National Toxicology Program
Toxicity Report Series
Number 25

NTP Technical Report
on Toxicity Studies of

Glutaraldehyde

(CAS No. 111-30-8)

Administered by Inhalation
to F344/N Rats and B6C3F$_1$ Mice

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Post Office Box 12233
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March 1993

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

Foreword

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- the National Center for Toxicological Research (NCTR) of the Food and Drug Administration; and
- the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control.

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TABLE OF CONTENTS

ABSTRACT 5

PEER REVIEW PANEL 8

SUMMARY OF PEER REVIEW COMMENTS 9

INTRODUCTION 11
Physical Properties, Production, Use, and Exposure 11
Biochemical Reactivity 12
Absorption, Disposition, Metabolism, and Excretion 12
Toxicity 14
Study Rationale and Design 18

MATERIALS AND METHODS 19
Procurement and Characterization of Glutaraldehyde 19
Vapor Generation System 20
Concentration Monitoring 21
Chamber Characterization 24
Toxicity Study Designs 26
Genetic Toxicity Studies 33
Statistical Methods 37
Quality Assurance 39

RESULTS 41
2-Week Inhalation Study in F344/N Rats 41
13-Week Inhalation Study in F344/N Rats 45
2-Week Inhalation Study in B6C3F_1 Mice 50
13-Week Inhalation Study in B6C3F_1 Mice 53
Respiratory Tract Histoautoradiographic Evaluations 57
Reproductive System Evaluations 58
Genetic Toxicity Studies 58

DISCUSSION 67

REFERENCES 71

TABLES
Table 1 Mean Chamber Concentrations of Glutaraldehyde in the 2-Week and 13-Week Inhalation Studies in F344/N Rats and B6C3F1 Mice 23

Table 2 Experimental Design and Materials and Methods in the 2-Week and 13-Week Inhalation Studies of Glutaraldehyde 31

Table 3 Survival and Weight Gain of F344/N Rats in the 2-Week Inhalation Study of Glutaraldehyde 42

TABLES (CONTINUED)

Table 4 Selected Histopathologic Lesions for F344/N Rats in the 2-Week Inhalation Study of Glutaraldehyde 43

Table 5 Survival and Weight Gain of F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde 45

Table 6 Selected Histopathologic Lesions of the Nasal Passages/ Turbinates for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde 49

Table 7 Survival and Weight Gain of $B6C3F_1$ Mice in the 2-Week Inhalation Study of Glutaraldehyde 51

Table 8 Selected Histopathologic Lesions for $B6C3F_1$ Mice in the 2-Week Inhalation Study of Glutaraldehyde 51

Table 9 Survival and Weight Gain of $B6C3F_1$ Mice in the 13-Week Inhalation Study of Glutaraldehyde 54

Table 10 Selected Histopathologic Lesions for $B6C3F_1$ Mice in the 13-Week Inhalation Study of Glutaraldehyde 56

FIGURES

Figure 1 Body Weights of F344/N Rats Exposed to Glutaraldehyde by Inhalation for 13 Weeks 46

Figure 2 Body Weights of $B6C3F_1$ Mice Exposed to Glutaraldehyde by Inhalation for 13 Weeks 55

PLATES

Legends 1-6 60

Plates 1-6 61

Legends 7-12 64

Plates 7-12 65

APPENDICES

Appendix A Organ Weights and Organ-Weight-to-Body-Weight Ratios A-1

Appendix B Hematology and Clinical Chemistry B-1

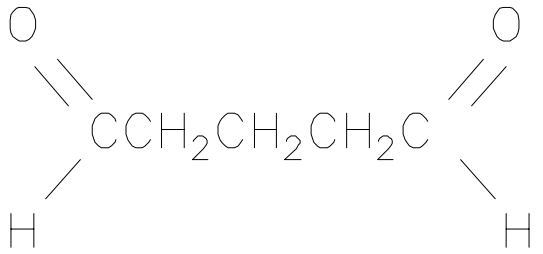
Appendix C Reproductive Tissue Evaluations and Estrous Cycle Characterization C-1

Appendix D Genetic Toxicology D-1

Appendix E Histoautoradiographic Evaluation of Respiratory Tract E-1

ABSTRACT

Glutaraldehyde



Molecular Formula	$C_5H_8O_2$
CAS Number	111-30-8
Molecular Weight	100.12
Synonyms	1,5-Pentanedial; Glutaral; Glutaric dialdehyde; 1,3-Diformylpropane

Glutaraldehyde is a potent sensory irritant with the capability to cross-link, or fix, proteins. It is used industrially as an antimicrobial agent and as a cold sterilant in hospitals, and it has a variety of other industrial uses. The toxicity of glutaraldehyde was evaluated in 2-week and 13-week inhalation exposure studies in F344/N rats and $B6C3F_1$ mice. In addition to histopathology, evaluations included clinical pathology and assessments of sperm morphology and estrous cycle length. *In vitro* genetic toxicity studies included assessments of mutagenicity in *Salmonella typhimurium* and in mouse lymphoma L5178Y cells and analysis of chromosomal aberrations and sister chromatid exchanges in Chinese hamster ovary cells. The ability of glutaraldehyde to induce sex-linked recessive lethal mutations was also studied *in vivo* in *Drosophila melanogaster*.

In 2-week inhalation studies, groups of five rats and five mice of each sex were exposed to glutaraldehyde by whole-body inhalation at concentrations of 0, 0.16, 0.5, 1.6, 5, and 16 ppm for 6 hours per day, 5 days per week. All rats and mice exposed to 5 or 16 ppm glutaraldehyde died before the end of the studies; all mice exposed to 1.6 ppm

also died. Rats exposed to 1.6 ppm did not gain weight. Deaths were attributed to severe respiratory distress. Mice appeared to be more sensitive than rats because the small airways of the nasal passage of mice were more easily blocked by cell debris and keratin. Lesions noted in the nasal passage and larynx of rats and mice included necrosis, inflammation, and squamous metaplasia. At higher exposure concentrations, similar lesions were present in the trachea of rats and mice and in the lung and on the tongue of rats.

In 13-week studies, groups of 10 rats and 10 mice of each sex were exposed to glutaraldehyde by whole-body inhalation at concentrations of 0, 62.5, 125, 250, 500, and 1000 ppb for 6 hours per day, 5 days per week. There were no exposure-related deaths in rats, but all mice exposed to 1000 ppb and two female mice exposed to 500 ppb died before the end of the study. Body weight gains were reduced in male rats exposed to 1000 ppb and in female rats exposed to 500 or 1000 ppb. Body weight gains of male mice exposed to 125, 250, or 500 ppb and female mice exposed to 250 or 500 ppb were reduced in a concentration-related manner. There was no clear evidence of systemic toxicity in rats or mice by histopathologic or clinical pathology assessments; however, exposure-related lesions in the respiratory tract were observed, and resembled those noted in the 2-week studies.

In rats, the most severe lesions occurred in the anterior portions of the nasal passages and involved both the respiratory and olfactory epithelium. Hyperplasia and squamous metaplasia were most commonly noted on the lateral wall of the nasal cavity and on the tips of the nasoturbinates. Lesions were most extensive in rats exposed to 1000 ppb, but were also noted in the 250 and 500 ppb groups and in one male exposed to 125 ppb.

In mice, histopathologic lesions in the respiratory tract were most severe in animals in the 1000 ppb group and consisted of minimal to mild squamous metaplasia of the laryngeal epithelium, suppurative inflammation in the anterior parts of the nasal cavity, and minimal squamous metaplasia on the tips of the nasoturbinates. Necrosis and inflammation were noted at lower concentrations, primarily in the anterior portion of the nasal passage.

In genetic toxicity studies, glutaraldehyde was mutagenic with and without S9 metabolic activation in *Salmonella typhimurium* strains TA100, TA102, and TA104. Glutaraldehyde was mutagenic in mouse L5178Y lymphoma cells in the absence of S9 and induced sister

chromatid exchanges in Chinese hamster ovary cells with and without S9. In one laboratory, chromosomal aberrations were induced in Chinese hamster ovary cells by glutaraldehyde in the absence of S9 only; no increase in chromosomal aberrations was observed with or without S9 in a second laboratory. Glutaraldehyde did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* treated as adults by feeding or injection or treated as larvae by feeding.

In summary, exposure of rats and mice to glutaraldehyde by inhalation for up to 13 weeks resulted in a spectrum of necrotic, inflammatory, and regenerative lesions confined to the upper respiratory tract. Mice were somewhat more sensitive than rats because the small airways of the nasal passage in mice were more prone to blockage with cellular debris, bacteria, and keratin. The no-observed-adverse-effect level (NOAEL) was 125 ppb for respiratory lesions in rats. An NOAEL was not reached for mice, as inflammation was found in the anterior nasal passage at concentrations as low as 62.5 ppb.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of glutaraldehyde on June 24, 1992, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members determine if the design and conditions of these NTP studies are appropriate and ensure that this toxicity study report presents the experimental results and conclusions fully and clearly.

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SUMMARY OF PEER REVIEW COMMENTS

On June 24, 1992, the Technical Reports Review Subcommittee of the Board of Scientific Counselors for the National Toxicology Program met in Research Triangle Park, NC, to review the draft technical report on toxicity studies of glutaraldehyde.

Dr. Frank Kari, NIEHS, introduced the short-term toxicity studies of glutaraldehyde by reviewing the uses of the chemical, rationale for the study, experimental design, and results.

Dr. Bailey, a principal reviewer, said the study was performed extremely well. He questioned the absence of inflammation in the anterior nares of mice exposed to 1000 ppb glutaraldehyde for 13 weeks, given the presence of inflammation at lower exposure concentrations. Dr. Elwell, NIEHS, stated that a rather diffuse inflammation accompanied the squamous exfoliation seen in the 1000 ppb group, but that it differed from the inflammatory changes seen at lower concentrations. He said the report would clarify this.

Dr. Silbergeld, another principal reviewer, said the report was well written and the experiments excellently conducted. She was, however, disappointed that the studies appeared to focus so heavily on the respiratory tract lesions, at the possible expense of finding changes in the brain, peripheral nervous system, and the immune system. She stated that a number of changes observed in immune system parameters were considered "secondary to generalized stress" and wondered whether these were, in fact, indicative of immunotoxicity. Dr. Thompson, NIEHS, said that decreases in lymphocytes in male rats were mild and similar changes did not occur in females; thus, the findings were not considered significant. Dr. Silbergeld commented that toxic effects were seen at concentrations well below the OSHA permissible exposure limit of 200 ppb.

Dr. Davidson questioned the high exposure concentrations in the 2-week studies. Dr. Kari replied that as a result of the reactivity of the chemical, literature values for the LC_{50} varied widely; therefore, a wide range of concentrations was selected for the short-term studies. Dr. J. Haartz, NIOSH, recommended that further details concerning the configuration and components of the inhalation exposure system be added to the report. Dr. Silbergeld and Dr. Hayden asked that an expanded comparison between the toxic effects of glutaraldehyde and formaldehyde be included in the discussion. Dr. Kari agreed to these suggestions. Dr. Carlson accepted the report on behalf of the peer review panel.

INTRODUCTION

Physical Properties, Production, Use, and Exposure

Glutaraldehyde (CAS 11-30-8) is a saturated, aliphatic dialdehyde that exists as a pungent oil at room temperature. It has a molecular weight of 100.12 and is highly soluble in water, ethanol, benzene, and ether. Glutaraldehyde is highly reactive and undergoes a variety of heterocyclic and homocyclic reactions to form mixtures of hydrates, pyrans, and various polymers. In aqueous solutions, there is a reversible equilibrium between the open-chain, cyclic, and polymeric forms (Whipple and Ruta, 1974). Consequently, its chemical activity, and presumably its toxicity, are not necessarily proportional to the stoichiometric amount of glutaraldehyde in solution. Commercially available preparations of glutaraldehyde are often dilute solutions (approximately 2%) to which various stabilizers and buffers have been added. Beauchamp *et al.* (1992) have critically reviewed the chemistry and toxicity of glutaraldehyde.

Glutaraldehyde is extremely reactive in cross-linking protein (Peters and Richards, 1977) and therefore has found wide use industrially as a cross-linking agent, tanning intermediate, and general biocide in a variety of clinical and commercial applications. It is widely used as a cold sterilant for surgical and laboratory supplies. Glutaraldehyde is also used for diverse specialty applications, including use as a fixative for electron microscopy and in the preparation of surgical grafts, bioprostheses, and dental materials (Okamura *et al.*, 1980; Golobov *et al.*, 1989).

Although recent production figures are unavailable (SRI, 1976; HSDB, 1990), estimates of occupational exposure, surveyed from 1981 to 1983, indicate that at least 318,000 people in the United States come into daily contact with glutaraldehyde in the workplace (NIOSH, 1990). Exposure occurs primarily among individuals involved in health services, including nurses and surgical staffs, laboratory technicians, inhalation therapists, and chemists (NIOSH, 1985). The permissible exposure limit for glutaraldehyde established by the Occupational Safety and Health Administration is 0.8 mg/m^3 (0.2 ppm); this ceiling concentration was based on the irritant effects to the eyes, nose, and throat that have been associated with short-term exposure to glutaraldehyde (OSHA, 1989).

Biochemical Reactivity

REACTION WITH PROTEINS

Many of the uses of glutaraldehyde are related to its ability to react with and to cross-link proteins (Peters and Richards, 1977; Beauchamp *et al.*, 1992). Glutaraldehyde can react with the α-amino groups of amino acids, the N-terminal amino groups of peptides, and the sulfhydryl group of cysteine (Habeeb and Hiramoto, 1968; Beauchamp *et al.*, 1992). The predominant site of reaction in proteins is the ε-amino group of lysine, although reaction may also occur with tyrosine, histidine, and sulfhydryl residues. Gels or precipitates are formed on treatment of some proteins with glutaraldehyde (Beauchamp *et al.*, 1992).

REACTION WITH DNA

Little information is available on the reactivity of glutaraldehyde with DNA or the components of DNA. Products are formed on reaction of glutaraldehyde with deoxyadenosine, deoxyguanosine, and deoxycytidine, but not with deoxythymidine (Hemminki and Suni, 1984; Beauchamp *et al.*, 1992); the adducts formed with deoxyadenosine are unstable, but those formed on reaction with deoxyguanosine are relatively stable. The position of substitution is believed to be the exocyclic amine group of these nucleosides (Beauchamp *et al.*, 1992).

The reaction of glutaraldehyde with DNA or RNA, as measured by increasing absorbance at 260 nm, occurs at temperatures above 60°C, at which the nucleic acids undergo melting (Hopwood, 1975). Intermolecular DNA-DNA cross-linking has not been reported, although DNA-protein cross-linking was detected in replicating human TK6 lymphoblasts treated with glutaraldehyde (St. Clair *et al.*, 1991a).

Absorption, Disposition, Metabolism, and Excretion

The uptake of glutaraldehyde has been investigated in a variety of biological systems. An *in vitro* dermal study using human tissues showed that 3.3% to 13.8% of the applied dose of glutaraldehyde penetrated the thin stratum corneum of the chest and abdomen, and that 2.8% to 4.4% of the dose penetrated the isolated epidermis (Reifenrath *et al.*, 1985). In a more recent *in vitro* study in skin samples of F344 rats, CD-1 mice, rabbits, guinea pigs, and humans, less than 1% of the applied glutaraldehyde penetrated the skin (Tallant *et al.*, 1990).

Material balance studies have been carried out *in vivo* in F344 rats and in New Zealand white rabbits (Ballantyne, 1986). Following intravenous administration of 0.075% and 0.75% solutions of [1,5-^{14}C]-glutaraldehyde to rats and rabbits (0.2 mL for rats and 2.5 mL for rabbits), the majority of the radiolabel was excreted as $^{14}CO_2$, with approximately 80% being exhaled in the first 4 hours. Urinary excretion of radiolabel ranged from 8% to 12% in the rat and 15% to 28% in the rabbit. Excretion of $^{14}CO_2$ as a percentage of total dose was less at the higher dose than at the lower dose, particularly in the rabbit. Following topical administration of 0.2 mL of 0.075%, 0.75%, or 7.5% solution to rats, the majority of the radiolabel was recovered at the application site, with approximately 5% of the dose being absorbed. In rabbits administered 2.5 mL of 0.75% or 7.5% solution of [1,5-^{14}C]-glutaraldehyde topically, between 32% and 53% of the dermal dose was absorbed and either excreted or found in tissues. Pharmacokinetic analyses of the levels of radioactivity in plasma were also performed following intravenous and dermal administration of [1,5-^{14}C]-glutaraldehyde to rats and rabbits (Ballantyne, 1986). The dermal absorption rate constants were low, ranging from 0.2 to 2.0 per hour in both species. Terminal half-lives calculated for glutaraldehyde were long, possibly due to a combination of binding to protein and slow excretion of metabolites. Urinary excretion of metabolites was reported, but none of the metabolites have been characterized (Ballantyne, 1986; Myers *et al.*, 1986; Beauchamp *et al.*, 1992).

Extensive metabolism of glutaraldehyde to CO_2 has been described in a number of *in vivo* and *in vitro* studies in which [1,5-^{14}C]-glutaraldehyde was used as tracer (Packer and Greville, 1969; Ballantyne, 1986; Myers *et al.*, 1986; Karp *et al.*, 1987). Although direct identification of the metabolites has not been accomplished, the probable metabolic pathway involves a series of oxidation, decarboxylation, and hydration reactions (Beauchamp *et al.*, 1992). The initial step is probably oxidation of glutaraldehyde to glutaric semialdehyde, followed by oxidation to glutaric acid, which can undergo further metabolism by synthesis of a Coenzyme A thioester. The glutaryl CoA produced is then oxidized by glutaryl CoA dehydrogenase to give glutaconyl CoA, which is then decarboxylated to crotonyl CoA (Besrat *et al.*, 1969). The crotonyl CoA is then converted by enoyl CoA hydratase to β-hydroxybutyryl CoA, which can subsequently be used for synthesis of acetoacetate or be degraded to acetate and then to CO_2.

Evidence that glutaraldehyde undergoes oxidation derives from *in vitro* studies in rat liver mitochondria in which an increase in oxygen consumption was measured (Packer and

Greville, 1969). The oxidation of glutaraldehyde involves the electron transport system and results in reduction of NAD^+ and consumption of two atoms of oxygen per molecule of glutaraldehyde (Smith and Packer, 1972). Glutaraldehyde was oxidized extensively to CO_2 in rat tissue slices, with the greatest activity occurring in the kidney and then the liver (Karp *et al.*, 1987); the activity was localized in the mitochondrial fraction of the kidney.

Glutaraldehyde has been found to be a poor substrate for the isoenzymes I, IIa, and IIb of human liver aldehyde dehydrogenase compared with other aliphatic aldehydes (Jones and Teng, 1983). Glutaraldehyde is also a poor substrate for an aldehyde dehydrogenase isolated from liver microsomes of rats treated with clofibrate (Antonenkov *et al.*, 1987) and for two isoforms of cytosolic aldehyde dehydrogenases isolated from rat liver (Pirozhkov and Panchenko, 1988). However, glutamic-γ-semialdehyde dehydrogenase (1-pyrroline-5-carboxylate dehydrogenase), an enzyme that had been considered an isoenzyme of human liver aldehyde dehydrogenase, has a high capacity for metabolism of glutaric semialdehyde, which is produced on oxidation of glutaraldehyde (Forte-McRobbie and Pietruszko, 1986). In addition, the NAD^+-dependent succinic semialdehyde dehydrogenase isolated from human brain mitochondria is capable of using glutaric semialdehyde as a substrate for oxidation, although at a lower rate than for succinic semialdehyde (Ryzlak and Pietruszko, 1988; Beauchamp *et al.*, 1992).

Toxicity

ANIMAL TOXICITY

The acute toxicity of glutaraldehyde, alone or diluted with water or corn oil, has been investigated in a variety of species. When administered to male and female rats for 4 hours by inhalation, the LC_{50} of glutaraldehyde reportedly ranged from 24 to 5000 ppm (Ballantyne, 1986; Sax and Lewis, 1989). This wide range undoubtedly reflects the large experimental error introduced into the studies because of the extreme difficulty of generating and detecting this very reactive chemical.

The oral LD_{50} in rats ranged from 1.30 mL/kg of a 50% aqueous solution to 12.3 mL/kg of a 1% solution (Ballantyne, 1986). In rats, the subcutaneous LD_{50} was 2390 mg/kg (Uemitsu *et al.*, 1976), the intraperitoneal LD_{50} was 17.9 mg/kg, and the intravenous LD_{50} was 15.3 mg/kg (Sax and Lewis, 1989). In mice, the oral LD_{50} was 100 mg/kg glutaraldehyde (Sax and Lewis, 1989) or 1300 mg/kg of a 25% olive oil solution (Ohsumi

and Kuroki, 1988). Uemitsu *et al.* (1976) determined an LD_{50} of 1430 mg/kg for glutaraldehyde administered subcutaneously to male mice. The intraperitoneal LD_{50} in mice was 13.9 mg/kg and the intravenous LD_{50} was 15.4 mg/kg (Sax and Lewis, 1989).

Glutaraldehyde is currently classified as a primary dermal irritant, and topical application to the skin of rabbits, mice, and guinea pigs and to the ears of mice caused moderate to severe irritation and/or allergic responses (Descotes, 1988; Stern *et al.*, 1989). Severe local inflammation and punctate necrosis were observed following an occluded-patch test on rabbit skin with 25% glutaraldehyde; the concentration threshold for glutaraldehyde-induced erythema was 1% (Ballantyne *et al.*, 1985). Other investigators have demonstrated the induction of a weak immunologic response in rabbits (Ranly *et al.*, 1985; Jaworsky *et al.*, 1987; Beauchamp *et al.*, 1992).

Subcutaneous administration of 25 or 125 mg/kg glutaraldehyde daily to male rats for 35 days was associated with an increase in white blood cell count, decreased levels of hemoglobin and lymphocytes, hypertrophy of white pulp in the thymus, atrophy of the thymus, and degeneration of renal tubules. Urine and blood chemistry parameters were within normal limits except for elevated serum urea nitrogen and urine total protein levels (Uemitsu *et al.*, 1976). After 11 weeks of exposure to 0.25% glutaraldehyde in drinking water, rats exhibited no evidence of damage to the peripheral or central nervous systems (Spencer *et al.*, 1978).

In inhalation studies in rodents, exposure to glutaraldehyde was associated with significant respiratory distress. A single 24-hour inhalation exposure of NMRI mice to 133 mg/L glutaraldehyde vapor resulted in toxic hepatitis (Varpela *et al.*, 1971). A single 8-hour inhalation exposure of rats to saturated glutaraldehyde vapors resulted in signs of toxicity and irritation, including excess lacrimation and salivation, audible breathing, and mouth breathing (Ballantyne, 1986). Single inhalation exposures of rats to glutaraldehyde vapors at chamber concentrations decreasing from 11 to 2 ppm over a 6-hour period caused sensory and respiratory tract irritation (Ballantyne *et al.*, 1985). In a repeated-dose inhalation study in which rats were exposed to 0 to 3.1 ppm glutaraldehyde for 6 hours per day for 9 days, significant mortality occurred at the highest concentration and depressed body weight gain, sensory irritation, and inflammation of the nasal mucosa were noted in rats in the 2.1 and 3.1 ppm exposure groups (Ballantyne *et al.*, 1985). Instillation of 20 to 40 mM glutaraldehyde into the nasal cavities of rats

caused epithelial changes characteristic of inhalation exposure to a number of irritating gases (St. Clair *et al.*, 1989, 1990).

In a subchronic inhalation study in which F344 rats were exposed to 0 to 194 ppb glutaraldehyde daily for 3 months, perinasal wetness and significantly decreased body weight gain were observed in animals in the 49 and 194 ppb exposure groups. However, no damage to the nasal mucosa occurred, and although activities of several serum enzymes (phosphokinase, lactate dehydrogenase, and hydroxybutyric dehydrogenase) were elevated, no histopathologic lesions were found in any organ (Greenspan *et al.*, 1985).

The cardiotoxic effects of glutaraldehyde were investigated in dogs following a single intravenous dose of 1 to 10 mg/kg. Glutaraldehyde caused prolongation of the Q-T interval, resulting in ventricular fibrillation (James and Bear, 1968).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No studies that investigated the effects of glutaraldehyde on female reproductive function and fertility were found in the literature. In a study that examined the effects of glutaraldehyde on male reproductive function using the dominant lethal assay, male mice were administered a single oral dose of 30 or 60 mg/kg glutaraldehyde and mated for the next 6 weeks with virgin females. There was no evidence of reduced fertility in treated males and no significant effects on embryonic/fetal viability were noted (Tamada *et al.*, 1978).

Studies assessing the incidence of spontaneous abortions and fetal malformations in Finnish hospital nurses and staff who had been exposed to glutaraldehyde used as a sterilizing agent found no significant increase in risk of either endpoint (Hemminki *et al.*, 1982, 1985). No information on birth defects was reported in these studies.

Investigations in animals showed a low occurrence of developmental toxicity as a result of glutaraldehyde exposure during gestation. Dosing during the organogenesis period of gestation (Days 7 through 12), however, revealed no adverse effects on embryonic development except at a dose of 30 mg/kg, which was also severely maternally toxic (Ballantyne, 1986). Oral administration of Sonacide® (acidified glutaraldehyde containing 2% glutaraldehyde w/v) to CD-1 mice during Days 6 through 15 of gestation was highly

toxic to pregnant dams at daily doses greater than 2.0 mL/kg (40 mg glutaraldehyde/kg); Sonacide® only affected fetuses at the highest dose, 5 mL/kg, at which the average fetal weight was significantly reduced and about 12% of the fetuses were malformed (Marks *et al.*, 1980). Oral doses of 25 and 50 mg/kg of glutaraldehyde given to albino rats on Days 6 to 15 of gestation were maternally toxic but not fetotoxic (Ballantyne, 1986). Preliminary results of a developmental toxicity study in which rabbits were administered 5, 15, or 40 mg/kg glutaraldehyde by gavage daily for 13 consecutive days indicate that the high dose was severely toxic to dams and caused a significant increase in implantation loss and reduction of the mean body weight of surviving fetuses (BASF Corp., 1990); the two lower doses were nontoxic.

CARCINOGENICITY

No adequate long-term bioassay studies on the potential carcinogenicity of glutaraldehyde have been reported in the literature.

GENETIC TOXICITY

Short-term genotoxicity tests with glutaraldehyde have yielded mixed responses, and early reviews of the genotoxicity of glutaraldehyde were generally negative (Wade *et al.*, 1982; Watts, 1984). However, results from more recent *in vitro* testing generally show the chemical to be genotoxic, with no requirement for S9 metabolic enzymes.

Positive results were reported for glutaraldehyde in a forward mutation assay using a specially constructed *Escherichia coli* WP2 uvrA strain that contained the plasmid pKM101 from *Salmonella typhimurium* (Kosako and Nishioka, 1982); however, negative results were obtained in a reversion assay using this same strain without the plasmid (Hemminki *et al.*, 1980). In addition, Hemminki *et al.* (1980) detected no alkylation potential for glutaraldehyde *in vitro* using 4-(*p*-nitrobenzyl)pyridine or deoxyguanosine as the target. Results from *S. typhimurium* mutation tests were also mixed. Negative results were reported by laboratories using low doses (less than 52 µg/plate) of glutaraldehyde in strains TA100, TA1535, TA1537, and TA98 (Sasaki and Endo, 1978; Slesinski *et al.*, 1983; Sakagami *et al.*, 1988a), but positive results were obtained when higher doses (up to 1000 µg/plate) were used with strain TA100 (Haworth *et al.*, 1983). These standard tester strains have G:C base pairs at the site of mutation. Use of the recently developed *S. typhimurium* strains TA102 and TA104, which have A:T base pairs at the target site and

which were reported to be sensitive to carbonyl compounds, gave strongly positive results for glutaraldehyde without S9 (Levin *et al.*, 1982; Marnett *et al.*, 1985). Positive results were obtained for induction of L-arabinose resistance in *S. typhimurium* strains BA13 and BA9 by glutaraldehyde-induced forward mutations at an A:T base pairing site, without S9 activation (Ruiz-Rubio *et al.*, 1985). Finally, glutaraldehyde was reported to be positive in the *S. typhimurium* umu test and in the *Bacillus subtilis* recombinant assay (Sakagami *et al.*, 1988a,b).

In other genotoxicity assays, glutaraldehyde was mutagenic in mouse lymphoma cells (McGregor *et al.*, 1988) and cultured human TK6 lymphoblasts (Gross *et al.*, 1991; St. Clair *et al.*, 1991b). Glutaraldehyde induced sister chromatid exchanges (SCEs) and chromosomal aberrations in Chinese hamster ovary (CHO) cells in the absence of S9 liver enzymes (Galloway *et al.*, 1985). Studies by Slesinski *et al.* (1983) had negative results in SCE and gene mutation tests in CHO cells, but these studies used much lower doses than the studies that showed positive results. Assessment of glutaraldehyde-induced unscheduled DNA synthesis (UDS) in primary hepatocyte cultures revealed a small, dose-related increase in DNA repair activity (Gross *et al.*, 1991; St. Clair *et al.*, 1991b).

In vivo, glutaraldehyde did not induce sex-linked recessive lethal mutations in male *Drosophila melanogaster*, treated either as larvae (Zimmering *et al.*, 1989) or as adults (Yoon *et al.*, 1985). Oral administration of glutaraldehyde did not induce UDS in hepatocytes of male rats (Mirsalis *et al.*, 1989) or dominant lethal mutations in mice (Tamada *et al.*, 1978).

Study Rationale and Design

Glutaraldehyde was selected for toxicological characterization because of the documented widespread occupational exposure to glutaraldehyde, evidence that glutaraldehyde is mutagenic in several short-term genotoxicity assays, and the structural relationship between glutaraldehyde and formaldehyde, a known nasal carcinogen in rats (Kerns *et al.*, 1983). Two-week and 13-week inhalation studies of glutaraldehyde were conducted in F344/N rats and B6C3F$_1$ mice. The toxicologic characterization included assessments of gross and histopathologic organ toxicity, with special emphasis on upper respiratory tract toxicity, hematology and clinical chemistry, and genetic and reproductive toxicity.

MATERIALS AND METHODS

Procurement and Characterization of Glutaraldehyde

Glutaraldehyde (CAS Number 111-30-8) was obtained in one lot (Lot 95296) from Union Carbide (South Charleston, WV) as a 50% aqueous solution. The chemical was further diluted with deionized water to 25% w/w glutaraldehyde prior to vapor generation.

Chemical analyses performed by Midwest Research Institute (Kansas City, MO) identified the chemical, a clear colorless liquid, as glutaraldehyde. Infrared, ultraviolet/visible, and ^{13}C nuclear magnetic resonance spectra were consistent with the structure of aqueous glutaraldehyde and with available literature references. Elemental analysis results for hydrogen agreed with theoretical values corrected for the determined water content, while the results for carbon were 2.33% high. Karl Fischer analysis indicated a water content of 45% and functional group titration by oximation with hydroxylamine indicated a glutaraldehyde content of 52% based on two aldehyde groups per molecule. No impurities greater than 0.79% relative to the major peak were observed by gas chromatography. Quantitative ^{13}C nuclear magnetic resonance analysis performed to estimate the relative amount of each of the isomeric forms of glutaraldehyde in equilibrium with water indicated a molar percentage of 6% glutaraldehyde, 15% hemihydrate, 8% dihydrate, and 71% as the cyclic hemiacetal (37% *cis* and 34% *trans*). Cumulative analytical data based on elemental analysis, Karl Fischer water analysis, functional group titration, and two gas chromatographic systems indicated a glutaraldehyde content of 50.0%, with only minor contamination from the polymeric forms of glutaraldehyde and other volatile impurities.

Stability studies performed by Midwest Research Institute indicated that 50% aqueous glutaraldehyde was stable when stored for 2 weeks, protected from light, at temperatures up to 25°C. At the study laboratory, the bulk chemical was stored in the original amber glass containers under a nitrogen blanket at approximately 5°C. Material diluted for vapor generation was also stored refrigerated under nitrogen in sealed glass jars. Subsequent chemical reanalyses by infrared spectroscopy and gas chromatography showed consistent purity levels throughout the studies; ultraviolet spectroscopy indicated that the amount of polymer present in the bulk material remained within acceptable limits.

Vapor Generation System

The glutaraldehyde vapor exposures were conducted using an automated data acquisition and control system. A central computer (Hewlett Packard 9816) monitored and controlled the basic chamber functions (*i.e.*, glutaraldehyde, airflow, vacuum, temperature, and relative humidity) of the test exposure. Animals were exposed and maintained in inhalation exposure chambers developed at Battelle Pacific Northwest Laboratories and commercially produced by Harford Division of Lab Products, Incorporated (Aberdeen, MD). Each chamber had an active mixing volume of 1.7 m^3.

A 25% w/w solution of aqueous glutaraldehyde was pumped into a rotating glass evaporation column by an FMI® pump (Fluid Metering Systems, Oyster Bay, NY). HEPA-filtered compressed air was heated and passed through the column from the base at a flow rate of approximately 3 cubic feet per minute. The glass column rotated at approximately 15 revolutions per minute and a glass rod extending the length of the column served to spread the liquid glutaraldehyde over the internal surface. This spreading enhanced the evaporation of the material and was necessary due to the low vapor pressure of glutaraldehyde. The air temperature was monitored with a thermocouple feedback system and was manually controlled to maintain the temperature of the air exiting the evaporator at approximately 60°C. The glass column and associated tubing were insulated to prevent heat loss. Excess, unvaporized material was collected at the base of the rotating column in an Erlenmeyer flask.

In the 2-week studies, the warm, vapor-laden air exiting the evaporation tube was passed through a water-cooled condensing column (18°C) to bring the temperature closer to the room temperature prior to distribution to the chambers. The room temperature vapor was then siphoned to the individual exposure chambers through flow meters connected to individual delivery lines.

No condenser was used in the 13-week studies. In these studies, the warm vapor was conveyed from the generator to a mixing chamber, where it was diluted with conditioned air and was monitored by a Miran infrared analyzer, prior to distribution to the individual delivery lines.

Great care was taken in the design of the generation system to ensure that the glutaraldehyde was not excessively heated. Glutaraldehyde solutions exhibit enhanced

bactericidal activity at elevated pH and, possibly, at elevated temperatures (Robert Egar, personal communication, Union Carbide Corporation, Terrytown, NY). This enhanced activity may be due to the formation of higher molecular weight glutaraldehyde polymers. If vaporized, these polymers might be more toxic than the parent compound. Evidence of polymeric forms of glutaraldehyde was detected in the 2-week, but not in the 13-week, studies.

In both the 2-week and 13-week studies, Air-Vac® pumps (Air-Vac Engineering, Milford, CT) connected at the junction of each delivery line and exposure chamber inlet provided the driving vacuum for delivering the glutaraldehyde vapor. Adjustment of exposure chamber concentrations was achieved by adjusting the driving pressure to the appropriate Air-Vac® pump.

The glutaraldehyde vapor reacted readily with the surfaces of the chamber, the animals' fur, excrement, and water present in the chambers. To reduce the absorption of the vapor from the chamber atmosphere, an airflow enhancement system was constructed. A portion of the air exiting the chamber was returned to the inlet by a fan and a stainless steel duct. The result was an effective air flow of approximately 72 cubic feet per minute through the chamber while maintaining the required 15 fresh air changes per hour. Increasing the mass flow of the air through the chamber minimized the influence of the animals.

Concentration Monitoring

Glutaraldehyde vapor concentration was monitored with an automated gas sampling gas chromatograph system equipped with a flame ionization detector (HP5840 in the 2-week studies, HP5890A in the 13-week studies; Hewlett-Packard, Palo Alto, CA). This system was used to measure glutaraldehyde concentration in the 5 exposure chambers, control chamber, exposure room, an on-line standard, and blank samples (nitrogen blank in the 2-week studies, carbon-filtered air blank in the 13-week studies). Sampling from multiple positions was accomplished by means of an automated 12-port stream select valve, fed by sampling lines from 12 sampling locations. In the 2-week studies, calibration of the on-line chamber monitor was based on a quantitative analysis of grab samples using gas adsorption tubes; quantitative analysis of these tube samples was performed on a separate, off-line gas chromatograph system calibrated with gravimetrically prepared glutaraldehyde standards. In the 13-week studies, the on-line gas chromatograph was

calibrated by high performance liquid chromatography (HPLC) analysis of bubbler (grab) samples from the chambers; the HPLC system was calibrated with gravimetrically prepared standards of glutaraldehyde.

In both studies, the concentration of glutaraldehyde in the chamber was then defined by the correlation between chamber concentrations determined by analysis of the grab samples and the on-line monitor peak area at the times of sample collection. Possible drift in the calibration of the on-line gas chromatograph was determined by monitoring an on-line standard. In the 2-week studies, acetone in nitrogen was used as the standard. In the 13-week studies, furfuryl alcohol in nitrogen, which elutes at approximately the same time as glutaraldehyde, was used as the standard because of the difficulty in generating a constant on-line standard concentration of glutaraldehyde.

Mean chamber concentrations of glutaraldehyde during the 2-week and 13-week inhalation studies were calculated from daily monitoring data (Table 1). The mean concentrations in all chambers for the 2-week studies were between 94% and 101% of target concentrations with relative standard deviations ranging from 5% to 17%; at least 59% of all individual concentration measurements were within 10% of target concentrations. During the 13-week studies, the mean chamber concentrations ranged from 99% to 105% of target concentrations with relative standard deviations between 12% and 20%; at least 63% of all individual concentration measurements were within 15% of the target concentrations. The reactivity of the test chemical with the exposure chamber and its contents made it difficult to control the concentration within the desired limits of ±15% relative standard deviation.

TABLE 1 Mean Chamber Concentrations of Glutaraldehyde in the 2-Week and 13-Week Inhalation Studies in F344/N Rats and B6C3F$_1$ Mice

Target Concentration	Mean ± SD	Target ± RSD[1]	Maximum	Minimum	Samples within Range[2] (%)
F344/N RATS					
2-WEEK STUDY					
0.0 ppm	-0.022 ± 0.017	_	0.00	-0.044	_
0.16 ppm	0.161 ± 0.018	100 ± 11	0.193	0.111	68
0.50 ppm	0.497 ± 0.082	99 ± 17	0.803	0.278	59
1.60 ppm	1.53 ± 0.233	96 ± 15	1.83	0.826	66
5.00 ppm	4.96 ± 0.226	99 ± 5	5.39	4.17	98
16.00 ppm	15.4 ± 0.916	97 ± 6	17.3	13.4	88
13-WEEK STUDY					
0.0 ppb	MDL[3]	_	MQL[4]	0.0	_
62.5 ppb	63.4 ± 10.4	102 ± 16	106	22.2	71
125 ppb	131 ± 25.9	105 ± 20	307	18.1	63
250 ppb	263 ± 43.5	105 ± 16	506	95.4	70
500 ppb	503 ± 73.7	100 ± 15	867	182	78
1000 ppb	990 ± 123	99 ± 12	1460	274	85
B6C3F$_1$ MICE					
2-WEEK STUDY					
0.0 ppm	-0.022 ± 0.017	_	0.0	-0.044	_
0.16 ppm	0.161 ± 0.017	101 ± 11	0.193	0.111	69
0.50 ppm	0.499 ± 0.081	100 ± 16	0.803	0.278	61
1.60 ppm	1.51 ± 0.229	94 ± 15	1.78	0.924	65
5.00 ppm	4.95 ± 0.237	99 ± 5	5.39	4.17	98
16.00 ppm	15.4 ± 0.752	96 ± 5	17.3	14.1	92
13-WEEK STUDY					
0.0 ppb	MDL[3]	_	MQL[4]	MDL[3]	_
62.5 ppb	63.2 ± 10.3	101 ± 16	106	22.2	72
125 ppb	130 ± 25.6	104 ± 20	307	18.1	64
250 ppb	261 ± 41.2	104 ± 16	412	95.4	71
500 ppb	502 ± 73.7	100 ± 15	867	182	78
1000 ppb	988 ± 121	99 ± 12	1430	274	86

[1] Target concentration ± relative standard deviation as a percent of target concentration.
[2] Samples were considered to be in range if they were within 10% of target concentrations during the 2-week studies and if they were within 15% of target concentrations in the 13-week studies.
[3] MDL = minimum detectable limit, 13 ppb.
[4] MQL = minimum quantifiable limit, 43 ppb.

Chamber Characterization

CONCENTRATION UNIFORMITY

During the 2-week studies, the uniformity of vapor concentration throughout each exposure chamber was measured prior to the start of the exposures and once during the study. The chamber uniformity data were within the limits (±5%) established by the NTP for all chambers during the 2-week study.

During the 13-week studies, difficulties were encountered in establishing uniform distributions of vapor concentration with animals in the chambers, and the variation in overall uniformity of concentration was greater than desired, with relative standard deviations ranging from 4% to 25%. Uniformity of vapor concentration was measured prior to the start of the study with animals in the 62.5 ppb and 1000 ppb chambers and once during the study in all chambers.

CONCENTRATION BUILDUP AND DECAY

During the 2-week studies, buildup and decay rates were measured prior to the start of the study without animals in the chambers and again at the beginning of the exposure regimen to determine if the presence of animals in the chamber would affect the rates. The time following the start of the exposure for the glutaraldehyde concentration to reach 90% of the final stable concentration in the chamber (T_{90}) and the time following the exposure for the chamber to decrease to 10% of the stable concentration (T_{10}) were determined. A value of 20 minutes was used as the T_{90} for the 2-week studies. The decay times were essentially the same (12 to 15 minutes) with and without animals. Buildup times were longer with animals present, ranging from 14 to 21 minutes without animals and from 19 to 35 minutes with animals present.

During the 13-week studies, all measurements were made with animals in the chambers because of their profound influence on the vapor concentration. Buildup times ranged from 8 to 42 minutes, while decay times ranged from 5 to 9 minutes in the prestart measurements. Because of the potential for loss of control of the exposure concentration during the measurement routine, buildup and decay measurements were not repeated after the start of the study. A value of 30 minutes was used for T_{90} during the 13-week studies.

STABILITY STUDIES

The stability and purity of the glutaraldehyde vapor in the exposure chambers were characterized using solid phase gas sampling tubes with thermal (13-week studies only) and solvent elution followed by gas chromatography analysis. Samples were taken both with and without animals present during the last hour of exposure in the 0.16 ppm and 16.0 ppm chambers and in the generator and distribution system prior to the 2-week studies, and in the mixing chamber and the 62.5 ppb and 1000 ppb chambers with animals present prior to the 13-week studies. Characterization of the methanol content and contents of other volatile compounds in the exposure chambers and the mixing chamber was performed by headspace analysis of samples collected in water bubblers in the 13-week studies.

Extensive formation and concentration of polymeric species occurred within the generation system used in the 2-week studies. The production/accumulation of polymeric species was shown by the composition of test material taken from the evaporation column at the end of the exposure day. The vapor generation system contained a water-cooled condenser which removed some glutaraldehyde as well as less volatile compounds. Enough fractionation occurred within the generator to significantly reduce the vapor concentration of polymeric species. A chamber purity of approximately 97.5% was indicated by the gas adsorption tubes for unoccupied chambers. One major impurity was found to have a gas chromatograph area of about 0.9% in the unoccupied chambers. The 0.16 ppm and 16.0 ppm occupied chambers showed purities of approximately 99.5%, and the largest detected impurity occurred at a level of approximately 0.5%.

The low concentrations of glutaraldehyde in the 13-week studies hindered the detection of impurities present at low percentages of the glutaraldehyde concentration. Methanol content in the mixing chamber was determined to be less than 0.1% w/w. Analysis by thermal desorption and gas chromatography of chamber samples trapped on gas sampling tubes indicated that no impurities were present in the exposure chambers at concentrations greater than 1%. Gas chromatographic analysis of solvent-eluted adsorbent samples from the exposure chambers indicated a glutaraldehyde purity of greater than 98%. Analysis of reservoir samples at the beginning and end of an exposure day demonstrated that glutaraldehyde was not altered during use as generation material.

Toxicity Study Designs

CORE INHALATION STUDIES

Male and female F344/N rats and B6C3F$_1$ mice used in these studies were obtained from Taconic Farms (Germantown, NY) for the 2-week studies and from Simonsen Laboratories (Gilroy, CA) for the 13-week studies. Rats and mice were approximately 4 to 5 weeks old at receipt; rats and mice were quarantined for 11 to 12 days and were about 6 to 7 weeks old when the studies began. Blood samples were collected from five animals per sex and species at the start and end of the studies. The sera were analyzed for antibody titers to rodent viruses and to *Mycoplasma pulmonis*; data from screenings performed in rats and mice (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b) showed no positive antibody titers. Additional details concerning study design and performance are listed in Table 2.

In the 2-week studies, groups of five rats and five mice of each sex were exposed to glutaraldehyde vapor through whole-body exposure at target concentrations of 0, 0.16, 0.5, 1.6, 5.0, and 16.0 ppm for 6 hours plus T_{90} (20 minutes) per day, 5 days per week, excluding weekends and holidays, for 12 exposure days. Based on the results of the 2-week studies, the high dose selected for the 13-week studies was 1000 ppb. In the 13-week studies, groups of 10 animals per sex per species were exposed to glutaraldehyde vapor through whole-body exposure at target concentrations of 0, 62.5, 125, 250, 500, and 1000 ppb for 6 hours plus T_{90} (30 minutes) per day, 5 days per week, excluding weekends and holidays, for 13 weeks.

Rats and mice were housed in individual cages within the exposure chambers. City water (Richland, WA) was available *ad libitum* during the 2-week studies and *ad libitum* except during the daily exposure period in the 13-week studies. During both the 2-week and 13-week studies, NIH-07 Open Formula diet in pellet form (Zeigler Brothers, Gardners, PA) was available *ad libitum*, except during the daily exposure period. Animal rooms were maintained at 75° ± 3°F and 55% ± 15% relative humidity, with 15 ± 3 room air changes per hour and 12 hours of light daily.

Complete necropsies were performed on all animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed prior to fixation. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with

hematoxylin and eosin. Histopathologic examinations were performed on selected tissues of all treated and control animals in the 2-week studies. In the 13-week studies, complete histopathologic examinations were performed on all control animals, on all animals in the highest dose group with at least 60% survivors, and on all animals, including those that died before the end of the study, in the higher dose groups. Target tissues (Table 2) were identified; these tissues, as well as gross lesions, were examined in all animals from the lower dose groups. All tissues examined microscopically are listed in Table 2.

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. For rats and mice, two additional sections of anterior nares were processed, sectioned, and stained for microscopic evaluation at NIEHS. These results are included in this report. Results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

SUPPLEMENTAL EVALUATIONS

Clinical Pathology

Clinical pathology studies were performed on designated male and female rats at 4 and 24 days and on core-study rats at the end of the study (Day 94). Ten rats per sex per dose group were used for these clinical pathology evaluations. At all time points, rats were anesthetized with CO_2:air (70:30), and blood samples were collected from the retroorbital sinus with heparinized capillary tubes. Blood was placed in plastic tubes containing potassium EDTA (Microtainers®, Beckton Dickinson, Rutherford, NJ) for hematologic analyses (approximately 0.5 mL) and in similar tubes devoid of an anticoagulant for biochemical determinations (approximately 1.0 mL). These latter samples were allowed to clot at room temperature for at least 30 minutes. The samples were then centrifuged and serum was removed. All hematologic and biochemical analyses were performed on the day of sample collection.

Automated hematologic determinations were performed with an Ortho ELT-8 (Ortho Instruments, Westwood, MA) hematology analyzer. The following variables were measured or calculated: erythrocyte, leukocyte, and platelet counts; hemoglobin concentration; hematocrit; mean cell volume; mean cell hemoglobin concentration; and mean cell hemoglobin. Leukocyte differentials and morphologic evaluation of blood cells were determined from blood smears stained with Wright-Giemsa. Smears made from preparations of equal volumes of new methylene blue and whole blood that had been incubated for at least 20 minutes were examined microscopically using a Miller disc for the quantitative determination of reticulocytes.

Clinical chemistry variables were measured with a Cobas Fara (Roche Diagnostic Systems, Inc., Montclair, NJ) chemistry analyzer. Assays included activities of sorbitol dehydrogenase, alanine aminotransferase, creatine kinase, and alkaline phosphatase and concentrations of total protein, albumin, globulin, urea nitrogen, creatinine, and total bile acids. Reagents for these assays were obtained from the manufacturer, with the exception of reagents for assays of sorbitol dehydrogenase and total bile acids, which were obtained from Sigma Chemical Company (St. Louis, MO).

Sperm Morphology and Vaginal Cytology Evaluations

At the conclusion of the 13-week studies, sperm morphology and vaginal cytology evaluations were performed in rats exposed to glutaraldehyde at 0, 62.5, 250, and 1000 ppb and in mice exposed to glutaraldehyde at 0, 62.5, 250, and 500 ppb. Methods were those described by Morrissey *et al.* (1988). Briefly, for the 12 days prior to sacrifice, the vaginal vaults of 10 females of each species and exposure group were lavaged, and the aspirated lavage fluid and cells were stained with Toluidine Blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (*i.e.*, diestrus, proestrus, estrus, or metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left epididymis was isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide.

After sperm motility estimates, each left cauda epididymis was placed in buffered saline solution (0.9%). Cauda were gently minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate buffered saline containing 10% dimethyl sulfoxide (DMSO). Homogenization-resistant spermatid nuclei were enumerated using a hemacytometer.

Histoautoradiographic Evaluation of Respiratory Tract

Additional rats and mice were included in the study to quantify cell proliferation in the respiratory tract following whole-body inhalation exposure to glutaraldehyde for four time points. Male and female F344/N rats and B6C3F$_1$ mice were exposed to 0, 62.5, 125, 250, 500, or 1000 ppb glutaraldehyde, and five rats and five mice per sex per exposure level were evaluated after 1 or 4 days or 6 or 13 weeks of exposure. Eighteen hours after the last glutaraldehyde exposure, and 2 hours before sacrifice, each animal was treated with tritiated thymidine, a DNA precursor used to detect cells in S-phase. Each animal received a single intraperitoneal injection of 2 µCi tritiated thymidine per gram body weight.

Each animal was anaesthetized with an overdose of intraperitoneally administered pentobarbital and exsanguinated via the abdominal aorta. All tissues, including gross lesions and the duodenum, complete respiratory tract, and tail (for animal identification), were fixed in 10% neutral buffered formalin. The nasal passages and larynx were flushed retrogradely with fixative via the trachea, the lungs were gently inflated with fixative, and the trachea was tied off. A tag was attached to the zygomatic arch to identify the heads, which were then decalcified in 5% formic acid with ion exchange resin. Tissue blocks were trimmed to provide the following set of sections: six transverse sections of the nose (Morgan, 1991), two transverse sections of the trachea, a frontal section of the carina, a longitudinal section of the left lung to include the major bronchus and longitudinal profiles of several tertiary airways, and a transverse section of the duodenum. The tissue blocks were embedded in paraffin wax, sectioned, and either stained with hematoxylin and eosin for histopathologic examination or prepared for light microscopic autoradiography.

Histopathologic findings were entered into the XYBION PATHTOX computerized database. The majority of findings were given a subjective score for severity, based on a scale of 1 to 5 as follows: 1=minimal, of doubtful biological significance; 2=mild, a clear lesion but very

limited in extent and/or severity; 3=moderately severe; 4=severe; and 5=very severe. Sections for autoradiography, selected on the basis of histopathology (see below) were de-paraffinized, dipped in Kodak autoradiography emulsion (NTB-2; Dupont), and exposed for 10 weeks at –20°C. Slides were developed in Kodak D-19 (Kodak, Rochester, NY) developer and stained with hematoxylin and eosin. Two sites were selected for histoautoradiographic analysis: (a) the pseudostratified respiratory epithelium of the dorsal atrioturbinate and (b) the squamous epithelial lining of the most anterior section of the nasal vestibule. Cells of these regions having more than 10 silver grains over the nucleus were counted as being in S-phase, and basement membrane lengths for the same regions were measured using a Zeiss Videoplan (Carl Zeiss Inc., New York, NY). Results were expressed as the number of labeled cells per millimeter basement membrane (Monticello *et al.*, 1990).

A summary of the results, the time course of selected histologic findings (Tables E1 through E4), and results of cell replication analyses in the nasal vestibule and dorsal atrioturbinate (Tables E5 and E6) are in Appendix E. These cell proliferation studies were performed in collaboration with Dr. Kevin Morgan and Elizabeth Gross of the Chemical Industry Institute of Toxicology (Gross *et al.*, 1992).

TABLE 2 Experimental Design and Materials and Methods in the 2-Week and 13-Week Inhalation Studies of Glutaraldehyde

EXPERIMENTAL DESIGN	
Study Laboratory	Battelle Pacific Northwest Laboratories, Richland, WA
Size of Study Groups	2-Week Studies: 5 males and 5 females of each species per dose group 13-Week Studies: Core Studies: 10 males and 10 females of each species per dose group Clinical Pathology Study (rats only): 10 males and 10 females per dose group Histoautoradiographic Studies: 5 males and 5 females of each species per dose group at each of four time points
Exposure Concentrations/ Duration	2-Week Studies: Duration: 6 hours plus 20 minutes per day, 5 days per week for 12 exposure days Exposure concentrations: Rats: 0, 0.16, 0.5, 1.6, 5.0, or 16.0 ppm Mice: 0, 0.16, 0.5, 1.6, 5.0, or 16.0 ppm 13-Week Studies: Duration: 6 hours plus 30 minutes per day, 5 days per week for 13 weeks Exposure concentrations: Rats: 0, 62.5, 125, 250, 500, or 1000 ppb Mice: 0, 62.5, 125, 250, 500, or 1000 ppb
Date of First Exposure	2-Week Studies: Rats: 3 August 1987 Mice: 4 August 1987 13-Week Studies: Rats: males, 18 September 1989; females, 19 September 1989 Mice: 19 September 1989
Date of Last Exposure	2-Week Studies: Rats: 18 August 1987 Mice: 19 August 1987 13-Week Studies: Rats: males, 18 December 1989; females, 19 December 1989 Mice: males, 20 December 1989; females, 21 December 1989
Type and Frequency of Observation	2-Week Studies: Observed two times per day, 7 days per week, for mortality/morbidity and up to three times per day for clinical signs of toxicity on each exposure day. Animals were weighed on Days 1 and 8 and at necropsy. 13-Week Studies: Observed two times per day. Clinical signs of toxicity were recorded weekly. Body weights were recorded at study initiation, weekly thereafter, and at necropsy.
Necropsy and Histologic Examinations	All animals received a complete necropsy. 2-Week Studies: All tissues from all animals were saved in 10% neutral buffered formalin. In addition to organs with gross lesions, histopathologic evaluations were conducted on the following tissues from all treated and control animals: larynx (transverse sections), lungs and attached tracheobronchial lymph nodes, nasal cavity (three sections), and trachea (longitudinal and transverse sections).

TABLE 2 Experimental Design and Materials and Methods in the 2-Week and 13-Week Inhalation Studies of Glutaraldehyde (continued)

Necropsy and Histologic Examinations (continued)	13-Week Studies: The protocol required that tissues be examined microscopically in all control animals, all animals in the highest dose group with at least 60% survivors, and all animals in the higher dose groups. In addition to any gross lesions, tissue masses, or suspect tumors, along with regional lymph nodes, tissues to be examined were: adrenal glands, bone (femur, diaphysis with marrow cavity and epiphyseal cartilage plate, articular cartilage and articular surface), brain (3 sections), clitoral glands, esophagus, eyes (if grossly abnormal), gallbladder (mice), heart, intestine (large: cecum, colon, rectum; small: duodenum, jejunum, ileum), kidneys, larynx, liver, lungs and mainstem bronchi, lymph nodes (bronchial, mandibular, mediastinal, mesenteric), mammary glands (including surface skin), muscle (thigh), nasal cavity and turbinates (3 sections), ovaries, pancreas, parathyroid glands, pituitary gland, preputial glands, prostate gland, salivary glands, seminal vesicles, spinal cord and sciatic nerve (if neurologic signs present), spleen, stomach (forestomach and glandular stomach), testes with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus. In rats at all lower dose levels, the following target organs were examined microscopically: nasal cavity and all gross lesions. In mice, target organs identified during examination of the high-dose group were examined at lower doses and included: larynx, nasal cavity, thymus, spleen, lymph nodes, bone marrow, and epididymides.
Supplemental Evaluations	Clinical Pathology (rats only): Clinical pathology studies were performed on designated male and female rats at 4 and 24 days and on core-study rats at the end of the study (Day 94). Hematology studies included erythrocyte count and morphologic assessment, hematocrit, hemoglobin concentration, mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), leukocyte count with differential, platelet count and morphologic assessment, reticulocyte count, and volume of packed red blood cells (VPRC). Clinical chemistry studies included alanine aminotransferase (ALT), albumin, albumin/globulin (A/G) ratio, alkaline phosphatase, creatine kinase (CK), creatinine, globulin, sorbitol dehydrogenase (SDH), total bile acids, total protein, and urea nitrogen (UN). Sperm Morphology/Vaginal Cytology: Sperm morphology/vaginal cytology studies were performed at the end of the 13-week studies. Male rats and mice exposed to 0, 62.5, 250, 500 (mice), or 1000 ppb (rats) were evaluated for necropsy body and reproductive tissue weights and spermatozoal data. Female rats and mice exposed to 0, 62.5, 250, 500 (mice), or 1000 ppb (rats) were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spend in the various stages. Histoautoradiographic Evaluation of Respiratory Tract: Rats and mice exposed to 0, 62.5, 125, 250, 500, or 1000 ppb for 1 or 4 days or 6 or 13 weeks were evaluated for incidence and severity of nasal lesions. Cell replication data for the nasal vestibule and dorsal atrioturbinate were also determined. Complete details are provided in Appendix E. These studies were performed in collaboration with the Chemical Industry Institute of Toxicology.

TABLE 2 Experimental Design and Materials and Methods in the 2-Week and 13-Week Inhalation Studies of Glutaraldehyde (continued)

ANIMALS AND ANIMAL MAINTENANCE	
Strain and Species	F344/N Rats B6C3F$_1$ Mice
Animal Source	2-Week Studies: Taconic Farms, Germantown, NY 13-Week Studies: Simonsen Laboratories, Gilroy, CA
Time Held Before Study	2-Week Studies: rats, 11 days; mice, 12 days 13-Week Studies: rats, 11 days (males), 12 days (females); mice, 12 days
Age When Placed on Study	2-Week Studies: rats, 7 weeks; mice, 7 weeks 13-Week Studies: rats, 6 weeks; mice, 6 weeks
Age When Killed	2-Week Studies: rats, 9 weeks; mice, 9 weeks 13-Week Studies: rats, 19 weeks; mice, 18 weeks
Method of Animal Distribution	Animals were weighed and randomized using a Xybion® computer program (partitioning algorithm).
Diet	NIH-07 Open Formula Pellets (Zeigler Brothers, Inc., Gardners, PA) available *ad libitum*, except during daily exposure period
Animal Room Environment	Rats and mice were housed in individual cages in the exposure chambers for all studies. Temperature was maintained at 75° ± 3°F and relative humidity at 55% ± 15%, with 15 ± 3 air changes per hour. Fluorescent light was provided for 12 hours per day.

Genetic Toxicity Studies

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Haworth *et al.* (1983) and Zeiger *et al.* (1992). Glutaraldehyde was sent to the laboratory as a coded aliquot. It was incubated with *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, TA1537, TA102, or TA104) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat and Syrian hamster liver) for 20 minutes at 37°C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following 2 days of incubation at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of glutaraldehyde. The high dose was limited by toxicity. All positive

assays were repeated under the conditions that elicited the positive response. If no positive responses were seen, all negative assays were repeated.

MOUSE LYMPHOMA MUTAGENICITY TEST PROTOCOL

The experimental protocol is presented in detail by McGregor *et al.* (1988). Glutaraldehyde was supplied as a coded aliquot. The high dose of 8 µg/mL was determined by toxicity. Mouse lymphoma L5178Y cells were maintained at 37°C as suspension cultures in Fischer's medium supplemented with *l*-glutamine, sodium pyruvate, pluronic F68, antibiotics, and heat-inactivated horse serum; normal cycling time was approximately 10 hours. To reduce the number of spontaneously occurring trifluorothymidine-resistant cells, subcultures were exposed once to medium containing THMG (thymidine, hypoxanthine, methotrexate, and glycine) for 1 day, to THG for 1 day, and to normal medium for 3 to 5 days. For cloning, horse serum content was increased and Noble agar was added.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6 x 10^6 cells in 10 mL of medium. Incubation with glutaraldehyde continued for 4 hours, at which time the medium plus glutaraldehyde was removed and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log-phase growth was maintained. After the 48-hour expression period, 3 x 10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine (TFT) for selection of TFT-resistant ($TK^{-/-}$) cells; 600 cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37°C in 5% CO_2 for 10 to 12 days. Because a clearly positive response was obtained in this assay, the experiments were not repeated with S9.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1985). Glutaraldehyde was sent to the laboratory as a coded aliquot. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs) both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and

positive controls and of at least three doses of glutaraldehyde; the high dose was limited by toxicity. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

In the SCE test without S9, CHO cells were incubated for 26 hours with glutaraldehyde in McCoy's 5A medium supplemented with fetal bovine serum, *l*-glutamine, and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing glutaraldehyde was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with glutaraldehyde, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no glutaraldehyde, and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with glutaraldehyde for 8.5 to 12 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with glutaraldehyde and S9 for 2 hours, after which the treatment medium was removed and the cells incubated for 8.5 to 12 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. One hundred first-division metaphase cells were scored at each dose level. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

DROSOPHILA MELANOGASTER SEX-LINKED RECESSIVE LETHAL TEST PROTOCOL

The assays for induction of sex-linked recessive lethal (SLRL) mutations were performed with adult flies as described in Yoon *et al.* (1985) and with larvae as described in Zimmering *et al.* (1989). Glutaraldehyde was assayed in the SLRL test by feeding (for

3 days) to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no response was obtained, it was retested by injection into adult males.

To administer glutaraldehyde by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament and the tip was broken off to allow delivery of the test solution. Injection was performed either manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 µL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector which automatically delivered a calibrated volume. Flies were anaesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of glutaraldehyde at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. Oral exposure was achieved by allowing Canton-S males to feed for 72 hours on a solution of glutaraldehyde in 5% sucrose. In the injection experiments, 24- to 72-hour-old Canton-S males were treated with a solution of glutaraldehyde and allowed to recover for 24 hours. A control group was also included. For the larval feeding experiment, Canton-S females and males were mated and eggs were exposed in vials with standard cornmeal food containing glutaraldehyde in solvent (5% ethanol) or solvent alone (Valencia *et al.*, 1989). Adult emergent males were mated at approximately 24 hours of age with two successive harems of three to five *Basc* females to establish two single-day broods. In the adult exposures, treated males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from successive matings were treated at successively earlier postmeiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event, and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution.) If a cluster was identified, all data from the male in question were discarded. Presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

Statistical Methods

ANALYSIS OF CONTINUOUS VARIABLES

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) or Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value. The extreme values chosen by the statistical test were subject to approval by NTP personnel. In addition, values indicated by the laboratory report as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF VAGINAL CYTOLOGY DATA

Because the data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels.

ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

A positive response in the *Salmonella typhimurium* assay was defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response was defined as an increase in revertants that was not dose related, not reproducible, or of insufficient magnitude to

support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

ANALYSIS OF MOUSE LYMPHOMA MUTAGENICITY DATA

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Caspary *et al.* (1988). All data were evaluated statistically for both trend and peak responses. Both responses had to be significant ($P<0.05$) for a chemical to be considered capable of inducing TFT resistance; a single significant response led to a "questionable" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

ANALYSIS OF CHO CELL CYTOGENETICS DATA

For the SCE data, statistical analyses were conducted on the slopes of the dose-response curves (Galloway *et al.*, 1985). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose, along with a trend P-value less than 0.025, was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.05$) in the absence of any responses reaching 20% above background led to a call of equivocal (Galloway *et al.*, 1985).

Chromosomal aberration data are presented as percentage of cells with aberrations. Statistical analyses were conducted on both the dose-response curve and individual dose points (Galloway *et al.*, 1985). For a single trial, a statistically significant ($P<0.05$) difference for one dose point and a significant trend ($P<0.005$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend, in the absence of a statistically significant increase at any one dose point, led to a conclusion of equivocal activity.

ANALYSIS OF *DROSOPHILA MELANOGASTER* DATA

Sex-linked recessive lethal data were analyzed by simultaneous comparison with the concurrent and historical controls using a normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered positive if the P-value was less than or equal to 0.01 and the mutation frequency in the tested group was greater that 0.10%, or if the P-value was less than or equal to 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if (a) the P-value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or (b) the P-value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P-value was greater than or equal to 0.10 or if the frequency in the treatment group was less than 0.10%.

Quality Assurance

The studies of glutaraldehyde were performed in compliance with the United States Food and Drug Administration's Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of Battelle Pacific Northwest Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of these studies. The operations of the Quality Assurance Unit were monitored by the NTP.

RESULTS

2-Week Inhalation Study in F344/N Rats

All males and females in the 5.0 ppm and 16.0 ppm groups died or were killed moribund before the end of the study (Table 3). All animals in the 16.0 ppm groups died on Day 4 of the study; 7 of 10 rats (3 males, 4 females) in the 5.0 ppm groups died on Day 5, and the remaining animals in the 5.0 ppm groups (two males, one female) died on Day 9. Body weight gains of rats in the 0.16 ppm and 0.5 ppm groups were similar to those of controls. Body weight gain for male rats in the highest surviving exposure group (1.6 ppm) was negligible. Female rats in the 1.6 ppm group showed a slight weight gain during the first week of exposure, but they had lost substantial body weight by the end of the study. Chemical-related clinical signs of toxicity were observed in the 1.6 ppm, 5.0 ppm, and 16.0 ppm groups and included labored breathing, ocular and/or nasal discharge, mouth breathing, and rough haircoat; respiratory difficulties and ocular and nasal discharge were observed immediately after exposure to 5.0 ppm or 16.0 ppm glutaraldehyde. No clinical signs were observed in rats exposed to glutaraldehyde at concentrations of 0.5 ppm or lower.

Exposure to glutaraldehyde was associated with decreases in the absolute organ weights for rats in the highest surviving concentration group (1.6 ppm); however, these differences could be explained by the lower final body weights of the animals in this exposure group.

At necropsy, exposure-related gross lesions were present only in rats that died or were sacrificed moribund. Lesions were limited to the respiratory tract and oral cavity and consisted of a crusted exudate at the anterior tip of the nares, a gray, thickened appearance to the laryngeal mucosa, and an exudate or crust on the surface of the tongue. In a few rats, the stomach and intestine were dilated with air; this dilation was considered to be the result of mouth breathing (clinical observation) and the subsequent swallowing of air.

TABLE 3 Survival and Weight Gain of F344/N Rats in the 2-Week Inhalation Study of Glutaraldehyde

Concentration (ppm)	Survival[1]	Mean Body Weight (grams) Initial[2]	Final	Change[3]	Final Weight Relative to Controls (%)[4]
MALE					
0	5/5	107	190	82	
0.16	5/5	108	193	85	102
0.5	5/5	108	183	75	97
1.6	5/5	107	109	2	58
5.0	0/5[5]	106	—	—	—
16.0	0/5[6]	109	—	—	—
FEMALE					
0	5/5	101	141	40	
0.16	5/5	100	144	45	102
0.5	5/5	101	138	38	98
1.6	5/5	98	83	–15	59
5.0	0/5[7]	101	—	—	—
16.0	0/5[6]	100	—	—	—

[1] Number surviving at 2 weeks/number of animals per group. For groups with no survivors, no final mean body weights or body weight changes are given.
[2] Body weights were measured on Day 1 of the study before the first exposure.
[3] Mean weight change of the survivors.
[4] (Treated group mean/control group mean) x 100.
[5] Day of death: 5, 5, 5, 9, 9.
[6] Day of death: all on Day 4.
[7] Day of death: 5, 5, 5, 5, 9.

Exposure-related histopathologic findings were limited to the nasal passages, larynx, trachea, lung, and tongue of both sexes of rats (Table 4). In the nasal cavity, necrosis and acute (neutrophilic) inflammation of the respiratory and olfactory epithelium were noted; hyperplasia and squamous metaplasia of the respiratory epithelium were also prominent. Nasal lesions were most severe in the respiratory mucosa over the tips of the nasal turbinates and on the lateral wall of the anterior portion (Level I) of the nasal cavity. These changes were generally of moderate to marked severity in the 5.0 and 16.0 ppm exposure groups and of mild to moderate severity in the 1.6 ppm exposure groups. At the 0.5 ppm exposure level, there was only minimal to mild hyperplasia and squamous metaplasia and no evidence of necrosis or inflammation.

TABLE 4 Selected Histopathologic Lesions for F344/N Rats in the 2-Week Inhalation Study of Glutaraldehyde[1]

	Concentration (ppm)					
	0	0.16	0.5	1.6	5.0	16.0
Nasal passages/turbinates						
Hyperplasia	–[2]	–	3/0	5/4	5/5	4/5
Squamous metaplasia	–	–	2/1	5/5	5/5	5/5
Necrosis/inflammation	–	–	–	5/5	5/5	5/5
Larynx						
Necrosis/inflammation	–	–	0/1	2/4	4/3	5/5
Squamous metaplasia	–	–	–	2/5	5/5	5/5
Trachea						
Necrosis/inflammation	–	–	–	–	3/1	5/5
Squamous metaplasia	–	–	–	–	–	5/4
Lung and bronchi						
Inflammation	–	–	–	–	–	5/5
Squamous metaplasia	–	–	–	–	–	5/5
Tongue						
Inflammation/erosion	–	–	–	–	–	4/1

[1] Data are given as male incidence/female incidence for five animals per group.
[2] (–) lesion not present

In the larynx and trachea of rats exposed to 16.0 ppm glutaraldehyde, there was moderate to marked inflammation with necrosis, erosion, and squamous metaplasia of the respiratory epithelium. At the 5.0 ppm exposure level, the tracheal changes were limited to minimal to mild inflammation. Laryngeal lesions were more extensive than tracheal lesions and were present in the lower exposure groups. At the 1.6 ppm exposure level, there was minimal to mild inflammation and squamous metaplasia of the respiratory epithelium in the larynx. Minimal inflammation in the larynx was present in 1 female rat from the 0.5 ppm exposure group. Exposure-related lesions in the lung were identified only in the 16.0 ppm groups and consisted of a moderate, acute inflammatory cell exudate in the mainstem bronchi and squamous metaplasia of the bronchial respiratory epithelium.

Histopathologic lesions in the tongue occurred in the 16.0 ppm exposure groups and consisted of minimal to mild, single or multiple erosions of the squamous epithelium on the dorsal surface; a minimal acute (neutrophilic) inflammatory cell infiltrate in the mucosa was associated with these erosions.

In 5 of 10 rats exposed to 1.6 ppm glutaraldehyde, mild to moderate thymic (lymphoid) atrophy was noted. This was the highest exposure group that survived to the end of the study and the thymic atrophy was considered to be secondary to stress, as suggested by the marked depression in body weight gain in this group.

Exposure concentrations for the 13-week studies were limited to 1000 ppb (1 ppm) because of the extent of respiratory tract lesions that occurred in animals receiving 1.6 ppm or greater in the 2-week studies.

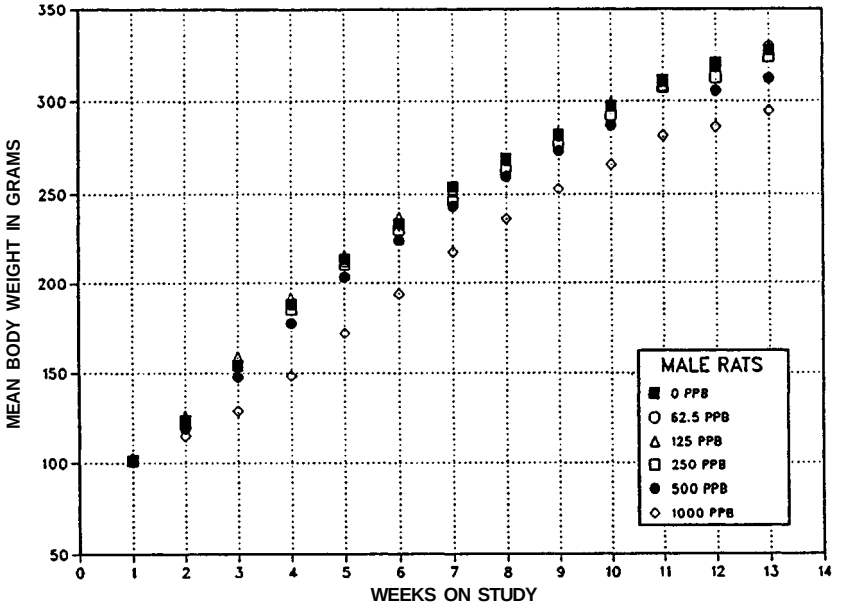
13-Week Inhalation Study in F344/N Rats

No exposure-related deaths were recorded during this study in rats; however, one female in the 250 ppb group was sacrificed moribund before the end of the study because of dental malocclusion (Table 5). Mean final body weights and body weight gains were significantly lower than control values for male and female rats exposed to 1000 ppb glutaraldehyde (Figure 1). Females exposed to 500 ppb also had a lower mean body weight gain than controls, but the mean final body weight for this group was similar to the control value.

TABLE 5 Survival and Weight Gain of F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde

Concentration (ppb)	Survival[1]	Mean Body Weight (grams) Initial[2]	Final	Change[3]	Final Weight Relative to Controls (%)[4]
MALE					
0	10/10	102	327	225	
62.5	10/10	102	329	227	101
125	10/10	102	328	226	100
250	10/10	101	323	222	99
500	10/10	101	312	211	95
1000	10/10	103	295	192	90
FEMALE					
0	10/10	92	192	100	
62.5	10/10	92	191	99	100
125	10/10	94	195	101	102
250	9/10[5]	91	185	93	97
500	10/10	95	184	89	96
1000	10/10	91	179	88	93

[1] Number of core-study animals surviving at 13 weeks/number of animals per exposure level.
[2] Body weight was measured before the first exposure. Subsequent calculations were based on animals surviving to the end of the study.
[3] Mean weight change of the animals in each group.
[4] (Treated group mean/control group mean) x 100.
[5] Week of death: 8. Sacrificed moribund due to malocclusion.



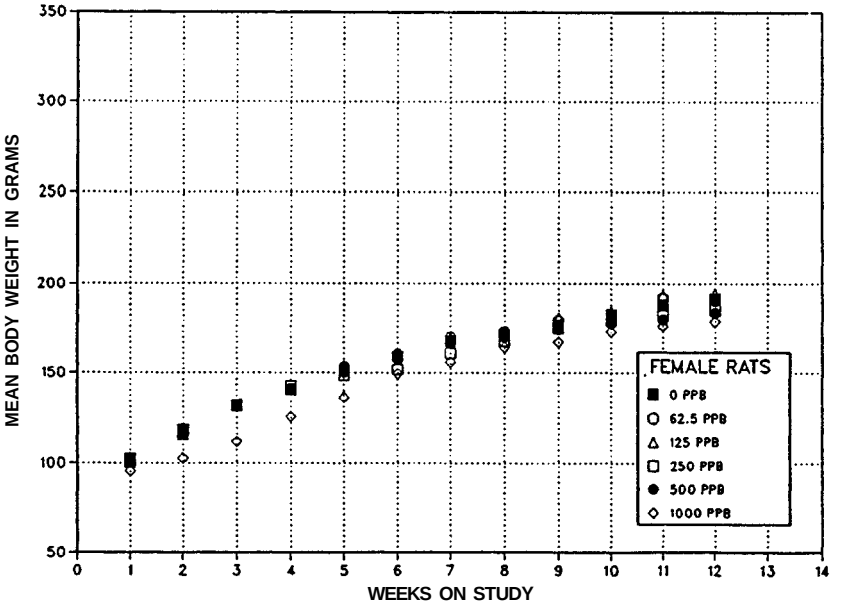


FIGURE 1 Body Weights of F344/N Rats Exposed to Glutaraldehyde by Inhalation for 13 Weeks

Clinical findings of toxicity included dyspnea and ruffled fur in all animals in the 1000 ppb group and emaciation in many animals (3 of 10 males, 8 of 10 females) in this same group during the first 5 weeks of the study; however, these animals exhibited no clinical signs during the latter portion of the study.

Hematology and clinical chemistry evaluations were conducted on Days 4 and 24 in supplemental-study animals and at the end of the 13 weeks in core-study animals. Findings are presented in detail in Appendix B.

No clinically significant changes in hematology or clinical chemistry parameters occurred on Day 4 following 3 days of exposure to glutaraldehyde. At 24 days, there were significant increases in total counts of segmented neutrophils in males in three of the four highest exposure groups (125, 250, and 1000 ppb) and in females in two of the three highest exposure groups (250 and 1000 ppb). In female rats, the increase in the highest exposure group, 1000 ppb, was accompanied by an increase in total leukocyte count. Additionally, at 24 days, there were minimal to mild, statistically significant increases in activities of alanine aminotransferase in male and female rats in the 250, 500, and 1000 ppb exposure groups and in alkaline phosphatase in female rats in the 500 and 1000 ppb exposure groups. Other findings included mild decreases in concentrations of total protein in males in the 125 to 1000 ppb exposure groups and in females in the 1000 ppb exposure group and of albumin in females in all groups except the 500 ppb exposure group; increases in urea nitrogen were noted in females in the 250 to 1000 ppb exposure groups. Changes in other hematology and clinical chemistry parameters in male and female rats at Day 94 of the study were sporadic and were not considered exposure related (Appendix B).

Statistically significant increases were observed in the relative weights of the heart, kidney, lungs, and testis of males exposed to 1000 ppb glutaraldehyde, the heart of females exposed to 1000 ppb, and the kidney of females exposed to 250 ppb, 500 ppb, or 1000 ppb glutaraldehyde (Appendix A, Table A1). However, these differences were small and were not considered to be toxicologically significant.

Exposure to glutaraldehyde at concentrations up to 1000 ppb caused treatment-related microscopic lesions in the nasal passages and/or turbinates of male and female rats (Table 6). Lesions occurred primarily in the anterior portions of the nose (Levels I and II) and affected the respiratory and the olfactory epithelium. The lateral wall of the nasal

cavity and the tips of the nasoturbinates were the most commonly affected portions of the respiratory mucosa, while the dorsal meatus was the most common location of the olfactory lesions. Hyperplasia of goblet cells was prominent on the nasal septum (Plates 1 and 2) and was generally present only at the highest exposure level. In the respiratory epithelium, hyperplasia and squamous metaplasia were present. Hyperplasia also occurred in the respiratory epithelium on the lateral wall (transitional/cuboidal epithelium) and the nasoturbinates; when the surface epithelial layers were flattened, the lesion was diagnosed as squamous metaplasia (Plates 3 and 4). Minimal inflammation (neutrophilic) occurred in most rats exposed to 1000 ppb, the highest concentration of glutaraldehyde.

Minimal to mild olfactory degeneration, characterized by a thinning of the nuclear layers of the olfactory epithelium, was present in several rats, but only at the highest exposure level. In addition, exposure-related lesions were noted in the two nasal sections taken anterior to the standard Level I section; these sections included the nasal vestibule and the most anterior portion of the nasal turbinates. Minimal to mild squamous exfoliation was present in the nasal vestibule of rats exposed to 250 ppb or greater. This lesion consisted of an accumulation of keratin, cell debris, and bacteria in the lumen of the nasal vestibule. Minimal focal erosions of the squamous mucosa were present in one female rat in the 500 ppb group and two females in the 1000 ppb group.

TABLE 6 Selected Histopathologic Lesions of the Nasal Passages/Turbinates for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde[1]

	Concentration (ppb)					
	0	62.5	125	250	500	1000
Male						
Respiratory epithelium						
Nasoturbinates/septum						
Hyperplasia	0	0	0	0	0	7 (1.7)
Hyperplasia, goblet cell	0	0	0	1 (1.0)	3 (1.0)	9 (1.4)
Squamous metaplasia	0	0	0	0	0	5 (2.0)
Inflammation	0	0	0	0	0	7 (1.0)
Lateral wall						
Hyperplasia	0	0	1 (1.0)	0	4 (1.0)	7 (1.7)
Squamous metaplasia	0	0	0	0	1 (1.0)	7 (2.5)
Olfactory epithelium						
Degeneration	0	0	0	0	0	1 (2.0)
Nasal vestibule/anterior nares						
Squamous exfoliation	0	0	0	1 (1.0)	4 (1.0)	9 (1.1)
Inflammation	0	1 (1.0)	0	0	0	3 (1.0)
Female						
Respiratory epithelium						
Nasoturbinates/septum						
Hyperplasia	0	0	0	0	0	4 (1.7)
Hyperplasia, goblet cell	0	0	0	0	0	8 (1.2)
Squamous metaplasia	0	0	0	0	0	5 (1.4)
Inflammation	0	0	0	1 (1.0)	0	5 (1.2)
Lateral wall						
Hyperplasia	0	0	0	1 (2.0)	2 (1.0)	8 (1.6)
Squamous metaplasia	0	0	0	1 (3.0)	0	8 (2.0)
Olfactory epithelium						
Degeneration	0	0	0	0	0	2 (1.5)
Nasal vestibule/anterior nares						
Squamous exfoliation	0	0	0	3 (1.3)	7 (1.1)	9 (1.7)
Inflammation	1 (1.0)	0	0	0	0	0
Erosion	0	0	0	0	1 (1.0)	2 (2.0)

[1] The incidence is the number of core-study animals with lesions for groups of 10 animals. Average severity (in parentheses) is based on the number of animals with lesions; 1=minimal, 2=mild, 3=moderate, 4=marked.

2-Week Inhalation Study in B6C3F$_1$ Mice

All mice exposed to glutaraldehyde at concentrations of 1.6 ppm, 5.0 ppm, or 16.0 ppm died or were killed moribund before the end of the study (Table 7). All mice in the 16.0 ppm groups died on Day 4, as did four of the five females in the 5.0 ppm group. All mice in the three highest exposure groups were dead by study Day 8. One control female died on study Day 8. Body weight gains were similar to those of controls for male mice in the 0.16 ppm and 0.5 ppm groups. Females in the 0.5 ppm group failed to gain weight during the first week of the study but recovered by the end of the study. No clinical signs of toxicity were seen in mice exposed to glutaraldehyde at concentrations of 0.5 ppm or less. Chemical-related clinical findings were limited to animals exposed to the three highest concentrations of glutaraldehyde (1.6 ppm, 5.0 ppm, 16.0 ppm) and included marked respiratory difficulties accompanied by ocular and nasal discharge and mouth breathing immediately after the first exposure. These animals did not appear to eat or drink during the study.

Female mice exposed to 0.5 ppm glutaraldehyde had significantly lower mean absolute and relative heart weights than controls, while males in the 0.5 ppm group had a significantly greater mean relative liver weight than controls. These findings were not considered biologically significant.

At necropsy, gross lesions attributed to the toxicity of glutaraldehyde were present in the external nares and larynx of mice in the 16.0 ppm exposure groups. Gross observations included a red crust at the anterior tip of the nares and a gray, thickened appearance of the laryngeal mucosa. Dilation of the stomach and intestine with air was seen in mice that died or were sacrificed moribund (mice in the 1.6, 5.0, and 16.0 ppm groups). This finding was considered to be the result of mouth breathing (clinical observation) and the subsequent swallowing of air.

Exposure-related histopathologic findings were limited to the nasal passages, larynx, and trachea in male and female mice (Table 8). In the nasal cavity of mice exposed to 1.6 to 16.0 ppm glutaraldehyde, there was necrosis and acute (neutrophilic) inflammation of the respiratory and olfactory epithelium and squamous metaplasia of the respiratory epithelium. These lesions were of minimal to mild severity and were located primarily in the anterior portion (Level I) of the nasal cavity. Typically, these lesions were present on the tips of the nasal turbinates and on the lateral wall of the nasal cavity.

TABLE 7 Survival and Weight Gain of B6C3F$_1$ Mice in the 2-Week Inhalation Study of Glutaraldehyde

Concentration (ppm)	Survival[1]	Mean Body Weight (grams) Initial[2]	Final	Change[3]	Relative to Controls (%)[4]
MALE					
0	5/5	24.8	26.2	1.3	
0.16	5/5	24.3	26.6	2.3	102
0.5	5/5	24.2	25.7	1.5	98
1.6	0/5[5]	24.2	—	—	—
5.0	0/5[6]	24.0	—	—	—
16.0	0/5[7]	24.0	—	—	—
FEMALE					
0	4/5[8]	19.3	23.2	4.0	
0.16	5/5	19.6	22.5	2.9	97
0.5	5/5	20.0	22.1	2.1	95
1.6	0/5[9]	19.4	—	—	—
5.0	0/5[10]	19.0	—	—	—
16.0	0/5[7]	19.3	—	—	—

[1] Number surviving at 2 weeks/number of animals per group. For groups with no survivors, no final mean body weights or body weight changes are given.
[2] Body weights were measured on Day 1 of the study before the first exposure. Subsequent calculations were based on animals surviving to the end of the study.
[3] Mean weight change of the survivors.
[4] (Treated group mean/control group mean) x 100.
[5] Day of death: 6, 8, 8, 8, 8.
[6] Day of death: 5, 6, 7, 7, 7.
[7] Day of death: all on Day 4.
[8] Day of death: 8.
[9] Day of death: 7, 8, 8, 8, 8.
[10] Day of death: 4, 4, 4, 4, 6.

TABLE 8 Selected Histopathologic Lesions for B6C3F$_1$ Mice in the 2-Week Inhalation Study of Glutaraldehyde[1]

	Concentration (ppm) 0	0.16	0.5	1.6	5.0	16.0
Nasal passages/turbinates						
Squamous metaplasia	–[2]	–	–	1/2	2/1	1/3
Necrosis/inflammation	–	–	–	4/4	4/2	5/5
Larynx						
Necrosis/inflammation	–	–	–	0/1	1/2	5/5
Squamous metaplasia	–	–	1/0	5/4	5/4	5/4
Trachea						
Necrosis/inflammation	–	–	–	–	–	3/2

[1] Data are given as male incidence/female incidence for five animals per group.
[2] (–) lesion not present

Laryngeal lesions consisted of inflammation and necrosis of the mucosa and squamous metaplasia of the respiratory epithelium. In some mice exposed to 16.0 ppm, the squamous metaplasia was not present because of the marked necrosis and inflammation of the respiratory mucosa. Squamous metaplasia was more prominent in the mice in the 1.6 and 5.0 ppm exposure groups; these mice survived longer than those in the 16.0 ppm groups. Treatment-related lesions in the trachea were present only at the highest exposure level, 16.0 ppm, and consisted of inflammation and necrosis of the respiratory mucosa.

Exposure concentrations chosen for the 13-week study were limited to 1000 ppb (1 ppm) because of the extent and severity of the respiratory tract lesions that occurred in mice exposed to glutaraldehyde at concentrations of 1.6 ppm or greater in the 2-week study.

13-Week Inhalation Study in B6C3F$_1$ Mice

All mice exposed to 1000 ppb and two females exposed to 500 ppb glutaraldehyde died before the end of the study (Table 9). Mean final body weights of male and female mice were reduced in a concentration-dependent manner. Male and female mice in the 250 and 500 ppb groups weighed significantly less than control animals. Final body weights for males and females exposed to 500 ppb glutaraldehyde were approximately 11% less than those of the controls.

Body weight gains were recorded for all groups except females exposed to 1000 ppb. However, a significant concentration-related decrease in mean body weight gain occurred in male mice in all exposure groups and in female mice in the 250 ppb and 500 ppb groups. Growth curves are shown in Figure 2; the irregular weight curve for males exposed to 1000 ppb is due to deaths of animals with low body weights, whose removal produced an increase in mean body weight at the next weighing. Chemical-related signs of toxicity were seen in 7 of 10 males and in 9 of 10 females in the 1000 ppb groups before death and included dyspnea and emaciation. Dyspnea was observed in 7 of 10 males and in 5 of 10 females in the 500 ppb groups early in the study, but it subsided after the first few weeks. Other clinical signs exhibited by animals exposed to 1000 ppb included abnormal posture, hypoactivity, ruffled fur, paraphimosis, and tachypnea.

Exposure-related increases in organ-weight-to-body-weight ratios were noted for the heart, kidney, and lungs of male and female mice, the liver of female mice, and the testes of male mice (Appendix A). However, the increases in relative organ weights were generally not accompanied by significant increases in absolute organ weights and probably reflected the typically higher relative organ weights in lighter-weight animals.

TABLE 9 Survival and Weight Gain of B6C3F$_1$ Mice in the 13-Week Inhalation Study of Glutaraldehyde

Concentration (ppb)	Survival[1]	Mean Body Weight (grams) Initial[2]	Final	Change[3]	Final Weight Relative to Controls (%)[4]
MALE					
0	10/10	22.5	34.2	11.8	
62.5	10/10	22.8	33.0	10.2	96
125	10/10	22.7	32.8	10.1	96
250	10/10	22.7	31.7	9.0	92
500	10/10	22.2	30.3	8.0	88
1000	0/10[5]	22.8	27.7	4.4	81
FEMALE					
0	10/10	18.9	29.5	10.7	
62.5	10/10	19.4	29.0	9.6	98
125	10/10	19.0	29.4	10.4	100
250	10/10	19.1	27.4	8.4	93
500	8/10[6]	19.4	26.3	7.0	89
1000	0/10[7]	19.4	—	—	—

[1] Number of core-study animals surviving at 13 weeks/number of animals per exposure level. For groups with no survivors on Day 84, no final mean body weights or body weight changes are given.

[2] Body weight was measured before the first exposure. Subsequent calculations were based on animals surviving to the end of the study.

[3] Mean weight change of the animals in each group.

[4] (Treated group mean/control group mean) x 100.

[5] Week of death: 1, 1, 1, 1, 1, 2, 3, 4, 5, 14. Final body weights were recorded on Day 84 when one survivor remained in this group; however, this animal died before scheduled necropsy. Statistical analyses were not performed due to the insufficient number of measurements.

[6] Week of death: 7, 8.

[7] Week of death: 1, 1, 1, 2, 2, 2, 2, 2, 2, 3.

Exposure-related gross observations were present in mice that died or were sacrificed moribund before the end of the study. Most commonly, dilation of the stomach and intestine was observed. This change was considered to be secondary to the dyspnea observed clinically and the subsequent swallowing of air. In three mice, the spleen appeared paler or smaller than normal as the result of lymphoid depletion. Lymphoid depletion in the spleen, as well as the lymphoid and cellular depletion in the lymph nodes, thymus, and bone marrow, and increased numbers of degenerative cells in the lumen of the epididymis, were considered to be secondary to the generalized stress associated with exposure in the mice that died during the study.

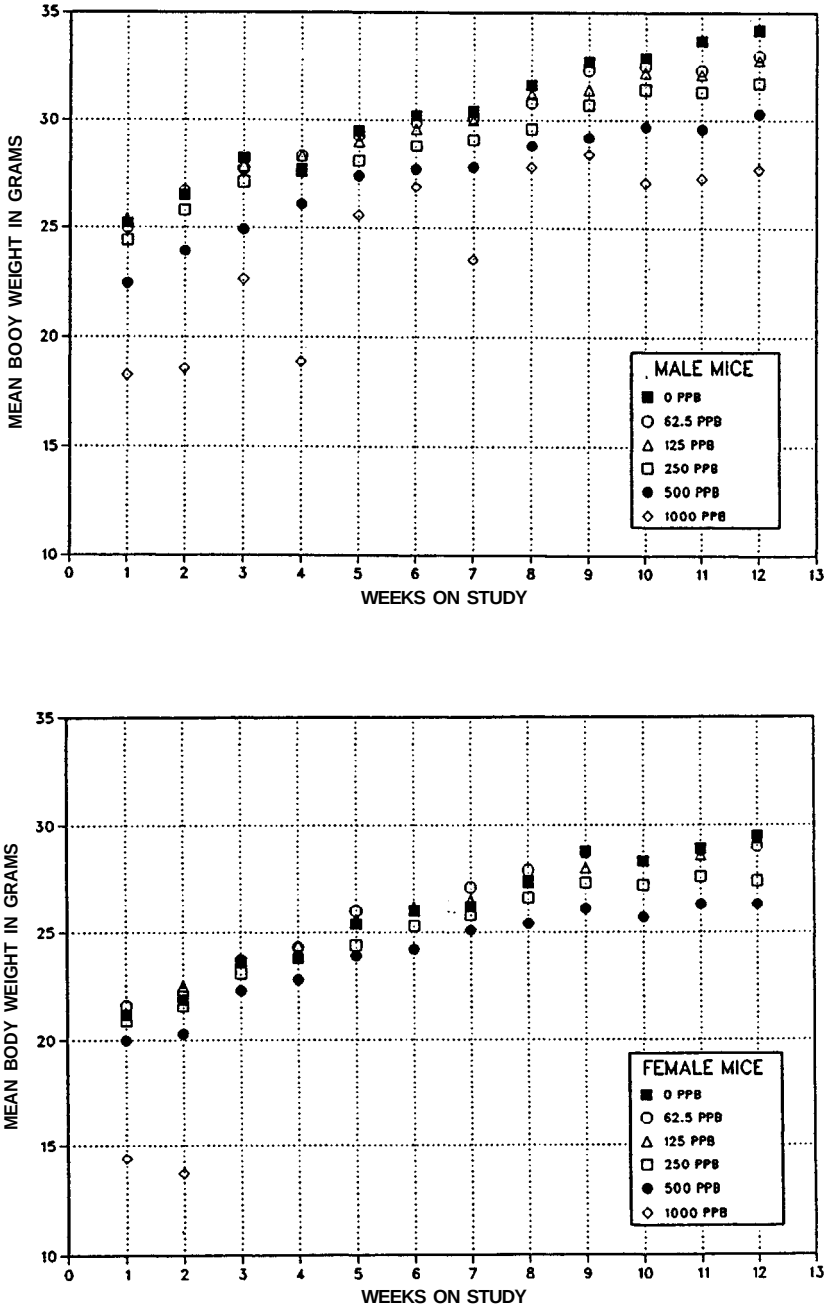


FIGURE 2 Body Weights of B6C3F$_1$ Mice Exposed to Glutaraldehyde by Inhalation for 13 Weeks

Exposure-related microscopic lesions occurred in the nasal passages and/or turbinates and the larynx (Table 10). Changes in the larynx were present only in the 1000 ppb groups of mice and consisted of necrosis and squamous metaplasia of the respiratory mucosa at the base of the epiglottis (Plates 5 and 6). Nasal lesions were seen primarily in Level I and to a much lesser extent in Level II. Lesions were also present in additional sections prepared from the tip of the nose which included the nasal vestibule and anterior portion (anterior to Level I) of the nasal turbinates.

TABLE 10 Selected Histopathologic Lesions for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Glutaraldehyde[1]

	Concentration (ppb)					
	0	**62.5**	**125**	**250**	**500**	**1000**
Male						
Nasal passages/turbinates						
Respiratory epithelium						
Inflammation	0	0	0	0	0	4 (1.0)
Squamous metaplasia	0	0	0	0	0	1 (2.0)
Nasal vestibule/anterior nares						
Squamous exfoliation	0	0	0	1 (1.0)	2 (1.0)	9 (2.8)
Inflammation	0	0	0	0	7 (1.1)	0[2]
Erosion	0	0	0	1 (1.0)	1 (1.0)	0
Larynx						
Squamous metaplasia	0	0	0	0	0	7 (1.6)
Necrosis	0	0	0	0	0	2 (1.0)
Female						
Nasal passages/turbinates						
Respiratory epithelium						
Inflammation	0	0	0	1 (1.0)	1 (1.0)	7 (1.4)
Squamous metaplasia	0	0	0	0	0	3 (1.0)
Nasal vestibule/anterior nares						
Squamous exfoliation	0	0	0	1 (1.0)	2 (2.5)	10 (2.8)
Inflammation	0	5 (1.0)	8 (2.0)	8 (1.6)	8 (2.5)	0[2]
Erosion	0	0	1 (1.0)	0	0	0
Larynx						
Squamous metaplasia	0	0	0	0	0	10 (1.6)
Necrosis	0	0	0	0	0	2 (1.0)

[1] The incidence is the number of core study animals with lesions from groups of 10. Average severity (in parentheses) is based on the number of animals with lesions; 1=minimal, 2=mild, 3=moderate, 4=marked.

[2] Inflammation was a component of "squamous exfoliation" and not diagnosed separately when the latter was present.

Squamous exfoliation, characterized by the accumulation of keratin, an inflammatory cell exudate, cell debris, and bacterial colonies in the lumen of the vestibule, occurred in mice exposed to 250 ppb or greater (Plates 7, 8, and 9). In the groups of mice that survived to the end of the study, the squamous exfoliation and inflammatory cell exudate in the nasal lumen were generally not observed. However, foci of acute (neutrophilic) inflammation were present in the nasal mucosa. Neutrophils were present in the lamina propria and throughout the squamous or respiratory epithelium in the most anterior portion of the nasoturbinates (Plate 10). In the standard nasal sections (Levels I to III), inflammation and squamous metaplasia of the respiratory mucosa were located on the lateral wall of the nasal cavity and on the tips of the nasoturbinates (Plates 11 and 12).

Respiratory Tract Histoautoradiographic Evaluations

A number of histopathologic lesions, generally of minimal to mild severity, were present in the mucosa of the nasal passages of rats and mice. The spectrum of morphologic changes (inflammation, erosion, squamous metaplasia, etc.) was similar to that seen at the end of the 13-week core studies. Because of the multiple early time points for sampling in this special histoautoradiographic study, the onset of lesions and the progression of severity was demonstrated (Tables E1-E4).

The squamous epithelium of the nasal vestibule, and to a lesser extent the respiratory epithelium of the atrioturbinate of the dorsal meatus, exhibited exposure-related increases in cell replication rates (Tables E5 and E6). When the results from individual animals were examined, there was an increased rate of cell replication in the squamous epithelium of the nasal vestibule in mice that also had neutrophilic infiltration of the mucosa; this was most evident at the 13-week time point and was more clear in females than in males. Pairwise comparisons between the subjective score for severity of neutrophilic infiltrate and the extent of the S-phase response at the 13-week time point in female mice indicated that the severity of the infiltrate did not correlate with the degree of S-phase response. However, increased cell replication occurred only in those animals in which the neutrophilic infiltrate was observed.

In rats, the exposure-related increase in cell replication was generally greater than that observed for mice. In contrast to the results obtained for mice, the increased cell replication in the nasal vestibule of rats occurred early and remained elevated or decreased slightly through the course of the study. In addition to increased replication in the

squamous epithelium of the vestibule, there was an equally prominent increase in replication in the respiratory epithelium of the dorsal atrioturbinate in rats. At this site, the increased replication corresponded more with the presence of squamous metaplasia of the respiratory epithelium than with inflammation.

Reproductive System Evaluations

Sperm morphology and vaginal cytology evaluations in rats revealed no significant differences between treated animals and controls for any of the parameters evaluated (Appendix C).

Reproductive system evaluations in mice showed significant differences in the amount of time spent in estrous stages for females in the 250 ppb and 500 ppb groups compared to controls. Female mice in these groups spent more time in estrus and diestrus and less time in metestrus and proestrus (500 ppb group only) than did controls. Glutaraldehyde exposure did not directly affect any parameters measured in male mice in these evaluations (Appendix C).

Genetic Toxicity Studies

Glutaraldehyde was tested for induction of mutations in *Salmonella typhimurium* in three laboratories (Appendix D, Table D1). In the first laboratory, positive results were obtained with strain TA100 with and without liver S9 from Aroclor 1254-induced male Sprague-Dawley rats or Syrian hamsters. In the second laboratory, no increase in mutations was observed in TA100 in the absence of S9 or with 10% induced hamster S9. A small increase in mutations was noted in TA100 in the presence of 10% induced rat S9, and results were considered equivocal. In both laboratories, negative results were obtained with TA1535, TA1537, and TA98 with and without S9. Complete data sets from these two studies are presented by Haworth *et al.* (1983). The third laboratory tested glutaraldehyde for induction of mutations in *S. typhimurium* strains TA100, TA102, and TA104. Results were clearly positive for all three strains with and without induced hamster or rat liver S9. Glutaraldehyde also induced mutations at the $TK^{+/-}$ locus of mouse L5178Y cells at a concentration of 8 µg/mL in each of two trials conducted in the absence of S9 activation (Appendix D, Table D2; McGregor *et al.*, 1988).

In one of two test laboratories, glutaraldehyde induced sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9; results from the second laboratory were weakly positive in the presence of S9 and negative without S9 (Appendix D, Table D3; Galloway *et al.*, 1985). Although the negative trial in the absence of S9 showed a significant increase in SCEs at the highest dose tested, the trial was called negative on the basis of the trend test, with a P-value greater than 0.025 (Galloway *et al.*, 1985). Under the current evaluation criteria (Galloway *et al.*, 1987), this trial would be considered equivocal. Glutaraldehyde was also tested in these same two laboratories for induction of chromosomal aberrations (Abs) in CHO cells (Appendix D, Table D4; Galloway *et al.*, 1985). The first laboratory reported negative results with and without S9, while the second laboratory found a weakly positive increase in Abs in the absence of S9. Higher doses were used in the second study, which may explain the apparently discordant results between laboratories. At the second laboratory, the trial conducted with S9 showed a dose-related increase in Abs, but this increase was not considered to be of sufficient magnitude to be considered positive under the older data evaluation system (Galloway *et al.*, 1985). Under the current system (Galloway *et al.*, 1987), this trial would be considered weakly positive.

Glutaraldehyde was tested for its ability to induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* treated as newly emerged adult flies by feeding or injection (Yoon *et al.*, 1985) or treated as larvae by feeding (Zimmering *et al.*, 1989). Results from all three tests were negative (Appendix D, Table D5).

In summary, glutaraldehyde was shown to be genotoxic *in vitro*, inducing mutations in bacterial cells, and producing mutations, sister chromatid exchanges, and chromosomal aberrations in mammalian cells. Its mutagenic activity in these assays was independent of S9 activation.

PLATE 1

Section through nasal septum of control rat showing the columnar respiratory epithelium (arrows) with occasional unstained goblet cells. Compare with similar section in Plate 2 from rat exposed to glutaraldehyde. 160x.

PLATE 2

Section through nasal septum of rat exposed to 1000 ppb glutaraldehyde. Note increased thickness of respiratory epithelium (arrows) and hyperplasia of goblet cells. 160x.

PLATE 3

Nasoturbinate of control rat for comparison with glutaraldehyde-exposed rat in Plate 4. Note the low columnar to cuboidal respiratory epithelium (arrow). 260x.

PLATE 4

Nasoturbinate of rat exposed to 1000 ppb glutaraldehyde. Squamous metaplasia is characterized by a thickened epithelium compared to control (Plate 3) and a superficial layer of a flattened, squamous epithelium (arrow). 260x.

PLATE 5

Larynx (base of epiglottis) of control mouse showing columnar, ciliated epithelium (arrow) for comparison with Plate 6. 260x.

PLATE 6

Larynx (base of epiglottis) of mouse exposed to 1000 ppb glutaraldehyde, showing squamous metaplasia of the respiratory epithelium (arrow). 260x.

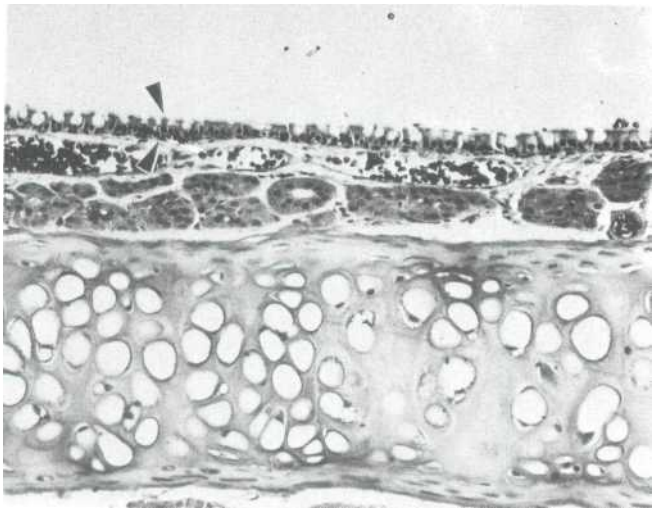


PLATE 1

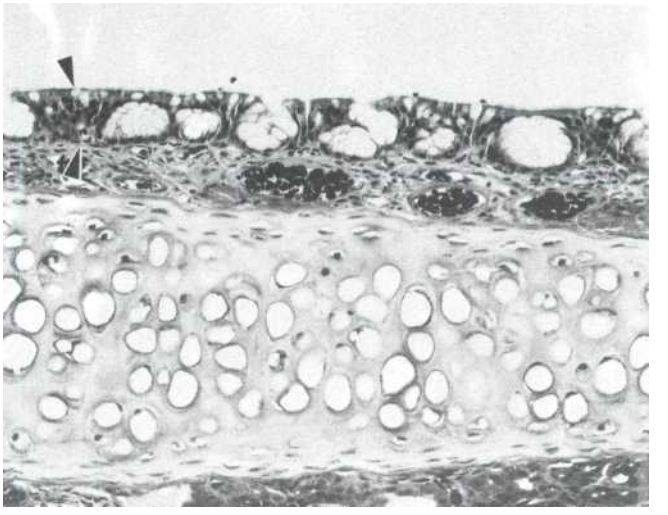


PLATE 2

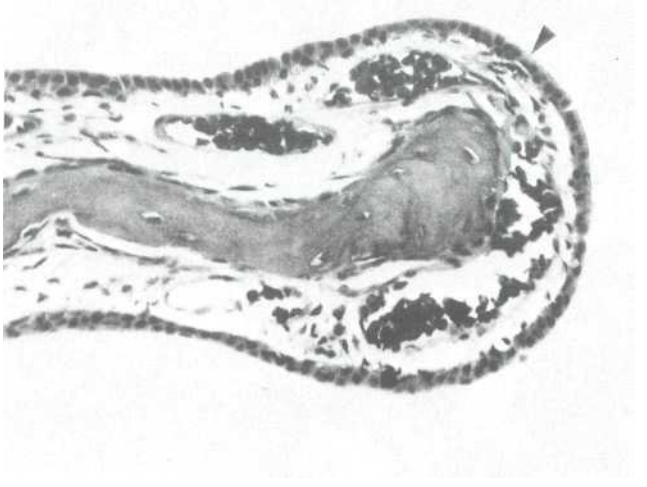


PLATE 3

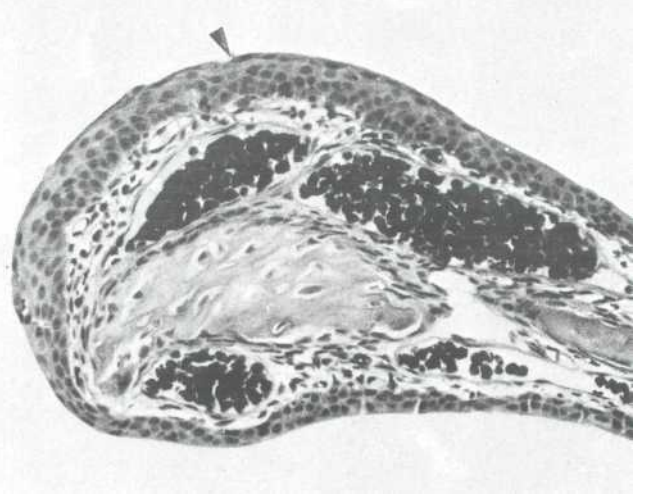


PLATE 4

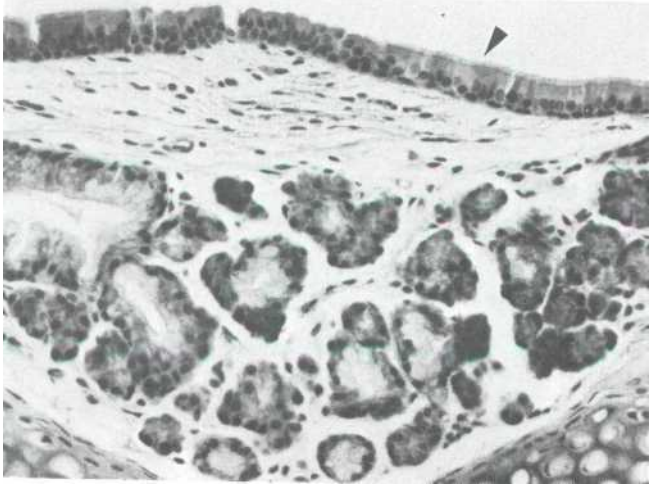


PLATE 5

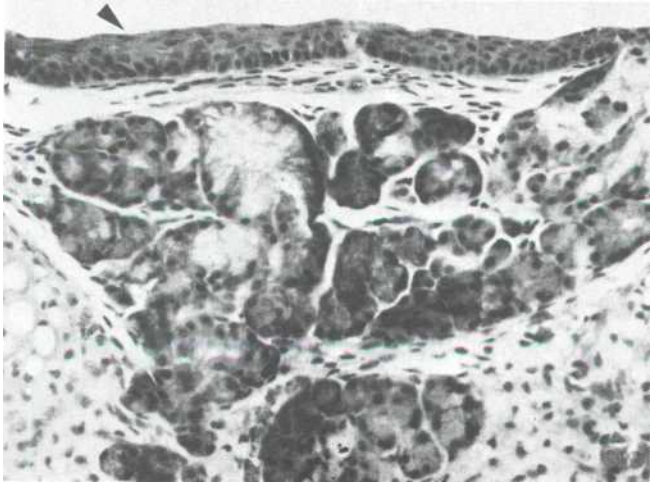


PLATE 6

PLATE 7

Nasal vestibule of mouse exposed to 1000 ppb glutaraldehyde. Lumen (L) of vestibule contains abundant keratin and cell debris (arrows). 70x.

PLATE 8

Detail of lumen contents in nasal vestibule shows numerous bacterial colonies (B) between darkly stained keratin fibers (arrows). 160x.

PLATE 9

Lumen (L) of anterior nares from mouse exposed to 1000 ppb glutaraldehyde contains darkly stained keratin fibers and cell debris. 260x.

PLATE 10

Tip of nasoturbinate from mouse exposed to 125 ppb glutaraldehyde, showing infiltration of neutrophils (arrows) throughout the mucosa. 320x.

PLATE 11

Tip of nasoturbinate from control mouse for comparison with Plate 12. 160x.

PLATE 12

Tip of nasoturbinate from mouse exposed to a concentration of 1000 ppb glutaraldehyde, showing inflammation in the mucosa and an exudate (E) of neutrophils in the lumen of the nasal cavity. Note mild squamous metaplasia (arrow) of respiratory epithelium. 160x.

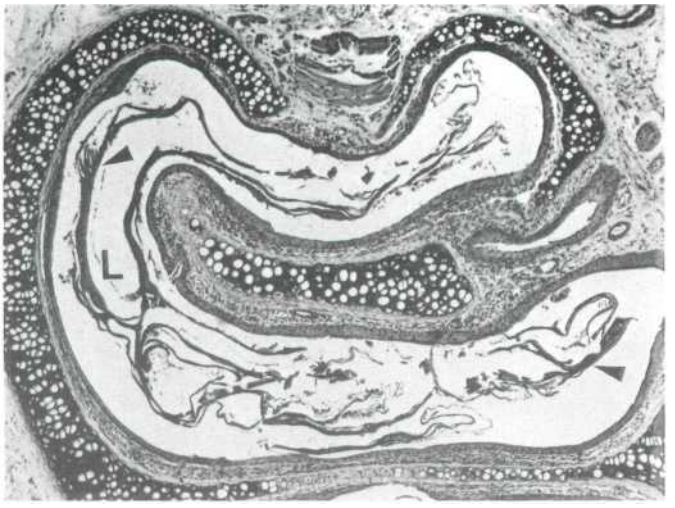


PLATE 7

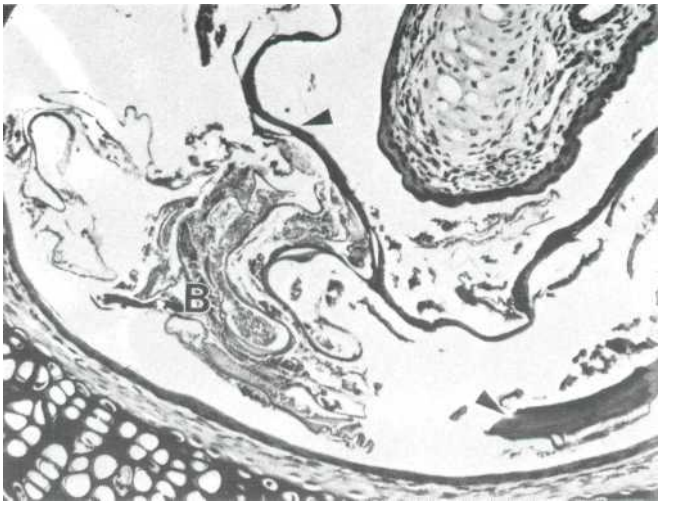


PLATE 8

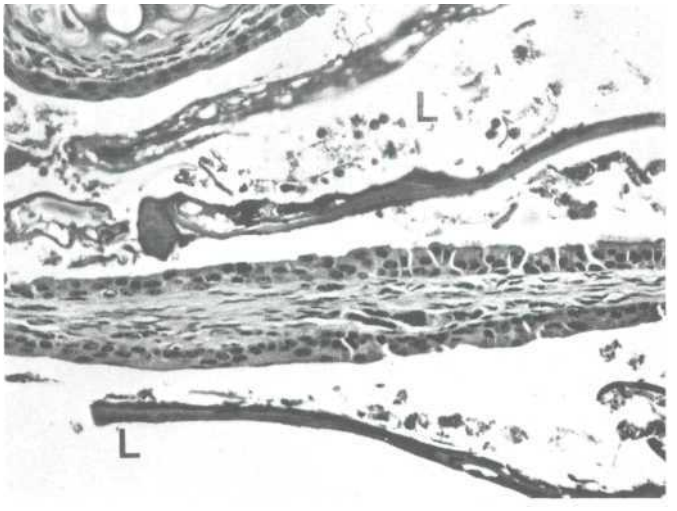


PLATE 9

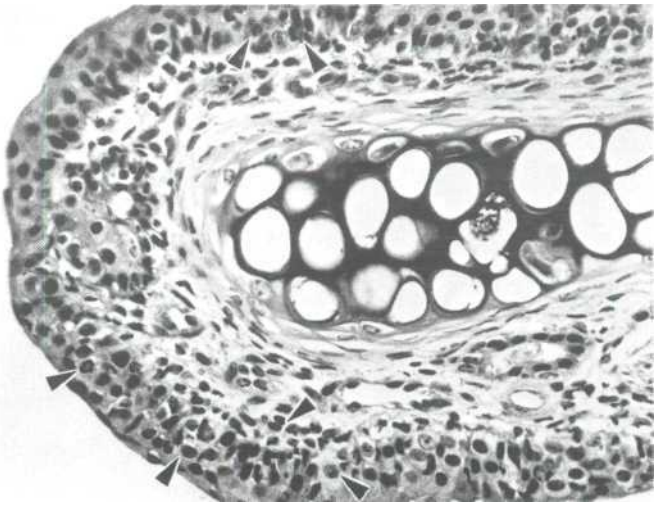


PLATE 10

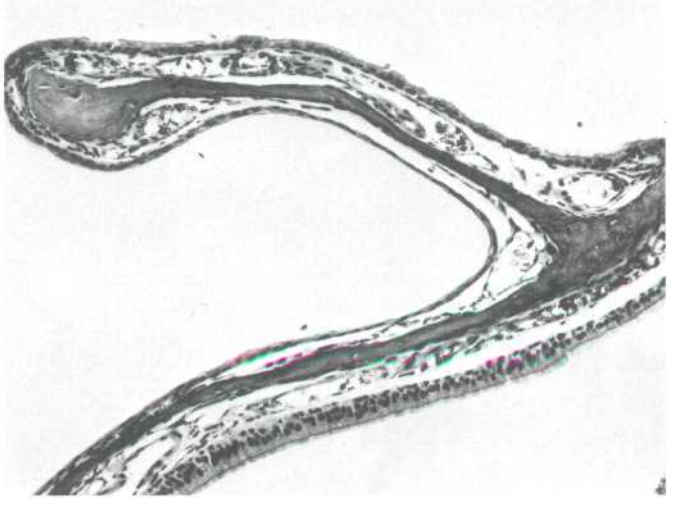


PLATE 11

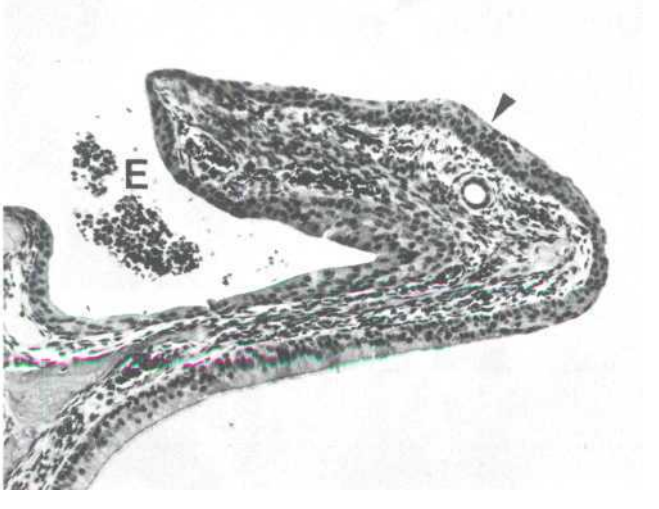


PLATE 12

DISCUSSION

The results of the 2-week and 13-week studies indicate that the respiratory tract is the major target for toxicity following exposure of rats and mice to glutaraldehyde by the inhalation route. There was no substantial evidence of systemic toxicity. At the higher doses used in the 2-week studies, there was marked toxicity in the upper respiratory tract in addition to mortality and the clinical finding of dyspnea. Minimal to mild squamous metaplasia, hyperplasia, and inflammation or necrosis of the larynx, nasal passages, or both were present in rats and mice after 2 weeks of exposure to glutaraldehyde at concentrations as low as 0.5 ppm. At higher exposure concentrations, the incidences and severity of the lesions increased and necrosis and inflammation were present at lower sites in the respiratory tract (including the trachea in rats and mice and the lung in rats); at the highest exposure concentration in rats (16.0 ppm), inflammation and erosions were present on the dorsal surface of the tongue. Based on the mortality observed in the 2-week studies, mice were more sensitive than rats to glutaraldehyde toxicity.

In rats, there was little evidence for increased toxicity following 13 weeks of exposure beyond that observed after the 2-week exposure; the no-observed-adverse-effect level (NOAEL) was 0.16 ppm in the 2-week study and 125 ppb (0.125 ppm) in the 13-week study. The primary site of glutaraldehyde toxicity in the 13-week study, the nasal cavity, was the same as that seen in the 2-week study at similar exposure concentrations.

In contrast, mice were more sensitive to the 13-week exposure. Mortality occurred at exposure levels as low as 500 ppb for females, and all mice exposed to 1000 ppb died during the study. Although the severity of the respiratory tract lesions in mice in the 13-week study was similar to the severity of the lesions seen in rats, a high number of deaths occurred only in mice. This may have been related to the accumulation of keratin and cell debris in the anterior tip of the nares of mice and possible occlusion of that portion of the airway in the nasal passage. The fact that more mice died than rats, which have a larger airway in the external nares, combined with the observations of respiratory difficulty (gaseous distension of the stomach and small intestine) in mice exposed to 1000 ppb glutaraldehyde, suggests that the early deaths in mice were due to nasal obstruction.

In the 13-week study, inflammation characterized by focal accumulation of neutrophils in the nares was observed in mice that received exposures as low as 62.5 ppb, particularly in female mice. Data collected from the animals killed at various times throughout the study as part of the collaborative effort with the Chemical Industry Institute of Toxicology indicated that the neutrophilic infiltrate became progressively more severe and was associated with increased epithelial cell replication in the anterior nasal passages (Appendix E, Tables E1 through E6). The neutrophil accumulation was present in female mice at all concentration levels of glutaraldehyde at which animals survived. Examination of Table E1 reveals the difficulty of interpreting this lesion in rats due to the background incidence of both suppurative and nonsuppurative nasal lesions.

Hematologic evaluations at 24 days showed that segmented neutrophil counts were increased in male and female rats. Changes in counts of segmented neutrophils can be produced by a variety of mechanisms, including stress, physiologic causes, and inflammation. Of these mechanisms, stress is often associated with decreases in segmented neutrophil counts, and physiologic leukocytosis is associated with increases in total counts of lymphocytes. Because changes in lymphocyte counts did not occur, the mature neutrophilia probably resulted from inflammation. The lack of immature forms (no left shift) is consistent with a mild response. These findings are probably associated with the exposure-related effects in the nares. Local inflammation with migration of neutrophils through the nasal respiratory epithelium is a prominent feature of formaldehyde toxicity in rats (Monticello *et al.*, 1991).

Increased activities of alkaline phosphatase and alanine aminotransferase (ALT) as were observed in rats at 24 days are generally associated with cholestasis and release of the enzymes from hepatocellular membranes and cytosol, respectively. These changes are consistent with mild, exposure-induced hepatobiliary damage. Lack of an increase in activity of sorbitol dehydrogenase and negative hepatic histopathology at the end of the study, however, may indicate an alternate mechanism. Five- to seven-fold increases in ALT activity have been reported in the liver tissue of feed-restricted rats. The increase probably results from enhanced gluconeogenesis (Schwartz *et al.*, 1973; Loeb and Quimby, 1989). This process would be consistent with the decreased weight gain in rats in the high-exposure groups and the mild, selective increase in enzyme activity. The minimal to mild decreases in concentrations of total protein and albumin and the increase in urea nitrogen (UN) were likely associated with secondary effects of the exposures. These include

decreased food intake (total protein and albumin) and catabolism of proteins and/or mild dehydration (UN).

The specific site and morphological effects of glutaraldehyde on the upper respiratory tract of rats and mice are consistent with those produced by exposure to other irritant chemicals administered by the inhalation route (Jiang *et al.*, 1983; Boorman *et al.*, 1987; Morgan and Monticello, 1990; NTP, 1991; NTP, 1992). When irritant chemicals are administered at higher concentrations, lesions have also been shown to occur in the lung and oral cavity (Jiang *et al.*, 1983; Boorman *et al.*, 1987; NTP, 1991).

A specific comparison of respiratory tract responses induced by glutaraldehyde versus formaldehyde is of interest because of the extensive pathology data available for formaldehyde. Intranasal instillation studies have demonstrated that on a molar basis, glutaraldehyde is 10 to 20 times more toxic (potent) than formaldehyde when delivered to the nasal mucosa as a single treatment in aqueous solution (St. Clair *et al.*, 1990). The present study and reported toxicity data for formaldehyde (Monticello, 1990; Heck *et al.*, 1990; Monticello *et al.*, 1991) reveal that when administered by inhalation, glutaraldehyde is about 20 times more potent than formaldehyde. The distribution of nasal lesions induced by inhalation of glutaraldehyde in the present study differed from that reported for formaldehyde (Morgan *et al.*, 1986a,b; Monticello *et al.*, 1991) in that the glutaraldehyde-induced lesions were more anterior in the nose, involving the squamous epithelium. No preneoplastic lesions like those occurring in animals receiving formaldehyde by inhalation for 13 weeks (focal hyperkeratosis and hyperplasia with cellular atypia and dysplasia) (Monticello, 1990; Morgan and Monticello, 1990) were detected in the present study of glutaraldehyde, although glutaraldehyde was genotoxic in *in vitro* studies in both bacterial and mammalian cells.

In summary, exposure of rats and mice to glutaraldehyde by inhalation for up to 13 weeks resulted in a spectrum of necrotic, inflammatory, and regenerative lesions confined to the upper respiratory tract. Mice were somewhat more sensitive than rats because the small airways of the nasal passage in mice were more prone to blockage with cellular debris, bacteria, and keratin. The NOAEL was 125 ppb for respiratory lesions in rats. An NOAEL was not reached for mice, as inflammation was found in the anterior nasal passage at concentrations as low as 62.5 ppb.

REFERENCES

ANTONENKOV, V. D., PIROZHKOV, S. V., AND PANCHENKO, L. F. (1987). On the role of microsomal aldehyde dehydrogenase in metabolism of aldehydic products of lipid peroxidation. *FEBS Lett.* **224**, 357-360.

BALLANTYNE, B. (1986). *Review of Toxicological Studies and Human Health Effects—Glutaraldehyde*. Union Carbide Corp., Danbury, CT.

BALLANTYNE, B., GARMAN, R. H., GREENSPAN, B. J., AND MYERS, R. C. (1985). Acute toxicity and irritancy of glutaraldehyde. *Toxicologist* **5**, 204. (Abstr.)

BASF CORPORATION (1990). *Substantial Risk Report on Glutaraldehyde*, EPA number 83HQ-0990-1008, US EPA/OPTS, Public Files 89-900000423 and 88-900000179.

BEAUCHAMP, R., ST. CLAIR, M. G., FENNELL, T., CLARKE, D., AND KARI, F. (1992). Toxicology of glutaraldehyde: A critical review. *CRC Crit. Rev. Toxicol.*, under review.

BESRAT, A., POLAN, C. E., AND HENDERSON, L. M. (1969). Mammalian metabolism of glutaric acid. *J. Biol. Chem.* **244**, 1461-1467.

BOORMAN, G. A., MONTGOMERY, C. A., JR., EUSTIS, S. L., WOLFE, M. J., MCCONNELL, E. E., AND HARDISTY, J. F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H. A. Milman and E. K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

BOORMAN, G. A., HICKMAN, R. L., DAVIS, G. W., RHODES, L. S., WHITE, N. W., GRIFFIN, T. A., MAYO, J., AND HAMM, T. E., JR. (1986). Serological titers to murine viruses in 90-day and 2-year studies. In *Complications of Viral and Mycoplasmal Infections in Rodents to Toxicology Research and Testing* (T. E. Hamm, Jr., Ed.), pp. 11-23. Hemisphere, New York.

BOORMAN, G. A., URIAH, L. C., GUPTA, B. N., AND BUCHER, J. R. (1987). Two-hour methyl isocyanate inhalation and 90-day recovery study in B6C3F$_1$ mice. *Environ. Health Perspect.* **72**, 63-69.

CASPARY, W. J., LEE, Y. J., POULTON, S., MYHR, B. C., MITCHELL, A. D., AND RUDD, C. J. (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Quality- control guidelines and response categories. *Environ. Mol. Mutagen.* **12** (Suppl. 13), 19-36.

CODE OF FEDERAL REGULATIONS (CFR) **21**, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.

DESCOTES, J. (1988). Identification of contact allergens: The mouse ear sensitization assay. *J. Toxicol. Cut. Ocul. Toxicol.* **7**, 263.

DEVRO INCORPORATED (1984). Glutaraldehyde oral toxicity tests indicate no related lesions. *Food Chem. News* **26**, 42.

DIXON, W. J., AND MASSEY, F. J., JR. (1951). *Introduction to Statistical Analysis*, 1st ed., pp. 145-147. McGraw-Hill Book Company, New York.

DUNN, O. J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

DUNNETT, C. W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

FORTE-MCROBBIE, C. M., AND PIETRUSZKO, R. (1986). Purification and characterization of human liver "high K_m" aldehyde dehydrogenase and its identification as glutamic γ-semialdehyde dehydrogenase. *J. Biol. Chem.* **261**, 2154-2163.

GALLOWAY, S. M., BLOOM, A. D., RESNICK, M., MARGOLIN, B. H., NAKAMURA, F., ARCHER, P., AND ZEIGER, E. (1985). Development of a standard protocol for in vitro cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. *Environ. Mutagen.* **7**, 1-51.

GALLOWAY, S. M., ARMSTRONG, M. J., REUBEN, C., COLMAN, S., BROWN, B., CANNON, C., BLOOM, A. D., NAKAMURA, F., AHMED, M., DUK, S., RIMPO, J., MARGOLIN, B. H., RESNICK, M. A., ANDERSON, B., AND ZEIGER, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* **10** (Suppl. 10), 1-175.

GOLOBOV, Y., FURSOV, B. A., MISHCHENKO, B. P., ZAITSEV, V. V., DYATLOV, V. A., ZAITSEV, L. V., ROZANOVA, I. B., TSEITLINA, E. A., SOLOMATINA *ET AL.* (1989). Effect of chemical treatment methods on calcification, hemocompatibility, and immunogenic properties of xenopericardial bioprosthesis of cardiac valves. *Dokl. Akad. Nauk Ukr. SSR, Ser. B: Geol., Khim. Biol. Nauki* **1**, 56.

GREENSPAN, B. J., BALLANTYNE, B., FOWLER, E. H., AND SNELLINGS, W. M. (1985). Subchronic inhalation toxicity of glutaraldehyde. *Toxicologist* **5**, 29. (Abstr.)

GROSS, E. A., MORGAN, K. T., MELLICK, P. W., AND KARI, F. W. (1991). Glutaraldehyde-induced pathology and cell proliferation in the rat nasal cavity. *Toxicologist* **11**, 182.

GROSS, E. A., MELLICK, P., KARI, F., AND MORGAN, K. T. (1992). Histopathology and cell replication in the nasal epithelium of rats and mice exposed to glutaraldehyde vapor. *Toxicologist* **12**, 399. (Abstr.)

HABEEB, A. F. S. A., AND HIRAMOTO, R. (1968). Reaction of proteins with glutaraldehyde. *Arch. Biochem. Biophys.* **126**, 16-26.

HAWORTH, S., LAWLOR, T., MORTELMANS, K., SPECK, W., AND ZEIGER, E. (1983). Salmonella mutagenicity test results for 250 chemicals. *Environ. Mutagen.* **5** (Suppl. 1), 3-142.

HAZARDOUS SUBSTANCES DATA BANK (HSDB) (1990). Glutaraldehyde. Toxnet computer file record 949, October 10, 1990.

HECK, H. D'A., CASANOVA, M., AND STARR, T. B. (1990). Formaldehyde toxicity - new understanding. *CRC Crit. Rev. Toxicol.* **20**, 397-426.

HEMMINKI, K., AND SUNI, R. (1984). Sites of reaction of glutaraldehyde and acetaldehyde with nucleosides. *Arch. Toxicol.* **55**, 186-190.

HEMMINKI, K., FALCK, K., AND VAINIO, H. (1980). Comparison of alkylation rates and mutagenicity of directly acting industrial and laboratory chemicals: Epoxides, glycidyl ethers, methylating and ethylating agents, halogenated hydrocarbons, hydrazine derivatives, aldehydes, thiuram, and dithiocarbamate derivatives. *Arch. Toxicol.* **46**, 277-285.

HEMMINKI, K., MUTANEN, P., SALONIEMI, I., NIEMI, M.-L., AND VAINIO, H. (1982). Spontaneous abortions in hospital staff engaged in sterilising instruments with chemical agents. *Br. Med. J.* **285**, 1461-1463.

HEMMINKI, K., KYYROENEN, P., AND LINDBOHM, M. (1985). Spontaneous abortions and malformations in the offspring of nurses exposed to anaesthetic gases, cytostatic drugs, and other potential hazards in hospitals, based on registered information of outcome. *J. Epidemiol. Community Health* **39**, 141.

HOPWOOD, D. (1975). The reactions of glutaraldehyde with nucleic acids. *Histochem. J.* **7**, 267.

JAMES, T. N., AND BEAR, E. S. (1968). Cardiac effects of some simple aliphatic aldehydes. *J. Pharmacol. Exp. Ther.* **163**, 300-308.

JAWORSKY, C., TAYLOR, J. S., EVEY, P., AND HANDEL, D. (1987). Allergic contact dermatitis to glutaraldehyde in a hair conditioner. *Cleve. Clin. J. Med.* **54**, 443-444.

JIANG, X. Z., BUCKLEY, L. A., AND MORGAN, K. T. (1983). Pathology of toxic responses to the RD50 concentration of chlorine gas in the nasal passages of rats and mice. *Toxicol. Appl. Pharmacol.* **71**, 225-236.

JONCKHEERE, A. R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

JONES, G. L., AND TENG, Y.-S. (1983). A chemical and enzymological account of the multiple forms of human liver aldehyde dehydrogenase. Implications for ethnic differences in alcohol metabolism. *Biochim. Biophys. Acta* **745**, 162-174.

KARP, W. B., KORB, P., AND PASHLEY, D. (1987). The oxidation of glutaraldehyde by rat tissues. *Pediatr. Dent.* **9**, 301.

KERNS, W. D., PAVKOV, K. L., DONOFRIO, D. J., GRALLA, E. J., AND SWENBERG, J. A. (1983). Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Res.* **43**, 4382-4392.

KOSAKO, M., AND NOSHIOKA, H. (1982). New forward mutation assay using low-concentration streptomycin resistance mutation in E coli strains with plasmid pKM 101. *DDRKA* **22**, 239-249.

LEVIN, D. E., HOLLSTEIN, M., CHRISTMAN, M. F., SCHWIERS, E. A., AND AMES, B. N. (1982). A new *Salmonella* tester strain (TA102) with A:T base pairs at the site of mutation detects oxidatibe mutagens. *Proc. Natl. Acad. Sci. USA* **79**, 7445-7449.

LOEB, W. F., AND QUIMBY, F. W. (EDS.) (1989). *The Clinical Chemistry of Laboratory Animals*. Pergamon Press, New York.

MARGOLIN, B. H., COLLINGS, B. J., AND MASON, J. M. (1983). Statistical analysis and sample-size determinations for mutagenicity experiments with binomial responses. *Environ. Mutagen.* **5**, 705-716.

MARKS, T. A., WORTHY, W. C., AND STAPLES, R. E. (1980). Influence of formaldehyde and Sonicide (potentiated acid glutaraldehyde) on embryo and fetal development in mice. *Teratology* **22**, 51.

MARNETT, L. J., HURD, H. K., HOLLSTEIN, M. C., LEVIN, D. E., ESTERBAUER, H., AND AMES, B. N. (1985). Naturally occurring carbonyl compounds are mutagens in *Salmonella* tester strain TA104. *Mutat. Res.* **148**, 25-34.

MARONPOT, R. R., AND BOORMAN, G. A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

MCGREGOR, D. B., BROWN, A., CATTANACH, P., EDWARDS, I., MCBRIDE, D., AND CASPARY, W. J. (1988). Responses of the L5178Y tk^+/tk^- mouse lymphoma cell forward mutation assay. II: 18 coded chemicals. *Environ. Mol. Mutagen.* **11**, 91-118.

MIRSALIS, M. M., TYSON, C. K., STEINMETZ, K. L, LOH, E.K., HAMILTON, C. M., BAKKE, J. P., AND SPALDING, J. W. (1989). Measurement of unscheduled DNA synthesis and S-phase synthesis in rodent hepatocytes following in vivo treatment: Testing of 24 compounds. *Environ. Mol. Mutagen.* **14**, 155-164.

MONTICELLO, T. M. (1990). Formaldehyde Induced Pathology and Cell Proliferation: A Thesis. Duke University, Durham, NC.

MONTICELLO, T. M., MORGAN, K. T., AND HURTT, M. E. (1990). Unit length as the denominator for quantitation of cell proliferation in nasal epithelia. *Toxicol. Pathol.* **18**, 24-31.

MONTICELLO, T. M., MILLER, F. J., AND MORGAN, K. T. (1991). Regional increases in rat nasal epithelial cell proliferation following acute and subchronic inhalation of formaldehyde. *Toxicol. Appl. Pharmacol.* **111**, 409-421.

MORGAN, K. T. (1991). Approaches to the identification and recording of nasal lesions in toxicology studies. *Toxicol. Pathol.* **19** No. 4 (Part 1), 337-351.

MORGAN, K. T., AND MONTICELLO, T. M. (1990). Formaldehyde toxicity: Respiratory epithelial injury and repair. In *Biology, Toxicology, and Carcinogenesis of the Respiratory Epithelium* (D. G. Thomassen and P. Nettesheim, Eds.), pp. 155-171. Hemisphere Publishing Corp., Washington, DC.

MORGAN, K. T., GROSS, E. A., AND PATTERSON, D. L. (1986a). Distribution, progression and recovery of acute formaldehyde-induced inhibition of nasal mucociliary function in F-344 rats. *Toxicol. Appl. Pharmacol.* **86**, 448-456.

MORGAN, K. T., JIANG, X. Z., STARR, T. B., AND KERNS, W. D. (1986b). More precise localization of nasal tumors associated with chronic exposure of F-344 rats to formaldehyde gas. *Toxicol. Appl. Pharmacol.* **82**, 264-271.

MORRISON, D. F. (1976). *Multivariate Statistical Methods*, pp. 170-179. McGraw-Hill Book Company, New York.

MORRISSEY, R. E., SCHWETZ, B. A., LAMB, J. C., IV, ROSS, M. D., TEAGUE, J. L., AND MORRIS, R. W. (1988). Evaluation of rodent sperm, vaginal cytology, and reproductive organ weight data from National Toxicology Program 13-week studies. *Fundam. Appl. Toxicol.* **11**, 343-358.

MYERS, D. R., PASHLEY, D. H., LAKE, F. T., BURNHAM, D., KALATHOOR, S., AND WATERS, R. (1986). Systemic absorption of 14C-glutaraldehyde from glutaraldehyde-treated pulpotomy sites. *Pediatr. Dent.* **8**, 134.

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (NIOSH) (1985). National Occupational Exposure Survey database search.

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (NIOSH) (1990). National Occupational Exposure Survey (1981 to 1983), unpublished provisional data as of July 1, 1990. Cincinnati, OH.

NATIONAL TOXICOLOGY PROGRAM (NTP) (1991). NTP Technical Report on Toxicity Studies of Formic Acid administered by inhalation to F344/N Rats and B6C3F$_1$ Mice. Toxicity Report Series No. 19. NIH Publication No. 92-3342. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

NATIONAL TOXICOLOGY PROGRAM (NTP) (1992). Draft NTP Technical Report on Toxicity Studies of 1,6-Hexanediamine Dihydrochloride Administered by Drinking Water and Inhalation to F344/N Rats and B6C3F$_1$ Mice. Toxicity Report Series No. 24. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION (OSHA) (1989). Air Contaminants. Final Rule, *Fed Regist.* **54**, 2332.

OHSUMI, T., AND KUROKI, K. (1988). Acute toxicity of glutaraldehyde. *Kyushu Shika Gakkai Zasshi* **42**, 470.

OKAMURA, K., CHIBA, C., IRIYAMA, T., ITOH, T., MAETA, H., IJIMA, H., MITSUI, T., AND HORI, M. (1980). Antigen depressant effect of glutaraldehyde for aortic heterografts with a valve, with special reference to a concentration right fit for the preservation of grafts. *Surgery* **87**, 170.

PACKER, L., AND GREVILLE, G. D. (1969). Energy-linked oxidation of glutaraldehyde by rat liver mitochondria. *FEBS Lett.* **3**, 112-114.

PETERS, K., AND RICHARDS, F. M. (1977). Chemical cross-linking: Reagents and problems in studies of membrane structure. *Ann. Rev. Biochem.* **46**, 523-551.

PIROZHKOV, S. V., AND PACHENKO, L. F. (1988). Role of aldehyde dehydrogenase in malonic dialdehyde metabolism in rat liver. *Biokhimiiya* **53**, 1443.

RANLY, D. M., HORN, D., AND ZISLIS, T. (1985). The effect of alternatives to formocresol on antigenicity of proteins. *J. Dent. Res.* **64**, 1225-1228.

RAO, G. N., HASEMAN, J. K., AND EDMONDSON, J. (1989a). Influence of viral infections on body weight, survival, and tumor prevalence in Fischer 344/NCr rats on two-year studies. *Lab. Anim. Sci.* **39**, 389-393.

RAO, G. N., PIEGORSCH, W. W., CRAWFORD, D. D., EDMONDSON, J., AND HASEMAN, J. K. (1989b). Influence of viral infections on body weight, survival, and tumor prevalence of B6C3F1 (C57BL/6N x C3H/HeN) mice in carcinogenicity studies. *Fundam. Appl. Toxicol.* **13**, 156-164.

REIFENRATH, W. G., PRYSTOWSKY, S. D., NONOMURA, J. H., AND ROBINSON, P. B. (1985). Topical glutaraldehyde-percutaneous penetration and skin irritation. *Arch. Dermatol. Res.* **277**, 242.

RUIZ-RUBIO, M., ALEJANDRE-DURAN, E., AND PUEYO, C. (1985). Oxidative mutagens specific for A:T base pairs induce forward mutations to *l*-arabinose resistance in *Salmonella typhimurium*. *Mutat. Res.* **147**, 153-163.

RYZLAK, M. T., AND PIETRUSZKO, R. (1988). Human brain "high K_m" aldehyde dehydrogenase: Purification, characterization, and identification as NAD^+-dependent succinic semialdehyde dehydrogenase. *Arch. Biochem. Biophys.* **266**, 386-396.

SAKAGAMI, Y., YAMASAKI, K., YOKOYAMA, H., OSE, Y., AND SATO, T. (1988a). DNA repair test of disinfectants by liquid rec-assay. *Mutat. Res.* **193**, 21-30.

SAKAGAMI, Y., YAMASAKI, H., OGASAWARA, N., YOKOYAMA, H., OSE, Y., AND SATO, T. (1988b). The evaluation of genotoxic activities of disinfectants and their metabolites by umu test. *Mutat. Res.* **209**, 155-160.

SASAKI, Y., AND ENDO, R. (1978). Mutagenicity of aldehydes in *Salmonella*. *Mutat. Res.* **54**, 251-252.

SAX, N. I., AND LEWIS, R. J. (EDS.) (1989). Glutaraldehyde. In *Dangerous Properties of Industrial Materials*, 7th ed., p. 1809. Van Nostrand Reinhold, New York.

SCHWARTZ, E., TORNABEN, J. A., AND BOXILL, G. C. (1973). The effects of food restriction on hematology, clinical chemistry and pathology in the albino rat. *Toxicol. Appl. Pharmacol.* **25**, 515-524.

SHIRLEY, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

SLESINSKI, R. S., HENGLER, W. C., GUZZIE, P. J., AND WAGNER, K. J. (1983). Mutagenicity evaluation of glutaraldehyde in a battery of in vitro bacterial and mammalian test systems. *Food Chem. Toxicol.* **21**, 621-629.

SMITH, L., AND PACKER, L. (1972). Aldehyde oxidation in rat liver mitochondria. *Arch. Biochem. Biophys.* **148**, 270-276.

SPENCER, P. S., BISCHOFF, M. C., AND SCHAUMBURG, H. H. (1978). On the specific molecular configuration of neurotoxic aliphatic hexacarbon compounds causing central-peripheral distal axonopathy. *Toxicol. Appl. Pharmacol.* **44**, 17-28.

ST. CLAIR, M. B., GROSS, E. A., AND MORGAN, K. T. (1989). Interaction of glutaraldehyde with nasal epithelium. *Toxicologist* **9**, 37. (Abstr.)

ST. CLAIR, M. B., GROSS, E. A., AND MORGAN, K. T. (1990). Pathology and cell proliferation induced by intra-nasal instillation of aldehydes in the rat: Comparison of glutaraldehyde and formaldehyde. *Toxicol. Pathol.* **18**, 353.

ST. CLAIR, M. B. G., BERMUDEZ, E., GROSS, E. A., RECIO, L., MORGAN, K. T., AND BUTTERWORTH, B. E. (1991a). Evaluation of the genotoxic and cytotoxic potential of glutaraldehyde. *Environ. Mol. Mutagen.* **17** (Suppl. 19), 71.

ST. CLAIR, M., BERMUDEZ, E., GROSS, E., BUTTERWORTH, B., AND RECIO, L. (1991b). Evaluation of the genotoxic potential of glutaraldehyde. *Environ. Mol. Mutagen.* **18**, 113-119.

STANFORD RESEARCH INSTITUTE (SRI) (1976). U.S. production: Glutaraldehyde. HSDB record, October 1990.

STERN, M. L., HOLSAPPLE, M. P., MCCAY, J. A., AND MUNSON, A. E. (1989). Contact hypersensitivity response to glutaraldehyde in guinea pigs and mice. *Toxicol. Ind. Health* **5**, 31.

TALLANT, M. J., FRANTZ, S. W., AND BALLANTYNE, B. (1990). Evaluation of the *in vitro* skin penetration of glutaraldehyde using the rat, mouse, rabbit, guinea pig and human skin. *Toxicologist* **10**, 256. (Abstr.)

TAMADA, M., SASAKI, S., KADONO, Y., KATO, S., AMITANI, M., OGASAHARA, Y., TAMURA, T., AND SATO, N. (1978). Mutagenicity of glutaraldehyde in mice. *Bobkin Bobai* **6**, 62.

UEMITSU, N., KAWASAKI, H., FURUHASHI, T., MIYOSHI, K., OHTAKA, T., NOMURA, A., HASEGAWA, T., SHIMIZU, Y., AND NAKAZAWA, M. (1976). Acute and subacute toxicity studies and local irritation study of glutaraldehyde. *Oyo Yakuri*, **12**, 11.

VALENCIA, R., MASON, J. M., AND ZIMMERING, S. (1989). Chemical mutagenesis testing in *Drosophila*. VI. Interlaboratory comparison of mutagenicity tests after treatment of larvae. *Environ. Mol. Mutagen.* **14**, 238-244.

VARPELA, E., OTTERSTRÖM, S., AND HACKMAN, R. (1971). Liberation of alkalinized glutaraldehyde by respirators after cold sterilization. *Acta Anaesthesiol. Scand.* **15**, 291-298.

WADE, M. J., FRIEDMAN, L., AND ROSS, V. C. (1982). Biochemical and toxicological effects of glutaraldehyde. *FDA By-Lines* **12**, 7-19.

WATTS, P. (1984). Non-mutagenicity of glutaraldehyde. *Food Chem. Toxicol.* **22**, 1024.

WHIPPLE, E. B., AND RUTA, R. (1974). Structure of glutaraldehyde. *J. Org. Chem.* **39**, 1666-1668.

WILLIAMS, D. A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

WILLIAMS, D. A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

YOON, J. S., MASON, J. M., VALENCIA, R., WOODRUFF, R. C., AND ZIMMERING, S. (1985). Chemical mutagenesis testing in *Drosophila*. IV. Results of 45 coded chemicals tested for the National Toxicology Program. *Environ. Mutagen.* **7**, 349-367.

ZEIGER, E., ANDERSON, B., HAWORTH, S., LAWLOR, T., AND MORTELMANS, K. (1992). Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* **19** (Suppl. 21), 2-41.

ZIMMERING, S., MASON, J. M., AND VALENCIA, R. (1989). Chemical mutagenesis testing in *Drosophila*. VII. Results of 22 coded compounds tested in larval feeding experiments. *Environ. Mol. Mutagen.* **14**, 245-251.

APPENDIX A

Organ Weights and Organ-Weight-to-Body-Weight Ratios

Table A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde A-2

Table A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for $B6C3F_1$ Mice in the 13-Week Inhalation Study of Glutaraldehyde A-3

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
n	10	10	10	10	10	10
Necropsy body wt	329 ± 8	337 ± 8	334 ± 6	332 ± 6	321 ± 10	303 ± 7*
Heart						
Absolute	0.990 ± 0.028	1.001 ± 0.027	0.964 ± 0.025	0.968 ± 0.014	0.984 ± 0.025	0.959 ± 0.017
Relative	3.01 ± 0.03	2.97 ± 0.05	2.88 ± 0.04	2.92 ± 0.04	3.07 ± 0.05	3.17 ± 0.05**
Right kidney						
Absolute	1.090 ± 0.029	1.159 ± 0.033	1.098 ± 0.034	1.137 ± 0.028	1.082 ± 0.040	1.072 ± 0.031
Relative	3.31 ± 0.03	3.44 ± 0.06*	3.28 ± 0.05	3.43 ± 0.06	3.37 ± 0.04	3.54 ± 0.05**
Liver						
Absolute	11.282 ± 0.414	11.680 ± 0.319	11.494 ± 0.262	11.569 ± 0.296	11.484 ± 0.483	10.685 ± 0.333
Relative	34.22 ± 0.62	34.67 ± 0.45	34.41 ± 0.46	34.83 ± 0.46	35.71 ± 0.59	35.27 ± 0.66
Lungs						
Absolute	1.633 ± 0.072	1.685 ± 0.059	1.681 ± 0.049	1.670 ± 0.056	1.665 ± 0.073	1.688 ± 0.073
Relative	4.95 ± 0.16	5.00 ± 0.14	5.04 ± 0.16	5.04 ± 0.17	5.18 ± 0.13	5.58 ± 0.22*
Right testis						
Absolute	1.390 ± 0.028	1.405 ± 0.025	1.394 ± 0.028	1.348 ± 0.030	1.365 ± 0.026	1.360 ± 0.022
Relative	4.23 ± 0.06	4.18 ± 0.08	4.17 ± 0.04	4.07 ± 0.11	4.27 ± 0.08	4.50 ± 0.06*
Thymus						
Absolute	0.310 ± 0.008	0.325 ± 0.016	0.305 ± 0.009	0.334 ± 0.010	0.346 ± 0.016	0.278 ± 0.012
Relative	0.94 ± 0.02	0.96 ± 0.04	0.91 ± 0.03	1.01 ± 0.03	1.08 ± 0.05*	0.92 ± 0.04
Female						
n	10	10	10	9	10	10
Necropsy body wt	196 ± 3	195 ± 4	198 ± 4	190 ± 5	187 ± 4	175 ± 5**
Heart						
Absolute	0.673 ± 0.015	0.682 ± 0.016	0.683 ± 0.018	0.683 ± 0.017	0.673 ± 0.013	0.667 ± 0.020
Relative	3.44 ± 0.06	3.51 ± 0.06	3.45 ± 0.07	3.60 ± 0.09	3.60 ± 0.06	3.82 ± 0.08**
Right kidney						
Absolute	0.671 ± 0.013	0.687 ± 0.024	0.685 ± 0.016	0.707 ± 0.013	0.678 ± 0.015	0.652 ± 0.027
Relative	3.43 ± 0.04	3.53 ± 0.08	3.45 ± 0.05	3.72 ± 0.05*	3.63 ± 0.08*	3.72 ± 0.08**
Liver						
Absolute	6.484 ± 0.152	6.584 ± 0.309	6.459 ± 0.171	6.418 ± 0.276	6.146 ± 0.151	5.888 ± 0.242
Relative	33.17 ± 0.62	33.74 ± 1.19	32.57 ± 0.62	33.68 ± 1.09	32.80 ± 0.36	33.54 ± 0.65
Lungs						
Absolute	1.192 ± 0.029	1.265 ± 0.055	1.297 ± 0.051	1.290 ± 0.082	1.182 ± 0.037	1.174 ± 0.055
Relative	6.11 ± 0.18	6.48 ± 0.20	6.54 ± 0.21	6.75 ± 0.32	6.31 ± 0.15	6.72 ± 0.25
Thymus						
Absolute	0.276 ± 0.008	0.271 ± 0.007	0.263 ± 0.012	0.257 ± 0.010	0.259 ± 0.005	0.227 ± 0.007[2]**
Relative	1.41 ± 0.03	1.40 ± 0.05	1.32 ± 0.05	1.35 ± 0.05	1.38 ± 0.02	1.27 ± 0.04[2]

1 Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).
2 n = 9.
* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.
** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of Glutaraldehyde[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
n	10	10	10	10	10	0
Necropsy body wt	36.1 ± 0.4	34.2 ± 0.6*	33.9 ± 0.8*	33.0 ± 0.5**	31.9 ± 0.8**	
Heart						
Absolute	0.161 ± 0.004	0.157 ± 0.003	0.162 ± 0.004	0.159 ± 0.005	0.150 ± 0.002	
Relative	4.46 ± 0.10	4.60 ± 0.08	4.79 ± 0.07*	4.82 ± 0.14*	4.72 ± 0.09*	
Right kidney						
Absolute	0.310 ± 0.007	0.327 ± 0.009	0.316 ± 0.008	0.318 ± 0.008	0.309 ± 0.007	
Relative	8.59 ± 0.16	9.56 ± 0.19**	9.34 ± 0.14**	9.65 ± 0.23**	9.72 ± 0.18**	
Liver						
Absolute	1.690 ± 0.037	1.616 ± 0.043	1.626 ± 0.039	1.570 ± 0.038*	1.569 ± 0.042*	
Relative	46.82 ± 0.94	47.26 ± 0.90	48.06 ± 0.56	47.62 ± 0.91	49.29 ± 0.99	
Lungs						
Absolute	0.217 ± 0.005	0.224 ± 0.007	0.223 ± 0.006	0.226 ± 0.006	0.218 ± 0.005	
Relative	6.02 ± 0.17	6.56 ± 0.20	6.60 ± 0.16*	6.87 ± 0.22**	6.87 ± 0.22**	
Right testis						
Absolute	0.120 ± 0.001	0.124 ± 0.002	0.125 ± 0.003	0.122 ± 0.002	0.121 ± 0.002	
Relative	3.32 ± 0.06	3.62 ± 0.09**	3.69 ± 0.06**	3.71 ± 0.09**	3.80 ± 0.08**	
Thymus						
Absolute	0.048 ± 0.002	0.049 ± 0.002	0.047 ± 0.002	0.044 ± 0.003	0.040 ± 0.003*	
Relative	1.32 ± 0.06	1.42 ± 0.04	1.38 ± 0.04	1.35 ± 0.09	1.24 ± 0.10	
Female						
n	10	10	10	10	8	0
Necropsy body wt	30.9 ± 0.3	31.1 ± 0.8	30.3 ± 0.6	28.7 ± 0.5**	27.3 ± 0.6**	
Heart						
Absolute	0.143 ± 0.002	0.143 ± 0.002	0.144 ± 0.002	0.137 ± 0.002	0.140 ± 0.004	
Relative	4.63 ± 0.06	4.62 ± 0.12	4.77 ± 0.09	4.78 ± 0.09	5.14 ± 0.12**	
Right kidney						
Absolute	0.203 ± 0.003	0.229 ± 0.006**	0.224 ± 0.004**	0.218 ± 0.003	0.218 ± 0.005	
Relative	6.59 ± 0.16	7.39 ± 0.20**	7.41 ± 0.12**	7.61 ± 0.16**	7.98 ± 0.13**	
Liver						
Absolute	1.538 ± 0.033	1.640 ± 0.044	1.627 ± 0.035	1.524 ± 0.033	1.444 ± 0.060	
Relative	49.78 ± 0.75	52.73 ± 0.54*	53.82 ± 0.83*	53.06 ± 0.71*	52.93 ± 1.77*	
Lungs						
Absolute	0.216 ± 0.003	0.236 ± 0.004**	0.229 ± 0.005	0.224 ± 0.005	0.215 ± 0.004	
Relative	7.00 ± 0.14	7.61 ± 0.14*	7.58 ± 0.18*	7.82 ± 0.20**	7.89 ± 0.10**	
Thymus						
Absolute	0.060 ± 0.003	0.059 ± 0.002	0.058 ± 0.003	0.055 ± 0.001	0.052 ± 0.003*	
Relative	1.94 ± 0.08	1.90 ± 0.04	1.91 ± 0.10	1.91 ± 0.05	1.92 ± 0.12	

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error). Data are not available for the highest dose group (1000ppb) since there were no survivors in this group at 13weeks.

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

APPENDIX B

Hematology and Clinical Chemistry Results

Table B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde B-2

Table B2 Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde B-5

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Manual hematocrit (%)						
Day 4	42.0 ± 0.3	41.2 ± 0.5	41.4 ± 0.3	41.9 ± 0.4	42.0 ± 0.3	42.8 ± 0.4
Day 24	47.4 ± 0.2	46.7 ± 0.3	46.3 ± 0.5	47.0 ± 0.4	46.5 ± 0.4	49.3 ± 0.7
Day 94	46.9 ± 0.3	46.3 ± 0.4	48.2 ± 0.3	46.3 ± 0.4	46.5 ± 0.1	47.2 ± 0.4
Volume of packed red cells (mL/dL)						
Day 4	42.0 ± 0.4	41.3 ± 0.5	41.7 ± 0.4	42.1 ± 0.4	42.4 ± 0.3	42.9 ± 0.3
Day 24	47.2 ± 0.3	46.4 ± 0.5	45.9 ± 0.5	46.6 ± 0.5	45.8 ± 0.4	49.7 ± 0.6
Day 94	46.7 ± 0.2	46.2 ± 0.3	48.3 ± 0.4	46.3 ± 0.4	46.4 ± 0.1	46.7 ± 0.4
Hemoglobin (g/dL)						
Day 4	13.3 ± 0.1	13.2 ± 0.2	13.4 ± 0.1	13.4 ± 0.1	13.5 ± 0.1	13.6 ± 0.1
Day 24	15.1 ± 0.1	14.9 ± 0.2	14.7 ± 0.1	15.0 ± 0.2	14.6 ± 0.1	16.0 ± 0.2
Day 94	14.9 ± 0.1	14.8 ± 0.1	15.3 ± 0.1**	15.0 ± 0.2[2]	15.2 ± 0.1*	15.2 ± 0.2
Erythrocytes (10^6/µL)						
Day 4	7.05 ± 0.09	7.04 ± 0.11	7.02 ± 0.08	7.12 ± 0.07	7.13 ± 0.08	7.21 ± 0.08
Day 24	8.09 ± 0.08	8.02 ± 0.10	7.88 ± 0.11	7.99 ± 0.10	7.91 ± 0.09	8.73 ± 0.11*
Day 94	8.83 ± 0.06	8.85 ± 0.06	9.17 ± 0.08**	8.85 ± 0.07	8.90 ± 0.03	8.97 ± 0.08
Reticulocytes (10^6/µL)						
Day 4	0.40 ± 0.02	0.41 ± 0.02	0.45 ± 0.03	0.45 ± 0.02	0.40 ± 0.02	0.42 ± 0.03
Day 24	0.28 ± 0.02	0.28 ± 0.02	0.30 ± 0.02	0.31 ± 0.01	0.28 ± 0.02	0.20 ± 0.03
Day 94	0.18 ± 0.01	0.15 ± 0.01	0.18 ± 0.01	0.18 ± 0.02	0.19 ± 0.01	0.18 ± 0.01
Nucleated erythrocytes (/100 leukocytes)						
Day 4	1.40 ± 0.37	2.10 ± 0.77	1.20 ± 0.49	0.600 ± 0.22	1.30 ± 0.45	0.80 ± 0.13
Day 24	1.10 ± 0.28	0.70 ± 0.30	1.10 ± 0.53	0.80 ± 0.42	0.90 ± 0.23	0.90 ± 0.23
Day 94	0.10 ± 0.10	0.30 ± 0.15	0.30 ± 0.15	0.30 ± 0.15	0.60 ± 0.27	0.60 ± 0.34
Mean cell volume (fL)						
Day 4	59.6 ± 0.3	58.8 ± 0.3	59.6 ± 0.3	59.3 ± 0.3	59.7 ± 0.5	59.7 ± 0.5
Day 24	58.2 ± 0.4	57.8 ± 0.3	58.0 ± 0.3	58.5 ± 0.3	57.9 ± 0.4	57.0 ± 0.3*
Day 94	52.9 ± 0.3	52.3 ± 0.2	52.6 ± 0.2	52.3 ± 0.3	52.1 ± 0.2*	52.3 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	18.9 ± 0.1	18.8 ± 0.1	19.1 ± 0.1	18.8 ± 0.1	18.9 ± 0.2	18.9 ± 0.2
Day 24	18.7 ± 0.1	18.5 ± 0.1	18.7 ± 0.1	18.7 ± 0.1	18.5 ± 0.1	18.4 ± 0.1
Day 94	16.9 ± 0.1	16.7 ± 0.1	16.7 ± 0.1	16.7 ± 0.1	16.7 ± 0.1	16.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	31.6 ± 0.1	32.1 ± 0.1	32.2 ± 0.1**	31.8 ± 0.1	31.9 ± 0.1	31.8 ± 0.1
Day 24	32.0 ± 0.1	32.0 ± 0.1	32.1 ± 0.1	32.2 ± 0.1	32.0 ± 0.1	32.2 ± 0.1
Day 94	31.9 ± 0.1	32.1 ± 0.1	31.8 ± 0.1	32.0 ± 0.1	32.1 ± 0.1	31.9 ± 0.1
Platelets (10^3/µL)						
Day 4	785.5 ± 13.6	800.3 ± 18.9	768.2 ± 17.5	780.8 ± 15.9	818.0 ± 14.9	759.6 ± 14.1
Day 24	678.3 ± 14.5	707.2 ± 10.2	733.7 ± 22.7	721.9 ± 12.9	762.7 ± 21.9	552.3 ± 25.0
Day 94	531.4 ± 12.9	529.4 ± 11.5	508.5 ± 14.7	527.5 ± 10.5	510.3 ± 8.5	527.8 ± 9.5
Leukocytes (10^3/µL)						
Day 4	7.68 ± 0.28	7.29 ± 0.39	7.86 ± 0.36	8.36 ± 0.29	8.94 ± 0.41*	8.18 ± 0.26
Day 24	7.41 ± 0.35	6.75 ± 0.38	7.36 ± 0.18	7.60 ± 0.42	7.67 ± 0.38	8.71 ± 0.50
Day 94	7.36 ± 0.59	7.68 ± 0.21[2]	8.75 ± 0.41	7.51 ± 0.48	6.31 ± 0.13**	6.77 ± 0.16*
Segmented neutrophils (10^3/µL)						
Day 4	0.92 ± 0.08	0.78 ± 0.11	0.93 ± 0.07	0.87 ± 0.09	1.13 ± 0.16	1.09 ± 0.12
Day 24	0.72 ± 0.05	0.90 ± 0.12	1.19 ± 0.06**	0.94 ± 0.11*	0.90 ± 0.10	2.48 ± 0.36**
Day 94	1.38 ± 0.17	1.32 ± 0.05[2]	1.22 ± 0.13	1.14 ± 0.20	0.98 ± 0.08	1.38 ± 0.09

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde (continued)

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male (continued)						
Lymphocytes (10^3/μL)						
Day 4	6.61 ± 0.22	6.45 ± 0.37	6.80 ± 0.39	7.34 ± 0.30	7.63 ± 0.35*	6.95 ± 0.17
Day 24	6.60 ± 0.34	5.79 ± 0.28	6.10 ± 0.14	6.59 ± 0.37	6.72 ± 0.36	6.19 ± 0.41
Day 94	6.35 ± 0.24[2]	6.00 ± 0.40	7.45 ± 0.36	6.33 ± 0.38	5.28 ± 0.17*	5.35 ± 0.21*
Monocytes (10^3/μL)						
Day 4	0.07 ± 0.02	0.03 ± 0.01	0.06 ± 0.04	0.12 ± 0.03	0.14 ± 0.04	0.10 ± 0.02
Day 24	0.04 ± 0.02	0.03 ± 0.01	0.03 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	0.02 ± 0.01
Day 94	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Eosinophils (10^3/μL)						
Day 4	0.07 ± 0.02	0.03 ± 0.01	0.06 ± 0.02	0.03 ± 0.01	0.04 ± 0.02	0.04 ± 0.01
Day 24	0.06 ± 0.01	0.03 ± 0.01*	0.04 ± 0.02	0.02 ± 0.01	0.02 ± 0.02*	0.03 ± 0.01*
Day 94	0.05 ± 0.02	0.01 ± 0.01	0.06 ± 0.02	0.01 ± 0.01[2]	0.05 ± 0.02	0.04 ± 0.01
Female						
Manual hematocrit (%)						
Day 4	43.6 ± 0.5	42.7 ± 0.4[2]	44.0 ± 0.4	44.2 ± 0.5	44.6 ± 0.4	44.7 ± 0.4[2]
Day 24	47.6 ± 0.3	47.5 ± 0.2	46.9 ± 0.4	48.1 ± 0.2	48.3 ± 0.4	48.8 ± 0.7
Day 94	47.0 ± 0.6	46.3 ± 0.4	47.4 ± 0.4	47.0 ± 0.4[2]	47.6 ± 0.2	47.0 ± 0.5
Volume of packed red cells (mL/dL)						
Day 4	44.0 ± 0.5	43.4 ± 0.5[2]	44.8 ± 0.5	44.7 ± 0.5	45.6 ± 0.6*	45.5 ± 0.5[2]*
Day 24	48.7 ± 0.3	49.0 ± 0.3	47.9 ± 0.5	48.8 ± 0.4	48.8 ± 0.5	49.7 ± 0.8
Day 94	47.3 ± 0.5	47.0 ± 0.6	48.2 ± 0.5	47.2 ± 0.6[2]	48.0 ± 0.3	48.1 ± 0.5
Hemoglobin (g/dL)						
Day 4	13.8 ± 0.2	13.8 ± 0.1[2]	14.1 ± 0.1	14.2 ± 0.2	14.2 ± 0.2	14.2 ± 0.2[2]
Day 24	15.5 ± 0.1	15.7 ± 0.1	15.3 ± 0.2	15.6 ± 0.1	15.5 ± 0.2	16.0 ± 0.3
Day 94	15.0 ± 0.2	14.9 ± 0.2	15.4 ± 0.2	15.0 ± 0.2[2]	15.2 ± 0.1	15.2 ± 0.2
Erythrocytes (10^6/μL)						
Day 4	7.36 ± 0.12	7.45 ± 0.06[2]	7.58 ± 0.11	7.53 ± 0.12	7.68 ± 0.12*	7.64 ± 0.13[2]
Day 24	8.25 ± 0.09	8.39 ± 0.08	8.18 ± 0.09	8.29 ± 0.07	8.37 ± 0.11	8.70 ± 0.19
Day 94	8.31 ± 0.09	8.29 ± 0.10	8.54 ± 0.10	8.31 ± 0.10[2]	8.51 ± 0.05	8.51 ± 0.12
Reticulocytes (10^6/μL)						
Day 4	0.35 ± 0.02	0.39 ± 0.03[2]	0.37 ± 0.03	0.40 ± 0.02[2]	0.37 ± 0.03	0.35 ± 0.02[2]
Day 24	0.19 ± 0.01	0.17 ± 0.01	0.20 ± 0.02	0.17 ± 0.02	0.18 ± 0.02	0.15 ± 0.03
Day 94	0.16 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.16 ± 0.01[2]	0.18 ± 0.01	0.16 ± 0.01
Nucleated erythrocytes (/100 leukocytes)						
Day 4	1.11 ± 0.26[2]	1.56 ± 0.44[2]	0.90 ± 0.28	1.50 ± 0.31	1.10 ± 0.38	1.56 ± 0.48[2]
Day 24	0.30 ± 0.15	0.40 ± 0.16	0.30 ± 0.15	0.10 ± 0.10	0.20 ± 0.13	0.30 ± 0.15
Day 94	0.90 ± 0.48	1.00 ± 0.39	0.50 ± 0.22	0.89 ± 0.42[2]	0.30 ± 0.21	0.80 ± 0.25
Mean cell volume (fL)						
Day 4	59.7 ± 0.4	58.3 ± 0.2[2]*	59.2 ± 0.3	59.5 ± 0.2	59.2 ± 0.3	59.7 ± 0.4[2]
Day 24	59.1 ± 0.4	58.4 ± 0.3	58.5 ± 0.5	58.7 ± 0.2	58.4 ± 0.3	57.3 ± 0.5**
Day 94	56.9 ± 0.1	56.7 ± 0.2	56.6 ± 0.2	56.9 ± 0.2[2]	56.4 ± 0.2*	56.5 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	18.8 ± 0.1	18.5 ± 0.1[2]	18.7 ± 0.1	18.8 ± 0.1	18.6 ± 0.2	18.7 ± 0.1[2]
Day 24	18.8 ± 0.1	18.7 ± 0.1	18.7 ± 0.1	18.8 ± 0.1	18.5 ± 0.1	18.5 ± 0.1
Day 94	18.0 ± 0.1	17.9 ± 0.0	18.1 ± 0.1	18.0 ± 0.1[2]	17.9 ± 0.1	17.9 ± 0.1

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde (continued)

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Female (continued)						
Mean cell hemoglobin concentration (g/dL)						
Day 4	31.4 ± 0.1	31.8 ± 0.2[2]	31.6 ± 0.1	31.7 ± 0.1	31.2 ± 0.2	31.3 ± 0.1[2]
Day 24	31.8 ± 0.1	31.9 ± 0.1	32.0 ± 0.1	32.1 ± 0.1	31.7 ± 0.1	32.3 ± 0.1*
Day 94	31.6 ± 0.1	31.6 ± 0.1	31.9 ± 0.2	31.7 ± 0.1[2]	31.7 ± 0.1	31.6 ± 0.1
Platelets (10^3/µL)						
Day 4	746.1 ± 24.6	732.1 ± 5.3[2]	716.0 ± 28.3	724.6 ± 16.4	712.9 ± 24.5	728.2 ± 29.7[2]
Day 24	712.0 ± 19.5	705.5 ± 26.7	679.8 ± 19.5	666.9 ± 17.8	644.9 ± 17.4*	571.3 ± 48.7*
Day 94	585.9 ± 25.1	648.7 ± 38.2	591.7 ± 24.9	565.4 ± 13.5[2]	586.2 ± 21.8	562.6 ± 34.0
Leukocytes (10^3/µL)						
Day 4	8.58 ± 0.53	6.44 ± 0.65[2]	8.32 ± 0.43	8.44 ± 0.53	8.94 ± 0.53	8.71 ± 0.45[2]
Day 24	8.25 ± 0.42	7.69 ± 0.27	8.03 ± 0.33	8.07 ± 0.37	7.69 ± 0.30	12.01 ± 0.53**
Day 94	7.99 ± 0.52	7.02 ± 0.48	6.75 ± 0.55	7.33 ± 0.48[2]	6.32 ± 0.33	7.86 ± 0.87
Segmented neutrophils (10^3/µL)						
Day 4	1.00 ± 0.15	0.75 ± 0.11[2]	0.81 ± 0.11	1.15 ± 0.19	0.85 ± 0.07	1.01 ± 0.11[2]
Day 24	0.75 ± 0.06	0.80 ± 0.07	0.77 ± 0.06	1.06 ± 0.12*	0.77 ± 0.10	3.11 ± 0.31**
Day 94	1.22 ± 0.14	0.94 ± 0.12	0.95 ± 0.13	0.96 ± 0.10[2]	0.87 ± 0.12	1.23 ± 0.13[2]
Lymphocytes (10^3/µL)						
Day 4	7.54 ± 0.54	5.65 ± 0.58[2]	7.47 ± 0.38	7.27 ± 0.45	7.98 ± 0.48	7.64 ± 0.45[2]
Day 24	7.41 ± 0.43	6.83 ± 0.23	7.24 ± 0.36	6.94 ± 0.34	6.83 ± 0.23	8.73 ± 0.51
Day 94	6.70 ± 0.41	6.03 ± 0.45	5.77 ± 0.47	6.35 ± 0.42[2]	5.42 ± 0.35	6.30 ± 0.65
Monocytes (10^3/µL)						
Day 4	0.00 ± 0.00	0.01 ± 0.01[2]	0.04 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01[2]
Day 24	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.05 ± 0.03
Day 94	0.02 ± 0.02	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00[2]	0.01 ± 0.01	0.01 ± 0.01
Eosinophils (10^3/µL)						
Day 4	0.05 ± 0.02	0.03 ± 0.02[2]	0.01 ± 0.01	0.01 ± 0.01	0.09 ± 0.03	0.05 ± 0.02[2]
Day 24	0.06 ± 0.02	0.05 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.06 ± 0.03	0.12 ± 0.04
Day 94	0.05 ± 0.03	0.05 ± 0.02	0.03 ± 0.02	0.03 ± 0.01[2]	0.01 ± 0.01[2]	0.04 ± 0.02

[1] Mean ± standard error for groups of 10 animals, unless otherwise specified. Day 4 and Day 24 data were collected from male and female rats in groups specifically designated for clinical pathology supplemental studies; after collection of the Day 24 sample, these animals were killed and discarded without necropsy. Data for Day 94 were collected from animals in the core study immediately before they were killed and necropsied.

[2] n = 9.

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.

** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE B2 Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Alanine aminotransferase (IU/L)						
Day 4	43 ± 1	44 ± 1	45 ± 1	45 ± 1	45 ± 1	45 ± 1
Day 24	37 ± 0	38 ± 1	38 ± 1	41 ± 1*	45 ± 4*	51 ± 3**
Day 94	51 ± 2	61 ± 5	68 ± 7	47 ± 4	52 ± 3	44 ± 2
Albumin (g/dL)						
Day 4	3.5 ± 0.1	3.5 ± 0.0	3.5 ± 0.0	3.5 ± 0.1	3.6 ± 0.0	3.7 ± 0.0
Day 24	4.0 ± 0.0	3.9 ± 0.0	3.8 ± 0.1	3.8 ± 0.0	3.9 ± 0.0	3.9 ± 0.1
Day 94	3.9 ± 0.0	3.9 ± 0.1	3.8 ± 0.1	3.8 ± 0.0	3.9 ± 0.1	3.8 ± 0.0
Albumin/globulin ratio						
Day 4	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.0	1.6 ± 0.0	1.8 ± 0.1	1.7 ± 0.1
Day 24	1.5 ± 0.0	1.6 ± 0.0	1.5 ± 0.0	1.5 ± 0.0	1.6 ± 0.1	1.7 ± 0.1
Day 94	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
Alkaline phosphatase (IU/L)						
Day 4	801 ± 27	835 ± 19	872 ± 19	799 ± 19	782 ± 23	771 ± 29
Day 24	538 ± 18	559 ± 15	579 ± 10	544 ± 36	561 ± 9	499 ± 34
Day 94	290 ± 7	287 ± 5	335 ± 20*	291 ± 6	305 ± 7	312 ± 9
Bile acids (µmol/L)						
Day 4	20.40 ± 1.73	16.20 ± 1.08	16.00 ± 1.37	16.30 ± 1.05	15.10 ± 0.64	19.80 ± 2.53
Day 24	30.20 ± 3.38	26.70 ± 2.16	24.00 ± 2.25	25.90 ± 2.60	29.90 ± 2.27	25.70 ± 1.41
Day 94	21.10 ± 1.83	22.40 ± 2.09	16.60 ± 1.01	23.80 ± 3.59	17.80 ± 0.63	19.22 ± 1.12[2]
Creatine kinase (IU/L)						
Day 4	237 ± 14	232 ± 18	380 ± 72	243 ± 20	248 ± 20	384 ± 82
Day 24	212 ± 32	183 ± 22	186 ± 27	206 ± 34	209 ± 21	230 ± 30
Day 94	85 ± 6[2]	111 ± 24	68 ± 5	106 ± 10	106 ± 15	71 ± 4
Creatinine (mg/dL)						
Day 4	0.63 ± 0.02	0.59 ± 0.01	0.63 ± 0.02	0.62 ± 0.01	0.56 ± 0.02**	0.57 ± 0.03*
Day 24	0.66 ± 0.03	0.68 ± 0.02	0.63 ± 0.02	0.59 ± 0.02	0.58 ± 0.01*	0.60 ± 0.03
Day 94	0.66 ± 0.02	0.68 ± 0.03	0.65 ± 0.03	0.63 ± 0.02	0.68 ± 0.02	0.61 ± 0.01
Globulin (g/dL)						
Day 4	2.1 ± 0.1	2.1 ± 0.1	2.0 ± 0.0	2.2 ± 0.0	2.0 ± 0.1	2.2 ± 0.1
Day 24	2.6 ± 0.1	2.5 ± 0.1	2.5 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	2.4 ± 0.1*
Day 94	2.9 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	2.8 ± 0.1
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 1	16 ± 0	15 ± 0	16 ± 0	15 ± 0	15 ± 1
Day 24	16 ± 1	16 ± 1	15 ± 1	17 ± 1	18 ± 2	18 ± 1
Day 94	20 ± 1	26 ± 3	23 ± 2	16 ± 0[2]*	19 ± 1	14 ± 0**
Total protein (g/dL)						
Day 4	5.6 ± 0.1	5.6 ± 0.0	5.5 ± 0.1	5.7 ± 0.1	5.6 ± 0.0	5.9 ± 0.1**
Day 24	6.6 ± 0.1	6.4 ± 0.1	6.3 ± 0.1**	6.4 ± 0.1*	6.3 ± 0.1*	6.3 ± 0.1*
Day 94	6.8 ± 0.1	6.9 ± 0.1	6.7 ± 0.0	6.8 ± 0.1	6.9 ± 0.1	6.6 ± 0.1
Urea nitrogen (mg/dL)						
Day 4	20.2 ± 1.0	21.8 ± 0.9	21.3 ± 0.5	23.4 ± 0.8*	22.0 ± 0.7	22.4 ± 0.8
Day 24	16.9 ± 0.5	17.1 ± 0.8	17.4 ± 0.6	16.5 ± 0.4	15.9 ± 0.4	16.1 ± 0.8
Day 94	20.5 ± 0.5	21.0 ± 0.8	22.2 ± 0.6	20.9 ± 0.6	21.5 ± 0.7	21.6 ± 0.5

TABLE B2 Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde (continued)

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Female						
Alanine aminotransferase (IU/L)						
Day 4	41 ± 1	43 ± 1	39 ± 1	40 ± 1	42 ± 1	39 ± 2
Day 24	31 ± 1	34 ± 2	34 ± 2	34 ± 1*	35 ± 2*	52 ± 5**
Day 94	44 ± 3	56 ± 6	52 ± 3	43 ± 2[2]	56 ± 6	50 ± 5
Albumin (g/dL)						
Day 4	3.6 ± 0.1	3.8 ± 0.1	3.5 ± 0.1	3.4 ± 0.0	3.6 ± 0.1	3.7 ± 0.1
Day 24	4.0 ± 0.0	3.9 ± 0.1*	3.8 ± 0.1*	3.8 ± 0.0*	4.0 ± 0.0	3.7 ± 0.1**
Day 94	3.9 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1[2]	4.0 ± 0.0	3.9 ± 0.1
Albumin/globulin ratio						
Day 4	1.8 ± 0.0	1.7 ± 0.1	1.7 ± 0.1	1.8 ± 0.1	1.8 ± 0.1	1.8 ± 0.0
Day 24	1.7 ± 0.0	1.6 ± 0.0	1.6 ± 0.0	1.6 ± 0.1	1.7 ± 0.0	1.7 ± 0.1
Day 94	1.3 ± 0.0	1.4 ± 0.0	1.3 ± 0.0	1.3 ± 0.1[2]	1.4 ± 0.0	1.4 ± 0.0
Alkaline phosphatase (IU/L)						
Day 4	685 ± 17	662 ± 9	708 ± 64	622 ± 15*	636 ± 17	650 ± 36
Day 24	416 ± 11	446 ± 8	475 ± 46	454 ± 15	488 ± 13**	490 ± 29**
Day 94	276 ± 15	258 ± 6	298 ± 11	266 ± 10[2]	273 ± 9	300 ± 10
Bile acids (µmol/L)						
Day 4	13.60 ± 1.03	11.44 ± 0.82[2]	13.20 ± 0.94	14.40 ± 0.87	15.00 ± 1.15	13.56 ± 0.97[2]
Day 24	15.20 ± 0.44	16.30 ± 0.60	14.20 ± 0.44	14.90 ± 1.09	16.50 ± 0.64	15.60 ± 1.85
Day 94	19.56 ± 2.08[2]	21.90 ± 4.42	20.60 ± 2.13	15.44 ± 0.73[2]	26.00 ± 4.75	15.80 ± 1.87
Creatine kinase (IU/L)						
Day 4	314 ± 35	274 ± 33[2]	244 ± 33	184 ± 10*	307 ± 49	420 ± 47
Day 24	203 ± 36	198 ± 37	123 ± 14*	99 ± 6[2]**	139 ± 19*	134 ± 19*[2]
Day 94	115 ± 14[2]	131 ± 20	85 ± 10	114 ± 14[2]	105 ± 14	108 ± 11
Creatinine (mg/dL)						
Day 4	0.65 ± 0.02	0.62 ± 0.02	0.59 ± 0.02*	0.60 ± 0.02	0.60 ± 0.02	0.55 ± 0.03**
Day 24	0.60 ± 0.03	0.63 ± 0.02	0.61 ± 0.02	0.56 ± 0.02	0.56 ± 0.02	0.54 ± 0.02
Day 94	0.69 ± 0.02	0.68 ± 0.02	0.71 ± 0.02	0.66 ± 0.02[2]	0.68 ± 0.02	0.67 ± 0.02
Globulin (g/dL)						
Day 4	2.1 ± 0.0	2.2 ± 0.1	2.0 ± 0.0	1.9 ± 0.0	2.0 ± 0.1	2.1 ± 0.0
Day 24	2.4 ± 0.1	2.4 ± 0.1	2.5 ± 0.0	2.4 ± 0.1	2.3 ± 0.0	2.1 ± 0.1*
Day 94	3.0 ± 0.0	3.0 ± 0.1	3.0 ± 0.1	3.1 ± 0.1[2]	3.0 ± 0.1	2.8 ± 0.1
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 1	16 ± 0	16 ± 1	15 ± 1	14 ± 1	12 ± 0**
Day 24	22 ± 1	23 ± 1	20 ± 1	20 ± 1	20 ± 1	17 ± 1**
Day 94	18 ± 1	21 ± 2	21 ± 2	18 ± 1[2]	21 ± 2	19 ± 1
Total protein (g/dL)						
Day 4	5.7 ± 0.1	5.9 ± 0.1	5.6 ± 0.1	5.4 ± 0.1*	5.6 ± 0.1	5.8 ± 0.1
Day 24	6.4 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	5.8 ± 0.1**
Day 94	6.9 ± 0.1	7.0 ± 0.2	7.0 ± 0.1	7.1 ± 0.1[2]	7.0 ± 0.1	6.7 ± 0.1
Urea nitrogen (mg/dL)						
Day 4	21.9 ± 0.7	22.2 ± 0.8	23.0 ± 0.9	24.4 ± 0.9	23.2 ± 0.6	22.4 ± 0.9
Day 24	17.3 ± 1.1	18.7 ± 0.9	19.2 ± 0.5	20.8 ± 0.8*	20.4 ± 0.6*	20.7 ± 0.9*
Day 94	20.7 ± 0.6	23.7 ± 0.6*	24.0 ± 0.7*	22.7 ± 1.0[2]	22.9 ± 1.1	22.5 ± 1.3

[1] Mean ± standard error for groups of 10 animals, unless otherwise specified. Day 4 and Day 24 data were collected from male and female rats in groups specifically designated for clinical pathology supplemental studies; after collection of the Day 24 sample, these animals were killed and discarded without necropsy. Day 94 data were collected from animals in the core study immediately before they were killed and necropsied.

[2] n = 9.

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

APPENDIX C

Reproductive Tissue Evaluations and Estrous Cycle Characterization

Table C1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde C-2

Table C2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde C-2

Table C3 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Inhalation Study of Glutaraldehyde C-3

Table C4 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Inhalation Study of Glutaraldehyde C-3

TABLE C1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde[1]

Study Parameters	0 ppb	62.5 ppb	250 ppb	1000 ppb
Weights (g)				
Necropsy body weight	329 ± 8	337 ± 8	332 ± 6	303 ± 7*
Left epididymis	0.266 ± 0.005	0.268 ± 0.007	0.268 ± 0.004	0.265 ± 0.006
Left cauda epididymis	0.169 ± 0.006	0.167 ± 0.004	0.170 ± 0.004	0.161 ± 0.004
Left testis	1.45 ± 0.03	1.46 ± 0.03	1.43 ± 0.02	1.40 ± 0.02
Spermatid measurements				
Spermatid heads (10^7/g testis)	8.53 ± 0.44	8.75 ± 0.34	8.53 ± 0.41	9.15 ± 0.30
Spermatid heads (10^7/testis)	12.26 ± 0.43	12.73 ± 0.55	12.17 ± 0.50	12.80 ± 0.33
Spermatid count (mean/10^{-4}mL suspension)	61.30 ± 2.14	63.63 ± 2.75	60.85 ± 2.50	64.00 ± 1.63
Spermatozoal measurements				
Motility (%)	94.92 ± 0.50	93.97 ± 0.49	95.74 ± 0.95	95.40 ± 0.69
Concentration (10^6/g cauda epididymal tissue)	983.3 ± 43.3	1066.9 ± 39.2	1036.7 ± 47.2	1020.0 ± 71.7

[1] Data presented as mean ± standard error; n=10. Differences from the control group for testicular weights are not significant by Shirley's test; epididymal and epididymal tail weights, spermatid measurements, and spermatozoal measurements are not significant by Dunn's test.

* Significantly different (P≤0.05) from the control group by Williams' test.

TABLE C2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Inhalation Study of Glutaraldehyde[1]

Study Parameters	0 ppb	62.5 ppb	250 ppb[2]	1000 ppb
Necropsy body weight (g)	196 ± 3	195 ± 4	190 ± 5	175 ± 5**
Estrous cycle length (days)	4.65 ± 0.15	5.00 ± 0.00	4.88 ± 0.16[3]	4.90 ± 0.16
Estrous stages as % of cycle				
Diestrus	39.2	39.2	33.3	36.7
Proestrus	15.0	18.3	18.5	18.3
Estrus	27.5	21.7	29.6	26.7
Metestrus	18.3	20.8	18.5	18.3

[1] Data presented as mean ± standard error; n=10, except where noted. Estrous cycle lengths are not significant by Dunn's test. By multivariate analysis of variance dosed groups do not differ significantly from controls in cycle length or in the relative length of time spent in the estrous stages.

[2] For the 250 ppb concentration group, n=9.

[3] Estrous cycle longer than 12 days or unclear in one of nine animals; data for this animal were not included in the mean.

** Significantly different (P≤0.01) from the control group by Williams' test.

TABLE C3 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Inhalation Study of Glutaraldehyde[1]

Study Parameters	0 ppb	62.5 ppb	250 ppb	500 ppb
Weights (g)				
Necropsy body weight	36.1 ± 0.4	34.2 ± 0.6*	33.0 ± 0.5**	31.9 ± 0.8**
Left epididymis	0.029 ± 0.001	0.027 ± 0.001	0.029 ± 0.001	0.026 ± 0.001*
Left cauda epididymis	0.016 ± 0.001	0.016 ± 0.001	0.015 ± 0.001	0.015 ± 0.001
Left testis	0.116 ± 0.001	0.117 ± 0.002	0.117 ± 0.001	0.114 ± 0.002
Spermatid measurements				
Spermatid heads (10^7/g testis)	10.60 ± 0.81	11.23 ± 1.24	10.63 ± 1.06	10.48 ± 0.97
Spermatid heads (10^7/testis)	1.23 ± 0.10	1.31 ± 0.14	1.24 ± 0.12	1.19 ± 0.11
Spermatid count (mean/10^{-4}mL suspension)	38.40 ± 3.01	41.05 ± 4.51	38.68 ± 3.85	37.13 ± 3.40
Spermatozoal measurements				
Motility (%)	95.34 ± 0.61	95.60 ± 0.65	94.84 ± 0.44	94.44 ± 0.78
Concentration (10^6/g cauda epididymal tissue)	1657 ± 141	1794 ± 107	1742 ± 118	1920 ± 106

1 Data presented as mean ± standard error; n=10, except where noted. Differences from the control group for testicular weights, epididymal tail weights, spermatid measurements, and spermatozoal measurements are not significant by Dunn's test.

* Significantly different (P≤0.05) from the control group by Williams' test (necropsy body weights) or Shirley's test (left epididymal weights).

** Significantly different (P≤0.01) from the control group by Williams' test.

TABLE C4 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Inhalation Study of Glutaraldehyde[1]

Study Parameters	0 ppb	62.5 ppb	250 ppb	500 ppb[2]
Necropsy body weight (g)	30.9 ± 0.3	31.1 ± 0.8	28.7 ± 0.5**	27.3 ± 0.6**
Estrous cycle length (days)	4.26 ± 0.11	4.50 ± 0.40	4.25 ± 0.11	4.21 ± 0.15[3]
Estrous stages as % of cycle[4]				
Diestrus	23.3	25.8	26.7	31.3
Proestrus	20.0	21.7	20.0	9.4
Estrus	31.7	31.7	34.2	37.5
Metestrus	25.0	20.8	19.2	21.9

1 Data presented as mean ± standard error; n=10, except where noted.

2 For the 500 ppb concentration group, n=8.

3 n = 7.

4 Evidence suggests animals in the 250 and 500 ppb concentration groups differed significantly (P<0.05, Wilk's Criterion) from the controls in the relative length of time spent in the estrous stages. Females in these two concentration groups spent more time in estrus and diestrus and less time in metestrus than did controls.

** Significantly different (P≤0.01) from the control group by Williams' test.

APPENDIX D

Genetic Toxicology

Table D1 Mutagenicity of Glutaraldehyde in *Salmonella typhimurium* D-2

Table D2 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by Glutaraldehyde D-6

Table D3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Glutaraldehyde D-8

Table D4 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Glutaraldehyde D-10

Table D5 Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by Glutaraldehyde D-11

TABLE D1 Mutagenicity of Glutaraldehyde in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at EG&G Mason Research Institute							
TA100	0	120 ± 6.9	116 ± 8.6	124 ± 10.4	76 ± 0.9	148 ± 4.4	122 ± 1.2
	3.3	133 ± 1.7	134 ± 3.8	126 ± 10.1		134 ± 2.4	
	10	140 ± 9.8	130 ± 10.1	132 ± 4.4	81 ± 4.7	135 ± 8.7	135 ± 2.9
	20		159 ± 7.2				
	33	192 ± 11.7	229 ± 8.4	124 ± 7.8	88 ± 8.5	178 ± 8.2	178 ± 13.3
	50		227 ± 23.6[3]				218 ± 13.6
	75						219 ± 1.8
	100	70 ± 8.6[3]		179 ± 5.5	146 ± 9.8	182 ± 12.8[3]	147 ± 11.3[3]
	150				163 ± 4.9[3]		
	200				75 ± 2.9[3]		
	333	Toxic		Toxic		75 ± 7.5[3]	
Trial summary		Equivocal	Positive	Equivocal	Positive	Equivocal	Positive
Positive control[4]		1496 ± 14.6	1949 ± 20.1	1326 ± 58.7	1337 ± 47.2	972 ± 24.8	1262 ± 69.9
TA1535	0	19 ± 2.5	19 ± 4.6	12 ± 1.5	10 ± 0.3	11 ± 3.9	11 ± 2.2
	3.3	29 ± 1.9	19 ± 1.5	10 ± 2.4	10 ± 0.7		
	10	27 ± 2.3	17 ± 0.6	10 ± 1.5	10 ± 0.9	9 ± 1.2	12 ± 1.3
	20		23 ± 1.5				
	33	22 ± 2.3	19 ± 1.3	9 ± 2.0	13 ± 2.0	9 ± 1.5	11 ± 1.8
	50		19 ± 3.0[3]				11 ± 1.5
	75						13 ± 1.3
	100	Toxic		14 ± 1.9	11 ± 0.9	9 ± 0.7[3]	13 ± 1.3
	150				10 ± 2.1		
	200				9 ± 1.7[3]		
	333	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1521 ± 10.7	1467 ± 30.2	170 ± 40.8	123 ± 17.7	38 ± 6.1	71 ± 6.4
TA1537	0	9 ± 0.9	9 ± 2.0	8 ± 1.2	11 ± 0.3	7 ± 1.0	10 ± 1.9
	3.3	8 ± 1.2	6 ± 0.0	8 ± 1.9		7 ± 0.3	
	10	11 ± 2.5	7 ± 1.2	9 ± 2.3	10 ± 1.2	8 ± 1.3	8 ± 2.3
	20		11 ± 0.7				
	33	10 ± 1.2	11 ± 0.9	8 ± 1.5	9 ± 1.7	11 ± 1.9	11 ± 0.9
	50		9 ± 2.0				9 ± 1.5
	75						8 ± 1.8
	100	Toxic		9 ± 1.0	11 ± 1.7	8 ± 1.2	15 ± 3.2
	150				15 ± 3.3		
	200				9 ± 0.9[3]		
	333	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		509 ± 84.3	447 ± 21.4	71 ± 6.1	129 ± 1.5	34 ± 8.1	125 ± 9.8

TABLE D1 Mutagenicity of Glutaraldehyde in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA98	0	25 ± 5.7	27 ± 5.6	28 ± 2.5	32 ± 2.8	27 ± 1.9	32 ± 3.2
	3.3	22 ± 0.7	28 ± 2.7	19 ± 2.1		23 ± 1.5	
	10	25 ± 1.5	32 ± 2.1	23 ± 4.3	30 ± 4.6	24 ± 1.2	28 ± 2.5
	20		30 ± 3.6				
	33	32 ± 3.3[3]	37 ± 7.4	26 ± 2.8	28 ± 3.2	34 ± 5.2	38 ± 2.7
	50		38 ± 4.1				42 ± 4.0
	75						54 ± 4.6
	100	Toxic		27 ± 2.5	28 ± 3.7	35 ± 0.9[3]	36 ± 3.8[3]
	150				44 ± 3.0		
	200				32 ± 1.5[3]		
	333	Toxic		16 ± 2.1[3]		Toxic	
Trial summary		Negative	Negative	Negative	Equivocal	Negative	Equivocal
Positive control		2245 ± 98.6	1434 ± 19.3	1121 ± 62.3	1093 ± 20.9	469 ± 32.3	1007 ± 55.1

TABLE D1 Mutagenicity of Glutaraldehyde in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at Case Western Reserve University							
TA100	0	92 ± 2.8	98 ± 2.6	117 ± 20.8	113 ± 6.4	85 ± 2.7	111 ± 9.1
	10		91 ± 3.6		116 ± 0.3		175 ± 10.0
	33	99 ± 2.7	93 ± 0.6	101 ± 12.0	114 ± 6.9	95 ± 2.6	137 ± 12.5
	100	96 ± 8.5	93 ± 6.2	98 ± 2.8	160 ± 17.0	114 ± 7.1	163 ± 6.6
	333	99 ± 6.4	87 ± 5.0	130 ± 12.0	Toxic	133 ± 8.7	Toxic
	1000	Toxic	95 ± 3.5	Toxic	4 ± 4.0	65 ± 9.5	3 ± 3.3
	3333	0 ± 0.0		0 ± 0.0		0 ± 0.0	
Trial summary		Negative	Negative	Negative	Negative	Equivocal	Equivocal
Positive control		307 ± 18.1	394 ± 78.3	2397 ±104.0	2104 ± 81.4	2363 ± 61.5	1230 ± 27.7
TA1535	0	10 ± 2.0	5 ± 1.2	9 ± 2.0	11 ± 0.6	10 ± 1.0	3 ± 0.3
	10		7 ± 0.6		9 ± 1.5		3 ± 1.2
	33	8 ± 2.7	6 ± 0.6	7 ± 0.3	9 ± 0.3	10 ± 1.3	8 ± 0.7
	100	9 ± 1.9	2 ± 0.3	7 ± 1.0	6 ± 0.9	10 ± 1.8	3 ± 0.6
	333	8 ± 1.7	2 ± 0.3	9 ± 2.5	5 ± 1.2	7 ± 0.3	3 ± 0.6
	1000	5 ± 1.5	0 ± 0.3	4 ± 1.2	2 ± 1.7	4 ± 0.3	3 ± 1.5
	3333	0 ± 0.0		0 ± 0.0		0 ± 0.0	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		97 ± 46.8	310 ± 33.8	39 ± 8.1	41 ± 3.8	37 ± 8.4	42 ± 4.3
TA1537	0	4 ± 1.2	3 ± 1.2	6 ± 2.6	8 ± 1.5	8 ± 1.9	8 ± 1.8
	10		4 ± 0.9		7 ± 2.0		5 ± 0.9
	33	2 ± 1.2	2 ± 0.3	7 ± 0.3	6 ± 0.3	7 ± 1.2	8 ± 1.2
	100	4 ± 0.9	2 ± 0.3	7 ± 2.4	3 ± 1.2	12 ± 2.3	5 ± 0.7
	333	4 ± 1.2	1 ± 0.3	6 ± 2.1	2 ± 0.9	10 ± 1.2	1 ± 0.6
	1000	1 ± 0.7	0 ± 0.3	5 ± 0.9	1 ± 0.6	8 ± 0.9	0 ± 0.3
	3333	0 ± 0.0		0 ± 0.0		0 ± 0.0	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		148 ± 19.5	72 ± 38.0	141 ± 4.4	163 ± 27.8	210 ± 58.3	72 ± 8.7
TA98	0	12 ± 1.2	11 ± 0.9	24 ± 1.5	21 ± 3.0	26 ± 1.9	17 ± 1.9
	10		13 ± 1.9		25 ± 1.8		17 ± 4.1
	33	14 ± 1.5	10 ± 0.3	23 ± 3.2	22 ± 1.8	27 ± 2.7	26 ± 2.7
	100	14 ± 1.5	7 ± 3.8	31 ± 5.5	22 ± 5.0	37 ± 11.3	13 ± 1.2
	333	15 ± 1.7	4 ± 1.2	33 ± 2.9	20 ± 2.1	43 ± 9.0	18 ± 1.5
	1000	8 ± 1.2	5 ± 1.5	19 ± 7.5	17 ± 2.7	Toxic	18 ± 0.6
	3333	0 ± 0.0		0 ± 0.0		0 ± 0.0	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		118 ± 11.8	150 ± 24.2	1775 ±121.2	1590 ± 52.8	2141 ± 79.2	561 ± 12.0

TABLE D1 Mutagenicity of Glutaraldehyde in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at Inveresk Research International									
TA102	0	257 ± 9.2	138 ± 10.4	—[5]	305 ± 21.0	327 ± 4.5	226 ± 27.0	279 ± 19.4	274 ± 9.0
	25	259 ± 5.0	187 ± 6.5	—	353 ± 24.8	322 ± 15.9	267 ± 21.6	346 ± 10.3	309 ± 53.3
	50	297 ± 25.9	214 ± 4.7	—	333 ± 6.5	364 ± 13.3	365 ± 5.9	394 ± 41.4	389 ± 34.3
	100	275 ± 7.3	278 ± 21.3	—	417 ± 5.0	441 ± 38.4	473 ± 27.0	485 ± 34.7	535 ± 15.2
	200	232 ± 11.3	192 ± 13.8	—	570 ± 19.7	741 ± 35.8	504 ± 58.2	379 ± 57.0	481 ± 39.8
	300	46 ± 29.7[3]	27 ± 1.8[3]	—	352 ± 17.3[3]	743 ± 23.2[3]	250 ± 24.5[3]	608 ± 8.7[3]	268 ± 68.4[3]
Trial summary		Negative	Positive	—	Positive	Positive	Positive	Positive	Positive
Positive control		634 ± 95.3	898 ± 38.0	—	443 ± 14.0	562 ± 25.8	478 ± 28.4	454 ± 3.0	497 ± 15.3
TA104	0	453 ± 13.3	338 ± 12.5	338 ± 8.7	482 ± 21.2	406 ± 7.5	—	417 ± 21.7	495 ± 15.7
	25	632 ± 10.8	321 ± 3.8	464 ± 36.1	726 ± 40.3	605 ± 36.6	—	506 ± 15.4	689 ± 50.0
	50	732 ± 13.5	452 ± 17.0	602 ± 6.6	893 ± 25.5	771 ± 19.9	—	543 ± 14.6	1003 ± 40.8
	100	1018 ± 21.1	600 ± 16.2	715 ± 22.2	1074 ± 56.5	1020 ± 21.0	—	1185 ± 121.4	1174 ± 31.8
	200	807 ± 44.3	815 ± 33.5	783 ± 28.9	754 ± 20.2	654 ± 123.9	—	667 ± 15.7	861 ± 51.0
	300	296 ± 68.7[3]	861 ± 14.4[3]	522 ± 110.3[3]	477 ± 55.0[3]	620 ± 65.0[3]	—	173 ± 23.2[3]	541 ± 107.8[3]
Trial summary		Positive	Positive	Positive	Positive	Positive	—	Positive	Positive
Positive control		232 ± 4.7[3]	653 ± 43.5	818 ± 50.4	1371 ± 21.4	1052 ± 14.9	—	1133 ± 60.4	976 ± 42.8
TA100	0	65 ± 5.5	82 ± 9.0	91 ± 3.6	84 ± 2.0	112 ± 3.6	90 ± 2.7	83 ± 1.7	94 ± 5.7
	25	106 ± 6.5	116 ± 4.9	108 ± 6.4	116 ± 6.2	122 ± 2.9	114 ± 4.3	119 ± 4.9	121 ± 4.2
	50	84 ± 7.4	135 ± 2.2	159 ± 5.4	139 ± 9.7	151 ± 1.7	146 ± 14.0	163 ± 3.5	180 ± 3.1
	100	131 ± 1.2	197 ± 27.4	330 ± 14.7	146 ±19.5	224 ± 18.2	261 ± 8.2	255 ± 3.8	259 ± 16.7
	200	149 ± 13.0	356 ± 18.1	355 ± 35.7	151 ±11.0	256 ± 19.6	296 ± 6.5	96 ± 9.0	177 ± 11.1
	300	89 ± 3.83	152 ± 4.43	117 ± 9.13	90 ± 3.53	158 ± 13.23	86 ± 6.83	85 ± 4.33	133 ± 10.73
Trial summary		Positive	Positive	Positive	Weakly positive	Positive	Positive	Positive	Positive
Positive control		182 ± 5.3	338 ± 12.5	455 ± 4.4	512 ± 15.5	1308 ± 105.9	1253 ± 78.9	408 ± 17.6	829 ± 38.0

[1] The detailed protocol and the data from the first two studies are presented in Haworth *et al*. (1983). The protocol for the third study (Inveresk Research International) is presented in Zeiger *et al.* (1992). 0 ±g/plate is the solvent control.

[2] Revertants are presented as mean ± standard error from three plates.

[3] Slight toxicity.

[4] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), 4-nitro-*o*-phenylenediamine (TA98), mitomycin C (TA102), and methyl methanesulfonate (TA104). The positive control for metabolic activation with all strains was 2-aminoanthracene, and 2-aminoanthracene/sterigmatocystin was used for TA102.

[5] Trial was not performed for this strain.

TABLE D2 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by Glutaraldehyde[1]

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[2]	Average Mutant Fraction
-S9						
Trial 1						
Distilled water						
		68	98	68	33	
		71	89	68	32	
		58	102	55	31	
		77	111	41	18	29
Ethyl methanesulfonate						
		70	91	312	149	
	250	80	84	353	147	148*
Glutaraldehyde						
	0.5	64	106	105	55	
		68	153	48	23	39
	1	62	96	87	47	
		83	154	80	32	40
	2	44	71	120	91	
		80	199	67	28	59*
	4	69	100	98	47	
		75	128	97	43	45
	8	29	26	236	270	
		67	22	285	142	206*
	16	Lethal				
		Lethal				

TABLE D2 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by Glutaraldehyde (continued)

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 2						
Distilled water						
		74	102	79	36	
		59	110	66	38	
		70	88	95	45	39
Ethyl methanesulfonate						
		75	55	725	324	
	250	63	62	615	325	324*
Glutaraldehyde						
	0.5	92	88	160	58	
		80	93	88	37	47
	1	86	98	92	36	
		66	90	57	29	32
	2	64	90	76	40	
		79	87	107	45	43
	4	89	64	187	70	
		72	69	89	41	56
	8	21	2	385	611	
		27	5	283	352	481*
	16	Lethal				
		Lethal				

[1] Study performed at Inveresk Research International. The experimental protocol and these data are presented in McGregor *et al.* (1988).

[2] Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF/1 x 10^6 cells treated); MF=mutant fraction.

* Significant positive response (P≤0.05).

TABLE D3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Glutaraldehyde[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)[2]
Study performed at Litton Bionetics, Inc.								
-S9								
Trial 1								
Summary: Positive								
Distilled water		50	1026	390	0.38	7.8	25.5	
Triethylenemelamine	15	50	1010	2079	2.05	41.6	25.5	441.53
Glutaraldehyde	0.36	50	1031	475	0.46	9.5	25.5	21.20*
	1.08	50	1034	400	0.38	8.0	25.5	1.77
	3.6	50	1028	539	0.52	10.8	25.5	37.94*
	10.8	0					25.5	
								P<0.001[3]
+S9								
Trial 1								
Summary: Weak positive								
Distilled water		50	1035	477	0.46	9.5	25.5	
Cyclophosphamide	1.5	50	1023	1348	1.31	27.0	25.5	185.92
Glutaraldehyde	1	50	1046	501	0.47	10.0	25.5	3.93
	3.6	50	1045	535	0.51	10.7	25.5	11.09
	10.8	50	1035	713	0.68	14.3	25.5	49.48*
								P<0.001
Trial 2								
Summary: Positive								
Distilled water		50	1026	394	0.38	7.9	26.0	
Cyclophosphamide	1.5	50	1052	1691	1.60	33.8	26.0	318.59
Glutaraldehyde	10	50	1028	451	0.43	9.0	26.0	14.24
	12.5	50	1019	560	0.54	11.2	26.0	43.11*
	15	50	1025	652	0.63	13.0	26.0	65.64*
								P<0.001

TABLE D3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Glutaraldehyde (continued)

Compound	Dose (µg/mL)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)
Study performed at Columbia University								
-S9								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1050	524	0.49	10.5	26.0	
Triethylenemelamine	0.015	50	1050	1437	1.36	28.7	26.0	174.24
Glutaraldehyde	0.5	50	1050	545	0.51	10.9	26.0	4.01
	1.6	50	1049	483	0.46	9.7	26.0	-7.74
	5	50	1048	531	0.50	10.6	26.0	1.53
	16	25	524	321	0.61	12.8	26.0	22.75*
								P=0.035
+S9								
Trial 1								
Summary: Weak positive								
Dimethylsulfoxide		100	2097	915	0.43	9.2	26.0	
Cyclophosphamide	1	100	2095	2593	1.23	25.9	26.0	183.66
Glutaraldehyde	1.6	50	1048	484	0.46	9.7	26.0	5.84
	5	50	1047	484	0.46	9.7	26.0	5.95
	16	100	2092	1167	0.55	11.7	26.0	27.85*
								P<0.001

[1] SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. The protocol and these data are published in Galloway *et al.* (1985).

[2] Percentage increase in SCEs/chromosome of culture exposed to glutaraldehyde relative to those of culture exposed to solvent.

[3] Significance was tested by the linear regression trend test vs. log of the dose.

* Positive (>20% increase over solvent control).

TABLE D4 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Glutaraldehyde[1]

-S9					+S9				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Study performed at Litton Bionetics, Inc.									
Trial 1 – Harvest time: 10.5 hours					**Trial 1** – Harvest time: 10.5 hours				
Summary: Negative					Summary: Negative				
Distilled water					Distilled water				
	100	3	0.03	3.0		100	8	0.08	6.0
Triethylenemelamine					Cyclophosphamide				
50	100	19	0.19	18.0	50	100	43	0.43	23.0
Glutaraldehyde					Glutaraldehyde				
0.3	100	0	0.00	0.0	1	100	2	0.02	2.0
1	100	1	0.01	1.0	3	100	2	0.02	2.0
3	100	1	0.01	1.0	10	100	5	0.05	5.0
10	0				15	0			
					30	0			
				P=0.843[2]					P=0.631
Study performed at Columbia University									
Trial 1 – Harvest time: 14.0 hours					**Trial 1** – Harvest time: 14.0 hours				
Summary: Weak positive					Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	1	0.01	1.0		100	1	0.01	1.0
Triethylenemelamine					Cyclophosphamide				
0.15	100	23	0.23	20.0	15	100	23	0.23	19.0
Glutaraldehyde					Glutaraldehyde				
1.6	100	4	0.04	4.0	1.6	100	1	0.01	1.0
5	100	6	0.06	5.0	5	100	4	0.04	3.0
16	100	12	0.12	11.0*	16	100	7	0.07	7.0*
				P=0.001					P=0.004

[1] Abs=aberrations. The protocol and these data are presented by Galloway *et al.* (1985).

[2] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose.

* Positive (P≤0.05).

TABLE D5 Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by Glutaraldehyde[1]

Route of Exposure	Dose (ppm)	Incidence of Deaths (%)	Incidence of Sterility (%)	No. of Lethal/No. of X Chromosomes Tested			
				Mating 1	Mating 2	Mating 3	Total[2]
Injection	3000	2	0	6/1792	1/1146	3/1688	10/4626 (0.22%)
	0			4/1125	3/1146	1/1133	8/3404 (0.24%)
Injection	4000	22	54	0/486	2/855	0/578	2/1919 (0.10%)
	0			1/1009	1/1297	0/865	2/3171 (0.06%)
Feeding	7500	27	37	2/1947	3/2194	1/1828	6/5969 (0.10%)
	0			3/1858	0/1832	1/2090	4/5780 (0.07%)
Feeding	10000	68	2	0/742	0/618	0/698	0/2058 (0.00%)
	0			2/724	1/706	1/443	4/1873 (0.21%)
Larva Feeding	3500	10	0	4/2694	2/2686	0/000	6/5380 (0.11%)
	0			2/2598	2/2630	0/000	4/5228 (0.08%)

[1] Study performed at Brown University. The protocol and the data from the adult feeding and injection studies are presented in Yoon *et al.* (1985). The protocol and the data from the larval feeding study are presented in Zimmering *et al.* (1989). Results were not significant at the 5% level (Margolin *et al.*, 1983).

[2] Total number of lethal mutations/total number of X chromosomes tested for three mating trials.

APPENDIX E

Histoautoradiographic Evaluation of Respiratory Tract

HISTOPATHOLOGY AND CELL REPLICATION IN THE NASAL EPITHELIUM OF RATS AND MICE EXPOSED TO GLUTARALDEHYDE VAPOR E-2

Table E1 Incidence of Nasal Lesions in the Histoautoradiographic Study of F344/N Rats Exposed to Glutaraldehyde by Inhalation E-3

Table E2 Incidence of Nasal Lesions in the Histoautoradiographic Study of $B6C3F_1$ Mice Exposed to Glutaraldehyde by Inhalation E-5

Table E3 Severity of Selected Nasal Lesions in the Histoautoradiographic Study of F344/N Rats Exposed to Glutaraldehyde by Inhalation E-7

Table E4 Severity of Selected Nasal Lesions in the Histoautoradiographic Study of $B6C3F_1$ Mice Exposed to Glutaraldehyde by Inhalation E-8

Table E5 Mean Cell Replication in F344/N Rats Exposed to Glutaraldehyde by Inhalation in the Histoautoradiographic Study E-9

Table E6 Mean Cell Replication in $B6C3F_1$ Mice Exposed to Glutaraldehyde by Inhalation in the Histoautoradiographic Study E-10

Histopathology and Cell Replication in the Nasal Epithelium of Rats and Mice Exposed to Glutaraldehyde Vapor

Both glutaraldehyde and formaldehyde, a potent nasal carcinogen in rats, are respiratory tract irritants and cross-linking agents. This study was undertaken to characterize acute and subchronic respiratory tract responses to glutaraldehyde. Male and female F344/N rats and B6C3F$_1$ mice were exposed to 0, 62.5, 125, 250, 500, or 1000 ppb glutaraldehyde for 6 hours per day for 1 or 4 days, or for 6 hours per day, 5 days per week for 6 or 13 weeks. Each animal received a 2-hour pulse of tritiated thymidine (intraperitoneal, 6.7Ci/mmole, 2μCi/gm) 18 hours after the last exposure. Nasal tissues were processed for autoradiography, and cell replication was assessed using unit length labeling index (ULLI; labeled cells/mm basement membrane). Acute and subacute glutaraldehyde-induced lesions, present in both species and sexes and confined to the anterior nasal passages, included epithelial erosions and inflammation and were associated with increases in cell replication. Increases in cell replication on the dorsal atrioturbinate over control values (ULLI≤4.53 cells/mm; Table E5) were most severe in male and female rats at 6weeks (ULLI 15.69 and 45.00 for male rats and 13.63 and 89.18 for female rats exposed to 500 and 1000 ppb; TableE5) and less severe in male and female mice, peaking at 4days (ULLI 5.37 and 6.60, respectively, at 500 ppb). A neutrophilic infiltrate in the squamous epithelium of the nasal vestibule was most severe in female mice, did not exhibit a no-effect concentration, became progressively more severe up to 13weeks, and was associated with increased ULLI; all individual animal responses were ULLI ≤50 (versus control mean ≤8.15; Table E6). Some high-concentration (500 and 1000 ppb) mice died due to glutaraldehyde-induced occlusion of the external nares. Results from these studies demonstrate that glutaraldehyde-induced lesions were located more anterior in the nose than, and differed in character from, those reported for formaldehyde. No evidence of preneoplastic changes previously reported for formaldehyde were observed (adapted from Gross *et al.*, 1992).

TABLE E1 Incidence of Nasal Lesions in the Histoautoradiographic Study of F344/N Rats Exposed to Glutaraldehyde by Inhalation[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Nonsuppurative rhinitis						
Day 1	5	5	4[2]	5	5	5
Day 4	5	5	5	5	5	5
Week 6	5	5	5	5	5	3[3]
Week 13	0	1	1	2	3	4
Squamous exfoliation						
Day 1	0	1	0[2]	1	3	5
Day 4	0	0	0	0	3	5
Week 6	0	0	0	0	3	3[3]
Week 13	0	0	0	2	2	2
Intraepithelial neutrophils						
Day 1	0	0	0[2]	1	2	5
Day 4	0	0	0	0	5	5
Week 6	1	0	1	2	4	3[3]
Week 13	5	3	5	5	4	5
Subepithelial neutrophils						
Day 1	0	0	0[2]	3	5	5
Day 4	1	0	2	1	5	5
Week 6	2	3	2	4	5	3[3]
Week 13	5	4	5	5	5	5
Epithelial erosions						
Day 1	0	0	0[2]	1	5	5
Day 4	0	0	0	1	2	5
Week 6	0	0	0	0	4	3[3]
Week 13	1	1	0	1	1	1
Squamous metaplasia						
Day 1	0	0	0[2]	3	1	1
Day 4	0	0	0	0	5	5
Week 6	0	0	0	0	4	3[3]
Week 13	1	0	0	0	5	5
Eosinophilic droplets						
Day 1	0	0	0[2]	0	0	0
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	0	0[3]
Week 13	0	0	0	0	0	0
Olfactory degeneration						
Day 1	0	0	0[2]	0	0	1
Day 4	0	0	0	0	0	4
Week 6	0	0	0	0	0	3[3]
Week 13	0	0	0	0	0	4

TABLE E1 Incidence of Nasal Lesions in the Histoautoradiographic Study of F344/N Rats Exposed to Glutaraldehyde by Inhalation (continued)

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Female						
Nonsuppurative rhinitis						
Day 1	5	5	5	5	5	5
Day 4	5	4	5	5	5	5
Week 6	3	4	4	2	5	2
Week 13	2	3	4	5	5	5
Squamous exfoliation						
Day 1	0	0	0	2	3	4
Day 4	0	0	0	3	5	5
Week 6	0	0	0	3	2	2[4]
Week 13	0	0	0[2]	0	2	4
Intraepithelial neutrophils						
Day 1	0	0	0	0	2	4
Day 4	1	0	0	2	5	5
Week 6	0	1	0	0	2	2[4]
Week 13	1	0	1	3	2	5
Subepithelial neutrophils						
Day 1	0	0	1	1	5	5
Day 4	2	0	0	4	5	5
Week 6	1	2	1	1	5	2[4]
Week 13	2	0	1	3	4	4
Epithelial erosions						
Day 1	0	0	1	0	4	5
Day 4	0	0	0	2	3	5
Week 6	0	0	0	0	4	1[4]
Week 13	0	0	0	0	0	1
Squamous metaplasia						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	1	5	5
Week 6	0	0	0	0	3	2[4]
Week 13	0	0	0	0	3	5
Eosinophilic droplets						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	0	0[4]
Week 13	0	0	0	0	0	0
Olfactory degeneration						
Day 1	0	0	0	0	0	1
Day 4	0	0	0	0	0	1
Week 6	0	1	1	1	1	2[4]
Week 13	0	0	1	0	0	1

[1] n=5 unless otherwise noted.
[2] n=4.
[3] n=3.
[4] n=2.

TABLE E2 Incidence of Nasal Lesions in the Histoautoradiographic Study of B6C3F$_1$ Mice Exposed to Glutaraldehyde by Inhalation[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Nonsuppurative rhinitis						
Day 1	3	0	0	0	0	2
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	0	–[2]
Week 13	0	0	1	0	1	–
Squamous exfoliation						
Day 1	0	0	0	0	4	5
Day 4	0	0	0	4	2	5
Week 6	0	0	2	0	0	–
Week 13	0	0	0	3	1	–
Intraepithelial neutrophils						
Day 1	1	0	1	0	1	5
Day 4	0	0	0	1	4	5
Week 6	0	0	0	1	1	–
Week 13	0	0	1	4	5	–
Subepithelial neutrophils						
Day 1	1	0	1	1	2	5
Day 4	0	0	0	2	4	5
Week 6	0	0	0	1	4	–
Week 13	0	1	2	5	5	–
Epithelial erosions						
Day 1	0	0	0	0	1	2
Day 4	0	0	0	0	1	2
Week 6	0	0	0	0	0	–
Week 13	0	0	0	1	3	–
Squamous metaplasia						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	1	4
Week 6	0	0	0	0	2	–
Week 13	0	0	0	0	1	–
Eosinophilic droplets						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	1	0
Week 6	0	0	0	0	0	–
Week 13	0	0	0	2	5	–
Olfactory degeneration						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	1	–
Week 13	0	0	0	0	1	–

TABLE E2 Incidence of Nasal Lesions in the Histoautoradiographic Study of B6C3F$_1$ Mice Exposed to Glutaraldehyde by Inhalation (continued)

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Female						
Nonsuppurative rhinitis						
Day 1	1	0	0	1	0	0
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	0	–
Week 13	1	0	0	1	0[3]	–
Squamous exfoliation						
Day 1	0	0	0	5	5	4
Day 4	0	0	2	5	5	5
Week 6	0	0	0	2	2	–
Week 13	0	0	0	1[3]	1[3]	–
Intraepithelial neutrophils						
Day 1	0	0	0	0	0	1
Day 4	0	1	0	1	5	4
Week 6	0	1	4	5	5	–
Week 13	0	4	5	4[3]	4[3]	–
Subepithelial neutrophils						
Day 1	0	0	1	0	2	3
Day 4	0	0	0	1	5	5
Week 6	1	1	4	5	5	–
Week 13	2	5	5	5	4[3]	–
Epithelial erosions						
Day 1	0	0	0	0	0	1
Day 4	0	0	0	0	0	2
Week 6	0	0	0	0	0	–
Week 13	0	0	0	0	0[3]	–
Squamous metaplasia						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	3	–
Week 13	0	0	0	0	1[3]	–
Eosinophilic droplets						
Day 1	0	0	0	0	0	0
Day 4	0	1	0	1	0	0
Week 6	1	0	0	1	1	–
Week 13	0	4	2	5	2[3]	–
Olfactory degeneration						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	1	0	0
Week 6	0	0	0	0	0	–
Week 13	0	0	0	0	0	–

[1] n=5 unless otherwise noted.
[2] All animals died before scheduled kill.
[3] n=4.

TABLE E3 Severity of Selected Nasal Lesions in the Histoautoradiographic Study of F344/N Rats Exposed to Glutaraldehyde by Inhalation[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Intraepithelial neutrophils						
Day 1	0	0	0	0.4	0.4	1.2
Day 4	0	0	0	0	1.4	2.6
Week 6	0.2	0	0.2	0.4	0.6	3.0
Week 13	1.2	0.8	1.0	1.2	1.2	1.6
Subepithelial neutrophils						
Day 1	0	0	0	0.8	1.8	2.6
Day 4	0.2	0	0.4	0.2	1.6	3.4
Week 6	0.4	0.6	0.6	0.8	2.0	3.7
Week 13	1.0	1.0	1.2	1.6	1.4	2.0
Squamous metaplasia						
Day 1	0	0	0	0.6	0.2	0.2
Day 4	0	0	0	0	1.2	2.2
Week 6	0	0	0	0	1.6	3.3
Week 13	0.2	0	0	0	2.0	3.0
Female						
Intraepithelial neutrophils						
Day 1	0	0	0	0	0.6	1.0
Day 4	0.2	0	0	0.4	2.2	3.4
Week 6	0	0.2	0	0	0.6	3.5
Week 13	0.2	0	0.4	1.0	0.8	1.4
Subepithelial neutrophils						
Day 1	0	0	0.4	0.2	2.4	2.8
Day 4	0.4	0	0	1.4	2.8	3.0
Week 6	0.6	0.4	0.4	0.4	2.2	4.5
Week 13	0.4	0	0.8	1.0	1.8	2.0
Squamous metaplasia						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0.2	2.0	3.0
Week 6	0	0	0	0	0.6	3.5
Week 13	0	0	0	0	1.2	2.0

[1] Mean for all animals; nonlesion included as 0 in calculation of mean value. Severity scale range: 0 to 5.

TABLE E4 Severity of Selected Nasal Lesions in the Histoautoradiographic Study of B6C3F$_1$ Mice Exposed to Glutaraldehyde by Inhalation[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Intraepithelial neutrophils						
Day 1	0.2	0	0.2	0	0.2	1.0
Day 4	0	0	0	0.2	1.8	2.8
Week 6	0	0	0	0.2	0.8	–[2]
Week 13	0	0	0.2	1.6	2.6	3.0
Subepithelial neutrophils						
Day 1	0.2	0	0.2	0.2	0.4	1.6
Day 4	0	0	0	0.4	1.8	3.2
Week 6	0	0	0	0.4	1.8	–
Week 13	0	0.2	0.8	2.2	2.8	3.0
Squamous metaplasia						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	0.2	0.8
Week 6	0	0	0	0	0.4	0
Week 13	0	0	0	0	0.2	2.0
Female						
Intraepithelial neutrophils						
Day 1	0	0	0	0	0	0.4
Day 4	0	0.2	0	0.4	1.0	0.8
Week 6	0	0.4	1.6	1.8	2.2	–
Week 13	0	2.0	2.4	3.2	3.8	–
Subepithelial neutrophils						
Day 1	0	0	0.2	0	0.4	1.2
Day 4	0	0	0	0.4	1.6	2.0
Week 6	0.2	0.4	2.0	2.4	2.6	–
Week 13	0.4	2.0	2.8	3.2	2.8	–
Squamous metaplasia						
Day 1	0	0	0	0	0	0
Day 4	0	0	0	0	0	0
Week 6	0	0	0	0	0.8	–
Week 13	0	0	0	0	0.5	–

[1] Mean for all animals; nonlesion included as 0 in calculation of mean value. Severity scale range: 0 to 5.
[2] All animals died before scheduled kill.

TABLE E5 Mean Cell Replication in F344/N Rats Exposed to Glutaraldehyde by Inhalation in the Histoautoradiographic Study[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Nasal vestibule[2]						
Day 1	9.36	10.91	10.44	15.73	13.44	20.85
Day 4	7.34	9.98	10.59	14.67	21.92	28.04
Week 6	6.52	8.82	6.61	12.37	15.00	15.53
Week 13	14.74	11.74	12.77	10.82	15.18	15.85
Dorsal atrioturbinate[3]						
Day 1	4.53	3.02	4.10	5.38	6.53	14.27
Day 4	4.11	3.66	3.70	5.12	4.51	21.98
Week 6	1.63	2.25	3.40	3.88	15.69	45.00
Week 13	3.30	3.65	6.71	4.37	9.71	36.29
Female						
Nasal vestibule						
Day 1	7.74[4]	8.94	11.56	11.97	14.73[4]	21.78
Day 4	12.09	7.83	10.23	14.52	23.92	37.84
Week 6	8.52	8.99	9.12	11.99	15.02	74.67
Week 13	9.08	12.04	14.04	16.29	19.78	15.85
Dorsal atrioturbinate						
Day 1	3.93[4]	1.93[5]	2.56[6]	3.05[6]	5.16[4]	20.66
Day 4	2.32	1.01	2.07	3.70	2.48	26.33
Week 6	3.14	3.07	3.46	3.83	13.63	89.18
Week 13	2.37	3.48	6.00	9.23	18.86	29.39

[1] Values are expressed as mean for five animals unless otherwise indicated; values are number of labeled cells/mm basement membrane.
[2] Lined by squamous epithelium.
[3] Normally lined by respiratory epithelium. Site of squamous metaplasia (see Table E3).
[4] n=4.
[5] n=2.
[6] n=3.

TABLE E6 Mean Cell Replication in B6C3F$_1$ Mice Exposed to Glutaraldehyde by Inhalation in the Histoautoradiographic Study[1]

	0 ppb	62.5 ppb	125 ppb	250 ppb	500 ppb	1000 ppb
Male						
Nasal vestibule[2]						
Day 1	4.52	4.38	3.32	4.65	7.04	13.01
Day 4	7.54	6.54	7.45	11.68	12.27	18.52
Week 6	6.38	5.87	7.03	8.35	15.35	–[3]
Week 13	5.40	5.61	7.71	11.12	17.76	–
Dorsal atrioturbinate[4]						
Day 1	1.54	2.03	1.57	0.95	2.99	2.22
Day 4	1.46	1.98	3.48	1.45	5.37	6.09
Week 6	0.31	0.93	1.13	0.85	2.68	–
Week 13	0.45	0.50	0.35	0.39	0.93	–
Female						
Nasal vestibule						
Day 1	5.82	4.58	3.92	2.52	2.89	5.56
Day 4	8.15	6.11	6.36	8.00	10.05	14.14
Week 6	6.40	8.06	11.56	14.59	21.00	–
Week 13	6.27	16.87	15.33	21.53	23.68	–
Dorsal atrioturbinate						
Day 1	0.82	0.65	0.43	1.31	1.03	1.20
Day 4	1.30	0.73	1.33	2.24	6.60	10.15
Week 6	0.80	0.59	4.03	2.66	4.41	–
Week 13	1.61	2.35	1.65	1.76	0.94	–

[1] Values are expressed as mean for five animals unless otherwise indicated; values are number of labeled cells/mm basement membrane.
[2] Lined by squamous epithelium.
[3] All animals died before scheduled kill.
[4] Normally lined by respiratory epithelium. Site of squamous metaplasia (see Table E4).