (1970, 1972) have investigated the retention or uptake of inhaled acetaldehyde by the respiratory tracts of human volunteers and dogs. These experiments were designed to determine uptake, not for steady-state inhalation conditions, but for brief periods of acute exposure (minutes) comparable to inhalation of puffs of cigarette smoke. Retention was defined as the percent difference between the amount of acetaldehyde inhaled in the brief exposure period and the amount exhaled. Dalhamn et al. investigated the retention of acetaldehyde (1 mg dose) in 2 second 35-ml puffs (once/min, 16 puffs) of cigarette smoke sucked into the mouth, or completely inhaled into the lungs (mouth or lung absorption) by human subjects. Mouth and lung (total respiratory tract absorption) retention of the dose averaged 60 percent and 99 percent, respectively. Egle conducted similar experiments in man, determining the total respiratory uptake when pure acetaldehyde vapor dispersed in laboratory air was inhaled. The average concentration inhaled was between 0.4 and

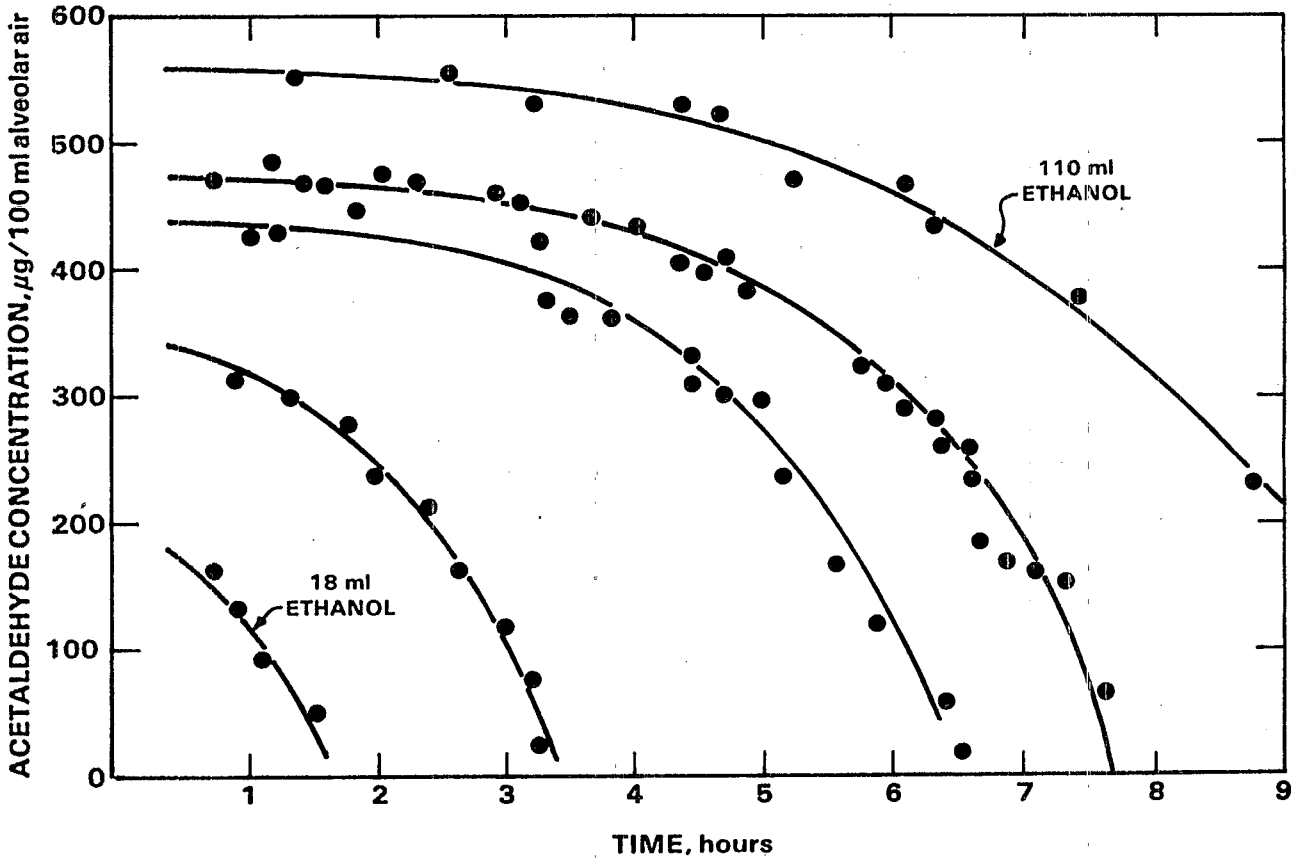

Figure 4-1. Acetaldehyde concentrations in alveolar air generated from the ingestion and metabolism of increasing oral doses of ethanol given to human volunteers.

Source: Freund (1967).

0.6 µg/ml air (200 to 600 ppm). The retention of acetaldehyde for 45- to 75-second exposures declined from 90 percent to 45 percent in a linear manner as the respiratory rate increased from 5 to 40 per min. There was no difference in retention for mouth breathing or nose breathing, but a direct relationship was found between duration of exposure and uptake, which was independent of respiratory rate. Similar results were obtained by Egle in dogs where it was possible experimentally to measure upper and lower respiratory tract retentions. Retention was observed to be higher in the upper tract (nose to endotracheal tube) than lower tract (bronchial tree and lungs). These studies demonstrate significant uptake of inhaled airborne acetaldehyde by both upper and lower respiratory tracts but they do not supply information of pulmonary uptake of acetaldehyde into alveolar blood and hence into the body. A large

component of the measured uptake may be presumed to be metabolized locally by the respiratory mucosal tissues.

4.3 DISTRIBUTION AND EXCRETION

4.3.1 Distribution

Acetaldehyde is known to distribute widely throughout the body tissues after exogenous administration or ethanol administration. Johannsson-Brittebo and Tjalve (1979) have demonstrated by whole-body autoradiography the extent of its distribution in mice using ^{14}C-acetaldehyde injected intravenously. Within 1.0 min, high radioactivity was present in heart muscle, the diaphragm, kidney cortex, gastrointestinal mucosa, the exocrine pancreas, the salivary and lacrimal glands, the bone marrow, the nasal and bronchial mucosa, brown fat, plexus chorioidus, Harder's gland, and the skeletal muscles. The radioactivity in the liver was low. After 5 min the radioactivity in the heart muscle, the diaphragm, and the skeletal muscles had decreased to low levels. Similar distribution pictures were also seen 30 min to 24 hr after administration; this later distribution picture is probably due to tissue metabolism and incorporation of radioactivity into the 2-carbon pool, since the capacity to metabolize acetaldehyde is an attribute of most tissues (Section 4.4). Eriksson and Sippel (1977) surveyed acetaldehyde tissue levels in rats arising from hepatic oxidation of several orally administered doses of ethanol. They found, using GC determination techniques, that levels were highest in liver (male > female), lower in cerebral blood, lower in peripheral blood from the tail, and very low in the brain. The near-absence of acetaldehyde in brain tissue in spite of high levels in cerebral blood suggests the existence of an efficient enzymatic blood barrier to acetaldehyde, perhaps in capillary linings, since there is no indication that the physicochemical properties of acetaldehyde would prevent it from diffusing freely into the brain. Similar studies have been reported by Westcott et al. (1980). The capacity of the brain to metabolize acetaldehyde has been demonstrated by Mukherji et al. (1975), although brain aldehyde dehydrogenase enzyme activities indicate the capacity to be low (Shiohara et al., 1984).

Hobara et al. (1985) determined tissue levels of acetaldehyde in rats immediately after a 1-hr inhalation exposure to high air concentrations of acetaldehyde (1 to 20 mM air; >2,5000 ppm). Acetaldehyde was found in all

tissues; peripheral blood levels were highest (1210 nmol/g); kidney, spleen, heart muscle, skeleton-muscle were much lower (183 to 345 nmol/g); and liver was lowest (55 nmol/g), presumably because of very rapid metabolism by this tissue.

Acetaldehyde crosses the placental barrier into the fetus. Blakley and Scott (1984) studied the kinetics of placental transfer of acetaldehyde in pregnant mice (10 d gestation) following intraperitoneal injection of 200 mg/kg. Maximum acetaldehyde concentrations 5 min later (as determined by GC methods) in embryo and yolk sac were 77 and 12 µg/g tissue respectively, as compared with 185 µg/ml in maternal blood and 176 µg/g maternal liver (Figure 4-2). Acetaldehyde was also found to be transferred across the placenta following ethanol administration. These results indicate that acetaldehyde is accessible to the embryo during the critical period of development.

4.3.2 Excretion

It has long been known that acetaldehyde disappears from the circulating blood very rapidly and exponentially, consistent with first-order kinetics of elimination, after intravenous, intraperitoneal or oral administration to experimental animals (Lubin and Westerfeld, 1945; Hald and Larsen, 1949; Westerfeld et al., 1949). The exponential disappearance of acetaldehyde from the blood after exogenous administration is much faster than the elimination of ethanol, which shows pseudo-zero-order kinetics (Wilkinson et al., 1976). Hence the kinetics of acetaldehyde are difficult to determine in the presence of ethanol, i.e., from blood acetaldehyde levels generated from the administration of ethanol.

For numerous technical reasons, acetaldehyde in blood and tissues has been very difficult to determine with accuracy and precision until the recent advent of gas-chromatograph methods (Lindros, 1978). For this reason and others, there are no extensive studies of the kinetics of acetaldehyde in recent years, however, some pertinent observations have been made incident to other studies. For example, Blakley and Scott (1984), in the course of exploring the kinetics of placental transfer of ethanol and acetaldehyde, administered 200 mg/kg acetaldehyde intraperitoneally to 10-day pregnant rats and determined blood and tissue levels by GC methods. Figure 4-2 shows the exponential and rapid nature of the disappearance of acetaldehyde from the blood with a half-life approximating 15 minutes and with complete clearance in

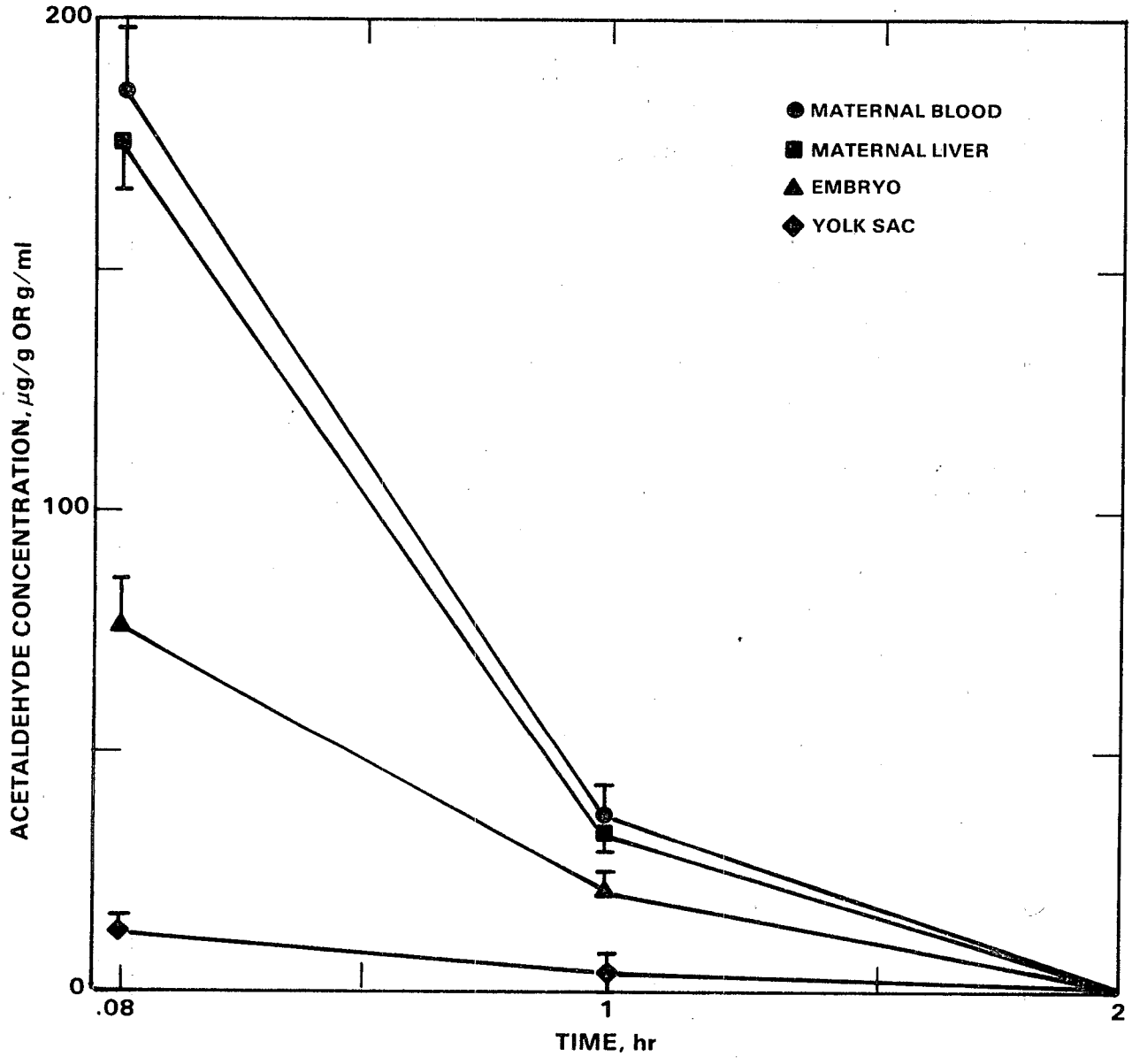

Figure 4-2. Acetaldehyde kinetics after intraperitoneal administration of 200 µg/kg to 10-day pregnant mice. The graph shows the exponential disappearance in blood, liver, embryo and yolk sac. Values are expressed as the mean ± SEM, n = 6 to 11.

Source: Blakley and Scott (1984).

2 hr. Similarly, Shiohara et al. (1984) exposed rats by inhalation to 0.3 mmol acetaldehyde/l air (7000 ppm) for 2 hr/d (4 x 30 min) for 7 d and determined blood levels by GC methods at termination of exposure. Mean acetaldehyde concentrations in the blood were 0.7, 0.2 and 0.1 mM at 5, 15, and 20 min after termination of final acetaldehyde inhalation, respectively. Acetaldehyde was not detected in the blood 40 min after termination of inhalation. These data again demonstrate the rapid first-order kinetics of body elimination of acetaldehyde of an assimilated dose from inhalation exposure. The half-life can be estimated at approximately 10 min with total body clearance within 40 min.

Figure 4-3, from studies of Hobara et al. (1985), further demonstrate the first-order elimination kinetics of acetaldehyde after inhalation exposure. These investigators exposed rats to very high inhalation concentrations of acetaldehyde (1 to 20 mM in air; >25,000 ppm) for 1 hr, at termination of which they determined blood acetaldehyde levels by head-space GC. When blood levels versus time were plotted on semilog paper, linear relationship was obtained (Figure 4-3) in accordance with first-order elimination kinetics. The half-life of elimination approximated only 3 min. Immediately following discontinuation of exposure, blood levels approximated 1.2 mM, i.e. relatively high levels expected only from acute high concentrations during exogenous exposure that exceed lung metabolism. These data provide further support for the suggestion that saturation kinetics are unlikely to occur with even massive exposure to acetaldehyde.

Acetaldehyde is extensively metabolized by most body tissues, with the liver a principal site of metabolism, particularly after oral administration (Section 4.4). A substantial first-pass effect after oral administration has been demonstrated for ethanol-generated acetaldehyde following oral ethanol dosage to man and rodents (Nuutinen et al., 1984; Eriksson and Sippel, 1977). Eriksson and Sippel (1977) estimate that 90 percent of ethanol-generated acetaldehyde in the liver of rats is metabolized in this organ and less than 5 percent is exhaled in breath. Acetaldehyde has not been demonstrated in urine. Perfusion studies have also indicated that liver has a very high capacity to metabolize acetaldehyde (Lindros et al., 1972). Hence the principal route to excretion of acetaldehyde is metabolism. While a compound that is so extensively metabolized is expected to exhibit Michaelis-Menten kinetics and saturation, the high body capacity to metabolize acetaldehyde results in first-order

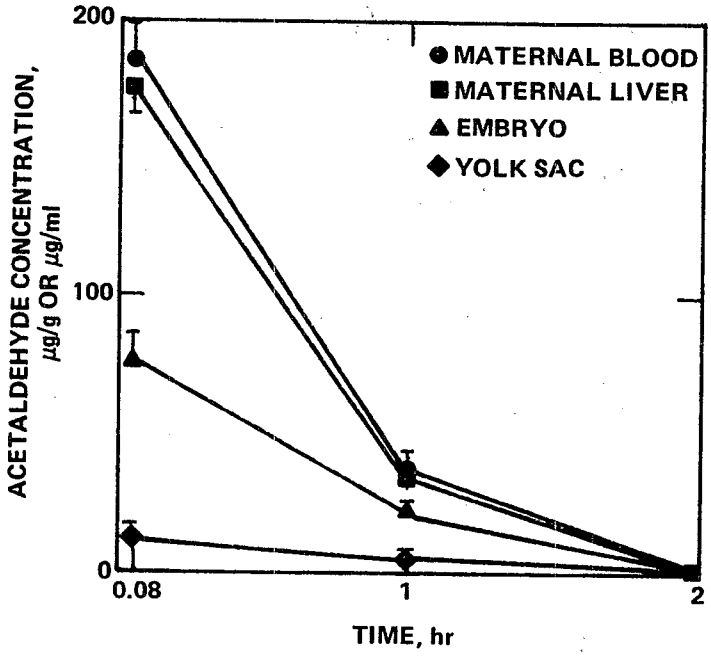

Figure 4-3. Kinetics of acetaldehyde in rats following termination of inhalation exposure for 1 hr (1 to 20 mM in air).

Source: Hobara et al. (1985).

elimination kinetics even with large assimilated doses or subchronic administration as noted above. However, quantitative studies at multiple dose levels designed to determine the kinetics of disposition of acetaldehyde are needed to more fully define the kinetics of the compound, for example, for the conditions of carcinogenicity assays.

4.4 METABOLISM

4.4.1 Quantitation of Metabolism

The extent to which a given dose of acetaldehyde is metabolized by mammalian species has not been fully defined experimentally. Complete balance studies with labeled or nonlabeled acetaldehyde administered by any route have not been reported. Hence dose-metabolism relationships for acetaldehyde are

not available. There is, however, sufficient reason to believe that acetaldehyde is extensively, if not completely, biotransformed by all mammalian species at exposure doses likely to be encountered in the workplace or ambient environment. Furthermore, there is no reason to believe that there are any qualitative differences of significance in the metabolism of acetaldehyde among mammalian species.

Figure 4-4 illustrates that acetic acid is the obligatory oxidation metabolite of acetaldehyde; acetate is further oxidized via the citric acid cycle with generation of ATP and production of cellular energy. Hence CO_2 is the principal end product of acetaldehyde metabolism. Acetate also enters the 2-carbon pool and synthetic pathways, leading to formation of amino acids, fatty acids, sterols, and nucleic acids, and thus is eventually incorporated into cellular constituents. At least for low assimilated doses, only a small percentage is lost intact from the body in exhaled air (<5 percent; Eriksson and Sippel, 1977; Forsander and Sekki, 1974). Unchanged acetaldehyde is not a normal constituent of urine, although acetate may be found in small amounts with acetaldehyde administration. Kallama and Hemminki (1983) injected rats with ^{14}C-acetaldehyde and assessed the urine for radioactivity, and they found less than 6 percent of the dose radioactivity. Acetate or acetate derivatives were identified as the main urinary radioactive metabolites, with 2 percent of radioactivity identified as isomeric cysteine adducts with acetaldehyde, 2-methylthiazolidiene-4-carboxylic acids (see Figure 4-7 and Section 4.4.3).

4.4.2 Enzymic Pathways

Figure 4-4 shows the principal enzyme pathways in the metabolism of acetaldehyde. The primary oxidative reaction by aldehyde dehydrogenase (ALDH) to acetate is followed by the oxidation of acetate by the citric acid cycle. The carnitine shuttle provides a mechanism for transport of acetic acid (and other fatty acids) from cytosolic aldehyde dehydrogenase activity into mitochondria for metabolism by the citric acid cycle.

The disposition of a dose of acetaldehyde given either intraperitoneally, intravenously, or perorally is very rapid and follows first-order kinetics (Section 4.3), indicating that enzyme capacity is not saturated even at high exposure doses. Furthermore, the activity of ALDH, the principal enzyme of aldehyde oxidative metabolism to acetate, is present in most tissues in 4 to 5 times higher activity than alcohol dehydrogenase (ADH), for example in the

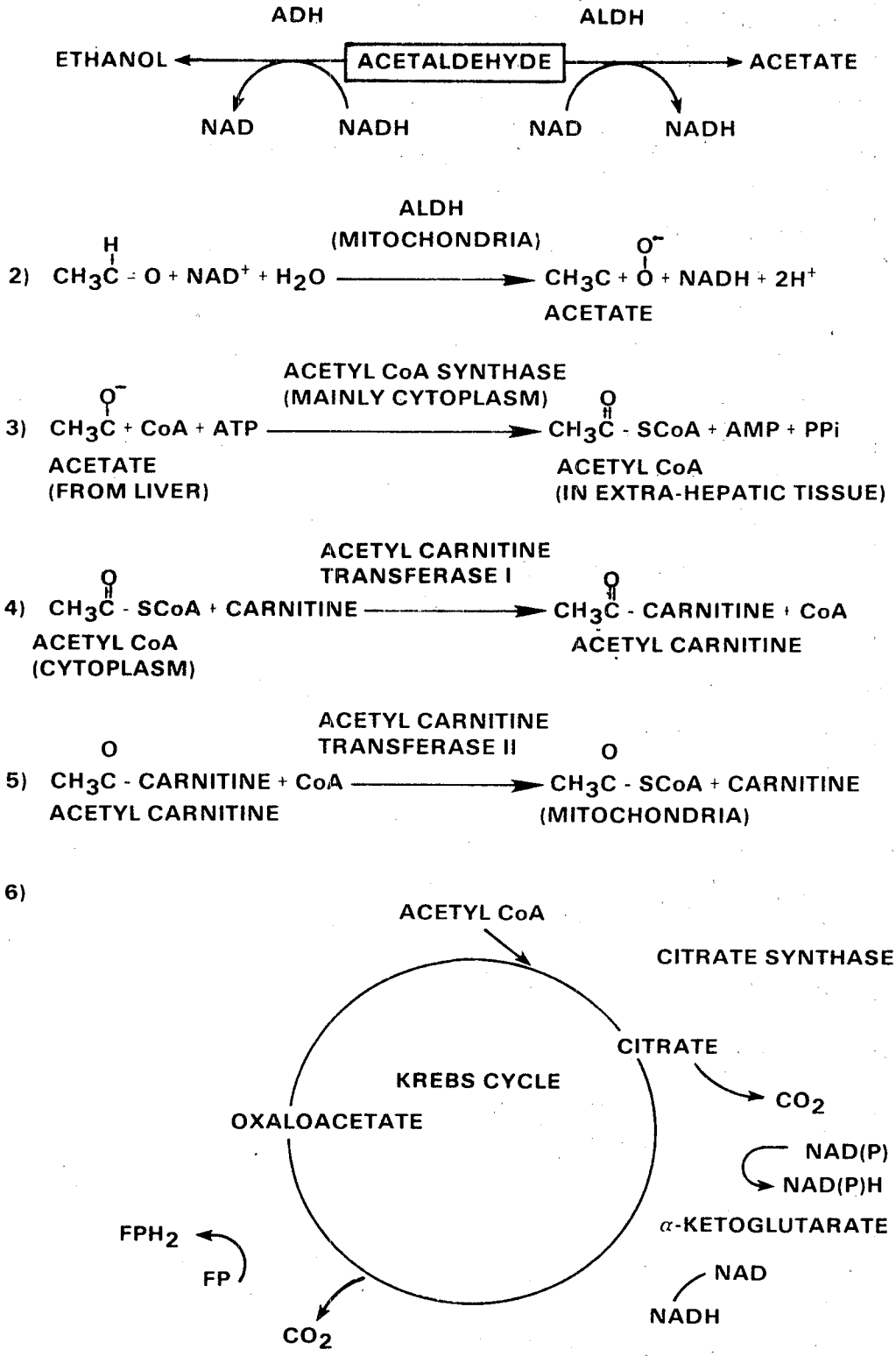

Figure 4-4. Primary pathway of acetaldehyde metabolism. See text for discussion.

Source: Kallama and Hemminki (1983).

liver (Buttner, 1965; Mizoi et al., 1979). However, an exogenous exposure dose of acetaldehyde can be a substrate for both enzymes, a metabolic route that is not available to ethanol-generated acetaldehyde. Thus in the absence of ethanol, acetaldehyde may undergo simultaneous oxidation and reduction by a dismutation process (Dalziel and Dickinson, 1965), as shown in Figure 4-4. In the process the coenzyme NAD that is reduced to NADH in the oxidation of acetaldehyde to acetate is continuously re-oxidized to NAD in the reduction of acetaldehyde to ethanol, and hence the availability of NAD with accumulation of NADH, normally a rate-limiting factor is bypassed. For example, Lindros et al. (1972) have shown in liver perfusion studies that the rate of acetaldehyde uptake was reduced from 15 to 3 μmol/g/min when pyrazole was added to block reduction of acetaldehyde to ethanol by ADH. Consequently, the metabolism of exogenous aldehyde is distinguished by the capacity of the organism to both oxidize and reduce acetaldehyde, whereas production of acetaldehyde from ethanol oxidation is the only significant route of metabolic elimination. Overall the metabolism is eventually oxidative because of the thermodynamic equilibrium of the ADH reaction towards oxidation and the reconversion of reduced acetaldehyde (ethanol) back to acetaldehyde.

Most authors report at least two kinetically distinct forms of ALDH (for example, human liver ALDH): a high K_m cytoplasmic form and a lower K_m mitochondrial form (Greenfield and Pietruszko, 1977). The equilibrium constant reported for the ADH enzyme(s) (Cornell et al., 1979) is

$$K_{eq} = \frac{(\text{acetaldehyde})\ (\text{NADH})\ (\text{H+})}{(\text{ethanol})\ (\text{NAD})} = 1.94 \times 10^{-11}\text{M}$$

and for ALDH enzyme(s) (Burton, 1955) is

$$K_{eq} = \frac{(\text{acetate})\ (\text{NADH})\ (\text{H+})}{(\text{acetaldehyde})\ (\text{NAD+})} = 1.4 \times 10^{-5}\text{M}$$

Since the equilibrium position of the ADH reaction actually favors ethanol formation, while the equilibrium position of the ALDH reaction to an even greater extent favors the conversion of acetaldehyde to acetate, the steady-state level of acetaldehyde must perforce be very low (10^6M lower) relative to both the ethanol and acetate concentrations. Furthermore, the experimental evidence indicates that the V_{max} of ALDH exceeds that of ADH (Dietrich and Siew, 1974), with also a high substrate affinity and conversion of acetaldehyde

to acetate even at very low substrate concentrations (Lundquist, 1981). The steady-state level of acetaldehyde is therefore determined primarily by the kinetics of ALDH, and the thermodynamics of the ALDH reaction dictates very low levels of acetaldehyde, below the K_m for other mammalian enzymes with acetaldehyde as a substrate.

In addition to the NAD-coupled dehydrogenases, a second group of enzymes metabolizing acetaldehyde has been traditionally described, i.e., the flavoprotein-type oxidases, aldehyde oxidase and xanthine oxidase (Gregory et al., 1972; Goodman and Meany, 1974; Weiner, 1980). These oxidases have low affinities for acetaldehyde and hence little acetaldehyde can be expected to be oxidized by these enzymes.

4.4.2.1 Tissue Distribution of Enzymes Metabolizing Acetaldehyde. The liver is known to have a high capacity to metabolize acetaldehyde because of a high ALDH content; however, the enzymes metabolizing acetaldehyde are ubiquitous in the body (Dietrich, 1966). The aldehyde dehydrogenases, as well as aldehyde and xanthine oxidases, are widely distributed in nature and have a broad substrate specificity (Jacoby, 1963). Most mammalian tissues exhibit aldehyde-oxidizing capacity. Although the highest activities are found in the liver, considerable activities are also found in the lung, kidneys, adrenals, gonads, brain, uterus and small intestine (Dietrich, 1966). Aldehyde dehydrogenase located in the red blood cell has been found to be responsible for the acetaldehyde metabolizing capacity of human and rodent blood (Pietruszko and Vallari, 1978; Tottmar et al., 1982; Nuutinen et al., 1984). The rate of uptake and oxidation of acetaldehyde in human blood has been reported to be about 2 μM/ml/min (Tottmar et al., 1982; Nuutinen et al., 1984). With a total volume of 5000 ml, the blood represents a significant metabolic capacity for acetaldehyde elimination (20 μM/min). Casanova-Schmitz et al. (1984) have demonstrated aldehyde dehydrogenases (as two isoenzymes differing in K_m and V_m) in rat nasal mucosa, the major target site for tumorigenesis found in inhalation carcinogenicity assays (Feron et al., 1982; Woutersen et al., 1984). However, repeated exposures to inhaled acetaldehyde (1500 ppm, 6 hr/d, 5 d) did not substantially affect the specific activities of ALDH in the nasal mucosa olfactory and respiratory mucosae. Casanova-Schmitz et al. postulate that ALDH may function as a defense mechanism in the respiratory mucosa, helping to minimize toxic injury resulting from high levels of airborne aldehydes. In further

support of this concept, Bogdanffy et al. (1986) have demonstrated by histochemical localization that the olfactory epithelium is virtually devoid of ALDH activity though the respiratory epithelium is high in activity. These investigators note that this distribution of the enzyme correlates with regional epithelial susceptibility to inhaled acetaldehyde, i.e., the greater resistance of the respiratory compared to the olfactory mucosa for lesion formation of animals exposed chronically to acetaldehyde (Appelman et al., 1982; Woutersen et al., 1984; Feron et al., 1982).

4.4.2.2 Other Pathways. Although oxidation of acetaldehyde by ALDH and aldehyde oxidases accounts for most if not all of acetaldehyde metabolism, several enzyme systems capable of condensation reactions with acetaldehyde have been described, as illustrated in Figure 4-5. The alpha-keto acids pyruvate and ketoglutarate, have been reported to react with acetaldehyde to form acetoin and ketol-hexanoate respectively (Westerfeld, 1949; Alkonyi et al., 1976). Studies in the rat have shown the formation of acetoin and its redox partner, 2,3-butanediol in at least the brain and testes of these animals after ethanol administration and after inhibition of ALDH with disulfiram (Veech et al., 1981). Butanediol has also been found in the blood of alcoholics (Turner et al., 1977; Felver et al., 1980).

4.4.2.3 Induction and Inhibition of ALDH. The question of whether high acetaldehyde substrate concentration or repetitive exposure can result in the induction of ALDH activity has not been clearly resolved. It has been reported that ethanol-generated acetaldehyde from chronic oral administration of ethanol to rats induces mitochondrial aldehyde dehydrogenase activity (Horton, 1971; Horton and Barrett, 1976). However, other investigators have failed to observe any significant induction (Greenfield et al., 1976; Koivula and Lindros, 1975; Redmond and Cohen, 1971). Casanova-Schmitz et al. (1984) found that repeated inhalation exposures of rats to acetaldehyde (1500 ppm, 6 hr/d, 5 d) did not result in the induction of ALDH in the nasal mucosa of rats. Shiohara et al. (1984), however, exposed rats to acetaldehyde by inhalation (7000 ppm) for 2 hr/d for 7 or 14 days and actually found a significant decrease in liver mitochondrial ALDH activity, although brain activity of mitochondrial ALDH remained unchanged. Phenobarbital treatment has been shown to cause induction of cytosolic ALDH, but this occurs only in some species of animals (Dietrich, 1971; Dietrich et al., 1972; Redmond and Cohen, 1971).

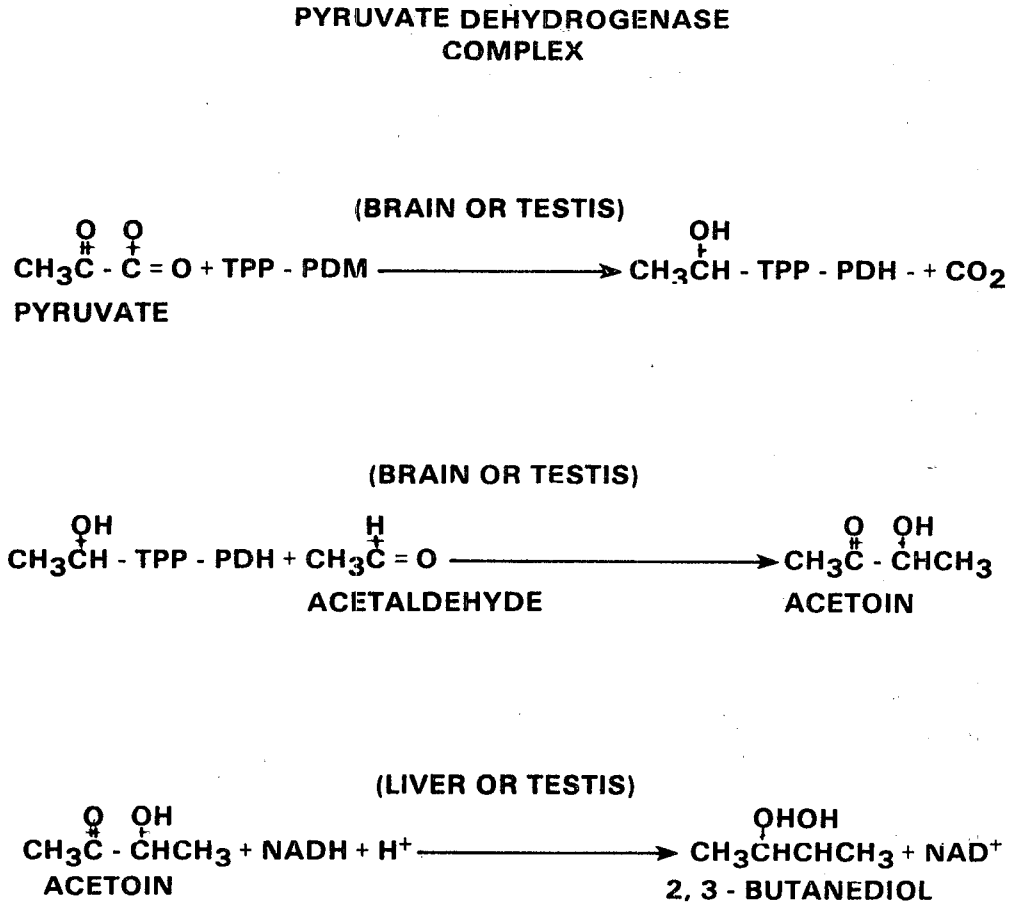

Figure 4-5. Alternate pathway of acetaldehyde metabolism. TPP-PDH is cofactor thiamine pyrophosphate-pyruvate dehydrogenase complex. See text for discussion.

Source: Alkonyi et al. (1976).

A large number of compounds have been found to inhibit ALDH and markedly increase the blood acetaldehyde level (reviewed by Maling, 1970), of which the best known are disulfiram (Antabuse) and calcium carbimide (Temposil), a derivative of cyanamide. The mechanism of the inhibition of ALDH by disulfiram is by competition of the compound with NAD for the enzyme (Dietrich and Hellerman, 1963).

4.4.3 Acetaldehyde - Adduct Formation

Because of the electrophilic nature of its carbonyl carbon, acetaldehyde is highly reactive and has been shown to nonenzymatically bind covalently with

many biologically important molecules. The results may include formation of new adducts, inhibition of critical enzymatic pathways, increases in the levels of existing molecules such as biogenic aldehydes, alterations or perturbations in intracellular and membrane functions, and cell death. Adduct formation falls into two general categories, namely, acetaldehyde adducts involving cellular macromolecules (proteins, nucleic acids and associated membrane structures), and adducts with relatively small molecules and monomers, e.g., glutathione, cysteine, etc.. A substantial portion of acetaldehyde binding with macromolecules is reversible, via labile Schiff bases, which, however, can progress to irreversible or stable binding with further reduction by biological reducing agents such as ascorbic acid (Tuma et al., 1984; Tuma and Sorrell, 1985). Tuma and Sorrell (1985) have proposed the reaction scheme shown in Figure 4-6 to describe the formation of stable acetaldehyde protein adducts via Schiff base intermediates. Schiff bases as the first reaction product are unstable adducts with several fates as shown in Figure 4-6. Schiff bases can dissociate to reform acetaldehyde and protein or undergo an exchange reaction with another amino group. Alternatively, Schiff bases can be stabilized by addition across the double bond either by reduction or nucleophilic addition of a strong nucleophile such as a thiol group. Reduction of the double bond would result in formation of N-ethyl lysine residues in protein. In addition, Schiff bases may react with thiol groups to form stable adducts in proteins. Interaction with a thiol group on the same polypeptide chain may result in an intra-chain covalent product, whereas if the thiol group was present in a different protein, a cross-link may occur.

However, Schiff bases are not the only reaction product possible with acetaldehyde. Other biologically relevant reactions may occur with other nucleophilic groups in proteins (e.g., guanidyl, phenolic). Thiols react with aldehydes to give thio- hemiacetals with, for example, reduced glutathione (GSH); acetaldehyde forms condensation reactions with catecholamines and other biogenic amines, and it forms thiazolidine derivatives with compounds exhibiting adjacent sulfhydryl and amino groups (for example, cysteine) (Figure 4-7). Some of these aspects of acetaldehyde adduct formation are reviewed below.

The concept that binding of aldehydes as well as other xenobiotics, drugs and their metabolites, to cellular macromolecules (proteins and nucleic acids) may be a mechanism of tissue toxicity, perhaps because of impairment of macromolecular function, has been frequently expressed (Hall et al., 1981; Bedford

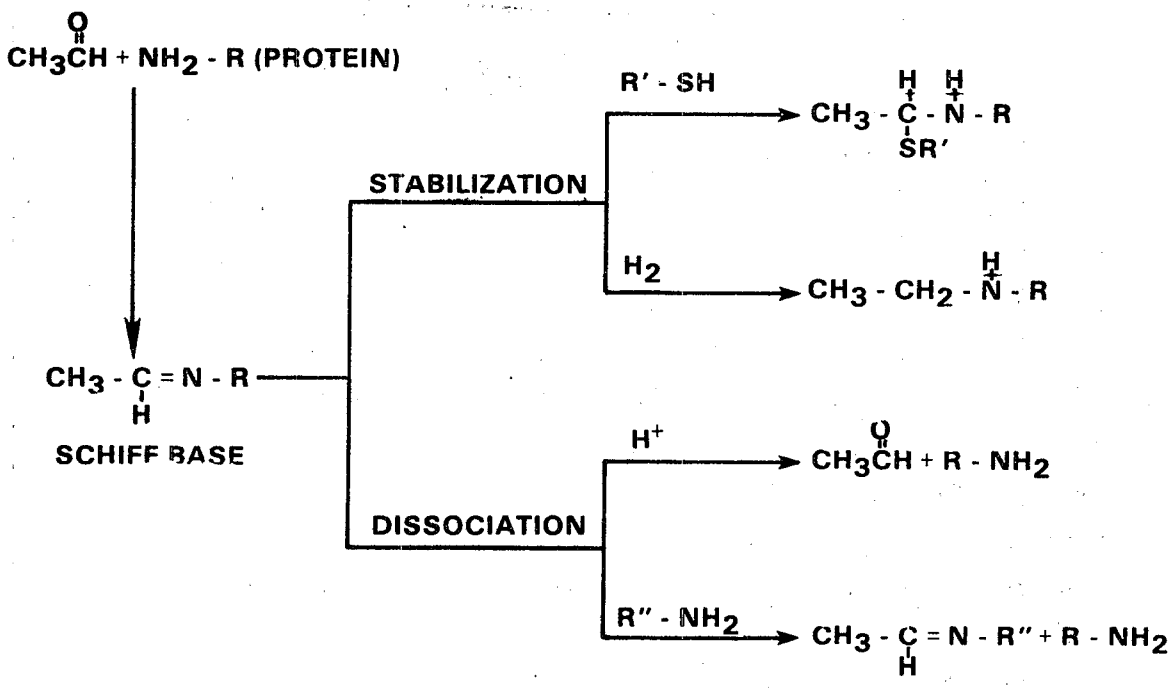

Figure 4-6. Formation of acetaldehyde-protein adducts via Schiff base intermediates.

Source: Tuma and Sorrell (1985).

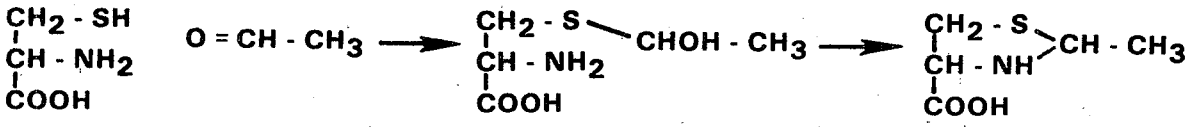

Figure 4-7. Formation of 2-methylthiazolidine-4-carboxylic acid from acetaldehyde and cysteine.

Source: Nagasawa et al. (1980).

and Fox, 1981; Jollow et al., 1973; Reynolds, 1967; Mirvish and Sidransky, 1971; Brodie et al., 1971; Gillette and Pohl, 1977). Acetaldehyde adducts formed during ethanol metabolism have been proposed for a causal role in alcohol liver injury (Tuma and Sorrell, 1985). Lam et al. (1986) have suggested that the acetaldehyde cytotoxicity of the respiratory tract, as evidenced by the development of hyperplasia and metaplasia in the nasal cavity of rats and hamsters exposed to acetaldehyde by inhalation (Appelman et al., 1982; Feron et al., 1982), would increase the rate of cell turnover and increase protein and nucleic adducts and cross-linking and thereby enhance the development of upper respiratory tract squamous cell carcinomas in rodents after preneoplastic changes had occurred (Woutersen et al., 1984; Feron et al., 1982). Acetaldehyde is known also to induce chromosomal damage in Chinese hamster ovarian cells (CHO) in culture (Obe and Ristow, 1977) and in human lymphocytes (Ristow and Obe, 1978; Obe et al., 1979; Bohlke et al., 1983). Thus, genotoxic effects of acetaldehyde might also be involved in the induction of respiratory tract tumors.

4.4.3.1 Protein-Adducts. Acetaldehyde has long been known to react nonenzymatically with protein to form stable derivatives. Mohammad et al. (1949) found that acetaldehyde (in fairly high concentration) reacts rapidly and irreversibly with bovine plasma albumin and amino acids at room temperature in aqueous solutions buffered at pH 7 to 8. These investigators showed that the reaction occurred principally at the amino groups of plasma albumin, and to a lesser extent with guanidyl groups. Furthermore, cross-linking between reactive groups of different protein molecules was observed. More recently, Donohue et al. (1983) have investigated adduct formation with bovine serum albumin with physiological concentrations (0.2 mM) of ^{14}C-labeled acetaldehyde in phosphate buffer, pH 7.4, at 37C. These workers observed both stable and unstable adducts (75 to 85 percent reversible). Stable binding was defined as the radioactivity which remained associated with albumin after rigorous precipitation, resolubilization and washing procedures. Cross-linking was not detected, but it was observed that reversible adducts could undergo secondary rearrangements to form stable bonds, as shown in Figure 4-6. Schiff base formation was considered a principal reaction product and lysine competitively decreased binding. Cysteine, and to a lesser extent glutathione, was a superior competitive compound presumably because of the ability of acetaldehyde to form a stable cyclic thiazolidine with cysteine (Figure 4-7; Nagasawa et al., 1980),

and with glutathione a less stable hemiacetal (Cederbaum and Rubin, 1976a,b). Stable binding to albumin, however, remained irreversible in the presence of these compounds. This group (Tuma et al., 1984) have also shown that biological reducing agents such as ascorbic acid increased stability of Schiff bases to secondary amines and enhanced stable acetaldehyde-adduct formation with albumin, polylysine polymers, lysine-rich histone and cytochrome C. Thus the covalent binding phenomenon appeared applicable to protein as well as other macromolecules.

In fact, acetaldehyde is known to form adducts with the protein globin chains of hemoglobin. Eriksson et al. (1977) observed that acetaldehyde bound to erythrocytes of rat blood in vitro and that the capacity to bind correlated with blood hemoglobin concentrations. These workers estimated binding in the ratio of 4 molecules acetaldehyde:1 molecule hemoglobin. Since rat hemoglobin has four sulfhydryl groups per molecule it was assumed that interaction occurred with these functional groups. Stevens et al. (1981) and Peterson and Nguyen (1985), using ^{14}C-labeled acetaldehyde (0.003 to 3.0 mM), have demonstrated rapid adduct formation with human hemoglobin in erythrocytes, hemolysate and isolated hemoglobin A in vitro in buffer pH 7.0 at 37C. After dialysis, 75 percent of total adduct formation was reversible while 15 to 20 percent was stable. The amount of adducts stable to dialysis was directly proportional to acetaldehyde concentration (Figure 4-8) and also a function of the number of pulses or exposure given at intermittent intervals. The amino acid residues of globin prepared from hemoglobin were identified (by amino acid analysis and radioactivity of the labeled adducts) and found distributed in derivatives corresponding to valine, lysine, and tyrosine. Normal individuals were found to have a low basal level of acetaldehyde-hemoglobin adducts, presumably from low levels of acetaldehyde production from intestinal bacteria and metabolism. However, individuals consuming ethanol, with a 5 to 50 mM blood acetaldehyde level, demonstrated increased level of hemoglobin adducts. Gaines et al. (1977) have found that exposure of hemoglobin to acetaldehyde can also result in cross-linking to give dimers and tetramers of hemoglobin.

Acetaldehyde also forms adducts with protein and lipids of cellular membranes. Gaines et al. (1977) demonstrated irreversible adduct formation and cross-linking of ghost membranes prepared from human erythrocytes in phosphate buffer, pH 8 at 2 to 4C. High-molecular-weight protein was formed by cross-linking. Nomura and Lieber (1981) assessed ^{14}C-acetaldehyde binding to rat

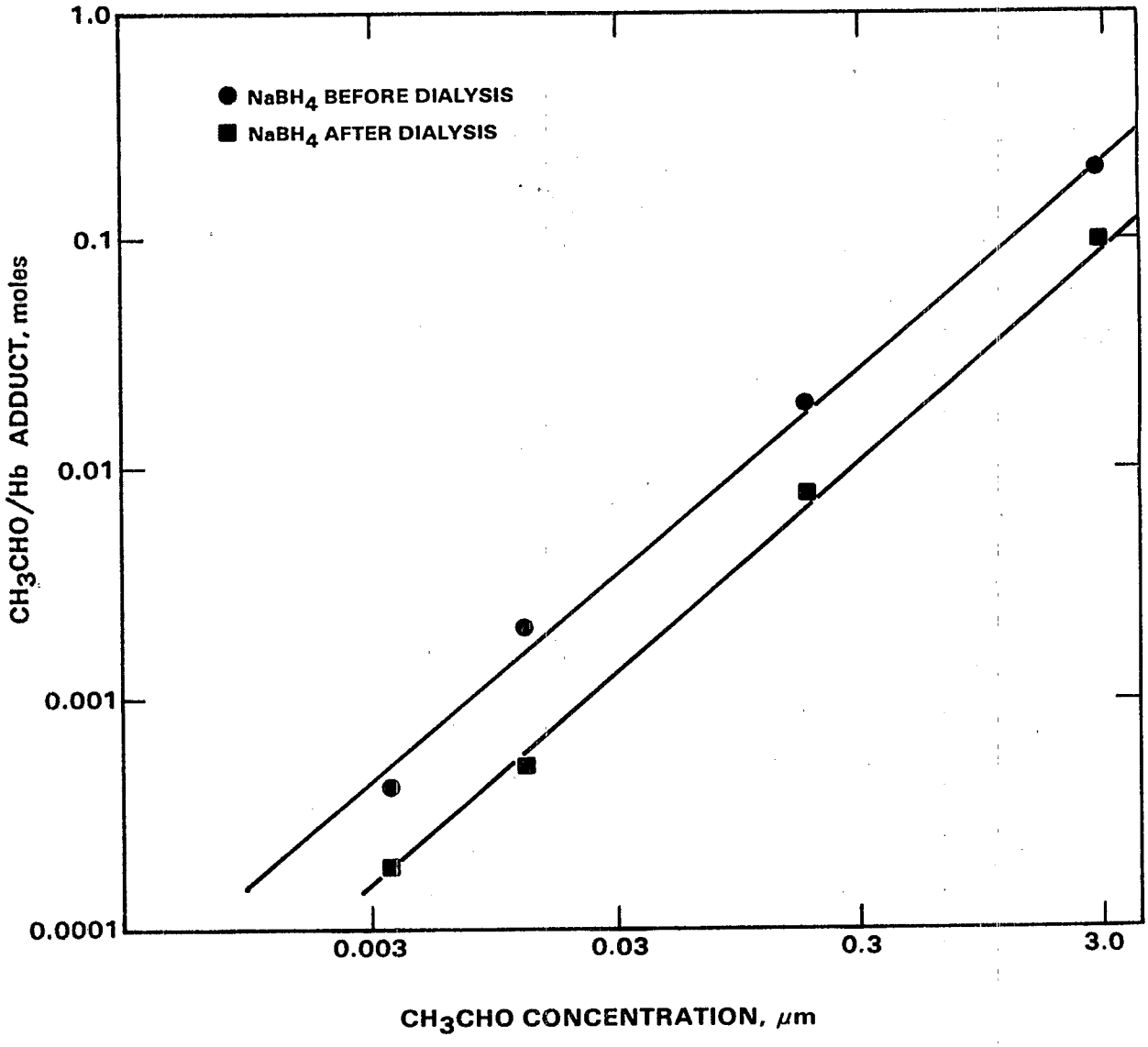

Figure 4-8. The relationship between acetaldehyde concentration of stable hemoglobin adduct formation. Conditions: 37C, pH 7.0 for 30 min. The reaction mixture was dialyzed and reduced with borohydride to form stable adducts.

Source: Stevens et al. (1981).

endoplasmic reticulum (microsomal membranes). Addition of aldehyde (200 μM) to microsomal preparations incubated at 37C, pH 7.4 for 60 min, resulted in binding which was not removed by dialysis or organic solvent extractions, indicating that the molecules which bound acetaldehyde was mostly protein rather than microsomal lipid. Binding was also observed from ethanol-generated acetaldehyde by microsomal oxidation of added ethanol. In this instance, binding was consistently greater than that of equivalent amounts of added

acetaldehyde, presumably because acetaldehyde produced at the surface of the membrane of the endoplasmic reticulum may have greater access to binding sites. Blocking of free amino groups and thiol groups with site-specific reagents (pyridoxal 5'-phosphate and p-hydroxymercuribenzoate) reduced binding, indicating the involvement of these functional groups. Kenney (1982, 1984), however, has reported the formation of Schiff base adducts between acetaldehyde and phospholipid from microsomal membranes. Rat liver microsomes were incubated with ^{14}C-acetaldehyde (0.2 mM) at pH 7.0 and 37C, and then treated with sodium borohydride to reduce the Schiff bases formed, and yielded on extraction stable phospholipid adducts. The adducts were identified as N-ethylphosphatidylethanolamine and N-ethylphosphatidylserine. Barry et al. (1984) used purified rat liver plasma membrane vesicles to assess the binding of acetaldehyde to the proteins and/or lipids of the rat liver plasma membrane. They found that acetaldehyde (<0.1 mM) bound to these lipoprotein membranes also, via Schiff base formation. The binding was concentration-dependent, and Scatchard plots indicated two classes of binding sites with dissociation constants of 2.1 and 140 μM; the high-affinity binding sites were saturated at acetaldehyde concentrations of less than 0.1 μM.

However, Barry et al. (1984) using isolated rat hepatocytes found no evidence that acetaldehyde plasma membrane binding at concentrations up to 10 μM acetaldehyde impaired cellular function (urea synthesis, gluconeogenesis, alanine transport, lactate dehydrogenase leakage). In contrast, it has been amply confirmed that hepatic cell mitochondrial function can be deranged by ethanol-generated acetaldehyde or by exogenous acetaldehyde (Cederbaum and Rubin, 1975; Hasumura et al., 1975, 1976; Koivula et al., 1975), at acetaldehyde levels of 1 to 3 mM. Furthermore, acetaldehyde, in a dose-dependent manner, inhibits incorporation of leucine and other amino acids into isolated liver slices (Perin et al., 1971), and in the perfused guinea pig heart (Schreiber et al., 1972) and isolated cardiac microsomes (Schreiber et al., 1974). Acetaldehyde (0.8 mM) has also been reported to inhibit cardiac membranal Na/P, ATPase activity (Williams et al., 1975), and ATPase activity of human muscle actomyosin (Puszkin and Rubin, 1975).

4.4.3.2 Nucleic Acids Adducts. Hemminki and Suni (1984) have demonstrated that acetaldehyde can form adducts with nucleosides and deoxynucleosides by nonenzymatic reactions in vitro. Acetaldehyde was reacted with guanosine in phosphate buffer, pH 6.5 at 37C for 20 hr, and the mixture then reduced with

sodium borohydride. Three stable adducts were isolated and by NMR spectrometry identified as 1) N^2-ethylguanosine, the principal adduct, and 2) N^2-(3 hydroxybutyl)guanosine, i.e. two diastereomeric compounds formed through aldol condensation of two acetaldehyde molecules. Without reduction with borohydride, acetaldehyde made reversible bonds, presumably Schiff bases, with exocyclic amino groups on adenine, cytosine and guanine bases.

Lam et al. (1986) have investigated the in vitro and in vivo interaction of exogenous acetaldehyde on DNA-protein cross-linking. Incubation of homogenates (20 min, 0C) of rat nasal respiratory mucosa with acetaldehyde (10 to 500 mM) resulted in a concentration-dependent decrease in extractability of DNA. DNA-protein cross-links, as a result of covalent binding, were measured by a decrease in the extractability of DNA from the proteins. The absent DNA can be quantitatively recovered from the proteins after proteolytic digestion. Acetaldehyde was incubated with the homogenates and subsequently DNA was isolated by extraction with chloroform/iso-amyl alcohol/phenol (14/1/25) solvent mixture followed by centrifuging. DNA in the aqueous and interface layers was determined before and after digestion with proteinase K to measure amount of total DNA extractable. A similar demonstration of nucleic acid protein cross-linking was shown with acetaldehyde interaction with calf thymus nucleohistones with consequent decrease of extractability of histone proteins. In vivo effects of acetaldehyde were investigated after acute 6-hr exposure of rats to 100 to 3000 ppm aldehyde in air, or to repetitive exposure to 1000 ppm for 6 hr/d for 5 days. Acute exposure was associated with a concentration-related increase of percentage interfacial DNA extracted from nasal respiratory mucosa (as shown in Figure 4-9), consistent with a decrease of extractability of DNA and evidence for cross-linking. An effect was not evident, however, for olfactory mucosa. With repetitive exposure (1000 ppm), the percentage of interfacial DNA from respiratory mucosa was not increased further, indicating maximal effect with a single acute exposure; for olfactory mucosa, though repetitive treatments did produce cross-linking, while an equivalent acute exposure did not. Lam et al. (1986) suggest that the hyperbolic shape of the dose-interfacial DNA curve for rat respiratory mucosa (Figure 4-9) may be due to acetaldehyde depression of respiratory rate and minute volume as observed by Babiuk et al. (1985), or to saturation of defense mechanisms at low exposure concentrations of acetaldehyde.

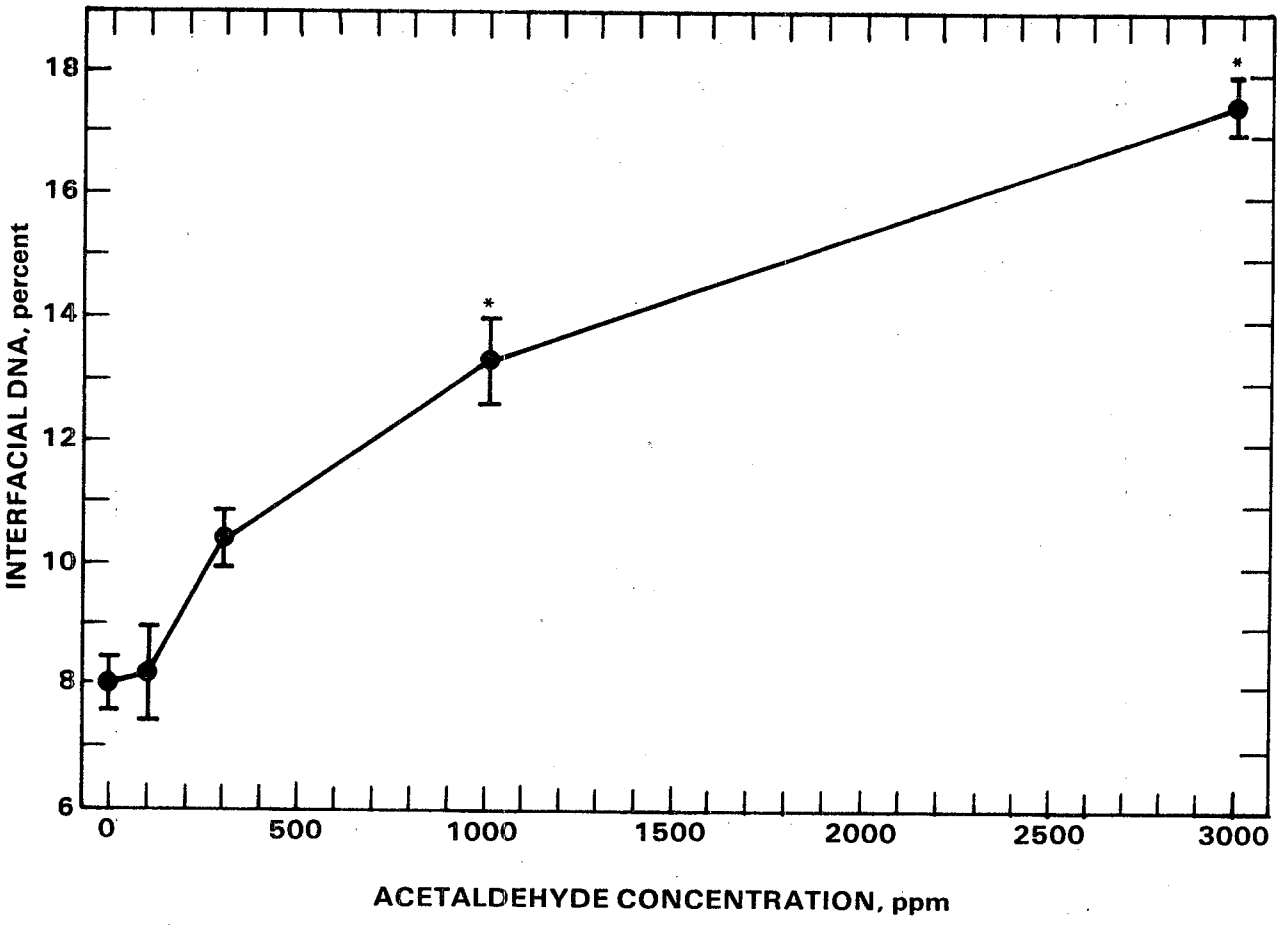

Figure 4-9. Percent interfacial DNA (as measure of cross-linking of DNA-protein) from the respiratory mucosa of rats exposed to 0, 100, 303, 1000 or 3016 ppm or acetaldehyde for 6 hr. Each point is means ± SEM, n = 3 animals per data point.

Source: Lam et al. (1986).

4.4.3.3 Adducts With Small-Molecular-Weight-Thiol Compounds. Aldehydes react nonenzymatically with thiols to give unstable thio-hemiacetals, and thiols with proximal amino groups may form stable thiazolidine derivatives (Nagasawa et al., 1980; Schubert, 1936, 1937; Cederbaum and Rubin, 1976a,b; Vina et al., 1980). Cederbaum and Rubin (1976a,b) found that cysteine, penicillamine, and mercapto-ethylamine protected against mitochondrial injury elicited by acetaldehyde added in vitro; reduced glutathione gave marginal protection, but other sulfhydryl compounds, dithiothreitol and N-acetylcysteine, did not. Similar observations have been made for these sulfhydryl compounds and for their

ability to protect against acetaldehyde-protein adduct formation induced by acetaldehyde (Donohue et al., 1983). Cysteine, N-acetylcysteine, penicillamine, L-cysteine and D,L-homocysteine have been reported to be protective against acetaldehyde toxicity in intact animals (Sprince et al., 1974; Morii et al., 1976; Macdonald et al., 1977). It has been suggested that cysteine complexes with acetaldehyde to form 2-methylthiazolidine-4-carboxylic acid (Figure 4-7), thereby trapping the aldehyde and preventing toxic effects. Both cysteine and penicillamine have a structure with proximal sulfhydryl and amino groups which are necessary for thiazolidine ring closure with acetaldehyde to occur. The thiazolidine derivative of penicillamine has been detected in the urine of rats given penicillamine and then given ethanol to metabolically generate acetaldehyde (Nagasawa et al., 1975).

Nagasawa and co-workers (1980) have examined the structural and other requirements for an acetaldehyde sequestering agent. They nonenzymatically reacted acetaldehyde with a series of polyfunctional thiol compounds in phosphate buffer, pH 7.5 at 37C. Only 1,2- or 1,3-disubstituted aminothiols, namely L-cysteine, D-penicillamine, 1-cysteinyl-1-valine, mercaptoethylglycine, and D,L-homocysteine formed stable thiazolidine derivatives. When tested in vivo in rats, only penicillamine was effective in decreasing blood acetaldehyde (generated by ethanol administration) by trapping acetaldehyde as the water-soluble 2,5,5-trimethylthiazolidine-4-carboxylic acid, which is then readily excreted by the kidneys. Cysteine and the other compounds were ineffective because of rapid in vivo metabolism. Kallama and Hemminki (1983), however, have detected the cysteine adduct in rat urine after administration of acetaldehyde.

Glutathione, which forms a hemiacetal with acetaldehyde (Ketterer, 1982), has a free SH and a free amino group which, however, are further apart than in cysteine, suggesting the necessity for the proximity of both ligands for thiazolidine formation. Glutathione, therefore, is less effective in sequestering acetaldehyde, and instead it has been shown that the thiol-hemiacetal formed is reversible. It has been suggested that the glutathione adduct forms a reservoir for acetaldehyde which can be released for conversion to acetic acid by aldehyde dehydrogenase, and thus restores cellular glutathione levels (Vina et al., 1980). Vina et al. (1980) have demonstrated that acetaldehyde (0.05 to 1.0 mM) added to incubating isolated rat hepatocytes decreased cellular glutathione in a concentration-dependent manner, as shown in Table 4-2, with maximum depletion occurring in 20 min and maintained for at least 60 min.

TABLE 4-2. EFFECT OF ACETALDEHYDE ON GLUTATHIONE (GSH) CONTENT OF ISOLATED HEPATOCYTES IN VITRO (FROM VINA ET AL., 1980).

Acetaldehyde conc. mM	GSH concn. after 60 min incubation μmol/g wet weight	% of control
0	2.5 ± 0.3(7)	100
0.05	1.8 ± 0.4(3)	70
0.10	1.6 ± 0.5(3)	64
1.00	1.4 (2)	57

Ethanol (10 and to 40 mM) also depleted glutathione, and since depletion was prevented by pyrazole (inhibitor of alcohol dehydrogenase), it can be assumed that depletion was due to metabolic generation of acetaldehyde. Large single doses of ethanol to intact mice and rats have been found to deplete hepatic glutathione content in a dose-related manner. Maximum depletion occurs 6 to 8 hr after ethanol administration and is maintained for at least 16 hr (Takada et al., 1970; Macdonald et al., 1977).

Reduced glutathione is the major nonprotein thiol of the cell. Hence, glutathione may be of some consequence in modulating acetaldehyde toxicity by hemiacetal adduct formation. Similarly, Braven and colleagues (Braven et al., 1967; Fenner and Braven, 1968) have suggested that free cellular cysteine represents a thiol-defense against the attack of acetaldehyde and other mutagens and carcinogens.

4.4.3.4 Other Adducts. Acetaldehyde is well known to form adducts nonenzymatically with a variety of small-molecular-weight compounds of physiological importance. The adducts produced are irreversible (or nearly so), owing to rapid internal cyclization steps from nascent or transient Schiff bases. Thus acetaldehyde forms adducts with biogenic amines; norepinephrine, serotonin, and dopamine (Truitt and Walsh, 1971); enkephalins and related peptides (Summers, 1985); and the cofactor tetrahydrofolate (Guynn et al., 1982). The role of these adducts in the toxicity of acetaldehyde are unclear. An extensive review of these and other adducts has been the subject of a recent symposium (Collins, 1985).

Acetaldehyde has been shown to inhibit specific enzyme activities possibly by adduct formation with the catalytic site or by other means. Inhibition has

been demonstrated for pyruvate dehydrogenase (Blass and Lewis, 1973; Alkonyi et al., 1976), isocitrate dehydrogenase (Fan and Plaut, 1974), phosphoenol-pyruvate carboxykinase (Baxter, 1976), and retinol and alcohol dehydrogenase (Grisolia et al., 1975).

4.5 SUMMARY

The principal routes of entry of acetaldehyde into the body are by gastrointestinal and inhalation absorption. Acetaldehyde, whether from exogenous sources or generated from ethanol metabolism, is known to be very rapidly and extensively metabolized oxidatively in mammalian systems to a normal endogenous metabolite, acetate, primarily by aldehyde dehydrogenases widely distributed in body tissues. Acetate enters the metabolic pool of intermediary metabolism and is used in cellular energy production (end products CO_2 and water) or in synthesis of cell constituents. In contrast to the situation for acetaldehyde generated from ethanol metabolism, there are few studies of the kinetics of acetaldehyde of exogenous origin, i.e. from environmental exposure or experimental dosing. It is known, however, that all mammalian species have a high capacity to rapidly and virtually completely metabolize acetaldehyde by most tissues in the body, including the gastrointestinal mucosa and respiratory mucosa and lungs, although hepatic capacity is the highest. After oral or inhalation administration, experimental evidence indicates that a substantial first-pass metabolism in the liver or respiratory organs occurs, effectively limiting acetaldehyde access to the systemic circulation. However, adequate studies have not been conducted to establish dose-metabolism relationships, or dose-blood concentration relationships.

Acetaldehyde readily crosses body compartmental membranes into virtually all body tissues, including the fetus, after administration or endogenous generation. Animal experiments have demonstrated a rapid exponential disappearance from circulating blood, consistent with first-order kinetics, with a short half-time of elimination of less than 15 min. Since less than 5 percent escapes unchanged in exhaled breath, and acetaldehyde is not known to be excreted into the urine, the elimination from the body is essentially by metabolism. While these observations suggest that the kinetics of acetaldehyde might best be described by nonlinear Michaelis-Menten kinetics, the high capacity of mammals to metabolize acetaldehyde indicates that even with very large assimilated dose, saturation kinetics will not be apparent.

Acetaldehyde is a highly reactive compound, and at high concentrations incident, for example, at the respiratory mucosa with inhalation exposure, it readily forms adducts nonenzymatically with membranal and intracellular macromolecules. Stable and reversible adduct formation including cross-linking have been demonstrated with proteins, nucleic acids (including DNA), and phospholipids. Moreover, even at physiological levels (10 to 150 μmol/l blood), acetaldehyde has been found to form adducts with cellular macromolecules. From these observations, it has been considered that acetaldehyde-adduct formation may play a role in the organ and cellular injury associated with acetaldehyde toxicities, and in the potential promoter or carcinogenic effect assigned to this compound. Acetaldehyde also readily reacts nonenzymatically with cysteine and glutathione to form stable and reversible adducts, respectively. Hence acetaldehyde may be a effective depleter of these important cellular nonprotein thiols, which represent a thiol defense against the attack of toxic aldehydes and other mutagens and carcinogens.

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5. MAMMALIAN TOXICITY

5.1 Acute Toxicity

The results of acute toxicity studies, by inhalation, oral, and the intravenous route, with acetaldehyde are shown in Table 5-1. The acute oral LD_{50} of acetaldehyde ranged from 1232 mg/kg to 5300 mg/kg. The LD_{50} for subcutaneous injection ranged from 560 mg/kg to 640 mg/kg. The acute inhalation LC_{50} was 20,000 ppm in rats exposed to acetaldehyde for 30 minutes. In another study, 4000 ppm for 4 hours killed some exposed rats. The following sections will discuss these acute toxicity studies in more detail.

5.1.1 Inhalation

The sensory irritant effect of acetaldehyde was studied in Swiss-Webster mice by recording the degree of respiratory rate depression (Kane et al., 1980). Groups of four animals received head-only exposures to varying concentrations of acetaldehyde for 10 minutes. From the concentration-response relationship, the RD_{50} (the concentration that produced a 50% decrease in respiration rate) was calculated to be 4946 ppm. In another study, the RD_{50} of acetaldehyde for mice was reported to be 2845 ppm (Barrow, 1982). Histopathology was not done in either of these studies. Also, RD_{50} values do not demonstrate or predict toxic effects, but indicate a biologic response to the chemicals, and are useful in comparing relative potencies of chemicals as irritants and in establishing threshold limit values (TLV's); therefore, these studies do not substitute for an inhalation toxicology study (Kane et al., 1979). The current TLV for acetaldehyde is 100 ppm (American Conference of Governmental Industrial Hygienists, 1980), and is between 0.1 and 0.01 times the cited RD_{50} values.

Changes in arterial blood pressure and heart rate were measured in anesthetized rats which were exposed to 0.5 - 30 µg/ml (278-16680 ppm) acetaldehyde (Egle, 1972). Significant increases were seen in blood pressure at 3.0 µg/ml (1668 ppm) and higher concentrations. Concentrations at 12 and 25 µg/ml (6672 and 13900 ppm) significantly increased heart rate (Table 5-2).

TABLE 5-1. ACUTE TOXICITY STUDIES OF ACETALDEHYDE

Species	Number and Sex Per Dose	Route of Administration	Dosage Eliciting Toxic Effect	Reference
Rats	-	oral	LD_{50} = 1900 mg/kg	Cited in Windholz et al. (1983)
	-	oral	LD_{50} = 1930 mg/kg	Cited in Lewis and Tatkin (1983)
	-	oral	LD_{50} = 5300 mg/kg	Omel'yanets et al. (1978)
	-	subcutaneous	LD_{50} = 640 mg/kg	Skog (1950)
	-	inhalation	LC_{50} = 20,000 ppm/30 min	Skog (1950)
	-	inhalation	LC_{Low} = 4,000 ppm/4 hours	Cited in Lewis and Tatkin (1983)
Mice	-	oral	LD_{50} = 1232 mg/kg	Cited in National Research Council (1977)
	-	subcutaneous	LD_{50} = 560 mg/kg	Skog (1950)
	4 males	inhalation	LC_{50} not determined RD_{50} = 4946 ppm	Kane et al. (1980)
	-	inhalation	LC_{50} not determined RD_{50} = 2845 ppm	Barrow (1982)
Guinea pigs	16 (both male and female)	intravenous	20 mg/kg	Mohan et al. (1981)

- Indicates that the data were not reported in the literature.

TABLE 5-2. EFFECTS OF INHALATION OF ACETALDEHYDE ON BLOOD PRESSURE AND HEART RATE IN RATS

Acetaldehyde Concentration (μg/ml)	Number of Rats	Number of Exposures	Blood Pressure % change ± S.E.	P	Heart Rate % change ± S.E.	P
0	7	24	2.5 ± 2.3	NS	1.6 ± 0.9	NS
1.0	9	38	0.5 ± 1.3	NS	1.4 ± 0.7	NS
3.0	9	38	5.5 ± 1.8	<.01	0.3 ± 0.9	NS
10.0	9	38	9.6 ± 1.5	<.01	0.8 ± 0.9	NS
12.0	6	18	16.6 ± 3.3	<.01	2.3 ± 1.0	.05
25.0	6	22	21.4 ± 4.2	<.01	3.4 ± 1.0	.01
30.0	8	24	24.0 ± 3.0	<.01	3.0 ± 1.0	NS

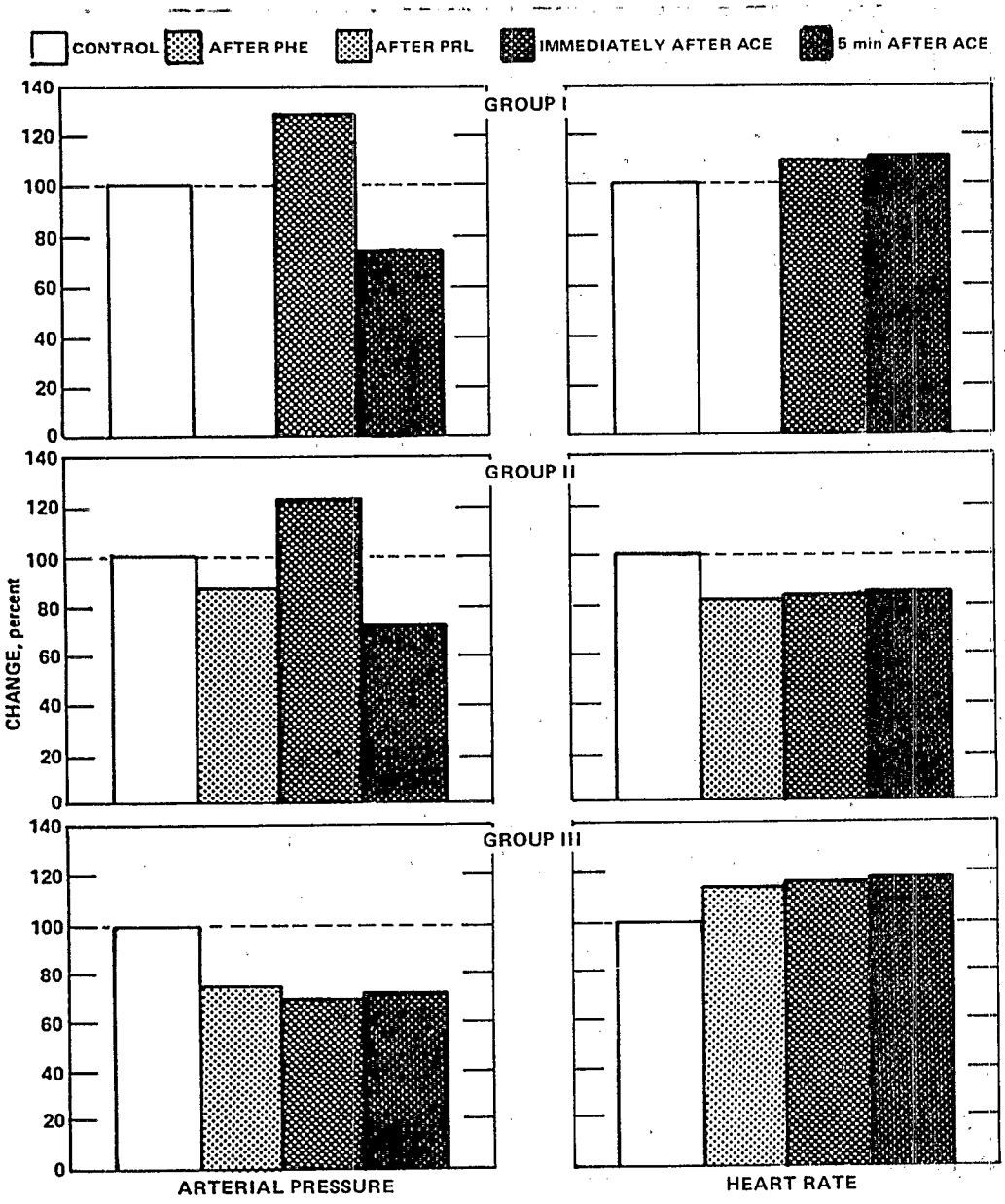
NS = not significant.

Source: Egle (1972).

5.1.2 Intravenous

A single intravenous dose of acetaldehyde (20 mg/kg) in guinea pigs caused an immediate increase in mean arterial blood pressure and heart rate (Mohan et al., 1981). Five minutes after the injection of the acetaldehyde, a lowering of the mean arterial pressure was observed (see Figure 5-1). Pre-treatment with phentolamine an alpha blocker and propranolol, a beta blocker prevented the increased mean arterial pressure and tachycardia, but not the prolonged hypotension. Only a single dose of acetaldehyde was used, so a dose-response relationship could not be established. Results are shown in Table 5-3. Therefore the effects of lower doses cannot be predicted. Histopathology was not performed in this study.

Egle et al. (1973) investigated the dose-response relationship of intravenous acetaldehyde on the cardiovascular system of anesthetized rats. The results of the study indicated that the sympathominetic effect of acetaldehyde at doses below 20 mg/kg caused a significant increase in blood pressure, while at higher doses stimulation of CNS higher centers caused bradycardia and hypotension. The first response was slightly reduced by adrenalectomy and strongly opposed by pretreatment with reserpine or phentolamine, indicating that the pressor effect of acetaldehyde is primarily due to vasoconstriction mediated by norepinephrine released from sympathetic nerve endings in vascular smooth muscle. Atropine reduced the hypotensive and cardioinhibitory effects of acetaldehyde, indicating that it exerts the effect via the vagus nerve;



UPPER PANELS: EFFECT OF 20 mg/kg ACETALDEHYDE (ACE) IN GROUP I ANIMALS.
MIDDLE PANELS: EFFECT OF 1 mg/kg OF PROPRANOLOL (PRL) FOLLOWED BY 20 mg/kg OF ACE IN GROUP II ANIMALS.
LOWER PANELS: EFFECT OF 1 mg/kg OF PHENTOLAMINE (PHE) FOLLOWED BY 20 mg/kg OF ACE IN GROUP III ANIMALS.
HORIZONTAL BROKEN IN LINES INDICATE MEAN CONTROL VALUES EXPRESSED AS 100%.

Figure 5-1. Arterial pressure and heart rate changes in guinea pigs after administration of acetaldehyde.

Source: Mohan et al. (1981).

TABLE 5-3. INFLUENCE OF VARIOUS DRUGS AND SURGICAL PROCEDURES UPON THE BLOOD PRESSURE RESPONSES TO INTRAVENOUS ACETALDEHYDE IN THE ANESTHETIZED RAT[a]

Drug or procedure	Direction of change	Acetaldehyde (mg/kg) 5	10	20	40
		Percent of change from resting blood pressure (mean ± SE)			
Control	↑	10.7 ± 1.6 (33/33)[b]	16.7 ± 2.2 (33/33)	22.7 ± 2.0 (15/33)	---
	↓	---	---	41.6 ± 5.4 (18/33)	53.9 ± 6.5 (15/15)
Adrenalectomy	↑	15.8 ± 3.8 (9/9)	9.2 ± 2.2 (6/9)	---	---
	↓	---	10.4 ± 4.7 (3/9)	23.1 ± 6.0 (9/9)	47.6 ± 2.4 (9/9)
Adrenalectomy + reserpine	↑	7.5 ± 1.6 (8/10)	7.2 ± 1.0 (6/10)	---	---
	↓	2.0 ± 0.0 (2/10)	15.2 ± 5.9 (4/10)	26.6 ± 6.1 (10/10)	55.2 ± 4.5 (10/10)
Phentolamine	↑	---	6.7 ± 1.9 (13/26)	---	---
	↓	5.5 ± 2.7 (15/15)	14.6 ± 2.7 (13/26)	41.2 ± 9.9 (12/12)	43.0 ± 6.0 (7/7)
Propranolol	↑	---	20.3 ± 4.0 (15/17)	---	---
	↓	4.3 ± 1.4 (16/16)	2.0 ± 2.0 (2/17)	38.9 ± 2.0 (10/10)	44.4 ± 5.8 (9/9)
Atropine	↑	8.2 ± 1.9 (18/18)	18.9 ± 2.1 (18/18)	30.2 ± 3.0 (8/10)	---
	↓	---	---	4.0 ± 1.1 (2/10)	11.0 ± 2.7 (10/10)
Vagotomy	↑	18.1 ± 3.3 (9/9)	39.7 ± 8.2 (9/9)	30.9 ± 2.2 (8/8)	36.0 ± 5.6 (12/12)
	↓	---	---	---	---

[a]Responses are calculated as percent of change from resting blood pressure. These values were taken from each experiment. Mean ± SE of the resting blood pressure in Table 1.

[b]Number in parentheses refer to the frequency of each response as a function of the number of observations.

Source: Mohan et al. (1981).

5.2 SUBCHRONIC TOXICITY

5.2.1 Intraperitoneal

One subchronic investigation of the effects of acetaldehyde, on the phospholipid composition of pulmonary surfactant, was found in the literature (Prasanna et al., 1981). Pulmonary surfactant is a lipoprotein complex with a high phospholipid content which prevents alveolar collapse during expiration by maintaining the stability and physical elasticity of the alveolar walls, by reducing the surface tension of the fluid lining the alveoli. The study was not designed as a subchronic safety evaluation: only a single dose was used, and the animals treated for only ten days.

Acetaldehyde was injected intraperitoneally (200 mg/kg) to six rats which had been pretreated with pyrazole (270 mg/kg/day) for ten days. Pyrazole was used to block the conversion of ethanol to acetaldehyde by inhibiting alcohol dehydrogenase activity. A saline control group was also pretreated with pyrazole for 10 days, but received no acetaldehyde. Ten days later, pulmonary surfactant material was harvested; the surface tension and phospholipid content of the lung lavage were measured. In the acetaldehyde-treated animals, the phospholipid concentration was significantly reduced and the maximum and minimum surface tension (dynes/cm) were significantly increased when compared to saline control values. Although this study has shown that acetaldehyde may alter pulmonary surfactant, only a single dose, was used (200 mg/kg/day for 10 days), and thus, the effects of low doses of acetaldehyde on the pulmonary surfactant remain uninvestigated. In addition, the observed decrease in pulmonary phospholipids may be due to the impairment of phospholipid synthesis or phospholipid secretion. Such changes in phospholipid synthesis or secretion are not specific to the lung and may be found in other tissues. Hence, the direct effect of acetaldehyde on pulmonary surfactant remains unclear.

It should also be noted that the authors used pyrazole to block the conversion of ethanol to acetaldehyde but did not use any agent to block the conversion of acetaldehyde to acetate. Thus, the degradative metabolism of acetaldehyde was not blocked, and exogenously administered acetaldehyde could be rapidly converted to acetate. Histopathological examinations were not performed to assess the effects of acetaldehyde on the microscopic anatomy of the lung.

5.3 CHRONIC TOXICITY

5.3.1 Inhalation

The chronic effects of inhalation of acetaldehyde were studied by Feron (1979). Male Syrian hamsters were exposed to 1500 ppm acetaldehyde vapor (7 hr/day, 5 days/week) for 52 weeks; control animals were exposed to air. Observation were made on general appearance, body weight, mortality, hematology, kidney function, organ weight, and gross and microscopic pathology of the respiratory tract. At the end of the treatment period, five randomly selected animals from each group were killed and autopsied. All remaining animals were allowed to recover for 20 weeks and sacrificed by week 72.

Exposure of hamsters to 1500 ppm acetaldehyde vapor produced growth retardation, slight anemia, increased urinary glutamic-oxaloacetic transaminase (UGOT) activity and protein content in the urine, increased kidney weights without renal pathology, and distinct histopathological changes in the nasal mucosa and trachea, including hyperplasia, squamous metaplasia, and inflammation. Thus, acetaldehyde vapor at 1500 ppm produced systemic effects in the hamster. However, since only male animals and only one dosage level were used, this study does not fulfill all requirements of a chronic safety evaluation. At least three dose levels should have been used so that dose-response relationship and a no observable effect level could be determined.

In a separate experiment (Feron, 1979), groups of 35 male and 35 female hamsters were treated intratracheally with acetaldehyde for a period of 52 weeks. The intratracheal instillations were given either weekly or fortnightly with 2 percent or 4 percent acetaldehyde. Interim sacrifice of three animals/sex/group were performed after 13, 26, and 52 weeks. All remaining animals were sacrificed after 104 weeks. Observations were made of general appearance, body weight, mortality, and gross and microscopic pathology of the respiratory tract.

Acetaldehyde had no effect on body weight and mortality. However, intratracheal instillation of acetaldehyde caused severe hyperplastic and inflammatory changes in the bronchioalveolar region of the respiratory tract. Under the conditions of this study, a no observable effect level of acetaldehyde administered by intratracheal instillation could not be demonstrated.

Feron et al. (1982) exposed male and female hamsters to acetaldehyde vapor for 7 hr/day, 5 days/week for 52 weeks to an average concentration of acetaldehyde of 2500 ppm during the first 9 weeks; 2250 ppm during weeks 10-20; 2000

ppm during weeks 21-29; 1800 ppm during weeks 30-44; and 1650 ppm during weeks 45-52. Animals exposed to air served as controls. The air control animals were divided by sex into two groups of 18 animals each; acetaldehyde-treated hamsters were divided, according to sex and body weight, into groups consisting of 30 animals each. The first treated group was exposed to air or acetaldehyde vapor only. The second treated group was exposed to air or acetaldehyde simultaneously with intratracheally instilled saline. At the end of the exposure period (week 52), three animals per sex were taken from each group for autopsy. All remaining animals were sacrificed after 81 weeks. Observations were made on general appearance, body weight, mortality, hematology, organ weight, and gross and microscopic pathology of the respiratory tract, tumors, and gross legions suspected of being tumors.

Hamsters exposed to acetaldehyde alone exhibited substantially lower body weights than their corresponding controls. By the end of the exposure period (week 52), the mortality rate among acetaldehyde-treated hamsters was comparable to that among controls. There was no significant differences in hematological and biochemical findings between air- and acetaldehyde-exposed animals. Distinct histopathological changes in the nose, trachea, and larynx were found in animals exposed to acetaldehyde.

There are significant deficiencies associated with this study. For example, the dosage was progressively reduced because of considerable growth retardation. Therefore, a no observable effect level could not be determined. In addition, the animals appear to have been gang-housed, since cannibalism was reported. Individual housing is more appropriate for chronic studies. Details concerning the carcinogenic effects of these investigations are discussed in Chapter 6.

Table 5-4 presents the results of chronic investigations of the toxic effects of acetaldehyde. All levels of the respiratory tract exhibited signs of pathology. Chronic inhalation of 1500 ppm of acetaldehyde caused growth retardation in hamsters. Neither the lowest effect level (LEL) nor no observed effect level (NOEL) were established because only single dosages of acetaldehyde were used. Thus, additional testing is necessary to determine those values.

TABLE 5-4. CHRONIC STUDIES WITH ACETALDEHYDE

Species	Number and Sex Per Dose	Dose Level Tested and Route of Administration	Duration	Results	Reference
Syrian hamsters	35M	1500 ppm inhalation	52 weeks and 20 weeks recovery	- Growth retardation - Increased UGOT - Histopathological changes in the nasal cavity and trachea	Feron (1979)
Syrian hamsters	35M	2% and 4% intratracheal	52 weeks and 20 weeks recovery	- Severe hyperplastice and inflammatory changes in the bronchioalveolar region - No effect on growth	Feron (1979)
Syrian hamsters	30M and 30F (treated) 18M and 18F (control)	2500 ppm - 1650 ppm inhalation 7 hr/day 5 days/week	52 weeks and 29 weeks	- Decreased body weight - Higher mortality during the recovery period - Histopathological changes in the nasal cavity, trachea, and larynx	Feron et al. (1982)

Source: Feron (1979); Feron et al. (1982).

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6. MUTAGENICITY

The purpose of this mutagenicity assessment is to evaluate studies which have been conducted to determine whether acetaldehyde has the potential to cause mutations in humans. Mutations in somatic cells may lead to the onset of cancer and possibly other diseases, whereas mutations in germ cells may be passed on to future generations and increase the incidence of genetic disease in the population. Chromosomal abnormalities in germ cells could also lead to embryonic and fetal deaths. This assessment, therefore, also includes an evaluation of whether the test agent reaches and produces genetic damage in mammalian germ cells.

Tests of acetaldehyde for genotoxicity have primarily measured cytogenetic end points, including sister chromatid exchanges (SCEs), chromosomal aberrations, and micronuclei in mammalian cell cultures.* Clastogenic activity has also been evaluated in plants. Studies in intact mammals are limited to two studies of SCEs in rodent bone marrow cells and a third study of chromosomal aberrations in rat embryos. Gene mutation studies are available in Drosophila, yeast, nematodes, and bacteria, but not in mammalian systems. Acetaldehyde has been studied for its ability to produce DNA strand breaks in cultured mammalian cells. There were no available studies regarding the ability of acetaldehyde to reach and damage DNA in mammalian germ cells in vivo. The genotoxicity studies on acetaldehyde are discussed below and are summarized in section 6.7. and Table 6-1.

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*These evaluations have been limited to papers in English or in English translation providing primary data and descriptions of protocols used. Several genotoxicity studies discussed in this report were conducted to determine whether ethanol is genotoxic via its first metabolite, acetaldehyde. These studies were evaluated whenever primary data for acetaldehyde itself were presented.

TABLE 6-1. MUTAGENICITY-GENOTOXICITY TESTING OF ACETALDEHYDE

Organism	Metabolic activation	Concentrations tested[a]	Reported result	Reference
GENE MUTATION				
Bacteria				
Salmonella typhimurium (reverse mutations)				
Plate test: Strains				
TA1535	±S9	2.3×10^{-4} mmol/plate (10 µg/plate)	-	Commoner, 1976
TA100	-S9	NR[b]	-	Laumbach et al., 1976
TA1535	±S9	10^{-5} to 2.5×10^{-3} mmol/plate (0.44 to 110 µg/plate)	-	Pool and Wiessler, 1981
TA1535, TA1538	-S9	0.18 mmol/plate (7,793 µg/plate)	?[c]	Rosenkranz, 1977
Preincubation method: Strains				
TA98, TA100, TA1535, TA1537	±S9	0.023 to 0.23 mmol/plate (33 to 10,000 µg/plate)	-	Mortelmans et al., 1986
TA104, TA102	-S9	up to 2.3×10^{-2} mmol/plate (1,000 µg/plate)	-	Marnett et al., 1985
Escherichia coli (reverse mutation)				
Liquid incubation: Strains				
WP2uvrA	-S9	"usually 0.02-10 mM" (0.9-441 µg/mL)	-	Heminki et al., 1980
	-S9	0.88 mM (38.8 µg/mL)	+ (no dose-relation)	Veghelyi et al., 1978
Yeast				
Saccharomyces cerevisiae (mitochondrial mutations)	-S9	534 mM (23,490 µg/mL) for 30, 60, and 90 min	?[d]	Bandas, 1982
Nematodes				
Caenorhabditis elegans (mutations affecting egg -laying)	-S9	18 and 178 mM (783 and 7,830 µg/mL) for 2 h	+ at 18 mM (no dose-relation)	Greenwald and Horvitz, 1980

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TABLE 6-1. (continued)

Organism	Metabolic activation	Concentrations tested[a]	Reported result	Reference
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)	-	
CHROMOSOMAL ABERRATIONS				
Plants				
Vicia faba (root-tips)		5 to 50 mM (220.5 to 2,205 µg/mL) for 24 h at 12°C	+DR[e] (breaks and translocations)	Rieger and Michaelis, 1960 Michaelis et al., 1959
Insects				
Drosophila 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	0.1 to 10 mM (4.4 to 441 µg/mL) for 12, 24, and 48 h	+DR (micronuclei)	Bird et al., 1982
		0.01 to 1 mM (0.44 to 44.1 µg/mL) for 12 and 24 h	+ (gaps, breaks, exchange-type aberrations, acentric fragments)	
			+?[f] (aneuploidy)	
Human lymphocytes	-S9	0.18 and 0.36 mM (7.83 and 15.7 µg/mL) for 24 h	+ (translocations, breaks, gaps in Fanconi anemia cells, but was not clastogenic in normal lymphocytes at same dosages)	Obe et al., 1979

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TABLE 6-1. (continued)

Organism	Metabolic activation	Concentrations tested[a]	Reported result	Reference
Human lymphocytes	-S9	0.09 to 1.08 mM (3.97 to 47.6 µg/mL) for 72 h	+DR (gaps, breaks, exchange-type aberrations)	Böhlke et al., 1983
Whole mammals				
Female rats/treated embryos (13th day of pregnancy)		0.02 mL of 178 mM (7,830 µg/mL) intra-amniotically	+ (gaps and breaks)	Barilyak and Kozachuk, 1983
SISTER CHROMATID EXCHANGE				
Mammalian cell culture				
Chinese hamster ovary cells (CHO)	-S9	0.09 and 0.18 mM (3.97 and 7.83 µg/mL) for 8 days	+DR	Obe and Ristow, 1977
CHO	-S9	0.045 to 0.27 mM (2 to 11.7 µg/mL) for 24 h	+DR	Obe and Beer, 1979
CHO	±S9	0.18 to 1.8 mM--plus S9 (0.78 to 78 µg/mL) 0.18 to 0.89 mM--minus S9 (0.78 to 39.4 µg/mL) for 1 h	+DR (response similar +/- S9)	de Raat et al., 1983
Human lymphocytes	-S9	0.09 to 0.36 mM (3.97 to 15.7 µg/mL) for 24 h; 15.4 µg/mL for 48 h	+DR	Ristow and Obe, 1978
Human lymphocytes	-S9	0.36 and 1.8 mM (15.7 µg/mL and 78 µg/mL) for 3 h	+ (the presence of aldehyde dehydrogenase + NAD slightly reduced the response)	Obe et al., 1986

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TABLE 6-1. (continued)

Organism	Metabolic activation	Concentrations tested[a]	Reported result	Reference
Human lymphocytes	-S9	0.09 to 0.18 mM (3.97 to 7.83 µg/mL) for 90 h	+DR	Jansson, 1982
Human lymphocytes	-S9	0.09 to 1.08 mM (3.97 to 47.6 µg/mL) for 72 and 96 h	+DR	Böhlke et al., 1983
Human whole-blood lymphocyte cultures	-S9	0.063 to 2 mM (2.8 to 88.2 µg/mL) for 48 h	+DR	Norrpa et al., 1985
Human lymphocytes	-S9	0.1 to 0.3 mM (4.4 to 13.2 µg/mL) for 47 and 70 h	+DR	He and Lambert, 1985
		0.6 to 2.4 mM (26.5 to 105.8 µg/mL) for 1 h	+DR	
Whole Mammals				
Male CBA mice		$2.5x10^{-7}$ and $5x10^{-7}$ mol/kg (0.011 and 0.022 mg/kg) (i.p. injection)	+9 (almost doubled background frequency at 0.02 mg/kg)	Obe et al., 1979
Male and female Chinese hamsters		$2.3x10^{-7}$ to $1.1x10^{-5}$ mol/kg (0.01 to 0.5 mg/kg) (i.p. injection)	+ (almost doubled background frequency at 0.5 mg/kg)	Korte and Obe, 1981

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TABLE 6-1. (continued)

Organism	Metabolic activation	Concentrations tested[a]	Reported result	Reference
OTHER END POINTS INDICATIVE OF DNA DAMAGE				
Bacteria				
Escherichia coli (polA$^+$/pol A$^-$ assay)	-S9	0.18 mmol (7,830 µg/plate)	wk[h]	Rosenkranz, 1977
Mammalian cell culture (DNA strand breaks)				
Rat hepatocytes	-S9	0.03 to 3 mM (1.3 to 132 µg/mL) for 3 h	-	Sina et al., 1983
Human lymphocytes	-S9	10 mM (441 µg/mL) for 4 h	-	Lambert et al., 1985
Human bronchial epithelial cells	-S9	up to 1 mM (up to 44.1 µg/mL) for 1 h	-	Saladino et al., 1985

[a] *In vitro* concentrations given as millimoles (mmol) or millimoles/L (mM); *in vivo* concentrations given as mol/kg body weight; numbers in parentheses indicate corresponding values in µg/mL or mg/kg body weight.
[b] NR = not reported.
[c] ? = Although a slight increase in TA1535 revertants (16 revertants in treated versus 4 revertants in controls) was reported, the very low background frequency renders the results inconclusive. Also, no dose-relation was demonstrated.
[d] ? = marginal response that is regarded as equivocal because it occurred in the presence of high toxicity and no dose-relation 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 control.
[h] wk = weakly positive or marginal response.

6.1. GENE MUTATION TESTS

6.1.1. Bacteria

Several authors have reported negative results in the standard Ames test (Laumbach et al., 1976; Pool and Wiessler, 1981; Commoner, 1976). Although Rosenkranz (1977) reported a slight increase in revertants in tester strain TA1535, the very low background frequency found in this study renders the results inconclusive. All of the Ames plate test results (Table 6-1), moreover, were somewhat equivocal because of one or more deficiencies in the experiments reported: appropriate concurrent controls being omitted, only one acetaldehyde concentration being tested, acetaldehyde being evaluated in only one tester strain, or the data being insufficient to determine whether an adequate test was conducted. In addition, the standard Ames plate test is not entirely suitable in this case because acetaldehyde is a volatile chemical and precautions were not taken to prevent its escape by evaporation.

The National Toxicology Program (NTP) evaluated the mutagenicity of acetaldehyde in four Salmonella tester strains (TA1535, TA1537, TA98, TA100) using a liquid preincubation procedure and reported negative results (Mortelmans et al., 1986). With this procedure, the liver activation system, bacteria, and test chemical are mixed and incubated in capped tubes for 20 minutes at 37°C. Melted top agar is then added, and the mixture is poured into petri dishes and incubated for 48 hours at 37°C. Two types of S9 mix were employed: Aroclor 1254-induced rat liver and Aroclor 1254-induced hamster liver. Concurrent negative and positive controls were used in this study. At least five concentrations (33 to 10,000 µg/plate) of acetaldehyde were examined by two different laboratories. Toxicity was reported at 10,000 µg/plate in TA100 and TA1535 and at 3,333 µg/plate in TA1537. Although incubation was carried out in capped tubes, it is possible that evaporation and some escape of the test material may

have occurred during preincubation and after plating. The NTP also tested a related compound, formaldehyde, in the same preincubation protocol and reported a positive response in tester strain TA100 with liver S9 mix (Haworth et al., 1983). The response was detected in a narrow concentration range of 75 to 150 µg/plate, after which toxicity occurred, and was never greater than three-fold over the background revertant count.

Acetaldehyde was also found to be nonmutagenic in the new Salmonella tester strains designated as TA102 and TA104 (Marnett et al., 1985). These strains were originally developed to detect the mutagenicity of peroxides and other oxidants and have been shown to be more sensitive to certain aldehydes and DNA cross-linking agents than the standard Salmonella tester strains (e.g., TA98, TA100, TA1535, TA1537, TA1538). TA102 and TA104 differ from the standard tester strains in that A:T bases rather than G:C bases are at the site of mutation (Levin et al., 1982): TA102 contains the mutation hisG428 on a multicopy plasmid; TA104 contains the same mutation in single copy on the chromosome. Acetaldehyde was tested at concentrations up to 1,000 µg/plate in a liquid preincubation procedure (tubes were incubated at 37°C for 20 min) without exogenous metabolic activation. In the same study, formaldehyde and several unsaturated aldehydes (acrolein, hexadienal, crotonaldehyde, and methacrolein) were found to produce dose-related mutational responses in strain TA102 and/or strain TA104.

In view of the positives in other test systems (discussed later), it is uncertain why acetaldehyde has consistently produced negative results in the bacterium Salmonella typhimurium. The negative findings may be due to the volatility of acetaldehyde, or perhaps Salmonella may be unresponsive or insensitive to acetaldehyde treatment.

Two papers have reported studies in Escherichia coli WP2 uvrA, both using

a liquid suspension procedure. One study reported negative results when capped tubes were incubated at 37°C for 18 hours, but the authors did not specify the concentrations tested except to indicate that six concentrations were evaluated, primarily in the range of 0.02 to 10 mM (Hemminki et al., 1980). The other study reported a mutagenic effect when 0.88 mM acetaldehyde was tested in stoppered tubes incubated at 0°C (Veghelyi et al., 1978). The lower incubation temperature used by Veghelyi et al. would reduce the evaporation of acetaldehyde during treatment. Nevertheless, the authors' conclusions in these two papers could not be evaluated because of the lack of detail provided regarding the results and methods. In the positive study no dose relation was shown, since only one concentration was tested.

6.1.2. Yeast

Bandas (1982) studied the mutagenic effects of acetaldehyde and ethanol on mitochondrial DNA (petite mutations) of the yeast Saccharomyces cerevisiae to determine whether ethanol itself or its metabolite, acetaldehyde, was genotoxic. A concentration of 3% acetaldehyde (534 mM) was added to cell cultures, and the cells were incubated for 30, 60, or 90 minutes. Although there was a twofold increase in the spontaneous frequency of petite mutants after a 90-minute treatment, there was over 96% cell killing at this dosage. This slight increase in petite mutants is regarded as questionable because it was detected at an extremely toxic dose and because the increase was not shown to be dose-related. In addition, the interpretation of an increase in mitochrondrial mutations is uncertain, because cytoplasmic mutations are less defined genetically than the nuclear mutations used in standard assays.

6.1.3. Nematodes

Greenwald and Horvitz (1980) tested acetaldehyde (0.1% or 1.0% for 2 hours) in the nematode (Caenorhabditis elegans) for its ability to produce mutations in

genes that affect the egg-laying system. A concentration of 1.0% (178 mM) acetaldehyde was too toxic for evaluation, but 0.1% (18 mM) increased the frequency of mutations (i.e., reduction in brood size) to 1 x 10^{-4}, relative to 6 x 10^{-6} in the untreated controls. No dose-relation was demonstrated in this study, and the interpretation of the result is also unclear because the organism is not typically used to screen chemicals for their mutagenic potential.

6.1.4. Drosophila

Acetaldehyde was evaluated in the sex-linked recessive lethal (SLRL) test in Drosophila melanogaster as a coded agent in a mutagenesis testing program sponsored by the National Toxicology Program (Woodruff et al., 1985). Tests for SLRLs in Drosophila have been used to detect induced mutations for 59 years, and are currently used in routine screening for mutagens (Lee et al., 1983). In this study, 53 chemicals were tested by adult feeding and adult injection for the induction of SLRL mutations in meiotic and post-meiotic germ cells of Canton S males. In the feeding experiment, a dose of 25,000 ppm, which produced 3% mortality, did not induce an increase in lethals (0.06% lethals at 25,000 ppm versus 0.07% lethals at 0 ppm). A total of 8,541 chromosomes were evaluated. It should be noted that other aldehydes (transcinnamaldehyde, crotonaldehyde, furfural, and formaldehyde) examined in this study were also not detected as mutagenic by adult feeding but produced sex-linked recessive lethals after an adult injection. In the injection experiment, acetaldehyde was also found to produce a significant increase in SLRLs (0.21% lethals versus 0.06% lethals in the 0 ppm control) at 22,500 ppm, a dose which caused 29% mortality. The criterion for a positive response in the SLRL test does not depend on showing a dose-response relationship, but requires the demonstration that differences between SLRL frequencies in treated and concurrent control groups are statistically significant at the 5% level (Margolin et

al., 1983; Woodruff et al., 1985). Formaldehyde was a more potent mutagen than acetaldehyde in the SLRL test (2,000 ppm formaldehyde given by injection produced 0.38% lethals versus 0.09% lethals in 0 ppm control).

Formaldehyde has been shown to produce large and small deletions in a Drosophila gene (Adh locus) (Benyajati et al., 1983). Some of these deletions were postulated to be caused by a slipped mispairing mechanism during DNA replication that resulted from the formation of DNA-DNA or DNA-protein cross-links. Since acetaldehyde has been shown to form DNA and DNA-protein cross-links (Ristow and Obe, 1978; Lam et al., 1986; Lambert et al., 1985) and produces SLRLs, a similar event may be involved in the mutagenicity of acetaldehyde.

6.2. CYTOGENETIC TESTS

Many studies on acetaldehyde's ability to induce sister chromatid exchanges (SCEs), and some of the chromosomal aberration tests, were conducted to determine whether acetaldehyde is the genotoxic intermediate in ethanol metabolism. The cytogenetic studies by different laboratories, without exception, demonstrate the ability of acetaldehyde to induce SCEs and chromosomal aberrations in mammalian systems. The lowest effective concentrations tested at which cytogenetic effects in mammalian cells in vitro were found, was in the range of approximately 0.1 to 1 mM. The ability of acetaldehyde to be a clastogen and an inducer of SCEs may be related to its DNA-DNA and/or DNA-protein cross-linking activity (Ristow and Obe, 1978; Lambert et al., 1985; Lam et al., 1986; see section 4.4.3. for a discussion of acetaldehyde cross-linking activity), since agents that cross-link DNA usually are clastogenic and induce SCEs (Latt et al., 1981; Preston et al., 1981).

6.2.1. Chromosomal Aberration Tests

6.2.1.1. Plants--Acetaldehyde has been reported to be clastogenic in cells of the root-tip meristem of Vicia faba (Rieger and Michaelis, 1960; Michaelis et

al., 1959). Chromatid breaks and translocations were reported after treatment with 5 to 50 mM acetaldehyde for 24 hours at 12°C. The frequency of chromosomal aberrations was increased in a dose-related manner. The clastogenicity of acetaldehyde was temperature-dependent, with more activity at lower temperatures than at higher ones. This finding suggests that the clastogenicity of acetaldehyde is influenced by the metabolic state of the cell; for example, oxidation of acetaldehyde to acetic acid may be reduced at lower temperatures. Alternatively, treatment at lower temperatures could reduce the evaporation of acetaldehyde during treatment, and thereby increase the effective exposure.

6.2.1.2. Drosophila--Acetaldehyde was evaluated by Woodruff et al. (1985) for its ability to induce reciprocal translocations after adult injection at 22,500 ppm. Results were negative in a test of 6,685 chromosomes. Formaldehyde also was negative for the induction of translocations. Crotonaldehyde was the only aldehyde in this study that produced reciprocal translocations.

6.2.1.3. Mammalian Cell Culture--The ability of acetaldehyde to produce chromosomal aberrations and micronuclei was studied in primary cultures of rat (Sprague-Dawley) skin fibroblasts (Bird et al., 1982). Another aldehyde, malonaldehyde, was also studied. Both acetaldehyde and malonaldehyde produced micronuclei in a dose-dependent manner. Acetaldehyde was tested in the concentration range of 0.1 mM to 10 mM for 12 hours, 24 hours, or 48 hours of exposure. The lowest effective concentration tested for micronuclei induction was 0.5 mM (2.4% cells with micronuclei versus 0.5% in control cultures after 12-hour treatment). Cells were treated with acetaldehyde at 0.01 mM to 1 mM and scored for chromosomal aberrations 12 hours and 24 hours later. At 12 hours, only 1 mM acetaldehyde produced effects: chromosome/chromatid breaks and gaps, exchange-type aberrations, and acentric fragments were observed. As the exposure time was increased to 24 hours, the frequency of chromosomal

aberrations also increased, with effects being detected at 0.1 and 1 mM. At 0.1 mM acetaldehyde, 20% of the cells had aberrations and at 1 mM, 40% of the cells had aberrations relative to 4% of cells with aberrations in the control cultures. Although increases in the frequency of aneuploid cells were reported for both aldehydes at concentrations of 0.1 mM and higher, no conclusions can be reached regarding the validity of these data because the total incidences of aneuploidy were reported rather than the incidences of hyperploidy and hypoploidy. This distinction is important because hypoploidy can be ascribable to technical artifacts (Dellarco et al., 1985; Galloway and Ivett, 1986). Nevertheless, this study demonstrates that acetaldehyde (and malonaldehyde) are clastogenic.

The DNA cross-linking activity of acetaldehyde in isolated calf thymus DNA (Ristow and Obe, 1978) stimulated Obe et al. (1979) to study the effects of acetaldehyde in peripheral lymphocytes from a patient with Fanconi anemia and from normal individuals to determine whether an increased frequency of chromosomal aberrations would be produced in humans in whom the repair of DNA cross-links is defective. Acetaldehyde, at 0.001% and 0.002% v/v (0.18 and 0.36 mM) for 24 hours, produced high frequencies of chromatid translocations, breaks, and gaps in cells from a human with Fanconi anemia. These effects were not shown to be dose-related. At similar concentrations, no clastogenicity was found in peripheral blood lymphocytes from three normal individuals. These negative findings, however, do not indicate that acetaldehyde does not produce chromosomal aberrations in normal human lymphocytes; a study by Böhlke and coworkers (discussed below) demonstrates that acetaldehyde produces chromosomal aberrations in human lymphocytes at concentrations higher than those used by Obe et al. (1979).

Acetaldehyde is oxidized to acetate via aldehyde dehydrogenase (ALDH). Böhlke et al. (1983) studied the effects of acetaldehyde on chromosomal aberration and SCE frequencies in lymphocytes from Germans possessing both ALDH isozymes I and II and from Japanese possessing either isozyme II or isozymes I and II. For both populations, a dose-related induction of SCEs (discussed later) and chromosomal aberrations was seen in lymphocytes treated with 0.09 mM to 1.08 mM acetaldehyde for 72 hours. Acetaldehyde concentrations of 0.72 and 1.08 mM produced a high number of gaps, breaks, and exchange-type aberrations in a dose-related manner. At 0.72 mM acetaldehyde, 18.9 ± 11.7 (SD) metaphases with chromosomal aberrations were found, and at 1.08 mM acetaldehyde the aberration frequency was increased to 31.1 ± 14.9 (SD) relative to 1.9 ± 1.6 (SD) aberrant cells in control cultures. There were no differences that could be related to the different ALDH phenotypes. These data suggest that differences in ALDH activity do not modulate the induction of cytogenetic abnormalities by acetaldehyde or that the different isozymes are not expressed in cultured lymphocytes. According to a recent article by He and Lambert (1985), no ALDH activity is detected in isolated lymphocytes. This finding is consistent with the observation reported by Böhlke et al. (1983).

6.2.1.4. Whole Mammals--Barilyak and Kozachuk (1983) injected female Wistar rats with 0.02 mL of 1% (178 mM) acetaldehyde intra-amniotically on day 13 of pregnancy and obtained embryonic cells 24 hours later for cytogenetic analysis. Treated rat embryos had a higher frequency of chromosomal aberrations (mostly chromatid gaps and breaks) than did controls (16.0 ± 1.5% [SE] metaphases with breaks versus 3.8 ± 0.8% [SE] in sham-treated controls). In the same study, 40% ethanol was not clastogenic to rat embryos. This study is discussed further in chapter 8 of this document (reproductive and developmental effects).

6.2.2. Sister Chromatid Exchange Tests

6.2.2.1. Mammalian Cell Culture--Obe and Ristow (1977) studied the ability of ethanol and its metabolite, acetaldehyde, to induce SCEs in Chinese hamster ovary (CHO) cells in vitro. The studies were conducted in the absence of exogenous metabolic activation; CHO cells have essentially no capacity to metabolize xenobiotics (Hsie et al., 1981). A dose-related increase in SCEs was found after daily treatments with 0.0005% and 0.001% v/v acetaldehyde for 8 days. Observed frequencies of SCEs were 13.56 and 28.35 SCE/cell at 0.0005% (0.09 mM) and 0.001% (0.18 mM) acetaldehyde, respectively; (4.69 SCE/cell were found in the control culture. Concentrations above 0.001% were too toxic for evaluation. After treatment for 7 or 8 days under the same treatment conditions, 0.1% (v/v) ethanol did not increase the frequency of SCEs.

Obe and Beer (1979) evaluated SCE frequencies in CHO cells after a 24-hour exposure to acetaldehyde at 0.00025 to 0.0015% v/v. The lowest effective concentration tested of acetaldehyde was 0.0005% v/v (0.09 mM), which produced 18.24 ± 0.49 (SE) SCE/cell (8.24 ± 0.36 [SE] SCE/cell in control culture). The maximum response was found at the highest concentration tested of acetaldehyde, 0.0015% v/v (0.27 mM), and produced 22.08 ± 0.53 (SE) SCE/cell. Formaldehyde was also tested in this study and appeared to be approximately twofold more potent at inducing SCEs than acetaldehyde when similar concentrations are compared. It should be cautioned, however, that this small difference in potency is confounded by such factors as differences in the volatility, possible differences in the persistence of DNA damage (He and Lambert, 1985), production of peroxides from acetaldehyde exposed to air (see section 6.4.), and differences in toxicity.

A study by de Raat et al. (1983) further supports the hypothesis that the induction of SCEs by ethanol is attributable to the formation of acetaldehyde.

These investigators found that ethanol produced approximately fourfold more SCEs in CHO (K1) cells in the presence of Aroclor 1254-induced rat liver S9 mix than in the absence of S9 mix. It is unlikely that ethanol is directly genotoxic; HPLC analysis of the absolute ethanol sample demonstrated the presence of aldehydes, ketones, and carboxylic acid, which could account for the small increases in the SCE frequency in the absence of S9 mix. Acetaldehyde, tested alone, produced a dose-related increase in SCEs at concentrations of 0.78 to 39.4 µg/mL for 1 hour in the absence of S9 mix. At the lowest effective concentration tested, 7.8 µg/mL (0.18 mM) acetaldehyde, 17.25 ± 4.27 (SD) SCE/cell (9.2 ± 3 (SD) SCE/cell in control) were produced; when the acetaldehyde concentration was increased to 39.4 µg/mL (0.89 mM), the SCE frequency increased to 50.5 ± 8.87 (SD) SCE/cell. The SCE response was similar with or without S9 mix.

It is surprising that liver S9 mix did not influence the genotoxicity of acetaldehyde in the de Raat et al. (1983) study. The kinetics of acetaldehyde metabolism by a liver homogenate apparently differs from the *in vivo* situation, in which acetaldehyde is readily converted into acetate by liver ALDH. In contrast to the result with acetaldehyde, S9 activation reduced the induction of SCEs by formaldehyde in Chinese hamster cells (Natarajan et al., 1983; Basler et al., 1985); this effect was more pronounced with longer incubation times. In the study by de Raat et al. (1983) it is possible that the 1-hour incubation period was insufficient to detect an effect of liver S9 mix.

To confirm that the SCEs induced by ethanol were due primarily to a metabolite of ethanol, de Raat et al. (1983) assayed absolute ethanol in the presence of varying amounts of the S9 fraction. Increasing amounts of S9 produced increasing frequencies of SCEs. The enhancing effects of the S9 metabolic activation system decreased when $NADP^+$ and glucose-6-phosphate were

omitted from the reaction mixture. The amount of acetaldehyde formed from ethanol in the presence and absence of S9 was measured spectrophotometrically. No acetaldehyde was detected in the ethanol without S9 mix. In the presence of S9, however, 15.8 g/L of ethanol produced up to 7.4 mg/L acetaldehyde after 45 minutes of exposure. The results are consistent with the hypothesis that the genotoxic effects of ethanol are due to acetaldehyde.

Ristow and Obe (1978) found acetaldehyde to be a strong inducer of SCEs in human whole-blood lymphocyte cultures. Cells were treated for 24 hours with 0.0005% to 0.002% v/v (0.09 to 0.36 mM) acetaldehyde. The highest concentration of acetaldehyde tested (0.36 mM) produced 14.18 SCE/cell, as compared to 4.02 SCE/cell in control cultures. When the cells were treated for 48 hours with 0.36 mM acetaldehyde, the SCE frequency was increased to 23.95 SCE/cell.

In recent studies by Obe et al. (1986), it was shown that the SCE-inducing activity of acetaldehyde is slightly reduced in human lymphocytes <u>in vitro</u> when ALDH (which needs nicotinamide adenine dinucleotide [NAD] as a cofactor) is added to the culture medium. For example, in one donor, 0.01% v/v (1.8 mM) acetaldehyde produced approximately 28 SCE/cell (versus 10 SCE/cell in control cultures) after a 3-hour treatment. When ALDH and NAD were added directly to the culture medium, the SCE frequency was reduced to 15 SCE/cell. Human lymphocytes from different donors treated with 0.002% v/v (0.35 mM) acetaldehyde for 3 hours (without added ALDH and NAD) produced approximately two- to threefold increases in SCE frequencies over the control levels.

Obe et al. (1986) also provided evidence that the SCE-inducing metabolite of ethanol is acetaldehyde. Treatment of human lymphocytes with ethanol (1% v/v) for 3 hours in the presence of alcohol dehydrogenase (and NAD) resulted in higher SCE frequencies (three- to sixfold) than when cells were treated with ethanol alone. The addition of ALDH and NAD reduced the SCE frequency by 1.6-

to twofold.

Jansson (1982) reported a dose-related increase in the frequency of SCEs in human peripheral lymphocytes from one donor after treatment of the cells in vitro with acetaldehyde concentrations from 0.0005% to 0.001% v/v. The maximum response was found at the highest concentration tested, 0.001% v/v (0.18 mM), and was approximately 28 SCE/cell relative to about 11 SCE/cell in control cultures. Although no increases in SCEs were observed with ethanol (0.1% to 2% v/v), the ethanol-treated cells were derived from a different donor and thus cannot be compared directly with the acetaldehyde results.

Bohlke et al. (1983) studied the induction of SCEs by acetaldehyde in peripheral lymphocytes of Japanese and Germans with different ALDH phenotypes. For both populations, a dose-related induction of SCEs (and chromosomal aberrations as discussed earlier) was seen in lymphocytes treated with 0.09 to 1.08 mM acetaldehyde. There were no differences that could be related to the different ADH phenotypes. The concentration of acetaldehyde that gave an SCE/cell response at least twice the background was 0.36 mM. The next highest concentration tested, 0.72 mM, produced SCE frequencies that ranged from 44.6 ± 4.2 (SD) to 52.8 ± 5.4 (SD) SCE/cell. Although 1.08 mM acetaldehyde induced more SCE/cell, relatively few second-division metaphases were found because acetaldehyde produced a marked delay of the cell cycle.

The genotoxicity of vinyl acetate also appears to involve acetaldehyde, which is produced by its enzymatic hydrolysis. Norrpa et al. (1985) observed dose-related increases in the frequencies of SCEs in human peripheral lymphocytes treated for 48 hours with 0.05 to 1 mM vinyl acetate. The increases were more pronounced in isolated lymphocyte cultures than in whole-blood cultures. A dose-dependent response was also observed in CHO cells after a 24-hour treatment with 0.125 to 1 mM vinyl acetate. Liver S9 mix enhanced the genotoxicity,

thus indicating that acetaldehyde might be responsible for the genotoxicity of vinyl acetate. Furthermore, gas chromatographic analysis of human whole-blood cultures treated with vinyl acetate for 20 minutes without S9 mix showed a rapid breakdown of vinyl acetate and the formation of acetaldehyde. At 20 minutes, approximately 4.5 mM acetaldehyde was formed from 5.4 mM vinyl acetate. A 48-hour treatment with 0.063 to 2 mM acetaldehyde alone produced a dose-related increase in SCEs in human whole-blood lymphocyte cultures without S9 mix. The increase in SCE frequency was about fivefold that of the control cultures (approximately 45 SCE/cell versus 85 SCE/cell in controls) at 0.5 mM acetaldehyde and ninefold (approximately 70 SCE/cell versus 85 SCE/cell in controls) at 2 mM.

He and Lambert (1985) also concluded that acetaldehyde is likely to be the active SCE-inducing compound in vinyl acetate-exposed cells in culture. This conclusion is supported by similarities in time-dependence and concentration-dependence of effects of vinyl acetate and acetaldehyde on SCE frequencies in human lymphocytes. The removal or repair of the SCE-inducing lesions appears to occur during the G_1 phase of the cell cycle (i.e., before the S phase) because a twofold higher SCE frequency was observed when cells in late G_1 (23 hours after mitogen stimulation) were exposed to acetaldehyde or vinyl acetate (0.1, 0.2, and 0.3 mM) compared to an early G_1 (agent added at the time of PHA stimulation) exposure. The duration of treatment also affected the SCE frequency. For example, a 24-fold higher concentration of acetaldehyde (2.4 mM) given for 1 hour was needed to approximate the SCE response produced by 0.1 mM acetaldehyde for 70 hours. These authors suggest that acetaldehyde has a slow turnover in human lymphocytes in vitro and may accumulate in cells by forming reversible Schiff bases and, when released, forming SCE-inducing cross-links. Their data also suggest the possibility that SCE-inducing lesions are persis-

tent over several cell cycles.

6.2.2.2. Whole-Mammal Bone Marrow Cells---In a study by Obe et al. (1979), a male CBA mouse was administered an intraperitoneal injection of 0.5 mL or 1 mL of 0.0001% v/v (0.01 or 0.02 mg/kg) acetaldehyde, and bone marrow slides were prepared 24 hours later. At these doses, the treated animals had 7.88 and 6.4 SCE/cell, respectively. There were no concurrent sham-treated negative controls for acetaldehyde, but negative control SCE frequencies in the ethanol treatment group ranged from 4.1 to 4.8 SCE/cell. A minimum of three animals per dose should be evaluated in in vivo SCE studies (Latt et al., 1981), but Obe et al. (1979) used only one animal per treatment (50 metaphases analyzed). Thus, the positive result reported in this study is considered merely suggestive of an effect rather than definitive. It is unclear why Obe et al. (1979) did not test acetaldehyde at doses greater than 0.02 mg/kg, because the LD_{50} of acetaldehyde given to mice by intraperitoneal injection is reported by the IARC (1985) to be 500 mg/kg.

Korte and Obe (1981) gave intraperitoneal injections of acetaldehyde (0.01, 0.1, or 0.5 mg/kg b.w.) to male and female Chinese hamsters from an inbred colony. Acetaldehyde exposures of 0.6 mg/kg or greater were lethal. Another group of animals was exposed to 10% v/v ethanol for 46 weeks. Acetaldehyde at 0.5 mg/kg almost doubled the background frequency of SCEs (3.5 SCE/cell in the bone marrow of control animals versus 6.1 SCE/cell in treated animals), while ethanol had no effect on the frequency of SCEs. Animals injected with 0.5 mg/kg acetaldehyde showed strong signs of intoxication.

6.3. OTHER STUDIES INDICATIVE OF DNA DAMAGE

Acetaldehyde has been evaluated for its genotoxicity in a test using DNA repair-deficient bacteria and by the alkaline elution technique for DNA strand breaks in mammalian cells.

6.3.1. Bacteria

Rosenkranz (1977) tested 10 µL (7,938 µg) of acetaldehyde per plate in the Escherichia coli polA assay. This test measures DNA damage that is expressed as the preferential inhibition of growth in a DNA repair-deficient strain compared to a normal repair-proficient strain. Although the growth inhibition of the DNA repair-deficient strain (polA$^-$) was only slightly greater than that of the DNA repair-proficient strain (polA$^+$), the volatility of acetaldehyde in aqueous media confounds the interpretation of the observed weak response.

6.3.2. Mammalian Cell Culture

The alkaline elution technique has been used to determine whether the DNA of cells exposed to acetaldehyde contains single-strand breaks. Single-strand breaks were not detected in any of the cell types (rat hepatocytes, human lymphocytes, and bronchial epithelial cells) studied (Sina et al., 1983; Lambert et al., 1985; Saladino et al., 1985). However, some agents that may produce genetic damage (such as SCEs) and form DNA-DNA and/or DNA-protein cross-links, do not necessarily cause single-strand breaks in DNA (Bradley et al., 1979).

Sina et al. (1983) reported no measurable DNA damage after 3 hours of treatment with 0.03, 0.3, and 3.0 mM acetaldehyde in rat hepatocytes using the alkaline elution assay. Lambert et al. (1985) similarly did not detect an increase in DNA strand breaks by alkaline elution analysis after incubating human lymphocytes with 10 mM acetaldehyde for 4 hours in vitro. However, Lambert et al. did demonstrate DNA-DNA cross-linking activity by acetaldehyde. For further discussion of cross-linking activity, see section 4.4.3. Saladino et al. (1985) tested both acetaldehyde and formaldehyde on the production of single-strand breaks and DNA-protein cross-linking activity in human bronchial

epithelial cells in vitro using the alkaline elution assay. At concentrations up to 1 mM for 1 hour, acetaldehyde did not produce detectable DNA damage or form DNA-protein cross-links, while formaldehyde produced both effects at 0.1 mM. Results obtained by Lam et al. (1986), however, suggest that acetaldehyde forms DNA-protein cross-links at higher concentrations than those used by Saladino et al. (1985). Perhaps the inability of Saladino et al. to detect DNA-protein cross-links after acetaldehyde treatment is attributable to the volatility of acetaldehyde and the low concentrations tested.

6.4. ORGANIC PEROXIDES AS IMPURITIES IN ACETALDEHYDE

Lam et al. (1986) reported the presence of organic peroxides (e.g., peroxyacetic acid) in acetaldehyde exposed to air in an open bottle or collected immediately after distillation in air. For example, acetaldehyde samples distilled in air contained 1.89 to 6.6 µmol organic peroxides/mL acetaldehyde after 1 to 5 days, respectively. Acetaldehyde stored at -4°C for 5 days contained 10.6 µmol organic peroxides/mL acetaldehyde. The authors indicated that although peroxides form readily in pure acetaldehyde, they form very slowly, if at all, in aqueous solutions of acetaldehyde. Thus, formation of peroxides should be minimal during genotoxicity testing, but may originate from the source of the test agent. None of the genotoxicity reports discussed in this section indicated whether precautions were taken to prevent the oxidation of acetaldehyde by air to form organic peroxides. The possibility of peroxide impurities in the test samples therefore cannot be ruled out. It is unlikely, however, that the formation of peroxides is wholly responsible for the observed responses. Studies by Norrpa et al. (1985), He and Lambert (1985), and Obe et al. (1986) provide evidence supporting the intracellular formation of acetaldehyde from ethanol or vinyl acetate (i.e., acetaldehyde is an SCE-inducing metabolite). Moreover, Lam et al. (1986) also provided supporting data that

acetaldehyde, distilled under nitrogen, forms DNA-protein cross-links in the rat nasal cavity (discussed in section 4.4.3.).

A survey of the literature revealed very little information on the mutagenicity of peroxyacetic acid. Negative responses were reported for unscheduled DNA synthesis in human fibroblasts (Coppinger and Thompson, 1983) and in the Ames test in Salmonella (Yamaguchi and Yamashita, 1980).

6.5. CHEMICAL INTERACTIONS IN THE MAMMALIAN GONAD

An important aspect of a mutagenicity evaluation is the assessment of the potential of the chemical to reach mammalian germinal tissue and cause heritable genetic damage (U.S. EPA, 1984). A survey of the published literature revealed no information on the ability of acetaldehyde to cause genetic damage in mammalian gonads or to cause other effects (e.g., abnormal sperm morphology, reduced fertility) on germinal tissue in vivo. In view of the evidence, however, that acetaldehyde induces heritable effects in the germ cells of Drosophila (Woodruff et al., 1985), it should be determined whether it reaches germ cells in whole mammals and produces genetic damage.

6.6. SUGGESTED MUTAGENICITY TESTING

As mentioned above, an important deficiency in the information available for characterizing the mutagenic hazards associated with acetaldehyde exposure is the lack of evidence on its ability to reach mammalian gonads and produce genetic damage. In view of acetaldehyde's ability to induce SCEs and chromosomal aberrations in somatic cells, it should be tested for cytogenetic damage in germ cells (e.g., the rodent dominant lethal test or cytogenetic analysis for chromosomal aberrations or SCEs). DNA-binding or cross-linking studies in gonads would provide evidence that exposure to acetaldehyde resulted in its transport to the germ cells. Although acetaldehyde clearly induces cytogenetic abnormalities in mammalian somatic cells and produces gene mutations in Droso-

phila, there is no information available on its ability to induce gene mutations in cultured mammalian cells. Thus, an in vitro mammalian cell gene mutation assay would further characterize acetaldehyde's ability to produce gene mutations.

6.7. SUMMARY AND CONCLUSIONS

Acetaldehyde has been shown by several different laboratories to induce sister chromatid exchanges in cultured mammalian cells (Chinese hamster cells and human peripheral lymphocytes) in a dose-related manner (Obe and Ristow, 1977; Obe and Beer, 1979; de Raat et al., 1983; Böhlke et al., 1983; Ristow and Obe, 1978; Jansson, 1982; Norrpa et al., 1985). The induced responses were observed at doses that did not severely affect cell proliferation. A recent study (He and Lambert, 1985) provided suggestive evidence that SCE-inducing lesions produced by acetaldehyde may be persistent over several cell generations. Lesions that persist could be more detrimental than those that are repaired rapidly. The in vitro responses did not require metabolic activation by a liver S9 preparation. One study showed that when S9 mix was used, the SCE response was similar to that in its absence (de Raat et al., 1983). Thus, the kinetics of in vitro metabolism/detoxification differs from the in vivo situation. However, acetaldehyde dehydrogenase (plus NAD), added directly to the cell cultures, resulted in the reduction of SCEs produced by acetaldehyde (Obe et al., 1986). The induction of SCEs by acetaldehyde has also been detected in bone marrow cells of whole mammals, namely mice and Chinese hamsters (Obe et al., 1979; Korte and Obe, 1981). The route of exposure in these studies was intraperitoneal injection, and it is uncertain whether similar responses would be observed if a route relevant to human exposure (e.g., inhalation or oral) were used.

In addition to its ability to induce SCEs, acetaldehyde has been shown to produce chromosomal aberrations (breaks, gaps, and exchange-type aberrations) and micronuclei in mammalian cell cultures in a dose-related manner (Bird et al., 1982; Böhlke et al., 1983). Chromosomal aberrations have also been detected in plants (Rieger and Michaelis, 1960). In Drosophila, chromosomal effects (i.e., reciprocal translocations) were not found after acetaldehyde treatment (Woodruff et al., 1985). The clastogenicity of acetaldehyde in whole mammals has not been sufficiently evaluated. In the one study that was available, female rats were injected intraamniotically on the 13th day of gestation, and the treated embryos had high frequencies of gaps and breaks (Barilyak and Kozachuk, 1983).

Although acetaldehyde did not produce reciprocal translocations in Drosophila, it was found to induce gene mutations (sex-linked recessive lethals) when administered by injection (Woodruff et al., 1985). Salmonella testing has been negative (Commoner, 1976; Laumbach et al., 1976; Pool and Wiessler, 1981; Marnett et al., 1985; Mortelmans et al., 1986). Because acetaldehyde is volatile, it is possible that loss of the chemical by evaporation occurred in these assays; alternatively, Salmonella may be unresponsive to acetaldehyde treatment. Positive results for gene mutations were reported in the nematode Caenorhabditis (Greenwald and Horvitz, 1980) but no dose relation was shown. An equivocal result was obtained for mitochrondrial mutations in yeast (Bandas, 1982). There were no available data on the ability of acetaldehyde to produce gene mutations in mammalian cells in vitro.

Acetaldehyde has yielded negative results in tests for DNA strand breaks in mammalian cells in vitro (Sina et al., 1983; Saladino et al., 1985; Lambert et al., 1985). However, if acetaldehyde produces SCEs and chromosomal aberrations by DNA-DNA or DNA-protein cross-linking, it may not necessarily produce

DNA strand breaks.

In conclusion, there is sufficient evidence that acetaldehyde produces cytogenetic damage (chromosomal aberrations, micronuclei, and sister chromatid exchanges) in cultured mammalian cells. Although there are only three studies in whole mammals, they suggest that acetaldehyde produces similar effects *in vivo*. Acetaldehyde produced gene mutations in *Drosophila* but not in *Salmonella*; no studies were found for cultured mammalian cells. Thus, the available evidence indicates that acetaldehyde is mutagenic and may pose a risk for somatic cells. Current knowledge, however, is inadequate with regard to germ cell mutagenicity because the available information is insufficient to support any conclusions about the ability of acetaldehyde to reach mammalian gonads and produce heritable genetic damage.

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7. CARCINOGENICITY

The purpose of this chapter is to evaluate the likelihood that acetaldehyde is a human carcinogen and to provide a basis for estimating its public health impact and evaluating its potency in relation to other carcinogens on the assumption that it is a human carcinogen. The evaluation of carcinogenicity depends heavily on animal bioassays and epidemiologic evidence. However, other factors, including mutagenicity, metabolism (particularly in relation to interaction with DNA), and pharmacokinetic behavior, have an important bearing on both the qualitative and the quantitative assessment of carcinogenicity. The available information on these latter subjects is reviewed in other chapters of this document, with key points incorporated into this evaluation as appropriate. This chapter presents an evaluation of the animal cancer bioassays with acetaldehyde, the epidemiologic evidence with direct acetaldehyde exposure, mechanistic considerations for risk estimation, and quantitative aspects of carcinogen risk assessment of acetaldehyde.

Although the scope of this chapter is restricted to external acetaldehyde exposure, it is recognized that exposure to other agents generates acetaldehyde internally (Chapter 4). Some examples are ethanol consumption, fermented foods, tobacco smoke, and intestinal bacteria. The largest of these potential sources of acetaldehyde is ethanol consumption.

While it is beyond the scope of this document to review the extensive literature on the carcinogenic effects of ethanol, that subject has relevance to the evaluation of acetaldehyde as a carcinogen. Chapter 4 describes some of the extensive evidence that the metabolism of ethanol involves acetaldehyde as the first step, which can proceed via more than one reaction pathway at high chronic ethanol doses. Since this metabolism occurs in all tissues, ethanol

consumption could be viewed as a means by which acetaldehyde is delivered to the epithelial tissues of the mouth, larynx, esophagus, and liver as a first-pass effect.

The question of whether ethanol consumption causes cancer at these sites is apparently unresolved. A number of prospective cohort studies showed excess incidence of, as well as mortality from, cancers of the buccal cavity, pharynx, larynx, esophagus, lungs, and liver in subjects who consumed high levels of alcoholic beverages and who also smoked (Schottenfeld and Fraumeni, 1982). It is noteworthy that smoking alone is a strong risk factor for all the cancers mentioned above except liver cancer. Most of these studies have not been able to adjust for the confounding effects of smoking; hence, it is uncertain how much effect can be attributed to alcohol consumption in causation of these cancers. Other chemical agents in alcoholic beverages, besides ethanol, could be potentially carcinogenic, so that no definite conclusions can be made regarding the carcinogenicity of ethanol and, hence, acetaldehyde derived from ethanol. Experiments in laboratory animals have failed to show carcinogenic responses to alcohol (U.S. DHEW, 1978).

There is a possibility that exposures to other carcinogenic agents could also generate acetaldehyde at tissue sites where malignancies develop. Several halogenated two-carbon carcinogens (ethylene dibromide, ethylene dichloride, vinyl chloride, vinylidene chloride, trichloroethylene) are believed to form two-carbon halogenated aldehydes as active intermediates. This evidence has been discussed in Health Assessment Documents on these agents.

7.1. ANIMAL STUDIES

7.1.1. Hamsters (Feron, 1979; Feron et al., 1982)

Feron (1979) studied the carcinogenic effect of the inhalation of acetaldehyde vapor in Syrian golden hamsters (Mesocricetus auratus). A total of 420 young male hamsters were divided into two equal groups. The first group (controls) was exposed to filtered and conditioned air, and the second group was exposed to acetaldehyde vapor at 1500 ppm 7 hours/day, for 5 days/week, for 52 weeks. Both groups were further divided into six groups of 35 hamsters each. These subgroups were treated with weekly intratracheal instillations consisting of 0.2 mL 0.9% NaCl solution in which benzo(a)pyrene (BaP) at concentrations of 0, 0.0625, 0.125, 0.25, 0.5, and 1.0 mg had been suspended. Following 52 weeks of treatment, five animals, randomly taken from each group, were killed and autopsied. The other animals were removed from the chamber for 26 weeks (rest period). The experiment was terminated after 78 weeks. The results are shown in Tables 7-1 and 7-2.

The animals exposed to acetaldehyde were more restless and had slightly reduced body weight gains--10% when compared to the controls. Up to 39 weeks, no differences were noted in mortality rates between exposed and control groups. Thereafter, the mortality of hamsters in the highest BaP-exposed group increased more rapidly than the other BaP-exposed and control groups (Table 7-1). The hemoglobin, hematocrit, and red blood count values were significantly lower in the exposed groups than in the controls. In addition, in the acetaldehyde-exposed groups, the urine contained more protein, and the kidney weights increased significantly as compared to the air-controlled groups.

With respect to nonneoplastic lesions, exposure of hamsters to 1500 ppm acetaldehyde vapor plus BaP instillation produced abnormalities in the respiratory tract and marked lesions in the nasal cavity. In animals killed at the

TABLE 7-1. CUMULATIVE MORTALITY OF MALE HAMSTERS GIVEN INTRATRACHEAL INSTILLATIONS OF BaP AND EXPOSED TO AIR OR ACETALDEHYDE VAPOR[a]

Total dose of BaP (mg)	Number of deaths at end of week							
	0	4	13	26	39	52[b]	65	78
Air								
0	0	0	0	1	1	2	2	5
3.25	0	0	1	1	1	2	3	5
6.5	0	0	0	0	1	2	2	3
13	0	0	0	0	0	3	6	7
26	0	0	1	2	2	2	8	11
52	0	0	0	0	0	4	15[c]	22[c]
Acetaldehyde								
0	0	0	0	0	1	8	11	12
3.25	0	1	2	2	2	2	2	7
6.5	0	0	0	1	1	2	3	4
13	0	0	0	0	2	5	6	7
26	0	0	1	1	1	3	5	11
52	0	0	0	0	2	15	25[c]	28[c]

[a]Each group initially consisted of 35 males.
[b]In week 52 all treatments were stopped and five animals of each group were killed for pathological examinations. These animals are not included in the table.
[c]$p < 0.001$, according to the chi-square test. Statistical analyses were done by the author.

SOURCE: Feron, 1979.

TABLE 7-2. TYPES AND INCIDENCES OF RESPIRATORY TRACT TUMORS IN MALE HAMSTERS AFTER 52 WEEKLY INTRATRACHEAL INSTILLATIONS OF BENZO(a)PYRENE (BaP) AND EXPOSURE TO AIR OR ACETALDEHYDE VAPOR

	Incidence of tumors											
	Air and BaP (mg)						1500 ppm Acetaldehyde and BaP (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
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
Bronochioli 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 the Fisher Exact Test. Statistical analysis was done by CAG.

SOURCE: Feron, 1979.

end of the 52-week exposure period, the normal respiratory and olfactory epithelia were replaced by keratinizing, stratified, squamous epithelia. In animals killed after a recovery period of 26 weeks, the lesions were clearly diminished or had disappeared completely. While these hyperplastic and metaplastic changes were observed in the nasal cavity, trachea, and laryngeal epithelium of all animals that were exposed to acetaldehyde, no lesions were observed in other parts of the respiratory tract.

Neoplastic alterations attributable to acetaldehyde exposure alone were not found, but respiratory tract tumors were observed in hamsters exposed at all dose levels of BaP (Table 7-2). Intratracheal instillation of 26 mg and 52 mg BaP produced tumors in the presence or absence of concurrent acetaldehyde exposure. It is of interest to note that intratracheal instillation of the highest BaP dose (52 mg, 1 mg/week, for 52 weeks) combined with acetaldehyde exposure produced twice the incidence of squamous cell carcinomas of the trachea (24/30 versus 11/28, $p = 0.002$) and bronchi (8/30 versus 4/28, $p = 0.20$) compared to the same dose of BaP without acetaldehyde. At lower BaP doses there were no corresponding differences. Tumors of the bronchi, bronchioli, and alveoli were evident at all dose levels of BaP, in the presence or absence of acetaldehyde, but they were mostly adenomas.

The following conclusions can be drawn from this experiment:

(1) The numbers of hamsters with respiratory tract tumors increased as the dose of BaP increased.

(2) At the highest dose level of BaP, the incidence of squamous cell carcinomas of the trachea was twice as high in hamsters exposed to acetaldehyde as in those exposed to air.

(3) A distinct shortening of the latency period (28 weeks versus 50 weeks) for the induction of neoplasms was observed with increasing doses of BaP, as described by the author.

(4) Acetaldehyde alone at a concentration of 1,500 ppm showed no effect beyond an increased mortality.

This experiment had some methodological limitations: only male hamsters were used, the duration of the exposure was only 1 year, only one dose level (1500 ppm) of acetaldehyde was used, and the study was terminated at 78 weeks. This dose level of acetaldehyde, which resulted in increased mortality and decreased body weight gain, might have exceeded the maximum tolerated dose (MTD). Several dose levels should have been used. The number of animals in each BaP group was small, and discontinuation of treatment with acetaldehyde might have caused regression in metaplastic lesions.

In the second part of the Feron (1979) study, 245 male and 245 female hamsters were divided into seven groups, each consisting of 35 males and 35 females, and were given various doses of BaP by intratracheal instillation for 52 weeks. The treatment schedule and dosages are presented in Table 7-3.

Subsequently, the animals were kept for an additional 52 weeks (recovery period). The experiment was terminated at 104 weeks. After 13, 26, and 52 weeks, three animals/sex/group were killed and autopsied. Observations were made of general appearance, body weight, mortality, and gross and microscopic pathology. In general, acetaldehyde treatment had no effect on body weight gain. The mortality of animals treated with the highest dose of acetaldehyde was slightly higher than that of controls (Table 7-4). The highest mortality rates observed in this experiment resulted mainly from respiratory tract tumors induced by diethylnitrosamine (DENA).

TABLE 7-3. 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% BaP 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% BaP 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 7-4. CUMULATIVE MORTALITY OF HAMSTERS GIVEN INTRATRACHEAL INSTILLATION OF 0.9% NaCl SOLUTION, ACETALDEHYDE, BaP, BaP + ACETALDEHYDE, DENA, OR DENA + ACETALDEHYDE[a]

Treatments[c]	Cumulative mortality[b] at end of week									
	0	4	13	26	39	52	65	78	91	104
Males										
0.9% NaCl solution	0	0	2	2	3	6	6	6	12	21
4 µL acetaldehyde	0	0	0	0	0	2	3	6	9	14
8 µL acetaldehyde	0	2	6	8	8	11	11	11	16	21
BaP	0	1	1	1	2	5	7	10	13	21
BaP+4 µL acetaldehyde	0	3	3	3	4	7	9	10	12	21
DENA	0	0	1	1	3	11	19[d]	26[d]	26[d]	26
DENA+4 µL acetaldehyde	0	1	1	1	2	17[d]	24[d]	26[d]	26[d]	26
Females										
0.9% NaCl solution	0	0	0	0	1	2	5	10	19	24
4 µL acetaldehyde	0	0	0	0	0	0	4	10	18	25
8 µL acetaldehyde	0	1	1	3	4	7	10	15	25[d]	26
BaP	0	0	1	1	1	2	7	14	19	25
BaP+4 µL acetaldehyde	0	0	0	1	3	3	3	7	17	25
DENA	0	0	0	0	1	14[e]	22[e]	26[e]	26[e]	26
DENA+4 µL acetaldehyde	0	1	1	1	1	17[e]	23[e]	26[e]	26[e]	26

[a]Each group initially consisted of 35 males and 35 females.
[b]At weeks 13, 26, and 52, three males and three females of each group were killed for pathological examination. These animals are not included in the table.
[c]Treatments were stopped at week 52.
[d]$p < 0.05$, according to the chi-square test. Statistical analyses were done by the author.
[e]$p < 0.01$, according to the chi-square test. Statistical analyses were done by the author.

SOURCE: Feron, 1979.

Various types of benign and malignant respiratory tract tumors were found in both male and female hamsters treated with BaP or BaP plus acetaldehyde (Table 7-5). Tracheal tumors occurred in 46% (22/48) of hamsters treated with BaP alone and in 59% (27/46) of hamsters given BaP plus acetaldehyde. In groups treated with acetaldehyde alone (2% or 4%), no tumors were observed in the larynx, trachea, and bronchi. However, large numbers of tracheal papillomas and lung adenomas were found in groups treated with acetaldehyde plus BaP or DENA. The carcinogenic effects of DENA on the various portions of the respiratory tract were not influenced by acetaldehyde, as concluded from the lack of clear difference in the incidences of respiratory tract tumors between the DENA and DENA-plus-acetaldehyde groups. These findings suggest that acetaldehyde is neither a primary carcinogen nor a promoter with BaP or DENA in hamsters under the conditions of this study. This experiment also had some methodological limitations in that the interim sacrifice included only three animals/sex/dose, and the duration of exposure was only 52 weeks.

In an extension of the above study, Feron et al. (1982) studied respiratory tract tumors in male and female hamsters exposed to high concentrations of acetaldehyde vapor alone or simultaneously with either BaP or DENA. In this study 504 male and 504 female hamsters were evenly distributed in two chambers, one a control chamber in which the animals were exposed to filtered air and conditioned air and the other a test chamber in to which acetaldehyde vapor was added. The animals were exposed 7 hours/day, 5 days/week, for 52 weeks to an average acetaldehyde concentration of 2500 ppm during the first 9 weeks, 2250 ppm during weeks 10 to 20, 2000 ppm during weeks 21 to 29, 1800 ppm during weeks 30 to 44, and 1650 ppm during weeks 45 to 52. The exposure levels were reduced several times because of considerable growth retardation and to avoid early mortality of the test animals. The animals were further divided as follows:

TABLE 7-5. TYPES AND INCIDENCES OF RESPIRATORY TRACT TUMORS IN HAMSTERS GIVEN INTRATRACHEAL INSTILLATIONS OF 0.9% NaCl SOLUTION, ACETALDEHYDE, BaP, BaP + ACETALDEHYDE, DENA, OR DENA + ACETALDEHYDE

	Incidence of tumors													
	0.9% NaCl		4 µL acetaldehyde		8 µL acetaldehyde		BaP		BaP+ 4 µL acetaldehyde		DENA		DENA+ 4 µL acetaldehyde	
Site and type of tumor	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
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

aA few animals were lost through autolysis or cannibalism.

SOURCE: Feron et al., 1979.

group 1 (18 hamsters of each sex)--no treatment; group 2 (18 hamsters of each sex)--52 weekly intratracheal instillations of 0.2 mL NaCl (0.9%) solution; group 3 (30 hamsters of each sex)--52 weekly intratracheal instillations of BaP (0.175%); group 4 (30 hamsters of each sex)--52 weekly intratracheal instillations of BaP (0.35%); group 5 (30 hamsters of each sex)--17 subcutaneous injections of DENA (0.0625%) given every 3 weeks. Following the 52-week treatment period there was a 29-week recovery period, after which all hamsters were killed for autopsy, i.e., at week 81 (Table 7-6).

Body weights were recorded every 2 weeks during the first 6 weeks and monthly thereafter. From week 4 onward, hamsters exposed to acetaldehyde had substantially lower body weights than those exposed to air (Table 7-7). During the post-exposure period (53 to 81 weeks), the significant ($p < 0.05$) differences in body weight between exposed and control hamsters generally diminished but did not disappear. Mortality was slightly higher in acetaldehyde-exposed hamsters than in controls (Table 7-8). There was an increase in mortality ($p < 0.05$) in animals treated with BaP and exposed to acetaldehyde or air over those exposed to acetaldehyde or air alone. In addition, mortality was higher in males treated with the highest dose of BaP. There was low mortality in the DENA-treated group exposed to air.

At the end of the exposure period of 52 weeks, a distinct nonneoplastic histopathological change, similar to those found in previous studies, was observed in acetaldehyde-exposed animals. The nasal changes consisted of thinning and degeneration of the layer of olfactory epithelium, and hyper- and metaplasia of the respiratory epithelium. No tumors were found in hamsters killed immediately at the end of the exposure period. All hamsters that were found dead or sacrificed at week 81 exhibited inflammatory, hyperplastic, and/or metaplastic changes in the nose and larynx. Incidences of respiratory

TABLE 7-6. TREATMENT PROTOCOL FOR HAMSTERS EXPOSED TO EITHER AIR OR ACETALDEHYDE VAPOR

Group	Number and sex of hamsters exposed via inhalation to acetaldehyde or air		Dosage and route of additional treatments to hamsters		
	Air	Acetaldehyde	Treatment	Dosage	Route
1	18 males 18 females	18 males 18 females	None	--	--
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	BaP	0.35 mg/week Total 18.2 mg	Intratracheal instillation
4	30 males 30 females	30 males 30 females	BaP	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

SOURCE: Feron et al., 1982.

TABLE 7-7. AVERAGE BODY WEIGHTS OF HAMSTERS EXPOSED TO AIR OR ACETALDEHYDE VAPOR AND TREATED INTRATRACHEALLY WITH BaP OR SUBCUTANEOUSLY WITH DENA

Treatment[a]			Average body weight (g) at the end of week							
Inhalation	Intratracheal instillation	Subcutaneous injection	0	4	14	26	42	52	66	80
			Males							
Air	--	--	85	96	102	106	101	102	98	102
Air	0.9% NaCl	--	84	95	106	112	106	110	113	116
Air	BaP (18.2 mg)	--	85	95	102	103	100	103	112	116
Air	BaP (36.4 mg)	--	85	96	101	105	105	107	106	113
Air	--	DENA	85	98	107	108	103	104	108	112
Acetaldehyde	--	--	85	87[b]	86[c]	90[c]	84[c]	87[b]	95	101
Acetaldehyde	0.9% NaCl	--	85	89	86[c]	87[c]	83[c]	87[c]	95[b]	98[b]
Acetaldehyde	BaP (18.2 mg)	--	84	89[c]	88[c]	86[c]	86[c]	91[b]	99[b]	101[c]
Acetaldehyde	BaP (36.4 mg)	--	84	84[c]	85[c]	83[c]	87[c]	89[c]	100	98[d]
Acetaldehyde	--	DENA	84	86[c]	87[c]	86[c]	84[c]	85[c]	92[c]	99[b]
			Females							
Air	--	--	86	108	115	119	113	119	115	113
Air	0.9% NaCl	--	86	108	119	116	120	119	120	118
Air	BaP (18.2 mg)	--	87	105	115	117	112	106	108	108
Air	BaP (36.4 mg)	--	87	101	116	116	110	106	108	110
Air	--	DENA	87	104	115	123	118	117	116	121
Acetaldehyde	--	--	86	97[d]	94[c]	96[c]	91[c]	94[c]	105	102[d]
Acetaldehyde	0.9% NaCl	--	87	96[d]	98[c]	101[c]	97[c]	97[c]	94[c]	94[d]
Acetaldehyde	BaP (18.2 mg)	--	86	94[b]	94[c]	97[c]	96[c]	95[c]	95[b]	103
Acetaldehyde	BaP (36.4 mg)	--	86	96	96[c]	100[c]	95[c]	99	98	101
Acetaldehyde	--	DENA	86	97	99[c]	103[c]	93[c]	92[c]	100[c]	103[b]

[a]At week 52, all treatments were stopped. All statistical analyses were done by the authors.
[b]$p < 0.01$, according to Student's t-test.
[c]$p < 0.001$, according to Student's t-test.
[d]$p < 0.05$, according to Student's t-test. The various groups of acetaldehyde-exposed animals were compared with the corresponding groups of air-exposed controls.

SOURCE: Feron et al., 1982.

TABLE 7-8. CUMULATIVE MORTALITY OF HAMSTERS EXPOSED TO AIR OR ACETALDEHYDE VAPOR AND TREATED INTRATRACHEALLY WITH BaP OR SUBCUTANEOUSLY WITH DENA

Treatment[a]			Number of animals/ group	Number of deaths at the end of week						
Inhalation	Intratracheal instillation	Subcutaneous injection		4	14	26	42	52	66	80
Males										
Air	--	--								
Air	0.9% NaCl	--	30[b]	0	1	1	2	4	5	7
Air	BaP (18.2 mg)	--	30	0	0	0	3	6	6	8
Air	BaP (36.4 mg)	--	30	0	0	0	3	3	6	11
Air	--	DENA	30	0	0	0	0	0	0	1
Acetaldehyde	--	--								
Acetaldehyde	0.9% NaCl	--	30[b]	0	0	0	2	6	8	11
Acetaldehyde	BaP (18.2 mg)	--	30	0	1	1	3	4	6	11
Acetaldehyde	BaP (36.4 mg)	--	30	0	1	1	5	12[c]	14[c]	20[c]
Acetaldehyde	--	DENA	30	0	0	0	0	0	4[c]	11[d]
Females										
Air	--	--								
Air	0.9% NaCl	--	30[b]	0	0	0	2	4	5	16
Air	BaP (18.2 mg)	--	30	0	1	5	9	13	16	21
Air	BaP (36.4 mg)	--	30	0	1	2	7	10	12	18
Air	--	DENA	30	0	1	1	3	3	6	11
Acetaldehyde	--	--								
Acetaldehyde	0.9% NaCl	--	30[b]	0	1	4[c]	7	9	13[c]	20
Acetaldehyde	BaP (18.2 mg)	--	30	0	0	2	4	5[c]	8[c]	17
Acetaldehyde	BaP (36.4 mg)	--	30	0	0	1	6	13	20[c]	23
Acetaldehyde	--	DENA	30	0	0	2	5	8	9	16

[a]At week 52, all treatments were stopped.
[b]Initially, both groups together comprised 36 males and 36 females. At week 52, six males and six females of each group were killed for interim information. These animals are not included in the table.
[c]$p < 0.05$, according to the chi-square test. All statistical analyses were done by the authors.
[d]$p < 0.001$, 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.

tumors in hamsters exposed to either air or acetaldehyde are presented in Table 7-9. Tumors were observed in both the nose (adenoma, adenocarcinoma, and aplastic carcinoma) and the larynx (carcinoma in situ, squamous cell carcinoma, and adeno-squamous carcinoma) of animals exposed to acetaldehyde vapor alone. The incidence of larynx tumors in control compared with exposed males was 0/20 versus 6/23 ($p = 0.017$) and in females was 0/22 versus 4/20 ($p = 0.043$). The neoplastic and nonneoplastic lesions in the larynx were mainly located on the true vocal cord or in the most anterior part of the larynx. None of the animals exposed to air alone demonstrated nasal or laryngeal tumors. No tracheal tumors were observed in hamsters exposed to acetaldehyde alone (Table 7-10). Further, it is of interest to note that the total respiratory tumors were increased at least threefold in either males or females in the highest acetaldehyde + BaP group as compared to acetaldehyde alone. The incidence of carcinomas in the trachea and bronchi was significantly ($p < 0.05$) higher in hamsters exposed to acetaldehyde and treated with high doses of BaP (36.4 mg) than in hamsters treated with the same dose of BaP but exposed to air (Table 7-11). The latency period for tracheobronchial carcinomas was much shorter after combined exposure than after treatment with 36.4 mg BaP alone indicated by the author. 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 (Tables 7-10 and 7-11). The present observation supports the previous conclusion of Feron (1979) that acetaldehyde treatment, together with high doses of BaP, results in pronounced increases in the incidence of tracheobronchial carcinomas.

7.1.2. Rats

7.1.2.1. Watanabe and Sugimoto (1956)--Watanabe and Sugimoto (1956) reported spindle-cell sarcoma in rats at the site of repeated acetaldehyde injection.

TABLE 7-9. INCIDENCE OF RESPIRATORY TRACT TUMORS IN HAMSTERS EXPOSED TO EITHER AIR OR ACETALDEHYDE VAPOR

Site	Type of tumor	Air		Acetaldehyde	
		Males	Females	Males	Females
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
	Total tumors	0/24	0/23	2/27	1/26
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
	Total tumors	0/20	0/22	6/23[a]	4/20[b]

[a] $p = 0.017$, according to the Fisher Exact Test. All statistical analyses were done by CAG.

[b] $p = 0.043$, according to the Fisher Exact Test. All statistical analyses were done by CAG.

SOURCE: Feron et al., 1982.

TABLE 7-10. SITES AND INCIDENCES OF RESPIRATORY TRACT TUMORS IN HAMSTERS EXPOSED TO AIR OR ACETALDEHYDE VAPOR AND TREATED INTRATRACHEALLY WITH BaP OR SUBCUTANEOUSLY WITH DENA[a]

Treatments				Numbers of animals with tumors of						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	--	--[h]	15							
Air	0.9% NaCl[c]	--	15	30[g] 0(0%)	0	0	0	0	0	0
Air	BaP(18.2 mg)[d]	--	29	4(14%)	0	0	2	1	2	5
Air	BaP(36.4 mg)[e]	--	30	19(63%)	0	1	8	3	13[j]	27
Air	--	DENA[f]	29	12(41%)	2	7	3	3	0	15
Acetaldehyde	--	--	15							
Acetaldehyde	0.9% NaCl[c]	--	14	29[g] 7(24%)[j]	2	6[j]	0	0	0	8
Acetaldehyde	BaP(18.2 mg)[d]	--	29	12(41%)[k]	2	8[j]	3	1	1	15
Acetaldehyde	BaP(36.4 mg)[e]	--	27	22(81%)	1	9[j]	14	5	3[j]	32
Acetaldehyde	--	DENA[f]	30	11(37%)	3	10	2	0	0	15
Females										
Air	--	--	14							
Air	0.9% NaCl[c]	--	14	28[g] 0(0%)	0	0	0	0	0	0
Air	BaP(18.2 mg)[d]	--	27	3(11%)	0	1	0	1	1	3
Air	BaP(36.4 mg)[e]	--	24	7(29%)	0	0	3	1	5	9
Air	--	DENA[f]	27	11(41%)	0	3	8	2	0	13
Acetaldehyde	--	--	15							
Acetaldehyde	0.9% NaCl[c]	--	14	29[g] 5(17%)	1	4	0	0	0	5
Acetaldehyde	BaP(18.2 mg)[d]	--	29	11(38%)	1	7[k]	4[k]	0	1	13
Acetaldehyde	BaP(36.4 mg)[e]	--	29	16(55%)	0	4	10	2	3	19
Acetaldehyde	--	DENA[f]	28	8(29%)	2	7	0[k]	0	0	9

[a]See Table 7-11 for types of tumors.
[b]A few animals were lost through cannibalism or autolysis.
[c]0.2 mL weekly during 52 weeks.
[d]52 weekly doses of 0.35 mg.
[e]52 weekly doses of 0.70 mg.
[f]Given subcutaneously in 17 three-weekly doses of 0.125 µL each.
[g]Animals killed at the end of the treatment period are not included in this table.
[h]No further treatment.
[i]Two animals had more than one type of pulmonary tumor.
[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 7-11. SITES, TYPES, AND INCIDENCES OF RESPIRATORY TRACT TUMORS IN HAMSTERS EXPOSED TO AIR OR ACETALDEHYDE VAPOR AND TREATED INTRATRACHEALLY WITH BaP OR SUBCUTANEOUSLY WITH DENA[a]

Site and type of tumor	Incidence of tumors							
	Inhalation of air				Inhalation of acetaldehyde			
	0.9% NaCl[bc]	BaP (18.2 mg)[d]	BaP (36.4 mg)[c]	DENA	0.9% NaCl[bc]	BaP (18.2 mg)[d]	BaP (36.4 mg)[e]	DENA[f]
Males								
Larynx	(20)[e]	(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
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 intratracheally in 52 weekly doses of 0.70 mg.
[f]Given subcutaneously in 17 three-weekly doses of 0.125 µL.
[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.

Of 14 out of 20 rats which survived the period between 489 and 554 days, only four rats (20% to 25%) developed sarcomas at the injection site. No conclusion can be drawn from this study because neither the total doses of acetaldehyde nor the tumor incidences in controls could be determined from the available data.

7.1.2.2. Woutersen and Appelman (1984); Woutersen et al. (1984, 1985)*--These investigators studied the carcinogenicity of acetaldehyde in 420 male and 420 female albino SPF Wistar rats (Cpb:WU, Wistar random) obtained from the TNO Central Institute (breeders of laboratory animals), Zeist, The Netherlands. After an acclimatization period of 3 weeks, these animals were randomly assigned to four groups of 105 males and 105 females each. The animals were then exposed by inhalation to atmospheres containing 0, 750, 1500, or 3000/1000 ppm acetaldehyde for 6 hours/day, 5 days/week, for 27 months. The concentration in the highest dose group was gradually reduced from 3000 to 1000 ppm because of severe growth retardation, occasional loss of body weight, and early mortality in this group. The animals of this group evinced symptoms of severe respiratory distress, including salivation, labored breathing, and mouth breathing. All animals were housed in inhalation chambers, five males and five females/ cage, both during and after exposure. For controls, an identical inhalation chamber was used. The GC-purity of the acetaldehyde was 99.8%, as specified by the supplier. Each batch was analyzed for its formaldehyde content. The concentration of formaldehyde was 60 ppm (v/v), indicating that the concentration of formaldehyde in 1500 ppm acetaldehyde atmosphere was at most 0.13 ppm. The experimental design is described in Table 7-12. The study

*The reference to Woutersen et al. (1984) is included merely for purposes of completeness, since it contains 15 months' interim results and is superseded by the later reports.

TABLE 7-12. EXPERIMENTAL DESIGN OF ACETALDEHYDE INHALATION STUDY IN WISTAR RATS

Group	Exposure level (ppm)	Number of animals	
		Males	Females
A	0	105	105
B	750	105	105
C	1,500	105	105
D	3,000	105	105

Each group of animals was divided into five subgroups consisting of the following numbers of animals:

Subgroups 1 + 2: each consisted of 5 males and 5 females, which were killed after 13 and 26 weeks of exposure, respectively, to obtain interim information.

Subgroup 3: 10 males and 10 females, killed after 52 weeks of exposure.

Subgroup 4[a]: 30 males and 30 females, intended for recovery; the animals were exposed for 52 weeks and killed after a recovery period of 26 weeks (subgroup 4A) and after a recovery period of 52 weeks (subgroup 4B).

Subgroup 5: 55 males and 55 females, intended for lifetime exposure (27 months).

[a]This "recovery study" was described in a separate report (Woutersen and Appelman, 1984) (see section 7.1.2.3. of this document).

SOURCE: Woutersen et al., 1985.

included: (1) interim kills after 13, 26, and 52 weeks; (2) one recovery group of 52 weeks' exposure; and (3) one group of lifetime (27 months') exposure. The present report considers the lifetime exposure and the results obtained after 13, 26, and 52 weeks.

The animals' body weights were recorded every week during the first month of the study, bi-weekly during the next 2 months, and monthly thereafter. Complete hematology, clinical chemistry, and urine analyses were done. The mean concentrations of acetaldehyde to which the rats were exposed during the study are summarized in Table 7-12.

Mortality was increased in each of the dose groups as compared to controls (Table 7-13), with a clear exposure-response relationship being evident. By day 715, all of the rats in the highest dose group had died. When the study was terminated at day 844, very few animals in the middle dose range were alive. All of the animals that were killed or had died were autopsied and subjected to detailed microscopic examination of tissues (nose, larynx, trachea, lung, kidneys, liver, spleen, pancrease, adrenals, heart, stomach, genital organs, etc.). The rats in the highest dose group showed signs of excitation, salivation, pilo-erection, and labored respiration. Several of the animals in the highest dose group showed blood around their nares. Despite a further reduction in the concentration of acetaldehyde, the number of animals showing the above conditions increased after 12 months. In almost every case, the rats in the highest dose group that died early or in moribund condition had partial or complete occlusion of the nose by excessive amounts of keratin and inflammatory exudate. Several male and female high-dose rats also showed acute bronchopneumonia, occasionally accompanied by tracheitis.

There were hyperplastic and metaplastic changes of the respiratory epithelium in all of the male and female rats in the highest dose group. These

TABLE 7-13. CUMULATIVE MORTALITY IN AN INHALATION CARCINOGENICITY STUDY OF ACETALDEHYDE IN RATS

Day of study	Males				Females			
	Acetaldehyde (ppm)				Acetaldehyde (ppm)			
	0	750	1500	3000	0	750	1500	3000
	(55)[a]	(55)	(55)	(55)	(55)	(55)	(55)	(55)
140	0	1	0	1	0	1	0	0
210	0	1	0	7[b]	0	1	0	1
356	0	3	1	14[d]	0	1	0	7[b]
412	2	4	4	22[d]	0	1	0	18[d]
468	3	4	6	28[d]	1	2	2	24[d]
524	3	6	9	38[d]	1	5	5	30[d]
580	3	9	15[c]	44[d]	3	6	7	32[d]
636	8	11	22[c]	48[d]	5	7	11	41[d]
715	18	26	27	55[d]	9	20[b]	21[b]	55[d]
813	30	38	38	55[d]	23	33	37[b]	55[d]
844	33	44[b]	46[c]	55[d]	27	38	44[d]	55[d]

[a]Numbers in parentheses represent initial numbers of animals. All statistical analyses were done by the authors.
[b]$p < 0.05$, according to the Fisher Exact Test. All comparisons were made with the controls.
[c]$p < 0.01$, according to the Fisher Exact Test. All comparisons were made with the controls.
[d]$p < 0.001$, according to the Fisher Exact Test. All comparisons were made with the controls.

SOURCE: Woutersen et al., 1985.

changes were accompanied by moderate to severe keratinization. In several animals, papillomatous hyperplasia and proliferation of atypical basal cells were found. At the mid-dose level, a few animals showed slight degenerative changes of the respiratory epithelium and olfactory epithelium (Table 7-14).

In conclusion, treatment-related nonneoplastic histopathological changes were found in the noses of all animals of all test groups, and in the vocal cord region of the larynx of several animals of the mid-dose and high-dose groups. There was slight focal flattening of tracheal epithelium of one male rat in the high-dose group. The trachea and lung of all other animals did not show lesions which could be related to acetaldehyde exposure. Mononuclear inflammatory cells, calcareous deposits, and foci of cellular alterations were found in small intestine, kidney, and liver.

A summary of the respiratory tract tumors observed in this study is presented in Table 7-15. Exposure to acetaldehyde increased the number of animals with tumors in an exposure-related manner in both male and female rats. In addition, there were exposure-related increases in the incidences of multiple respiratory tract tumors. It is of interest to note that only one benign tumor was found and two types of malignant nasal tumors were seen (Table 7-16). Adenocarcinomas were increased significantly ($p < 0.01$) in both male and female rats at all exposure levels, whereas squamous cell carcinomas were increased significantly in male rats at middle and high doses and in female rats only at high doses. These squamous cell carcinoma incidences showed a clear dose-response relationship. The incidence of adenocarcinoma was highest in the mid-exposure (1500 ppm) group in both male and female rats, but this was probably due to the high mortality and competing squamous cell carcinomas at the highest exposure level. In the low-exposure group, the adenocarcinoma incidence was higher in males than in females. These tumors varied in size from

TABLE 7-14. SUMMARY OF RESPIRATORY TRACT HYPERPLASTIC AND PRENEOPLASTIC LESIONS IN RATS

Organ examined and site and type of lesion observed	Incidence of lesions							
	Males				Females			
	Acetaldehyde (ppm)				Acetaldehyde (ppm)			
	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]
Presence of 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]
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. All statistical analyses were done by the author.
[b]$p < 0.01$, according to the Fisher Exact Test. All comparisons were made with the controls.
[c]$p < 0.05$, according to the Fisher Exact Test. All comparisons were made with the controls.

SOURCE: Woutersen et al., 1985.

TABLE 7-15. SUMMARY OF RESPIRATORY TRACT TUMORS IN RATS

Parameter	Incidence of tumors							
	Males				Females			
	Acetaldehyde (ppm)				Acetaldehyde (ppm)			
	0	750	1500	3000	0	750	1500	3000
Animals examined	55	54	55	53	54	55	55	55
Animals with tumors	1	17	40	31	0	8	36	39
Animals with single tumors	1	17	39	25	0	8	35	33
Animals with multiple tumors	0	0	1	6	0	0	1	6
Animals with benign tumors	0	0	0	0	0	1	0	0
Animals with malignant tumors	1	17	40	31	0	7	36	39
Animals with metastatic tumors	0	0	1	2	0	0	0	1
Total tumors	1	17	41	37	0	8	37	45
Total benign tumors	0	0	0	0	0	1	0	0
Total malignant tumors	1	17	41	37	0	7	37	45
Total metastatic tumors	0	0	1	2	0	0	0	1

SOURCE: Woutersen et al., 1985.

TABLE 7-16. SITES, TYPES, AND INCIDENCES OF RESPIRATORY TRACT TUMORS IN RATS

Organ examined and site and type of lesion observed	Incidence of lesions							
	Males				Females			
	Acetaldehyde (ppm)				Acetaldehyde (ppm)			
	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]	30[b]	20[b]	0	6[c]	26[b]	20[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]	14[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 the Fisher Exact Test. All statistical analyses were done by CAG.
[c]$p < 0.05$, according to the Fisher Exact Test. All statistical analyses were done by CAG.

SOURCE: Woutersen et al., 1985.

small nests of atypical neoplastic cells to large osteolytic tumors growing outside the nose. Most of these tumors consisted of sheets or cords of densely packed cells having large hyperchromic nuclei with one or two large nucleoli. In addition, some adenocarcinomas consisted of cells having scant cytoplasm and large ovoid nuclei with prominent chromatin. The olfactory epithelium was considered to be the site of origin for these adenocarcinomas.

The squamous cell carcinomas varied in size, filling one or both sides of the nasal cavity, and destroying turbinates, invading the nasal bones, and extending into subcutis and brain.

Although both adenocarcinomas and squamous cell carcinomas frequently invaded the surrounding areas, including bones, subcutis vessels, and nerve bundles, metastases were seen in only four animals. Two adenocarcinomas had metastasized to the cervical lymph nodes and one adenocarcinoma and one squamous cell carcinoma to the lung. Several common neoplasms (of the adrenals, pituitary, mammary gland, and uterus) outside the respiratory tract were also found. These tumors are not shown in Table 7-16 because they were not significantly different in experimental group versus controls. The incidences of these tumors were low in the high-dose group as compared to the other dose groups or to the controls. Since these tumors tend to develop in older rats, the low incidence in the high-dose group could be expected due to the high and early mortality in this group.

7.1.2.3. Woutersen and Appelman (1984)--This study, referred to as a "recovery study" (see subgroup 4A in Table 7-12) was part of a lifetime carcinogenicity study of acetaldehyde, and was performed to investigate the process of regeneration of damaged nasal mucosa. Two groups each of 30 male and 30 female albino Wistar rats were exposed to acetaldehyde at several concentrations for 52 weeks. Ten males and 10 females per dose group were killed after a recovery

period of 26 weeks, and 20 males and 20 females per dose group were killed after a recovery period of 52 weeks. Details of the procedures used were given in section 7.1.2.2. (Woutersen et al., 1985). In spite of the termination of acetaldehyde exposure after 52 weeks, mortality was higher in males in all treated groups and in females in the highest dose group (Table 7-17). It is of interest to note that the number of animals that died during the first 26 weeks of the recovery period was similar to the number of animals that died in the lifetime study. Moreover, during this period the number of nasal tumors was almost the same as in the lifetime study. These findings indicate that after 52 weeks of exposure to acetaldehyde, proliferative epithelial lesions of the nose may develop into tumors even without continued acetaldehyde exposure. Restoration of the olfactory epithelium was evident in the low-dose group, and to a lesser degree in the mid-dose group, and was absent in animals in the highest dose group. These findings suggest that the olfactory epithelium, after damage by acetaldehyde, may regenerate, provided that the mucosa is not completely devoid of basal cells and that Bowman's glands in the animals have not been totally destroyed (Woutersen et al., 1985).

Recent studies indicate that acetaldehyde is mutagenic and induces cross-links between DNA strands and between DNA and protein at very high concentrations *in vitro* (see Chapter 6 and IARC, 1985). It also has been shown to increase sister chromatid exchanges in cultured human lymphocytes and in cultured ovarian cells of Chinese hamsters (Obe and Ristow, 1977; Ristow and Obe, 1978). Acetaldehyde should thus be considered genotoxic, and therefore a potentially carcinogenic agent. On the other hand, it has been suggested that acetaldehyde may be capable of deactivating free cysteine in bronchial epithelial cells, thereby suppressing the "thiol defense" of the epithelium against the attack of mutagens and carcinogens (Braven et al., 1967; Fenner and Braven, 1968).

TABLE 7-17. COMPARISON OF MORTALITY AND INCIDENCES OF NEOPLASTIC LESIONS IN THE NOSE OF WISTAR RATS THAT DIED OR WERE KILLED DURING WEEKS 52 THROUGH 78 OF THE WOUTERSEN ET AL. (1984b, 1985) RECOVERY AND LIFETIME EXPOSURE STUDIES

	Recovery study[a]								Carcinogenicity study[b]							
	Males				Females				Males				Females			
	Acetaldehyde (ppm)								Acetaldehyde (ppm)							
Parameter	0	750	1500	3000	0	750	1500	3000	0	750	1500	3000	0	750	1500	3000
Initial number of rats	30	30	30	30	30	30	30	30	55	55	55	55	55	55	55	55
Effective number of rats[c]	29	29	30	24	28	30	29	24	55	52	54	41	55	54	55	48
Number of intercurrent deaths	0	3	7	18	1	3	5	12	2	3	12	22	1	5	5	19
Adenocarcinomas	0	1	4	4	0	0	4	6	0	1	7	11	0	1	5	7
Squamous cell carcinomas	0	0	1	10	0	0	0	4	0	0	2	8	0	1	0	9
Total animals with nasal tumors(%)[d]	0 (0)	1 (3.4)	5 (16.7)	12 (50.0)	0 (0)	0 (0)	4 (13.8)	8 (33.3)	0 (0)	1 (1.9)	9 (16.7)	16 (39.0)	0 (0)	2 (3.7)	5 (9.1)	15 (31.25)
Deaths without tumor	0	2	2	6	1	3	1	4	2	2	3	6	1	3	0	4

[a]Animals exposed to acetaldehyde for 52 weeks.
[b]Animals exposed to acetaldehyde for a lifetime (2 months).
[c]Animals still alive at the start of the recovery period.
[d]No statistically significant differences in eight individual comparisons between recovery and lifetime exposure studies.

SOURCE: Adapted from Woutersen and Appelman, 1984 and Woutersen et al., 1985.

Such mechanisms might have enhanced the effect of acetaldehyde on the formation of BaP-initiated tumors. The investigators concluded that acetaldehyde was a carcinogen with weak initiating and strong promoting (cocarcinogenic) activity. To date, no cell transfomation studies have been done. A recent study on the HRRT kidney cell line (Eker and Sanner, 1986) indicates that acetaldehyde and formaldehyde are both able to initiate cell transformation. However, formaldehyde was 100 times more potent than acetaldehyde on a molar basis. These differences of potencies were similar to those found for cytotoxic effects in human bronchial epithelial cells (Saladino et al., 1985) and induction of tumors in rats (Woutersen et al., 1985). Further investigation into the mechanisms of the action of acetaldehyde is needed.

7.2. EPIDEMIOLOGIC STUDIES

7.2.1. Bittersohl (1974)

This is the only known epidemiologic study involving acetaldehyde exposure. The study was conducted in an aldol and aliphatic aldehyde factory in the German Democratic Republic. The work force in this factory is potentially exposed to various chemicals, primarily acetaldol (70%), with smaller but variable amounts of acetaldehyde, butylaldehyde, crotonaldehyde, "large" condensed aldehydes such as hexantriol, hexantetrol, and ethyl-hexanol, and traces of acrolein, in solution with 20% to 22% water.

The investigator conducted a morbidity survey to study the incidence of total cancer in this factory. The observation period extended from 1967 to 1972. A cohort of 220 people who were actively employed in the factory during the observation period was studied. Approximately 150 of these 220 individuals were employed for more than 20 years in the factory. Records of "the industrial poly-chemic and tumor investigation physicians practices" were used to obtain information on the occurrence of cancer among this population. Air

sampling was performed in the reduction process worksite for various chemicals, including acetaldehyde.

Nine cases of cancer among the male employees were identified during the 6-year study period. (Two female cancer cases were excluded from the analysis because the author felt that the latency period for these cases was too short for their cancers to have been the result of the industrial chemical exposure.) 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 German Democratic Republic during the same time period was 1,200 per 100,000 population.

Analysis by latency showed that eight cases had an average latency period of 26 years with a range of $\pm$ 4 years, while one case (buccal cavity carcinoma) had a latency period of 13 years. Out of the nine cases, five belonged to the 55 to 59 year age group, while the remaining four were over 65 years old.

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
- One adenocarcinoma of the cecum.

All of these cases had a history of smoking. One individual smoked 30 cigarettes per day (buccal cavity carcinoma with latency period of 13 years), while the remaining eight smoked between 5 and 10 cigarettes per day.

The author conducted an air sample analysis in the reduction process worksite where a leak was suspected (time not specified). Acetaldehyde concentrations were found to range from 1 to 7 mg/m^3, which was far below the recommended "MAK value" (not explained) of 100 mg/m^3 for this chemical. When the

combined concentration was calculated by the "usual" formulae (the author fails to give either the formulae used or the reference) the concentration level was far below the recommended "MAK value." (The value for the combined concentration was not given.)

This study has quite a few limitations. As reported, it suggests an increased risk in the incidence of total cancer in the factory employees that is five times higher than that of the general population of the German Democratic Republic. This, however, is a crude rate and is not age-adjusted. Hence, one should not give much credence to this apparent fivefold increase. The study purports to cover a period from 1967 to 1972, but the exact dates are not given. At a maximum, the study covered only 6 years of observation. No criteria for inclusion or exclusion of subjects in the cohort are mentioned, but it is presumed that the author considered only those employees who had worked for more than 20 years (rate calculation 9/150, not 9/220). The sample size is small, and distributions by age and sex are not presented for the study population. The author mentions the exclusion of two female cancer cases, but fails to mention how many individuals out of the 150 were females. Former employees, such as retired and terminated individuals, were not traced at all, thus raising a possibility that some cancers may have been missed, which may have led to underestimation of the total cancer incidence in the exposed population. Confounding by other chemicals and by smoking are also not considered, although the author does mention that there was no confounding by asbestos in this factory. Only current smoking histories were available. Past patterns of smoking, such as whether the amount of cigarettes smoked currently and in the past is the same or different, or how long the person smoked, are not known. In addition, no smoking histories were available for the remaining cohort. It is of interest to note that cigarette smoking is a strong risk factor for cancers of

the bronchi and the buccal cavity, and is also considered (to a lesser extent) a risk factor for cancer of the stomach. These three cancers constitute eight out of the nine cases in this cohort.

Air samples were collected from the reduction process area, where a leak was suspected, but were not collected from the condensation process area, where a leak was known to have occurred. The investigator also fails to describe when the leak first occurred and how long it lasted. Furthermore, no details on air sample collection, frequency of collection, and analysis of the samples are described. Hence, it is difficult to judge the extent and duration of exposures to acetaldehyde and other chemicals in this study cohort.

Because of these limitations, this study is considered inadequate for the purpose of drawing any conclusions regarding the possible carcinogenicity of acetaldehyde.

7.3. MECHANISTIC CONSIDERATIONS FOR RISK ESTIMATION

7.3.1. Possible Mechanisms of Acetaldehyde Carcinogenesis

The exact mechanism by which aldehydes, and particularly acetaldehyde, cause cancer in rodents is not clear. Chemical and toxicological comparisons may be helpful in characterizing what is known. Acetaldehyde is structurally related to formaldehyde; both are mutagenic and both have been shown to be carcinogenic in two species of rodents (Kerns et al., 1983; Woutersen et al., 1984; Feron et al., 1982). Recently, some evidence has emerged concerning the direct interaction of aldehydes with DNA.

The first evidence of DNA protein cross-links was observed *in vivo* for formaldehyde (Casanova-Schmitz et al., 1984) with similar evidence indicating a likelihood of cross-linking for acetaldehyde (Lam et al., 1986). For formaldehyde the formation of DNA-protein cross-links occurred in the nasal mucosa and nasal olfactory region of Fischer 344 rats at concentrations (6 to 15 ppm)

that were similar to those that induced nasal cancer in the rats (Kerns et al., 1983). For acetaldehyde, nasal tumors were induced in rats at concentrations of 750 to 3000 ppm with DNA-protein cross-links observed at an exposure concentration of 3000 ppm acetaldehyde. Researchers have observed that cross-linking of DNA to proteins may result in deletions and some types of chromosomal damage (Benyajjafi et al., 1983, Natarajan et al., 1983), and that acetaldehyde at high concentrations increases the rate of cell turnover in the nasal mucosa, which can be attributed to the cytotoxicity of the compound (Feron et al., 1982).

An increased cell turnover rate also increases the rate of synthesis of DNA, thereby increasing the availability of sites in the DNA for reaction with acetaldehyde. It follows, therefore, that acetaldehyde could also enhance the proliferation of initiated cells. Thus, covalent reactions with DNA-associated proteins and cytotoxicity are probably involved in the development of upper respiratory tract cancer induced by acetaldehyde.

7.3.2. Metabolism of Acetaldehyde

Mammalian metabolism, kinetics of disposition, and covalent binding of acetaldehyde have been reviewed extensively in Chapter 4. In this section, an attempt is made to summarize information that has a potential bearing on the quantitative risk assessment.

1. Acetaldehyde is known to be metabolized very rapidly and extensively to a normal endogenous metabolite, acetate, mainly by means of acetaldehyde dehydrogenases, which are present in many body tissues (Bogdanffy et al., 1986). Acetate further enters the two-carbon metabolic pool, is utilized in the synthesis of cellular constituents, and is ultimately metabolized to carbon dioxide and water.

2. Adequate studies of the kinetics of acetaldehyde of exogenous origin are not available; thus, a dose-metabolite relationship, dose-blood concentra-

tion, or dose-organ concentration cannot be established.

3. Animal experiments have demonstrated a rapid, exponential disappearance of acetaldehyde from the circulating blood, consistent with first-order kinetics, with a half-time for elimination of less than 15 minutes. Since less than 5% of acetaldehyde escapes unchanged in exhaled breath, and acetaldehyde is not known to be excreted in the urine, the elimination of acetaldehyde from the body occurs essentially by means of metabolic processes. These observations suggest that the kinetics of acetaldehyde metabolism might best be described by nonlinear Michaelis-Menten kinetics. Further, the high capacity of mammals to metabolize acetaldehyde indicates that even with very large assimilated doses, "saturation" kinetics will not be apparent. However, quantitative studies at multiple dose levels, showing the kinetics and disposition of acetaldehyde at cancer bioassay concentrations, are not available to confirm the hypothesis.

4. Acetaldehyde readily reacts and forms adducts nonenzymatically with cellular constituents, such as protein, DNA, and phospholipids. With inhalation exposure, acetaldehyde cross-links DNA and protein in the nasal olfactory and respiratory mucosa region of experimental animals (Lam et al., 1986).

7.3.3. Significance of the DNA-Adduct in Carcinogenicity and Low-Dose Extrapolation

Although acetaldehyde is much less reactive and less toxic than formaldehyde, it is known that, under certain conditions, acetaldehyde can form adducts (Tuma and Sorrel, 1985) and cross-links with DNA (Ristow and Obe, 1978), with proteins (Mohammad et al., 1949), and with tetrahydrofolate (LaBume and Guynn, 1985) *in vitro*. Initial evidence of increasing amounts of DPX (DNA-protein cross-link *in vivo*), as a measure of a decrease in extractable DNA from protein, was provided in a study by Lam et al. (1986) using homogenates of nasal respiratory mucosa. Fischer 344 rats were exposed for a single day to selected

concentrations of acetaldehyde (0, 100, 300, 1000, or 3000 ppm) for 6 hours and to 1000 ppm, 6 hours/day, for 5 days. Upon sacrifice, the nasal, respiratory, and olfactory mucosa were removed and measured for DNA extractability using a procedure similar to the one used for the formaldehyde study in the same laboratory (Casanova-Schmitz et al., 1984). The decrease in DNA extractability in the rat nasal mucosa induced by acetaldehyde in vivo was a nonlinear function of the inhaled acetaldehyde concentration (Tables 7-18 and 7-19). As noted in Tables 7-18 and 7-19, DNA cross-linking was obtained with formaldehyde with the maximum incorporation of formaldehyde into DNA, as DPX, observed in a single exposure at 6 ppm for 6 hours/day (Casanova-Schmitz et al., 1984). For acetaldehyde, DPX was not detected in statistically significant amounts in the rat nasal mucosa at 100 or 300 ppm. While the amount of DPX formed was elevated at 300 ppm, it increased significantly at 1000 ppm. DPX was not detected in statistically significant amounts (Table 7-19) in the olfactory mucosa after a single 6-hour exposure to acetaldehyde at 1000 or 3000 ppm. However, significant increases of DPX in the olfactory mucosa were observed after repeated exposure to 1000 ppm acetaldehyde, 6 hours/day, for 5 days. The authors hypothesized a possible explanation for the increase in DPX in the olfactory mucosa after repeated exposure: the DPX increase may be due to cytotoxicity, which induces a rapid cell turnover and causes the DNA to be more susceptible to DPX formation, if acetaldehyde, like formaldehyde, binds preferentially to single-strand regions of DNA. Another possible explanation is that DPX increases may be due to differences in the level of aldehyde dehydrogenases in the nasal mucosa compared to the olfactory mucosa, since through histological localization, aldehyde dehydrogenase activity appears to be less in olfactory mucosa than in nasal mucosa (Bogdanffy et al., 1986).

TABLE 7-18. PERCENT OF INTERFACIAL DNA FROM THE NASAL RESPIRATORY MUCOSA OF RATS EXPOSED TO ALDEHYDES FOR 6 HOURS

Formaldehyde concentration (ppm)	Percent interfacial DNA[a,c]	Acetaldehyde concentration[b] (ppm)	Percent interfacial DNA[a,d]
0	8.1 ± 0.4 (6)	0	8.05 ± 0.44 (6)
		100	8.21 ± 0.76 (4)
6	12.5 ± 1.4 (4)[b]	300	10.42 ± 0.46 (4)
		1000	13.31 ± 0.68 (4)[b]
		3000	17.51 ± 0.49 (4)[b]

[a]Mean ± SE; the numbers of groups are given in parentheses (three animals per group).
[b]Significantly ($p < 0.05$) greater than the value for the groups not exposed to acetaldehyde.
[c]From Casanova-Schmitz, 1984.
[d]From Lam et al., 1986.

TABLE 7-19. PERCENT OF INTERFACIAL DNA FROM THE OLFACTORY MUCOSA OF RATS EXPOSED TO ACETALDEHYDE FOR ONE DAY OR FIVE DAYS

Acetaldehyde concentration (ppm)	Exposure length (days)[a]	Percent interfacial DNA[d]
0	1	12.61 ± 0.74 (3)
1000 ± 22	1	11.09 ± 0.64 (4)
3016 ± 1233	1	12.66 ± 0.98 (4)
1000 ± 22	5	16.31 ± 0.78 (4)[c]

[a]Rats were given a single 6-hour exposure to air or to acetaldehyde at indicated concentrations, or five 6-hour exposures on consecutive days to acetaldehyde at 1000 ppm.
[b]Mean ± SE; the numbers of groups are given in parentheses (three animals per group).
[c]Significantly ($p < 0.05$) greater than the olfactory mucosal value for the groups exposed for one day (6 hours) to either 0 or 1000 ppm.

SOURCE: Lam et al., 1986.

Although the relationship of DNA-protein cross-linking in carcinogenesis is not fully understood, there are a number of indications that the formation of DPX in target tissues, with formaldehyde as well as with acetaldehyde, may play a role in the observed carcinogenic effects. A positive correlation between a number of polycyclic aromatic hydrocarbons of widely differing carcinogenic potencies and the extent of reaction of reactive metabolites with DNA has been noted (Hoel et al., 1983). A similar correlation also has been observed for binding of β-propiolactone and other alkylating agents to DNA (Hoel et al., 1983). In addition, evidence has been obtained that cross-links formed by formaldehyde caused various small deletions to occur in a sequenced Drosophila gene. The authors suggest that this response can be explained by a slipped pairing mechanism during replication that could be due to the formation of DPX (Benyajjafi et al., 1983).

Given the many hypotheses, additional studies of DNA-protein cross-linking would be crucial to the development of a consensus regarding improved mechanistically based risk assessment for acetaldehyde. The utility of the available data on DPX formed by acetaldehyde needs further investigation before it is used for dose adjustment in quantitative risk assessment. A similar data base on formaldehyde, in which formation of DPX was observed, has been critically reviewed by several panels of experts. A panel convened by EPA at the request of EPA's Science Advisory Board identified several methodological limitations in the Casanova-Schmitz et al. (1984) study, but noted that it was an important step toward attempting to assess the intracellular dose delivery of externally applied formaldehyde. The panel further stated that at its present level of development and validation, the study does not represent an adequate basis for quantitative risk assessment (Life Systems Inc., 1986).

7.4. QUANTITATIVE RISK ESTIMATION

7.4.1. Introduction

This quantitative section deals with the incremental unit risk for acetaldehyde in air and the potency of acetaldehyde relative to other chemicals that the Carcinogen Assessment Group (CAG) of the U.S. Environmental Protection Agency has evaluated as potential or known human carcinogens. The incremental unit risk estimate for an air pollutant is defined as the additional lifetime cancer risk occurring in a hypothetical population in which all individuals are exposed continuously from birth throughout their lifetimes to a concentration of 1 ppm or 1 $\mu g/m^3$ of the agent in the air they breathe. This calculation is done to estimate, in quantitative terms, the impact of the agent as a carcinogen. Unit risk estimates are used for two purposes: 1) to compare the carcinogenic potency of several agents with each other, and 2) to give a crude indication of the population risk that might be associated with exposures to air contaminated with these agents, if the actual exposures are known. The data used for the quantitative estimate for acetaldehyde are from the Woutersen and Appelman (1984) and the Woutersen et al. (1984, 1985) rat inhalation studies showing an exposure-related increase in nasal cancers. Neither the hamster studies (Feron, 1979; Feron et al., 1982) nor the single epidemiologic study by Bittersohl (1974) was considered satisfactory for this estimation. The many problems associated with the epidemiologic study have been discussed in section 7.2.1. The Feron (1979) study was an intratracheal instillation study, not an inhalation study. The Feron et al. (1982) hamster inhalation study used a 52-week exposure to a significantly higher acetaldehyde level than the 750 ppm used in the lifetime and recovery rat inhalation study. Furthermore, the hamster study used only one exposure group to acetaldehyde alone, there were only 30 animals per group, and the surviving animals were sacrificed at 81

weeks. By contrast, the rat study had three exposure groups plus a control with 85 animals of each sex in each exposure group. Furthermore, there were both 1-year and 2-year exposures in the rat study, which lasted a full 2 years.

7.4.2. Quantitative Risk Estimates Based on Animal Data

7.4.2.1. Procedures for Determination of Unit Risk from Animal Data--In animal studies it is assumed, unless evidence exists to the contrary, that if a carcinogenic response occurs at the dose levels used in the study, responses will also occur at all lower doses with an incidence determined by the extrapolation model. This is known as a nonthreshold assumption and such a model is called a nonthreshold model.

There is no solid scientific basis for any mathematical extrapolation model which relates carcinogen exposure to cancer risks at the extremely low concentrations which must be dealt with when evaluating environmental hazards. For practical reasons, such low levels of risk cannot be measured directly.

Based on observations from epidemiologic and animal cancer studies, and because most dose-response relationships have not been shown to be supralinear in the low-dose range, the linear nonthreshold model has been adopted as the primary basis for animal-to-human risk extrapolation to low levels of the dose-response relationship. The upper-limit risk estimates made with this model should be regarded as conservative, representing the most plausible upper limit for the risk, i.e., the true risk is not likely to be higher than the estimate, but it could be lower.

The mathematical formulation the CAG has chosen to describe the linear nonthreshold dose-response relationship at low doses is the linearized multistage model. This model employs enough arbitrary constants to be able to fit almost any monotonically increasing dose-response data. It is called a linearized model because it incorporates a procedure for estimating the largest pos-

sible linear slope (in the 95% confidence limit sense) at low extrapolated doses that is consistent with the data at all dose levels of the experiment.

In addition to the curve fitting described above, extrapolation from animals to humans incorporates several other procedures which increase the uncertainty of the estimate. Apart from the major uncertainty of cross-species extrapolation, the CAG procedure has traditionally modeled the response from the most sensitive species and sex (if sex responses are not significantly different, the sexes are pooled). Also, responses from different tumors showing a significant increase are added to determine total response of animals with at least one tumor type. Taken together this methodology is thought to provide a conservative upper limit of incremental risk. However, this estimate is not described as the 95% upper limit, because of the additional procedures associated with the methodology.

7.4.2.1.1. Description of the low-dose animal-to-human extrapolation model.

Two forms of the linearized multistage model will be used to analyze the Woutersen data. The first form is the quantal model, in which the data are summarized by the percent of animals responding with significant tumors following treatment with a continuous exposure of the toxicant. This is often called the Crump multistage model (Crump et al., 1977) following that author's compression of the original Armitage and Doll (1961) multistage model into a polynomial. It is described below:

Let P(d) represent the lifetime risk (probability) of cancer at dose d. The Crump multistage model has the form

$$P(d) = 1 - \exp\left[-(q_0 + q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$q_i \geq 0, \; i = 0, 1, 2, \ldots, k$$

Equivalently,

$$P_t(d) = 1 - \exp\left[-(q_1 d + q_2 d^2 + \ldots + q_k d^k)\right]$$

where

$$P_t(d) = \frac{P(d) - P(0)}{1 - P(0)}$$

is the extra risk over background rate at dose d, or the effect of treatment.

The point estimate of the coefficients q_i, i = 0, 1, 2, ..., k, and consequently the extra risk function $P_t(d)$ at any given dose d, is calculated by the maximizing the likelihood function of the data. (In the section calculating the risk estimates, $P_t(d)$ will be abbreviated as P).

In fitting the dose-response model, the number of terms in the polynomial is chosen equal to k up to k = 6. The model with the value of k estimating the smallest upper-limit incremental unit risk and still providing an adequate ($p > 0.01$) fit to the data is retained and the corresponding q_1^* is employed.

The point estimate, q_1 and the 95% upper confidence limit of the extra risk $P_t(d)$ are calculated by using the computer program GLOBAL83, developed by Howe (1983, unpublished). At low doses, upper 95% confidence limits on the extra risk and lower 95% confidence limits on the dose producing a given risk are determined from a 95% upper confidence limit, q_1^*, on parameter q_1. Thus, the value q_1^* is taken as an upper bound of the potency of the chemical in inducing cancer at low doses. It represents the 95% upper-limit incremental

unit risk consistent with a linear nonthreshold dose-response model.

The second form of the linearized multistage model was also developed by Crump (Crump and Howe, 1984) but is potentially superior to the first form in three significant aspects:

1. It allows for a time-dependent or variable dose pattern.
2. It adjusts for intercurrent mortality.
3. It is capable of estimating risk at any time for any dosing pattern.

This form of the model also uses the theory of multistage carcinogenesis developed by Armitage and Doll (1961), but uses it in its more generalized form. The Armitage-Doll multistage model assumes that a cell is capable of generating a neoplasm when it has undergone k changes in a certain order. The rate, r_i, of the ith change is assumed to be linearly related to D(t), the dose at age t, i.e., $r_i = a_i + b_i D(t)$, where a_i is the background rate, and b_i is the proportionality constant for the dose. It can be shown that the probability of cancer by age t is given by

$$P(t) = 1 - \exp\,[-H(t)]$$

where

$$H(t) = \int_0^t \int_0^{u_k} \cdots \int_0^{u_2} \{[a_1 + b_1 D(u_1)] \cdots [a_k + b_k D(u_k)]\}\, du_1 \cdots du_k$$

is the cumulative incidence rate by time t.

When H(t) or the risk of cancer is small, P(t) is approximately equal to H(t). When only one stage is dose-related, all proportionality constants are zero except for the proportionality constant for the dose-related stage.

A computer program, ADOLL1-83, has been developed by Crump and Howe (1983) to implement the computational aspect of the model. In this program, the model is generalized to estimate tumor induction time I by replacing the time factor t by t-I. The best-fitting model is defined as the one that has the maximum likelihood among various models with different numbers of stages and the stage affected by the exposure.

Two possible problems arise with the time-dependent form of the model relating to the Woutersen rat inhalation data. The first is that the model requires the ability to differentiate between tumors that are fatal and those that have been found at death. The rat tumor data have not been categorized that way, and the assumption has been made in most cases that the tumors were fatal (see Table 7-22 in section 7.4.2.2.3.2.). The second is that the model is not designed to adjust for possibly different timing patterns between the two competing tumor types--the nasal adenocarcinomas associated more with the lower exposures and the squamous cell carcinomas which predominate at the higher exposures. Thus, because both models have possible complications, the results of each are presented and compared.

7.4.2.1.2. Interpretation of quantitative estimates. For several reasons, the unit risk estimate is only an approximate indication of the risk in populations exposed to known concentrations of a carcinogen. First, there are important host factors, such as species differences in uptake, metabolism, and organ distribution of carcinogens, as well as species differences in target site susceptibility, immunological responses, hormone function, and disease states. Second, the concept of equivalent doses for humans compared to animals is virtually without experimental verification regarding carcinogenic response. Finally, human populations are variable with respect to genetic constitution and diet, living environment, activity patterns, and other cultural factors.

The unit risk estimate can give a rough indication of the relative potency of a given agent compared with other carcinogens. The comparative potency of different agents is more reliable when the comparison is based on studies in the same test species, strain, and sex, and by the same route of exposure.

The quantitative aspect of the carcinogen risk assessment is included here because it may be of use in the regulatory decision-making process, e.g., in setting regulatory priorities or evaluating the adequacy of technology-based controls. However, it should be recognized that the estimation of cancer risks to humans at low levels of exposure is uncertain. At best, the linear extrapolation model used here provides a rough but plausible estimate of the upper limit of risk; i.e., it is not likely that the true risk would be much more than the estimated risk, but it could very well be considerably lower. The risk estimates presented in subsequent sections should not be regarded as accurate representations of the true cancer risks even when the exposures are accurately defined. The estimates presented may, however, be factored into regulatory decisions to the extent that the concept of upper limits of risk is found to be useful.

7.4.2.1.3. Alternative methodological approaches. The methods presented in the Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986b) and followed by the CAG for quantitative assessment are consistently conservative, i.e., they avoid underestimating risks. The most important part of the methodology contributing to this conservatism is the linear nonthreshold model. There are a variety of other extrapolation models that could be used, most of which would give lower risk estimates. The appendix following this chapter presents four of these, the one-hit, the log-Probit, the logit, and the Weibull, and compares both their maximum likelihood estimates (MLEs) and the upper confidence limits with those of the Crump two-stage model.

These extrapolation models and estimates are presented for comparison only. It is the EPA's position that for quantitative risk extrapolation "in the absence of adequate information to the contrary, the linearized multistage procedure will be employed" (U.S. EPA. 1986b). Furthermore, while MLEs may be calculated for all these models, for the linearized multistage model, the MLEs may be very unstable at low exposures because of constraints on the parameters. For this model "an established procedure does not yet exist for making "most likely" or "best" estimates of risk within the range of uncertainty defined by the upper and lower limit values" (U.S. EPA, 1986b). Thus, because of the instability of the MLEs in the linearized multistage model, a comparison with the MLEs from other models may not be meaningful.

With respect to the choice of animal bioassay data as the basis for extrapolation, the present approach is to use the most sensitive responder. Alternatively, the average responses of all the adequately tested bioassays could be used. Again, with the superiority of the Woutersen studies over the other studies, all efforts will be concentrated on deriving the best estimates from these.

7.4.2.2. Calculation of Cancer Unit Risk Estimates Based on the Woutersen and Appelman (1984) and Woutersen et al. (1984, 1985) Rat Inhalation Study--

7.4.2.2.1. Results of the Study. The details of this rat inhalation study have been presented in sections 7.1.2.2. and 7.1.2.3. The design of the entire study is presented in Table 7-12. Pertinent results are summarized below:

1. Acetaldehyde vapor exposure causes both adenocarcinomas (AC) and squamous cell carcinomas (SCC) to the nasal tract of rats in an exposure-related manner. The AC originated from olfactory epithelium, while the SCC originated from respiratory epithelium. The AC are caused at lower exposures

than the SCC (Table 7-16), and both show exposure-response trends. For the mid- and high-exposure groups, there are some animals with each type, and these tumors represent competing causes of death for the animal. However, very few animals with these tumors developed metastases. In the calculation procedure, the tumors will be combined. Qualitatively, male and female rats are affected the same manner, but the males have a somewhat higher response at the low- and mid-exposure levels. Because of this quantitative difference, males and females are analyzed separately.

2. Acetaldehyde vapor exposure does not appear to cause benign tumors of the nasal tract, but it did cause degeneration of the olfactory epithelium at all dose levels. It also caused degenerative changes of the respiratory epithelium only in the high-exposure group.

3. Acetaldehyde vapor exposure does not affect any other organ directly with the exception of the larynx and, to a minor degree, the trachea. Lesions in the larynx are characterized by minimal to slight hyperplasia, metaplasia, and keratinization in the vocal cord region of several animals in the mid- and high-exposure groups.

4. Animals in the high-exposure group (3000 ppm) suffered severe growth retardation, respiratory distress, and high early mortality. At 4 months, exposure concentrations were reduced to 2150 ppm and then adjusted periodically throughout the study.

5. With respect to stopping exposure, during the first 26 weeks of recovery the nasal tumor rates and death rates of the recovery group were essentially the same as those of the lifetime exposure group. During the second half of the recovery period (26 to 52 weeks after treatment stopped), however, both the low- and mid-exposure recovery groups had significantly decreased nasal tumor rates, while the high-exposure recovery group continued to exhibit the

same high nasal tumor rates as the lifetime exposure groups.

The findings indicate exposure-response nasal cancer effects in lifetime-exposure as well as recovery groups. It is for this reason that the studies will be combined for analysis, where possible.

7.4.2.2.2. Dose equivalence from rat to human. It is assumed that ppm in air is equivalent from rats to humans. Besides simplicity, there are several other reasons for taking this approach:

1. It is consistent with the Agency methodology used for estimating carcinogenic risks from formaldehyde (U.S. EPA, 1986a). This dose equivalence was chosen for formaldehyde following review by two panels (IRMC, 1984; Consensus Workshop on Formaldehyde, 1984), as explained in the formaldehyde document. Supporting evidence is also provided by a third expert panel (Life Systems Inc., 1986), as explained in section 7.3.3. Since formaldehyde and acetaldehyde are so structurally similar and cause such similar cancer effects in rats, a strong reason would be required to deviate from this well-reviewed methodology at this point.

2. Actual target dose measurements for acute acetaldehyde exposure do not present enough confidence that the results would be similar under conditions of chronic exposure. If percent interfacial DNA as a measure of acetaldehyde binding to DNA is to be used as a measure of delivered dose in quantitative risk assessment, then measurements following chronic exposure are needed. The results of the Lam et al. (1986) study (section 4.4.3., Figure 4-8, also discussed in section 7.3.3.), showed that percent interfacial DNA from the olfactory mucosa of rats exposed to acetaldehyde depended on whether the rats were exposed for 1 or for 5 days. The results of the 1-day exposure showed no increase in percent interfacial DNA, while the 5-day exposure results showed a significant increase. However, the only 5-day exposure was to 1000 ppm acet-

aldehyde; no information can be derived about the shape of the acetaldehyde concentration-percent interfacial DNA curve. A different situation holds for the results in the respiratory mucosa. The 1-day results appear to show a nonlinear trend, supported by the limited 5-day study results, but at this stage it is felt that these results do not present consistent enough evidence for use in quantitative risk assessment. Starr and Buck (1984) have calculated a risk assessment for formaldehyde based on formaldehyde-induced DNA adduct formation in nasal respiratory mucosa. Their results showed that the maximum likelihood estimates in the Crump multistage model were about 50 times larger using administered versus delivered dose, with corresponding upper-limit estimates about 2.5 times as large. These results, however, do not directly relate to acetaldehyde. Even though formaldehyde and acetaldehyde produce similar effects on the nasal respiratory mucosa, they have different cancer effects on the nasal olfactory mucosa, making the Lam et al. (1986) results much more difficult to interpret.

3. It is consistent with Agency methodology used for estimating cancer risk via inhalation of epichlorohydrin, which also acts as a carcinogen at the site of initial contact, causing similar nasal cancer effects in rats.

4. Alternatively mg/surface area of nose could have been used, but this was not done, since rats, unlike humans, are obligatory nasal breathers, and it was thought that the uncertainty associated with breathing patterns would not have increased the accuracy of the estimation.

7.4.2.2.3. Analysis of data.

7.4.2.2.3.1. Crump multistage model (quantal form). Analysis with the quantal form of the linearized multistage model is presented here for comparison with quantitative risk estimations calculated for other chemicals and with the estimation from the time-to-tumor form to be calculated next. As explained

in section 7.4.2.1.1., the quantal form is not as flexible as the time-dependent, variable-dose form; as a result, the model can accommodate data only in certain summarized forms. These forms and the upper-limit incremental risk estimates are presented in Tables 7-20 and 7-21. Briefly, the upper-limit incremental estimates of q_1^*, based on (1) the number of animals with nasal tumors in the lifetime study only, and (2) the number of animals in the lifetime study plus the recovery group, were computed separately. In order to adjust for the high early mortality in the high-exposure group, two separate approaches were used. In the first approach, the variable-exposure, high-exposure group was eliminated from the analysis. This approach has been used before and is felt to be justified when the maximum tolerated dose has been exceeded. In the second approach, the high-exposure group is included in the calculations, but the animals dying during the first 52 weeks of exposure (before the first tumor appeared) are eliminated as not having had sufficient latent period to develop a tumor. Elimination of these early deaths has the effect of making the nontumor mortality of the exposure groups much more comparable, since nearly all of the first-year deaths occurred in the high-exposure group.

A recent modification to the procedure of determining the upper-limit incremental unit risk has been incorporated into this analysis (Crump 1986a, b). This modification involves choosing the smallest upper-limit incremental unit risk estimate from the set of Crump models up to k = 6 (see section 7.4.2.1.1.) that adequately fit the data.

In the study being analyzed, exposures to the animals were 6 hours/day, 5 days/week, for 1 or 2 years. In order to extrapolate to low continuous exposures, an adjustment to a lifetime continuous exposure equivalent had to be made. This was done in two ways. First, for the lifetime-exposure animals,

TABLE 7-20. CRUMP LINEARIZED MULTISTAGE MODEL ESTIMATES OF UPPER-LIMIT INCREMENTAL UNIT CANCER RISK BASED ON VARIOUS COMBINATIONS OF THE WOUTERSEN RAT INHALATION STUDY (MALES)

Study and group	Number of animals with nasal tumors/ effective number of animals (%) — Nominal exposure concentrations (ppm) 0	750	1500	3000	Upper-limit incremental unit risk of q_1^* $(ppm)^{-1}$	Model with smallest q_1^*
A. Lifetime study only						
1. Drop high-exposure group	1/55(2)	17/55(31)	40/55(73)	--	3.3×10^{-3}	k = 2
2. Include high-exposure group; exclude deaths in first year	1/55(2)	17/52(33)	40/54(74)	31/41(76)	3.8×10^{-3}	k = 2
Continuous-exposure equivalent (ppm)	0	129.8[a]	256.8	279.0[c]		
B. Lifetime study plus recovery group						
1. Drop high-exposure group	1/85(1)	20/85(24)	49/85(58)	--	2.5×10^{-3}	k = 2
2. Include high exposure group; exclude deaths in first year	1/84(1)	20/81(25)	49/84(58)	47/65(72)	2.1×10^{-3}	k = 2
Continuous-exposure equivalent[d] (ppm)	0	130.3[b]	255.1	279.0[c]		

[a]Continuous-exposure equivalent = 727 x 5/7 x 6/24 = 129.8.
[b]Continuous-exposure equivalent = [727(55/85) + 735(30/85)] x 5/7 x 6/24 = 130.3.
[c]Estimated on the basis of 104-week exposures. Exposures given in Table 7-22.
[d]Recovery-group exposures considered to be lifetime exposures.

TABLE 7-21. CRUMP LINEARIZED MULTISTAGE MODEL ESTIMATES OF UPPER-LIMIT INCREMENTAL UNIT CANCER RISK BASED ON VARIOUS COMBINATIONS OF THE WOUTERSEN RAT INHALATION STUDY (FEMALES)

Study and group	Number of animals with nasal tumors/ effective number of animals (%) — Nominal exposure concentrations (ppm) 0	750	1500	3000	Upper-limit incremental unit risk of q_1^* $(ppm)^{-1}$	Model with smallest q_1^*
A. Lifetime study only						
1. Drop high-exposure group	0/55	7/55(13)	36/55(65)	--	8.8×10^{-4}	k = 2
2. Include high-exposure group; exclude deaths in first year	0/55(0)	7/54(13)	36/55(65)	39/48(81)	9.2×10^{-4}	k = 2
Continuous-exposure equivalent (ppm)	0	129.8[a]	256.8	279.0[c]		
B. Lifetime study plus recovery group						
1. Drop high-exposure group	0/85(0)	7/85(8)	43/85(51)	--	5.2×10^{-4}	k = 2
2. Include high exposure group; exclude deaths in first year	0/83(0)	7/84(8)	43/84(51)	50/72(69)	4.1×10^{-4}	k = 2
Continuous-exposure equivalent[d] (ppm)	0	130.3[b]	255.1	279.0[c]		

[a]Continuous-exposure equivalent = 727 x 5/7 x 6/24 = 129.8.
[b]Continuous-exposure equivalent = [727(55/85) + 735(30/85)] x 5/7 x 6/24 = 130.3.
[c]Estimated on the basis of 104-week exposures. Exposures given in Table 7-22.
[d]Recovery-group exposures considered to be lifetime exposures.

the nominal exposure was adjusted by the factor (5/7) x (6/24). Second, when the recovery group was included with the lifetime study, the 1-year exposure of the recovery group was assumed to have been continued for a lifetime. The reasoning behind this assumption is that the latent period for observation of the tumor from acetaldehyde exposure is at least 1 year in these rats, and the effects of exposure during the second year would be only minimally apparent in this 2-year study. Footnote b of Tables 7-20 and 7-21 shows that the 30 recovery-group animals in the 750-ppm nominal exposure group were actually exposed to an average of 735 ppm during their 6-hour exposure. When combined with the 727-ppm actual exposure of the lifetime-exposure group, the weighted average was 130.3 ppm.

The results of the analyses are presented in Table 7-20 for the males and Table 7-21 for the females. Estimates for the males are four to five times as high as estimates for the females. Furthermore, inclusion of the recovery group decreases the estimates by about 25% to 40%. This is due mainly to the lower tumor rates occurring in the recovery groups versus the lifetime-exposure group during weeks 79 to 104 of the study. Both approaches, adjusting for high early mortality, provide similar estimates indicative of the small effect which a high-exposure group has on a linear estimate, especially when the lower-exposure groups have significant responses.

7.4.2.2.3.2. Multistage model with adjustments for variable exposure and nontumor differential mortality. As discussed in Section 7.4.2.1., the Woutersen data with the recovery group, variable exposures and high early nontumor mortality in the high-exposure group require the adjustments of a more sophisticated analysis than the type presented previously. This is provided by the second form of the linearized multistage model discussed in section 7.4.2.1.1. In order to apply this model, however, the data must be in a form in which for

each animal the following is known:

1. time of death
2. whether there was a tumor at death
3. whether the tumor was incidental to the death or was fatal.

In the Woutersen reports, the data are not in this form. By combining mortality and tumor pathology tables, however, we may arrive at approximate times of death and whether or not the tumor was fatal or was an incidental finding at death. These grouped individual data are presented, by sex, in Table 7-22, which indicates whether the groups were exposed for a lifetime or for 52 weeks and then allowed to recover. The controls for the lifetime and recovery exposure groups are combined, since both groups of controls were untreated for their entire lifetimes. The totals and numbers with tumors differ slightly from the numbers in Table 7-17 because of the addition of data on the scheduled sacrifice at 52 weeks. Also, three of the nasal tumors, two AC and one SCC, have been determined to be incidental because they were diagnosed as either "early" or "small." All nasal cancers diagnosed as other than "early" or "small" were defined as fatal, even though they might have been found in animals killed as part of a scheduled sacrifice. This was done because the nature of nasal tumors is such that breathing is usually severely restricted and eventually occluded.

The data in Table 7-22 have been incorporated into the model and the parameters estimated using ADOLL1-83. The choice of model (number of stages and which stage was active or exposure-related) was determined by that which gave the highest likelihood. These likelihood results, as presented in Table 7-23, show the higher likelihoods for two- and three-stage models with the first-stage exposure related for both males and females. These results are consistent both with the results of the quantal models (Tables 7-20 and 7-21)

TABLE 7-22. INDIVIDUAL DEATH TIMES AND NASAL TUMORS (NUMBER OF ANIMALS WITH EITHER AC OR SCC) FOR THE LIFETIME EXPOSURE AND RECOVERY GROUPS, BY SEX, IN THE WOUTERSEN RAT INHALATION STUDY, EXCLUDING 13- AND 26-WEEK INTERIM SACRIFICES
(S = SCHEDULED SACRIFICE; R = RECOVERY--EXPOSURE TO ACETALDEHYDE FOR FIRST 52 WEEKS; TS = SCHEDULED TERMINAL SACRIFICE)

	Males				Females			
			Nasal tumors				Nasal tumors	
Group	Week	Deaths	Incidental	Fatal	Week	Deaths	Incidental	Fatal
Controls, combined lifetime plus recovery	40	1	0	0	52(S)	10	0	0
	52	3	0	0	58	1	0	0
	52(S)	10	0	0	80	2	0	0
	88	15	0	0	87	2	0	0
	111	15	0	1	196	4	0	0
	121(TS)	22	0	0	111	18	0	0
	78(R,S)	18	0	0	121(TS)	28	0	0
	91(R)	1	0	0	40(R)	2	0	0
	104(R,TS)	18	0	0	55(R,S)	1	0	0
	Total	94	0	1	78(R,S)	10	0	0
					91(R)	5	0	0
					104(R,TS)	12	0	0
					Total	95	0	0
750 ppm nominal; actual was 727 ppm for lifetime (121 weeks)	20	1	0	0	20	1	0	0
	51	2	0	0	52(S)	10	0	0
	52(S)	10	0	0	67	4	0	0
	55	1	0	1	88	15	0	2
	70	2	0	0	111	18	0	3
	99	38	0	12	121(TS)	17	0	2
	121(TS)	11	0	4	Total	65	0	7
	Total	65	0	17				
Recovery; 750 ppm nominal; actual was 735 ppm for 1-52 weeks; 0 ppm for 53-104 weeks	50	1	0	0	52(S)	10	0	0
	65	3	0	1	63	1	0	0
	78(S)	9	1	0	71	2	0	0
	91	6	0	1	78(S)	10	0	0
	104(TS)	11	0	0	91	3	0	0
	Total	30	1	2	104(TS)	14	0	0
					Total	30	0	0

(continued on the following page)

TABLE 7-22. (continued)

Group	Males				Females			
			Nasal tumors				Nasal tumors	
	Week	Deaths	Incidental	Fatal	Week	Deaths	Incidental	Fatal
1500 ppm nominal; actual was 1438 ppm for lifetime (121 weeks)	40	1	0	0	58	2	0	2
	52(S)	10	0	0	52(S)	9	0	0
	58(S)	5	0	4	71	4	0	4
	71	7	0	5	90	15	0	9
	90	14	0	10	109	16	0	10
	109	11	0	8	118	7	0	4
	118	8	0	6	121(TS)	11	0	7
	121(TS)	9	0	7	Total	64	0	36
	Total	65	0	40				
1500 ppm nominal; actual was 1412 ppm for 1-52 weeks; 0 ppm for 53-104 weeks	65	7	0	5	40	1	0	0
	78(S)	10	0	1	65	5	0	4
	91	7	0	3	78(S)	9	0	0
	104(TS)	6	0	0	91	3	0	2
	Total	30	0	9	104(TS)	10	0	1
					Total	28	0	7
3000 ppm nominal; actual exposures were as follows: 0-20 3033 21-30 2167 31-34 2039 35-43 1433 44-45 1695 45-51 1472 55-99 977 99- 0	20	1	0	0	30	1	0	0
	30	6	0	0	40	6	0	0
	40	7	0	0	52(S)	9	0	1
	52(S)	8	0	0	58	17	0	12
	58	14	0	7	71	6	0	3
	71	11	0	9	84	11	0	11
	84	9	0	9	96	13	0	12
	96	6	0	5	102	1	0	1
	102	1	0	1	Total	64	0	40
	Total	63	0	31				
Recovery; same exposure as above for 1-52 weeks; 0 ppm for 53-104	40	6	0	0	40	6	0	0
	65	18	2	10	65	12	0	8
	78(S)	1	0	1	78(S)	6	0	0
	91	3	0	2	91	1	0	1
	104(TS)	1	0	1	104(TS)	4	0	2
	Total	29	2	14	Total	30	0	11

TABLE 7-23. LIKELIHOOD BY NUMBER OF STAGES AND BY EXPOSURE-RELATED STAGE OF THE VARIABLE EXPOSURE FORM OF THE LINEARIZED MULTISTAGE MODEL FOR THE WOUTERSEN RAT INHALATION STUDY. BY SEX.

Exposure-related stage	Number of stages				
	2	3	4	5	6
Males					
1	-6.37[a]	-7.56	-9.33	-10.73	-12.34
2	-18.31	-11.54	-13.93	-15.66	-17.17
3	--	-44.69	-21.17	-22.88	-24.68
4	--	--	--[b]	--[b]	--[b]
5	--	--	--	-79.69	--
Females					
1	-26.57	-26.38[a]	-27.49	-28.41	-30.58
2	-52.80	-32.09	-33.45	-34.48	-36.05
3	--	-66.83	-42.53	-42.67	-43.96
4	--	--	-90.48	--[b]	--[b]

[a]Least negative value represents fit of maximum likelihood.
[b]Did not converge in allocated computing time.

and the actual observations from the recovery group, which developed tumors after cessation of exposure. The rates of tumors in the first 6-month recovery period were the same as the rates in the lifetime-exposure group, indicating an effect of an early-stage carcinogen.

Based on the results in Table 7-23, the models chosen to estimate the incremental unit risks were the two- and three-stage models, both with the first stage active. These results are presented in Table 7-24. Here the estimates were calculated for males and females separately. Estimates based on either model are very close: for males, $q_1^* = 4.0 \times 10^{-3}$ $(ppm)^{-1}$ for the two-stage model with the first stage active versus 3.9×10^{-3} $(ppm)^{-1}$ for the three-stage model with the first stage active. Also, estimates of q_1^* were nearly identical whether exposure was for the first half or for the full lifetime. Estimates for females were somewhat lower, but were also consistent with respect to two- versus three-stage and half versus full lifetime exposure.

7.4.2.2.3.3. Final upper-limit unit risk estimate. Comparison of the q_1^* estimates from the quantal and variable exposure input forms of the linearized multistage model (Tables 7-20 and 7-21 versus Table 7-24) shows very little difference in the results. The reasons that there is so little difference between the estimates are as follows:

1. The adjustment required for high early mortality affects the high-exposure group, but this group has a relatively small effect on low-exposure risk, and consequently, on q_1^*.

2. The adjustment required for variable exposures (in the low- and mid-exposure groups, this represents the recovery group only) has little effect, since the recovery and lifetime exposure groups had generally similar nasal cancer response.

TABLE 7-24. ESTIMATE OF UPPER-LIMIT INCREMENTAL UNIT RISK $(ppm)^{-1}$ FOR TWO- AND THREE-STAGE MODELS WITH THE FIRST STAGE ACTIVE (EXPOSURE-RELATED) FOR THE WOUTERSEN DATA. BY SEX AND EXPOSURE LENGTH.

Model	Length of exposure (weeks)	Upper-limit incremental unit risk for lifetime continuous exposure to 1 ppm acetaldehyde (latent period from start of exposure to death with tumor)	
		Males	Females
Two-stage, first stage active	0-52	4.029×10^{-3}	2.87×10^{-3}
	0-104	4.030×10^{-3}	2.88×10^{-3}
		(51.1 weeks)	(49.1 weeks)
Three-stage, first stage active	0-52	3.867×10^{-3}	2.70×10^{-3}
	0-104	3.877×10^{-3}	2.72×10^{-3}
		(39.1 weeks)	(36.3 weeks)

As a result, for the final upper-limit incremental unit risk estimate, we choose the value

$$q_1^* = 4.0 \times 10^{-3}(\text{ppm})^{-1}$$

based on the variable exposure input form of the model. This estimate is applicable whether exposure is during the first half only or for the full lifetime.

Expressing this unit risk in terms of $\mu g/m^3$ requires the transformation:

$$\frac{1\ \mu g\ \text{acetaldehyde}}{m^3\ \text{air}} = \frac{10^{-3}\ \text{ppm}}{1.2\ (\text{m.w. acetaldehyde/m.w. air})}$$

$$= \frac{10^{-3}\ \text{ppm}}{1.2(44.05)/28.8} = 5.4 \times 10^{-4}\ \text{ppm}$$

Thus, in terms of $\mu g/m^3$

$$q_1^* = 4.0 \times 10^{-3}(\text{ppm})^{-1} \times \frac{5.4 \times 10^{-4}\ \text{ppm}}{\mu g/m^3} = 2.2 \times 10^{-6}\ (\mu g/m^3)^{-1}$$

7.4.3. Relative Potency

One of the uses of unit risk is to compare the potency of carcinogens. To estimate the relative potency, the unit risk slope factor is multiplied by the molecular weights, and the resulting number is expressed in terms of $(\text{mmol/kg/day})^{-1}$. This is called the relative potency index.

Figure 7-1 is a histogram representing the frequency distribution of the potency indices of 58 chemicals evaluated by the CAG as suspect carcinogens.

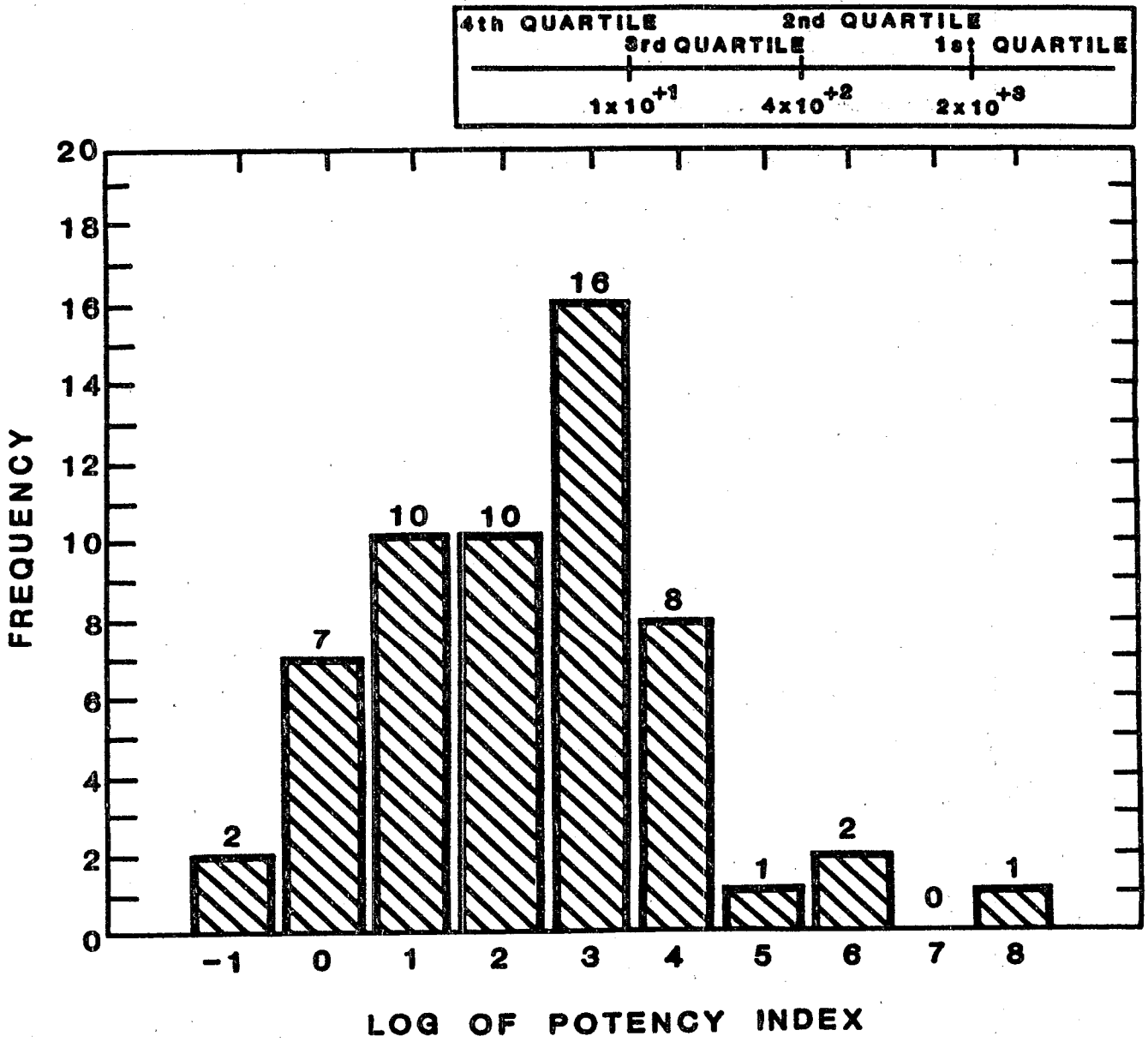

Figure 7-1. Histogram representing the frequency distribution of the potency indices of 58 suspect carcinogens evaluated by the Carcinogen Assessment Group.

The actual data summarized by the histogram are presented in Table 7-25. Where positive human data are available for a compound, they have been used to calculate the index. Where no human data are available, animal oral studies and animal inhalation studies have been used, in that order. In the present case, the Woutersen rat inhalation study was used.

The potency index for acetaldehyde based on this study is 0.34 (mmol/kg/day)$^{-1}$. This is derived as follows: the upper-limit slope estimate is $q_1^* = 4.0 \times 10^{-3}$ ppm^{-1} or $2.2 \times 10^{-6}(\mu g/m^3)^{-1}$. To convert this to mg/kg/day we assume a breathing rate for a 70-kg human of 20 m^3/day. This transforms the upper-limit incremental unit risk estimate to

$$q_1^* = 2.2 \times 10^{-6} \ (\mu g/m^3)^{-1} \times \frac{1000 \ \mu g}{mg} \times \frac{70 \ kg}{20 \ m^3/day}$$

$$= 7.7 \times 10^{-3} \ (mg/kg/day)^{-1}$$

Multiplying by the molecular weight of 44.05 gives a potency index of 3.4×10^{-1}. Rounding off to the nearest order of magnitude gives a value of 10^{-1}, which is the scale presented on the horizontal axis of Figure 7-1. The index of 0.34 lies near the bottom of the fourth quartile of the 58 suspect carcinogens that the CAG has evaluated, placing acetaldehyde as the second weakest of these carcinogens.

Two other chemicals, formaldehyde and epichlorohydrin, produce tumor responses that are similar to those resulting from acetaldehyde exposure. The relative potency of formaldehyde is approximately 25 times greater than acetaldehyde on a per mole basis when the formaldehyde risks are based on SCC only. When the formaldehyde risks are based on both SCC and the benign polypoid adenomas, the relative potency of formaldehyde is approximately 250 times as

TABLE 7-25. RELATIVE CARCINOGENIC POTENCIES AMONG 58 CHEMICALS EVALUATED BY THE CARCINOGEN ASSESSMENT GROUP AS SUSPECT HUMAN CARCINOGENS

Compounds	CAS Number	Level of evidence[a] Humans	Animals	Grouping based on EPA criteria	Slope[b] $(mg/kg/day)^{-1}$	Molecular weight	Potency index[c]	Order of magnitude (log_{10} index)
* Acetaldehyde	75-07-0	I	S	B2	$7.7x10^{-3}$	44	$3x10^{-1}$	-1
Acrylonitrile	107-13-1	L	S	B1	0.24(W)	53.1	$1x10^{+1}$	+1
* Aldrin	309-00-2	I	S	B2	16	369.4	$6x10^{+3}$	+4
Allyl chloride	107-05-1	I	S	B2	$4.7x10^{-4}$	76.5	$4x10^{-2}$	-1
Arsenic	7440-38-2	S	I	A	15(H)	149.8	$2x10^{+3}$	+3
B[a]P	50-32-8	I	S	B2	11.5	252.3	$3x10^{+3}$	+3
Benzene	71-43-2	S	S	A	$2.9x10^{-2}$(W)	78	$2x10^{0}$	0
Benzidene	92-87-5	S	S	A	234(W)	184.2	$4x10^{+4}$	+5
* Beryllium	7440-41-7	I	S	B2	8.4(W)	9	$8x10^{+1}$	+2
1,3-Butadiene	106-99-0	I	S	B2	1.8(I)	54.1	$1x10^{+2}$	+2
Cadmium	7440-43-9	L	S	B1	6.1(W)	112.4	$7x10^{+2}$	+3
Carbon tetrachloride	56-23-5	I	S	B2	$1.30x10^{-1}$	153.8	$2x10^{+1}$	+1
* Clordane	57-74-9	I	S	B2	1.3	409.8	$5x10^{+2}$	+3

(continued on the following page)

TABLE 7-25. (continued)

Compounds	CAS Number	Level of evidence[a] Humans	Level of evidence[a] Animals	Grouping based on EPA criteria	Slope[b] $(mg/kg/day)^{-1}$	Molecular weight	Potency index[c]	Order of magnitude (log_{10} index)
Chlorinated ethanes								
1,2-Dichloroethane (Ethylene dichloride)	107-06-2	I	S	B2	$9.1x10^{-2}$	98.9	$9x10^{0}$	+1
Hexachloroethane	67-72-1	I	L	C	$1.42x10^{-2}$	236.7	$3x10^{0}$	0
1,1,2,2-Tetrachloroethane	79-34-5	I	L	C	0.20	167.9	$3x10^{+1}$	+1
1,1,2-Trichloroethane	79-00-5	I	L	C	$5.73x10^{-2}$	133.4	$8x10^{0}$	+1
Chloroform	67-66-3	I	S	B2	$8.1x10^{-2}$	119.4	$1x10^{+1}$	+1
Chromium VI	7440-47-3	S	S	A	41(W)	100	$4x10^{+3}$	+4
Coke Oven Emissions		S	S	A	2.16(W)	NA	NA	NA
DDT	50-29-3	I	S	B2	0.34	354.5	$1x10^{+2}$	+2
3,3-Dichlorobenzidine	91-94-1	I	S	B2	1.69	253.1	$4x10^{+2}$	+3
1,1-Dichloroethylene (Vinylidene chloride)	75-35-4	I	L	C	1.16(I)	97	$1x10^{+2}$	+2
Dichloromethane (Methylene chloride)	75-09-2	I	S	B2	$1.4x10^{-2}$(I)	84.9	$1x10^{0}$	0
* Dieldrin	60-57-1	I	S	B2	20	380.9	$8x10^{+3}$	+4
2,4-Dinitrotoluene	121-14-2	I	S	B2	0.31	182	$6x10^{+1}$	+2
Diphenylhydrazine	122-66-7	I	S	B2	0.77	180	$1x10^{+2}$	+2
Epichlorohydrin	106-89-8	I	S	B2	$9.9x10^{-3}$	92.5	$9x10^{-1}$	0
Bis(2-chloroethyl)ether	111-44-4	I	S	B2	1.14	143	$2x10^{+2}$	+2

(continued on the following page)

TABLE 7-25. (continued)

Compounds	CAS Number	Level of evidence[a] Humans	Level of evidence[a] Animals	Grouping based on EPA criteria	Slope[b] $(mg/kg/day)^{-1}$	Molecular weight	Potency index[c]	Order of magnitude (log_{10} index)
Bis(chloromethyl)ether	542-88-1	S	S	A	9300(I)	115	$1x10^{+6}$	+6
Ethylene dibromide (EDB)	106-93-4	I	S	B2	41	187.9	$8x10^{+3}$	+4
Ethylene oxide	75-21-8	L	S	B1	$3.5x10^{-1}$(I)	44.1	$2x10^{+1}$	+1
* Heptachlor	76-44-8	I	S	B2	4.5	373.3	$2x10^{+3}$	+3
* Heptachlor epoxide	1024-57-3	I	S	B2	9.1	389.32	$4x10^{+3}$	+4
Hexachlorobenzene	118-74-1	I	S	B2	1.67	284.4	$5x10^{+2}$	+3
Hexachlorobutadiene	87-68-3	I	L	C	$7.75x10^{-2}$	261	$2x10^{+1}$	+1
Hexachlorocyclohexane								
technical grade					2.0	290.9	$6x10^{+2}$	+3
alpha isomer	319-84-6	I	S	B2	2.7	290.9	$8x10^{+2}$	+3
beta isomer	319-85-7	I	L	C	1.5	290.9	$4x10^{+2}$	+3
gamma isomer	58-89-9	I	S-L	B2-C	1.1	290.9	$3x10^{+2}$	+3
Hexachlorodibenzodioxin	34465-46-8	I	S	B2	$6.2x10^{+3}$	391	$2x10^{+6}$	+6
Nickel refinery dust		S	S	A	0.84(W)	240.2	$2x10^{+2}$	+2
Nickel subsulfide	0120-35-722	S	S	A	1.7(W)	240.2	$4x10^{+2}$	+3
Nitrosamines								
Dimethylnitrosamine	62-75-9	I	S	B2	25.9(not by q_1^*)	74.1	$2x10^{+3}$	+3
Diethylnitrosamine	55-18-5	I	S	B2	43.5(not by q_1^*)	102.1	$4x10^{+3}$	+4
Dibutylnitrosamine	924-16-3	I	S	B2	5.43	158.2	$9x10^{+2}$	+3
N-nitrosopyrrolidine	930-55-2	I	S	B2	2.13	100.2	$2x10^{+2}$	+2
N-nitroso-N-ethylurea	759-73-9	I	S	B2	32.9	117.1	$4x10^{+3}$	+4

(continued on the following page)

TABLE 7-25. (continued)

Compounds	CAS Number	Level of evidence[a] Humans	Animals	Grouping based on EPA criteria	Slope[b] (mg/kg/day)$^{-1}$	Molecular weight	Potency index[c]	Order of magnitude (log_{10} index)
N-nitroso-N-methylurea	684-93-5	I	S	B2	302.6	103.1	$3x10^{+4}$	+4
N-nitroso-diphenylamine	86-30-6	I	S	B2	$4.92x10^{-3}$	198	$1x10^{0}$	0
* PCBs	1336-36-3	I	S	B2	7.7	324	$2x10^{+3}$	+3
Tetrachlorodibenzo-p-dioxin (TCDD)	1746-01-6	I	S	B2	$1.56x10^{+5}$	322	$5x10^{+7}$	+8
* Tetrachloroethylene (Perchloroethylene)	127-18-4	I	S	B2	$5.1x10^{-2}$	165.8	$8x10^{0}$	+1
Toxaphene	8001-35-2	I	S	B2	1.13	414	$5x10^{+2}$	+3
Trichloroethylene	79-01-6	I	S	B2	$1.1x10^{-2}$	131.4	$1x10^{0}$	0
2,4,6-Trichlorophenol	88-06-2	I	S	B2	$1.99x10^{-2}$	197.4	$4x10^{0}$	+1
Unleaded gasoline vapor		I	S	B2	$3.5x10^{-3}$	110[d]	$4x10^{-1}$	0
Vinyl chloride	75-01-4	S	S	A	2.3	62.5	$1x10^{+2}$	+2

[a]S = Sufficient evidence; L = Limited evidence; I = Inadequate evidence.
[b]Animal slopes are 95% upper-bound slopes based on the linearized multistage model. They are calculated based on animal oral studies, except for those indicated by I (animal inhalation), W (human occupational exposure), and H (human drinking water exposure). Human slopes are point estimates based on the linear nonthreshold model. Not all of the carcinogenic potencies presented in this table represent the same degree of certainty. All are subject to change as new evidence becomes available. The slope value is an upper bound in the sense that the true value (which is unknown) is not likely to exceed the upper bound and may be much lower, with a lower bound approaching zero. Thus, the use of the slope estimate in risk evaluations requires an appreciation for the implication of the upper bound concept as well as the "weight of evidence" for the likelihood that the substance is a human carcinogen.
[c]The potency index is a rounded-off slope in (mmol/kg/day)$^{-1}$ and is calculated by multiplying the slopes in (mg/kg/day)$^{-1}$ by the molecular weight of the compound.
[d]The molecular weight is based on the weighted average of the compounds present in gasoline. Some variation may be expected among samples.
NA = not applicable.
* = currently under review.

great as that of acetaldehyde. With regard to relative potency, acetaldehyde is much closer to epichlorohydrin, which also causes nasal cancers in rats. On a per mole basis, epichlorohydrin is approximately three times as potent as acetaldehyde.

7.5. SUMMARY

Acetaldehyde has been tested for carcinogenicity in hamsters by intratracheal instillation and inhalation and in rats by subcutaneous injection and inhalation exposure. In inhalation studies, acetaldehyde induced inflammatory changes, hyperplasia, and metaplasia of the nasal, laryngeal, and tracheal epithelium. In intratracheal studies in hamsters, acetaldehyde enhanced the development of benzo(a)pyrene-initiated tracheobronchial carcinomas, but there was no evidence of acetaldehyde enhancing the development of diethynitrosamine-initiated respiratory tract tumors (Feron, 1979; Feron et al., 1982). In one rat study, spindle cell carcinomas were produced at injection sites by repeated subcutaneous injection (Watanabe and Sugimoto, 1956), but the experiment was considered inadequate for evaluation because of the small number of animals and the lack of a control group. One lifetime inhalation study (27 months) was done in rats (Woutersen et al., 1985). In this study, the acetaldehyde exposure increased the number of animals with nasal tumors, both adenocarcinomas and squamous cell carcinomas, in an exposure-related manner. In addition, exposure-related increases in the incidence of multiple respiratory-tract tumors were noted. Adenocarcinomas were increased significantly in both male and female rats at all dose levels, whereas squamous cell carcinomas were increased significantly in male rats at the middle and high doses and in female rats at the high dose only. In the Woutersen and Appelman (1984) study, which was referred to as a "recovery study," the animals were exposed to acetaldehyde for 52 weeks, followed by a recovery period of 26 weeks. In this study the

nasal tumor response was the same as in the lifetime study (Woutersen et al., 1985). These findings indicate that even though the exposure to acetaldehyde was discontinued, proliferative epithelial hyperplasia and metaplasia of the respiratory tract may develop into tumors.

The only epidemiologic study involving acetaldehyde exposure showed an increased crude incidence rate of total cancer in the workers as compared to the general population. This apparent incidence increase cannot be validated as a real incidence increase because these crude rates were not age-adjusted. This study also has several other methodological limitations. The cohort was very small and age and sex distribution was not provided. Criteria for inclusion in the cohort were not specified. The workers were also exposed to several chemicals other than acetaldehyde. All of the incident cancer individuals were smokers. No adjustment for possible confounders such as other chemicals, smoking, etc. was carried out. Hence, the study is considered inadequate to support any positive or negative conclusions about the causal association of acetaldehyde with human cancer.

The upper-limit incremental unit cancer risk for acetaldehyde has been quantitatively estimated from nasal cancers in the Woutersen and Appelman (1984) and the Woutersen et al. (1984, 1985) rat inhalation studies. Because the studies contained both lifetime and first-half lifetime exposure groups, incremental risk estimates can be made for both types of human exposure. The estimates, derived from the male rat tumor data, yield an upper-limit incremental unit risk estimate of $q_1^* = 4.0 \times 10^{-3}$ $(ppm)^{-1}$ or $q_1^* = 2.2 \times 10^{-6}$ $(\mu g/m^3)^{-1}$ for a lifetime continuous exposure. For a first-half lifetime continuous exposure the estimates are only slightly less than those based on a full lifetime continuous exposure. In terms of relative potency, on a per mole basis, acetaldehyde is the second weakest of the 58 chemicals that the CAG has evaluated as

suspect carcinogens, and is only about 1/25 to 1/250 as potent as formaldehyde.

7.6. CONCLUSIONS

Positive evidence for the carcinogenicity of acetaldehyde has been provided by instillation and inhalation studies in hamsters and inhalation studies in rats. In these studies, acetaldehyde produced nasal and laryngeal tumors in rats and hamsters of both sexes. The rat inhalation study, Woutersen and Appelman (1984) and Woutersen et al. (1984, 1985), has been used for quantitative risk extrapolation because it was a lifetime (27 months) study, used three exposure levels, and contained both full and partial lifetime exposure groups. For quantitative estimation, it was considered superior to the hamster study (Feron et al., 1982), which was of shorter exposure duration (52 weeks) and used only one level of exposure to acetaldehyde (1500 ppm).

In the chronic inhalation studies, acetaldehyde caused nasal tumors in rats, and primarily laryngeal tumors in hamsters. A possible explanation for this difference between the species may be that rats are obligatory nose-breathers, while hamsters may also breathe through the mouth. In rats, both acetaldehyde and its related compound, formaldehyde, have been shown to produce nasal squamous cell carcinomas (Swenberg et al., 1980). Acetaldehyde, unlike formaldehyde, has also induced the formation of nasal adenocarcinomas (Woutersen et al., 1985). The concentrations of acetaldehyde that produced nasal cancer in rats ranged from 750 to 3000 ppm, whereas the concentrations of formaldehyde were only 6 to 15 ppm. The evidence thus suggests that the carcinogenic potency of acetaldehyde is about 1/25 to 1/250 that of formaldehyde, on a per mole basis. The only epidemiologic study of workers exposed to acetaldehyde was considered inadequate to support any conclusions as to the carcinogenicity of acetaldehyde.

Acetaldehyde is an alkylating agent that has induced DNA damage and mutations in bacteria and yeast mitochondria. It has also induced chromosomal aberrations in plants and mammalian cells. An increased incidence of sister chromatid exchange in bone marrow has been observed in mice and hamsters treated with acetaldehyde *in vivo*. The positive animal bioassay studies provide sufficient evidence for the carcinogenicity of acetaldehyde in animals, whereas the one available epidemiologic study provides inadequate evidence because of methodology and data limitations. On the basis of the accumulated evidence, acetaldehyde is a probable human carcinogen, classified in Group B2 using the EPA's guidelines for carcinogen risk assessment (U.S. EPA, 1986b), with the carcinogenic potency estimated to be the second weakest of 58 chemicals evaluated by the CAG.

APPENDIX: COMPARISON OF RESULTS BY VARIOUS EXTRAPOLATION MODELS

The estimates of unit risk from animals presented in Chapter 7 of this document are all calculated by the use of the linearized multistage model. The reasons for its use have been detailed therein. Essentially, it is part of a methodology that estimates a conservative linear slope at low extrapolation doses and is consistent with the data at all dose levels of the experiment. It is a nonthreshold model which holds that the upper limit of risk predicted by a linear extrapolation to low levels of the dose-response relationship is the most plausible upper limit for the risk.

Other models have also been used for risk extrapolation. Four other nonthreshold models are presented here: the one-hit, the log-Probit, the logit, and the Weibull. The one-hit model is characterized by a continuous downward curvature, but is linear at low doses. It can be considered the linear form or first stage of the multistage model because of its functional form. Because of this and its downward curvature, it will always yield estimates of low-level risk which are at least as large as those of the multistage model. Further, whenever the data can be fitted adequately by the one-hit model, estimates from the two procedures will be comparable.

The other three models, the log-Probit, the logit, and the Weibull, are often used to fit toxicologic data in the observable range, because of the general "S" curvature. The low-dose upward curvatures of these models usually yield lower low-dose risk estimates than those of the one-hit or multistage models.

The log-Probit model was originally proposed for use in biological assay problems such as the assessment of the potency of toxicants and drugs, and has usually been used to estimate such values as percentile lethal dose or percen-

tile effective dose. Its development was strictly empirical, i.e., it was observed that several log dose-response relationships followed the cumulative normal probability distribution function. In fitting the cancer bioassay data, assuming an independent background, this function becomes:

$$P(D;a,b,c) = c + (1-c)\ \Phi\ (a+b\ \log_{10}D) \quad a,b > 0 < c < 1$$

where P is the proportion responding at dose D, c is an estimate of the background rate, a is an estimate of the standardized mean of individual tolerances, b is an estimate of the log dose-Probit response slope, and Φ represents the cumulative normal distribution function.

The logit or log-dose logistic model, like the log-Probit, also has a long history in the analysis of quantal data. Its form, also with an independent background, c, is

$$P(D;a,b,c) = c + (1-c)[\exp(a+b\ \log_{10}D)+1]^{-1}$$

The overall shape of the logit model, like the log-Probit model, is sigmoid, but it approaches the low extremes more slowly, and as a result, yields higher risk estimates at the low exposures. At low doses and low background rates, its form is approximately log linear in dose-response.

The one-hit model arises from the theory that a single molecule of a carcinogen has a probability of transforming a single noncarcinogenic cell into a carcinogenic one. It has the probability distribution function:

$$P(D;a,b) = 1-\exp-(a+bd) \quad a,b > 0$$

Finally, a model from the theory of carcinogenesis arises from the multi-hit model applied to multiple target cells. This model has been termed here the Weibull model. It is of the form

$$P(D;b,k) = 1-\exp-(bd^k) \quad b,k > 0$$

For the power of dose only, the restriction $k > 0$ has been placed on this model. When $k > 1$, this model yields low-dose estimates of risks that are usually significantly lower than the estimates of either the multistage or one-hit models, which are linear at low doses. All four of these models usually project risk estimates significantly higher at the low exposure levels than those from the log-Probit.

The estimates of risk based on various added exposures for the above models are given below for the Woutersen and Appelman (1984) and the Woutersen et. al., (1984, 1985) rat inhalation data. Both maximum likelihood estimates and 95% upper confidence limits are presented. All estimates incorporate Abbott's correction for independent background rate.

The results (Tables A-1 and A-2) show that the estimates of incremental risk for the log-Probit model are all less than those for the other models. The one-hit model yields the highest estimates, slightly higher than those of the multistage (two-stage) model. The Weibull model yields risk estimates between those of the two-stage and the logit models. The Carcinogen Assessment Group feels that estimates based on the linearized multistage model represent the plausible upper limits of risk.

TABLE A-1. ESTIMATES OF LOW-EXPOSURE RISK FROM FEMALE WISTAR RATS, FROM THE WOUTERSEN AND APPELMAN (1984) AND THE WOUTERSEN ET AL. (1984, 1985) INHALATION DATA. DERIVED FROM FIVE DIFFERENT MODELS. (All estimates incorporate Abbott's correction for independent background rates.)

Continuous exposure (ppm)	Maximum likelihood estimates of additional risk					95% upper confidence limit of additional risk				
	Crump multistage model[b]	One-hit model	Weibull model	Log-probit model	Logit model	Crump multistage model[b]	One-hit model	Weibull model	Log-probit model	Logit model
Lifetime exposure group only										
0.1	9.0×10^{-5}	4.4×10^{-4}	1.2×10^{-6}	0	5.2×10^{-9}	3.8×10^{-4}	5.3×10^{-4}	7.5×10^{-6}	0	4.0×10^{-8}
1	9.1×10^{-4}	4.4×10^{-3}	7.1×10^{-5}	2.2×10^{-16}	1.8×10^{-6}	3.8×10^{-3}	5.3×10^{-3}	3.3×10^{-4}	5.1×10^{-15}	1.1×10^{-5}
10	1.0×10^{-2}	4.3×10^{-2}	4.1×10^{-3}	3.2×10^{-6}	6.5×10^{-4}	3.8×10^{-2}	5.2×10^{-2}	1.3×10^{-2}	2.8×10^{-5}	2.4×10^{-3}
100	2.2×10^{-1}	3.6×10^{-1}	2.1×10^{-1}	1.8×10^{-1}	1.9×10^{-1}	3.4×10^{-1}	4.1×10^{-1}	3.2×10^{-1}	3.0×10^{-1}	3.0×10^{-1}
Lifetime exposure plus recovery group										
0.1	2.5×10^{-5}	3.4×10^{-4}	2.6×10^{-7}	0	4.6×10^{-4}	2.1×10^{-4}	3.9×10^{-4}	1.5×10^{-6}	0	3.1×10^{-8}
1	2.6×10^{-4}	3.4×10^{-3}	2.2×10^{-5}	1.1×10^{-16}	1.5×10^{-6}	2.1×10^{-3}	3.9×10^{-3}	9.5×10^{-5}	2.7×10^{-15}	7.4×10^{-6}
10	3.9×10^{-3}	3.3×10^{-2}	1.9×10^{-3}	1.3×10^{-6}	4.8×10^{-4}	2.1×10^{-2}	3.9×10^{-2}	5.4×10^{-3}	1.0×10^{-5}	1.6×10^{-3}
100	1.5×10^{-1}	2.8×10^{-1}	1.4×10^{-1}	1.3×10^{-1}	1.3×10^{-1}	2.3×10^{-1}	3.3×10^{-1}	2.2×10^{-1}	2.0×10^{-1}	2.0×10^{-1}

[a]Model with k = 2 provided lowest upper-limit estimate of incremental risk for all values to k = 6 for both male and female data.

Animal exposures: 0, 750 ppm, 1500 ppm, 3000 ppm (initial, adjusted downward), 6 hours/day, 5 days/week, 1 and 2 years

Data	Lifetime exposure group only				Lifetime exposure plus recovery group			
Continuous equivalent exposure	0	129.8	256.8	279.0	0	130.3	255.1	279.0
No. of tumors/No. alive at 12 months	1/55	17/52	40/54	31/41	0/55	7/54	36/55	39/48

TABLE A-2. ESTIMATES OF LOW-EXPOSURE RISK FROM FEMALE WISTAR RATS, FROM THE WOUTERSEN AND APPELMAN (1984) AND THE WOUTERSEN ET AL. (1984, 1985) INHALATION DATA. DERIVED FROM FOUR DIFFERENT MODELS.[a]
(All estimates incorporate Abbott's correction for independent background rates.)

Continuous exposure (ppm)	Maximum likelihood estimates of additional risk				95% upper confidence limit of additional risk			
	Crump multistage model[b]	Weibull model	Log-probit model	Logit model	Crump multistage model[b]	Weibull model	Log-probit model	Logit model
Lifetime exposure group only								
0.1	1.6×10^{-7}	1.5×10^{-11}	0	2.5×10^{-14}	7.2×10^{-5}	1.3×10^{-10}	0	2.3×10^{-13}
1	1.6×10^{-5}	2.3×10^{-8}	0	3.1×10^{-10}	7.3×10^{-4}	1.4×10^{-7}	0	2.1×10^{-9}
10	1.6×10^{-3}	3.6×10^{-5}	4.9×10^{-14}	3.9×10^{-6}	8.5×10^{-3}	1.4×10^{-4}	7.2×10^{-13}	1.7×10^{-5}
100	1.6×10^{-1}	5.6×10^{-2}	3.7×10^{-2}	4.7×10^{-2}	1.9×10^{-1}	1.0×10^{-2}	7.6×10^{-2}	8.8×10^{-2}
Lifetime exposure plus recovery group								
0.1	1.2×10^{-7}	1.9×10^{-12}	0	2.3×10^{-14}	4.1×10^{-5}	1.5×10^{-11}	0	2.0×10^{-13}
1	1.2×10^{-5}	4.9×10^{-9}	0	2.5×10^{-11}	4.2×10^{-4}	2.9×10^{-8}	0	1.6×10^{-9}
10	1.2×10^{-3}	1.3×10^{-5}	5.9×10^{-14}	2.7×10^{-6}	5.1×10^{-3}	5.0×10^{-5}	7.7×10^{-13}	1.1×10^{-5}
100	1.1×10^{-1}	3.2×10^{-2}	2.1×10^{-2}	2.8×10^{-2}	1.3×10^{-1}	5.9×10^{-2}	4.4×10^{-2}	5.3×10^{-2}

[a]Fit is too poor with one-hit model.
[b]Model with k = 2 provided lowest upper-limit estimate of incremental risk for all values to k = 6 for both male and female data.

Animal exposures: 0, 750 ppm, 1500 ppm, 3000 ppm (initial, adjusted downward), 6 hours/day, 5 days/week, 1 and 2 years

Data	Lifetime exposure group only				Lifetime exposure plus recovery group			
Continuous equivalent exposure	0	129.8	256.8	279.0	0	130.3	255.1	279.0
No. of tumors/No. alive at 12 months	0/55	7/54	36/55	39/48	0/83	7/84	43/84	50/72

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8. REPRODUCTIVE AND DEVELOPMENTAL EFFECTS

The female and male reproductive toxicity and developmental effects of acetaldehyde are reviewed in this chapter. The literature review concentrates primarily on investigations in which acetaldehyde was the agent administered. Only cursory attention is paid to studies utilizing ethanol even though acetaldehyde, as a major metabolite, would potentially be available to exert toxicity. Also omitted are studies in which drugs (e.g., disulfiram, an acetaldehyde dehydrogenase inhibitor) were administered in conjunction with ethanol, presumably to elevate acetaldehyde levels. However, acetaldehyde exposure via ethanol intake should not be ignored as an additional risk factor in conjunction with other sources of environmental and occupational exposures.

8.1. FEMALE REPRODUCTIVE AND DEVELOPMENT EFFECTS OF ACETALDEHYDE

There are no studies reporting on the effects of acetaldehyde on aspects of female reproduction aside from pregnancy (e.g., cyclicity, oocyte toxicity, etc.). Thus, the focus of this section is on the developmental effects of acetaldehyde.

Data in this area have been primarily drawn from studies in rats and mice or *in vitro* embryo preparations in these species. The majority of investigations have concentrated on the teratogenic properties of acetaldehyde. This emphasis has been motivated by attempts to define the proximate teratogen (ethanol or acetaldehyde) in the fetal alcohol syndrome. In reviewing the *in vivo* studies, two experimental flaws were consistently apparent; namely, the use of relatively small sample sizes (under 10 litters per dose) and the failure to use litter as the unit of analysis in analyzing fetal data. In addition, studies that evaluated the teratogenic properties of acetaldehyde routinely utilized intraperitoneal or intravenous routes of administration. The

interpretation of the data from these investigations must be qualified in the absence of kinetic studies comparing these routes to the actual occupational/environmental routes of exposure. Finally, only three studies provide any information regarding maternal toxicity. One investigation in mice reported no effects on maternal weight gain (O'Shea and Kaufman, 1979). Of the two reports in rats, one found no effects on maternal weight (Dreosti et al., 1981) while the second reported an unspecified "relative" reduction in maternal weight and food and fluid consumption (Padmanabhan et al., 1983). In the absence of data, the contribution of acetaldehyde-induced maternal toxicity cannot be determined.

There has been only one study in which the effects of acetaldehyde upon preimplantation events were evaluated (Checiu et al., 1984). Female rats were injected with 150 to 200 nM (i.v.) acetaldehyde on day 3 postconception* and then sacrificed on day 4. Embryos were recovered from the oviducts and uterine horns. Litter size was not affected; however, the manner in which this variable was assessed was not defined. The authors reported a retarded rate of segmentation and blastulation and an increase in cellular fragmentation in the blastocyst. Although the sample sizes in this study were adequate (~25 litters/group), these data were not analyzed with litter as the statistical unit. However, the effects appear sufficiently pronounced to assume an acetaldehyde-induced effect on the preimplantation embryo.

Peripheral support for acetaldehyde-induced preimplantation damage (Checiu et al., 1984) is provided by a study by Kawamoto (1981), who noted an increased lethality in chicken eggs that were injected with acetaldehyde during the early

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*For the majority of developmental papers, day 1 of pregnancy was defined as the morning, following mating the previous evening, in which sperm were seen in a vaginal lavage or a copulatory plug was observed.

stages of incubation. The influence of acetaldehyde on preimplantation events in the human is unknown. Veghelyi and Osztovics (1979) have suggested that the fertilized ovum either survives or is killed by acetaldehyde exposure. They state that "it [acetaldehyde] does not affect (or else kills it) the ovum in the follicle." This opinion is based upon clinical observation of a single alcoholic patient.

The data in rats strongly suggest that acetaldehyde is a teratogen; the data in mice are much less conclusive. Summaries of the relevant studies appear in Tables 8-1 and 8-2, respectively.

Although all of the rat studies have yielded positive data, the majority of work has been conducted in one laboratory (Sreenathan and colleagues). The research by Sreenathan utilized a number of end points of fetal growth in addition to assessing malformations. These measures included fetal and placental weight, crown-rump length (CRL), tail length, and transumbilical distance (TUD). In the initial study (Sreenathan et al., 1982), dams were injected with acetaldehyde (50 to 100 mg/kg, i.p.) on days 10, 11, 12, or 10 to 12 of gestation and then sacrificed on day 21. Acetaldehyde produced significant increases in fetal resorptions, retardation in growth (reflected in the measures described above), delayed skeletogenesis, and an increase in malformations. Malformations included digital anomalies and cranial and facial malformations. Placental weight was also depressed. However there was no evidence of dose-response trends.

A second study (Padmanabhan et al., 1983) extended the dosing period to encompass days 8 to 15 of gestation. The authors now observed a dose-dependent increase in resorptions and fetal death and dose-response trends in the other indicators of intrauterine growth retardation. Acetaldehyde produced a variety of malformations in the face, fore- and hindlimbs, as well as a slowed rate of

TABLE 8-1. DEVELOPMENTAL EFFECTS OF ACETALDEHYDE IN RATS[a]

Dose	Route	Period	Results	Comments	References
50, 75, 100 mg/kg	i.p.	Admin: D10, 11, or 12 or D10-12; sac: D21	↑Resorp; variable↑ in litters with malformations; ↓FW; ↓CRL; ↓TUD; ↓placental wt: ↓rate skeletogenesis	Small sample size, 5-8 litters/treatment; no dose-response trend. Multiple days of treatment do not increase severity of effect.	Sreenathan et al., 1982
			↓Volume of labyrinthine zone of placenta which correlates positively with ↓FW morphological lesions in placenta		Sreenathan and Padmanabhan, 1984; Sreenathan et al., 1984a
			Unspecified "relative" reduction in maternal food and fluid consumption and weight		
50, 75, 100, 150 mg/kg	i.p.	Admin: D8-15; sac: 21	↑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 trends	Padmanabhan et al., 1983; Sreenathan et al., 1984b
150-200 nM (44 ng/kg)	i.v.	Admin: D4 sac: D5	Delayed segmentation and blastulation; ↑cell fragmentation within blastocyst; litter size unaffected	Sample sizes adequate (>25 litter/group)	Checiu et al., 1984

(continued on the following page)

TABLE 8-1. (continued)

Dose	Route	Period	Results	Comments	References
0.02 mL of 1 or 10% per embryo	Intra-amniotic	Admin: D13	High embryolethality; +malformations; + craniocaudal size; +chromosomal anomalies	Sample size marginal (10-12 litters/group)	Barilyak and Kozachuk, 1983
50 or 100 mg/kg x 3 (30-min intervals)	i.p.	Admin: D16; sac: D16	+^{3}H-thymidine uptake by fetal brain and liver	Dosing regime poorly described, small sample sizes (6-8 litters/group)	Dreosti et al., 1981
0.5 mL of 3% (v/v) x 2 (approx. 53 mg/kg/inj.)	i.p.	Admin: throughout preg.; sac: D20	↓FW; + resorp; ↓litter size		

[a]Abbreviations used in tables.

Admin = Day of pregnancy acetaldehyde was administered
Sac = Day of pregnancy that dam was sacrificed
CRL = Crown-rump length
FW = Fetal weight
MW = Maternal weight
PC = Protein content
Resorp = Resorptions
TUD = Transumbilical distance

TABLE 8-2. DEVELOPMENTAL EFFECTS OF ACETALDEHYDE IN MICE[a]

Dose	Route	Period	Results	Comments	References
200 mg/kg x 5 in 10 hr period	i.p.	Admin: D10; sac: D18	No effect on resorp or FW	Small sample size, 8-12 litters/group	Blakley and Scott, 1984a
200 mg/kg	i.p.	Admin: D10; sac: 5 min-24 hr post-inj.	Maximal acetaldehyde levels within 5 min; undetectable by 2 hr	Acetaldehyde does gain access to fetus	Blakley and Scott, 1984b
2, 4, 6% (approx. 60-480 mg/kg)	i.p.	Admin: D9; sac: 1-24 hr post-inj.	No histopathological changes seen in fetus (light microscopy)	No actual data on acetaldehyde presented; authors suggest rapid clearance of single dose may explain negative findings	Bannigan and Burke, 1982
320 mg/kg	i.p.	Admin: single inj. D6, 7, 8, or 9; two inj. D6, 7, 8, or 9 (30 min apart); two inj. D6, 7, 8, or 9 (6 hr apart) sac: D17	Nonsignificant ↓FW; slight ↑in % fetuses with head and limb defects	Small sample size, between 5-8 litters/treatment; 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
0.1 mL (2% solution) (approx. 80 mg/kg)	i.v.	Admin: D5, 6, or 7, 5-7, 6-8, or 6-7; sac: D9 or D11	Day 9 ↑resorp; ↑no. embryos failing to turn to fetal position; ↓CRL and PC; ↑no. malformed	Small sample sizes, approx. 4-6 litters/treatment; no D12 control groups; no consistent trends seen with ↑no. days of dosing	O'Shea and Kaufman, 1981
1 or 2% (approx. 40-80 mg/kg)	i.v.	Admin: D7-9; 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 occurrence of terata difficult to evaluate due to small no. litters/treatment. In fact, no. of D10 litters reported in text disagree with N in tables.	O'Shea and Kaufman, 1979

[a]Abbreviations used in tables.

Admin = Day of pregnancy acetaldehyde was administered
Sac = Day of pregnancy that dam was sacrificed
CRL = Crown-rump length
FW = Fetal weight
MW = Maternal weight
PC = Protein content
Resorp = Resorptions
TUD = Transumbilical distance

ossification. The delays in ossification were on the order of 1 to 2 days (described in greater detail for the low-dose group in Sreenathan et al., 1984c). The placentas of the treated fetuses were grossly reduced in weight and exhibited thick fibrinotic material around the margins. Lesions were especially apparent in the labyrinthine zone of the placenta, where maternal-fetal exchange occurs.

Sreenathan et al. (1984a) and Sreenathan and Padmanabhan (1984) reported a more detailed examination of the morphology of the placentas of fetuses evaluated in a previous study (Sreenathan et al., 1982). Placental lesions that were observed at day 21 occurred at all doses and irrespective of the day(s) of treatment during organogenesis. Although there was no correlation between total placental weight and fetal weight, the volume of the labryinthine zone was positively correlated with fetal weight. The authors suggest that acetaldehyde damage to this zone interferes with maternal-placental nutrient exchange, resulting in the retarded growth.

There are additional data that support the hypothesis that acetaldehyde interferes with placental function. Henderson et al. (1981, 1982) have examined the effects of acetaldehyde (155-465 μM) on amino acid uptake in vitro by villous fragments of rat placentas obtained at day 20. Preincubation with acetaldehyde for 2 hours resulted in a decrease in the uptake of aminoisobutyric acid (AIB) and cycloleucine. Alanine, leucine, and lysine uptake were unimpaired. The authors also showed that this "apparent" decrease was the result of actual impaired cellular uptake and not enhanced efflux. Asai et al. (1985) have demonstrated that acetaldehyde can interfere with L-alanine transport systems in microvillous brush border membrane vesicles prepared from human placenta. This system removes the complications of internal compartmentalization and metabolism seen with cultures of placental fragments.

Fisher et al. (1981a, b; 1984) have also found a decrease in AIB uptake in human term placenta incubated in vitro with acetaldehyde (200 μM, lowest effective dose). These authors note that such disruptions in placental function may create a state of fetal malnutrition that is independent of maternal nutritional status. Such a state may be a factor in intrauterine growth retardation.

The in vitro data used to support a role for placental dysfunction in acetaldehyde-induced intrauterine growth retardation must be interpreted with some caution (e.g., Henderson et al., 1981, 1982; Asai et al., 1985; Fisher et al., 1981a, b, 1984). Those studies examined the status of term placentas. It remains to be determined what relationship exists between alterations observed in these structures to potential acetaldehyde-induced modifications in preplacental structures present during organogenesis (Beck, 1981). However, similar qualifications cannot be applied to the placental lesions reported by Sreenathan et al. (1984a) and Sreenathan and Padmanabhan (1984), since the morphological effects observed at day 21 were in females treated with acetaldehyde during organogenesis.

Support for a direct, teratogenic effect of acetaldehyde in rats may be afforded by the work of Barilyak and Kozachuk (1983). In their study, laparotomies were performed on female rats on day 13 of pregnancy and either a 1% or a 10% solution (0.02 mL) of acetaldehyde was injected, intra-amniotically, into the fetuses on one side of the uterus. Some females were sacrificed 24 hours later, and the fetuses were removed and processed for chromosomal analyses. The remaining females were sacrificed on Day 20, and the litters were analyzed for developmental effects. The 10% solution caused the death of all fetuses on the uterine side of injection and high fetal mortality on the non-injected side (78%). At 1% acetaldehyde, fetal lethality was 69% and 33% for

the injected and the non-injected horns, respectively. At this dose, 80% of the surviving fetuses collected from the injected horn had cranial and facial malformations (14% from the non-injected side). Skeletal ossification was also "disturbed," a term not defined by the authors. Craniocaudal length (mm) was also significantly reduced. Acetaldehyde also produced marked clastogenic effects causing increases in chromosomal aberrations (see Section 6.2.2).

Only one study has examined the effects of acetaldehyde exposure throughout gestation (Dreosti et al., 1981). These authors administered acetaldehyde (0.5 mL, 3% v/v, i.p.) twice daily throughout pregnancy and sacrificed females on day 20. Based upon term maternal body weights, this dose translates to approximately 53 mg/kg/injection. These authors reported that, in the absence of maternal toxicity (as reflected in maternal weights), acetaldehyde produced an increase in fetal resorption sites, a decrease in litter size, and a decrease in fetal weight. Since the surviving fetuses appeared morphologically normal, Dreosti et al. (1981) suggest that acetaldehyde-induced dysmorphogenesis may be so severe as to compromise survival in those fetuses so affected. These authors also reported that acetaldehyde, administered on day 16 of gestation, reduces the incorporation of ^{3}H-thymidine into DNA in fetal brain and liver.

As noted earlier, the teratologic data on mice are equivocal. At doses that were equivalent or higher than those employed in the rat studies (see Table 8-2), acetaldehyde appears to produce no effects (Bannigan and Burke, 1982; Blakley and Scott, 1984a), a low level of terata (Webster et al., 1983), or pronounced developmental toxicity (O'Shea and Kaufman, 1979, 1981). Many of these investigators injected acetaldehyde for a single day and/or at earlier time periods than in the rat studies. This last distinction may be slight. Since the mouse exhibits more rapid, early development, the periods of organogenesis in which acetaldehyde was administered probably overlap in the rat and

mouse studies. The toxicity associated with single versus multiple days of treatment may be a more critical factor. Given the rapid clearance of acetaldehyde from the mouse fetus (Blakley and Scott, 1984b), it is possible that a single exposure may be insufficient to produce adverse developmental effects.

In the one laboratory reporting more pronounced effects (O'Shea and Kaufman, 1979, 1981), acetaldehyde was administered intravenously. In the initial study, acetaldehyde was administered on days 6 to 8 of pregnancy with dams examined on days 10 and 19. In the subsequent investigation, acetaldehyde was administered on days 6, 7, or 8 or in various combinations (e.g., days 6 to 8) with evaluations conducted on days 10 or 12. In both studies, acetaldehyde produced an increase in total resorptions, a decrease in the number of embryos that had rotated into the fetal position, retarded growth, and an increase in the number of fetuses with malformations. Neural tube defects were the most prominent malformations. However, these effects were not magnified by multiple injections (O'Shea and Kaufman, 1981). The research also had a number of flaws. No control data were reported for day 12 comparisons in the second study. Moreover, the day 10 control data across the two studies are not in close agreement. The control litters in the initial study (O'Shea and Kaufman, 1979) had a lower resorption rate (9.8% versus 15%), and the embryos had greater CRL (2.8 mm versus 2.4 mm) and protein content (192 µg versus 141 µg). The small sample sizes employed probably contributed to the variability seen across the two studies.

Several studies have examined the direct embryotoxic properties of acetaldehyde utilizing whole embryo cultures (rat and mouse). The majority of these data demonstrate that acetaldehyde can produce growth retardation and terata *in vitro* (Table 8-3). In all instances, evaluations have been conducted on cultures of embryos recovered between days 7 and 10 of gestation. In the initial

TABLE 8-3. DEVELOPMENTAL EFFECTS OF ACETALDEHYDE ON EMBRYO CULTURES[a]

Species	Dose	Age	Results	Comments	References
Mice	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
Mice	.4 μM-400 mM (17.6 μg/L-1.76 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 lowest dose (.4 μM)	Higuchi and Matsumoto, 1984
Rat	5-100 μM (0.22-4.4 mg/L)	Explant: D10 Examine: 28 hr later	Embryolethality at 100 μM; growth retard. (↓DNA, ↓PC, ↓CRL, ↓head length)	No dose-response trends for growth retardation	Campbell and Fantel, 1983
Rat	4.5 μM-45 mM (0.20-1980 mg/L)	Explant: D9.5 Examine: immed. (?)	Embryolethality; growth retard. (↓no. somites, ↓PC, ↓cranio-caudal length); ↓malformations	Sample size not provided; dose-response trends present	Popov et al., 1982
Rat	100, 260 μM (4.4-11.44 mg/L)	Explant: D10 Examine: over 48-hr period	No effects at these doses on PC, DNA, somite no., or morphology	Author suggests differences between his data and Campbell and Fantel may be result of using different strains of rats	Priscott, 1985
	800 mM (35.2 mg/L)		Embryolethality		

[a]Abbreviations used in tables.

Admin = Day of pregnancy acetaldehyde was administered
Sac = Day of pregnancy that dam was sacrificed
CRL = Crown-rump length
FW = Fetal weight
MW = Maternal weight
PC = Protein content
Resorp = Resorptions
TUD = Transumbilical distance

report using cultured mouse embryos, Thompson and Folb (1982) reported dose-related alterations in a number of developmental measures in 8-day embryos (see Table 8-3). However, effects at day 9 were very inconsistent. Toxicity was seen in the middle-dose group, but developmental stimulation was observed in the high-dose group. Such variability in response makes it difficult to interpret these data in any meaningful fashion. Subsequent work with mouse embryo cultures (Higuchi and Matsumoto, 1984) has demonstrated that acetaldehyde is embryotoxic. These authors reported not only embryolethality at 40 μM, but also an increase in malformations (cranial, facial, and limb defects) and growth retardation at even the lowest dose employed (0.4 μM). Although the number of cultures/dose upon which the data analyses were based was small, the presence of dose-response trends for the majority of measures provides some reassurance as to the validity of the data. These authors stated that these in vitro results were quite consistent with observations that they had made in a previous in vivo study (cited in Higuchi and Matsumoto, 1984).

Two studies employing rat embryo cultures have also shown that acetaldehyde produces growth retardation and malformations (Popov et al., 1982; Campbell and Fantel, 1983). The lowest effective dose at which effects were seen was 25 μM (Campbell and Fantel, 1983). In contrast to these findings, Priscott (1985) failed to find any acetaldehyde-embryotoxicity at doses of up to 260 μM. He suggested that the differences between his findings and those of Campbell and Fantel (1983) may be the result of employing different strains of rats.

The majority of embryo culture data does implicate acetaldehyde as a toxicant in these systems. However, this conclusion must be viewed with some caution. In the only in vivo study in which acetaldehyde was administered and embryo concentration determined, no embryotoxicity was noted at concentrations

(175 μM) that well exceeded those reported to be effective *in vitro* (Blakley and Scott, 1984a, b). The Blakley and Scott data (1984a) show that acetaldehyde concentrations, *in vivo*, peak by 5 minutes and then rapidly disappear. Thus, the dynamic detoxifying processes *in vivo* may more efficiently remove this readily oxidized agent, irrespective of initial concentration. This issue needs to be clarified before a fuller understanding of the *in vitro* data can be obtained.

The applicability of these data to human risk assessment is unclear. Questions remain as to differences that apparently exist between the rat and mouse and the relevance of data that have been derived from studies employing intraperitoneal or intravenous routes of administration.

While all of the rat data are positive, the mouse data are equivocal. The reason for this species difference is not readily apparent. In the negative mouse studies, animals were injected with acetaldehyde on a single day of organogenesis, whereas the positive mouse studies included multiple days of injection (O'Shea and Kaufman, 1979, 1981). The rapid clearance of acetaldehyde by the mouse fetus (Blakley and Scott, 1984b) may necessitate more prolonged exposure to produce developmental effects. In the rat studies, positive effects were seen with both single and multiple days of treatment. However, in at least one case, dose-response effects were seen only with more prolonged exposure (Padmanabhan et al., 1983). Clarification of species differences in pharmacokinetics of acetaldehyde is essential to resolving this conflict. Special attention should be paid to the maternal-fetal unit and the placenta in such investigations.

Studies that employ intraperitoneal injections of acetaldehyde provide the opportunity for local uptake of the agent at concentrations that may well exceed those attained and maintained with occupational or environmental routes

of exposure. Moreover, given the ubiquitous nature of ALDH (including placenta and fetus), it is quite likely that acetaldehyde would be rapidly eliminated following such exposures. The extrapolation of risk to the developing human cannot be based upon the current data. However, the data are suggestive enough to support the conduct of appropriate studies to ascertain the developmental toxicity of acetaldehyde before final risk estimations are derived.

8.2. MALE REPRODUCTIVE EFFECTS OF ACETALDEHYDE

There are no published whole-animal studies in which the direct effects of acetaldehyde on the male reproductive system are described. Rather, inferences have been drawn from studies in which ethanol was the agent under investigation. Often, drugs which alter acetaldehyde or ethanol metabolism have been included in such studies. These drugs themselves are not without male reproductive effects. As was true for the teratology studies, the interest has been in determining the primary toxicant associated with reproductive impairment seen in males who consume alcohol. Ethanol has been demonstrated to interfere with androgen synthesis and regulation (pituitary/hypothalamic and testicular levels), produce testicular atrophy, reduce fertility, and impair sexual behavior. If acetaldehyde was the primary toxicant, then these effects would be associated with acetaldehyde exposure.

The pharmacokinetics of testicular acetaldehyde as a result of ethanol administration may bear little resemblance to the levels that might be obtained following direct acetaldehyde treatment. Acetaldehyde is rapidly metabolized by aldehyde dehydrogenase (ALDH), which is present in most organs, including the testes. In the testes, ALDH is ubiquitous, and is evenly distributed between the germinal and Leydig cell compartments. It is primarily present in the cytosolic fraction and to a lesser extent in the mitochonodrial fraction (Anderson et al., 1985). ALDH is also found in the epididymis and accessory

organs (Messiha, 1980, 1981, 1983). In light of this marked metabolic capacity, it is problematic as to whether acetaldehyde would attain and be maintained at toxic levels in the reproductive organs following its direct administration. Therefore, data on acetaldehyde derived from studies on ethanol are, for the most part, inappropriate to draw definitive conclusions regarding acetaldehyde-mediated reproductive toxicity.

The primary support for acetaldehyde-induced reproductive dysfunction is derived from in vitro studies examining the influence of acetaldehyde on androgen production. These investigations have employed a number of models, including the perfused testes (Cobb et al., 1978, 1980; Boyden et al., 1981), testicular homogenates (Badr et al., 1977), dispersed testicular cell cultures (e.g., Cicero et al., 1980a, b), isolated Leydig cells (Santucci et al., 1983), and testicular microsomal fractions (Johnson et al., 1981). The majority of these studies have demonstrated that acetaldehyde significantly depresses HCG-stimulated testosterone production; however, the exact mechanism is unknown. This effect has been reported in a number of species, including mice (Badr et al., 1977), rats (Cicero and Bell, 1980; Cicero et al., 1980a, b), and dogs (Boyden et al., 1981). Moreover, this depression occurs at levels of acetaldehyde that can be obtained from blood following ethanol consumption.

Only one study has examined the reproductive effects of acetaldehyde aside from endocrine influences. Anderson et al. (1982) assessed the effects of acetaldehyde on sperm capacitation. These authors demonstrated that acetaldehyde did not alter the in vitro fertilizing capacity of mouse spermatozoa. Although these data suggest that acetaldehyde is not directly toxic to mature sperm, the relevance of this culture system to in vivo fertilization is unclear. Furthermore, these negative results do not eliminate a role for acetaldehyde in the testes or epididymis during other stages of sperm development.

In summary, definitive conclusions cannot be drawn at this time as to the potential male reproductive toxicity that might result from direct acetaldehyde exposure. However, the *in vitro* data strongly suggest the possibility for such toxicity and support the need for such data to be generated in *in vivo* systems.

8.3. REFERENCES

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