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TOXICOLOGICAL REVIEW

OF

ACROLEIN

(CAS No. 107-02-8)

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

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Washington, DC

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CONTENTS – TOXICOLOGICAL REVIEW OF ACROLEIN (CAS No. 107-02-8)

FOREWORD v

AUTHORS, CONTRIBUTORS, AND REVIEWERS vi

1. INTRODUCTION 1

2. CHEMICAL AND PHYSICAL INFORMATION
RELEVANT TO ASSESSMENTS 2

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS 5
3.1. ABSORPTION AND DISTRIBUTION 5
3.2. METABOLISM AND EXCRETION 6
3.3. PHYSIOLOGICALLY-BASED TOXICOKINETIC MODELS 7

4. HAZARD IDENTIFICATION 9
4.1. STUDIES IN HUMANS--EPIDEMIOLOGY, CASE REPORTS, CLINICAL
CONTROLS 9
4.1.1. Acute Exposures (<24 hours) 9
4.1.2. Exposures (> 24 Hours 11
4.2. ACUTE STUDIES IN ANIMALS—ORAL AND INHALATION 11
4.2.1. Lethality Studies 11
4.2.2. Sensory Irritation 13
4.2.3. Other Effects 16
4.3. PRECHRONIC AND CHRONIC STUDIES AND CANCER BIOASSAYS IN
ANIMALS--ORAL AND INHALATION 17
4.3.1. Noncancer Toxicity 17
4.3.1.1. Inhalation Studies 17
4.3.1.2. Oral Administration 29
4.3.1.3. Dermal Administration 32
4.3.2. Cancer Assessment 32
4.3.2.1. Inhalation Exposure 32
4.3.2.2. Oral Administration 34
4.3.2.3. Injection Studies 34
4.3.2.4. Initiation and Promotion Studies 34
4.4. REPRODUCTIVE/DEVELOPMENTAL STUDIES-ORAL
AND INHALATION 35
4.5. OTHER STUDIES 38
4.5.1. *In Vitro* Toxicity 38
4.5.2. Intraperitoneal/Intragastric/Intravenous Toxicity 41
4.5.3. Genotoxicity 42
4.5.3.1. DNA Adduct Formation, Sister Chromatid Exchange
and DNA-Protein Cross-links 42

4.5.3.2. Mutagenic Effects of Acrolein in *Drosophila melanogaster* . . . 43
4.5.3.3. Tests for Gene Mutation in Mammalian Cell Cultures 47
4.5.3.4. Tests for Gene Mutation in Bacterial Cells 48
4.5.4. Mechanistic Studies 50
4.6. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION---ORAL AND INHALATION 53
4.6.1. Oral Administration 53
4.6.2. Inhalation Exposure 55
4.7. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER 57
4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES 60
4.8.1. Possible Childhood Susceptibility 60
4.8.2. Possible Gender Differences 60
4.8.3. Other 61

5. DOSE-RESPONSE ASSESSMENTS 62
5.1. ORAL REFERENCE DOSE (RfD) 62
5.1.1. Choice of Principal Study and Critical Effect--with Rationale and Justification 62
5.1.2. Methods of Analysis 64
5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs) . . 64
5.1.4. Previous Oral Assessment 64
5.2. INHALATION REFERENCE CONCENTRATION (RfC) 65
5.2.1. Choice of Principal Study and Critical Effect 65
5.2.2. Methods of Analysis 67
5.2.3. RfC Derivation 67
5.2.4. Previous Inhalation Assessment 69
5.3. CANCER ASSESSMENT 69

6. MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE 69
6.1. HUMAN HAZARD POTENTIAL 69
6.2. DOSE RESPONSE 71

7. REFERENCES 73

APPENDIX A. Summary of External Peer Review Comments and Disposition 94

FOREWORD

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

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

For other general information about this assessment or other questions relating to IRIS, the reader is referred to EPA's IRIS Hotline at 301-345-2870.

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Summaries of the external peer reviewers' comments and the disposition of their recommendations are in Appendix A.

1. INTRODUCTION

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

The RfD and RfC provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious noncancer effects during a lifetime. The inhalation RfC is analogous to the oral RfD, but provides a continuous inhalation exposure estimate. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg-day. The *unit risk* is the quantitative estimate in terms of either risk per µg/L drinking water or risk per $\mu g/m^3$ air breathed. Another form in which risk is presented in a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for acrolein has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986a), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986b), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996), *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a), *Draft Revised Guidelines for Carcinogen Assessment* (U.S. EPA, 1999), *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988), (proposed) *Interim Policy for Particle Size and Limit Concentration Issues in Inhalation Toxicity* (U.S. EPA, 1994a), *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b), *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995), *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998b, 2000a), *Science Policy Council Handbook*: *Risk Characterization* (U.S. EPA, 2000b), *Benchmark Dose Technical Guidance Document* (U.S.

EPA, 2000c) and *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures* (U.S. EPA 2000d).

The literature search strategy employed for this compound was based on the CASRN and at least one common name. At a minimum, the following data bases were searched: RTECS, HSDB, TSCATS, CCRIS, GENE-TOX, DART/ETIC, EMIC, TOXLINE, CANCERLIT, and MEDLINE. Any pertinent scientific information submitted by the public to the IRIS Submission Desk was also considered in the development of this document.

2. CHEMICAL AND PHYSICAL INFORMATION RELEVANT TO ASSESSMENTS

Acrolein is also known as acrylaldehyde, acrylic aldehyde, allyl aldehyde, ethylene aldehyde, 2-propenal, and prop-2-en-1-al (Izard and Libermann, 1978). Trade names include aqualin, aqualine, biocide, magnacide, magnacide B, and Slimicide (Ghilarducci and Tjeerdema, 1995). Some relevant physical and chemical properties are listed below (HSDB, 2003; unless otherwise referenced).

CASRN: 107-02-8
Empirical formula: C_3H_4O
Structure: C=C-C=O
Molecular weight: 56.06 g/mol
Vapor pressure: 274 mm Hg @ 25°C
Vapor density: 1.94 (Air = 1)
Specific gravity: 0.8389 @ 20°C
Boiling point: 52.5°C at 760 mm Hg
Melting point: -88°C
Water solubility: 208 g/L @ 20°C
Log K_{ow} (octanol / water partition coefficient): -0.01 (high water solubility)
Log K_{oc} (organic carbon / water partition coefficient): 0.5 (low adsorption to soil)
pH: 6.0 (max); a 10% solution in water @ 25°C
Eye irritation: in the range of 0.09 ppm for five minutes (Weber-Tschopp et al., 1977)
Odor threshold: 0.160 ppm (Amoore and Hautala, 1983)
Conversion factor: 1 ppm = 2.3 mg/m^3; 1 mg/m^3 = 0.44 ppm

At room temperature acrolein is a colorless to yellowish flammable liquid with a disagreeable, choking odor. It is extremely acrid and is irritating to mucous membranes (ACGIH, 1991). Reported values for the odor thresholds include 0.21 ppm (0.5 mg/m^3) (Leonardos et al., 1969) and 0.16 ppm (0.4 mg/m^3) (Amoore and Hautala, 1983).

The principal use of acrolein is as an intermediate in the synthesis of acrylic acid, which is used to make acrylates, and of DL-methionine, an essential amino acid used as an animal feed supplement. Other derivatives of acrolein are glutaraldehyde, pyridines,

tetrahydrobenzaldehyde, allyl alcohol and glycerol, 1,4-butanediol and 1,4-butenediol, 1,3-propanediol, DL-glyceraldehyde, flavors and fragrances, polyurethane and polyester resins. Acrolein is unstable and polymerizes (especially under light or in the presence of alkali or strong acid) to form diacryl, a plastic solid (Merck Index, 1966).

The most important direct use of acrolein is as a biocide. As an herbicide, acrolein is used to control algae, aquatic weeds and mollusks in recirculating process water systems. Acrolein also controls the growth of microorganisms in liquid fuel, the growth of algae in oil fields, the formation of slime in paper manufacture. It is used to promote cross-linking of protein collagen in leather tanning, and as a tissue fixative for histological samples (IARC, 1995).

Due to its high vapor pressure and water solubility, acrolein is expected to be highly mobile when released into the environment, although degradation processes are likely to limit its transport. Acrolein is released to the environment through manufacturing processes and its use as an intermediate for glycerine, methionine, glutaraldehyde and other organic chemicals. It is also released into the environment through exhaust gas from combustion processes, including tobacco smoke, emissions from forest fires, and auto exhaust. Acrolein has also been detected in sugar cane molasses, souring salted pork, the fish odor of cooked horse mackerel, the volatiles in white bread, the volatile components of chicken breast muscle, the aroma volatiles of ripe arctic bramble berries and the products from heating animal fats and vegetable oils (HSDB, 2003).

If released to air, a vapor pressure of 274 mm Hg at 25°C indicates acrolein will exist solely in the vapor-phase in the ambient atmosphere. Vapor-phase acrolein will be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals, ozone, and nitrate radicals; the half-lives for these reactions in air are estimated to be 20 hours, 15 days, and 28 days, respectively. Acrolein in hexane solvent showed moderate absorption of UV light >290 nm, which indicated potential for photolytic transformation under environmental conditions (HSDB, 2003). Other reports for half-life are on the order of 4 to 20 hours with removal from the atmosphere primarily by reaction with hydroxyl radicals (Grosjean, 1990; Atkinson, 1985).

If released to soil, acrolein is expected to have very high mobility based upon an estimated Koc of 3 (log Koc = 0.5). Volatilization from moist soil surfaces is expected to be an important fate process based upon a Henry's Law constant of 1.22E-4 atm-m^3/mole. Acrolein may volatilize from dry soil surfaces based upon its vapor pressure (HSDB, 2003).

If released into water, acrolein is not expected to adsorb to suspended solids and sediment based upon the estimated Koc (HSDB, 2003). In deionized water at a concentration of 0.5 mg/ml, there was no decomposition of acrolein at 4 and 24 hours, but at 6 mg/ml, losses were reported of 0.5% by 4 hours and 3.9% by 24 hours (Parent et al., 1993). Lijinsky and Reuber (1987) measured loss of acrolein at 18% after 6 days at a temperature of 5°C, and 27% after 3 days at 22°C.

The half-life of acrolein in natural unsterilized water was 29 hours compared with 43 hours in sterilized (thymol-treated) water. Volatilization from water surfaces is expected to be

an important fate process based upon the compound's Henry's Law constant. Estimated volatilization half-lives for a model river and model lake are 4.4 hours and 4.6 days, respectively. An estimated bioconcentration factor (BCF) of 3 suggests the potential for bioconcentration in aquatic organisms is low (HSDB, 2003).

Occupational exposure to acrolein may occur through inhalation and dermal contact with this compound at workplaces where acrolein is produced or used. The half-life of acrolein in drinking water suggests some potential for water to be a source of exposure to humans. Howard et al. (1991) estimated groundwater half-lives of 11 days under aerobic conditions and 14-56 days under anaerobic conditions. However, limited studies indicate that it has rarely been detected in drinking or well water (Glaze et al., 1989; Staples et al., 1985), and the short half-lives of acrolein in surface waters make long range aquatic transport unlikely (CICAD, 2002).

Exposure of the general population occurs primarily through atmospheric contact (HSDB, 2003). EPA reported mean ambient acrolein concentrations of 14.3 mg/m^3 (6.2 ppb), ranging from 8.2 to 24.6 mg/m^3 (3.6 to 10.7 ppb), for two urban locations based upon data from 1961 to 1980 (U.S. EPA, 1993). Acrolein has been detected in exhaust gases from both gasoline engines (0.05-27.7 mg/m^3) and diesel engines (0.12-0.21 mg/m^3) (IARC, 1995).

Concentrations in indoor air may exceed outdoor levels 2- to 20-fold times (Environment Canada, 2000). Levels between 2.3 and 275 mg/m^3 have been reported in smoky indoor environments such as bars and restaurants (IARC, 1995). In residences where wood stoves were used, concentrations from 0.7-6.0 mg/m^3 have been reported (IARC, 1995). IARC (1995) noted that the acrolein concentrations in the smoke from various cigarettes ranged from 3-220 mg/cigarette. Levels as high as 463-684 mg/cigarette were reported in Japan (Kuwata et al., 1979). Jones et al. (1999) reported concentrations of acrolein in mainstream smoke (defined as smoke that is directly exhaled from the smoker) ranging from 10 – 140 mg per cigarette, and estimated concentrations in sidestream smoke (i.e., smoke emitted from the smoldering tobacco between puffs) in the range of 100 – 1700 mg per cigarette.

EPA's Toxic Release Inventory (TRI) lists the release of acrolein at on-site and off-site facilities for all industries in the US in calendar year 2000 as follows: Total Air Emissions - 208,108 lbs; Surface Water Discharges - 643 lbs; Underground Injection - 201,020 lbs; Releases to Land - 404 lbs; Total On-site Releases - 410,175 lbs; Total Off-site Releases - 410 lbs; Total On- and Off-site Releases - 410,585 lbs (TRI, 2003).

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

3.1. ABSORPTION AND DISTRIBUTION

Respiratory uptake studies with acrolein in dogs indicate that acrolein is retained at rates of 75-80% in the upper respiratory tract (URT) with a lesser rate of retention (65-70%) for the lower respiratory tract. At inhaled concentrations of 176-264 ppm (400-600 mg/m^3), 80-85% was retained in the total respiratory track at varying ventilation rates, suggesting little distribution elsewhere (Egle, 1972). Acrolein's strong reactivity with tissues is proposed to result in little systemic distribution (Beauchamp et al., 1985). This hypothesis is supported by the results from McNulty et al. (1984) who observed no reduction in liver glutathione (GSH) following inhalation of acrolein by rats, indicating that inhaled acrolein does not reach the liver to any great extent.

Deposition efficiency of inhaled acrolein (nominal concentrations of 0, 0.9, 4.5 and 9.1 ppm or 0, 2.1, 10.4, and 20.9 mg/m^3) in the upper respiratory tract of the anesthetized male F344 rat was examined by Morris (1996). During nose-only exposures of the surgically-isolated URT for 40 minutes, steady-state concentrations were not attained or maintained during the exposure, and uptake slowly decreased, suggesting limited uptake at these concentrations and durations.

Evidence for systemic absorption of acrolein from the gastrointestinal tract was reported by Draminski et al. (1983), who identified low levels of acrolein-derived conjugates in the urine of rats after ingestion of a single dose of 10 mg/kg body weight. This dose, however, resulted in 50% mortality and would be expected to cause severe gastrointestinal damage under these conditions. Damage to the stomach lining, especially endothelial cells (Patel and Block, 1993), may allow some absorption to occur. The likelihood of significant absorption from the gastrointestinal tract at lower concentrations is uncertain.

The distribution of [2,3-14C]acrolein administered to Sprague-Dawley rats (5/sex/group) after intravenous (iv) or oral gavage was evaluated by Parent et al. (1996a, 1998). Doses were 2.5 mg/kg (iv and oral), 2.5 mg/kg after 14 consecutive days of unlabeled acrolein (oral), and 15 mg/kg (oral). Radiolabel in expired air, urine, and feces was measured at 4, 12, and 16 and 24 hours post-dosing, then every 24 hours for the next 6 days. Data in the report demonstrate that the large majority of label (>96%) was recovered in excreta within the first 24 hours. Tissue concentrations (including blood) of radioactivity were minimal (<1.2% from the iv dosing and <0.7% from the oral dosing) and time course of excretion for all groups was similar except for delayed excretion in the high-dose group. Radiolabel measured in excreta and in tissues was associated with various acrolein metabolites and not attributed to parent compound. The radiolabel in feces was later determined to be associated with a homopolymer of acrolein, which was apparently formed in the gastrointestinal tract (Parent et al., 1998). These studies indicate little systemic distribution of acrolein.

The high reactivity of acrolein is due to the polarization of the double bond by the aldehyde group, and the resulting increased potential for nucleophilic addition. Because acrolein

readily reacts with sulfhydryl and amino groups on proteins, it is unlikely to distribute systemically, and thus its adverse effects are characterized in terms of cytotoxicity at the site of entry. Additional evidence of the reactivity of acrolein can be seen in conflicting data reported in the literature for the *in vitro* mutagenic potential of acrolein. In a series of Ames assays, Parent et al. (1996b) resolve many of the different outcomes by considering the presence or absence of non-DNA nucleophiles from the S9 activation mixture, in the test chemical solution, or in the plating solutions. If non-DNA nucleophiles were present, acrolein would rapidly and indiscriminately react with any available species and not reach the DNA target.

While the possibility of some transport of acrolein or a metabolite of acrolein to systemic sites remains, the critical target sites, as noted in the toxicology section, are those at the point of contact, the respiratory system, the gastrointestinal tract, mucous membranes, and skin.

3.2. METABOLISM AND EXCRETION

Absorbed acrolein reacts directly with protein and non-protein sulfhydryl groups, and with primary and secondary amines found in proteins and nucleic acids (Ghilarducci and Tjeerdema, 1995). In proteins, it preferentially attacks free SH groups of cysteine residues, ϵ-amino groups of lysine residues and histidine residues (Esterbauer et al., 1991). Uchida et al. (1998a,b) has shown that, *in vitro*, acrolein binds to serum albumin and low-density lipoproteins. Acrolein's role as a lipid peroxidation byproduct and possible mediator in various human diseases has been recently reviewed by Uchida (1999). It is well-documented that the conjugation of the β-carbon of acrolein with sulfhydryl groups is rapid and essentially irreversible (Esterbauer et al., 1976), and leads to thiazolidine derivatives and a decrease in glutathione (GSH) stores without an increase in oxidized GSH (GSSG). This pathway results in an acrolein-GSH adduct which is then further metabolized by both high- and low-affinity forms of mitochondrial and cytosolic aldehyde and alcohol dehydrogenase (Mitchell and Peterson, 1989); one resultant product has been identified as 3-hydroxypropylmercapturic acid (Clapp et al., 1969; Kaye and Young, 1970). This product has been isolated from urine of rats after subcutaneous injection of acrolein (Kaye, 1973) and after inhalation and intraperitoneal (ip) injection of Wistar rats (Linhart et al., 1996). The reduction of the acrolein-GSH adduct by alcohol dehydrogenase to 3-hydroxypropylmercapturic acid was postulated as a potentially important pathway (Mitchell and Peterson, 1989). There is increasing evidence that aldehydes such as acrolein are generated endogenously during the process of lipid peroxidation (Esterbauer et al., 1991); the rate constant for reaction of acrolein with cysteine at pH 7.4 was 220 M^{-1} sec^{-1} compared to 121 with GSH. Among all α, β-unsaturated aldehydes, acrolein is the strongest electrophile, which accounts for its high reactivity with nucleophiles (Witz, 1989). Thiol adducts of acrolein are considerably more stable than adducts formed by all other α, β-unsaturated aldehydes (Esterbauer et al., 1991).

When radiolabeled acrolein was administered by gavage (0.82 mg/kg) to one lactating goat, incorporation of radioactivity appeared to follow incorporation of metabolites into normal biosynthetic pathways (Sharp et al., 2001).

Elucidation of the major pathways of metabolism has been greatly enhanced by the

studies of Parent and colleagues. Parent et al. (1998) synthesized and characterized the potential metabolites of acrolein in the feces and urine of rats administered acrolein either orally or intravenously. The pathways of metabolism proposed by Parent et al. (1998) are illustrated in Figure 1. The main pathway appears to be an addition of GSH to the activated double bond, followed by processing to mercapturic acid derivatives, the three compounds at the bottom of the figure, which are then excreted in the urine after either oxidation or reduction of the aldehyde, with reduction predominating. Another pathway of metabolism is that of epoxidation of the double bond followed by attack of GSH on the epoxide. A third pathway involves addition of water to acrolein to form 3-hydroxypropionaldehyde, which is further oxidized to malonic acid and ultimately oxalic acid. Some of these compounds can be incorporated into normal metabolic pathways. For example, glycidaldehyde can be hydrated to glyceraldehyde (Patel et al., 1980).

None of the unconjugated metabolites resulting from the epoxidation of acrolein, such as those reported by Patel et al. (1980), were found in the excreta by Parent et al. (1998). A polar and a nonpolar fraction were extracted with a molecular weight range of 2,000-20,000 Da (Parent et al., 1998). They concluded that these compounds were either homopolymers of acrolein, or that the polyacrolein in this fraction was originally a copolymer with a natural polymer, either a protein or polysaccharide.

Marinello et al. (1984) incubated [^{14}C]acrolein with purified cytochrome P450 in the absence of NADPH and observed the binding of label. GSH inhibited the binding of label to hepatic microsomes by 90%. Binding to microsomes was substantially enhanced in the presence of NADPH. Addition of the P450 inhibitor, SKF-525A, in the presence of NADPH prevented binding of label.

Incubation of Wistar liver microsomes with 5 mM acrolein for 30 seconds resulted in a two-fold stimulation of GSH transferase and 0.1 mM for 30 minutes reduced GSH protection against lipid peroxidation (Haenen et al., 1988).

3.3. PHYSIOLOGICALLY-BASED TOXICOKINETIC MODELS

No physiologically-based toxicokinetic models are available for acrolein.

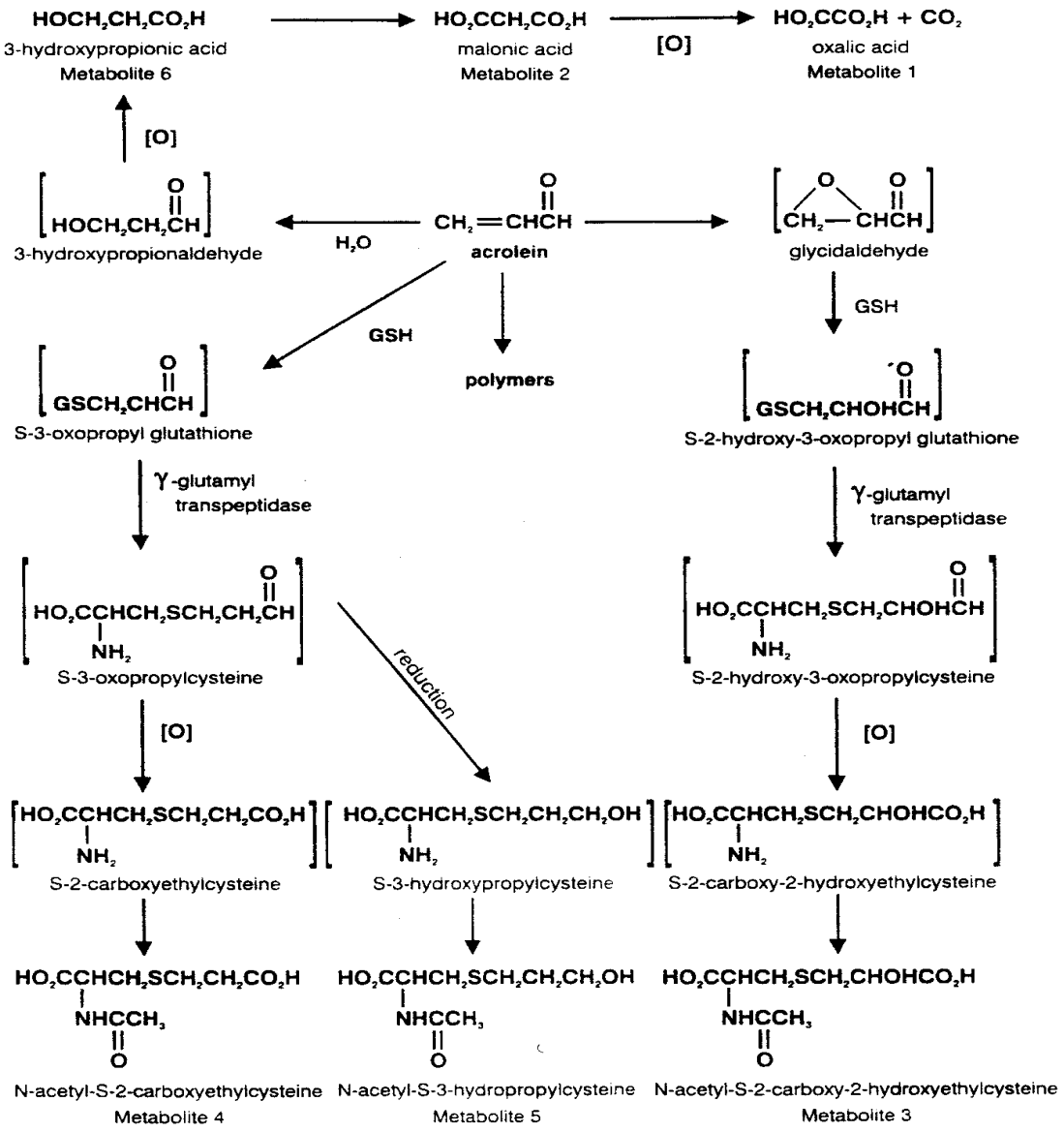

Figure 1. Proposed metabolism of acrolein in rats. The structures in brackets represent postulated intermediates.

Source: Reprinted from Toxicol Sci (1998) by Parent et al., with permission of the Society of Toxicology.

4. HAZARD IDENTIFICATION

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

EPA has an interim policy on the use of third-party studies submitted by regulated entities (U.S. EPA, 2001). For these purposes, EPA is considering "third party studies" as studies that have not been conducted or funded by a federal agency pursuant to regulations that protect human subjects. Under the interim policy, the Agency will not consider or rely on any such human studies (third-party studies involving deliberate exposure of human subjects when used to identify or quantify toxic endpoints such as those submitted to establish a NOAEL or NOEL for systemic toxicity) in its regulatory decision making, whether previously or newly submitted. Some of the supporting studies discussed in this Toxicological Review are third-party studies; however, the scientific and technical strengths and weaknesses of these studies were described before this Agency policy was articulated. In addition, the studies cited provide data that suggest and inform a public health concern for acrolein, but were not designed or used as principal studies in the derivation of any quantitative value for acrolein based on NOAELs or LOAELs. The Agency is requesting that the National Academy of Sciences conduct an expeditious review of the complex scientific and ethical issues posed by EPA's possible use of third-party studies that intentionally dose human subjects with toxicants to identify or quantify their effects.

4.1.1. Acute Exposures (<24 hours)

A clinical study by Weber-Tschopp et al. (1977) provides the most comprehensive description of acute effects in humans. Three experiments were performed using male and female student volunteers: (1) a continuous exposure at constantly increasing acrolein concentrations, (2) short exposures to successively increasing concentrations, and (3) a 1-hour exposure to a constant concentration.

In experiment (1), 31 males and 22 females were exposed to acrolein for 40 minutes during which the acrolein concentration was gradually increased to 0.6 ppm (1.4 mg/m^3) during the first 35 minutes, then remained constant. The standard deviation in the acrolein concentration used was 0.023 ppm (3.8%). Groups of unexposed students were used as controls. The subjects had to fill out a questionnaire for the first 5 minutes. After that, the eye blinking frequency of two subjects was measured as well as the breathing frequency of a third subject during the entire exposure. The incidence (not stated) of complaints about eye irritation after 35 minutes of exposure was significantly higher ($p<0.01$) than controls beginning at 0.09 ppm (0.21 mg/m^3) and was increasing even at 0.6 ppm (1.4 mg/m^3). Nasal irritation was significantly higher ($p<0.01$) than controls beginning at 0.26 ppm (0.6 mg/m^3) and was increasing even at 0.6 ppm (1.4 mg/m^3). Throat irritation increased significantly through 0.43 ppm (1 mg/m^3). The eye blink frequency increased significantly beginning at 0.26 ppm (0.6 mg/m^3) ($p<0.01$).

In experiment (2), 17 males and 25 females were exposed, in groups of five, for 1 ½ minutes to successive concentrations of 0, 0.15, 0.3, 0.45, and 0.6 ppm (0, 0.3, 0.7, 1.0, and 1.4 mg/m^3). After one minute of exposure they were administered a questionnaire. Between each exposure they were allowed to recuperate in a clean room for 8 minutes. As in the first experiment, eye blink frequency and respiration rate were measured. The same controls as for the first experiment were used. Eye and nasal irritation was significantly higher ($p<0.05$) than controls beginning at 0.3 and 0.6 ppm (0.7 and 1.4 mg/m^3), respectively. Throat irritation was not evident.

In experiment (3), 21 males and 25 females were distributed into three groups and exposed for 60 minutes to a constant acrolein concentration of 0.3 ppm (0.7 mg/m^3). As in the other two experiments, eye blink frequency and respiration rate were measured. In controls, measurements of eye blink and breathing frequency, and subjective symptoms of irritation were assessed at the beginning of exposure. Each of the effects increased significantly ($p<0.01$) during the first 20-30 minutes of exposure compared to controls, after which the irritation effects reached a plateau. Eye blink frequency reached a steady rate after 10 minutes of exposure. During exposure there was a decrease in the average respiration rate (16 individuals) after 40 minutes ($p<0.01$). Each individual that demonstrated an increase in eye blink frequency also reported a sharp increase in eye irritation. Throat irritation, not a significant response in the previous two experiments, was increased compared to controls after 10 minutes of exposure.

It was concluded by the investigators that the average threshold of sensation lies in the range of 0.09 (eye irritation) to 0.30 ppm (respiration rate, throat irritation) with nasal irritation at 0.15 ppm (0.35 mg/m^3). No adaptation to these effects was observed.

According to the review by Esterbauer et al. (1991), a level of 5.5 ppm (12.6 mg/m^3) resulted in painful eye and nose irritation after 20 seconds, and 22 ppm (51 mg/m^3) was immediately intolerable. In one case report, exposure to 153 ppm (352 mg/m^3) for 10 minutes was fatal.

Sim and Pattle (1957) exposed volunteers (12 males/group) to 0.8 and 1.2 ppm (1.88 and 2.80 mg/m$^{3)}$ acrolein for 10 and 5 minutes, respectively. Acrolein concentration was determined via reaction with hydroxylamine hydrochloride, followed by back titration to pH 4.5. Volunteers were exposed simultaneously in 100 m^3 exposure chambers with no restrictions on movement or smoking within the chamber. The vapor was described by the volunteers as "extremely irritating" to all exposed mucosal surfaces, with lacrimation occurring within 20 and 5 seconds in the low and high exposures, respectively. Ten minutes of low-dose exposure was described as "only just tolerable," and high-dose exposure for more than 5 minutes "would have been extremely distressing." The comments by the volunteers were subjective, and it does not appear that any other endpoints were monitored. The effects of exposure to acrolein were considerably more apparent than exposure to much higher concentrations of several other aldehydes.

In one of two additional case reports, a 27-month-old boy was exposed to probable high levels of acrolein (and other chemicals) from burning vegetable oil for one hour (Mahut et al., 1993). No exposure measurements were reported. Initial acute respiratory failure regressed in a

few hours, but in the months following exposure diffuse bronchiectasis developed. In the second case report, a chemical worker was exposed to a sudden release of acrolein from a rupture in the workplace. The principal effect was chemical pneumonia and eye irritation, both of which resolved with treatment (Champeix et al., 1966).

In summary, based upon the available human data, levels as low as 0.09 ppm (0.02 mg/m^3) for 5 minutes may elicit subjective complaints of eye irritation with increasing concentrations leading to more extensive eye, nose and respiratory symptoms.

4.1.2. Exposures (> 24 Hours)

No chronic studies of humans exposed to acrolein are available.

The only study relating to cancer was a nested case control study by Ott et al. (1989), in which individuals were classified as having been exposed to one of a large number of chemicals in the work environment. The study investigators reported non-Hodgkin's lymphoma (52 cases), multiple myeloma (20 cases), nonlymphocytic leukemia (39 cases), and lymphocytic leukemia (18 cases) within a cohort of employed men from two chemical manufacturing facilities and a research and development center. Exposure odds ratios were examined in relation to 111 work areas, 21 specific chemicals, and 52 chemical activity groups. Odds ratios of 2.6 (2 cases) for non-Hodgkin's lymphoma, 1.7 (1 case) for multiple myeloma, and 2.6 (3 cases) for nonlymphocytic leukemia were reported for workers exposed to acrolein. None of the lower 95% confidence limits exceeded 1.0. Because of a lack of a statistically significant increase in the cancer endpoints and the likelihood of confounding by concomitant exposure to other chemicals in the workplace, the results must be considered equivocal.

4.2. ACUTE STUDIES IN ANIMALS—ORAL AND INHALATION

4.2.1. Lethality Studies

Ballantyne et al. (1989) examined the effects of 1- and 4-hour exposures to acrolein in male and female Sprague-Dawley rats (5/sex/exposure). Animals were exposed to 14, 22, 24, 31, and 81 ppm (32, 50, 55, 71, and 186 mg/m^3)[1] acrolein for 1 hour or 4.8, 7.0, 9.1, and 12.1 ppm (11, 16, 20.8, and 27.7 mg/m^3) for 4 hours. One- and 4-hour LC_{50} values of 65 and 25.8 mg/kg, respectively, were calculated for the combined sexes. Clinical signs of sensory irritation and toxicities were observed at all exposure concentrations. Lachrymation, perinasal and periocular wetness and encrustation, mouth and audible breathing, decreased breathing rate, and hypoactivity were observed during exposure in all animals. Signs of respiratory distress and hypoactivity were observed for post-exposure days 1-6. Body weights of survivors decreased during the first post-exposure week but the weight was regained during the second week.

A necropsy of animals that died during the post-exposure period revealed perinasal and

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[1]Conversion to mg/m^3: 1 ppm = 2.3 mg/m^3

perioral encrustation, mottled discoloration of the lungs and liver, clear fluid in the trachea and thoracic cavity, gas-filled stomach and intestine, and opaque or cloudy corneas. Histology of the lungs revealed congestion and intra-alveolar hemorrhage, fibrin disposition in the smaller airways, and necrosis and exfoliation of the bronchiolar epithelium. Death was attributed to lung injury. Histopathology was not performed on surviving animals.

In another study examining acute exposure effects of acrolein in rats, Crane et al. (1986) exposed Sprague-Dawley rats to acrolein at concentrations ranging from 580-41,550 ppm (1,330-95,268 mg/m^3) for exposure durations ranging from 2.8 to 36.5 minutes until animals were incapacitated. The time-to-incapacitation endpoint (i.e., when rats could no longer perform a coordinated act of walking in a rotating cage, and exhibiting stumbling, sliding, or tumbling) was recorded for each animal. Exposure was then continued until animals expired and the time of death was reported. After incapacitation, death occurred very quickly (in 1.9-19.7 minutes). Prior to death, animals exhibited clinical signs of respiratory distress, agitation, and convulsions. Ocular effects were not noted.

Mortality and clinical signs have also been reported in other species. Groups of 50 mice, 20 guinea pigs, and 5 rabbits were exposed to 2,279 ppm (5,225 mg/m^3) of acrolein vapor for 13, 25, and 27 minutes, respectively, until the animals died (Salem and Cullumbine, 1960). In addition, the same species were exposed to 2,019 ppm (4,624 mg/m^3) of an acrolein aerosol for 13, 24, and 26 minutes, respectively, until the animals died. Initial exposure to both forms of acrolein caused increased activity which was attributed to compound-related irritation. Respiration then slowed and animals convulsed just prior to death. Of the nine aldehydes tested, acrolein had the highest relative toxicity.

Beeley et al. (1986) examined the effects of acute acrolein exposure in female New Zealand rabbits. Animals (18/group) were exposed to 375 or 489 ppm (860 or 1,121 mg/m^3) for 15 minutes. Animals were sacrificed at 3 days post-exposure, and lung and trachea were removed and examined for histopathological changes. Five animals in the 860 mg/m^3 exposure group and 8 animals in the 1,121 mg/m^3 exposure group died during the 3 day post-exposure period. The surviving animals exhibited edema, necrosis of the lung parenchyma, and damage to the bronchial linings of the large airways. Acute inflammatory reactions were found in conjunction with areas of necrosis.

To assess the potential of acrolein to impair escape, a signal avoidance task was developed in which a baboon's ability to escape from a chamber containing the noxious gas was monitored (Kaplan, 1987). Male juvenile baboons (1/group) were exposed to 12, 25, 95, 100, 250, 505, 1,025, or 2,780 ppm (28, 57, 218, 229, 573, 1,158, 2,350, or 6,374 mg/m^3) for 5 minutes. After exposure, animals were allowed to exit the chamber by depressing a lever. Escape time, i.e., the time it took for the animal to select the correct lever and exit the chamber, was measured. Acrolein exposure did not inhibit escape time. However, irritant effects of the gas were noted at each concentration tested, and the severity of the effects increased with increasing concentration. Irritant effects manifested from blinking and closing of the eyes and rubbing the nose/eyes at lower concentrations to salivation, nasal discharge, violent shaking of the head, and nausea at higher concentrations. However, the exposures at which the more

serious effects occurred were not reported by the study authors. Animals exposed to 2,350 and 6,374 mg/m^3 acrolein expired after 24 and 1.5 hours, respectively. Severe pulmonary edema and hemorrhage were the significant histological changes observed in these two animals.

An acute oral LD_{50} was reported as 29 mg/kg in Sprague-Dawley rats administered acrolein by gavage (Bioassay Systems Corp., 1981c); male rats were somewhat more sensitive with an LD_{50} of 25 mg/kg compared to females with an LD_{50} of 33 mg/kg. In contrast, LD_{50} s of 10.3 (males) and 11.8 mg/kg (females) were reported in another gavage study with acrolein with a stated purity of 97% (Microbiological Assoc., 1989). In male CD-1 mice the LD_{50} was 14 mg/kg (Bioassay Systems Corp., 1981d). In female mice, the LD_{50} was determined to be 18 mg/kg (Bioassay Systems Corp., 1981e). The acute dermal LD_{50} in New Zealand white rabbits was 231 mg/kg with females somewhat more sensitive (223 mg/kg) than males (240 mg/kg) (Bioassay Systems Corp., 1981f).

4.2.2. Sensory Irritation

Alterations in respiratory function have been used as an indicator of sensory irritation. Murphy et al. (1963) exposed male guinea pigs (n=10) to 0.6 ppm (1.4 mg/m^3) acrolein for 2 hours. The study authors reported that expiratory flow resistance and tidal volume increased and respiratory rate decreased. These adverse responses were rapid and reached a maximum within 30 to 60 minutes. In a second experiment, male guinea pigs were exposed to 0.1, 0.2, 0.35, 0.6 or 1 ppm (0.2, 0.5, 0.8, 1.4, or 2.3 mg/m^3) for 2 hours. Respiratory flow resistance during inspiration and expiration was significantly increased and respiratory rates decreased at levels of 0.35 to 1 ppm (0.8 to 2.3 mg/m^3). The study authors also reported that several drugs (atropine, aminophylline, isoproterenol, and epinephrine) partially or completely reversed increased flow resistance. Statistically significant increases in respiratory resistance and tidal volume coupled with decreases in respiration rate and minute volume were observed in guinea pigs exposed to 17 ppm (40 mg/m^3) for 60 minutes (Davis et al., 1967).

One measure of the potency of a sensory irritant is the exposure concentration at which respiratory rate is depressed by 50% (RD_{50}). Table 1 shows RD_{50}s for mice and rats. A comparison of rat and mouse values indicates that mice are more sensitive than rats to sensory irritation. Respiratory rate depression following acrolein exposure recovers rapidly, usually within 10 minutes (Cassee et al., 1996a; Nielsen et al., 1984; Steinhagen and Barrow, 1984). However, the recovery rate decreases as acrolein concentration increases. Cassee et al. (1996a) reported that 24 hours after exposure of Wistar rats to 1.7, 11.1 and 31.9 ppm (3.9, 25.4, and 73 mg/m^3), breathing patterns were comparable to pre-exposure values, indicating that the effect was not persistent. The decrease in breathing frequency was maximal between 1 and 3 minutes of exposure with desensitization occurring only with the two lower concentrations. Kane and Alarie (1977) reported that 4 daily consecutive 3-hour exposures to 0.5 and 1.7 ppm (1.1 and 3.9 mg/m^3) caused further decreases in respiratory rate, which suggests that animals become sensitized to the irritant effect. However, when animals were exposed to 0.17 ppm (0.39 mg/m^3) acrolein 3 hr/day for 3 days and then exposed to 0.44-11.2 ppm (1.0-26.7 mg/m^3) acrolein for 10 minutes, there was a decrease in response compared to controls, i.e., the control RD_{50} was 1.7 ppm (3.9 mg/m^3) compared to 3 ppm (6.8 mg/m^3) in pre-exposed animals.

TABLE 1. RD_{50}s for Rats and Mice

Species	RD_{50}	Reference
F-344 Rats (male)	6.0 ppm (13.7 mg/m^3)	Babiuk et al., 1985
Wistar Rats (male)	4.6 ppm (10.5 mg/m^3)	Bergers et al., 1996
Wistar Rats (male)	9.2 ppm (21.7 mg/m^3)	Cassee et al., 1996b
Swiss Webster Mice (male)	1.7 ppm (3.9 mg/m^3)	Kane and Alarie, 1977
Ssc:CF-1 Mice (male)	2.9 ppm (6.6 mg/m^3)	Nielsen et al., 1984
B6C3F1 Mice (male)	1.41 ppm (3.2 mg/m^3)	Steinhagen and Barrow, 1984
Swiss Webster Mice (male)	1.03 ppm (2.4 mg/m^3)	

Davis et al. (1967) examined the respiratory irritant effect of acrolein in normal and tracheotomized guinea pigs. Groups of normal and tracheotomized guinea pigs were exposed to 17 ppm (39 mg/m^3) acrolein for one hour. Normal animals exhibited clinical signs of sensory irritation, i.e., depressed respiratory rate as described by Murphy et al. (1963). However, tracheotomized animals did not exhibit respiratory rate depression. A similar finding was reported by Kane and Alarie (1977). Davis et al. (1967) theorized that tracheotomized animals lacked receptors for irritant responses that were present in the intact animal.

To further understand the mechanism through which acrolein elicits its irritant effect, Lee et al. (1992) examined the effect of capsaicin treatment of the cervical vagi followed by acrolein exposure in rats. Capsaicin treatment selectively blocked C-fiber afferent nerves and inhibited the respiratory rate depression normally observed during acrolein exposure. In addition, bilateral vagotomy also inhibited the respiratory rate depression. These results are consistent with a mode of action in which acrolein activates C-fiber afferent nerves.

Since acrolein exposure in the workplace is usually concurrent with other chemicals, particularly aldehydes, studies have been undertaken to examine the effects of acrolein exposure with pre-exposure and co-exposure to other chemicals. Babiuk et al. (1985) examined the effects of pre-exposure to 15 ppm (34 mg/m^3) formaldehyde 6 hr/day, for 9 days followed by exposure to acrolein for 10 minutes on the 10th day. The study authors reported that the RD_{50} in pre-exposed animals increased to 29.6 ppm (68.1 mg/m^3) compared to 6 ppm (13.8 mg/m^3) in the controls. This would suggest that pre-exposure to lower concentrations of sensory irritants desensitizes animals to sensory irritation effects of acrolein. However, co-exposure to acrolein with other aldehyde sensory irritants, acetaldehyde and formaldehyde, resulted in a more pronounced decrease in respiratory rate in male Wistar rats than exposure to acrolein only (Cassee et al., 1996a). Groups of four rats were exposed to a mixture of the three at concentrations which were expected to result in a decrease in breathing frequency (DBF) between 10 and 35% for each. The observed DBF for the mixture was more pronounced than the DBF for each chemical separately, but was less than the sum of the DBFs for the single

chemicals. Model prediction indicated that the combined effect was consistent with a competition for a common receptor, i.e., the trigeminal nerve.

The clinical signs and sensory irritation reported in the above mentioned animal studies indicate that the respiratory system is a principal target following acute exposure to acrolein. Further studies provide additional evidence. Kilburn and McKenzie (1978) exposed Syrian golden hamsters to 6 ppm (14 mg/m^3) acrolein for 4 hours, which caused a > 50% exfoliation of ciliated cells in the bronchi. The cells were pale and swollen at 24 and 48 hours post-exposure. The basal lamina was indented or penetrated by proliferating basal cells. After 96 hours there were areas of irregular epithelium with early stratification and hyperplasia. There was no recruitment of polymorphonuclear leukocytes (PMN) to the trachea or intrapulmonary airways; however, acrolein administered absorbed on carbon or simultaneous with carbon was chemotactic for PMN leukocytes. Formaldehyde behaved similarly to acrolein.

Acrolein has been reported to deplete the neuropeptides calcitonin-gene related peptide (CGRP) and substance P in the trachea of rats (Springall et al., 1990). Female Wistar rats exposed to 22, 81 or 249 ppm (51, 186, or 571 mg/m^3) for 10 minutes exhibited a dose-dependent decrease in these two sensory neuropeptides. The study authors suggested that the neuropeptide decrease could be responsible for the observed vasodilation and bronchoconstriction that follows irritant exposure. Roemer et al. (1993) reported that respiratory tract cell proliferation in male Sprague-Dawley rats occurred following an acute 6-hour exposure to 0.2 and 0.6 ppm (0.46 and 1.4 mg/m^3) acrolein.

Bronchial hyperresponsiveness following acrolein exposure has also been reported. Leikauf (1991) and Leikauf et al. (1989a) exposed guinea pigs to 0.31-1.26 ppm (0.71-2.9 mg/m^3) acrolein for 2 hours and determined bronchial responsiveness with an acetylcholine challenge up to 24 hours after exposure. The effective dose of acetylcholine sufficient to double specific resistance (ED_{200}) was decreased at all post-exposure times. The authors interpreted these results as suggestive evidence that asthmatics may be predisposed to an asthmatic attack following acrolein exposure. In addition, thromboxane B_2, the inactive form of the potent vasoconstrictor thromboxane A_2, and prostaglandin $F_{2\alpha}$ were increased immediately after exposure, and neutrophils were increased 24 hours after exposure. In a subsequent study, Leikauf et al. (1989b) reported that acrolein exposure resulted in an increase in leukotriene C4 (LTC4) in bronchoalveolar lavage fluid in guinea pigs. It was also determined that hyperresponsiveness to acetylcholine following acrolein exposure could be abated if guinea pigs were pretreated with 5-lipooxygenase inhibitors and leukotriene receptor antagonists, which suggests that the sulfidopeptide leukotrienes play a causal role in acrolein-induced bronchial hyperresponsiveness.

Acute exposure of Swiss-Webster mice to acrolein (0.3 or 0.6 µg/ml; 300 or 600 mg/m^3) decreased pulmonary compliance, pulmonary resistance, tidal volume and respiratory frequency (Watanabe and Aviado, 1974); pretreatment with a beta-adrenergic blocking agent indicated that lung effects were not mediated through adrenergic receptors. Similarly, chronic exposure (30 minutes, daily, 5 weeks) to a lower concentration reduced pulmonary compliance.

4.2.3. Other Effects

Antibacterial Defenses: Several studies have assessed the effects of acrolein exposure on pulmonary antibacterial defenses. Jakab (1977) exposed Swiss CD-1 mice (18-24 animals/group) to 1-2 ppm (2.3-4.6 mg/m^3) acrolein for 4 or 24 hours following a 0.5-hour bacterial challenge to *Staphylococcus aureus* and *Proteus mirabilis*. After 24 hours of exposure, there was a statistically significant increase in the number of surviving bacteria (both *S. aureus* and *P. mirabilis*) in exposed animals compared to controls. In a second study, Astry and Jakab (1983) exposed female Swiss mice (6/group) to 0.5, 3, 6.2, 7.5, or 9 ppm (1.1, 6.8, 14.2, 17.2, or 20.6 mg/m^3) for 8 hours following a 45-minute bacterial challenge to *S. aureus*. Exposure to 0, 0.5, 3.0, 6.2, 7.6, and 9.1 ppm (0, 1.1, 6.8, 14.2, 17.2, and 20.6 mg/m^3) resulted in survival of 3.2, 5.0, 12.8, 33.9, 35, and 40% of bacteria, respectively. The study authors reported significantly greater percent of surviving bacteria at exposures $\geq$ 3 ppm (6.8 mg/m^3). Exposure to 0.09 ppm (0.21 mg/m^3) acrolein for 3 hours following exposure to *Klebsiella pneumonia* had no effect on percent bacteria killed compared to controls in female CD1 mice (Aranyi et al., 1986). These studies suggest that acrolein exposure can inhibit pulmonary antibacterial defenses.

Cardioinhibitory Effects: Egle and Hudgins (1974) examined possible cardioinhibitory effects of acrolein exposure in male Wistar rats. Animals (6-11/group) were exposed to concentrations of acrolein ranging from 4-2,181 ppm (9.2-5,000 mg/m^3) for 1 minute. Animals were assessed for changes in blood pressure and heart rate. The principal effects observed were significant increases in blood pressure and heart rate with exposure concentration, with statistically significant increases in heart rate occurring at exposures $\geq$ 50 mg/m^3. However, exposure to 1,100 and 2,200 ppm (2,500 and 5,000 mg/m^3) acrolein generally caused a decrease in heart rate. Intravenous studies in Wistar rats with several aldehydes indicated that the relative pressor potency of acrolein was higher than that of formaldehyde, acetaldehyde and proprionaldehyde.

Biochemical Changes: Biochemical changes have also been reported following inhalation exposure to acrolein. Alabert et al. (1971) found significant alterations in NAD/NADH ratios in liver, lung, and brain of rats exposed to high concentrations of acrolein. Murphy (1965) reported that liver alkaline phosphatase and tyrosine transaminase activities were increased 3.1- and 3.6-fold, respectively, in male Holtzman rats exposed to 8 ppm (18.3 mg/m^3) acrolein for 4 hours; a dose-response relationship was also observed upon injection of acrolein. Cassee et al. (1996b) examined changes in the nasal epithelium of male Wistar rats exposed to 0, 0.25, 0.67, or 1.40 ppm (0, 0.45, 1.2, or 2.5 mg/m^3) acrolein by nose only exposure for 6 hours. No effects on cell proliferation or treatment-related lesions were observed for this duration of exposure. Likewise, non-protein sulfhydryl levels were similar to controls. However, exposure to 0.67 or 1.4 ppm (1.2 or 2.5 mg/m^3) acrolein significantly decreased GSH reductase activity in a dose-dependent manner.

Glutathione and P450 Levels: When male rats were given a single i.p. dose of acrolein (89 µmoles/kg) and sacrificed at 30 min, 4 and 24 hours, hepatic GSH was decreased 51% only at the 4-hour period (Witz, 1989). Levels returned to normal at 24 hours. However, cytochrome P450 levels were 61-71% of controls at 24 hours. Walk and Hausmann (1989) found that acute

inhalation exposure of rats to acrolein (0.7 to 4 ppm; 1.6 to 9.2 mg/m^3) resulted in a decrease in the total glutathione (GSH and GSSG) pool of nasal and olfactory epithelia and in the trachea and lungs. These decreases were accompanied by complex changes in GSH enzyme activities. After a 4-hour exposure of rats to acrolein (1 to 15 ppm; 2.3 to 34.5 mg/m^3), a dose-dependent decrease in the total GSH pool was observed in nasal olfactory and respiratory epithelia (Hausmann and Walk, 1989). Activities of GSH peroxidase, GSH reductase, and GSH transferase increased slightly in olfactory epithelium, but decreased in respiratory epithelium as exposures increased.

Eye Irritation: Eye lesions were reported in New Zealand white rabbits when acrolein was placed on the everted lower lids and examined for different time periods up to 7 days post-exposure (Bioassay Systems Corp., 1981a).

Skin Irritation: Acrolein was determined to be a skin irritant after 0.5 ml was place on intact and abraded skin of six male New Zealand white rabbits with erythema and edema scored after 24 and 72 hours (Bioassay Systems Corp., 1981b).

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

4.3.1. Noncancer Toxicity

Acrolein, like other aldehydes, is a known sensory irritant (Lyon et al., 1970; Cassee et al., 1996a,b) producing both nasal and eye irritation. Breathing frequency which is depressed upon initial exposure has been shown in Wistar rats to partially or fully recover during post-exposure. Sensory irritation and depressed breathing frequency are regarded as defense mechanisms for penetration to the lower respiratory tract. Acrolein was the most potent of 15 saturated and unsaturated aldehydes in sensory irritation potential as measured by the reflex decrease in respiratory rate in B6C3F1 and Swiss-Webster mice (Steinhagen and Barrow, 1984). The relationship of the RD_{50} and other structure-activity properties of acrolein in relation to other sensory irritants has been documented by Alarie et al. (1998).

4.3.1.1. Inhalation Studies

Several studies have found that subacute exposure of guinea pigs, rats, and mice to acrolein causes pulmonary inflammation, decreases in respiratory rate, and nasal lesions, effects also seen upon acute exposure. The effects of inhaled acrolein on laboratory animals are shown in Table 2.

TABLE 2. Effects of Inhaled Acrolein on Laboratory Animals

Species	Exposure Duration	Concentration (ppm)	Principal Effects	Reference
Rat				
Wister, male	6 h/day for 3 days	0, 0.25, 0.67, and 1.4	Nasal necrosis of respiratory epithelium and increased proliferation up to 0.67 ppm; 1.4 ppm group not evaluated.	Cassee et al. (1996b)
S-D, male	6 h/day 5 days/wk for 3 weeks	0, 0.1, 1.0, and 3.0	No effect on macrophage killing of inhaled *K. pneumonia*.	Sherwood et al. (1986)
S-D, male	6 h/day 5 days/wk for 3 weeks	0, 0.1, 1.0, and 3.0	Nasal exfoliation, erosion necrosis of respiratory epithelium and squamous metaplasia at 3 ppm; no effects on lungs or in local pulmonary antibody responsiveness to *L. monocytogenes.*	Leach et al. (1987)
F-344, male	6 h/day 5 days/wk 62 days (except for weekends for 12.4 weeks)	0, 0.4, 1.4, and 4.0	1. High mortality at 4 ppm. 2. Increase in lung collagen at 1.4 and 4 ppm ($p<0.05$). 3. Elastin content in 4 ppm group twice controls. 4. Bronchial necrosis and pulmonary edema at 4 ppm. 5. Parenchymal restriction at 0.4 ppm and obstructive lesions at 4.0 ppm. 6. No cytogenic or sperm abnormalities	Kutzman (1981); Kutzman et al. (1985); Costa et al. (1986)
Dahl, female (selected for susceptibility or resistance to salt-induced hypertension)	6 h/day 5 days/wk for 61-63 days (excluding weekends for 12.4 weeks)	0, 0.4, 1.4, and 4.0	1. All susceptible 4 ppm rats died after 11 days and 60% of resistant rats survived to end of study. 2. Lungs of susceptible rats had severe airway necrosis with edema and hemorrhage but only proliferative changes with resistant rats. 3. No differences in histopath between rat groups at lower doses. 4. No effect of exposure on blood pressure changes.	Kutzman et al. (1984, 1986)
SPF-OFA, male	Not explicitly stated, but up to 77 days	0, 0.55	1. Decrease in alveolar macrophage. 2. No effects on reproductive potential.	Bouley et al. (1975, 1976)
S-D, male	7 h/day for 3 consecutive days	1.7	1. Olfactory degeneration in all exposed rats. 2. Ulceration of respiratory epithelium in 4/10.	Teredesai and Stinn (1989)

Species	Exposure Duration	Concentration (ppm)	Principal Effects	Reference
S-D, male	6 h/day for 1 or 3 days	0, 0.2, and 0.6	Proliferative nasal and tracheal cells in epithelia at both concentrations.	Roemer et al. (1993)
Mouse				
Swiss-Webster, male	6 h/day for 5 days	1.7	1. Lesions of moderate severity in respiratory epithelium except for severe squamous metaplasia. 2. Lesions (ulceration and necrosis) of moderate severity in olfactory epithelium with squamous metaplasia mild. 3. Incomplete recovery after 72 hours.	Buckley et al. (1984)
Swiss, female	4 h/day for 4 days	2.5	Coexposure to acrolein and carbon black increased pulmonary killing of *P. mirabilis* and impaired elimination of *L. monocytogenes*. Killing of *S. aureus* was suppressed on first post-exposure day, but returned to normal on seventh day.	Jakab (1993)
CDI, female	3 h/day for 5 consecutive days	0.1	Decreased ($p<0.01$) in percent killing of *S. zooepidemicus* and *K. pneumonia.*	Aranyi et al. (1986)
White albino, male	6 h/day, for one 5-day period or 6h/day for two 5-day periods	various	1. Lung lesions (but no mortality) in mice exposed for two 5-day periods (concentration unknown). 2. LC_{50} of 66 ppm in group exposed for 6 hours. 3. 91% mortality in mice exposed to 50 ppm for 5 days.	Philippin et al. (1970)
FVB/N, male	6 h/day 5 days/wk for 3 weeks	3.0	Acrolein-induced excessive macrophage accumulation was associated with mucus hypersecretion.	Borchers et al. (1999b)
Guinea Pig, Rabbit				
Guinea pigs	7.5 h/day 2 consecutive days	1.6	1. Pulmonary inflammation. 2. Prolonged increase in airway sensitivity to substance P.	Turner et al. (1993)
Rabbits, New Zealand, female	15 min	375 and 489	1. Mortality at both concentrations. 2. Extensive lung damage at both concentrations.	Beeley et al. (1986)

Species	Exposure Duration	Concentration (ppm)	Principal Effects	Reference
Multiple				
S-D rat, male and female Beagle dogs, male Princeton or Hartley guinea pigs, male and female Squirrel monkeys, male	8 h/day 5 days/wk for 6 weeks	0.7 or 3.7	1. No concurrent controls. 2. No nasal histopathology. 3. Excessive salivation and eye irritation in dogs and monkeys at 3.7 ppm. 4. Chronic lung inflammatory changes and occasional emphysema in all animals at 0.7 ppm.	Lyon et al. (1970)
	24 h/day for 90 consecutive days	0.22, 1.0, and 1.8	1. Ocular and nasal discharges in dogs and monkeys at 1 ppm; severe at 1.8 ppm. 2. Squamous metaplasia of trachea in all monkeys at 1.8 ppm. 3. Two dogs at 1.8 ppm had confluent bronchopneumonia. 4. Evidence of pulmonary inflammation (guinea pigs at 1 ppm) and fecal liver necrosis (rats and guinea pigs at 1 ppm). 5. Nonspecific inflammatory changes in a variety of tissues in both rats and guinea pigs at 1.8 ppm.	
Syrian golden hamsters, male and female Wister rats, male and female Dutch rabbits, male and female	6 h/day 5 days/wk for 13 weeks	0, 0.4, 1.4, and 4.9	1. Mortality in rats at 4.9 ppm. 2. Necrotizing rhinitis in rats at 4.9 ppm and squamous metaplasia at 1.4 ppm with neutrophilic infiltration. 3. Lungs of hamsters unaffected. Severe nasal lesions in hamsters at 4.9 ppm, and tracheal hyperplasia in all female hamsters at 4.9 ppm. 4. Nasal and tracheal lesions similar to rat and hamster in rabbits at 4.9 ppm; no nasal lesions at lower doses.	Feron et al. (1978)

RATS: Male Wistar rats (5-6/group) were exposed 6 hr/day, for 3 consecutive days, in a nose-only exposure chamber to acrolein at concentrations of 0, 0.25, 0.67, or 1.4 ppm (0, 0.6, 1.5, or 3.2 mg/m^3) (Cassee et al., 1996b). Variation in exposure concentration was 13%. Rats were examined for nasal lesions (6 levels of the nasal tract examined) immediately after the last exposure. **Histopathology:** After one 6-hour exposure, no treatment-related histopathological lesions were found in any of the treatment groups. Only the histopathology of the 0.25 and 0.67 ppm (0.6 and 1.5 mg/m^3) groups were reported following three days of exposure; effects at 1.4 ppm (3.2 mg/m^3) were not reported. After 3 days, slight to moderate effects were observed from acrolein exposure in two of the four histopathology categories evaluated. In the category for disarrangement, necrosis, thickening and desquamation in the respiratory/transitional epithelium, 4/5 animals exposed to 0.25 ppm (0.6 mg/m^3) were observed to have slight effects (characterized as mainly disarrangement) and 1/5 developed a moderate level of effect. In the 0.67 ppm (1.5 mg/m^3) group, 3/6 were classified as slightly affected and 3/6 rats developed a moderate degree of response. For rhinitis, 1/5 of the 0.25 ppm (0.6 mg/m^3) rats developed a moderate response, and only 1/6 of the 0.67 ppm (1.5 mg/m^3) rats had a response and it was scored as a slight response. For the other two categories, single cell necrosis or atrophy of the olfactory epithelium, no effects were observed in either the 0.25 or 0.67 ppm (0.6 or 1.5 mg/m^3) group. **Proliferation:** After one 6-hour exposure, no treatment-related proliferative effects were found in any of the treatment groups. A proliferative response was defined as basal cell proliferation and/or an increased number of mitotic figures in respiratory/transitional epithelium. After 3 days, 3/5 male rats at 0.25 ppm (0.6 mg/m^3) developed a slight focal proliferative response and 2/5 showed no response. In the 0.67 ppm (1.5 mg/m^3) group, 2/6 rats developed a slight response and 4/6 developed a moderate response. The concentrations of acrolein associated with the proliferation indices were considerably lower than those of formaldehyde and acetaldehyde. Cell proliferation data was expressed as the number of positive-stained cells per millimeter basement membrane. Proliferative effects were not reported for the 1.4 ppm (3.2 mg/m^3) exposure group. **Enzymatic Changes**: Among biotransformation enzymes measured in homogenates of nasal tissue, glutathione S-transferase activity was significantly depressed in the 1.4 ppm (3.2 mg/m^3) exposure group ($p<0.01$) while formaldehyde dehydrogenase and aldehyde dehydrogenase activities were significantly increased ($p<0.05$). No changes were reported in the other dose groups, or for glutathione peroxidase activity in any of the dose groups. Non-protein sulfhydryl (NPSH) depletion was not observed in this study. No biochemical effects were observed in olfactory tissue. The LOAEL in this study is 0.25 ppm (0.6 mg/m^3). The duration-adjusted LOAEL is 0.25 ppm x 6/24 x 3/7 = 0.03 ppm or 0.07 mg/m^3.

In a study designed to evaluate the effect of acrolein on bacterial defense systems, male Sprague-Dawley rats were exposed to 0.1, 1.0 or 3.0 ppm (0.23, 2.3 or 6.9 mg/m^3) acrolein at 6 hr/day, 5 days/week for 3 weeks (Sherwood et al., 1986). No change was noted in the clearance of ^{35}S-*Klebsiella pneumonia* at any of the concentrations. Alveolar macrophage lysozyme and 5'-nucleotidase of acrolein-exposed rats were significantly increased at all exposure concentrations ($p<0.05$ at the low and intermediate concentration, and $p<0.01$ at the high concentration), while alkaline phosphatase showed a non-statistically significant increase. Phagocytosis was significantly increased at the low and intermediate concentrations ($p<0.01$), but not at the 3.0 ppm (6.9 mg/m^3). However, these changes had no apparent effect on macrophage killing of inhaled bacteria and were not indicative of extreme chemical toxicity.

Four groups of 40 male Sprague-Dawley rats were exposed by inhalation to target concentrations of 0, 0.1, 1.0, and 3.0 ppm (0, 0.23, 2.3 and 6.9 mg/m^3) acrolein 6 hr/day, 5 days/week for 3 weeks (Leach et al., 1987). Mean body weights were lower in the high-dose group, although differences were not statistically significant. There were no statistically significant effects of acrolein on immune responsiveness as measured by a hemolytic plaque assay performed on lung-associated lymph node cells. The ability of spleen- and lung-associated lymph nodes to respond to the T cell mitogen, PHA, and the B cell mitogen, STM, as well as resistance to infection by *L. monocytogenes* were not affected by acrolein exposure. Microscopic examination of the nasal turbinates of the high-dose group revealed acrolein-induced exfoliation, erosion and necrosis of the respiratory epithelium as well as squamous metaplasia. No effects were reported in the lungs of the high-dose group or at any location at the lower concentrations.

Kutzman (1981) and Kutzman et al. (1985) exposed male Fischer 344 rats (50/group) via inhalation to acrolein at 0, 0.4, 1.4, or 4.0 ppm (0, 0.9, 3.2 or 9.2 mg/m^3) 6 hr/day, 5 days/week for 62 exposure days (consecutive weekdays, except for weekends, for 12.4 calender weeks) to principally relate lung function with lung pathology. The duration-adjusted concentrations were 0, 0.07, 0.25 and 0.9 ppm (0, 0.16, 0.57, and 2.0 mg/m^3). Of the 50 animals/group, 24 were assessed for pulmonary function, 8 for pathology only, 10 for cytology, and 8 for reproductive function. Ten males and 8 females served as controls. Eight females per group were exposed to assess reproductive potential; weight gain and mortality were also evaluated. There was no histopathology for females. Cytological endpoints included sister chromatid exchanges (SCE) and cell proliferation kinetics. All examinations (with the exception of the cytology studies) were measured 6 days after final exposure to reduce the effect of acute exposure upon results. This recovery period undoubtedly allowed for compensatory changes. Sperm was examined for morphological abnormalities. Histopathology was performed on lung, peribronchial lymph node, nasal turbinates, brain, kidney, liver, spleen, testes, and heart (8 male rats from each dose group except 3 only from the 4.0 ppm or 9.2 mg/m^3 group). Of the 24 animals/group examined for pulmonary function, the right lung was subsequently used for biochemical analyses and the left lung processed for pathological examination.

Mortality (32/57) was observed only in males at the highest concentration, with many displaying severe acute bronchopneumonia. Body weights were significantly lower in the high-dose males and females during the first 10 days after which they gained weight; females never achieved their starting weight throughout the study. Lung hydroxyproline per mg protein (as an index of lung collagen) was increased 113 and 137% above controls ($p<0.05$) in the 1.4 and 4.0 ppm (3.2 and 9.2 mg/m^3) groups, respectively. Lung elastin per mg protein did not change significantly in the two lower dose groups but was increased to 174% of control levels ($p<0.05$) in the group exposed to 4.0 ppm (9.2 mg/m^3). Histologically, the 4.0 ppm (9.2 mg/m^3) surviving animals demonstrated bronchiolar epithelial necrosis and sloughing, bronchiolar edema with macrophages, and focal pulmonary edema. Rats from the 0.4 and 1.4 ppm (0.9 and 9.2 mg/m^3) groups did not exhibit pulmonary lesions attributable to acrolein exposure. Changes in the non-respiratory organs appeared incidental. The severity of the pulmonary lesions was scored for the left lung with a concentration-related increase in severity noted. No adverse histopathology was noted in other tissues examined. The only finding in the nasal turbinates was an apparent dose-

dependent increase in submucosal aggregates. In addition, no cytogenetic nor sperm abnormalities were observed nor was there any treatment-related effect on reproductive performance. In this latter aspect of the study, exposed male rats were mated with unexposed females for 6 days and also exposed females were mated with unexposed males.

Pulmonary function testing and the morphometric and compositional analyses in the male Fisher 344 rats (24 rats/exposure group on the sixth post-exposure day) from the Kutzman et al. (1985) studies was reported by Costa et al. (1986). Results indicated that at 0.4 ppm (0.9 mg/m^3), parenchymal tissue density was significantly increased (+15%) along with significantly increased maximal expiratory flow volume (MEFV), together inferring some degree of parenchymal restriction. Lung composition was similar to controls. The animals in the 1.4 ppm (3.2 mg/m^3) group did not differ functionally from controls. Parameters measured in the 4 ppm (9.2 mg/m^3) group, however, suggested obstructive lesions causing impaired ventilation in both the small and large airways. Internal surface areas of the lung were elevated (6 to 29%) in all exposure groups indicating hyperinflation ($p<0.001$). While the diffusing capacity for carbon monoxide was elevated significantly ($p<0.001$) in all exposure groups compared to controls, when normalized for lung volume these increase largely disappeared. The investigators conjectured that the functional effects from the restrictive lesions (0.4 ppm; 0.9 mg/m^3) and obstructive lesions at 4 ppm (9.2 mg/m^3) canceled in the 1.4 ppm (3.2 mg/m^3) group. Based upon an adequate number of animals evaluated by acceptable methodology at a time point at which acute effects are minimized, these data support the level of 0.4 ppm (0.9 mg/m^3) as a LOAEL associated with minimal effects, with more substantial lung damage occurring at elevated concentrations. Since only a single time point was evaluated, it is difficult to gauge the role of adaptation in the effects observed.

Female Dahl rats (which are derived from the Sprague-Dawley rat) that have been selected for either susceptibility (DS) or resistance (DR) to salt-induced hypertension were exposed to filtered air at 0.4, 1.4, and 4.0 ppm (0.9, 3.2 and 9.2 mg/m^3) acrolein (Kutzman et al., 1984, 1986). Ten DS and 10 DR rats/group were exposed 6 hr/day, 5 days/week for 61-63 days (consecutive weekdays, except for weekends, for 12.4 calender weeks). A 0.4% NaCl commercial diet was provided during non-exposure hours. Animals were necropsied one week after final exposure or 13.3 weeks after the first exposure. All of the DS rats exposed to 4.0 ppm (9.2 mg/m^3) acrolein died within the first 11 days of exposure, while 60% of the DR animals survived to the end of exposure. Neither dose-dependent blood pressure changes or altered behavioral characteristics were evident following acrolein exposure. Measures of lung connective tissue, hydroxyproline and elastin, as well as several serum chemistry parameters, alkaline phosphatase, phosphorus, SGOT and SGPT were significantly increased ($p<0.05$) in the DR rats following exposure to 4.0 ppm (9.2 mg/m^3) acrolein. There was a marked difference in the pulmonary pathology observed in DS and DR rats exposed to 4.0 ppm (9.2 mg/m^3) acrolein. The lungs of the DS rats exhibited severe airway epithelial necrosis with edema and hemorrhage, while surviving high-dose DR rats developed primarily a proliferative change. These included collections of intra-alveolar macrophages with foamy cytoplasm, terminal bronchiolar hyperplasia, squamous metaplasia of tracheal epithelium and terminal bronchial epithelium, as well as interstitial pneumonitis in 4/6 survivors. Pathologic changes in the two lower dose groups were similar, but less severe. Collections of intra-alveolar macrophages with foamy

cytoplasm were present in 7/10 DS rats and 5/10 DR rats in the 0.4 and 1.4 ppm (0.9 and 3.2 mg/m^3) and were adjacent to acutely damaged terminal bronchioles. Differences between the DS and DR groups at the 2 lower doses were minimal and not dose-dependent. Reasons for the difference in susceptibility of DS and DR rats to 4.0 ppm (9.2 mg/m^3) acrolein are unclear.

Bouley et al. (1975, 1976) exposed male SPF OFA rats (110/group) via inhalation to 0 and 0.55 ppm (0 and 1.3 mg/m^3) acrolein. Daily length of exposure and duration of the exposure were not explicitly stated, although measurements were reported for exposures up to 77 days. Body weights decreased to slightly less than 80% of controls by 60 days of exposure. Signs of nasal irritation (sneezing) were consistently observed in exposed rats between the 7th and 21st day of exposure. Sneezing subsequently disappeared despite continuing exposure. Exposed rats also exhibited a significant decrease in the number of alveolar macrophages. No differences were noted in liver weight after 22 days of exposure although liver/body weight ratios were decreased in exposed animals after day 15. Lung/body weight ratios were unchanged after day 15 or 32, but were significantly elevated ($p<0.002$) after 77 days. There was no effect on hepatic alcohol dehydrogenase after 15 days of exposure. Serum alkaline phosphatase was unchanged at days 15, 32 and 77. On the other hand, serum acid phosphatase was increased on day 15 ($p<0.001$), but not on days 32 and 77. An LD_{50} inhaled dose of *Salmonella enteritidis* resulted in a higher death rate in treated animals than controls at 18 days, but not at 63 days. Results were negative in a reproduction study involving 21 females and 3 males. In this portion of the study, mating was started on the 4th day after exposure was initiated and female rats were sacrificed on the 26th day after exposure began. There were no significant differences between control and exposed animals in the number of pregnant animals or number and mean weight of foetuses. While a large number of animals were exposed in the study and numerous measurements were made, use of only one exposure concentration and lack of histopathology greatly limit the usefulness of this study.

In an abstract, Teredesai and Stinn (1989) reported that exposure of male Sprague-Dawley rats to 1.7 ppm (3.9 mg/m^3) acrolein for 7 hr/day for 3 successive days caused ulceration of the respiratory epithelium in 4/10 rats and olfactory degeneration in all rats. Proliferative responses in nasal and tracheal epithelia were observed in male Sprague-Dawley rats exposed at levels of 0.2 and 0.6 ppm (0.5 and 1.4 mg/m^3) acrolein for 6 hr/day on 1 or 3 successive days (Roemer et al., 1993); significant cell proliferative changes were also noted with formaldehyde alone, but at 2 ppm (4.6 mg/m^3) and higher.

MICE: Male Swiss-Webster mice were exposed via inhalation 6 hr/day for 5 consecutive days to 1.7 ppm (3.9 mg/m^3) acrolein, the estimated concentration resulting in a 50% decrease in respiration (RD_{50}) (Buckley et al., 1984). Eight to 10 animals were sacrificed for pathologic examination immediately post exposure and an approximately equal number were sacrificed 72 hours later. The nasal region was sectioned at 5 levels for examination. Changes were labeled as none, slight, minimal, moderate or severe. For respiratory epithelium, exfoliation, inflammation, erosion, ulceration and necrotic changes were considered to be moderate. Squamous metaplasia was considered severe. For olfactory epithelium, ulceration and necrosis were considered to be moderate while squamous metaplasia and serous exudate mild. No effects were reported in the lungs. Recovery after 72 hours was minimal to moderate, suggesting that

the recovery period was insufficient for complete repair of lesions. During single exposures to acrolein, the reflex decrease in respiration was virtually eliminated by tracheal cannulation, providing additional evidence that the critical site for irritant effects of acrolein is the nasal region rather than the deep lung (Kane and Alarie, 1977).

The effect of coexposure to acrolein and carbon black upon lung defenses was evaluated by Jakab (1993). Female Swiss mice were exposed using a nose-only inhalation chamber, 4 hr/day for 4 days to carbon black (10 mg/m^3), acrolein (2.5 ppm; 5.7 mg/m^3), or the two combined. Twenty-four animals per group were assayed for resistance to *Staphylococcus aureus* and *Proteus mirabilis* at 1, 4, and 7 days post-exposure. For *Listeria monocytogenes*, 30 animals/group were utilized, with 6 animals/group sacrificed at 3, 6, 10, and 13 days post exposure. For influenza virus, 30 mice/group were used and 6 mice/group were sacrificed at 3, 6, 8, and 11 days post exposure with an additional group of six lavaged for quantitative cell counts and determination of lung lavage albumin concentrations. *S. aureus* was used for the alveolar macrophage (AM) surveillance phagocytic system, *P. mirabilis* for the dual phagocytic system composed of AMs and inflammatory polymorphonuclear leucocytes (PMNs), *Listeria monocytogenes* for the lymphokine-mediated arm of the acquired cellular immune response, and the influenza A virus for the cytotoxic T-cell mediated effector mechanism of cellular immunity.

Intrapulmonary killing of *S. aureus* was suppressed on the first day post-exposure to acrolein, with a return to normal by day 7. Coexposure enhanced pulmonary killing of *P. mirabilis,* which correlated with a significant increase in accessory phagocytic PMNs recovered from the lungs. Elimination of *L. monocytogenes* and influenza A virus from the lungs was impaired. Exposure to acrolein or carbon black alone had no effect upon lung defenses. Effects noted were likely due not only to the ability of carbon black to carry acrolein into the deep lung, but ingestion of particles by macrophages resulting in enhanced cellular penetration of acrolein. In an earlier study, Astry and Jakab (1983) found that an underlying viral pneumonia in mice compounded the pulmonary toxicity of 3 or 6 ppm (6.9 or 12.8 mg/m^3) acrolein in that antibacterial (challenge with *S. aureus*) defense mechanisms were suppressed.

Aranyi et al. (1986) exposed female CD1 mice via inhalation to 0.10 ± 0.22 ppm (0.23 ± 0.50 mg/m^3) acrolein, 3 hr/day for 5 consecutive days. To evaluate resistance to infection, the animals were simultaneously challenged with *Streptococcus zooepidemicus* for measurements of mortality and ^{35}S-*Klebsiella pneumonia* (noncapsulated) for determination of *in vivo* bacteriocidal activity of alveolar macrophages. A non-significant increase in mortality from 6/140 among controls to 11/140 in exposed mice was recorded. The percent bacteria killed showed a small, but significant decrease from 84.3 to 76.6 ($p < 0.01$).

Philippin et al. (1970) examined the inhalation effects of acrolein in mice exposed for 6 hours and for 2 weeks (6 hours/day). Groups of white albino male mice were exposed (1) for 6 hours, (2) for two 5-day periods (not known if consecutive) at 6 hr/day, and (3) for one 5-day period. At the conclusion of the two 5-day exposures (47 mice/group), there was no mortality at 6, 15, and 25 ppm (2.6, 34, and 58 mg/m^3); there was 91% mortality when 34 mice were exposed to 50 ppm (116 mg/m^3) for 5 consecutive days. There was no mortality at the lowest concentration tested (31 ppm; 71 mg/m^3) in the 6-hours-only exposure group. The acute LC_{50}

was determined to be 66 ppm (152 mg/m^3). Primary lung lesions observed in the groups (15 mice each examined for histopathology) exposed for two 5-day periods and sacrificed 24 hours after the last exposure were atelectasis and inflammatory responses with edema.

Borchers et al. (1998) obtained evidence that exposure of male Sprague-Dawley rats to 3 ppm (6.9 mg/m^3) acrolein for 6 hr/day, 5 days/week for 2 weeks were associated with mucus hypersecretion in isolated tracheal preparations from increases in MUC5ac gene expression. These investigators (Borchers et al., 1999b) later exposed FVB/N male mice to 3.0 ppm (6.9 mg/m^3) acrolein for 6 hr/day, 5 days/week for 3 weeks and found a significant and persistent increase in macrophages in bronchoalveolar lavage fluid and evidence that acrolein-induced excessive macrophage accumulation is associated with mucus hypersecretion.

MULTI-SPECIES: Groups of 15 Sprague-Dawley rats, 7-8/sex; Princeton or Hartley guinea pigs, 7-8/sex; 2 male purebred beagle dogs; and 9 male squirrel monkeys (*Saimiri sciurea)* were exposed to acrolein, 8 hr/day, 5 days/week for 6 weeks at concentrations of either 0.7 or 3.7 ppm (1.6 or 8.4 mg/m^3) (Lyon et al., 1970). According to the first author (Lyon, 2001), there were no concurrent controls in this study; control data (including histopathology) were obtained at a different time point. Histopathological examinations were stated to have been carried out on all dogs and monkeys and about one-half of the rats and guinea pigs. Nasal histopathology was not conducted. No deaths occurred and all animals appeared to be normal during exposure to 0.7 ppm (1.6 mg/m^3) acrolein. Lung sections from all animals exposed to 0.7 ppm (1.6 mg/m^3) showed chronic inflammatory changes and occasional emphysema. These changes were more prominent in dogs and monkeys. The inflammatory changes, consisting of interstitial infiltration of round cells, were mild and ranged from focal to diffuse, and while some infiltrates were peribronchial in distribution, no alteration of the respiratory epithelium or of the peribronchial smooth muscle was noted. In the 3.7 ppm (8.4 mg/m^3) exposure groups, dogs and monkeys salivated excessively and blinked frequently during the first week, and during the next four weeks the dogs experienced continued eye irritation. Two monkeys died, but it is unclear if these deaths were related to exposure because the condition of the monkeys upon arrival was not discussed. Nonspecific inflammatory changes were noted in sections of lung, liver, and kidney from all species. Focal calcification of renal tubular epithelium was noted in some of the rats and monkeys. Significant morphological changes, considered by the investigators to be related to acrolein exposure, consisted of squamous metaplasia and basal cell hyperplasia of the trachea of dogs and monkeys, and necrotizing bronchitis and bronchiolitis with squamous metaplasia of the lungs from 7 of the 9 monkeys, including 2 that died early in the study. The lung changes were commonly present in the bronchi rather than the bronchioles; the necrosis of the bronchial mucosa was associated with varying degrees of repair and regeneration of the epithelium. Bronchopneumonia was noted in dogs. There was no mention of any histopathological effects in control animals. Although data from control animals was under-reported, it appears that 0.7 ppm (1.6 mg/m^3) was associated with lung injury in all species evaluated.

Lyon et al. (1970), using identical group sizes, species (Sprague-Dawley rats, Hartley- or Princeton-derived guinea pigs, beagle dogs and squirrel monkeys) and strains as described above in the 6-week study also carried out 90-day continuous inhalation exposures (24 hr/day) at concentrations of 0, 0.21, 0.23, 1.0, and 1.8 ppm (0, 0.5, 0.5, 2.3, and 4.1 mg/m^3). The two lower

exposures were combined as one experiment at 0.22 ppm (0.5 mg/m^3). The same histopathological protocol was followed. All animals appeared normal during the 0.22 ppm (0.5 mg/m^3) exposure.

Monkeys and Dogs: Two monkeys died as a result of apparent infections. Histopathology demonstrated inflammatory effects in the eyes of dogs and monkeys, although no detail regarding number of animals or degree of inflammation was given. Ocular and nasal discharges were reported in the dogs and monkeys exposed to 1.0 ppm (2.3 mg/m^3), the latter keeping their eyes closed for extended periods. Morphological changes observed in tracheas of monkeys and lungs of dogs were considered related to exposure. At 1.0 ppm (2.3 mg/m^3) focal inflammatory reactions were reported in the lungs, liver, and kidneys of dogs. At 1.8 ppm (4.1 mg/m^3) the dogs and monkeys experienced severe irritation as evidenced by excessive salivation and ocular discharge. All monkeys in the 1.8 ppm (4.1 mg/m^3) group showed squamous metaplasia and 6/9 monkeys presented with basal cell hyperplasia of the trachea. The lungs from the two dogs at this concentration showed confluent bronchopneumonia. Lungs from 2/4 dogs in the 0.22 ppm (0.5 mg/m^3) group demonstrated moderate emphysema and focal splenic hemorrhage. The other two dogs showed hyperplasia of the thyroid. It is not clear if these observations from the 0.22 ppm (0.5 mg/m^3) group were treatment-related since there was no discussion of the condition of the control dogs. The investigators, however, did consider the lung effects in dogs (at all exposure levels) to be treatment-related.

Rats and Guinea Pigs: While weight gain was significantly ($p<0.005$) lower in rats exposed to 1.0 and 1.8 ppm (2.3 and 4.1 mg/m^3), no statistically significant differences in weight gain were noted in the other three species. In the 1 ppm (2.3 mg/m^3) groups, guinea pigs showed various degrees of pulmonary inflammation and occasional focal liver necrosis while rats (3/9) had occasional pulmonary hemorrhage and focal liver necrosis. In the 1.8 ppm (4.1 mg/m^3) exposure groups nonspecific inflammatory changes were observed in sections of brain, heart, lung, liver, and kidney of all animals.

Given the similarities in lung effects across species seen during the repeated and continuous exposures as well as in other studies, 1.0 ppm (2.3 mg/m^3) can be considered a LOAEL.

Feron et al. (1978) exposed four equal groups, each consisting of 20 Syrian golden hamsters, 12 Wistar rats, and 4 Dutch rabbits (equal numbers of each sex) to 0, 0.4, 1.4, and 4.9 ppm (0, 0.9, 3.2, and 11 mg/m^3) acrolein, 6 hr/day, 5 days/week for 13 weeks in whole-body exposure chambers. Duration-adjusted values are 0, 0.07, 0.25, and 0.9 ppm (0, 0.16, 0.57, and 2.0 mg/m^3). Histopathology was performed on all major organs/tissues, including three transverse sections of the nasal cavity.

Rats: Of the three species, rats seemed to be the most sensitive to the effects of acrolein. Mortality (6/24) occurred in the 4.9 ppm (11 mg/m^3) group and animals kept their eyes closed. No adverse clinical observations were reported for the other concentration groups. Hematological and serum enzyme levels were within the normal range. Body weight gain was significantly ($p<0.001$) depressed at 4.9 ppm (11 mg/m^3) and at 1.4 ppm (3.2 mg/m^3) ($p<0.05$).

The decrease in weight gain appeared related to decreased food consumption. Of several rats that died, hemorrhage, perivascular and alveolar edema of the lung were seen. Focal broncho-pneumonia, bronchitis, hyper- and metaplasia of the bronchial and bronchiolar epithelium, increased numbers of mucous-producing cells in the bronchioles, macrophage accumulation, and focal interstitial pneumonitis were observed in surviving rats in the 4.9 ppm (11 mg/m^3) group. Incidence of nasal lesions was not reported for any of the exposure groups. Only one male rat of the 0.4 ppm (0.9 mg/m^3) group showed any evidence of histopathological effects (metaplastic and inflammatory changes of slight severity) in the nasal tract. Incidence data in the 1.4 ppm (3.2 mg/m^3) group were not reported. Squamous metaplasia and neutrophilic infiltration (moderate severity) of the nasal mucosa were observed in the 1.4 ppm (3.2 mg/m^3) group. In the 4.9 ppm (11 mg/m^3) group, necrotizing rhinitis was occasionally seen in the dorsomedial part of the nasomaxillary region, with normal epithelium being partly replaced by stratified squamous epithelium, and in some cases showing keratinization. Neutrophil infiltration was invariably observed, but substantial neutrophilic exudation was seen in only a few animals. The trachea of rats in the 4.9 ppm (11 mg/m^3) group was described as "severely damaged," with nodules of granulation tissues protruding into the lumen. Given the apparent concentration-related increase in severity of nasal lesions (i.e., slightly to severely affected), it is reasonable to consider 0.4 ppm (0.9 mg/m^3) as a minimal LOAEL (i.e., an exposure level close to the expected NOAEL). Even though only 1/12 rats at this concentration demonstrated minimal metaplastic and inflammatory changes, these effects were consistent with the pathology demonstrated at the higher concentrations in which severity was increased. The duration-adjusted LOAEL is 0.4 ppm (0.9 mg/m^3) x 6/24 x 5/7 = 0.07 ppm (0.16 mg/m^3).

Hamsters: There was one death among the hamsters, but it was not related to treatment. Body weight gain was depressed only in the 4.9 ppm (11 mg/m^3) group and there was no evidence of decreased food intake. Lungs were unaffected by exposure. Only minimal inflammatory changes were seen in the nasal cavity at 1.4 ppm (3.2 mg/m^3); the nasal lesions observed in the 4.9 ppm (11 mg/m^3) group were similar (severe) to those seen in the rat. Hyperplasia and metaplasia in the trachea occurred in a few males and all females at 4.9 ppm (11 mg/m^3). In females at 4.9 ppm (11 mg/m^3), there were statistically significant increases in red blood cell packed cell volume, hemoglobin (Hb) content, and in numbers of lymphocytes accompanied by a decrease in the number of neutrophilic leucocytes. All serum enzyme activities were within normal ranges. The NOAEL based on nasal lesions is 0.4 ppm (0.9 mg/m^3) with a LOAEL of 1.4 ppm (3.2 mg/m^3).

Rabbits: Body weight gain (males and females combined) was significantly depressed (<0.05) in only the 4.9 ppm (11 mg/m^3) group. Decreased weight gain appeared to be related to diminished food intake. No effects were detected in the nasal region in the low- and mid-dose groups. Nasal lesions in the 4.9 ppm (11 mg/m^3) group was similar to those in the rat, but less severe. Tracheal effects were seen only in the high-dose group, primarily hyperplasia and metaplasia.

Based on the severity of respiratory tract lesions in the rat compared to diminished responses in the rabbit and hamster in the 4.9 ppm (11 mg/m^3) groups, the rat is considered the most sensitive species of the three with a minimal LOAEL for nasal lesions of 0.4 ppm (0.9

mg/m^3).

GUINEA PIGS: Turner et al. (1993) found that exposure of guinea pigs for 7.5 hours per day for 2 consecutive days to 1.6 ppm (3.7 mg/m^3) acrolein resulted in pulmonary inflammation and epithelial damage. Even after 28 days post-exposure there was a prolonged increase in airway sensitivity to aerosolized substance P, a sensitivity which may have been enhanced by an acrolein-induced reduction in neutral endopeptidase in bronchoalveolar lavage fluid.

RABBITS: In a study designed to evaluate the effects of corticosteroids on mortality and lung histopathology of female New Zealand rabbits exposed to acrolein, animals (18/group) were exposed to 375 and 489 ppm (862 and 1125 mg/m^3) for 15 minutes (Beeley et al., 1986). Although treatment with methylprednisolone reduced mortality (no significant differences between the two groups), there was no evidence of an improvement in lung histopathology (hemorrhagic necrosis, edema).

4.3.1.2. Oral Administration

RATS: Arumugam et al. (1997) demonstrated that acrolein treatment results in severe depletion of liver cytosolic GSH. In a study that clearly shows acrolein-induced damage to cellular function, Arumugam et al. (1999b) exposed male Wistar rats, 5 animals/group, daily for 45 days to distilled water or acrolein in distilled water (2.5 mg/kg BW) via intubation. The authors did not specify as to whether the dosing was continuous or 5 days/week. Observation of clinical symptoms and histopathology were not part of the protocol. Electron microscopic examination revealed a loss of mitochondrial lamellae of the cristae in the treated livers compared to normal architecture in controls. This was accompanied by a 41% decrease in mitochondrial GSH ($p<0.001$) as well as the activities of the citric acid cycle enzymes, isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, malate dehydrogenase, succinate dehydrogenase, NADH dehydrogenase, cytochrome c oxidase ($p<0.001$), and levels of cytochrome a, b, c_1, and c. The decreases in the activities of the citric acid cycle enzymes ranged from 30 to 56%. The activities of GSH peroxidase and superoxide dismutase were increased significantly ($p<0.001$). Because GSSG was unchanged, GSH depletion was presumed to result from conjugation with acrolein. Superoxide anion radicals generated by mitochondria under physiological and pathological conditions are converted to hydrogen peroxide by superoxide dismutase. The authors state that hydrogen peroxide is cleared from mitochondria only by GSH peroxidase, and if the GSH cofactor has been depleted due to conjugation with acrolein, the resulting higher peroxide levels may lead to increased lipid peroxidation, mitochondrial damage, and the observed decreased enzyme activities.

Limitations of the study for evaluation of chronic effects are the (1) use of a 45-day exposure period rather than a longer one, (2) use of only one dose level, and (3) lack of histopathology of the stomach which would have ascertained if intubation of acrolein damaged stomach lining. Given the significant depletion of citric acid cycle enzymes, longer-term exposure to acrolein could compromise an animal's ability to survive.

Administration of acrolein in water by oral gavage at 0.05, 0.5, and 5.0 mg/kg to male and female Sprague-Dawley rats (30/sex/group) daily, 5 days/week for 13 weeks did not result in any significant observed toxic effects (Bioassay Systems Corp., 1981g). No mortality or clinical, hematological, or urinalysis effects were ascribed to treatment. Histopathological results were similar to controls. Histotopathology was conducted on 12 rats/group at the 5.0 mg/kg/day dose level and controls only. This study provided the dose selection used by Parent et al. (1992c) in the chronic gavage study described below.

Parent et al. (1992c) administered acrolein in water daily via gavage to Sprague-Dawley rats, 70/sex/group, at dose levels of 0, 0.05, 0.5, and 2.5 mg/kg BW. There was no indication in the report that the "daily" dosing was limited to 5 days/week. Dosing volume was 10 ml/kg. Ten animals from each group were sacrificed after one year and the remainder after two years. An extensive array of tissues was examined microscopically, including stomach tissue. Although it was not explicitly stated that both the glandular stomach and forestomach were examined, it is unlikely that both parts of the stomach were not examined. Daily observations were made and various clinical, hematological and urinary parameters were measured after 3, 6, 12, and 18 months of treatment and immediately prior to termination. There were no significantly increased incidences of microscopic lesions in the treated rats, whether neoplastic or non-neoplastic. Food consumption and body weights were unaffected by treatment. With the exception of a statistically significant depression of creatinine phosphokinase (creatine kinase) levels at all dose levels and at most time intervals (except 12 months), clinical chemistry parameters, hematology and urinalysis measurements were unaffected by treatment. The most definitive responses reported were treatment-related increases in early cumulative mortality. Data were provided in the form of survival curves. Among high-dose males, survival was significantly reduced after one year ($p<0.05$), and marginally reduced among mid-dose males (p value not reported). Among high-dose males, a trend test for survival during the first year indicated a highly significant ($p=0.003$) decrease; however, the statistical differences are nullified when the survival data for two years are included in the analysis. Survival among females during the first year corresponded closely to those obtained for males. A statistically significant decrease in survival ($p<0.05$) was reported in the high-dose group, while a decrease in survival in the mid-dose group was marginally significant (p value not reported). A highly significant trend toward reduced survival ($p<0.001$) in the high-dose group was also reported. Unlike responses in males, the significant associations between dosing and survival persisted in females through the end of the study. After two years, a statistically significant reduction in survival was noted based on four different statistical tests for the mid-dose group and in 3/4 statistical tests in the high-dose group (p values not reported). Although the differences in survival were statistically significant in females after two years, it should be noted that the differences were relatively small. No differences in survival were seen in the low-dose groups. There was no apparent cause cited for the early mortalities. There were 11 confirmed accidental deaths due to gavage error and 17 possible. However, even after censoring these data early mortality remained. There was no information or discussion in the report relating to forestomach hemorrhage as a possible cause. No obvious dose-related clinical symptoms were observed.

MICE: In a study designed to evaluate the potential carcinogenicity of acrolein (Parent et al., 1991b), Swiss albino CD-1 mice (70/sex/group) were dosed via gavage (acrolein in

distilled water and stabilized with hydroquinone) with 0, 0.5, or 2.0 mg/kg-day for 18 months. A separate group (75/sex) was similarly dosed at 4.5 mg/kg-day. All animals were sacrificed at 18 months. The primary effect was increased mortality ($p<0.05$) only in high-dose males of the 4.5 mg/kg-day group; mortality in mid- and low-dose groups was less than controls. There were no dose-related adverse histopathological or clinical findings.

Pretreatment of male Charles River mice with high oral doses of L-ascorbic acid, L-cysteine, and an alpha adrenergic-blocking agent gave a high degree of protection against the lethality of orally-administered acrolein administered once after which animals were followed for 72 hours (Sprince et al., 1979).

RATS and MICE: In a 13-week daily gavage study of acrolein (in 0.5% methyl cellulose) conducted for the National Toxicology Program (NTP), 10 F344 rats/sex/dose were administered 0.75, 1.25, 2.5, 5.0, and 10 mg acrolein/kg; 10 B6C3F1 mice/sex/dose were administered 0, 1.25, 2.5, 5.0, 10 and 20 mg/kg. Dose volume was 5 ml/kg for rats and 10 ml/kg for mice. Treatment resulted in similar dose-related effects in both sexes of rats: hemorrhage and necrosis and chronic-active inflammation of the forestomach and glandular stomach and secondary changes associated with acrolein-induced mortality in high-dose animals (NTP, 1995; Pathology Working Group, 1997). Hemorrhage of the glandular stomach was also confirmed in 5 and 10 mg/kg males and 10 mg/kg females. Abnormal breathing and nasal/eye discharge were among the clinical findings in high-dose rats. Nearly all high-dose animals died or were removed from study because of gastrointestinal toxicity. Forestomach squamous epithelial hyperplasia was observed in male rats at 2.5 mg/kg and higher (no-observed-adverse-effect level, NOAEL, of 1.25 mg/kg-day) and in females at 1.25 mg/kg and higher (NOAEL of 0.75 mg/kg-day).

There were no clinical signs of toxicity in mice. The forestomach lesions in mice were similar to those in the rat. Glandular stomach lesions were only seen in the 10 and 20 mg/kg males and in the 20 mg/kg females. Statistically significant increases in absolute and relative liver weights were seen in male mice at 10 mg/kg without attendant hepatic histopathology. Forestomach squamous epithelial hyperplasia was observed in one male mouse at the lowest dose of 1.25 mg/kg (i.e., no NOAEL for the male mice), and in female mice at 2.5 mg/kg-day and higher (NOAEL of 1.25 mg/kg-day).

DOGS: Six male and 6 female beagle dogs/group were administered acrolein (0.1% aqueous) in gelatin capsules at doses of 0, 0.1, 0.5, and 1.5 mg/kg-day, 7 days/week for 53 weeks (Parent et al., 1992a). At week 4, the high dose was increased to 2 mg/kg-day. Blood and biochemical measurements were made at pretest and at 3-month intervals. At termination, all dogs were subjected to full necropsy and histological examination. Body weights and food consumption were not significantly affected by treatment. A primary effect noted was a dose-dependent increase in the frequency of vomiting shortly after dosing which can limit the dose retained. The frequency decreased with time indicating adaptation. Serum albumin, calcium and total protein levels were significantly depressed (p values not given) in high-dose animals throughout the study. Measurements for the other exposure groups were not listed. Some variability in red blood cell parameters and coagulation times were noted, but the significance of

these effects was not obvious. It was reported that clinical signs, with the exception of vomiting, were evenly distributed among groups. At termination, gross necropsy indicated vascular congestion and mucosal reddening of the gastrointestinal tract of both males and females, but it is unclear if these effects were treatment-related. While the study was well-designed and the methodology adequately reported, incomplete reporting of results limits its usefulness in a quantitative dose-response assessment.

4.3.1.3. Dermal Administration

The toxicity of acrolein dissolved in water + ethanol in rabbits was evaluated by dermal application of 7, 21, and 63 mg acrolein/kg-day, 5 days/week for 3 consecutive weeks according to a FIFRA study design (Bioassay Systems Corp., 1982a). Observations included slight to significant reduction in body weight, moderate to severe skin irritation, and histopathologic lesions in skin and lungs.

4.3.2. Cancer Assessment

4.3.2.1. Inhalation Exposure

Feron and Kryusse (1977) exposed groups of 36 Syrian golden hamsters of both sexes to acrolein vapor at measured levels of 0 and 4.0 ppm (0 and 9.2 mg/m^3), 7 hr/day, 5 days/week for 52 weeks. Six animals per group were sacrificed at 52 weeks and the remainder at 81 weeks. Overall mortality was 38% in exposed hamsters and 33% in controls. Histological changes were observed in the anterior half of the nasomaxillary turbinates, consisting of epithelial metaplasia, but not hyperplasia. In addition, exposure resulted in abnormal behavior and growth retardation. The only respiratory tract tumor observed was a small tracheal papilloma in an acrolein exposed female. The exposure period for this study was short for a cancer bioassay and sacrifice at 81 weeks may have been insufficient to allow for latency.

In a study by Le Bouffant et al. (1980), 20 female Sprague-Dawley rats/group were exposed to 8 ppm (18.3 mg/m^3), 1 hr/day, 5 days/week for either 10 or 18 months. No tumors or metaplasias were reported. Use of only one exposure concentration and less than lifetime exposure duration limits inferences that can be drawn from this study.

4.3.2.2. Oral Administration

There have been three long-term cancer bioassays by the oral route: male F-344 rats via drinking water (Lijinsky and Reuber, 1987); CD-1 mice via drinking water (Parent et al., 1991b); and Sprague-Dawley rats via drinking water (Parent et al., 1992c).

Male Fischer 344 rats (20/group) were administered acrolein in the drinking water at concentrations providing average daily doses of 0, 1.9, 5.0, or 12.5 mg/day, 5 days/week for 104-124 weeks (Lijinsky and Reuber, 1987). On the remaining 2 days, tap water was provided. High-dose animals stopped drinking the solution before the other groups. Drinking water solutions were prepared weekly and stored at unspecified refrigerator temperatures until

dispensed. Each cage of four rats was given a measured amount (80 ml) of drinking water over the span of the study. The daily dose per kg BW could not be calculated from the data given.[2] The maximum tolerated dose was not determined. Major organs and tissues were reported as being examined histopathologically (if there were any non-proliferative lesions they were not reported). One group of 20 females also received the highest dose on the same schedule as the males. Adrenocortical tumors (5/20) and hyperplastic nodules of the adrenal cortex (2/20) were found only in females in the high concentration group. The increased incidence of adrenocortical tumors was considered by the authors to be marginally significant as judged by the Fisher's exact test (p=0.091) and significant for adrenocortical tumors plus hyperplastic nodules (p=0.022). According to the authors, this type of tumor is rare in untreated female Fischer 344 rats; there was one reported in concurrent controls. The historical incidence is approximately 4.8% based on the findings of Solleveld et al. (1984) for untreated female F-344 rats allowed to die naturally. Significant increases in tumor incidence were not found in male rats. There was no treatment-related mortality. Lijinsky and Reuber (1987) also exposed rats to acrolein diethylacetal, acrolein oxime, and allyl alcohol, agents that can be expected to be hydrolyzed to acrolein in the stomach acids, with negative results. A reevaluation of the tumors in this study (Parent et al., 1992c) is described in Section 4.7.

Lijinsky and Reuber (1987) also exposed hamsters to acrolein, but the does proved to be too toxic to complete the cancer bioassay. A single, 1 mg dose via gavage in corn oil killed all of the animals within a few hours; hamsters reportedly drank too little water to make the study feasible.

Four groups of 70-75 male and 70-75 female Swiss albino CD-1 mice, eight weeks of age, were administered 0, 0.5, 2.0, or 4.5 mg acrolein/kg BW via gavage in deionized water daily for 18 months, followed by sacrifice of survivors at the end of the treatment period (Parent et al., 1991b). Dosing levels were chosen based on a range-finding study demonstrating severe stomach lesions at higher dosing levels. Body weight gains were decreased and mortality increased in males, especially at the high dose. All mice killed at the end of treatment, as well as those found dead or moribund, were necropsied. Tissues from major organs were examined histologically. No treatment-related increase in tumor frequency was observed. The study was near lifetime duration for mice and the maximum tolerated dose (MTD) appeared to be achieved. Thus, acrolein appears unlikely to be carcinogenic in mice by gavage.

Parent et al. (1992c) also gavaged 560 Sprague-Dawley rats about 6 weeks of age (70/sex/group) daily with 0, 0.05, 0.5 and 2.5 mg acrolein/kg in water (10 ml/kg). Ten rats/sex/group were sacrificed at 1 year for various clinical measurements. The remainder of the animals were treated for 102 weeks followed by sacrifice. Dosing solutions were prepared daily from stock solutions (prepared daily) and analyzed weekly by gas chromatography. Stability studies indicated losses at <10% after storage for 3 hours at room temperature. The only statistically significant changes noted in treated animals were consistent depression of creatine

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[2] Parent et al. (1992c), assuming that each of the four rats/cage drank the same amount of water, estimated a daily dose of 50 mg/kg BW at the highest concentration, which exceeds the LD_{50} for rats. This suggests a lower rate of intake or that acrolein in solution may not have been as stable in solution as reported.

phosphokinase levels (significance unknown) and consistent increases in early cumulative mortalities in both males and females. There was no significantly increased incidence of either neoplastic or non-neoplastic microscopic lesions in treated rats. Analyses of survival took into account confirmed and possible accidental deaths (28 total). Decreased survival of high- and mid-dose males during the first year was highly and marginally significant, respectively; however, this trend did not persist into the second year. Unlike survival in male rats, decreased survival in females during the first year persisted until the end of the second year. Based upon results of this two-year exposure in which mortality indicated a maximum tolerated dose (MTD) was achieved, it can be concluded that there was no evidence for carcinogenicity in an adequately designed and conducted study. While the doses/kg BW used in this study are most likely much lower than those used by Lijinsky and Reuber (1987), Parent et al. have raised concerns about the conclusions reported in the Lijinsky and Reuber (1987) study, and that dose levels may have been lower than the original authors assumed (see Section 4.7).

4.3.2.3. Injection Studies

The earliest reported study investigating the potential carcinogenicity of acrolein was reported by Steiner et al. (1943). Fifteen female partly-inbred albino mice received subcutaneous injections (0.2 mg/kg) of acrolein weekly for 24 weeks. No sarcomas developed at the site of injection. The use of only one dose level and a small number of animals limits any conclusions.

4.3.2.4. Initiation and Promotion Studies

Cohen et al. (1992) exposed 30 male Fischer 344 rats/group to acrolein, 2 mg/kg by i.p. injection twice weekly as part of a larger initiation/promotion study. All groups were sacrificed 53 weeks from the start of the study. No increases in tumor incidence were reported in groups exposed to acrolein alone for either 6 or 21 weeks (severe toxicity occurred during the 21 week study). Exposure to acrolein for 6 weeks followed by administration of uracil (3% by weight) for an additional 20 weeks resulted in the induction of 18 papillomas and one carcinoma, a significantly greater incidence ($p<0.05$) than following exposure to uracil alone (8/30). While it appears that acrolein may have some tumor initiating capability, it should be noted that the incidence of papillomas and nodular hyperplasias combined, was significantly greater in the uracil only group compared with the group initiated with acrolein ($p<0.05$).

A group of 15 “S” strain mice (sex and age unspecified) received 10 weekly skin applications of a 0.5% solution of acrolein in acetone at a total dose of 12.6 mg/animal (Salaman and Roe, 1956). Starting 25 days after the first application of acrolein, the mice received weekly skin applications of 0.17% croton oil for 18 weeks; for the second and third applications the concentration was reduced to 0.085%. When croton oil and acrolein were administered together, each compound was given alternately at 3 or 4 day intervals. Tumor incidence was evaluated at the end of the croton oil treatment. Four skin papillomas were reported in 4 of 19 control animals that received croton oil alone. A total of 3 papillomas were noted in 2 of the 15 mice treated with acrolein and croton oil. The data suggest that acrolein lacks potential for initiation of skin tumors. Small numbers, however, limit any definitive conclusions.

Feron and Kryusse (1977) exposed groups of 30 male and 30 female Syrian golden hamsters, about 6 weeks of age, to 0 or 4 ppm (0 or 9.2 mg/m^3) acrolein for 52 weeks, together with either weekly intratracheal installations of 0.175 or 0.35% benzo[a]pyrene (BP) in 0.9% saline, or subcutaneous injections of 0.0675% *N*-nitrosodiethylamine (DENA) in saline once every 3 weeks (total dose, 2 µL/animal). The experiment was terminated at 81 weeks, and all survivors were killed and autopsied. An increased incidence of papillomas, adenomas, adenocarcinomas and squamous-cell carcinomas of the respiratory tract were found in acrolein-exposed male and female hamsters treated with BP and DENA. Exposure to acrolein vapor alone resulted in only one respiratory tumor (female).

Conclusions that can be drawn from the Feron and Kryusse (1977) inhalation and initiation/promotion studies are limited because of the use of only one dose level, although they did report toxic responses at concentrations of 9.2 mg/m^3. Because respiratory tract tumors typically occur in hamsters administered BP or DENA, the evidence is insufficient to suggest that acrolein is a cofactor in carcinogenesis.

4.4. REPRODUCTIVE/DEVELOPMENTAL STUDIES-ORAL AND INHALATION

***In vivo*:**

Kutzman (1981) exposed female and male Fischer 344 rats (8/group) via inhalation to acrolein at 0, 0.4, 1.4, or 4.0 ppm (0, 0.9, 3.2 or 9.2 mg/m^3) 6 hr/day, 5 days/week for a total of 62 exposure days. The duration of exposure was 12.4 weeks and the animals were evaluated 13.3 weeks after initiation of exposure. Exposed and control male rats were mated with unexposed females for 6 days and also exposed females were mated with unexposed and exposed males. Parameters evaluated were corpora lutea, viable embryos, early and late deaths, and pre-implantation losses. There were no treatment-related effects on reproductive performance.

The only other inhalation reproduction study reported to date was performed by Bouley et al. (1976). Three male and 21 female SPF OFA rats were exposed continuously to acrolein at a measured concentration of 1.3 mg/m^3 (0.6 ppm) and then mated on the fourth day of exposure. Exposures were continued for an additional 22 days when the females were sacrificed. The exposure did not cover the entire period of spermatogenesis. No significant differences in the number of and mean weight of fetuses (no data given) were reported. While the results were negative, the minimal results reporting limits conclusions that can be drawn from this study.

Claussen et al. (1980) intravenously injected New Zealand white rabbits on day 9 of gestation with 3, 4.5, or 6 mg/kg acrolein. Embryolethal effects increased in a dose-dependent manner, but few malformations were noted. After direct injection into the rabbit embryos at doses of 10, 20 and 40 µL, resorptions and malformations increased in a dose-dependent manner. The highest dose by both routes showed that direct embryo injection of acrolein induced malformation at doses 50-60 times lower than those inducing embryolethal effects via intravenous injection.

In a two-generation gavage study, four groups of 30 male and 30 female Sprague-Dawley rats were gavaged daily with 70 doses of acrolein at levels of 0, 1, 3, or 6 mg/kg in a dosing

volume of 5 ml/kg (Parent et al., 1992b). Rats within each dosing group (F_0 generation) were then assigned to a 21-day period of cohabitation. Dosing continued for females through cohabitation, gestation, and lactation. A similar regime was carried out for F_1 generation offspring, resulting in F_2 generation pups. Mortality was significant (at 6 mg/kg) in both males and female of the F_0 and F_1 generations with the pattern continuing with F_1 mid-dose animals, most of the latter showing signs of respiratory distress and histopathological lesions in the lungs and stomach. Reproductive parameters (i.e., mating performance and fertility indices) were unaffected. Erosions of glandular mucosa and hyperplasia/hyperkeratosis of the forestomach were the most frequent stomach lesions observed. Significant depressions in body weight gains were noted in the high-dose groups and achieved statistical significance in the mid-dose animals on several occasions. No treatment-related gross or microscopic effects were observed in the reproductive tissues of any of the F_0 or F_1 animals. There were no statistically significant differences among the groups in the number of F_1 litters with gross abnormalities for the pups (F_2) during lactation or gross lesions identified in the pups at necropsy. The data provide evidence that acrolein is not a selective reproductive toxicant, but does produce toxicological effects at doses as low as 3 mg/kg-day.

The respiratory effects in the Parent et al. (1992b) gavage study raises the question about possible reflux and/or regurgitation and aspiration of gavage solution, which can occur with volatile or highly irritating chemicals. There is also the possibility of incorrect tube placement, or esophageal or gastric perforation. The authors note the development of stomach lesions suggesting that the dose was being delivered to the stomach, but provide no further discussion of the possible amount of chemical that may have been aspirated to the lungs. In light of studies indicating no systemic distribution (Parent et al. 1996b, 1998), the respiratory effects noted in this gavage study may be due to aspiration of gavage solution.

Pregnant New Zealand white rabbits (20/sex/group) were dosed via gavage with 0, 0.1, 0.75, or 2.0 mg/kg-day for days 7 through 19 of presumed gestation and subjected to caesarean sectioning on day 29 (Parent et al., 1993). Three deaths were observed, but were considered a result of misdosing or aspiration. Transient effects on feed consumption and body weight gains were noted. Resorptions were elevated in the high-dose group, but the effect was not statistically significant. Fetal malformations were distributed evenly among the groups and were consistent with historical control data. Higher doses in a range-finding study (0, 0.5, 1.0, 2.0, 4.0 and 6.0 mg/kg-day) produced high incidences of maternal mortality (at 4 and 6 mg/kg), spontaneous abortion, resorption, clinical signs, gastric ulceration, and/or sloughing of the gastric mucosa. Thus, acrolein was not found to be a developmental toxicant or teratogen at maternally nontoxic doses.

***In vitro*:**

Rat conceptuses were explanted from the uterus on day 10.5 of gestation, transferred to culture bottles and treated with acrolein at concentrations ranging from 100 to 250 µM (Schmid et al., 1981). Slight, but statistically significant inhibition of growth was reported at 100 and 150 µM. A concentrations of 200 µM resulted in drastic inhibition of growth and differentiation and no gross structural defects, but 250 µM completely arrested differentiation and growth. These findings indicate that acrolein is lethal to embryos in a narrow dose range, but has no teratogenic

potential.

Slott and Hales (1986), however, reported 100% mortality in embryos cultured in a standard medium containing an acrolein concentration of 140 µM, and teratogenic effects at 80-120 µM. In a serum-free medium acrolein was 100% lethal to embryos at 20 µM and was teratogenic in the range of 5-15 µM. The EC_{50} for malformations in the serum medium was 137 µM, whereas that for embryolethality was 115 µM. In a further study, Slott and Hales (1987a) reported that acrolein induced 64 and 100% mortality at acrolein concentrations of 120 and 160 µM, respectively. At concentrations of 80 and 120 µM, 50 and 100% of the embryos were malformed, respectively. In addition, both concentrations of acrolein produced growth retardation manifested by significant decreases in the yolk sac diameter, crown-rump and head lengths, number of somites, and morphological score. Concurrent exposure to 100 or 500 µM GSH markedly protected embryos against all of these effects, but GSH addition 2 hours after the beginning of acrolein exposure offered little protection. Because addition of GSH resulted in little change in concentration in the yolk sack or embryos, protection was believed to be primarily due to interaction between acrolein and GSH in the culture medium. The embryotoxicity of acrolein, on the other hand, was significantly enhanced by addition of glutathione sulfoxamine (10 or 100 µM), an inhibitor of GSH synthesis (Slott and Hales, 1987b).

Stahlmann et al. (1985) tested acrolein in a mouse limb bud culture system. Concentrations of acrolein between 3 and 10 mg/ml (56 and 178 µM) induced a significant impairment of limb bud differentiation with explants from 12 day old mouse embryos. Scapula and paw skeleton were more affected than ulna and radius. With limbs from 11-day-old embryos similar effects were reported at even lower concentrations. A contact time of 20-40 minutes was sufficient to induce abnormal development.

Mirkes et al. (1984) evaluated the role of acrolein in cyclophosphamide (CP) teratogenesis in a culture medium containing day 10 rat embryos. The dechloro derivative of cyclophosphamide (D-CP), breaks down upon activation to acrolein and dechlorophosphamide mustard (D-PM). D-CP was teratogenic and resulted in decreases in growth parameters, whereas D-PM did not. When embryos were exposed to acrolein alone (0.45 to 18 µM), all concentrations produced abnormal flexion in some embryos, but effects did not resemble those induced by D-CP. Complete lethality was produced at 8.9 µM. This suggests that the high reactivity of acrolein limits its entry to sensitive sites, but when D-CP is transported into the cell, yielding acrolein, teratogenicity can result.

Using the embryo culture, Hales (1989) reported that while phosphoramide mustard and acrolein are both teratogenic, they had differing effects on developing limbs, indicating different targets and/or mechanisms of action.

No evidence of acrolein-induced teratogenicity was found in chicken eggs treated on day 3 with acrolein at doses of 0.001-10 µmol and examined on day 14 of incubation. The LD_{50} was estimated to be 0.05 µmol (Kankaanpää et al., 1979). In a similarly designed study, however, Korhonen et al. (1983) reported malformation in chicks at doses of 0.05 µmol/egg. Chibber and Gilani (1986) also reported increases in malformations in chicks at doses as low as 0.001 mg/egg

(0.02 μmol) when dosed at 48 hours of incubation and examined on day 13.

In summary, acrolein can induce teratogenic and embryotoxic effects if administered directly to the embryos or fetuses, but had no selective reproductive, developmental or teratogenic effects in a gavage study in the rat. In the rabbit, there were no teratogenic effects following iv administration. An inhalation study found no effect of acrolein on reproductive performance in exposed male and female rats. The high reactivity of acrolein may limit its ability to reach critical sites in the developing embryo.

4.5. OTHER STUDIES

This section focuses upon the results of *in vitro* tissue and cell culture experiments, including the genotoxic potential of acrolein and its conjugates in *Salmonella*.

4.5.1. *In Vitro* Toxicity

Heart: Perfusion of rat hearts with 0.01-0.03 mM acrolein led to cessation of beating within 15 minutes; no lesions were detected, but creatine kinase was reported to be inactivated (Sklar et al., 1991). Rat neonatal myocytes were unaffected by exposure to 0.01 mM acrolein, but stopped beating within 2 hours during exposure to 0.05 mM acrolein with accompanying cell lysis and release of lactic dehydrogenase (Toraason et al., 1989). While acrolein was shown to act as an inhibitor of mitochondrial electron transport, the effective concentrations for a 50% inhibition (0.39-0.80 mM) are probably too great to invoke a direct action on electron transport as a primary mechanism for cardiotoxicity of acrolein (Biagini et al., 1990).

Pulmonary Cells: Patel and Block (1993) observed that acrolein exposure results in alterations in plasma membrane-dependent transport in cultured pulmonary endothelial cells, leading to decreased availability of precursor amino acids used in GSH and protein synthesis. Joseph et al. (1994) reported that acrolein at concentrations of 5-50 μM resulted in disruption of actin cytoskeletal fibers in cultured pulmonary artery endothelial cells. The damage was postulated as possibly due to cross-linking of sulfhydryl groups.

Survival of human alveolar macrophages was significantly decreased following 24 hours exposure to acrolein concentrations of 25 μM or greater (Li et al., 1997). Incubation of type II alveolar macrophages cultured for 24 hours with acrolein led to a near-zero adenosine triphosphate (ATP) concentration at 50 μM and a significant increase ($p<0.001$) in LDH at an acrolein concentration of 25 μM. These effects were considerably muted when lung slices were used (Monteil et al., 1999).

Cytotoxicity of acrolein, as measured by the decrease in colony forming efficiency (CFE) of cultured human bronchial fibroblasts, was not observed at 1 μM acrolein, but CFE decreased to less than 50% following 7-8 days incubation at 3 μM (Krokan et al., 1985); intracellular thiol content was decreased and inhibited the DNA repair enzyme O^6-methylguanine-DNA methyltransferase, but had no effect on activity of uracil-DNA glycosylase. Cell survival was significantly decreased at lower acrolein levels than those that reduced thiol levels. Grafström et

al. (1988) and Grafström (1990) also reported that less than 3 μM acrolein was required to decrease-colony forming efficiency 50% in human bronchial epithelial cells. The 1-hour LD50 determined by trypan blue exclusion (a measure of cell permeability) was about 20 μM. A small, but significant increase in single strand breaks and DNA protein cross-links occurred at a concentration of 30 μM acrolein. Fibroblasts (origin not stated) derived from patients with Xeroderma pigmentosum (XP) were more sensitive to the cytotoxic effects of acrolein than were cells from normal individuals (Curren et al., 1988).

Acrolein enhanced responsiveness of human bronchi sections to carbachol following 20 minutes exposure at 0.1 μM (Marthan et al., 1996). After 60 minutes responsiveness was depressed, indicating a toxic effect. The effects appeared to follow a time-concentration C x T relationship, with a maximum response at a C x T (μM x min) of slightly less than 10 and a depressed response at a C x T of 60.

Liver: Acrolein induced a rapid dose-related depletion of GSH in rat hepatocyte cultures at concentrations of 25-500 μM after 2 hours (Zitting and Heinonen, 1980); at 500 μM, recovery did not occur and the integrity of cell membranes was impaired. Similarly, Silva and O'Brien (1989) reported that five minutes exposure to 25 μM acrolein resulted in an approximately 25% decrease in viability of cultured rat hepatocytes, while 50 μM resulted in a greater than 50% decrease (significance levels not given).

Several studies have evaluated the effects of inhibiting aldehyde dehydrogenase (ALDH) on the toxicity of acrolein. Oxidation of acrolein by hepatic ALDH is a detoxification reaction (Rikans, 1987). ALDH metabolizes acrolein to the less reactive acrylic acid. Silva and O'Brian (1989) showed that incubating rat hepatocytes with inhibitors of ALDH resulted in an increased toxicity and greater depletion of GSH. The administration of ALDH inhibitors, cyanamide or disulfiram, caused substantial inhibition of acrolein oxidation by the hepatic mitochondrial and cytosolic low Km ALDHs (Rikans, 1987). A significant increase in lipid peroxidation ($p<0.01$), and a depletion of GSH ($p<0.04$) occurred within 5 minutes of exposure to 100 μM acrolein to cultured liver cells (Watanabe et al., 1992). Dogterom et al. (1988) observed an increase in acrolein toxicity evidenced by increased cell death following disulfiram inhibition of ALDH and exposure of cultured rat liver cells to 0.4 mM acrolein, but there was an unexplained decrease in lipid peroxidation. Acrolein alone can inhibit ALDH. Incubation of rat liver hepatocytes with 30 μM acrolein resulted in an irreversible inhibition of high affinity ALDH with a 91 and 33% reduction in mitochondrial and cytosolic ALDH activities (Mitchell and Petersen, 1988). N-acetylcysteine protected against acrolein-induced toxicity in isolated hepatocytes, possibly by maintaining sulfhydryl levels (Dawson et al., 1984).

Brain: Recent evidence has established that increased lipid peroxidation is intimately involved in the pathogenesis of Alzheimer's disease and represents a marker of oxidative stress (Calingasan et al., 1999). Lovell et al. (2001) obtained evidence in brains (10) obtained from Alzheimer's patients at autopsy (8 age-matched controls) that acrolein is increased in brains of Alzheimer's patients. In hippocampal neuron cultures, acrolein was neurotoxic in a time- and concentration-dependent manner and disrupted calcium homeostasis.

Acrolein was found to be a potent inhibitor of ADP-induced mitochondria state 3 and calcium-induced respiration in whole brain mitochondria obtained from adult male Sprague-Dawley rats. Acrolein did not affect basal levels of state 3 respiration, did not alter activity of complexes I-V or mitochondrial calcium transporter activity, and did not induce cyctochrome c release (Picklo and Montine, 2001). Inhibition was prevented by GSH and N-acetylcysteine. These results were similar to those obtained using isolated rat hepatic mitochondria in which phosphate and glutamate transport were inhibited (Zollner, 1973). In isolated mitochondria from rat heart, acrolein did inhibit complex II-linked state 3 and uncoupled respiration (Biagini et al., 1990).

Following a 24-hour exposure of acrolein to cultured neuroblastoma cells, the concentrations of acrolein required to induce a 50% change in cytotoxic endpoints from controls were as follows: sloughed cells (1 μM), neurite formation (7.6 μM), viability of sloughed cells (5.3 μM), total cell number (580 μM), and viability of harvested neuroblastoma cells (30 μM) (Koerker et al., 1976). Neuronal survival was decreased to about 50% following 24-hour exposure to 600 μM acrolein and less than 25% following 48-hour exposure (Smith et al., 1990b).

Treatment of hippocampal cultures taken from gestation day 18 rat embryos with acrolein led to a time- and concentration-dependent decrease in cell survival as well as a concentration-dependent increase in intracellular calcium (Lovell et al., 2000). When cortical neuron or astrocyte cultures were similarly treated, there was an impairment of glutamate uptake.

Skin fibroblasts: A one-hour exposure of xeroderma pigmentosum cells to acrolein caused depletion of GSH and free protein thiols to a quantitative extent similar to that in normal skin fibroblasts without causing changes in the thiol redox state (Dypbukt et al., 1993).

Tumor cells: Acrolein was shown to be highly cytotoxic in two lung carcinoma cell lines and in a glioblastoma cell line (Rudra and Krokan, 1999); in one of the lung carcinoma cell lines, toxicity was partially reversed by vitamin E. Acrolein was shown to reduce AP-1 activation in human lung adenocarcinoma cells of the A549 cell line (Biswal et al., 2000). There was also an elevation in CYP2E1 and a >9-fold elevation in redox-related gene activity. In an earlier study of the A549 cell line (Horton et al., 1997), acrolein's ability to alter the proliferation of cells *in vitro* was dependent on cell density and total cell number.

Transformed cells: Four concentrations of acrolein failed to induce malignant transformation in C3H/10T1/2 mouse embryo fibroblasts (Bioassay Systems Corp., 1982c).

Immunotoxicity: Topical administration of acrolein to the shaved skin of female guinea pigs (15) was shown not to result in positive skin reactions; positive controls were used (Susten and Breitenstein, 1990).

Miscellaneous: Survival of human umbilical artery cells was unchanged by exposure to 10 μM acrolein, but reduced to 17 and 11% of controls by 50 and 100 μM acrolein (Pino and Lyles, 1995). Inhibition of P450 in rat microsomal preparations by acrolein has been

demonstrated by Gurtoo et al. (1981). Myeloperoxidase isolated from human neutrophils was shown to convert L-threonine into acrolein (Anderson et al., 1997); activated neutrophils required the myeloperoxidase-H_2O_2-chloride system to produce acrolein in high yield. It was suggested that activated phagocytes have the potential to cause tissue damage at sites of inflammation.

Toxicity of conjugates: The toxicity of acrolein-thiol conjugates as well as acrolein mercapturates to human lung adenocarcinoma A549 cells was examined by Ramu et al. (1996). These conjugates were incubated with cells following a 2-hour exposure of cells treated with diethyl maleate (DEM) to deplete GSH. There was a dose-dependent inhibition of cell growth following treatment with acrolein, S-3-oxopropyl N–acetyl cysteine and its sulfoxide, with the sulfoxide also resulting in plasma membrane damage. A 24-hour (but not 2-hour) exposure of cells to S-3-oxopropyl GSH also resulted in growth inhibition. Pretreatment with DEM increased the inhibition of cell growth seen with acrolein. Quantitative, pH, and rate considerations suggested that β-elimination of acrolein was not the sole mechanism of toxicity of S-3-oxopropyl N–acetyl cysteine and its sulfoxide. Acrolein itself has been demonstrated to inhibit NF-κB activation of A549 cells, consistent with formation of acrolein-NF-κB conjugates (Horton et al., 1999). NF-κB is a transcription factor controlling a number of genes, including those involved in proliferation and apoptosis.

Perry et al. (1995) evaluated the toxicity of several acrolein derivatives to A549 cells. No significant toxicity was observed with S-3-hydroxypropyl N-acetyl cysteine or S-3-oxopropylGSH. S-3-oxopropyl N-acetyl cysteine caused growth inhibition that was reversed by GSH and N-acetyl cysteine.

Eisenbrand et al. (1995) speculated that acrolein-GSH could potentially function as transport molecules for 2-alkenals, such as acrolein, if they reach tissues low in GSH and GST. These investigators found that, in the absence of GSH, acrolein-GSH conjugate decomposed slowly into aldehyde and GSH. The toxicological importance of GSH lies in its role as a substrate in detoxifying conjugation reactions catalyzed by GSH transferase and as a substrate for GSH peroxidase, which protects against membrane damage (Zitting and Heinonen, 1980). Incorporation of the GST isozyme P1-1 (Pi class) into Hep G2 cells was found to increase their resistance to acrolein toxicity, suggesting that GSTs may play a role in cellular detoxication (Berhane et al., 1994). Comparison of the specific activities of GST A1-1 (alpha class), M1-1 (Mu class), and P1-1 indicated that the rate of reaction was in the order of A1-1< M1-1< P1-1.

4.5.2. Intraperitoneal/Intragastric/Intravenous Toxicity

Intraperitoneal administration of acrolein (0.5 to 6 mg/kg BW) to male F344 rats was found to result in urinary bladder hyperplasia; hyperplasia was not observed upon intragastric administration at lethal levels (Sakata et al., 1989). The nephrotoxicity of a 1:1 acrolein-GSH adduct in the rat was examined by Horvath et al. (1992). Male Sprague-Dawley rats were given the adduct intravenously at 0.5 or 1 mmole/kg. In addition to gross and histologic changes in the kidney, glucosuria and proteinuria, an elevation in serum urea nitrogen was observed. The nephrotoxicity was inhibited by acivicin, a γ-glutamyltranspeptidase inhibitor, indicating that

metabolism through the first step in the renal mercapturic acid synthesis pathway is required.

4.5.3. Genotoxicity

4.5.3.1. DNA Adduct Formation, Sister Chromatid Exchange and DNA-Protein Cross-links

Munsch et al. (1973) found that acrolein (2×10^{-5} to 8×10^{-4}M) inhibited partially purified regenerating rat liver DNA polymerase, but not DNA polymerase I from *E. coli*. The site of action was at the -SH groups and was presumed to oxidize the -SH groups. In a confirmatory study, Munsch et al. (1974) observed that (^{3}H) acrolein was bound to regenerating rat liver DNA polymerase 10 to 20 times more than to *E. coli* DNA polymerase I, the latter having no -SH groups at its active center. Acrolein has also been demonstrated to inhibit transcriptional activity of isolated liver nuclei from male Wistar rats and bacterial RNA polymerase (Moulé et al., 1971).

Acrolein at concentrations of 5, 15, and 20 µM, but not lower doses, induced significant increases in sister chromatid exchanges in cultured human lymphocytes (Wilmer et al., 1986). Acrolein was reported to induce formation of deoxyguanosine adducts at concentrations of 10 µM, but not 4 or 7 µM, in *Salmonella* tester strain TA104 (Foiles et al., 1989). Lack of response at the lower doses suggests the presence of a saturable repair mechanism. Increasing adduct formation with dose was seen in tester strain TA100 at concentrations of 4 mM and greater. The responses are in agreement with increases in mutagenicity at about the same doses in these two tester strains. Using the same methodology, deoxyguanosine adduct formation increased progressively at concentrations ranging from 0.1 to 1 mM in Chinese hamster ovary cells (CHO) (Foiles et al., 1990). In studies by Chung et al. (1984), reaction of acrolein with deoxyguanosine or DNA under physiological conditions led to the formation of cyclic 1,N^2-propanodeoxyguanosine and its adducts (Chung et al., 1984). Smith et al. (1990c) were able to determine the structure of an adduct formed from calf thymus DNA following acrolein exposure as 1,N^6-propanodeoxyadenosine. Several putative adducts were observed in DNAs isolated from acrolein-treated human fibroblasts. One of these adducts was tentatively identified as the cyclic 1,N^2-hydroxypropanodeoxyguanosine product, 3-(2′-deoxyribosyl)-5,6,7,8-tetrahydro-8-hydroxypyrimido[1,2-*a*]purine-10-one (Wilson et al., 1991). Chenna et al. (1992) reported that reaction of acrolein with thymidine resulted in one major product, N^3-(3″-oxopropyl)thymidine. Reaction of acrolein with 2-deoxyuridine under physiological conditions formed N^3-(3″-oxopropyl)-2′-deoxyuridine. This product was reduced to give N^3-(3″-hydroxypropyl)-2′-deoxyuridine (Chenna and Iden, 1993). At neutral pH, acrolein reacts with guanosine and cytosine and adenine derivatives to yield several cyclic adducts (Sodum and Shapiro, 1988).

Using a ^{32}P-postlabeling method, Nath et al. (1996) found evidence of DNA-acrolein adducts (1,N^2-propanodeoxyguanosine) in liver DNA of unexposed humans and untreated F344 rats, suggesting that they may be prevalent background lesions. Using the same technique, Nath and colleagues (Penn et al., 2001) found this adduct in aortic DNA of white leghorn cockerels exposed to 1 or 10 ppm (2.3 or 23 mg/m^3) acrolein for 6 hours. When cockerels were exposed to

1 ppm (2.3 mg/m^3) acrolein for 6 hr/day for 8 weeks to examine arteriosclerotic plague formation potential, there was no effect of exposure on plaque development.

DNA-protein cross-links were increased between calf thymus DNA and histone at acrolein concentrations of about 25 µM, based upon graphic data, although numbers were not reported (Kuykendall and Bogdanffy, 1992). Costa et al. (1997) reported significant ($p<0.05$) increases in DNA-protein cross-links in human Burkitt's lymphoma cells exposed 4 hours at concentrations greater than 150 µM acrolein. It is uncertain if the differences in sensitivity reported are due to differences in methodology or cell type. Kozekov et al. (2001) cross-linked two DNA strands with the principal adduct of acrolein.

Inhalation exposure of male F344 rats to 2 ppm (4.6 mg/m^3) acrolein for 6 hours did not cause detectable DNA-protein cross-linking in the nasal respiratory mucosa whereas cross-linking was observed under *in vitro* conditions (Lam et al., 1985). It was hypothesized that acrolein reacted preferentially with sulhydryl-containing nucleophiles.

DNA single strand breaks were induced by acrolein in cultures of Namalva cells, a human lymphoblastic cell line poor in deactivating enzymes and low in GSH and in GST activity, at much lower concentrations than needed in primary rat hepatocytes (Eisenbrand et al., 1995). Acrolein caused a higher extent of DNA single-strand breaks (SSB) in XP cells (which normally have < 5% of excision repair capacity of normal cells) than normal cells (Dypbukt et al., 1993). Exposure to acrolein followed by incubation in fresh medium resulted in continued formation of DNA SSB in normal cells without further accumulation in XP cells.

4.5.3.2. Mutagenic Effects of Acrolein in *Drosophila melanogaster*

Effects of acrolein on somatic mutations in *Drosophila* are shown in Table 3. Vogel and Nivard (1993) reported positive effects only with inhalation at toxic exposure levels, and not in feeding studies of larvae. Sierra et al. (1991), on the other hand, reported positive effects in feeding studies under similar conditions. Reasons for the difference in findings are uncertain, although they could be due to the use of 48-hour cultures by Vogel and Nivard (1993) compared with 72-hour cultures by Sierra et al. (1991).

Effects of acrolein on sex-linked recessive lethals (SLRLs) in *Drosophila* are shown in Table 4. No effects of acrolein were reported for SLRL induction in feeding studies, but highly significant increases were noted in injection studies at high mM concentrations (Sierra et al., 1991). No effect on percent lethals, either by injection (200 ppm) or feeding (3,000 ppm) was observed in Canton-S wild-type males (Zimmering et al., 1985). Mutations in excision repair deficient *Drosophila* (*mus201*) induced a greater incidence of SLRLs than in repair efficient females. Based upon statistical analysis to evaluate hypermutability, it was concluded by the authors that acrolein induces lesions that are partially repaired by excision repair mechanisms. Since cyclic adducts can be repaired by excision mechanisms (Vogel, 1989), and the only acrolein-derived lesions reported to date are cyclic adducts (Foiles et al., 1989; Smith et al., 1990b; Wilson et al., 1991), it appears that at least some of the SLRLs are derived from cyclic adducts. The response of *Drosophila* to acrolein with *mus308* mutation, which is thought to play

a role in repair of cross-linking adducts, was no different than normal mice (Commendador et al., 1992; Barros et al., 1994a). These results thus provide additional support for the likelihood that cyclic adducts are the predominant forms induced by acrolein.

Barros et al. (1994b) tested the effects of metabolic modification upon induction of SLRLs. Diethyl maleate, a GSH-depleting agent, induced an increase in SLRLs in feeding studies with acrolein-exposed *D. melanogaster (Berlin K and Muller-5* strains). Phenobarbital, a cytochrome P450 inducing agent, eliminated response to acrolein via injection. Iproniazid and 1-phenylimidazole, potent inhibitors of cytochrome P450 oxidative enzymes of *Drosophila*, had no effect on SLRL induction by injection of acrolein. These results support the hypothesis that acrolein is a direct mutagen. Moreover, acrolein is deactivated by enzymatic activity induced by phenobarbital. The results also indicate that sensitivity to acrolein by the oral route is relatively low. This may be a function of its reactivity, with little reaching the reproductive organs by way of food.

TABLE 3. Effects of acrolein on somatic mutations in *Drosophila melanogaster*

Sex	Conc (mM)	Dose Method	Endpoint	% Spots	Average clone size	Reference
both	0	food	eye spots	4.2	2.9	Vogel and Nivard (1993)
both	10	food	eye spots	3.0 (-)	2.4	
both	20	food	eye spots	4.1 (-)	3.8	
both	80	food	eye spots	4.0 (-)	2.1	
both	0	inhalation	eye spots	4.7	4.1	
both	500 ppm	inhalation	eye spots	8.9 (+)	4.1	
both	1,000 ppm	inhalation	eye spots	4.4	4.0	
both	2,000 ppm	inhalation	eye spots	lethal	-----	
male	0	food	eye spots	1.4	5.0	Sierra et al. (1991)
male	5	food	eye spots	5.5 (+)	4.1	
male	10	food	eye spots	3.8 (+)	4.7	
male	20	food	eye spots	11.6 (+)	5.7	
female	0	food	eye spots	3.3	7.0	
female	5	food	eye spots	7.4 (+)	11.2	
female	10	food	eye spots	7.4 (+)	4.4	
female	20	food	eye spots	16.6 (+)	6.9	
both	0	food	wing spots	17.5	1.3	
both	5	food	wing spots	21.1 (-)	1.6	
both	10	food	wing spots	29.3 (+)	1.8	
both	20	food	wing spots	29.6 (+)	3.0	

A statistical analysis was conducted according to Frei and Wurgler (1988): +,S positive; -, inactive

TABLE 4. Effects of acrolein in the induction of sex-linked recessive lethal (SLRL) mutations in *D. melanogaster* exposed for 5 hr (feeding) and by injection

Concentration (mM)	Dose Method	Stage of Meiosis	Other Treatments	Percent Lethals	Reference
0.00 (broods pooled)	food	post	none	0.5	Sierra et al. (1991)
0.50	food	post	none	0.33	
1.00	food	post	none	0.16	
2.50	food	post	none	0.15	
5.00	food	post	none	0.16	
10.00	food	post	none	0.50	
5.00 (24-hr exposure)	food	post	none	0.19	
0.00	injection	post	none	0.17	
2.00	injection	post	none	0.31	
3.00	injection	post	none	0.39*	
3.00	injection	post	none	0.92***	
5.00	injection	post	none	0.61***	
5.00	injection	post	none	1.01***	
7.00	injection	post	none	0.60***	
7.00	injection	post	none	0.34	
0.00	injection	post	*mus*201	0.35	Barros et al. (1994a)
2.00	injection	post	*mus*201	0.82**	
3.00	injection	post	*mus201*	0.99***	
5.00	injection	post	*mus*201	1.06***	
0.00	injection	post	*mus*308	0.24	
1.00	injection	post	*mus*308	0.41*	
2.00	injection	post	*mus*308	0.25	
3.00	injection	post	*mus*308	1.41**	
5.00	injection	post	*mus*308	0.31	
0.00	injection	post	DEM	0.08	Barros et al. (1994b)
0.00	injection	pre	DEM	0.09	
3.00	injection	post	DEM	0.50*	
5.00	injection	post	DEM	0.46*	
5.00	injection	pre	DEM	0.14	
7.00	injection	post	DEM	0.20	
0.00	injection	post	PB	0.27	
0.00	injection	pre	PB	0.10	
3.00	injection	post	PB	0.30	
3.00	injection	pre	PB	0.03	
5.00	injection	post	PB	0.18	

Concentration (mM)	Dose Method	Stage of Meiosis	Other Treatments	Percent Lethals	Reference
5.00	injection	pre	PB	0.00	
0.00	injection	post	PHI + IPR	0.10	
0.00	injection	pre	PHI + IPR	0.12	
0.30	injection	post	PHI + IPR	0.18	
0.30	injection	pre	PHI + IPR	0.17	
0.50	injection	post	PHI + IPR	0.36*	
0.50	injection	pre	PHI + IPR	0.13	
0.70	injection	post	PHI + IPR	0.37*	
0.70	injection	pre	PHI + IPR	0.17	

*mus*201 maternal excision repair deficiency
*mus*308 hypersensitive to cross-linking agents
PB (phenobarbital): Induces xenobiotic metabolism
DEM (diethylmaleate): Glutathione-depleting agent
PHI (1-phenylimidazole): Inhibitor of cytochrome P450
IPR (ipronazid): Inhibitor of cytochrome P450
* ($p<0.05$), **($p<0.01$), ***($p<0.001$)

4.5.3.3. Tests for Gene Mutation in Mammalian Cell Cultures

Although acrolein has been shown to induce DNA adducts in a variety of cell types as well as mutagenesis in *Drosophila* and microorganisms under certain conditions, there is limited information regarding the ability of acrolein to induce mutations in normal mammalian cells. Acrolein was shown to be highly mutagenic to human fibroblast cells that were deficient in DNA repair (cells from xeroderma pigmentosum patients). While a positive dose-response was observed between 0.2 and 0.8 µM acrolein in the repair deficient cells, acrolein did not induce an increase in the mutant frequency of normal fibroblasts (Curren et al., 1988). Acrolein was also mutagenic in V79 cells deficient in DNA repair (Smith et al., 1990a). Normal V79 cells were not tested. *In vitro* chromosomal studies of acrolein have produced weakly positive findings in Chinese hamster ovary (CHO) cells (Au et al., 1980) and in cultured human lymphocytes (Wilmer et al., 1986). Chromosomal aberrations were not detected in CHO cells either in the presence or absence of metabolic activation (Bioassay Systems Corp., 1982d) or in rat bone marrow cells (Bioassay Systems Corp., 1982e). More recently Parent et al. (1991a) failed to detect mutagenic effects of acrolein using the sensitive Chinese hamster ovary hypoxanthine-guanine phosphoribosyl transferase (HGPRT) forward mutation assay system both with and without exogenous activation, even at toxic dose levels. These results confirmed earlier findings in which acrolein was found not to induce mutations at the HGPRT locus in CHO cells (Bioassay Systems Corp., 1982b). Kawanishi et al. (1998) conducted a molecular analysis using *supF* shuttle vector plasmids for the spectrum of mutations that acrolein may induce in human fibroblast cells. The majority of the mutations were base substitutions (76%) followed by deletions and insertions (24%). Single base substitutions were most frequently found (46%), multiple base substitutions accounted for 18%, and tandem (two adjacent) base substitutions

were 12%. Of the base substitutions, G:C to T:A transversions accounted for 44% of the total and G:C to A:T transitions for 24%.

4.5.3.4. Tests for Gene Mutation in Bacterial Cells

Results are summarized in Table 5. In tests for frameshift mutagens without metabolic activation (TA98 and TA1538), TA98 gave some positive responses while TA1538 was negative. The only positive response for TA98 with S-9 activation was reported by Claxton (1985). With this exception, metabolic activation generally resulted in negative responses in all strains. Tests for base repair and point mutations (TA100, TA104 and TA1535) were positive in some tests with TA100, in most tests with TA104, but not with TA1535. TA104 has been reported to be more sensitive to carbonyl compounds (Marnett et al., 1985). Among strains sensitive to cross-linking (TA102, TA2638, WP2 and *Escherichia coli* HB101), TA2638 and HB101 were positive in the only study reported for each strain, while TA102 and WP2 strains were negative. The *Escherichia coli* strains JTG10 and AR1157, which are lacking in GSH synthetase, are sensitive to induction of mutations as well as induction of cytotoxicity at very low concentrations.

VanderVeen et al. (2001) has shown that when acrolein reacts with guanine residues in *S. typhimurium* to form 8-hydroxypropanodeoxyguanosine, the latter was not mutagenic in *S. typhimurium*. Acrolein was similarly nonmutagenic in *E. coli* (Yang et al., 2001).

It is clear from the studies reported that acrolein is highly reactive and cytotoxic. Acrolein has been shown to be mutagenic in some test systems within a narrow range of concentrations. Sensitivity to mutational effects is increased by GSH depleting agents and decreased by addition of metabolic activation, indicating that acrolein is a direct acting agent. While acrolein is capable of alkylating DNA and DNA bases (Maccubbin et al., 1990) and is known to inhibit purified DNA methylase activity from liver and bladder (Cox et al., 1988), it may never reach the target tissues of whole animals other than those at the site of insult. Even in the *in vitro* assays cited, acrolein is so reactive that special techniques must generally be employed to reduce cytotoxicity to induce positive effects. Parent et al. (1996b) have suggested that the reactivity of acrolein precludes its reaching target cells at a sufficient concentration to initiate the carcinogenic process.

TABLE 5. Tests for gene mutation in bacterial systems

Species/Strain	Result[a] +S9	Result[a] -S9	Test Type	Reference
Salmonella typhimurium TA98	-	-	Reverse mutation	Basu and Marnett (1984)
	+	+		Claxton (1985)
	-	-		Haworth et al. (1983)
	-	-		Florin et al. (1980)
	-	+		Lijinsky and Andrews (1980)
	-	-		Loquet et al. (1981)
	-	±		Parent et al. (1996b)
Salmonella typhimurium TA100	-	-		Basu and Marnett (1984)
	toxic	+		Eder et al. (1993)
	-	-		Florin et al. (1980)
		+		Foiles et al. (1989)
	±	-		Haworth et al. (1983)
	-	-		Lijinsky and Andrews (1980)
	-	-		Loquet et al. (1981)
	-	+		Lutz et al. (1982)
	-	+		Parent et al. (1996b)
Salmonella typhimurium TA102	-	-		Jung et al. (1992)
	-	-		Parent et al. (1996b)
	-	-		Watanabe et al. (1998)
Salmonella typhimurium TA104	0	+		Foiles et al. (1989)
	-	+		Hoffman et al. (1989)
	0	+		Marnett et al. (1985)
	-	-		Parent et al. (1996b)
Salmonella typhimurium TA1535	-	-		Florin et al. (1980)
	-	±		Hales (1982)
	-	-		Haworth et al. (1983)
	-	-		Lijinsky and Andrews (1980)
	-	-		Loquet et al. (1981)
	-	-		Parent et al. (1996b)
Salmonella typhimurium TA1537	-	-		Florin et al. (1980)
	-	-		Haworth et al. (1983)
	-	-		Lijinsky and Andrews (1980)
	-	-		Parent et al. (1996b)
Salmonella typhimurium TA1538	-	-		Basu and Marnett (1984)
	-	-		Lijinsky and Andrews (1980)
	-	-		Parent et al. (1996b)

Species/Strain	Result[a] +S9	-S9	Test Type	Reference
Salmonella typhimurium TA2638	0	+		Watanabe et al. (1998)
*Salmonella typhimurium his*D3052/*nop*KM101	-	-		Basu and Marnett (1984)
Salmonella typhimurium TA1535	0	-	SOS (*umu*) induction assay	Benamira and Marnett (1992)
Escherichia coli PQ37	0	+	SOS repair	Eder et al. (1993)
Escherichia coli HB101pUC13	0	+	DNA-histone cross-links	Kuykendall and Bodanffy (1992)
Escherichia coli WP2 *uvr*A	-	±	Reverse mutation	Hemminki et al. (1980)
	±	-		Parent et al. (1996b)
Escherichia coli WP2/pKM101	0	-		Watanabe et al. (1998)
Escherichia coli WP2 *uvrA*/pKM101	0	-		Watanabe et al. (1998)
Escherichia coli JTG10	0	+		Nunoshiba and Yamamoto (1999)
Escherichia coli AB1157	0	+		
Escherichia coli WP2	0	-		Aikawa and Miwa (1993)
Escherichia coli WP2(*urv*)A155	0	-		
Escherichia coli ZA159(*uvrB*)	0	-		

[a] + , ≥2 x background rate or statistically significant ($P < 0.05$); ± , equivocal; - , negative; 0 , not tested.

4.5.4. Mechanistic Studies

A number of *in vitro* and *in vivo* studies demonstrated that acrolein has the potential to: (1) perturb the environments of human and laboratory animal cells in which GSH plays an important role, (2) suppress host defense mechanisms, and (3) elicit pro-inflammatory processes.

GSH depletion in isolated rat hepatocytes incubated with 0.25-0.5 mM acrolein caused lipid peroxidation and impaired integrity of cell membranes (Zitting and Heinonen, 1980). Depletion of GSH at 3-25 µM acrolein has also clearly been established in cultured endothelial cells (Patel and Block, 1993), and in human bronchial epithelial cells at 3 µM acrolein (Grafström et al., 1988). There was a dose-related decrease in plasma membrane surface -SH groups in human polymorphonuclear leukocytes and rat pulmonary alveolar macrophages when incubated at acrolein concentrations from 1 to 1000 µM (Witz et al., 1987). GSH protects cells by removing reactive metabolites such as electrophilic carbonium ions. Thus, GSH depletion deprives the cell of its natural defense against ubiquitous reactive metabolites and leaves the thiol groups in critical proteins vulnerable to attack by oxidation, cross-linking, and the formation of mixed disulfides or covalent adducts. For example, cellular constituents of the antioxidant defense system, including ascorbic acid, α-tocopherol, GSH peroxidase, and catalase in rat lung were decreased following inhalation exposure of male Wistar rats to 1 or 2 ppm (2.3 or 4.6 mg/m^3) acrolein (Arumugam et al., 1999a). This led to enhanced lipid peroxidation,

which produced extensive lung damage as indicated by elevated levels of the biochemical markers – angiotensin converting enzyme, LDH, protein, and lactate in the bronchioalveolar lavage.

In vivo exposure resulted in GSH depletion in nasal respiratory mucosa (McNulty et al., 1984). This is likely due to the highly reactive nature of acrolein, which reacts by virtue of its allylic function with GSH and similar compounds (Zitting and Heinonen, 1980). Meacher and Menzel (1999), using cultured adult rat type II alveolar cells, demonstrated with a fluorogenic reagent that the depletion of GSH by 1-5 μmol/L of acrolein follows the nonenzymatic rate constant for the forward reaction. In addition, rates of GSH depletion by other alkenals and alkanals correlated with LD50 values for each compound, leading the authors to conclude that structure-activity relationships are useful for predicting toxicity of aldehydes.

Adams and Klaidman (1993) reported that acrolein and its GSH adduct glutathionyl-propionaldehyde can directly induce oxygen radical formation *in vitro*. The enzymes, xanthine oxidase and ALDH, were found to interact with this adduct to produce $O_2 \cdot^-$ and HO·. Acrolein was also oxidized by xanthine oxidase to produce acroleinyl radical $O_2 \cdot^-$.

It would appear that when a reactive chemical, such as acrolein, comes in contact with a cell, its first site of attack is the plasma membrane. Srivastava et al. (1992) have, in fact, reported that in *in vitro* studies, acrolein interaction at low concentration inhibited rat liver plasma membrane enzymes (i.e., ATPases) to varying degrees and attacked membrane surface proteins, suggesting at least a superficial change could lead to changes in ion transport and membrane potential. Pompella et al. (1991), on the other hand, determined that alkylation of macromolecules by acrolein is not a major factor in liver cell injury. Although acrolein was observed to rapidly bind to cytosolic soluble proteins and membrane-bound thiols *in vitro*, acrolein avoided membrane-bound thiols *in vivo*, even after GSH depletion. Gurtoo et al. (1981) have obtained convincing evidence that acrolein binds to cytochrome P450 resulting in its denaturation.

The role of acrolein in suppressing host defense mechanisms is also an area of increasing research interest. Using cultured human alveolar macrophages, Li et al. (1997) demonstrated that acrolein *in vitro* inhibited the release of the cytokines IL-1β, TNF-α, and IL-12, and induced apoptosis and necrosis in human alveolar macrophages. Subsequently, Li and Holian (1998) provided preliminary information that inhibition of the transcription factor for many cytokine genes, NF-κB, may be responsible for the inhibition of cytokine release as well as acrolein induced apoptosis in alveolar macrophages. Most recently, Li et al. (1999) found that acrolein inhibited phosphorylation of the principal regulator of NF-κB. The activated form of NF-κB is of relevance to genes encoding cytokines involved in immune and proinflammatory responses, including viral genomes such as human immunodeficiency virus, type 1 (Müller et al., 1993). NF-κB also plays a central role in expression of adhesion molecules in human vascular endothelial cells (Collins et al., 1995). Since acrolein *in vitro* acts as an inhibitor of NF-κB activity, immunomodulation by acrolein should be regarded as an area for further investigation, particularly at environmental levels.

Acrolein also has been demonstrated to inhibit in a dose-dependent manner the *in vitro* synthesis of prostaglandin E_2 in rat resting and zymosan-stimulated alveolar macrophages (Grundfest et al., 1982). This resulted in a relative increase in release of thromboxane B_2, the inactive form of the potent vasoconstrictor, thromboxane A_2. GSH protected the macrophages from acrolein-induced changes in arachidonic acid metabolism. Acrolein was found to increase bronchial reactivity to intravenously administered acetylcholine in guinea pigs with a maximum at 2-4 hour postexposure. Upon bronchoalveolar lavage, thromboxane B_2 and prostaglandin $F_{2\alpha}$ was shown to be increased immediately after exposure followed by an influx of neutrophils 24 hours later (Leikauf, 1991). Prostaglandin $F_{2\alpha}$ has been demonstrated to increase bronchial reactivity in asthmatics (Mathe et al., 1973). Evidence suggests that acrolein-induced bronchial hyperresponsiveness may be the result of damage to epithelial cells (Costa et al., 1986).

Using freshly isolated rat tracheal smooth muscle myocytes, Hyvelin et al. (2000, 2001) found that acrolein modulates the Ca^{++} signaling pathway by increasing production of inositol triphosphate and does not directly affect the muscarinic cholinoceptor or inositol triphosphate receptor sensitivity. This extends previous work (Ben-Jebria et al., 1993, 1994) in which it was reported that acrolein exposure increased the reactivity of human bronchial and rat tracheal rings to muscarinic agents in a dose-dependent manner.

The mode of action whereby acrolein produces nasal irritation in Fischer 344 rats has been investigated by Morris et al. (1999). At 20 ppm (46 mg/m^3) for 50 minutes, acrolein induced vasodilation and plasma protein extravasation into nasal tissues; both responses were inhibited by capsaicin. Vasodilation, but not protein extravasation, was also elevated over controls at 2, 5, and 10 ppm (4.6, 11.4, and 22.9 mg/m^3). Inhibition by capsaicin was regarded as evidence of C-fiber involvement. While there was evidence of tachykinin release, substance P and neurokinin were not thought to be involved. On the other hand, exposure of female Wistar rats to 22, 81, and 249 ppm (50.4, 185.5, and 570.2 mg/m^3) acrolein for 10 minutes resulted in a significant decrease in nerves of the trachea immunoreactive for substance P (less so for calcitonin gene-related peptide) with the effect spreading further down the respiratory tract with increasing dose (Springall et al., 1990). There appeared to be no evidence of nerve damage.

It is clear that GSH plays a major role in acrolein toxicity. The depletion of GSH and the formation of acrolein GSH adducts resulting in an increase in reactive oxygen species is undoubtedly a major factor in the induction of toxic and mutagenic effects. Although membrane binding, inhibition of regulatory proteins, and modulation of cytokine release at the gene transcription level have been demonstrated, their importance at low levels of exposure is still uncertain.

Bronchitis, asthma, and cystic fibrosis, marked by inflammation and mucus hypersecretion, can be caused or exacerbated by airway pathogens or irritants including acrolein, an aldehyde present in tobacco smoke. To determine whether acrolein and inflammatory mediators alter mucin gene expression, steady-state mRNA levels of two airway mucins, MUC5AC and MUC5B, were measured (by RT-PCR) in human lung carcinoma cells (NCI-H292). MUC5AC mRNA levels increased after >/=0.01 nM acrolein, 10 µM prostaglandin E2 or 15-hydroxyeicosatetraenoic acid, 1.0 nM tumor necrosis factor-alpha

(TNF-alpha), or 10 nM phorbol 12-myristate 13-acetate (a protein kinase C activator). In contrast, MUC5B mRNA levels, although easily detected, were unaffected by these agonists, suggesting that irritants and associated inflammatory mediators increase mucin biosynthesis by inducing MUC5AC message levels, whereas MUC5B is constitutively expressed. When transcription was inhibited, TNF-alpha exposure increased MUC5AC message half-life compared with control level, suggesting that transcript stabilization is a major mechanism controlling increased MUC5AC message levels. Together, these findings imply that irritants like acrolein can directly and indirectly (via inflammatory mediators) increase airway mucin transcripts in epithelial cells (Borchers et al., 1999a).

4.6. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION---ORAL AND INHALATION

4.6.1. Oral Administration

No human studies are available regarding exposure by the oral route. Several animal studies are available.

Gavage studies (13 weeks) in F344 rats and B6C3F1 mice (NTP, 1995) demonstrated dose-response increases in gastrointestinal effects. Hemorrhage, necrosis, and chronic inflammation of the forestomach and glandular stomach with increased mortality was observed in the high-dose groups of both species (10 mg/kg-day in rats; 20 mg/kg-day in mice), and in both males and females. Hemorrhage of the glandular stomach was also seen at the next two lower dose levels in males and the next lower level in females. When Sprague-Dawley rats were administered acrolein in water by gavage at much lower doses for 2 years (Parent et al., 1992c), early mortality occurred. However, there was no gross or histopathological evidence of stomach lesions or histopathological evidence in other organs. Mortality occurred at a much lower dose (0.5 mg/kg-day) and early mortality was significant even after correction for gavage error. When this protocol was applied to CD-1 mice (Parent et al., 1991b), the primary effect was mortality in high-dose animals (4.5 mg/kg-day), again without any adverse clinical or histopathological findings.

Wistar rats were gavaged with 2.5 mg/kg-day for a period of 45 days, but clinical observation and histopathology were not part of the protocol (Arumugam et al., 1999b). At the end of dosing, there was significant reduced activities of citric acid cycle enzymes and cytosolic and mitochondrial GSH in the liver, as well as oxidative damage to mitochondrial membrane integrity. These effects may contribute to an increased mortality due to progressive mitochondrial damage over time.

Early mortality observed in the F344 and Sprague-Dawley rats is considered to be the critical effect that occurred at the lowest dose level (i.e., in the Parent et al., 1992c study). At higher dose levels in F344 rats and B6C3F1, gastrointestinal damage accompanies increased mortality.

Reasons for no reported observations of stomach lesions in Sprague-Dawley female rats

at the highest dose (2.5 mg/kg) of the Parent et al. (1992c) study compared with forestomach squamous epithelial hyperplasia observed in female F344 rats in the NTP (1995) study at 1.25 mg/kg-day are not readily apparent, but may relate to differences in strain sensitivity or vehicle. The vehicle dose volume was 5 ml/kg in the NTP (1995) study and 10 ml/kg in the Parent et al. (1992c) study, and there may have been reduced local gastric mucosal irritation and pathology by virtue of dilution. There were also differences in the vehicle solution and, possibly, the stability of the dosing solutions. Parent et al. (1992c) conducted stability studies on acrolein in water, and monitored the stability of their dosing solutions (reporting losses of less than 10% for 3 hours at room temperature). They used a stabilizing agent, 0.25% hydroquinone, in the stock solution, and prepared dosing solutions daily. The NTP (1995) study used a dose vehicle of 0.5% methylcellulose in deionized water, and no information was available on stability or stabilizing agents.

For the mouse results, there is a similar divergence between the absence of reported forestomach lesions in the CD-1 mice at 4.5 mg/kg in the Parent et al. (1991b) study compared with effects observed in female B6C3F1 at 2.5 mg/kg in the NTP (1995) study. Species differences and dose volume again may have accounted for the observed differences in response. Dose volume in the NTP (1995) study for mice was 10 ml/kg, and was unspecified in the Parent et al. (1991b) study.

An explanation for the early cumulative mortality in the absence of other significant effects is not provided by Parent et al. There is mention of the significant decrease in creatinine phosphokinase. Creatinine phosphokinase (CPK), also referred to as creatine kinase (CK), is a widespread enzyme that catalyzes the reversible oxidation of creatine (by adenosine triphosphate (ATP)) to creatine phosphate. CK occurs as three different isoenzymes, each composed of two polypeptide chains, B (brain derived) and M (muscle derived). Skeletal muscle and cardiac muscle have a very high CK content but different isozyme ratios, with very low percentage (less than 5%) of CK-MB in skeletal muscle and a higher percentage (20-30%) of CK-MB in heart. Brain, prostate, thyroid, gut and lung have predominantly CK-BB; plasma has predominantly CK-MM with less than 6% CK-MB. Usually, the heart is the only tissue in which the amount of CK-MB exceeds 5%. Serum CK is a very sensitive indicator of target tissue damage, with elevated serum levels within 4-6 hours post injury. If not progressive, CK serum levels decline to normal within 24 hours. Illness of the nervous system, heart, or musculature can also produce elevated serum CK levels (Hayes, 1994). Low serum CK levels have been associated with impaired energy metabolism or reduced skeletal muscle function from phosphate depletion (Brautbar et al., 1983), connective tissue disease including rheumatism (Wei et al., 1981; Lee et al., 2000) or alcoholic liver disease (Nanji and Blank, 1981).

The research demonstrating acrolein's high reactivity, low systemic distribution, toxicity at the point of entry, pronounced decreases in citric acid cycle enzymes and in liver GSH, depression of serum CK levels, and increased mitochondrial damage in the Wistar rat are suggestive of pathologies that could potentially be responsible for early mortality. In the absence of gastrointestinal histopathology, one could postulate that there was sufficient subclinical gastrointestinal toxicity to interfere with normal metabolic processes and possibly absorption of essential nutrients sufficient to lead to early mortality. Further research is needed

to support a more definitive understanding of acrolein's mode of action.

4.6.2. Inhalation Exposure

Acute Exposures:

In the few clinical studies that have examined the effects of low-level acrolein exposure, it is clear that measured levels considerably lower than 1 ppm (2.3 mg/m^3) elicit subjective complaints of eye and nasal irritation and a decrease in the respiratory rate (Weber-Tschopp et al., 1977; Sim and Pattle, 1957). Such effects should be considered adverse based upon longer-term studies in laboratory animals at higher concentrations that have demonstrated more severe nasal lesions as well as pronounced adverse effects on lung function leading to lethality.

Acrolein was reported by male and female volunteers (53) as causing eye irritation beginning at concentrations of 0.09 ppm (0.2 mg/m^3) and higher after 35 minutes, nasal irritation at concentrations of 0.26 ppm (0.6 mg/m^3) and higher, and a decreased respiratory rate at 0.6 ppm (1.4 mg/m^3) after 35 minutes (Weber-Tschopp et al., 1977). In an inhalation study by Sim and Pattle (1957) male volunteers (12) reported 0.8 ppm (1.9 mg/m^3) for 10 minutes acrolein as extremely irritating. It was not clear how the acrolein was administered in the latter study, by mask or in a chamber.

Signs of respiratory distress and irritation were noted in rats exposed to as low as 4.8 ppm (11 mg/m^3) for one hour (Ballantyne et al., 1989). These clinical indicators were not observed when rats were exposed to levels of 0.25 to 1.4 ppm (0.6 to 3.2 mg/m^3) for 6 hours or 6 hr/day for 3 days (Cassee et al., 1996b). Nor were there any acrolein-induced histopathological nasal lesions after 6 hours of exposure. Other exposure studies of laboratory animals involved much higher concentrations with expected results of lethality associated with respiratory distress.

The limited information from studies with human volunteers suggests that levels below 1 ppm (2.3 mg/m^3) can be expected to elicit subjective signs of nasal and eye irritation and affect the breathing rate. The limited human data as well as data from animal studies at higher concentrations and longer durations suggest that clinical symptoms of distress (and histopathological lesions in the case of laboratory animals) become more pronounced as exposure increases.

Long-Term Exposures:

No chronic exposure human or laboratory animal studies are available.

Two 90-day animal studies and four 60-day or more exposure studies have been reported. Feron et al. (1978) dosed groups of Syrian golden hamsters, Wistar rats, and Dutch rabbits 30 hours/week for 90 days to 0, 0.4, 1.4, and 4.9 ppm (0, 0.9, 3.2, and 9.2 mg/m^3) acrolein in whole-body exposure chambers. At the highest dose, mortality occurred in rats, while ocular and nasal irritation, growth depression and histopathologic changes in the respiratory tract were seen in all three species. At the intermediate dose, squamous metaplasia and neutrophilic infiltration of the nasal mucosa was seen in the rat, whereas in hamsters, minimal inflammatory changes were seen in the nasal cavity. No effects were detected in the nasal region in the mid and low-dose rabbits.

Slight inflammatory effects were reported in the nasal mucosa of one of 12 rats in the 0.4 ppm (0.9 mg/m^3) group. Thus, the LOAEL (minimal) for rats, the most sensitive species, was 0.4 ppm (0.9 mg/m^3) for slight inflammatory changes of the nasal mucosa. The NOAEL for hamsters was determined to be 0.4 ppm (0.9 mg/m^3), and the LOAEL was 1.4 ppm (3.2 mg/m^3) based on inflammatory changes in the nasal cavity. The NOAEL for rabbits was determined to be 1.4 ppm (3.2 mg/m^3) with a LOAEL of 4.9 ppm (9.2 mg/m^3).

Additional evidence in support of a minimal LOAEL of 0.4 ppm (0.9 mg/m^3) from Feron et al. (1978) is provided by the studies of Kutzman and colleagues (Kutzman, 1981; Kutzman et al., 1985; Costa et al., 1986) and Cassee et al. (1996b). Kutzman and colleagues exposed male Fischer 344 rats (50/group) via inhalation to acrolein at 0, 0.4, 1.4, or 4.0 ppm (0, 0.9, 3.2 or 9.2 mg/m^3) 6 hr/day, 5 days/week for 62 exposure days (consecutive weekdays, except for weekends, for 12.4 calender weeks). When rats were evaluated on the 6th day postexposure, some evidence of functional deficits was found at 0.4 ppm (0.9 mg/m^3) and more substantial damage at the highest concentration (4 ppm; 9.2 mg/m^3). The Cassee et al. (1996b) 3-day nose-only study in the rat reported slight nasal effects at lower concentrations (0.25 ppm; 0.6 mg/m^3) than in the Feron et al. (1978) whole-body inhalation study. The Cassee et al. (1996b) study was designed to evaluate the severity of effects from mixtures versus single chemical exposure, and used a higher resolution analysis to detect any interactions. The observed effects at lower levels in the Cassee et al. (1996b) study may be due to the higher resolution analysis of the nasal tract, i.e., six levels of sampling compared to only three by Feron et al. (1978). Alternatively, the nose-only exposure chamber may have delivered more dose or had a different dosimetric distribution to the nasal epithelium as compared to exposure in the whole-body chambers used by Feron et al (1978). In a whole body chamber, rats may bury their noses in their fur during daytime sleeping postures resulting in the animals receiving less exposure than assumed. Because the Feron et al. (1978) study was much longer in duration, it is also possible that some adaptation to the irritant effects of acrolein occurs with increasing duration, or that cessation of exposure for 2 days each week provided a period during which partial recovery from nasal effects might occur. Collectively, the principal study and supporting studies (Kutzman, 1981; Kutzman et al., 1985; Costa et al., 1986; Feron et al., 1978; Cassee, 1996b) provide support for a minimal LOAEL of 0.4 ppm (0.9 mg/m^3).

Lyon et al. (1970) exposed rats, guinea pigs, dogs, and monkeys to 0, 0.22, 1.0 and 1.8 ppm (0, 0.5, 2.3, and 4.1 mg/m^3) acrolein for 24 hr/day for 90 days. A LOAEL of 1 ppm (2.3 mg/m^3) could be derived based upon inflammation in several organs of one or more of the species; however, there was a principal deficiency in this study because of the absence of concurrent control groups, making it unclear whether or not the changes were directly related to an exposure to acrolein.

Acrolein is highly reactive and can induce toxicity in a variety of ways. An increase in reactive oxygen species resulting from reaction with and depletion of glutathione is considered to be the primary mechanism of toxicity (Zitting and Heinonen, 1980; Arumugam et al., 1999a). Reactions with cell membrane proteins and inhibition of regulatory proteins may also play a role.

As a result of acrolein's high degree of reactivity during inhalation, deposition occurs

primarily in the nasal mucosa with the accompanying pathological effects. As concentrations increase, penetration and toxicity occur deeper within the respiratory system. Effects in other organs such as the liver were occasionally reported (Lyon et al., 1970), but only at concentrations higher than those affecting the respiratory system, and the mechanism(s) for the effects are uncertain given acrolein's high reactivity. Therefore, the nasal mucosa is considered to be the critical target site, with a minimal LOAEL of 0.4 ppm (0.9 mg/m^3) in the most sensitive species, the rat (Feron et al., 1978). The data were not sufficient to derive a NOAEL.

4.7. WEIGHT-OF-EVIDENCE EVALUATION AND CANCER CHARACTERIZATION

Under the Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999), the potential carcinogenicity of acrolein cannot be determined because the existing "data are inadequate for an assessment of human carcinogenic potential for either the oral or inhalation route of exposure."

There are no adequate human studies of the carcinogenic potential of acrolein. Collectively, experimental studies provide inadequate evidence that acrolein causes cancer in laboratory animals. Specifically, two inhalation bioassays in laboratory animals are inadequate to make a determination because of protocol limitations. Two gavage bioassays failed to show an acrolein-induced tumor response in two species of laboratory animals. The finding of suggestive evidence of an extra-thoracic tumorigenic response in a drinking water study in female rats was not supported in a reanalysis of the data by an independently-convened pathology working group. Questions were also raised about the accuracy of the reported levels of acrolein in the drinking water from this study. A skin tumor initiation-promotion study was negative, and the findings from an intraperitoneal injection study were of uncertain significance. Although acrolein has been shown to be capable of inducing sister chromatid exchange, DNA cross-linking and mutations under certain conditions, its highly reactive nature and the lack of tumor induction at portals of entry make it unlikely that acrolein reaches systemic sites at biologically-significant exposure levels. The observations of positive mutagenic results in bacterial systems occurred at high concentrations near the lethal dose.

This evaluation replaces the cancer assessment for acrolein added to the IRIS data base in 1988. Under the Risk Assessment Guidelines of 1986 (EPA/600/8-87/045) applied at that time, acrolein was classified as a possible human carcinogen (Category C). The 1988 classification for acrolein was based on the increased incidence of adrenal cortical adenomas in female rats and carcinogenic potential of an acrolein metabolite, its mutagenicity in bacteria, and its structural relationship to probable or known human carcinogens. The updated cancer characterization considered new study results and reevaluated previous studies.

Human Studies:

Ott et al. (1989) reported a series of nested case-control studies in relation to various work areas, specific chemicals, and chemical activity groups. An odds ratio of 2.6 for nonlymphocytic leukemia was found for workers who had exposure to acrolein during employment. The small number of cases (3) and the likelihood of exposure to other chemicals, however, provide inadequate evidence of acrolein-induced leukemia or of the carcinogenic potential of acrolein.

Laboratory Animal Studies:

Two cancer bioassays failed to show an increase in tumor incidence when rats (Parent et al., 1992c) and mice (Parent et al., 1991b) were administered acrolein by gavage. In both studies the maximum tolerated dose was demonstrated by a significant increase in mortality.

Although administration of acrolein in drinking water to female F344 rats (Lijinsky and Reuber, 1987) resulted in an elevation of adrenocortical tumors (only in females) over 104-124 weeks (total dose=115 mmoles), the increase was only significant when the tumors were combined with hyperplastic nodules. This incidence of adrenal lesions appeared to exceed the historical control range for female F344 rats reported by Goodman et al. (1979) and Solleveld et al. (1984). However, because of the difference in findings between the Parent et al. (1992c) and Lijinsky and Reuber (1987) studies, an independent pathology working group (PWG) was convened to reevaluate the cortical tumors reported by Lijinsky and Reuber (1987). According to the PWG (cited in Parent et al., 1992c), the "slightly elevated incidence of pheochromocytomas (3/20; 15%) in the treated females were well within limits for historical controls (3/34; 9%) and were of no biological significance," and "it is the opinion of the PWG that there is no evidence of any carcinogenic effect of acrolein on the adrenal glands of female rats in this study." The PWG noted that the slides evaluated were taken from archived tissue blocks because the original slides for the high-dose females were not available for re-examination and only one of the original control slides was available. Parent et al. (1992c) identify additional weaknesses in the Lijinsky and Reuber (1987) studies that brings into question the dose levels and the overall conclusions. They reexamined the Lijinsky and Reuber (1987) reported intake levels, and calculated an estimated daily dose of 50 mg/kg BW for the high-dose group under the assumption that each of the four rats/cage in the group drank an equal share of the 80 ml delivered in the drinking water container. This dose, however, exceeds the LD_{50} for rats, and would have been ingested for five days a week for 132 weeks. Parent et al. (1992c) suggest that the acrolein in the drinking water solution might not have been as stable as Lijinsky and Reuber (1987) assumed, or that intake levels were lower than reported. An additional question was raised as to why Lijinsky and Reuber (1987) observed no increases in adrenal tumors from comparable studies with the acrolein parent compounds – diethylacetal, acrolein oxime, and allyl alcohol – compounds that are expected to be hydrolyzed to acrolein in the stomach acids.

Evidence that acrolein may have some tumor-initiating activity was shown in the study by Cohen et al. (1992). Intraperitoneal injection of acrolein, 2 mg/kg BW for either 6 or 21 weeks into male Fischer 344 rats did not induce cancer, but 6 weeks treatment with acrolein, followed by 20 weeks of uracil in the diet induced urinary bladder papillomas in 18 of 30 rats

compared with 8 of 30 administered uracil alone. A caveat in this study is that the incidence of nodular
hyperplasias (considered to be precursors to papillomas) was considerably lower in the acrolein pretreated group (10 of 30) compared with the solvent control/uracil only group (21 of 30). When the incidence of nodular hyperplasias and papillomas were combined, there were no significant differences between the two groups. Acrolein was too toxic to evaluate its tumor promoting potential, and the impact of its cytotoxicity on conclusions about its tumor initiating potential can not be determined from this study alone.

Based upon the (1) negative findings in 2/3 oral exposure studies that evaluated the carcinogenic potential of acrolein, (2) questionable findings in one study with positive results (Lijinsky and Reuber, 1987), and (3) uncertainty about the significance of the i.p. study for initiating potential (Cohen et al., 1992), the oral exposure data is considered inadequate to determine acrolein's carcinogenic potential.

Acrolein did not produce a carcinogenic response in two inhalation studies, one in hamsters (Feron and Kryusse, 1977) and one in rats (Le Bouffant et al., 1980). The use of only one exposure concentration and less than lifetime exposure duration limits inferences that can be drawn from these studies about the carcinogenic potential of acrolein from an inhalation exposure.

Genotoxicity Studies:

In vitro, acrolein has been shown to induce DNA adducts in a variety of cell types as well as mutagenesis in *Drosophila* and microorganisms under certain conditions, but there is only limited information regarding the ability of acrolein to induce mutations in normal mammalian cells. In mammalian cell *in vitro* assays, acrolein has been shown to induce sister chromatid exchange, DNA cross-linking, and binding to DNA polymerase. Even in the *in vitro* assays, acrolein is so reactive that special techniques must generally be employed to reduce cytotoxicity and induce positive effects. While mutagenic activity has occasionally been shown, positive results generally occurred only in a narrow, near lethal, dose range.

There have been conflicting results reported in the literature for *in vitro* mutagenicity. In a series of Ames assays, Parent et al. (1996b) proposed an explanation for the conflicting data by considering the presence or absence of non-DNA nucleophiles from the S9 activation mixture, in the test chemical solution, or in the plating solutions. They suggested that, in the presence of non-DNA nucleophiles, acrolein will rapidly and indiscriminately react with any available species and not reach the DNA target.

Mode of Action:

Acrolein and its GSH adduct directly induce oxygen radical formation *in vitro* (Adams and Klaidman, 1993) that could induce DNA damage. Extensive lung damage due to lipid peroxidation after inhalation exposure of rats to 1 or 2 ppm (2.3 or 4.6 mg/m^3) acrolein was demonstrated by Arumugam et al. (1999a); also antioxidant levels were significantly decreased. The highly reactive nature of acrolein, however, and the studies supporting the lack of systemic distribution of acrolein suggest that acrolein is not likely to reach potential target sites at a

sufficient concentration to initiate a carcinogenic process in mammalian species. According to Beauchamp et al. (1985), acrolein administered by the inhalation route is retained primarily in the upper respiratory tract because of its reactivity. Some evidence for systemic uptake following oral exposure was noted by Draminski et al. (1983), however, the large doses used (10 mg/kg) would be expected to induce cellular damage, which may allow some absorption. Tissues at the site of contact are, therefore, expected to be most highly exposed, and no evidence of tumor induction in the respiratory tract, skin or gastrointestinal tract has been reported. Studies by Parent et al. (1996a, 1998) indicate little systemic distribution to tissues.

4.8. SUSCEPTIBLE POPULATIONS AND LIFE STAGES

4.8.1. Possible Childhood Susceptibility

The results from animal studies indicate that ingested or inhaled acrolein does result in adverse developmental or teratogenic effects. The only indication from case histories, clinical studies or epidemiology studies of an increased susceptibility of children to acrolein toxicity is for children who have respiratory conditions that are marked by inflammation and mucus hypersecretion such as bronchitis, asthma, or cystic fibrosis.

A number of epidemiological and clinical studies support an association between air pollutants and increased prevalence of respiratory symptoms and emergency room visits (Leikauf, 2002). Some air pollutants are asthmagens, i.e., they can induce asthma and evoke asthma symptoms through immunologic mechanisms. Others do not induce asthma, but can augment the symptoms and exacerbate asthma. More research is needed to fully characterize the antigenic or asthma inducing potential of acrolein. Acrolein is one of the 33 Hazardous Air Pollutants (HAPs) of greatest concern for exposure and health effects, and one of the compounds that does have the potential to exacerbate asthma (TRI, 2003; Leikauf, 2002). *In vitro* mechanistic studies also indicate that irritants like acrolein can directly and indirectly (via inflammatory mediators) increase airway mucin transcripts in epithelial cells (Borchers et al., 1999a). Bronchial hyperresponsiveness and increases in inflammatory mediators following acrolein exposure have also been reported in a number of animal studies (Leikauf, 1991; Leikauf et al., 1989a).

Because children have higher rates of asthma compared to adults, and children tend to have more severe asthma symptoms due to their relatively smaller airways, children may have an increased susceptibility to adverse effects from an agent that can exacerbate asthma.

4.8.2. Possible Gender Differences

There are no human data and only limited, equivocal animal data on gender differences in response to acrolein.

No sex-related differences in toxicological responses to acrolein were reported in dogs exposed for up to 53 weeks orally to 2 mg/kg-day acrolein (Parent et al., 1992a). Parent et al. (1992c) found that female Sprague-Dawley rats intubated daily with 2.5 mg/kg acrolein had a

statistically significant ($p < 0.001$) decrease in survival over a two-year period compared to male rats, which showed some evidence of dose-related mortality during the first year of treatment, but not the second. On the other hand, the Parent et al. (1991b) study in mice showed increased mortality in high-dose males only.

Sex-related differences were absent in rats, guinea pigs, monkeys and dogs exposed 40 hours/week for 6 weeks, or continuously for 90 days to inhaled concentrations of acrolein up to 3.7 ppm (8.5 mg/m^3) (Lyon et al., 1970). Feron et al. (1978) reported no sex differences in responses among rats, hamsters and rabbits exposed to inhaled acrolein 30 hours/week for 13 weeks. LC_{50} values were nearly identical for male and female Sprague-Dawley rats following 1- and 4-hour inhalation exposures to acrolein (Ballantyne et al., 1989). Kutzman et al. (1985), however, reported that 32 of 57 male Fischer 344 rats exposed 6 hours/day, 5 days/week for 12.4 weeks to 4.9 ppm (9.2 mg/m^3) inhaled acrolein died compared with none of 8 exposed females.

4.8.3. Other

As noted in Section 4.5, depletion of GSH increases sensitivity to acrolein cytotoxicity and the induction of mutations. Also, male Wistar rats intubated with acrolein for 45 days had decreased GSH levels leading to mitochondrial damage in liver. Individuals with metabolic defects, such as decreased ability to synthesize GSH, would be expected to be more sensitive to the toxicity of acrolein. Differences in cytochrome P450 activity may affect sensitivity in humans, although this possibility has not been tested.

As discussed in section 4.8.1, acrolein is a respiratory irritant that can exacerbate asthma. Individuals who are asthmatics or who suffer from chronic bronchitis or other chronic pulmonary diseases are considered to be at an increased risk of respiratory symptoms from acrolein inhalation exposure.

Inhalation studies in Sprague-Dawley rats selected for either susceptibility (DS) or resistance (DR) to salt-induced hypertension reported a marked difference in the pulmonary pathology observed in DS and DR rats exposed to the highest dose (4.0 ppm; 9.2 mg/m^3) of acrolein. The lungs of the DS rats exhibited severe airway epithelial necrosis with edema and hemorrhage, while surviving high-dose DR rats developed primarily a proliferative change. Pathologic changes in the two lower dose groups were similar, but less severe. Differences in other respiratory measures between the DS and DR groups at the two lower doses were minimal and not dose-dependent. Reasons for the difference in susceptibility of DS and DR rats at the high dose of acrolein are unclear (Kutzman et al., 1984, 1986). These results suggest that people with hypertension may be more sensitive to respiratory effects from high exposures to acrolein.

5. DOSE-RESPONSE ASSESSMENTS

5.1. ORAL REFERENCE DOSE (RfD)

5.1.1. Choice of Principal Study and Critical Effect--with Rationale and Justification

There are no chronic human studies suitable for dose-response assessment.

Long-term studies with Sprague-Dawley rats and CD-1 mice (Parent et al., 1991b; 1992c), dogs (Parent et al., 1992a), and Fisher-344 rats and $B6C3F_1$ mice (NTP, 1995) indicate that mortality and stomach lesions are the predominant effects from oral exposure. The two-year gavage study with the Sprague-Dawley rat (Parent et al., 1992c) is considered to be the most suitable for developing an RfD. This study was a lifetime study that used an adequate number of animals (70/sex/group), compared with only 10/sex in the 13-week NTP (1995) study. Parent et al. (1992c) reported a statistically significant increase in mortality for female Sprague-Dawley rats over the two-year span of the study at doses as low as 0.5 mg/kg-day. Based on this reported mortality as the critical effect, the frank effect level (FEL) in rats was determined to be 0.5 mg/kg-day, and the NOAEL to be 0.05 mg/kg-day. The FEL is defined as "a level of exposure or dose which produces irreversible, adverse effects at a statistically- or biologically-significant increase in frequency or severity between those exposed and those not exposed" (IRIS, 2003).

The NTP (1995) 13-week gavage study provides supporting evidence that treatment causes early mortality. The NTP doses were higher than in the Parent et al. (1992c) study, and mortality was accompanied by the occurrence of observable glandular stomach and forestomach lesions. The stomach lesions observed at doses as low as 0.75 mg/kg-day in mice were not observed in the Parent et al. (1992c) study. Reasons for no reported observations of stomach lesions in Sprague-Dawley female rats at the highest dose (2.5 mg/kg) of the Parent et al. (1992c) study compared with forestomach squamous epithelial hyperplasia observed in female F344 rats in the NTP (1995) study at 1.25 mg/kg-day are not readily apparent, but may relate to differences in strain sensitivity or vehicle. The vehicle dose volume was 5 ml/kg in the NTP (1995) study, and 10 ml/kg in the Parent et al. (1992c) study for rats, and there may have been reduced local gastric mucosal irritation and pathology by virtue of dilution. There were also differences in the vehicle solution and, possibly, the stability of the dosing solutions. Parent et al. (1992c) conducted stability studies on acrolein in water, and monitored the stability of their dosing solutions (reporting losses of less than 10% for 3 hours at room temperature). They used a stabilizing agent, 0.25% hydroquinone, in the stock solution, and prepared dosing solutions daily. The NTP (1995) study used a dose vehicle of 0.5% methylcellulose in deionized water, and no information was available on stability or stabilizing agents.

For the mouse results, there was a similar divergence between the absence of reported forestomach lesions in the CD-1 mice at 4.5 mg/kg in the Parent et al. (1991b) study compared with effects observed in female B6C3F1 at 2.5 mg/kg in the NTP (1995) study. Species differences and dose volume again may have accounted for differences in response. Dose

volume in the NTP (1995) study for mice was 10 ml/kg, and was unspecified in the Parent et al. (1991b) study.

The Parent et al. (1992a) dog study (6 animals/sex/group) was deemed unsuitable as a principal study because of uncertain amounts of retained dose following vomiting from administration of acrolein. Dogs were administered acrolein (0.1% aqueous) in gelatin capsules at doses of 0, 0.1, 0.5, and 1.5 mg/kg-day, 7 days/week for 53 weeks to 6 beagle dogs/group. After four weeks the high-dose was increased to 2.0 mg/kg-day. At termination all dogs were subjected to full necropsy and histological examination. Body weights and food consumption were not significantly affected by treatment. The most commonly reported effect was a dose-dependent increase in the frequency of vomiting. The incidence, however, decreased greatly with duration of treatment. Observed treatment-related lesions on gross necropsy included vascular congestion and mucosal reddening of the gastrointestinal tract. The results of this study are difficult to evaluate. Although there were some alterations in blood parameters, they were unsupported by pathology evaluation. Some of the clinical parameters may have been changed as a result of vomiting. Moreover, adaptation appears to occur, as noted by the decreased vomiting with duration of exposure. Lack of changes in food consumption and body weight also suggest that any effects noted were mild.

A rat study by Arumugam et al. (1999b) provides support and a plausible explanation for the mortality increases reported in the Parent et al. (1992c) study. Arumugam et al. (1999b) exposed male Wistar rats, 5 animals/group, daily to acrolein via intubation (2.5 mg/kg BW) for 45 days. Damage to mitochondria, through loss of mitochondrial lamellae of the cristae, was demonstrated along with a decrease in the availability of GSH, a substrate for glutathione peroxidase, and a decrease in activities of citric acid cycle enzymes, resulting in decreased energy production in liver cells. The duration of the study was less than subchronic in duration and included only a single dose level. Also, the incidence of mortality, if any, was not reported in this study. These results indicate that at least some uptake occurs from the oral route, however, the stomach was not examined by light microscopy.

With regard to the relevance of gavage bolus dose to human exposures, the concentration of the administered dose can affect the time course and degree of severity of toxicity at the point of entry. Rats have both a forestomach and a glandular stomach, while humans have only a glandular stomach. The glandular stomach is more resistant than the forestomach to pH changes and irritation. The residence time in the forestomach (of approximately 2 hours) is sufficiently long compared to the reaction time for toxicity with airway tissue observed in inhalation studies (i.e., microseconds) so that the dose to the glandular stomach may be much lower than that to the forestomach (TERA, 1998). The dog is a better model for glandular stomach toxicity than the rat; however, Parent et al. (1992a) administered acrolein (0.1% aqueous) in gelatin capsules to beagle dogs, so the dose concentration to the glandular tissue is not known. In lieu of studies that provide data on glandular stomach toxicity, the Parent et al. (1992c) rat study remains the most suitable choice.

5.1.2. Methods of Analysis

The development of a RfD was based upon a NOAEL for mortality as determined from the Parent et al. (1992c) study, and the application of uncertainty factors. A benchmark dose approach was unsuitable for RfD development because the data in the Parent et al. (1992c) study were presented graphically, with statistical evaluation at one- and two-year time points, but no numerical values. Moreover, the NOAEL derived from the Parent et al. (1992c) study and used as the basis for the RfD is from a statistically significant increase in mortality, a frank effect. A benchmark dose analysis would not be appropriate when the dose-response is for early cumulative mortality.

5.1.3. RfD Derivation—Including Application of Uncertainty Factors (UFs)

The NOAEL for mortality of 0.05 mg/kg-day from the Parent et al. (1992c) study was used as the point of departure for calculating the RfD. A total uncertainty factor of 100 was applied to this point of departure: 10 for interspecies extrapolation (UF_A) and 10 for susceptible human subpopulations (UF_H).

A default UF_A of 10 was applied to account for interspecies differences between laboratory animals and humans. No information was available to support a change from the default.

A default UF_H of 10 was applied for intraspecies uncertainty to account for human variability and sensitive subpopulations, i.e., to account for human variability in the severity or range of response from any given acrolein exposure amongst different individuals.

A UF_D was not applied because the data base for acrolein was considered complete. The available oral data base includes chronic toxicity studies in the rat and mouse, an oral reproductive toxicity study in Sprague-Dawley rats and an oral developmental toxicity study in New Zealand white rabbits. The findings from the oral reproductive and developmental toxicity studies are supported by an inhalation reproductive toxicity study of acrolein in Fisher 344 rats that revealed no reproductive or developmental effects. Acrolein's high reactivity at the point of contact and the evidence for minimal systemic distribution of acrolein obviates the need for additional repeat dose studies.

The RfD is based on a NOAEL from a chronic study, which obviates the need for an uncertainty factor for LOAEL to NOAEL extrapolation or for subchronic to chronic extrapolation.

Application of a total uncertainty factor of 100 to the NOAEL of 0.05 mg/kg-day results in a reference dose (RfD) of 5×10^{-4} mg/kg-day.

5.1.4. Previous Oral Assessment

A RfD for acrolein was not previously available on IRIS.

5.2. INHALATION REFERENCE CONCENTRATION (RfC)

5.2.1. Choice of Principal Study and Critical Effect

Studies on the effects of chronic exposure to inhaled acrolein are unavailable. In the previous IRIS assessment, the principal study was the Feron et al. (1978) study, and the Kutzman (1981) and Kutzman et al. (1985) studies were cited as co-principal. The Kutzman studies (along with Costa et al., 1986) are considered supporting because of the reported lack of adverse histopathology in either the nose or lung in rats exposed to 0.4 ppm (0.9 mg/m^3) on the sixth day of postexposure, as well as some evidence of a functional pulmonary deficit (parenchymal restriction) at this concentration

In the current assessment, the Feron et al. (1978) study was considered the most suitable study for the development of a RfC. Based upon the results of slight nasal effects in 1 of 12 rats, a minimal LOAEL of 0.4 ppm (0.9 mg/m^3) is derived. In this study, 6 Wistar rats/sex/concentration, 10 Syrian golden hamsters/sex/concentration, and 2 Dutch rabbits/sex/concentration were exposed 6 hr/day, 5 days/week for 13 weeks to 0, 0.4, 1.4, or 4.9 ppm (0, 0.9, 3.2, or 11 mg/m^3) acrolein in a whole-body exposure chamber. Incidence data were not reported, but histopathological changes in the nasal cavity, lung, larynx, and trachea were graded as slightly, moderately, or severely affected. Hematological parameters were unaffected by acrolein in rats. Body weight gain was significantly inhibited at the high dose in rats, and less so at the intermediate concentration, but food consumption appeared to be decreased in these groups as well. At the intermediate concentration, both male and female rats showed significantly retarded weight gain ($p<0.05$). Three male and 3 female rats died during exposure at the highest dose. No other deaths considered to be treatment-related were reported in any of the species or exposure groups.

Histopathologic changes described as "slightly affected" were found in the nasal cavity of 1 of 12 rats exposed to 0.4 ppm (0.9 mg/m^3). Severity increased at the higher levels of exposure. No nasal lesions were reported in other species at 0.4 ppm (0.9 mg/m^3). The severity of nasal lesions was concentration-related in all 3 species, most clearly so in the rat. In the 4.9 ppm (11 mg/m^3) groups of all 3 species, slightly to markedly increased lesions were reported in the nasal cavity and trachea; moderate to marked effects were seen in the bronchi and lungs of rats and rabbits (but not hamsters). Based upon the concentration-related severity of lesions, the rat is clearly the most sensitive species, with hamsters and rabbits intermediate in sensitivity.

Although the Feron et al. (1978) study was adequately designed, the incidence of nasal lesions for treated groups was not reported. However, grading of histopathology allowed determination of NOAELs, LOAELs and FELs for the 3 species, determination of the critical target site, and a comparison of sensitivity among the 3 species tested. Other limitations of this study include: (1) an exposure duration of 3 months rather than lifetime, (2) histopathological examination of only three sections of the nasal cavity, (4) lack of characterization of the type of nasal lesions by sex, and (5) only 6 rats/sex were exposed.

Nasal and point of contact effects have been observed in clinical studies with short-term exposure. Exposure to acrolein, at levels as low as 0.09 ppm (0.02 mg/m^3) for 5 minutes, may elicit subjective complaints of eye irritation with increasing concentrations leading to more extensive eye, nose and respiratory symptoms (Weber-Tschopp et al., 1977; Sim and Pattle, 1957).

Studies by Kutzman and colleagues (Kutzman, 1981; Kutzman et al., 1985; Costa et al., 1986) support the Feron et al. (1978) results, with additional evidence of lung deficits on day six post-exposure in male rats following 62 total days of exposure spread over 12.4 weeks. The nasal region had only minimal evidence of submucosal lymphoid aggregates at 0.4 ppm (0.9 mg/m^3). Although the degree of involvement increased to moderate at higher concentrations, more extensive damage to the nasal epithelium was not observed. The absence of extensive damage may have been partly due to adaptation that might have occurred during the 6 days from the last day of exposure to evaluation.

Additional support for acrolein's respiratory effects and association with increased mortality is provided by Kutzman et al. (1984). Dahl rats (derived from the Sprague-Dawley rat) that were either susceptibility (DS) or resistance (DR) to salt-induced hypertension had increased mortality (100% and 40%, respectively) when exposed in whole body inhalation chambers to the highest dose of 0.4, 1.4, and 4.0 ppm (0.9, 3.2, and 9.2 mg/m^3) acrolein dose levels. Dose-response increases in the severity of epithelial lesions occurred in both species with the DS rats being more sensitive, and demonstrating a different pathological response at the high-dose.

Respiratory distress and irritation were observed by Cassee et al. (1996b) following a 3-day inhalation exposure of male Wistar rats to acrolein via nose-only inhalation at levels lower than 0.4 ppm (0.9 mg/m^3). Cassee et al. (1996b) examined the nasal effects of inhalation exposure of formaldehyde, acetaldehyde, and acrolein on male Wistar rats (5-6/group) exposed 6 hr/day, for 3 consecutive days, in a nose-only exposure chamber to acrolein at concentrations of 0, 0.25, 0.67, or 1.40 ppm (0, 0.6, 1.5, or 3.2 mg/m^3). The Cassee et al. (1996b) study was designed to evaluate the severity of effects from mixtures versus single chemical exposure, and analyzed six levels of the nasal tract for histopathological and biochemical changes immediately after the last exposure. After one 6-hour exposure, no treatment-related histopathological lesions were found in any of the treatment groups. After 3 days, 4/5 animals exposed to 0.25 ppm (0.6 mg/m^3) were observed to have slight effects (characterized as mainly disarrangement) and 1/5 developed a moderate level of effect. In the 0.67 ppm (1.5 mg/m^3) group, 3/6 were classified as slightly affected and 3/6 rats developed a moderate degree of response. The LOAEL in this study is 0.25 ppm (0.6 mg/m^3).

The occurrence of lesions at lower doses in the Cassee et al. (1996b) study than used in the Feron et al. (1978) study may be: (1) a consequence of nose-only exposure where, unlike whole-body exposure, the animals cannot minimize exposure by burying their noses in their fur, so that animals receive a full and uninterrupted dose; or (2) due to a higher resolution evaluation from the use of extended sectioning (6 sections) of the nasal tract compared to only 3 in the Feron et al. study.

Cassee et al. (1996b) does not discuss the persistence or reversibility of the observed histopathological changes in the low-dose group with exposures greater than 3 days (e.g., adaptive response). An adaptive response in nonprotein sulfhydryl levels after 3 days of exposure was observed and is discussed. It is possible that an adaptative response to the irritant effects of acrolein occurs over time. Conversely, cessation of exposure for 2 days each week in the Feron et al. (1978) study might have provided a period during which partial recovery from nasal effects could occur. Because the Feron et al. (1978) study was much longer in duration, it is possible that some adaptation to the irritant effects of acrolein occurs with increasing duration, or that cessation of exposure for 2 days each week provides a period during which partial recovery from nasal effects might have occurred.

The rationale for the choice of the Feron et al. (1978) study over the Cassee (1996b) study includes: (1) the higher number of test animals [12 (6/sex) vs. 6 male only]; (2) the longer duration [5 days/week for 13 weeks vs. 3 days]; (3) the testing of multiple species and both sexes in the Feron et al. (1978) study; and (4) the better characterization of multiple endpoints and the dose-response. Feron et al. (1978) evaluated many different end points and demonstrated dose-response for all 3 dose groups in all 3 species tested. The Feron et al. (1978) study also evaluated a dose-response over a 12-fold increase from low- to high-dose. The Cassee (1996b) study used about a 6-fold increase in dose level from low- to high-dose. Collectively, the principal study and supporting studies (Kutzman, 1981; Kutzman et al., 1985; Costa et al., 1986; Feron et al., 1978; Cassee, 1996b) provide support for a minimal LOAEL of 0.4 ppm (0.9 mg/m^3) (i.e., a LOAEL close to the expected NOAEL).

5.2.2. Methods of Analysis

The nasal cavity is considered the most sensitive target site for the pathological effects of acrolein, in part because it is the first point of contact in inhalation exposures. A benchmark dose approach was not possible because nasal pathology incidence data were not provided. Therefore, the approach used to derive the RfC was the determination of a LOAEL with application of uncertainty factors.

5.2.3. RfC Derivation

The endpoint used to derive the RfC was based upon the results in the Feron et al. (1978) study, which identified a minimal LOAEL of 0.4 ppm (0.92 mg/m^3) based on evidence of nasal histopathology in the Wistar rat (1/12). The LOAEL was adjusted from the dosing regimen of 0.9 mg/m^3 for 6 hr/day, 5 days/week for 13 weeks to a continuous exposure as follows:

$$\begin{aligned} LOAEL_{ADJ} &= 0.9\ mg/m^3 \times 6/24 \times 5/7 \\ &= 0.16\ mg/m^3 \end{aligned}$$

A Regional Gas Dose Ratio (RGDR) for a Category 1 gas with extrathoracic respiratory effects was then derived using a calculated ventilation rate of 0.20 m^3/day for an average Wistar rat (average of male and female ventilation rates), and a default value of 20 m^3/day for humans

along with default extrathoracic region surface area values of 15.0 cm^2 for the rat, and 200 cm^2 for humans (U.S. EPA, 1994b). The resulting equation is as follows:

$$RGDR = \frac{\text{Ventilation rate (rat) / surface area (rat)}}{\text{Ventilation rate (human) / surface area (human)}}$$

$$= \frac{0.20/15.0}{20/200}$$

$$= 0.14$$

Applying the RDGR of 0.14 to the adjusted LOAEL of 0.16 mg/m^3 yields a LOAEL dosimetically adjusted to a human equivalent concentration (HEC) of 0.02 mg/m^3.

The $LOAEL_{HEC}$ was used as the point of departure for calculating the RfC. A total uncertainty factor of 1,000 was applied to this point of departure: 3 ($10^{1/2}$) for extrapolation from animal to humans (UF_A), 10 for intrahuman variability (UF_H), 10 for subchronic to chronic duration (UF_S), and 3 ($10^{1/2}$) for use of a minimal LOAEL (UF_L).

A UF_A of 3 ($10^{1/2}$) was used for interspecies extrapolation, since this factor embodies two areas of uncertainty: pharmacokinetics and pharmacodynamics. In this assessment, the pharmacokinetic component was addressed by the calculation of the human equivalent concentration (HEC) according to the procedures in the RfC methodology (U.S. EPA, 1994b). Accordingly, only the pharmacodynamic area of uncertainty remains as a partial factor for interspecies uncertainty ($10^{0.5}$ or approximately 3).

A default UF_H of 10 was applied to for intraspecies uncertainty to account for human variability and sensitive subpopulations, i.e., to account for human variability in the severity or range of response from any given acrolein exposure amongst different individuals.

A UF_S of 10 was applied for adjustment from subchronic to chronic duration because the principal study involved a 13-week dosing period and because there are insufficient inhalation data to preclude an increase in severity (or incidence) with an increase in exposure duration from subchronic to chronic.

A UF_L of 3 ($10^{1/2}$) was applied for use of a minimal LOAEL of 0.4 ppm (0.9 mg/m^3) in lieu of a NOAEL. Although the severity of the nasal effect at the 0.4 ppm (0.9 mg/m^3) level was minimal and in only 1 of 12 animals in the Feron et al. (1978) study, a 3-day study in the male Wistar rat by Cassee et al. (1996b) also reported slight nasal effects in the respiratory/transitional epithelium from a nose-only inhalation exposure at 0.25 ppm (0.6 mg/m^3). With the Cassee et al. (1996b) results and the observed increase in the severity of the effects in the Feron et al. (1978) study as dose increases, the 0.4 ppm (0.9 mg/m^3) was designated a minimal LOAEL instead of a NOAEL.

A UF_D was not applied because the data base for acrolein was considered complete. The available inhalation data base includes subchronic toxicity studies in multiple species, and an inhalation reproductive toxicity study of acrolein in Fisher 344 rats that revealed no reproductive or developmental effects. Acrolein's high reactivity at the point of contact and the evidence for minimal systemic distribution of acrolein obviates the need for additional studies of repeat-dose toxicity or reproductive/developmental toxicity.

Application of a total uncertainty factor of 1,000 (3 x 10 x 10 x 3) to the $LOAEL_{HEC}$ of 0.02 mg/m^3 yields a RfC of 2×10^{-5} mg/m^3.

5.2.4. Previous Inhalation Assessment

The RfC of 2×10^{-5} mg/m^3 derived in this assessment is the same as the value entered on IRIS in 1991. The previous RfC was based on squamous metaplasia and neutrophilic infiltration of nasal epithelium as reported in the subchronic rat inhalation studies of Kutzman (1981) and Feron et al. (1978), and application of a total UF of 1,000.

5.3. CANCER ASSESSMENT

A dose-response assessment for carcinogenicity is precluded because there is inadequate evidence to establish a link between exposure to acrolein and cancer.

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

6.1. HUMAN HAZARD POTENTIAL

Acrolein is a colorless to yellowish flammable liquid at room temperature with a disagreeable, choking odor. It is extremely acrid and irritating to mucous membranes. Acrolein and its derivatives are used as an intermediate in the synthesis of acrylic acid for making acrylates, and of DL-methionine, an essential amino acid. It is used as a herbicide and to control algae aquatic weeds and molluscs in recirculating process systems, growth of microorganisms in liquid fuel, growth of algae in oil fields, and the formation of slime in paper manufacture. It is also used to promote protein cross-linking in leather tanning, and as a tissue fixative for histological preparations.

Acrolein is released to the air as a result of manufacturing processes, through incomplete combustion of petroleum fuels, as a component of cigarette smoke, and as a photooxidation product of hydrocarbon pollutants (ATSDR, 1990). Combustion of fuels represents the major source of emissions of acrolein to the atmosphere.

Inhaled acrolein is retained primarily in the upper respiratory tract (Egle, 1972) because of its high solubility and reactivity. No direct evaluations of uptake via oral administration have

been reported. Draminski et al. (1983) identified a low level of acrolein derived conjugates in the urine of rats following oral dosing, and Arumugam et al. (1999b) reported toxicological effects in the liver of rats exposed by daily intubation (acrolein in water) for 45 days. These results indicate that at least some uptake occurs from the oral route; however, the stomach was not examined by light microscopy.

The main pathway of metabolism for acrolein is the addition of GSH to the activated double bond followed by processing to mercapturic acid. A second pathway is that of epoxidation of the double bond followed by attack on the epoxide by glutathione. A third pathway is addition of water to acrolein to form 3-hydroxypropionaldehyde, which can be further metabolized and ultimately incorporated into normal metabolic pathways (Parent et al., 1998).

Data are not available to evaluate the toxicological effects in humans from chronic exposure to acrolein. Acute duration studies (Weber-Tschopp et al., 1977; Esterbauer et al., 1991) have documented that acrolein can cause pronounced eye and nasal irritation. Inhalation studies in laboratory animals indicate that the principal target sites for acrolein toxicity are the nasal membranes (Feron et al., 1978) and the lung (Lyon et al., 1970; Kutzman, 1981; Kutzman et al., 1985), i.e., the initial sites of contact. When acrolein was administered to laboratory animals by gavage, the principal sites affected were the stomach (Parent et al., 1992a; NTP, 1995) and liver (Arumugam et al., 1999b).

At present the carcinogenicity of acrolein cannot be determined by the inhalation route because of a lack of human data and lack of adequate chronic bioassays in laboratory animals. For oral exposures, two chronic oral bioassays, one with rats (Parent et al., 1992c) and one with mice (Parent et al., 1991b), reported negative results. Marginally positive effects were reported in one other chronic oral study in rats (Lijinsky and Reuber, 1987), but these results were questioned following reevaluation of the tissues at a later date by a pathology work group. Questions were also raised about the validity of the assumptions that supported the reported dose and uptake levels in the Lijinsky and Reuber (1987) study. A weak tumor initiating effect was reported in an intraperitoneal injection study (Cohen et al., 1992), but results were negative in a skin tumor initiation study (Salaman and Roe, 1956).

Because of acrolein's reactivity, toxicity can be induced by more than one mode of action. A major mode of action, however, has been shown to be related to depletion of GSH. Reaction of acrolein with GSH deprives the cell of its natural defense against reactive oxygen species (Arumugam et al., 1999a,b). Moreover, the acrolein GSH adduct has been shown *in vitro* to directly induce oxygen radical formation (Adams and Klaidman, 1993).

Based upon EPA's draft revised guidelines for carcinogen risk assessment (U.S. EPA, 1999), the "data are inadequate for an assessment of human carcinogenic potential by either the inhalation or oral routes of exposure."

6.2. DOSE RESPONSE

Quantitative estimates of the noncancer risk from either oral or inhalation routes of exposure were developed from animal data since no adequate human data are available. A RfD of 5 x 10^{-4} mg/kg-day was derived from a study in Sprague-Dawley rats (Parent et al., 1992c) based upon a NOAEL (with increased mortality as the critical effect) of 0.05 mg/kg-day, and adjusted by a 10-fold interspecies uncertainty factor and a 10-fold uncertainty factor for intraspecies (human) variability in sensitivity for response to acrolein.

Confidence in the principal study is medium. Several supporting studies involving other species also indicated that mortality increases sharply with an elevated dose. The research demonstrating acrolein's high reactivity, low systemic distribution, toxicity at the point of entry, pronounced decreases in serum creatinine phosphokinase (creatine kinase), citric acid cycle enzymes, and liver GSH; and increased mitochondrial damage in the Wistar rat are suggestive of interference with normal metabolic processes or possibly absorption of essential nutrients sufficient to lead to early mortality, although further research is needed to support a definitive mode of action. In the NTP (1995) study there were glandular stomach and forestomach lesions at higher doses that likely played a role in the observed mortality. Confidence in the data base is judged high with chronic exposure studies in two species. Moreover, two studies (Parent et al., 1992b; Parent et al., 1993) provide evidence that reproductive and developmental effects are not critical endpoints although only one species was tested for reproductive effects (rat) and for developmental effects (rabbit). While the possibility of some transport of acrolein or a metabolite of acrolein to systemic sites remains, the critical target sites are at the point of contact, e.g., the respiratory system, the gastrointestinal tract, mucous membranes, and skin. The high reactivity of acrolein and the lack of significant systemic distribution obviates the need to examine reproductive/developmental effects in a second species. The overall confidence in this RfD assessment is medium-to-high; a variety of studies across different durations of exposure and in several different laboratory animal species has been consistent in demonstrating that in the absence of mortality there are no clear indications of adverse effects.

A RfC of 2 x 10^{-5} mg/m^3 was derived from the results of a 13-week inhalation study with rats, hamsters and rabbits (Feron et al., 1978). The critical endpoint was lesions in the upper respiratory tract and lung. A minimal LOAEL of 0.4 ppm (0.9 mg/m^3) was based on lesions of slight severity in the nasal epithelium of rats following 13 weeks exposure at this level. Severity of the lesions increased with exposure concentration. This 0.4 ppm (0.9 mg/m^3) concentration was a NOAEL for hamsters and rabbits in the same study, although at the 4.9 ppm (11 mg/m^3) level severity of nasal lesions was similar across all 3 species.

The RfC was derived by duration adjusting the LOAEL of 0.9 mg/m^3 from 30 hour/week exposure to continuous exposure of 0.16 mg/m^3. Applying an RGDR for a Category 1 gas of 0.14 (U.S. EPA, 1994b) to convert dose/unit surface area of the extrathoracic region in the rat to that in humans, resulted in an equivalent human concentration (HEC) for continuous exposure of 0.02 mg/m^3. A total UF of 1,000 was applied (3 for interspecies extrapolation of a dosimetrically adjusted dose, 10 for intrahuman variability, 10 for subchronic to chronic extrapolation, and 3 for use of a minimal LOAEL). Support for the use of a minimal LOAEL is

provided by NOAELs for 2 of the 3 species tested in the same study at the same dose level. The resulting adjustment of the minimal $LOAEL_{HEC}$ concentration of 2×10^{-2} mg/m^3 by a total uncertainty factor of 1,000 yields a RfC of 2×10^{-5} mg/m^3.

The confidence in the principal study is judged medium. Although the principal study (3 species) was adequately designed and examined a wide range of endpoints, it had several shortcomings: (1) only 3 sections of the nasal cavity were examined, (2) there was low sample size, and (3) a lack of incidence data. Support for the minimal LOAEL is provided by subchronic studies in 2 other species (rabbit and hamster) and a 3-day study (Cassee et al., 1996b) in the rat in which nasal lesions of similar type and severity were observed. The primary limitation in the data base is the lack of a chronic inhalation study and the attendant uncertainty relating to incidence/severity of nasal lesions at subchronic/chronic exposure levels lower than 0.4 ppm (0.9 mg/m^3). The high reactivity of acrolein at the point of contact, the lack of significant systemic distribution demonstrated in studies with the dog and rat, and the lack of effects in oral studies lessens the priority for an evaluation of reproductive/developmental endpoints in a two-generation inhalation study. Additional evaluation of immunological endpoints is warranted especially focusing on potential contribution to asthma or compromise in respiratory response. Thus, confidence in the data base is judged low to medium. Overall, confidence in the RfC is judged medium.

As stated previously, the data are inadequate for an assessment of the human carcinogenic potential from exposure to acrolein that would precede any evaluation of a cancer dose-response.

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APPENDIX A: SUMMARY OF EXTERNAL PEER REVIEW COMMENTS AND DISPOSITION

The support document and IRIS summary for acrolein have undergone both internal peer review performed by scientists within EPA and a more formal external peer review performed by scientists in accordance with EPA guidance on peer review (U.S. EPA, 1998b, 2000a). Comments made by the internal reviewers were addressed prior to submitting the documents for external peer review and are not part of this appendix. The three external peer reviewers were tasked with providing written answers to general questions on the overall assessment and on chemical-specific questions in areas of scientific controversy or uncertainty. The reviewers made a number of editorial suggestions to clarify specific portions of the text. These changes were incorporated in the document as appropriate and are not discussed further. A summary of significant comments made by the external reviewers and EPA's response to these comments follows:

(1) General Questions for Peer Reviewers

A. Are you aware of any other data/studies that are relevant (i.e., useful for the hazard identification or dose-response assessment) for the assessment of the adverse health effects, both cancer and noncancer, of this chemical?

Comments: One reviewer identified a number of pharmacokinetic and mechanistic studies that should be reviewed and cited. The other reviewers were not aware of additional studies.

Response: The studies identified were reviewed and discussed in the Toxicological Review.

B. For the RfD and RfC, has the most appropriate critical effect been chosen (i.e., that adverse effect appearing first in a dose-response continuum)? Points relevant to this determination include whether or not the choice follows from the dose-response assessment, whether the effect is considered adverse, and if the effect and the species in which it is observed in a valid animal model for humans.

Comments: One reviewer requested any additional information on the cause of the early mortality in the Parent et al. (1992c) study be discussed. A second reviewer agreed with the selection of the Parent et al. (1992c) study and the critical effect. The third reviewer did not answer the question regarding the RfD. For the RfC, one reviewer agreed with the choice of the Feron et al. (1978) study and nasal effects as appropriate choices given the totality of the data base, but recommended that the low dose be considered a NOAEL and not a minimal LOAEL. Another reviewer also agreed with the choice of critical study and effect, but was concerned with the low dose as a minimal LOAEL. A third reviewer also agreed with the choices and indicated a clear dose-response in the principal study.

Response: The mortality data of Parent et al. (1992c) was re-examined and some minor additional detail was provided, although the cause of the early mortality was still not clear. Given the weight-of-evidence that inhalation exposure causes nasal effects and the clear indication of nasal effects given in the shorter-term nose-only study by Feron and colleagues (Cassee et al., 1996b) at an even lower concentration, the low dose in the Feron et al. (1978) study was categorized as a minimal LOAEL.

C. Have the noncancer assessments been based on the most appropriate studies? These studies should present the critical effect/cancer (tumors or appropriate precursor) in the clearest dose-response relationship. If not, what other study (or studies) should be chosen and why?

Comments: One reviewer thought the NTP (1995) gavage study should be used as the principal study or at least co-critical with the Parent et al. (1992c) gavage study given the "serious difficulties" (i.e., early mortality) with the latter study. A second reviewer thought the use of the Parent et al. (1992c) study was appropriate for the oral RfD considering the decrease in survival. The third reviewer provided no comments on the RfD portion of this question. With respect to the RfC portion, two reviewers felt that use of the Feron et al. (1978) study was appropriate. The third reviewer also felt the principal study was appropriate, but suggested that the results of Kutzman et al. (1985) and Costa et al. (1986) provide a more technically defensible basis for the LOAEL.

Response: Kutzman et al. (1985) and Costa et al. (1986) were re-considered and judged to provide support for the findings of Feron et al. (1978). The text was revised accordingly. EPA recognized the NTP (1995) gavage study as an important study; however, EPA continued to call it a "supportive" study rather than a co-principal study because it does not directly alter the quantitative determination of the reference dose. The text, however, was revised to further emphasize the importance of the NTP (1995) study results showing that acrolein causes early mortality and increased incidence of glandular stomach and forestomach lesions in the F344 strain.

D. Studies included in the RfC under the heading "Supporting/Additional studies" are meant to lend scientific justification for the designation of critical effect by including any relevant pathogenesis in humans, any applicable mechanistic information, any evidence corroborative of the critical effect, or to establish the comprehensiveness of the data base with respect to various endpoints (such as reproductive/developmental toxicity studies). Should other studies be included under the "Supporting/Additional" category? Should some studies be removed?

Comments: One reviewer suggested that Kutzman et al. (1984) be evaluated as a supporting study since lung pathology was evident at 0.4 ppm (0.9 mg/m^3). A second reviewer focused on the IRIS summary and suggested all studies, except for Feron et al. (1978) be moved from section I.B.2 and that the Cassee et al. (1996b) study be discussed more fully. The third reviewer suggested that the results of the 13-week gavage study conducted by Bioassay Systems

Corporation be included in the IRIS summary; the description of additional studies in the RfC was comprehensive.

Response: The Kutzman et al. (1984) study was re-examined and included in the IRIS summary in the Additional Studies section. A description of the Kutzman (1991), Kutzman et al. (1985) and Costa et al. (1986) studies was kept in section I.B.2. of the IRIS summary because the results were supportive of those of Feron et al. (1978) as were the results of Cassee et al. (1996b), the discussion of which was revised to relate better to the findings of Feron et al. (1978).

E. For the noncancer assessments, are there other data that should be considered in developing the uncertainty factors or the modifying factor? Do you consider that the data support the use of different (default) values than those proposed?

Comments: One reviewer recommended that the wording of the uncertainty factor text be revised to make it consistent with previous discussions on IRIS and to consider an uncertainty factor of 3 to reflect an incomplete data base (i.e., a lack of adequate reproductive and a second species developmental oral toxicity study). A second reviewer had no substantive comments in this area. The third reviewer suggested that if mortality, without a mechanistic explanation as to why it occurred, is retained as the critical effect, then a modifying factor of 10 should be included; this reviewer considered the uncertainty factors for the RfC appropriate.

Response: The uncertainty factor section was revised for consistency with past discussions. Although the criteria established for a complete data base call for a second species oral study for developmental effects, the nature of the data base suggests that a second study is not needed. The suggestion to include a modifying factor of 10 because of mortality was also judged not necessary. The observation in the Wistar rat that oral dosing results in substantial decreases in the activities of citric acid cycle enzymes, perturbs mitochondrial membrane integrity and decreases GSH, provides a plausible basis for why longer-term dosing could result in mortality.

F. Do the confidence statements and weight-of-evidence statements present a clear rationale and accurately reflect the utility of the studies chosen, the relevancy of the effects to humans, and the comprehensiveness of the data base? Do these statements make sufficiently apparent all the underlying assumptions and limitations of these assessments? If not, what needs to be added?

Comments: For the RfD, one reviewer rated the data base as medium-to-high, and the overall confidence in the RfD as high based upon the breadth of endpoints examined in the principal study. The reviewer suggested adding text to the confidence statement that the principal and supporting studies include chronic exposure in two species, but noting the absence of a second species developmental toxicity. A third reviewer rated the study confidence as low based upon deficiencies in the Parent et al. (1992c) study including lack of reporting of mortality incidence data and clinical signs data, plus no reasonable explanation for the treatment related mortality; the data base as low-to-medium; and the RfD as low-to-medium. For the RfC, two

reviewers agreed with the confidence ratings. One reviewer rated the study as low-to-medium based upon low sample size, inadequately quantified histopathological data, and acute data that does not support the study results. This reviewer rated the data base as low because of the absence of chronic data or developmental or reproductive studies, and the overall confidence in the RfC as low-to-medium.

Response: The Agency agrees that the confidence in the principal study would be higher if there was a clearer basis for the early mortality, although the supporting studies add confidence to the validity of this endpoint. Confidence in the principal study was thus rated as medium. The confidence in the data base was considered high due to the variety of studies at different durations and in different species. Although only one species was tested for reproductive and developmental effects, the critical target sites (discussed in Section 4) are at the point of contact (e.g., the respiratory system, the gastrointestinal tract, mucous membranes, and skin) and there is little evidence of systemic distribution. The high reactivity of acrolein and the lack of significant systemic distribution obviates the need to examine reproductive/developmental effects in a second species. The overall confidence in the RfD was thus considered medium-to-high. The text was revised to include the above rationale. For the RfC, the Agency agrees that the confidence in the principal study would be higher had the histopathology been more highly resolved and the sample sizes increased. The study was, however, in 3 species and examined a wide range of endpoints, and thus was rated medium. The data base was rated low-to-medium because of the absence of a chronic study. The absence of inhalation studies for reproductive or developmental effects was considered of less import because of the results from oral studies, and the rationale that there is little acrolein systemic distribution because of its high reactivity with tissues at the portal of entry. The confidence in the overall RfC was therefore medium.

(2) Chemical-specific Comments

A. Given the consistent nature of the irritative effects of acrolein across species, it is reasonable to focus upon 0.09 ppm (eye irritation) in the Weber-Tschopp et al. (1977) study as basis for the threshold level of concern for acute effects described in section 4.6.2 in the Toxicological Review? If not, why not?

Comments: One reviewer had no objection to using the wording "a threshold level of concern," but recommended reconciling some discrepancies in the discussions in sections 4.1 and 4.6.2. A second reviewer mistakenly concluded that discussion of this study was related to development of the RfC, and felt the focus on a 'threshold level of concern' was inappropriate. The third reviewer objected to using this study because (1) the results were published in German, (2) the English translation was difficult to understand, (3) number of subjects was low, (4) not clear how close the actual concentrations were to the target concentrations, and (5) the study was 25 years old.

Response: Given the comments from the external peer reviewers, the discussion was revised to remove the quantitative calculations and the wording 'threshold level of concern.' The study was, however, retained and placed in context of identifying an approximate level of

exposure above which subjective complaints would be expected during acute exposures. There is no reason to discount a study published in German with a relatively large number of subjects (53, 42, and 46 individuals in the three experiments, respectively) who elicited subjective complaints. The English translation provided by the Chemical Manager was considered sufficient to judge the merits of the study. The measured levels of acrolein in this study were within ± 10% of target concentrations.

B. Histopathological evidence of nasal lesions in Wistar rats (Feron et al., 1978) exposed to 0.4 ppm (0.9 mg/m^3) for 13 weeks was chosen as the critical effect for RfC derivation although the Cassee et al. (1996b) study in the Wistar rat indicated nasal lesions, albeit slight, at 0.25 ppm (0.6 mg/m^3) after only 3 days. How can this apparent inconsistency be explained? Each study indicated that effects increased in severity with increasing concentration.

Comments: One reviewer did not see any inconsistency because the duration-adjusted LOAEL from the Feron et al. (1978) study was 0.07 ppm (0.16 mg/m^3) compared to 0.06 ppm (0.14 mg/m^3) for the Cassee et al. (1996b) study. It was also indicated that some adaptation could have taken place during the two days per week in the Feron et al. (1978) study that exposure did not take place or that severity increases with increasing exposure duration. A second reviewer suggested that the apparent inconsistency may relate to nose-only (Cassee et al., 1996b) versus whole-body (Feron et al., 1978) exposure in relation to stress. Also, given the daytime exposures and the normal sleeping position of the animals, the animals may have inhaled less than the measured concentrations if they kept their noses buried in their fur. The third reviewer felt that the Cassee et al. results were confounding and suggested they be reconciled.

Response: The results of the Feron et al.(1978) and Cassee et al. (1996b) studies were considered not to be confounding. The points raised by the second reviewer were considered sufficient to explain why animals in the Cassee et al. (1996b) study had nasal effects at effects lower than the minimal ones noted in the Feron et al. (1978) study. The comparison of the duration-adjusted LOAELs as a basis was considered less than satisfactory in that the calculation for the Cassee et al. (1996b) study did not include a 3/7 (exposure days/weekdays) factor in the derivation; thus the duration-adjusted LOAEL including this factor would have resulted in 0.03 ppm (0.07 mg/m^3), approximately half that of the similar value (0.07 ppm or 0.16 mg/m^3) calculated for Feron et al. (1978). The discussions were therefore revised in both the Toxicological Review and IRIS summary to reflect the comments raised by the reviewers.

C. Evidence of restrictive lung function at 0.4 ppm (0.9 mg/m^3) was found in male F344 rats by Costa et al. (1986) who measured function 6 days <u>post-exposure</u>. Should this study thus be elevated to a co-principal study along with the Feron et al. (1978) study? Or should it stand as the principal study, with a derivation of a new RfC based on the lung as a critical effect? Or would it be more useful to derive RfCs based on both nasal lesions and lung function and present both values? [Note: a LOAEL(HEC) for the thoracic region = 0.3 mg/m^3].

Comments: One reviewer felt that the Costa et al. (1986) publication should serve as the primary study for deriving a RfC. The pulmonary function parameters at 0.4 ppm (0.9 mg/m^3) were statistically significant relative to controls and the increases in internal lung surface area and the correlated increase in diffusing capacity for carbon monoxide were found to increase in a dose-dependent manner. Use of this study "provides quantifiable effects that appear technically more defensible than the 'slightly affected' nasal tissue of a single rat in the 0.4 ppm (0.9 mg/m^3) Feron et al. (1978) study." A second reviewer would not use the Costa et al. (1986) study as a co-principal study because: (1) lung function measurements can be highly variable, (2) it is not known if the increase in MEFV was transient and reversible, (3) there was no confirmatory histopathological evidence, (4) tidal volume, breathing frequency, and pulmonary resistance did not differ between the 0.4 ppm (0.9 mg/m^3) group and controls, and (5) Costa et al. (1986) themselves noted that the interpretation of the pulmonary function tests are limited in the absence of other supporting pathologic or functional data. The third reviewer felt that there are too many unresolved issues to utilize the Costa et al. (1986) as a principal or co-principal study.

Response: The Agency agrees that the Costa et al. (1986) study of pulmonary function should not serve as a principal or co-principal study. However, the results of the study do provide more substantial support for 0.4 ppm (0.9 mg/m^3) as a minimal LOAEL based on the Feron et al. (1978) study alone. Costa et al. (1986) did show that 0.4 ppm (0.9 mg/m^3) resulted in a significant ($p<0.001$) increase in internal lung surface and diffusing capacity for carbon monoxide compared to controls using state-of-the-art measurement techniques with acceptable standard deviations for the parameters measured. The number of animals used (24) in each exposure group provided an acceptable number for comparison purposes with controls. As a result, this study was considered a supporting study. Although the animals in this study were those evaluated for other purposes in Kutzman (1981) and Kutzman et al. (1985) the findings (i.e., no effects at 0.4 ppm or 0.9 mg/m^3) are described under additional studies in the IRIS summary.