GR

ORIGINAL SUBMISSION

DuPont Tate & Lyle BioProducts

198 Blair Bend Drive
Loudon, TN 37774-6562

July 28, 2009

RECEIVED
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Division of Biotechnology and GRAS Notice Review

Office of Food Additive Safety (HFS-225)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Pkwy
College Park, MD 20740

RE: **GRAS EXEMPTION CLAIM: 1,3-PROPANEDIOL**

Dear Sir or Madam,

DuPont Tate & Lyle Bio Products is pleased to submit the attached GRAS Exemption Claim for 1,3-propanediol for use as a food ingredient as an alternate to 1,2-propanediol. 1,3-Propanediol has been determined by DuPont Tate & Lyle Bio Products to be Generally Recognized as Safe (GRAS), consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination is based on scientific procedures and has been evaluated by experts qualified by scientific training and experience to assess the safety of 1,3-propanediol under the conditions of its intended use in food. Therefore, the use of 1,3-propanediol in food as described in this GRAS Exemption Claim is exempt from the requirement of premarket approval.

The attached GRAS Notification provides a review of the information related to intended uses, manufacturing and safety of DuPont Tate & Lyle's 1,3-propanediol product. Included with this GRAS Notification submission you'll find three ***(3)*** hard copies of the GRAS Notification dossier which include the signed Expert Panel Consensus Statement and cited references.

DuPont Tate & Lyle Bio Products looks forward to the Agency's review of the accompanying dossier and is prepared to respond to any questions that arise in the review process. We are confident in our safety determination of 1,3-propanediol for its intended use as a food ingredient as an alternate to 1,2-propanediol and trust that the Agency will concur with this conclusion.

Respectfully submitted,

David Wood
Product Director
DuPont Tate & Lyle Bio Products
198 Blair Bend Road
Loudon, Tennessee 37774

I. GRAS EXEMPTION CLAIM

A. Claim of Exemption From the Requirement for Premarket Approval Pursuant to Proposed 21 CFR §170.36(c)(1) [62 FR 18938 (17 April 1997)]

As defined herein, 1,3-propanediol has been determined by DuPont Tate & Lyle Bio Products, to be Generally Recognized As Safe (GRAS), consistent with Section 201(s) of the Federal Food, Drug, and Cosmetic Act. This determination is based on scientific procedures as described in the following sections, under the conditions of its intended use in food. Therefore, the use of 1,3-propanediol in food as described below is exempt from the requirement of premarket approval.

Signed,

8/12/09

Date

David Wood
DuPont Tate & Lyle Bio Products
198 Blair Bend Road
Loudon, Tennessee
37774

B. Name and Address of Notifier

David Wood
DuPont Tate & Lyle Bio Products
198 Blair Bend Road
Loudon, Tennessee
37774

C. Common Name of the Notified Substance

1,3-propanediol

D. Conditions of Intended Use in Food

1,3-Propanediol is intended for use as a food ingredient as an alternate to 1,2-propanediol. The maximum individual intended food use levels of 1,3-propanediol in food, discussed in greater detail in Section IV.B herein, are the same as the current GRAS use of 1,2-propanediol, as specified in 21 CFR 184.1666, except that DuPont Tate & Lyle BioProducts wishes to exclude

from this notification the following categories: animal food (feed), infant formula, and ingredients used in infant formula. This exclusion is for business considerations only.

E. Basis for the GRAS Determination

Pursuant to 21 CFR § 170.30, the composition of 1,3-propanediol intended for use in foods by DuPont Tate & Lyle Bio Products, as defined herenin has been determined to be GRAS based on scientific procedures. This determination is based on data that is generally available in the public domain, pertaining to the safety of 1,3-propanediol and is supported by a consensus among a panel of experts who are qualified by scientific training and experience to evaluate the safety of 1,3-propanediol as a component of food[1] [see Appendix, Tab 1, entitled, "**EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF 1,3-PROPANEDIOL**".

F. Availability of Information

The data and information that serve as the basis for this GRAS Notification and that are referenced herein have been sent to the U.S. Food and Drug Administration (FDA) with this notification.

II. DETAILED INFORMATION ABOUT THE IDENTITY OF THE SUBSTANCE

A. Identity

1,3-propanediol is a flammable, colorless, stable liquid. DuPont Tate & Lyle utilizes a fermentation process in the manufacturing of 1,3-propanediol. The organism utilized in the production of the 1,3-propanediol is a genetically modified microorganism, derived from a non-pathogenic strain of *Escherichia coli* K-12. *E. coli* K-12 has a history of safe use, including the commercial production of insulin (NovoNordisk), phenylalanine (NutraSweet), and various industrial enzymes. The strain has exempt status under EPA-TSCA and is viewed to have low potential impact on human health, based on EPA risk assessment (EPA, 1997). DuPont Tate & Lyle's *E. coli* K-12 strain has been genetically modified to produce 1,3-propanediol.

Common or Usual Name: 1,3-propanediol

Chemical Name: 1,3-propanediol

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[1] The Panel consisted of Donald H. Hughes, Ph.D., Robert Nicolosi, Ph.D., C.N.S. (University of Massachusetts Lowell) and David Bechtel, Ph.D., DABT.

Chemical Abstracts Service 504-63-2

The structural formula of 1,3-propanediol is provided below in Figure 1.

Figure 1: Structural formula of 1,3-propanediol

B. Method of Manufacture

1,3-propanediol is manufactured by fermentation in accordance with food current Good Manufacturing Practices (cGMP; 21CFR110) through a multi-step fermentation, separation, and distillation process using a genetically modified and non-pathogenic strain of *Escherichia coli* K-12. The fermentation organism is heat deactivated with a high-temperature water stream in a heat exchanger. The contact raises the temperature (b)(4) for a period of several minutes. This temperature is sufficient to achieve complete deactivation of the biomass as measured by viability assays. The fermentation biomass is then separated out through a series of membrane separation steps. The 1,3-propanediol moves with fermentation broth from the separation area and is separated out by a 3-step process involving (i) ion exchange to remove charged impurities; (ii) evaporation to remove water; and (iii) 4 stages of distillation to remove impurities. No solvents are used in the separation process.

1. Raw Material Specifications

a *Escherichia coli* K-12

The strain utilized in the production of 1,3-propanediol is a genetically modified microorganism, derived from a non-pathogenic strain of *Escherichia coli* K-12. *E. coli* K-12 has a history of safe use, including the commercial production of insulin (NovoNordisk), phenylalanine (NutraSweet), and various industrial enzymes. The strain has exempt status under EPA-TSCA and is viewed to have low potential impact on human health, based on the EPA risk assessment (EPA, 1997).

DuPont Tate & Lyle's *E. coli* K-12 strain has been genetically modified to produce 1,3-propanediol. The nonpathogenicty and non-toxic nature of the genetically modified organism has been confirmed by DuPont Tate & Lyle. Additional information is provided in the Appendix (Tab 2).

b Culture Medium

Corn sugar is the primary raw material. Corn sugar, commonly called D-glucose or dextrose, is Generally Recognized as Safe in accordance with 21CFR 184.1857 for use with no limitation other than current good manufacturing practice. The feed to the production of 1,3-propanediol is a lower dry solids stream of a FCC compliant D-glucose finished product.

A variety of vitamins, common salts, and inorganic acids are used in the culture medium. All components of the culture medium meet food grade specifications or are of adequate purity for food fermentation processes.

c Biocatalyst

Genetically modified *Escherichia coli* K12

d Other ingredients?

A polypropylene glycol anti-foaming agent is used to control foaming in the fermentation process.

2. Manufacturing Process for 1,3-propanediol

a Fermentation

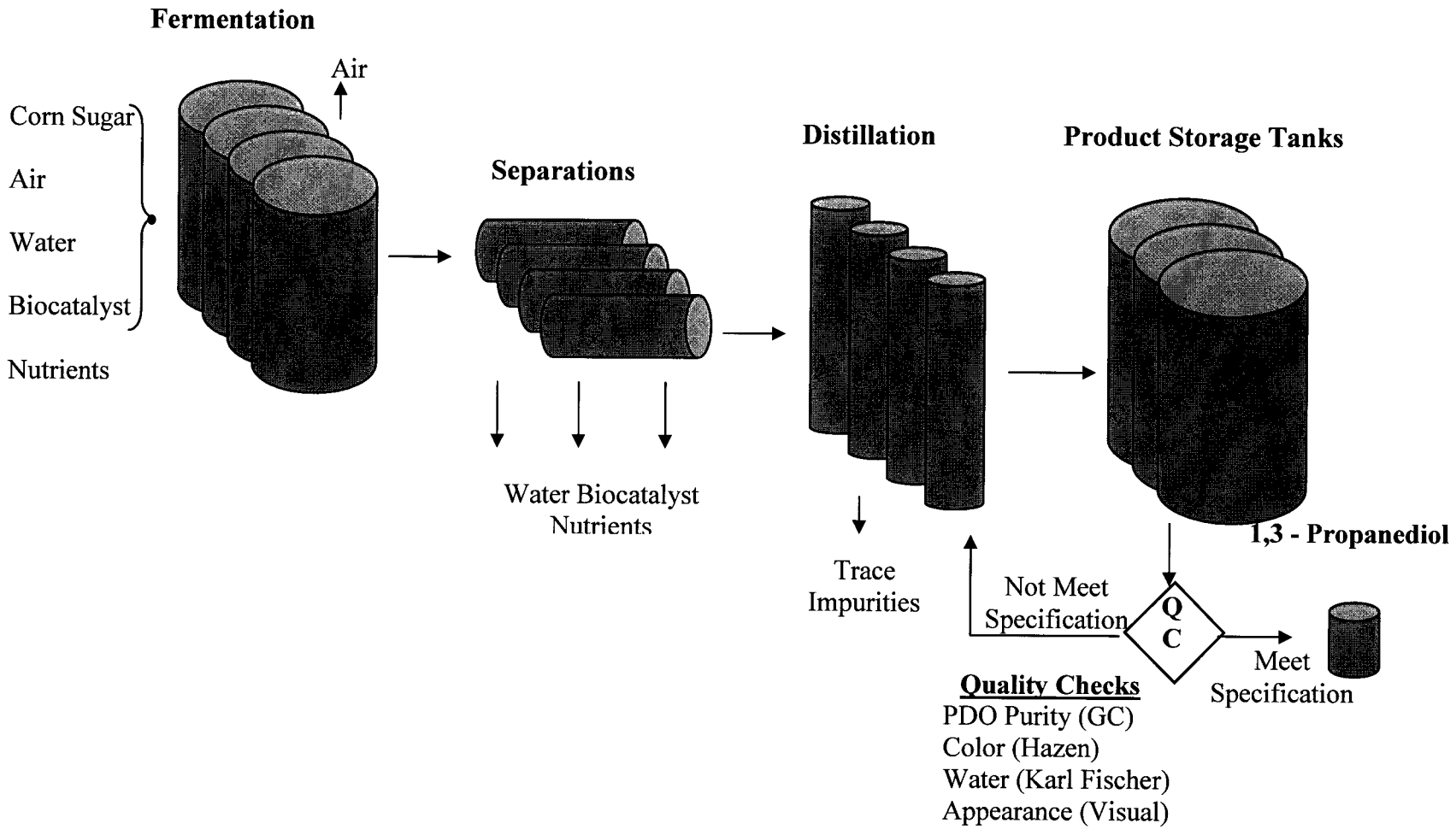
The production of 1,3-propanediol commences with fermentation of corn sugar by the genetically modified and non-pathogenic strain of *Escherichia coli* K-12. Standard media and aseptic lab practices are used to prepare the inoculums for the production process. The inoculums are aseptically transferred to pre-sterilized, production scale fermentors. Samples from production fermentors are routinely examined for contamination.

b Separation and Refining

Following fermentation, the fermentation organism is heat deactivated with a high-temperature water stream in a heat exchanger. The fermentation biomass is then separated out through a series of membrane separation steps. The 1,3-propanediol moves with fermentation broth from the separation area and is separated out by a 3-step process involving (i) ion exchange to remove charged impurities; (ii) evaporation to remove water; and (iii) 4 stages of distillation to remove impurities. No solvents are used in the separation process.

Distilled 1,3-propanediol undergoes quality control checks to determine purity, color, water, and appearance. 1,3-propanediol is returned for further distilliation until specifications are met, at which time it is stored and transported in vessels suitable for food storage. An overview of the manufacturing process for 1,3-propanediol is shown in Figure 2.

Figure 2: 1,3-Propanediol Process Flow Chart



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3. Chemical and Physical Characteristics of 1,3-propanediol

The chemical and physical characteristics of 1,3-propanediol are presented in Table 1.

Table 1: Chemical and Physical Characteristics of of 1,3-propanediol

Property	Value	Reference
Physical State	Liquid	
Melting Point	-26.7°C	Daubert, 1989
Boiling Point	214.4°C	Daubert, 1989
Density	1.055 g/cm^3 @ 20°C	Clayton, 1994
Vapor Pressure	5.88 x 10^{-2} hPa @ 25°C 100 hPa @ 149.9°C	Daubert, 1989; Lide, 2003
Water Solubility	Infinitely soluble	Riddick, 1986
Partition coefficient n-octanol/water (log value)	-1.332	Wilfried, 1992
Henry's Law Constant	4.416 x 10^{-9} atm-m^3 mole^{-1} @ 25°C	U.S. EPA, 2006

C. Specifications for Food-Grade Material

1,3-propanediol is manufactured in accordance with current Good Manufacturing Practices (cGMP; 21CFR110) and meets appropriate food-grade specifications. In order to ensure a consistent product, DuPont Tate & Lyle Bio Products has established numerous physical and chemical analyses for the final preparation. An analysis of 5 non-consecutive, representative lots of 1,3-propanediol (Table 2) demonstrates compliance with final product chemical and physical specifications. Certificates of Analysis are provided in the Appendix (Tab 3). Additional analysis is provided in the Appendix (Tab 4). Complete anlaysis revealed the presence of materials commonly found in the diet (*e.g.*, acetic acid, lactic acid, glycerol). Trace levels of other impurities were noted, primary among these 1,3-dipropylene glycol, alcohols, and pantolactone, generally at levels in the low parts per million. All of these impurities are anticipated to be without toxic effects at these levels. In-house data confirm the bacteriostatic/baceteriocidal nature of neat 1,3-propanediol, thus analysis for microorganisms was not conducted and is not part of the specifications and Certificate of Analysis. Although heavy metals are not anticipated to be present given the manufacturing process utilized, additional metal data was generated to confirm the absence of these metals in 1,3-propanediol (see Tab 4).

Table 2 Chemical and Physical Specifications plus Analyses of 1,3-propanediol

Lot Number	1,3-propanediol (%)	Hazen Color	Water (ppm)	Appearance
Accept/Reject Properties	Min: 99.80 Max: 100	Min: 0 Max: 10	Min: 0 Max: 1000	Pass
ZA80514ADZ	99.97	4.0	26.700	Pass
ZA80429ADR	99.97	3.2	74.200	Pass
ZA80426ADZ	99.97	3.2	74.200	Pass
ZA80223ADM	99.99	4.6	73.600	Pass
ZA80422ADZ	99.97	3.7	47.600	Pass

Further analysis of 5 non-consecutive, representative lots of 1,3-propanediol was performed to determine the level of various organic byproducts and metals. These data are presented in the Appendix (Tab 4).

4. Stability

Recommended storage conditions are 0-50 °C under a nitrogen blanket. With proper storage conditions, a 2-year shelf life is expected.

III. SELF-LIMITING LEVELS OF USE

The use of 1,3-propanediol will be based on the maximum use levels of 1,2-propanediol in specific food categories established by FDA. This ingredient is proposed for use in foods as a "1 for 1" replacement at levels not to exceed current Good Manufacturing Practice for 1,2-propanediol. Current Good Manufacturing Practice results in maximum levels, as served, of 5% for alcoholic beverages, 24% for confections and frostings, 2.5% for frozen dairy products, 97% seasonings and flavorings, 5% for nuts and nut products, and 2.0% for all other food categories except that DuPont Tate & Lyle BioProducts wishes to exclude from this notification the following categories: animal food (feed), infant formula, and ingredients used in infant formula. This exclusion is for business considerations only.

IV. BASIS FOR GRAS DETERMINATION

A. Documentation to Support the Safety of 1,3-propanediol

The determination that 1,3-propanediol is GRAS is on the basis of scientific procedures; the information supporting the general recognition of the safe use of 1,3-propanediol includes:

- published scientific data assessing the safety of 1,3-propanediol;
- supporting unpublished data demonstrating that 1,3-propanediol was neither mutagenic nor genotoxic; likewise, it does not exhibit teratogenic properties;
- the safety of the source organism;
- comparison of the pharmacokinetics and toxicity of1,3-propanediol to that of 1,2-propanediol, for which it is currently proposed as an alternative; and
- data pertaining to the identity, intended use, and estimated intake of 1,3-propanediol.

A summary of these data is presented herein. Moreover, these data were reviewed by a panel of experts, qualified by scientific training and experience to evaluate the safety of 1,3-propanediol as a component of food, who concluded that the proposed uses of 1,3-propanediol are safe and suitable and would be GRAS based on scientific procedures [see Appendix, Tab 1, entitled, "**EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF 1,3-PROPANEDIOL**".

B. Estimated Intake of 1,3-propanediol

As mentioned, 1,3-propanediol is intended for use in a variety of food products as an alternate to 1,2-propanediol. The proposed maximum food uses and use levels are the same as those currently considered GRAS for 1,2-propanediol, as specified in 21 CFR 184.1666 except that DuPont Tate & Lyle BioProducts wishes to exclude from this notification the following categories: animal food (feed), infant formula, and ingredients used in infant formula. Specifically, 1,3-propanediol is proposed for use in place of 1,2-propanediol as follows: as an anticaking agent and free-flow agent, antioxidant, dough strengthener, emulsifier, flavoring agent and adjuvant, formulation aid, heat transfer fluid-incidental food contact, humectant, pharmaceutical excipient, processing aid, solvent and vehicle, stabilizer and thickener, surface-active agent, or texturizer. This ingredient is proposed for use in foods as a "1 for 1" replacement at levels not to exceed current Good Manufacturing Practice for 1,2-propanediol. Current Good Manufacturing Practice results in maximum levels, as served, of 5% for alcoholic beverages, 24% for confections and frostings, 2.5% for frozen dairy products, 97% for seasonings and flavorings, 5% for nuts and nut products, and 2.0% for all other food categories except animal food (feed), infant formula, and ingredients used in infant formula. DuPont Tate &

Lyle BioProducts wishes to exclude these cateogires from this notification for business consierations only.
As part of The Center for the Evaluation of Risks to Human Reproduction (CERHR) of the National Toxicology Program (NTP)'s evaluation of 1,2-propanediol, it was estimated that adults (70 kg bw) are exposed to approximately 34 mg 1,2-propanediol/kg bw/day through food products (NTP, 2004). Data for per capita daily intake of propylene glycol in food products was estimated by the United Nations Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives by reviewing the annual volume of production.

Given that 1,3-propanediol is proposed for use in the majority of the applications and at those use levels that are currently considered GRAS for 1,2-propanediol, it can be assumed if total substitution of 1,3-propanediol for 1,2-propanediol occurs, that exposure to 1,3-propanediol through these food uses would be comparable. Thus, assuming a complete replacement of 1,2-propanediol with 1,3-propanediol, the maximum exposure to 1,3-propanediol would be approximately 34 mg/kg b.w./day. Given that DuPont Tate & Lyle BioProducts wishes to exclude from this notification animal food (feed), infant formula, and ingredients used in infant formula, and that complete replacement across all food products is not anticipated, actual exposure is expected to be less than this value.

C. Metabolic Fate of 1,3-propanediol

No traditional ADME studies were available in the scientific literature. A metabolic pathway for 1-3 propanediol is shown in Figure 3 (Pollitt *et al.,* 1987).

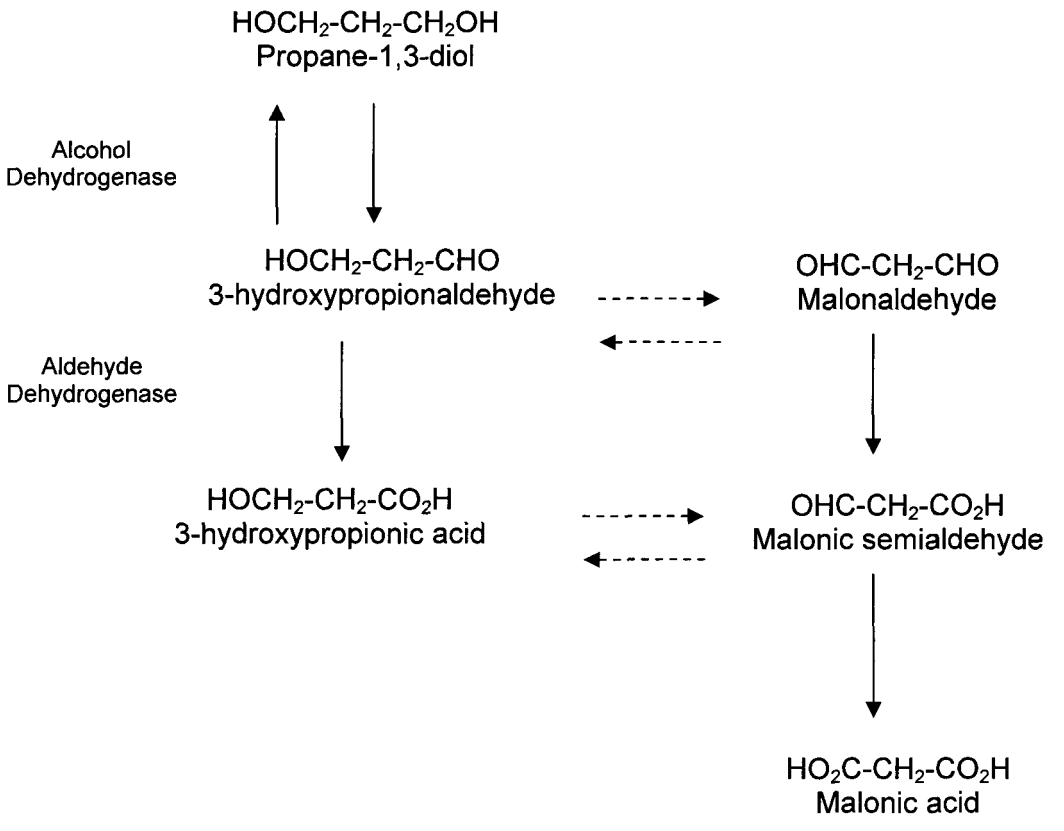

Figure 3: Metabolic Pathway of 1,3-Propanediol

As shown above, some studies indicate that malonaldehyde, a reactive cross-linking agent, can be formed as one of the products of 1,3-propanediol metabolism (Summerfield and Tappel, 1984). However, malonaldehyde would be expected to be a short-lived metabolic intermediate, with further metabolism to 3-hydroxypropionic acid and malonic acid (Gingell *et al.*, 2000).

There is limited available evidence pertaining to the carcinogenicity of malonaldehyde in experimental animals, and no relevant epidemiological data. As a result, malonaldehyde is considered not classifiable as to its carcinogenicity in humans (IARC, 1999). Malonaldehyde is not known to be a human carcinogen according to the 11th Report on Carcinogens (NTP, 2005).

There are no quantitative data demonstrating what percentage of 1,3-propanediol is metabolized to form malonaldehyde, or what portion of malonaldehyde that is formed would be further metabolized to non-toxic substances, however, the metabolism of 1,3-propanediol to malonaldehyde is not expected to represent an inordinate risk beyond that seen from consumption of malonaldehyde in meat, poultry, and fish products, or the endogenous formation of malonaldehdye *in vivo* during prostaglandin synthesis. Importantly, *in vitro* and *in vivo* genotoxicity assays conducted with 1,3-propanediol (Section D) demonstrate an absence of genotoxic activity. Likewise, no evidence of oxidative stress or DNA cross-linking (*e.g.,* hematopoiesis parameters and sperm production) or toxicity was observed in the 90-day study of 1,3-propanediol in rats at a dose level of up to 1000 mg/kg/day, the maximum dose tested. Such effects would have likely been apparent if malonaldehyde were generated in a significant amount since evidence of toxicity was reported in 90-day studies in rats with lower doses of malonaldehyde (125 mg/kg b.w./day, NTP, 1988).

D. Preclinical Studies Pertaining to the Safe Consumption of 1,3-propanediol

The safety of 1,3-propanediol under the proposed conditions of use is supported by a number of toxicological studies conducted in rats, specifically acute, subacute, subchronic, and developmental toxicity studies, as well as, mutagenicity studies conducted using *Salmonella typhimurium* (with or without metabolic activation), chromosomal aberration assays in Chinese hamster fibroblast cells, and an *in vivo* mouse micronucleus study. Summaries of these studies are presented in the following sections.

1 *Acute Toxicity Studies*

A single dose [0, 10.8, 13.0, 15.6, or 18.7 mL/kg body weight (bw)] of 1,3-propanediol was administered to male and female Wistar rats at doses by oral gavage. Within a few hours of treatment, signs of sluggishness, sedation, and ataxia were observed, however treatment groups were not specified. Unconsciousness was frequently observed; most deaths occurred between 8- and 23-hours post-dose. After 7 days of treatment, surviving rats recovered gradually and looked healthy at the end of the observation period. No treatment-related gross alterations were observed upon macroscopic examination. Based on these data, the oral LD_{50} of 1,3-propanediol was estimated to be approximately 14.9 mL/kg bw (Spanjers and Til, 1979). In a second unpublished rat study, the oral LD_{50} of 1,3-propanediol was estimated to be approximately 10 mL/kg bw, equivalent to 10.5 g/kg bw (Coombs and Clark, 1977).

2 *Subacute Toxicity Studies*

A 2-week oral toxicity study with 1,3-propanediol in rats (Crl:CD(SD)) was conducted. Groups of rats (5/sex/group) received 1,3-propanediol by gavage daily for 14 days at doses of 100, 250, 500, and 1000 mg/kg/day (10 mL/kg). A concurrent control group (5/sex) received the vehicle, deionized water. All animals survived to scheduled termination. No remarkable clinical signs were observed in any group. No treatment-related effects were observed in mean body weights, body weight gains, or food consumption values. There were no treatment-related differences in organ weights, findings at necropsy or microscopic findings of selected tissues (lungs, liver, kidneys, stomach, and testes). The no-observed-effect level (NOEL) of orally administered 1,3-propanediol was determined to be 1000 mg/kg/day (Mertens, 1997).

3 *Subchronic Toxicity Studies*

In a 90-day study, male and female Crl:CD(SD)BR rats were orally administered 1,3-propanediol or vehicle control at doses of 0, 100, 300, or 1,000 mg/kg. No compound-related effects were observed in clinical observations, food and water consumption, mortality, gross pathology and histopathology. Spermatogenic endpoints (mean testicular and epididymal sperm numbers, sperm production rate, and sperm motility and morphology) were unaffected. Under the conditions of the study, the NOEL for systemic toxicity of 1,3-propanediol administered orally *via* gavage to male and female rats for 90 consecutive days was 1000 mg/kg/day, the highest dose tested. Although the study was not designed to measure DNA cross-linking, if such cross-linking did occur, it did not cause any adverse functional effect on hematopoietic or spermatogenic systems, which because of the active DNA transcription and translation processes, might be expected to be susceptible. The authors also noted that there were no apparent pathological or toxicological effects on the liver, the organ in which the putative cross-linking agent malondialdehyde is most likely formed, as evidenced by the absence of histopathology and clinical chemistry changes (Gingell *et al.*, 2000).

4 *Developmental Toxicity Studies*

The reproductive and developmental toxicity of1,3-propanediol was assessed by Mitterer (1992). Twenty pregnant Sprague Dawley rats received 1,3-propanediol at 250 or 1000 mg/kg/day on gestation day (GD) 6 through 15. The control group (25 females) received 0.8% hydroxypropyl-methylcellulose under the same test conditions. On GD 20, the ovaries and uterus were removed and a macroscopic examination of the internal organs was conducted. All animals survived to scheduled termination. There were no treatment-related effects on clinical signs, food consumption, or water consumption at any dose level. Body weight gain was slightly but not significantly inhibited between GD 6 and 9 in the 1000 mg/kg group. There were no treatment-related observations recorded at necropsy for any of the groups. Gestation

parameters, fetal sex distribution, mean placental weights, and mean fetal weights were unaffected by treatment. The total incidence of fetal skeletal retardation was significantly higher in the 1000 mg/kg group compared to the control groups on an individual fetal basis, but was not different when compared on a per litter basis. The incidence of retarded/delayed ossification of the skull was significantly higher in the treatment groups compared to the control group. However, according to the authors, these differences were not considered to be treatment-related because the values for the control group were lower than the historical control data, and the increase among treated groups was within the range of historical control data. There were no skeletal or visceral malformations. No malformed or dead fetuses were seen. The authors concluded that the NOEL for maternal and fetal toxicity was greater than 1000 mg/kg bw, the highest dose tested, based on the lack of clear treatment-related effects on any parameter. Therefore, the authors concluded that 1,3-propanediol did not possess teratogenic properties.

5 *Mutagenicity/Genotoxicity Studies*

1,3-Propanediol was reported to produce negative results in the Ames assay using *Salmonella typhimurium* strains strains TA 1535, TA 1537, TA 98, TA 100, and TA 102 with or without metabolic activation (Wollny, 1994a). Gudi and Brown (2001) reported that 1,3-propanediol failed to produce chromosomal aberrations in Chinese hamster V79 cells with or without metabolic activation, while Volkner (1994) reported that 1,3-propanediol elicited positive clastogenic results without metabolic activation. In the Volkner study, the highest dose level (5000 ug/ml) could not be assessed due to cytotoxicity. The possibility that the recorded positive result at the next lower dose (2500 ug/ml) was due to less than optimal chromosome preparations could not be excluded. The Gudi and Brown study did not see cytotoxicity or clastogenicity at 2500 or 5000 ug/ml. Wollny (1994b) reported that 1,3-propanediol did not induce gene mutations at the hypoxanthine guanine phosphoribosyl transferase (HPRT) locus in V79 cells.

Importantly, no genotoxic effects were seen in an *in vivo* mouse micronucleus test. Male and female Hsd/Win: NMRI mice received the following orally: single dose with 2150 mg/kg bw of 1,3-propanediol (7/sex/group), physiological saline (negative control; 6/sex/group) or 31.6 mg/kg of cyclophosphamide (positive control; 6/sex/group). After 24 and 48 hours of treatment, animals were euthanized and femur bone marrow was collected for examination. Based upon the data of the initial assay, a single dose of 2150 mg/kg 1,3-propanediol was not considered to induce chromosome mutations. A second part of the study examined the reproducibility of the statistically significant positive response at the 48-hour sampling time and assessed a possible dose relation in the increase of micronucleated polychromatic erythrocytes. Male and female mice (6/sex/group) orally received the negative or positive control or a single dose of 1,3-

propanediol at 1000, 1470, or 2150 mg/kg. There were no signs of treatment-related toxicity with 1,3-propanediol. However, a statistically significant increase in micronucleated polychromatic erythrocytes (PCEs) with micronuclei was noted at 48 hours in the combined sexes (*vs.* negative control), which did not exclude a weak clastogenic effect. In the second assay, no statistically significant effects were observed at any dose. No dose-related increases in the number of micronucleated polychromatic erythrocytes were observed.

F. Other Data Pertaining to the Safety of 1,3-propanediol

1 Compariative Data on 1,2-Propanediol

As 1,3-propanediol is currently proposed as an alternative to 1,2-propanediol, the metabolism and safety of 1,2-propanediol is compared to 1,3-propanediol in Table 3.

Table 3: Comparison of 1,3-propanediol and 1,2-propanediol

1,3-propanediol	**1,2-propanediol**
Oral LD_{50} approximately 10 g/kg	Oral LD_{50} more than 8 g/kg
Metabolism to CO_2/H_2O	Metabolism to CO_2/H_2O
Clearance is rapid	Half-life approximately 2 to 4 hours
Non-mutagenic	Non-mutagenic
Not reprotoxin	Not reprotoxin
Oral rat NOEL is greater than 1000 mg/kg (13 weeks) (highest dose tested)	Oral rat NOEL is between 1700 to 2100 g/kg (104 weeks)

G. Summary and Basis for GRAS Conclusion

The safety of 1,3-propanediol has been established in the literature. The results of a 90-day subchronic toxicity study available in the published scientific literature demonstrates that 1,3-propanediol produced no systemic toxicity at a level of 1000 mg/kg/day, the highest dose tested. These data are supported by several unpublished studies of 1,3-propanediol. The results of other supporting preclinical toxicity studies demonstrate that 1,3-propanediol was not mutagenic or genotoxic. In a developmental toxicity study of 1,3-propanediol, the no-observed-effect-level (NOEL) for maternal and fetal toxicity was greater than 1000 mg/kg bw, the highest dose tested.

Based on the lack of clear treatment-related effects on any of the measured parameters, it was concluded that 1,3-propanediol did not possess teratogenic properties.

The organism utilized in the production of the of 1,3-propanediol is a genetically modified microorganism, derived from a non-pathogenic strain of *Escherichia coli* K-12. *E. coli* K-12 has a history of safe use, including the commercial production of insulin (NovoNordisk), phenylalanine (NutraSweet), and various industrial enzymes. The strain has exempt status under EPA-TSCA and is viewed to have low potential impact on human health, based on an EPA risk assessment (EPA, 1997). DuPont Tate & Lyle's *E. coli* K-12 strain has been genetically modified to produce 1,3-propanediol. The nonpathogenicty and non-toxic nature of the genetically modified organism has been confirmed by DuPont Tate & Lyle. Additional information is provided in the Appendix (Tab 2).

Assuming a complete replacement of 1,2-propanediol with 1,3-propanediol, the intake of 1,3-propanediol through the intended food uses of 1,3-propanediol is estimated to be approximately 34 mg/kg bw/day. This maximum-case intake provides nearly a 30-fold margin lower than the 1000 mg/kg bw/day NOEL dose reported in rats. Given the comparable toxicity profile for these ingredients, this level of intake is considered safe. Therefore, DuPont Tate & Lyle Bio Products has concluded that 1,3-propanediol is GRAS under the intended conditions of use on the basis of scientific procedures. This determination is further supported by an Expert Panel evaluation of the safety of 1,3-propanediol under the intended conditions of use.

REFERENCES

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APPENDIX

1,3-PROPANEDIOL GRAS NOTIFICATION

Submitted: May, 2009

Notifier: DuPont Tate & Lyle Bio Products
198 Blair Bend Road
Loudon, Tennessee
37774

EXPERT PANEL CONSENSUS STATEMENT REGARDING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF 1,3-PROPANEDIOL

(3 1)

DuPont Tate & Lyle Bio Products
August, 2009

EXPERT PANEL CONSENSUS STATEMENT CONCERNING THE GENERALLY RECOGNIZED AS SAFE (GRAS) STATUS OF 1,3-PROPANEDIOL

The undersigned, an independent Panel of recognized Experts (hereinafter referred to as the Expert Panel), qualified by scientific training and relevant international experience to evaluate the safety of food and food ingredients, was commissioned by DuPont Tate & Lyle Bio Products to determine the Generally Recognized as Safe (GRAS) status of 1,3-propanediol in various food use applications. 1,3-Propanediol is proposed for use as an alternate to 1,2-propanediol in the same applications and at the same use levels that are currently considered GRAS for 1,2-propanediol, as specified in 21 CFR 184.1666, 582.1666 and 582.4666. Specifically, 1,3-propanediol is proposed for use in place of 1,2-propanediol as follows: as an anticaking agent and free-flow agent, antioxidant, dough strengthener, emulsifier, flavoring agent and adjuvant, formulation aid, heat transfer fluid- incidental food contact, humectant, pharmaceutical excipient, processing aid, solvent and vehicle, stabilizer and thickener, surface-active agent, or texturizer, as an ingredient in animal feed. This ingredient is proposed for use in foods as a "1 for 1" replacement at levels not to exceed current good manufacturing practice for 1,2-propanediol. Current good manufacturing practice results in maximum levels, as served, of 5% for alcoholic beverages, 24% for confections and frostings, 2.5% for frozen dairy products, 97% seasonings and flavorings, 5% for nuts and nut products, and 2.0% for all other food categories.

A comprehensive search of the scientific literature for safety and toxicity information on 1,3-propanediol was conducted and made available to the Expert Panel. This GRAS determination summarized the product, technical and safety information necessary for evaluation by this Expert Panel to evaluate the safety of 1,3-propanediol in the proposed food uses at the intended use levels. The Expert Panel independently evaluated the materials submitted by DuPont Tate & Lyle Bio Products, and other materials deemed appropriate. Following their independent and critical evaluation, the Expert Panel members conferred by telephone and unanimously agreed to the decisions described herein. A summary basis for the discussion of GRAS status is provided below and is elaborated in the Expert Report.

- The results of 90-day subchronic toxicity study available in the published scientific literature demonstrate that 1,3-propanediol produced no systemic toxicity at a level of 1000 mg/kg/day, the highest dose tested. These data are supported by several unpublished studies of 1,3-propanediol;

- The results of other supporting preclinical toxicity studies demonstrate that 1,3-propanediol was not genotoxic;

- In a developmental toxicity study of 1,3-propanediol, the no-observed-effect-level (NOEL) for maternal and fetal toxicity was greater than 1000 mg/kg b.w. the highest dose tested. Based on the lack of clear treatment-related effects on any of the measured parameters, it was concluded that 1,3-propanediol did not possess teratogenic properties;

- Batch analysis data demonstrate that the manufacturing process for 1,3-propanediol results in a product that reproducibly meets specifications; and

- Assuming a complete replacement of 1,2-propanediol with 1,3-propanediol, the maximum exposure to 1,3-propanediol would be approximately 34 mg/kg b.w./day, or approximately 30-times less than the NOEL of 1000 mg/kg b.w./day observed in the 90-day rat study. This 30-fold safety margin provides adequate assurance of safety considering 1) the NOEL represents the highest dose tested, with the true NOEL somewhere greater than this value; and 2) complete replacement of 1,2-propanediol by 1,3-propanediol is a worst-case assumption and in fact would not occur, resulting in actual consumer exposures considerably below the 34 mg/kg b.w/day value.

As 1,3-propanediol is currently proposed as an alternative to 1,2-propanediol, the Expert Panel carefully assessed the pharmacokinetic data and compared their toxicity profile. According to the oral rat NOEL for 1,3-propanediol is greater than 1000 mg/kg (13 weeks) and that for 1,2-propanediol is between 1.7 to 2.1 g/kg (104 weeks). The LD_{50} for 1,3-propanediol is approximately 15 mL/kg and for 1,2-propanediol is more than 8 g/kg. Both 1,3-propanediol and 1,2-propanediol are non-mutagenic, not reprotoxic, and are metabolized to CO_2/H_2O. The clearance of 1,3-propanediol is rapid and the half-life of 1,2-propanediol is approximately 2 to 4 hours.

After a critical independent evaluation of the available information pertaining to safety of 1,3-propanediol, 1,3-propanediol can reasonably be anticipated to be safe in the intended applications. Its use would entail a reasonable certainty of no harm and may be considered GRAS by scientific procedures when used as a food ingredient under the conditions specified. 1,3-Propanediol is proposed for use as an alternate to 1,2-propanediol in the applications and at those use levels that are currently considered GRAS for 1,2-propanediol, as specified in 21 CFR 184.1666, 582.1666 and

582.4666. The undersigned members of the Expert Panel conferred and unanimously determined that the use of 1,3-propanediol, meeting food-grade specifications, and manufactured according to current Good Manufacturing Practice (cGMP; 21 CFR 110), is Generally Recognized as Safe (GRAS) for use as the following: as an anticaking agent and free-flow agent, antioxidant, dough strengthener, emulsifier, flavoring agent and adjuvant, formulation aid, heat transfer fluid- incidental food contact, humectant, pharmaceutical excipient, processing aid, solvent and vehicle, stabilizer and thickener, surface-active agent, or texturizer, as an ingredient in animal feed.

Donald H. Hughes, Ph.D. — 1/21/09 Date

Robert Nicolosi, Ph.D., C.N.S. — 1/15/09 Date
Director
Center for Health and Disease Research
University of Massachusetts Lowell, Lowell, MA

David Bechtel, Ph.D., DABT — 1/22/09 Date
Vice President & Senior Scientific Consultant
CANTOX U.S. Inc., Bridgewater, NJ

TOXICITY AND PATHOGENICITY DATA OF 1,3-PROPANEDIOL GENETICALLY MODIFIED ESCHERICHIA COL K12 STRAIN

(B 2)

Summary of Studies

Study	Test Design	Year	Result
Acute oral toxicity (rat)	Modified OPPTS 885.3050	2000	Not acutely toxic up to 5.6×10^9 CFU/ml
Acute oral pathogenicity (rat)	Modified OPPTS 885.3050	2000	Not pathogenic up to 5.6×10^9 CFU/ml
Skin irritation (rabbit)	Draize Test	2000	Mild skin irritant
Skin sensitization (guinea pig)	LLNA	2000	Not a skin sensitizer

Summary of Acute Oral Pathogenicity Study

Endpoint	Result
Body Weights and BW Gains	No statistically significant changes
Clinical Observations	No mortality/ no signs of toxicity
Gross Pathology	No compound related observations
Bacterial Clearance	Detected in feces on day 1 in 4/10 animals at 4.1×10^2 to 8.7×10^3 CFU/g; not detected at days 2, 3, and 4, and 3 weeks
Colonization/Infectivity Potential	Not detected in blood or tissues after 3 weeks

CERTIFICATES OF ANALYSES

(TAB 3)

DuPont Tate & Lyle BioProducts

CERTIFICATE OF ANALYSIS

SEND TO:

ATTN:

PRODUCT: Zemea#Propanediol 440lb drum

PO #:
ORDER #:
DELIVERY #:
DATE SHIPPED:

REPORT DATE: 07/25/2008
CARRIER ID: -

ANALYTICAL DATA
INSPECTION LOT 50000119827
LOT NUMBER ZA80514ADZ
MFG DATE 05/14/2008

1,3 Propanediol	%	99.97
Hazen Color		4.0
Water by Karl Fisher	PPM	26.700
Appearance, Suspende		PASS

REPORTED BY:

000031

DuPont Tate & Lyle Bioproducts, LLC
Michael J. Barder,Ph.D.
Quality Control Manager
198 Blair Bend Drive
Loudon, TN 37774
865 408-1964

David W. Wood
Product Director
198 Blair Bend Drive
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DuPont Tate & Lyle BioProducts

CERTIFICATE OF ANALYSIS

SEND TO:

ATTN:

PRODUCT: Zemea#Propanediol 440lb drum

PO #:
ORDER #:
DELIVERY #:
DATE SHIPPED:

REPORT DATE: 07/25/2008
CARRIER ID: -

ANALYTICAL DATA
INSPECTION LOT 50000115842
LOT NUMBER ZA80429ADR
MFG DATE 04/29/2008

1,3 Propanediol	%	99.97
Hazen Color		3.2
Water by Karl Fisher	PPM	74.200
Appearance, Suspende		PASS

REPORTED BY:

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DuPont Tate & Lyle BioProducts

CERTIFICATE OF ANALYSIS

SEND TO: ATTN:

PRODUCT: Zemea#Propanediol 440lb drum

PO #:
ORDER #:
DELIVERY #:
DATE SHIPPED:

REPORT DATE: 07/25/2008
CARRIER ID: -

ANALYTICAL DATA
INSPECTION LOT 50000115381
LOT NUMBER ZA80426ADZ
MFG DATE 04/26/2008

1,3 Propanediol	%	99.97
Hazen Color		3.2
Water by Karl Fisher	PPM	74.200
Appearance, Suspende		PASS

REPORTED BY:

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DuPont Tate & Lyle BioProducts

CERTIFICATE OF ANALYSIS

SEND TO: ATTN:

PRODUCT: Zemea#Propanediol 440lb drum

PO #:
ORDER #:
REPORT DATE: 07/25/2008 DELIVERY #:
CARRIER ID: - DATE SHIPPED:

ANALYTICAL DATA
INSPECTION LOT 50000100381
LOT NUMBER ZA80223ADM
MFG DATE 02/23/2008

1,3 Propanediol	%	99.99
Hazen Color		4.6
Water by Karl Fisher	PPM	73.600
Appearance, Suspende		PASS

REPORTED BY:

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DuPont Tate & Lyle BioProducts

CERTIFICATE OF ANALYSIS

SEND TO:

ATTN:

PRODUCT: Zemea#Propanediol 440lb drum

PO #:
ORDER #:
DELIVERY #:
DATE SHIPPED:

REPORT DATE: 07/25/2008
CARRIER ID: -

ANALYTICAL DATA
INSPECTION LOT 50000114480
LOT NUMBER ZA80422ADZ
MFG DATE 04/22/2008

1,3 Propanediol	%	99.97
Hazen Color		3.7
Water by Karl Fisher	PPM	47.600
Appearance, Suspende		PASS

REPORTED BY:

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ADDITIONAL ANALYSIS OF 1,3-PROPANEDIOL (TAB 4)

Item	Lot Number (values in ppm)				
	ZA70320BRA 3/20/2007	ZA70523BRA 5/23/2007	ZA70727ARA 7/28/2007	ZA70915BRA 9/15/2007	ZA71224CRA 12/24/2007
pH, Neat	7.52	7.12	7.92	7.33	6.31
Mono ROH & weak org acids	7	0	11	44	75
Acetic acid	0	0	11	4	0
EtOH	0	0	0	0	0
IPA	0	0	0	0	0
MeOH	3	0	0	0	9
Propanol	4	0	0	0	0
GlyCHO	0	0	0	35	44
Lactic acid	0	0	0	5	22
Glycerol	2.4	0	38	0	0
124 BTO	10	3	0	0	5
Poly ROH	34	3	38	0	5
1,2-dipropylene glycol	10	29	0	23	7
1,3-dipropylene glycol	0	0	0	0	0
Pantolactone	17	29	0	7	6
Ti	<1	<1	<1	<1	<1
V	<1	<1	<1	<1	<1
Cr	<1	<1	<1	<1	<1
Mn	<1	<1	<1	<1	<1
Co	<1	<1	<1	<1	<1
Ni	<1	<1	<1	<1	<1
Total N	2.72	2.31	3.65	2.05	2.22
Fe	<1	<1	<1	<1	<1
b color	0.03	0.08	0.13	0.08	0.03

Results demonstrate the high purity of 1,3-propanediol and compliance with specifications. As shown above, complete anlaysis revealed the presence of materials commonly found in the diet (*e.g.*, acetic acid, lactic acid, glycerol). Trace levels of other impurities were noted, primiary among these 1,3-dipropylene glycol, alcohols, and pantolactone, generally at levels in the low parts per million. All of these impurities are anticipated to be without toxic effects at these levels. In-house data confirm the bacteriostatic/baceteriocidal nature of 1,3-propanediol, thus analysis for microorganisms was not conducted. Although heavy metals are not anticipated to be present given the manufacturing process utilized, additional metal data was generated to confirm the absence of these metals for non-food use.

1,3-Propanediol (CAS RN 504-63-2) Test Plan
Extended High Production Volume Program

Chemical Name: 1,3-Propanediol

CAS Number: 504-63-2

Name of Consortium American Chemistry Council's 1,3-Propanediol Panel

Date: December 21, 2007

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1,3-Propanediol (CAS RN 504-63-2) Test Plan
Extended High Production Volume Program

Table 1 Test Plan

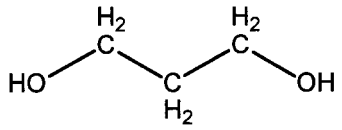
1,3-Propanediol CAS RN: 504-63-2		Information	OECD Study	GLP	Other Study	Estimation Method	Acceptable	SIDS Testing Required	Reference
	STUDY	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	**Y/N**	
PHYSICAL AND CHEMICAL DATA									
1.0	Melting Point	Y	N	N	Y	N	Y	N	6
2.0	Boiling Point	Y	N	N	Y	N	Y	N	6
3.0	Vapor Pressure	Y	N	N	Y	N	Y	N	6, 15
4.0	Partition Coefficient	Y	N	N	N	Y	Y	N	29
5.0	Water Solubility	Y	N	N	Y	N	Y	N	18
ENVIRONMENTAL FATE AND PATHWAY									
6.0	Photodegradation	Y	N	N	N	Y	Y	N	25
7.0	Stability in Water	Y	N	N	N	N	Y	N	--
8.0	Transport and Distribution	Y	N	N	N	Y	Y	N	26
9.0	Biodegradation	Y	Y	Y	N	N	Y	N	1
ECOTOXICITY									
10.0	Acute Toxicity to Fish	Y	Y	Y	Y	N	Y	N	3, 19
11.0	Toxicity to Algae	Y	Y	Y	N	N	Y	N	10
12.0	Acute Toxicity to Daphnia	Y	N	N	Y	N	Y	N	14
TOXICITY									
13.0	Acute Toxicity	Y	N	N	Y	N	Y	N	5, 11, 22
14.0	Genotoxicity *In Vitro or In Vivo* (Chromosome Aberration Test)	Y	Y	Y	N	N	Y	N	8, 13, 28, 31
15.1	Genotoxicity *In Vitro* (Bacterial Test)	Y	Y	Y	N	N	Y	N	30
15.2	Genotoxicity *In Vitro* (Mammalian Cells)	Y	Y	Y	N	N	Y	N	8, 28, 31
16.0	Repeated Dose Toxicity	Y	Y	Y	Y	N	Y	N	7, 12, 16, 20, 21
17.0	Reproductive Toxicity	Y	Y	Y	N	N	Y	N	7, 12
18.0	Developmental Toxicity / Teratogenicity	Y	Y	Y	N	N	Y	N	17

000039

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 504-63-2
IUPAC Name: 1,3-Propanediol
Molecular Formula: $C_3H_8O_2$
Structural Formula:



Molecular Weight: 76.10
Synonyms: Trimethylene glycol
1,3-Dihydroxypropane
1,3-Propanediol
1,3-Propylene glycol
1,3-Propylenediol
2-(Hydroxymethyl)ethanol
2-Deoxyglycerol
beta-Propylene glycol
omega-Propanediol

1.2 Purity/Impurities/Additives

1,3-Propanediol is sold as technical grade with a minimum purity of >99.5%.

1.3 Physico-Chemical properties

Table 2 Summary of physico-chemical properties

Property	Value	Reference
Physical state	Liquid	
Melting point	-26.7 °C	Daubert and Danner, 1989
Boiling point	214.4 °C	Daubert and Danner, 1989
Density	1.055 g/cm^3 @ 20°C	Clayton and Clayton, 1994
Vapour pressure	5.88 x 10^{-2} hPa @ 25°C 100 hPa @ 149.9°C	Daubert and Danner, 1989 Lide, 2003
Water solubility	Infinitely soluble	Riddick *et al.*, 1986
Partition coefficient n-octanol/water (log value)	-1.332	Wilfried, 1992
Henry's law constant	4.416 x 10^{-9} atm-m^3 mole^{-1} @ 25°C	U.S. EPA, 2006a

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

The estimated total volume of 1,3-propanediol production in North America in 2005 was 10,000-30,000 tons. 1,3-propanediol is a flammable, colorless, stable liquid. 1,3-Propanediol is produced commercially via either an ethylene oxide hydroformylation process or a biochemical fermentation route.

Between 75 and 90% of 1,3-propanediol is used in a polymerization process to manufacture Polytrimethylene terephthalate (PTT). 1,3-Propanediol may also be a component of deicing fluids, engine coolants, heat transfer fluids, chemical intermediates, personal care products, or process solvents.

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Because of its primary use in the production of polymers, environmental exposure to 1,3-propanediol occurs principally through accidental spills. Other non-polymer uses of 1,3-propanediol at this time are considered minimal.

2.2.2 Photodegradation

The indirect photodegradation of 1,3-propanediol by reaction with hydroxyl radicals in the atmosphere is estimated to occur with a half-life of approximately 1.099 days (12-hr day; 1.5E06 OH/cm^3,) (Reliability = 2, U.S. EPA, 2006b). The rate constant is 9.7 E-12 cm^3/(molecule•sec).

2.2.3 Stability in Water

1,3-Propanediol does not react with water; the only functionality other than carbon-carbon and carbon-hydrogen bonds is the hydroxyl groups which do not hydrolyze.

2.2.4 Transport between Environmental Compartments

The environmental distribution of 1,3-propanediol has been examined using the Level III fugacity model (Reliability = 2, U.S. EPA, 2006c). 1,3-Propanediol is used in several different processes. Therefore, potential release scenarios were examined as outlined in the following table:

Table 3 Environmental Distribution

Distribution	% Predicted in Air	% Predicted in Water	% Predicted in Soil	% Predicted in Sediment
Equal (Air, Water, Soil)	0.22	43.1	56.6	< 0.1
100% to Air	0.64	22.9	76.5	< 0.1
100% to Water	< 0.1	99.9	< 0.1	0.15
100% to Soil	< 0.1	16.1	83.9	< 0.1

2.2.5 Biodegradation

In a biodegradation assay according to OECD Guide-line 301 B "Ready Biodegradability: CO_2 Evolution Test", 1,3-propanediol was readily biodegradable meeting the 10-day window with 71% biodegradation after 28 days (Reliability = 1, Berti and Wolstenholme, 2007). 1,3-Propanediol was examined as one of a number of chemicals in a BOD5 (5-day test) and COD assay (Reliability = 2, Bridie *et al.*, 1979a). The BOD5 values from two tests were 11% (unacclimated inoculum) and 16% (acclimated inoculum) of the ThOD. The COD value from the first test was 98% of the ThOD. While providing additional evidence of biodegradation, this BOD5 study is not considered to define the extent of biodegradation of 1,3-propanediol. Therefore, based on the results of the more robust study conducted to modern guidelines, 1,3-propanediol is 'readily biodegradable'.

2.2.6 Bioaccumulation

No experimental data on bioaccumulation are available. With a measured log K_{ow} of -1.332, according to the Hansch and Leo Method (Reliability = 2, Wilfried, 1992; Hansch and Leo, 1995), a low bioaccumulation potential is expected.

2.3 Human Exposure

2.3.1 Occupational Exposure

There are no occupational exposure limits for 1,3-propanediol.

2.3.2 Consumer Exposure

The residual 1,3-propanediol in PTT polymer is very minimal, and consumer exposure is negligible. Other non-polymer uses of 1,3-propanediol at this time are considered minimal, with little possibility for exposures.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Acute Toxicity

Studies in Animals

Inhalation

To evaluate the "approximate lethal concentration (ALC)," six male rats were exposed nose-only for one, four-hour period to an aerosol of the 1,3-propanediol at a single concentration of 5.0 mg/L (1600 ppm; based on gravimetric analysis) (Kegelman, 1997; Reliability = 2). The mass median aerodynamic diameter for the aerosol was 3.2 µm. The animals were observed for clinical signs and body weights were measured over 14 days. All rats survived until scheduled sacrifice on day 14. Wet fur/perineum and ocular discharge were noted in rats immediately following the exposure (only clinical signs observed). One rat had transient body weight loss but all animals gained weight over the 14-day observation period. The ALC of 1,3-propanediol under the conditions of this study was > 5 mg/L (1600 ppm).

Oral

An acute oral toxicity study was performed by administering 1,3-propanediol via gavage to rats (5/sex) at doses of 9.0, 10.8, 13.0, 15.6 or 18.7 ml/kg body weight (Spanjers and Til, 1979; Reliability = 2). Sluggishness, ataxia, and sedation were observed in the rats within a few hours post-dose (the signs by treatment group were not reported). Most of the deaths (0, 10, 20, 50 and 100% for the 9.0, 10.8, 13.0, 15.6 or 18.7 ml/kg body weight doses, respectively) occurred between 8 and 23 hours post-dose although one female and one male died 4 and 7 days post dose, respectively. No individual animal observations were reported. After Day 7, the survivors recovered and looked healthy at study termination on Day 14. No treatment-related macroscopic findings were present at scheduled sacrifice. The LD_{50} of orally administered 1,3-propanediol was determined to be approximately 14.9 ml/kg bw which was equivalent to 15.8 g/kg bw.

A second acute oral toxicity test was conducted with 1,3-propanediol (Coombs and Clark, 1977; Reliability = 2). For the preliminary test, 1,3-propanediol was administered by oral gavage to rats (1/sex/group) at volumes of 0.63, 1.25, 2.5, 5.0 and 10.0 ml/kg body weight. All animals survived until Day 9. The definitive LD_{50} test was performed with 4 rats/sex administered 1,3-propanediol by gavage at 10.0 ml/kg bw. Piloerection was observed in some animals 24 hours post-dose. Two females died on Day 2. The remaining animals survived until Day 9, study termination. No individual animal observations were reported. The LD_{50} of orally administered 1,3-propanediol was determined to be approximately 10 ml/kg bw which was equivalent to 10.5 g/kg bw.

Dermal

The acute dermal LD_{50} of 1,3-propanediol was determined to be >4 ml/kg bodyweight, equivalent to >4200 mg/kg bw (Coombs and Clark, 1977; Reliability = 2). Undiluted 1,3-propanediol was applied at 1.0, 2.0 or 4.0 ml/kg bw to the shaved backs of Wistar rats

(2/sex/group) under occlusive conditions for 24 hours. After 24 hours, the bandage was removed, the test site washed and the animals observed over the next 9 days. All animals survived to scheduled sacrifice.

3.1.2 Irritation

Skin Irritation

Studies in Animals

The available studies (Reliability = 2) evaluating skin irritation of 1,3-propanediol are summarized in the following table:

Species	Exposure Conditions	Result	Reference
Rabbit	0.5 ml; undiluted; 24 hours; occluded; abraded and non-abraded skin	Mildly irritating*	Coombs and Clark, 1977
Rabbit	0.5 ml; undiluted; 24 hours; occluded; abraded and non-abraded skin	Slightly irritating**	van Beek, 1979

*An initial mild erythema and edema reaction was observed on the abraded and non-abraded skin of 7/8 rabbits; no irritation was observed by 3 days after the exposure period.
** All animals exhibited very slight to well defined erythema 24 hours after application which persisted in the majority of the animals at the 72-hour reading. Edema was not observed in any animals.

Eye Irritation

Studies in Animals

The available studies (Reliability = 2) evaluating eye irritation of 1,3-propanediol are summarized in the following table:

Species	Exposure Conditions	Result	Reference
Rabbit	0.2 ml; undiluted; single instillation; rinsed and not rinsed	Practically non-irritating*	Coombs and Clark, 1977
Rabbit	0.1 ml; undiluted; single instillation; not rinsed	Non-irritating**	van Beek, 1979

* A mild transient reddening and swelling of the conjunctivae was observed in three rabbits (two rinsed eyes and one eye not rinsed) initially and persisted in one rabbit at 24 hours.
**A mild transient conjunctival redness was observed in 4/6 rabbits at 24 hours. All signs of irritation cleared after 48 hours.

3.1.3 Sensitization

Studies in Animals

Skin

Species	Method	Exposure Conditions	Result	Reference
Guinea Pig	Magnusson and Kligman (Maximization)	Intradermal Induction: 2.5% Topical Induction: 100% Topical Challenge: 50%	Not a Sensitizer	Coombs and Clark, 1977 Reliability = 2
Guinea Pig	Landsteiner/ Draize	Intradermal Induction #1: 0.05 mL 25% Intradermal Inductions (#2-10): 0.1 ml 25% Intradermal Challenge: 0.05 ml 10%	Not a Sensitizer	Til and Keizer, 1979 Reliability = 2

3.1.4 Repeated Dose Toxicity

Studies in Animals

Inhalation

A 2-week inhalation toxicity study with 1,3-propanediol (Reliability = 1) was conducted with male rats (Scott, 1998; Scott *et al.*, 2005). In this study, groups of male rats (10/group) were exposed by nose-only inhalation to either vapor only or a vapor/aerosol mixture of 1,3-propanediol in air at target concentrations of 0, 60, 600 or 1800 mg/m^3 (mean aerosol concentrations were 0, 0, 610 and 1800 mg/m^3 and mean total concentrations were 0, 41, 650 and 1800 mg/m^3, respectively) for 9 exposures, 6 hours each over a 14-day period. A vehicle control group (10 male rats) was exposed to air under the same conditions. The day after the last exposure, five rats/group were euthanized and necropsied while the remaining animals were retained for a recovery period for 18 days then necropsied. Prior to necropsy of all test animals, urine was collected overnight and blood samples were collected the next day. All rats survived to scheduled termination and no treatment-related clinical signs of toxicity were observed during the study. No exposure-related changes in body weight, body weight gain, hematology, clinical chemistry or urinalysis were observed. No treatment-related changes were observed in findings at necropsy, organ weights, or at microscopic examinations. Under the conditions of this study, the NOEL for repeated inhalation exposure to 1,3-propanediol in male rats was 1800 mg/m^3.

Oral

A 2-week oral toxicity study with 1,3-propanediol (Reliability = 1) was conducted with male and female rats (Mertens, 1997). In this study, groups of rats (5/sex/group) were administered 1,3-propanediol by gavage daily for 14 days at concentrations of 100, 250, 500 and 1000 mg/kg/day. A concurrent control group (5/sex) was administered the vehicle, deionized water. The dose volume for all groups was 10 ml/kg. All animals survived to scheduled termination. No remarkable clinical signs were observed in any group. No treatment-related effects were observed in mean body weights, body weight gains or food consumption values of the treated groups as compared to the control group. There were no treatment-related differences in organ

weights, findings at necropsy or microscopic findings of selected tissues (lungs, liver, kidneys, stomach, testes). The NOEL of orally administered 1,3-propanediol under the conditions of this study was determined to be 1000 mg/kg/day.

A 90-day oral toxicity study with 1,3-propanediol (Reliability = 1) was conducted with male and female rats according to the EPA TSCA 798.2650 and OECD 408 guidelines (Kirkpatrick, 1999; Gingell *et al.*, 2000). In this study, rats (10/sex/group) were administered 1,3-propanediol by oral gavage daily for 91 or 92 days at concentrations of 100, 300 and 1000 mg/kg/day. A concurrent control group (10/sex) was administered the vehicle, deionized water. The dose volume for all groups was 10 ml/kg. All animals survived to scheduled termination. No treatment-related clinical signs, body weight or weight gain changes, or food consumption differences were observed in any group. There were no ocular changes or treatment-related macroscopic findings observed in any of the treated animals at scheduled necropsy. There were no treatment-related effects observed in mean organ weight data or at microscopic examination. The NOEL of orally administered 1,3-propanediol under the conditions of this study was determined to be 1000 mg/kg/day.

3.1.5 Mutagenicity

In vitro Studies

1,3-Propanediol was tested in the bacterial reverse mutation assay (OECD TG 471 and EEC B.14) with *Salmonella typhimurium* TA1535, TA1537, TA98, TA100, and TA102 at concentrations up to 5000 µg/plate with and without metabolic activation in a plate incorporation assay followed by a pre-incubation assay (Wollny, 1994a). The cytotoxic concentration was >5000 µg/plate. Positive controls produced the expected result. 1,3-Propanediol was not mutagenic under the conditions of the assay

A series of mammalian cell mutation assays with Reliability of 1 have been conducted with 1,3-propanediol. These are summarized in the following table:

CHO V79 – Chromosomal Aberration Assay	OECD Test Guideline 473; EEC B.10 and OPPTS 870.5550	625, 1250, 2500 and 5000 µg/mL	Negative with and without metabolic activation	Gudi and Brown, 2001
CHO V79 – Chromosomal Aberration Assay	OECD Test Guideline 473; EEC B.10	375, 1250, 2500, 3750 and 5000 µg/mL	Negative with metabolic activation; Positive without metabolic activation	Volkner, 1994
CHO V79 – HGPRT assay	OECD Test Guideline 476; EEC Directive 87/302	250, 1000, 2500 and 5000 µg/mL	Negative with and without metabolic activation	Wollny, 1994b

In vivo Studies

A mouse micronucleus study was performed with 1,3-propanediol (Reliability = 1) according to the OECD Directive 92/69/EEC, Method B.12 (Krauser, 1995). Male and female Hsd/Win: NMRI mice were orally dosed a single time with 2150 mg/kg of 1,3-propanediol (7/sex/group), physiological saline (negative control; 6/sex/group) or 31.6 mg/kg of cyclophosphamide (positive control; 6/sex/group). The volume administered was 10 ml/kg body weight. Animals were euthanized 24 and 48 hours after treatment and femur bone marrow was collected. In a repeat assay, performed to examine the reproducibility and possible dose-response of the initial assay results, male and female mice (6/sex/group) were orally dosed a single time with 1,3-propanediol (concentrations of 1000, 1470 or 2150 mg/kg), physiological saline (negative control) or 31.6 mg/kg of cyclophosphamide (positive control) and euthanized 24 and 48 hours after treatment. All animals, in both assays, survived to scheduled sacrifice and no treatment-related toxic symptoms were observed in the animals treated with 1,3-propanediol. Based upon the results of the initial assay, 1,3-propanediol was not considered to induce chromosome mutations in mice by damage to chromosomes or the mitotic apparatus after a 24- and 48-hour interval following a single oral dose at 2150 mg/kg. However, a statistically significant increase in micronucleated polychromatic erythrocytes (PCEs) with micronuclei at 48 hours in the sexes combined, as compared to the negative control, did not exclude a weak clastogenic effect. In the second assay, no statistically significant test substance-related increases in PCEs were observed in male or female mice, separately or combined, at any dose. No dose-related increase in the number of micronucleated polychromatic erythrocytes was present. The positive control animals exhibited a significant and clear increase in the number of micronucleated polychromatic erythrocytes as compared to the negative control animals and validated the study. Under the conditions of this study, 1,3-propanediol was considered to be non-mutagenic.

In conclusion, based on the overall weight of evidence, 1,3-propanediol is not mutagenic *in vivo*.

3.1.6 Toxicity for Reproduction

Studies in Animals

Effects on Fertility

There were no effects at any dose on reproductive organs or on spermatogenic endpoints (sperm count, sperm production rate, sperm morphology, or sperm motility) in the 90-day oral toxicity study of 1,3-propanediol in rats described above (Kirkpatrick, 1999; Gingell *et al.*, 2000).

Developmental Toxicity

A developmental toxicity study with pregnant rats orally administered 1,3-propanediol (Reliability = 1) was conducted according to OECD Test Guideline 414 (Mitterer, 1992). Female rats were dosed with 1,3-propanediol at 250 or 1000 mg/kg bw by oral gavage on gestation day (gd) 6 through 15. A concurrent vehicle control group (25 females), administered 0.8% hydroxypropyl-methylcellulose, was treated under the same test conditions. On gd 20, the ovaries and uterus were removed and a macroscopic examination of the internal organs was conducted. All animals survived to scheduled termination and no treatment-related effects were observed for clinical signs of toxicity, food consumption or water consumption at any dose. Body weight gain was slightly (-32%) but not significantly (at $p \leq 0.01$) inhibited between

gestation days 6 and 9 in the high-dosed dams (1000 mg/kg bw). There were no treatment-related observations recorded at necropsy for any of the dams. Gestation parameters were unaffected by treatment. There was no treatment-related effect on fetal sex distribution, mean placental weights and mean fetal weights or length. The total incidence of fetal skeletal retardation was statistically significantly increased in the 1000 mg/kg bw/day group as compared to the control when compared on individual fetal basis but was not different when compared on a per litter basis (the more appropriate comparison). The incidence of retarded/delayed ossification of the skull was significantly increased in the treatment groups as compared to the control. These differences were not considered to be treatment-related because the values for the control group were lower than the historical control data and the increase among treated groups was within the range of historical control data. There were no skeletal or visceral malformations. Under the conditions of this study, the NOEL for maternal and fetal toxicity was 1000 mg/kg bw based on the lack of clear treatment-related effects in any parameter. 1,3-Propanediol did not possess teratogenic properties.

3.2 Summary of Human Health Hazards

1,3-Propanediol has very low toxicity in acute and subchronic toxicity studies. In rats, the acute oral LD_{50} was 10.5-15.8 g/kg and the acute dermal LD_{50} was >4200 mg/kg. 1,3-Propanediol is slightly irritating to rabbit skin and non-irritating to the eye. 1,3-Propanediol was not a skin sensitizer in the guinea pig maximization test. There was no systemic toxicity following repeated dosing of rats via gavage or repeated exposure to aerosol with NOELs for subchronic exposure of 1000 mg/kg bw/day (90-day) and 1800 mg/m^3 (14-day), respectively.

1,3-Propanediol was not mutagenic to *Salmonella typhimurium* (bacterial reverse mutation assay) *in vitro,* with or without metabolic activation. Two studies in mammalian cells (chromosomal aberration) were negative with metabolic activation and a positive finding without metabolic activation was not repeated in a second study. An *in vivo* mouse micronucleus study was negative with and without metabolic activation. Overall, 1,3-propanediol does not have a mutagenic potential *in vivo* based on available data.

1,3-Propanediol had no effect on male or female reproductive organs following repeated oral dosing for 90 days. In addition, there were no effects on sperm evaluations conducted at the end of this 90-day study. In a gavage study exposing pregnant rats to 1,3-propanediol from gestation days 6 to 15 at doses as high as 1000 mg/kg bw/day, there was no clear treatment-related maternal toxicity, developmental or embryo toxicity or teratogenicity. Overall, based on the available animal studies, 1,3-propanediol is not toxic to reproduction or development and is not teratogenic.

There are no known effects of 1,3-propanediol exposure in humans.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

Acute toxicity to fish (Reliability = 1, Samel, 2000) yielded a 96-hour LC_{50} and NOEC for Fathead minnow (*Pimephales promelas*) of > 9720 mg/L (the highest concentration tested). The study followed OECD Test Guideline 203, EEC Method C.1 and EPA TSCA EG-9 using a static renewal design. Nominal exposure concentrations ranged from 750 to 10,000 mg/L. The mean measured concentrations were 720 to 9720 mg/L. No mortality or sublethal effects were seen in any of the test concentration or the dilution water control fish. 1,3-Propanediol was examined as one of a number of chemicals in acute toxicity studies to the Goldfish, *Carassius auratus* (Bridie *et al.*, 1979b; Reliability = 2). The study was terminated after 24 hours when no mortality was observed at the highest concentration tested. Therefore, the 24-hour LC_{50} value was >5000 mg/L.

Acute toxicity to *Daphnia magna* (Kuhn *et al.*, 1989; Reliability = 2) yielded a 48-hour EC_{50} of 7417 mg/L, EC_0 of 2603 mg/L and an EC_{100} of > 10,000 mg/L. Details of the test concentrations used were not provided (test solutions were selected to provide 3 or 4 effect concentrations between 0 and 100% immobility). The study followed Din 38412, Part II (Daphnia short-term test) using a static design.

Acute toxicity to the green algae, *Scenedesmus subspicatus* CHODAT, (Kamp, 1997; Reliability = 1) yielded a NOEC of ≤ 500 mg/L. The EbC_{10} and EbC_{50} values for biomass production were 700 and 1600 mg/L, respectively. The ErC_{10} and ErC_{50} values for growth rate production were 400 and >10,000 mg/L, respectively. The study followed OECD Test Guideline 201. Nominal exposure concentrations ranged from 0.5 to 10 g/L. Analytical recoveries of 1,3-propanediol in the test solutions ranged from 92.6% to 102%. Since the test substance remained stable within the 72-hr exposure period, the biological results were based on the nominal values of the test concentration.

4.2 Summary of Environmental Hazards

1,3-Propanediol is of low toxicity to aquatic organisms with LC_{50} and EC_{50} values ranging from 1600 to >9700 mg/L in fish, daphnia and algae.

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ATTACHMENT I--FINAL RISK ASSESSMENT OF ESCHERICHIA COLI K-12 DERIVATIVES

(February 1997)

I. INTRODUCTION

Escherichia coli is one of a number of microorganisms which are normal inhabitants of the colons of virtually all warm-blooded mammals. *E. coli* belongs to the taxonomic family known as *Enterobacteriaceae*, which is one of the best-defined groups of bacteria. The strain *E. coli* K-12 is a debilitated strain which does not normally colonize the human intestine. It has also been shown to survive poorly in the environment, has a history of safe commercial use, and is not known to have adverse effects on microorganisms or plants. Because of its wide use as a model organism in research in microbial genetics and physiology, and its use in industrial applications, *E. coli* K-12 is one of the most extensively studied microorganisms.

History of Commercial Use and Products Subject to TSCA Jurisdiction

E. coli K-12 has a history of safe use. Its derivatives are currently used in a large number of industrial applications, including the production of specialty chemicals (e.g., L-aspartic, inosinic, and adenylic acids) and human drugs such as insulin and somatostatin (Dynamac, 1990). Further, *E. coli* can produce a number of specialty chemicals such as enzymes which would be regulated under TSCA. An insulin-like hormone for use as a component of cell culture media, resulting from a fermentation application in which *E. coli* was used as the recipient, has already been reviewed under TSCA (Premanufacture Notice P87-693). EPA recently reviewed a submission (94-1558) for use of *E. coli* K-12 to produce indigo for use as a dye. In general, *E. coli* K-12 is one of the most extensively studied bacteria, and has been used in genetic studies in laboratories worldwide.

Experience with the use of *E. coli* is reflected in its classification under the National Institutes of Health Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines, U.S. Department of Health and Human Services, 1986). With the exception of strains which are known to be pathogenic, *E. coli* is considered a Class 1 Agent under the NIH Guidelines. Class 1 consists of all organisms which are not either human or animal pathogens. Most experiments involving *E. coli* K-12 have been exempted from the NIH Guidelines based on an analysis of safety,

except in certain circumstances (see Appendix C-II of the NIH Guidelines). Moreover NIH, under section III-D-4 of the NIH Guidelines, exempts transfers of genetic material between species that exchange DNA by known physiological processes with the genus *Escherichia*. Included in this exemption are exchanges between *Escherichia* and the closely related genera of *Shigella*, *Salmonella*, *Enterobacter*, *Citrobacter*, *Klebsiella*, *Erwinia*, *Pseudomonas aeruginosa*; also included are the species *Pseudomonas putida*, *Pseudomonas fluorescens*, *Serratia marcescens*, and *Yersinia enterocolitica*.

II. IDENTIFICATION AND TAXONOMY

A. Overview

E. coli belongs to the family *Enterobacteriaceae*. All *Enterobacteriaceae* are defined as Gram-negative, non-sporeforming rods that are facultative anaerobes. During the 1960's and 1970's, large amounts of information were generated regarding the phenotypic characteristics of the *Enterobacteriaceae*. The reasons for this increase in knowledge were two-fold. First, beginning in the early 1970's, a number of methods became available for the identification of enteric bacteria. These methods were based on biochemical or phenotypic reactions and could be performed with minimum labor and cost. Second, a major shift in nosocomial infections from Gram-positive to Gram-negative bacteria occurred in hospital patients during the 1960's and the early 1970's. Therefore, clinical microbiology laboratories, faced with the pressing need for accurate identification systems for enteric bacteria, carried out an extensive characterization of the members of this group of bacteria, including *E. coli*.

B. Taxonomy and Characterization

Escherichia coli is a member of the family *Enterobacteriaceae* and has been described by Brenner (1984). *Escherichia* is a Gram-negative rod which can be motile by peritrichous flagella or nonmotile. *Escherichia* is also a facultative anaerobe which has both a respiratory and a fermentative type of metabolism, and commonly occurs in the intestinal tract of humans and other animals.

E. coli K-12 was originally isolated from a convalescent diphtheria patient in 1922 (Bachmann, 1972). Because it lacks virulence characteristics, grows readily on common laboratory media, and has proven to be a valuable tool for microbial physiology and genetics research, it has become the standard bacteriological strain used in microbiological research and teaching. *E. coli* K-12 is now considered an enfeebled organism

as a result of being maintained in the laboratory environment for over 70 years (Williams-Smith, 1978).

E. coli can be readily differentiated from closely related bacteria by a number of standard tests. Classically, this has been accomplished by testing for production of indole from tryptophan, production of acid from glucose media using the dye methyl red as an indicator, lack of production of acetoin as a metabolic endproduct (also known as the Voges-Proskauer reaction), and the inability to utilize citrate as a sole source of carbon. Collectively, these reactions are known as the IMViC battery. The IMViC battery was developed for the analysis of water samples where it was important to differentiate *E. coli*, which was found to be always associated with fecal contamination of water, from other closely related bacteria which could be found naturally in water sources. Further refinements of the IMViC tests are used today and are available as commercial test kits.

C. Related Species of Concern

Taxonomically, the four species of the genus *Shigella* are closely related to *E. coli*. *Shigella* species cause diarrhea in humans and are classified as Class 2 agents under the NIH Guidelines. The *Shigella* species and *E. coli* share a high level of DNA sequence homology and many protein and polysaccharide capsular antigens. [These capsular antigens can be used to distinguish between *E. coli* strains and the pattern of capsular antigens determine the organism's "serotype" (Smith 1977).] The two genera can be distinguished based on the fact that *E. coli* has a unique colony morphology when grown on certain differential laboratory media (Jawetz et al., 1987). Commercially prepared kits for distinguishing between these organisms are available.

Most *E. coli* serotypes are benign and may even contribute to normal function and nutrition in the gastrointestinal tract. A few *E. coli* serotypes are pathogens.

E. coli K-12 strains in use today are from standard culture collections (Bachmann, 1972), such as the American Type Culture Collection and are not recent environmental isolates. As a result, these K-12 strains are well-characterized and should be expected to remain as pure cultures under standard microbiological practices. K-12 strains are distinguishable from both *Shigella* and other Escherichia (Cooke, 1974, Orskov 1978, Schmidt 1973).

III. HAZARD ASSESSMENT

The Proceedings of the Falmouth Workshop held in June 1977 served as a primary source for this assessment (Gorbach, 1978).

A. Human Health Hazards

The potential of K-12 strains to present risks to human health are examined in this assessment by analyzing K-12 in terms of (1) the phenotypic traits relevant to colonization of the colon, and (2) toxin production.

1. Colonization and Pathogenicity

E. coli is an inhabitant of the human colon, and it is thought that the primary means through which humans acquire their intestinal flora is through ingestion. Workers in fermentation facilities would not be expected to ingest *E. coli* under standard good practice, which prohibits the ingestion of food in work areas; however, some inhaled bacteria could be swallowed.

In order to evaluate K-12's potential to colonize the human intestine the following should be addressed: (1) the characteristics relevant to *E. coli* colonization of the human colon, and how K-12 compares to other *E. coli* in terms of these traits, and (2) data relevant to colonization potential of K-12 strains.

The binding of an *E. coli* to the mucosal surface of the colon requires two factors. The first factor is the production of a specific glycocalyx or fimbriae from the surface of the bacterium. This specific glycocalyx recognizes a specific lectin on the surface of the enterocyte lining of the human colon. The glycocalyxes appear to bind to structures such as the mucus glycoproteins elaborated from the goblet cells of the intestine. In Gram-negative bacteria, the polysaccharide chains arising from the core of the lipopolysaccharides in the outer membrane appear to be the major ones which affect binding to the colon.

E. coli K-12 is defective in at least three cell wall characteristics. The outer membrane has a defective lipopolysaccharide core which affects the attachment of the O-antigen polysaccharide side chains (Curtiss, 1978). Second, it does not have the type of glycocalyx required for attachment to the mucosal surface of the human colon (Edberg, 1991) as a result of the altered O-antigen properties noted above. Finally, K-12 strains do not appear to express capsular (K) antigens, which are heat-labile polysaccharides important for colonization and virulence (Curtiss, 1978).

K-12, thus, is not able to colonize the human intestinal tract under normal conditions, even after ingestion of billions of organisms (Anderson, 1975, Cohen et al., 1979., Levy and Marshall, 1981; Levy et. al, 1980, Smith, 1975). As noted above, K-12 is defective in cell wall components relevant to the ability

to recognize and adhere to the mucosal surface of colonic cells (Curtiss, 1978). The normal flora in residence in the colon thus can easily exclude K-12, and prevent it from colonizing the human colon.

A number of experiments have been conducted to measure the ability of K-12 to colonize in comparison to other *E. coli*. These experiments basically fall into two categories. First, those in which the normal human and/or animal flora was substantially reduced in order to provide the best opportunity for K-12 to colonize. The second category, known as co-colonization experiments, were to determine which strains could out-compete which other strains (Anderson, Gillespie and Richmond 1973; Anderson 1974; Burton et al. 1974; Anderson 1975; Smith 1975; Freter 1978; Laux, Cabelli and Cohen 1982; Myhal, Laux and Cohen 1982; Levine et al. 1983).

For the first category of experiments, normal flora were reduced through treatment with antibiotics or the experiments were performed using germ-free mice. In the second category, normal flora were reduced and two strains of *E. coli* were introduced to determine whether one strain could establish itself at the expense of the other.

These sets of experiments indicate that K-12 is a poor colonizer. Most strains of *E. coli*, including K-12, can colonize the intestine when the normal flora is substantially reduced. K-12 thus can colonize individuals whose normal intestinal flora is reduced through antibiotic therapy or is affected by other variables that can affect colonization such as anti-motility drugs. Similarly, *E. coli* K-12 can colonize germ-free mice, but is quickly displaced when the mice are fed other *E. coli* (Curtiss, 1978). In co-colonization experiments, *E. coli* K-12 has consistently been outcompeted by other *E. coli*. These studies indicate that K-12 is a poor colonizer and that indigenous intestinal microorganisms have a large competitive advantage over K-12 strains.

Aside from K-12's inherent decreased ability to colonize the gut, the culture conditions under which a K-12 strain is grown decrease the ability of the strain to colonize the gastrointestinal tract. Indeed, organisms grown under laboratory or fermentation conditions in general are not particularly competitive in comparison to microorganisms isolated from the environmental niche of the organism. Similarly, *E. coli*, which had been grown in continuous-flow cultures, were poor colonizers in mice with a normal cecal flora (Freter et al. 1983).

The medium in which *E. coli* are introduced to the GI tract is also important. Even in germ-free mice, the survival of K-12 strains is minimal unless the cultures of bacteria are introduced

in a basic buffer such as bicarbonate, since K-12 strains are killed by stomach acidity (Freter et al., 1983).

These two considerations further reduce the probability that K-12 strains grown under commercial fermentation conditions will be able to colonize the GI tract of workers. Such cultures would not be metabolically adapted to the human gut, and the microorganisms would not usually be grown in basically buffered fermentation media.

In order to cause disease, a microorganism must colonize a site of the human body and express virulence characteristics. On the basis of colonization alone, *E. coli* K-12 is innately defective as a pathogen, and a very low likelihood of acting as a pathogen of humans or animals.

The ability of certain *E. coli* serotypes to cause disease appears to be associated with certain specific capsular antigens. These serotypes of *E. coli* cause diarrhea, urinary tract infection, bacteremia and neonatal meningitis (Silverblatt and Weinstein 1979). It appears that colonization of the colon by the pathogenic serotype is a precondition for these illnesses (Moxon, Glode, Sutton 1977; Silverblatt and Weinstein 1979). The likelihood of these types of infections occurring with K-12 is thus very low for two reasons. First, since *E. coli* K-12 is a very poor colonizer it is unlikely to establish itself in the colon. Second, K-12 lacks adhesion and other virulence factors necessary for pathogenesis (Curtiss, 1978, Gorbach 1978, Edberg, 1991).

2. Toxin Production

A number of toxins affecting humans have been identified in *E. coli*: shiga-like toxins (SLT-I and II) and heat labile and heat stable toxins. The related genus *Shigella* produces shiga toxin (ST). These toxins are factors through which pathogenic serotypes cause diarrhea.

E. coli K-12 appears to lack the ability to produce significant quantities of toxins that affect humans (Edberg, 1991). There are two studies on production of toxins in K-12 strains. O'Brien and Holmes (1987) state that all strains of *E. coli* K-12 examined produced low levels of SLT as detected by monoclonal and polyclonal antibodies; however, DNA probes for SLT-I did not hybridize with DNA from these strains. These conflicting results are still unexplained. Edberg (1992) noted that strain K-12 was found to be in the low level toxin producing category ($2X10^2$ - $6X10^2$ CD50 per ml of sonic lysate). However, it is unclear whether this cell line-based assay is measuring an actual toxin or a protein that may exert activity against the cell.

No records of K-12 enterotoxin-induced disease for fermentation workers were located in the literature. This is not surprising since the K-12 would have to colonize and invade the tissue of the GI tract of workers in order to deliver the toxin to its site of action.

3. Conclusions

E. coli K-12 is not considered a human or animal pathogen nor is it toxicogenic. Any concerns for *E. coli* K-12 in terms of health considerations are mitigated by its poor ability to colonize the colon and establish infections.

B. Environmental Hazards

1. Hazards to Animals

A number of experiments with *E. coli* K-12 have been conducted in animal models (Burton et al. 1974; Freter *et al.*, 1983; Cohen and Laux, 1985; Laux *et al.* 1982; Myhal *et al.*).

These experiments demonstrate that *E. coli* K-12 will not under normal conditions colonize the GI tract of animals. A number of researchers have tried in vain to implant K-12 in the GI tract of laboratory animals under normal conditions (Freter 1978, Curtiss 1978). Negative results have been noted in mice, pigs, chickens, pigs and calves. K-12 is also unlikely to behave as a pathogen
(Gorbach 1978).

2. Hazards to Plants

There are no data that suggest that *E. coli* K-12 strains have adverse effects on plants. The genus *Escherichia* is not considered a plant pest species by the U.S. Department of Agriculture (7 CFR 330, et seq.).

3. Hazards Posed to Other Microorganisms

No evidence exists relative to hazardous effects of *E. coli* K-12 strains on other microorganisms in the environment (Sayre, 1991).

4. Conclusions

E. coli K-12 strains are very unlikely to pose a hazard to either animals, plants, or other microorganisms.

IV. EXPOSURE ASSESSMENT

A. Worker Exposure

E. coli K-12 is considered an exempt host system under the NIH Guidelines. This microorganism also falls under the Class 1 Containment under the European Federation of Biotechnology guidelines (Frommer et al., 1989).

No data were available for assessing the release and survival specifically for fermentation facilities using *E. coli*. Therefore, the potential worker exposures and routine releases to the environment from large-scale, conventional fermentation processes were estimated on information available from eight premanufacture notices submitted to EPA under TSCA Section 5 and from published information collected from non-engineered microorganisms (Reilly, 1991). These values are based on reasonable worst-case scenarios and typical ranges or values are given for comparison.

During fermentation processes, worker exposure is possible during laboratory pipetting, inoculation, sampling, harvesting, extraction, processing and decontamination procedures. A typical site employs less than 10 workers/shift and operates 24 hours/day throughout the year. NIOSH has conducted walk-through surveys of several fermentation facilities in the enzyme industry and monitored for microbial air contamination. These particular facilities were not using recombinant microorganisms, but the processes were considered typical of fermentation process technology. Area samples were taken in locations where the potential for worker exposure was considered to be potentially greatest, i.e., near the fermentor, the seed fermentor, sampling ports, and separation processes (either filter press or rotary drum filter). The workers with the highest potential average exposures at the three facilities visited were those involved in air sampling. Area samples near the sampling port revealed average airborne concentrations ranging from 350 to 648 cfu/m^3. Typically, the Chemical Engineering Branch would not use area monitoring data to estimate occupational exposure levels since the correlation between area concentrations and worker exposure is highly uncertain. Personal sampling data are not available at the present time. Thus, area sampling data have been the only means of assessing exposures for previous PMN biotechnology submissions. Assuming that 20 samples per day are drawn and that each sample takes up to 5 minutes to collect, the duration of exposure for a single worker will be about 1.5 hours/day. Assuming that the concentration of microorganisms in the worker's breathing zone is equivalent to the levels found in the area

sampling, the worst-case daily inhalation exposure is estimated to range up to 650 to 1200 cfu/day. The uncertainty associated with this estimated exposure value is not known (Reilly, 1991).

B. Environmental and General Exposure

1. Fate of the Organism

The natural habitat of *E. coli* is the large bowel of mammals. However, *E. coli* K-12 has lost the ability to colonize the gut and cannot survive in the bowel for very long. The ability of *E. coli* to survive under environmental conditions is also limited. In one study, *E. coli* HB101 introduced into non-sterile soil in saline declined to levels below detection (1-20 cells/g soil) after 21 days. *E. coli* also declines rapidly in seawater. There are of course many factors that determine the survival of these organisms in the environment and it is unlikely that all the introduced *E. coli* would die. However, it is reasonable to assume that over the long term, populations of *E. coli* K-12 in soil would be very low. *E. coli* released to air would not be expected to survive well because of the low nutrient levels and drying conditions (LaVeck, 1991).

2. Releases

Estimates of the number of *E. coli* organisms released during production are tabulated in Table 1 (Reilly, 1991). The uncontrolled/untreated scenario assumes no control features for the fermentor off-gases, and no inactivation of the fermentation broth for the liquid and solid waste releases. The containment criteria required for the full exemption scenario assume the use of features or equipment that minimize the number of viable cells in the fermentor off-gases. They also assume inactivation procedures resulting in a validated 6-log reduction of the number of viable microorganisms in the liquid and solid wastes relative to the maximum cell density of the fermentation broth.

TABLE 1. Estimated Number of Viable *E. coli* Organisms Released During Production

Release Media	Uncontrolled/ Untreated (cfu/day)	Full Exemption (cfu/day)	Release (days/yr)
Air Vents	$2x10^{8}$ - $1x10^{11}$	$<2x10^{8}$ - $1x10^{11}$	350
Rotary Drum Filter	250	250	350
Surface Water	$7x10^{16}$	$7x10^{10}$	90
Soil/Landfill	$7x10^{18}$	$7x10^{12}$	90

Source: Reilly, 1991

These are "worst-case" estimates which assume that the maximum cell density in the fermentation broth for bacteria is 10^{11} cfu/ml, with a fermentor size of 70,000 liters, and the separation efficiency for the rotary drum filter is 99 percent.

3. Air

Specific data which indicate the survivability of *E. coli* in the atmosphere after release are currently unavailable. Survival of vegetative cells during aerosolization is typically limited due to stresses such as shear forces, desiccation, temperature, and UV light exposure. Survival of *E. coli* K-12 after release is expected to be poor because its normal habitat is the human intestinal tract. However, as with naturally-occurring strains, human exposure may occur via inhalation as the organisms are dispersed in the atmosphere attached to dust particles, or lofted through mechanical or air disturbance.

Air releases from fermentor off-gas could potentially result in nonoccupational inhalation exposures due to point source releases. To estimate exposures from this source, the sector averaging form of the Gaussian algorithm described in Turner (1970) was used. For purposes of this assessment, a release height of 3 meters and downward contact at a distance of 100 meters were assumed. Assuming that there is no removal of organisms by controls/equipment for off-gases, potential human inhalation dose rates are estimated to range from 3.0×10^3 to 1.5×10^6 cfu/year for the uncontrolled/untreated scenario and less than that for systems with full exemptions. It should be noted that these estimates represent hypothetical exposures under reasonable worst case conditions (Versar, 1992).

4. Water

The concentrations of *E. coli* in surface water were estimated using stream flow values for water bodies receiving process wastewater discharges from facilities within SIC Code 283 (drugs, medicinal chemicals, and pharmaceuticals). The surface water release data (cfu/day) tabulated in Table 1 were divided by the stream flow values to yield a surface water concentration of the organism (cfu/l). The stream flow values for SIC Code 283 were based on discharger location data retrieved from the Industrial Facilities Dischargers (IFD) database on December 5, 1991, and surface water flow data retrieved from the RXGAGE database. Flow values were obtained for water bodies receiving wastewater discharges from 154 indirect (facilities that send their waste to a POTW) and direct dischargers facilities that have a NPDES permit to discharge to surface water). Tenth

percentile values indicate flows for smaller rivers within this distribution of 154 receiving water flows and 50th percentile values indicate flows for more average rivers. The flow value expressed as 7Q10 is the lowest flow observed over seven consecutive days during a 10-year period. The use of this methodology to estimate concentrations of *E. coli* in surface water assumes that all of the discharged organisms survive wastewater treatment and that growth is not enhanced by any component of the treatment process. Estimated concentrations of *E. coli* in surface water for the uncontrolled/untreated and the full exemption scenarios are tabulated in Table 2 (Versar, 1992).

TABLE 2. *E. coli* Concentrations in Surface Water

Flow	Receiving Stream Flow (MLD*)		Organisms (cfu/l)	
	Mean	7Q10	Mean	7Q10
Uncontrolled/Untreated				
10th Percentile	156	5.60	4.5×10^{8}	1.25×10^{10}
50th Percentile	768	68.13	9.11×10^{7}	1.03×10^{9}
Full Exemption				
10th Percentile	156	5.60	4.5×10^{2}	1.25×10^{4}
50th Percentile	768	68.13	9.11×10^{1}	1.03×10^{3}

*MLD = million liters per day
Source: Versar, 1992

5. Soil

Since *E. coli* is not a normal inhabitant of the soil, its survival would not be expected under these conditions. According to LaVeck (1991), *E. coli* HB101 introduced into non-sterile soil in saline declined to levels below detection (1-20 cells/g soil) after 21 days. Based on this study, and other studies cited in the section on environmental hazards and LaVeck (1991), survival of various strains of *E. coli* could range from 0 - 10^4 cells/gram of soil.

6. Summary

Although direct monitoring data are unavailable, worst case estimates do not suggest high levels of exposure of *E. coli* K-12 to either workers or the public resulting from normal fermentation operations.

V. INTEGRATION OF RISK

A. Discussion

E. coli K-12 is a well-studied bacterium which has been the subject of extensive research in microbial physiology and genetics and commercially exploited for a variety of industrial uses. The natural habitat of the parent species, *E. coli,* is the large bowel of mammals. *E. coli* K-12 has a history of safe use. Its derivatives are currently used in a large number of industrial applications, including the production of specialty chemicals (e.g., L-aspartic, inosinic, and adenylic acids) and human drugs such as insulin and somatostatin. An insulin-like hormone for use as a component of cell culture media, resulting from a fermentation application in which *E. coli* was used as the recipient, has already been reviewed under TSCA (Premanufacture Notice P87-693).

E. coli K-12 was originally isolated from a convalescent diphtheria patient in 1922 (Bachmann, 1972). Because it lacks virulence characteristics, grows readily on common laboratory media, and has proven to be a valuable tool for microbial physiology and genetics research, it has become the standard bacteriological strain used in microbiological research and teaching. *E. coli* K-12 is now considered an enfeebled organism as a result of being maintained in the laboratory environment for over 70 years (Williams-Smith, 1978). As a result, K-12 strains are unable to colonize the intestines of humans and other animals under normal conditions.

E. coli K-12 strains are not likely to pose a risk to human or animal health, to plants, or to other microorganisms. *E. coli* K-12 has been utilized for 70 years, often in industrial settings with high volumes and cell densities. Moreover, *E. coli* K-12 has been employed extensively in research laboratories. In the industrial setting with the use of appropriate industrial practices for handling microorganisms, and with good laboratory practices in the research setting, the potential for K-12 strains to colonize the human colon is quite low.

Likewise, the ecological risks associated with the use of *E. coli* K-12 are low. Industrial fermentation uses of this organism are expected to result in a low number of microorganisms released from the fermentation facility. Given its natural habitat of the large bowel of mammals, *E. coli* will not likely survive for long periods in soil, water, or air. *E. coli* K-12 has lost the ability to colonize the gut and has been shown to have poorer survival characteristics in soil and water than other *E. coli*. The ability of *E. coli* to survive under environmental conditions

is thus very limited. *E. coli* K-12 has no known survival mechanisms in the environment, such as the ability to produce spores.

In conclusion, the use of *E. coli* K-12 under contained conditions in fermentation facilities present low risk.

B. Recommendations

E. coli K-12 is recommended for the tiered exemption.

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TRADE SECRET

FINAL REPORT

Study Title

H-24783: *In Vitro* Mammalian Chromosome Aberration Test

Testing Guideline
US EPA Health Effects Test Guidelines, OPPTS 870.5550 (1998)
Commission Directive 87/302/EEC, EEC Part B
OECD Guideline for the Testing of Chemicals, Guideline 473

Authors

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Report Completion Date
June 13, 2001

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Performing Laboratory Study Number
AA41BC.391.BTL

DuPont Project ID
DuPont-5683

Work Request Number
13615

Service Code
531

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards except for the item documented below. The item listed does not impact the validity of the study.

The test substance was characterized by the Sponsor prior to the initiation of this study. Although characterization was not performed under Good Laboratory Practice Standards, the accuracy of the data is considered sufficient for the purposes of this study.

The identity, strength, purity and composition or other characteristics to define the control substances have not been determined by the testing facility. The control articles have been characterized as per the Certificates of Analysis on file with the testing facility.

The stability of the control substances has not been determined by the testing facility.

Analyses to determine the uniformity (as applicable), or concentration of the test or control mixtures were not performed by the testing facility. The Sponsor has indicated that they have not performed these analyses.

The stability of the test or control substances in the test and control mixtures, respectively, has not been determined by the testing facility. The Sponsor has indicated that they have not performed these analyses.

Applicant/Sponsor: E. I. du Pont de Nemours and Company
Wilmington, DE 19898 U.S.A.

BioReliance Study Director: _____ 13 June 2001
Ramadevi Gudi, Ph.D. Date

Applicant/Sponsor: _____ _____
DuPont Representative Date

Quality Assurance Statement

Study Title: H-24783: In Vitro Mammalian Chromosome Aberration Test

Study Number: AA41BC.391.BTL

Study Director: Ramadevi Gudi, Ph.D.

This study has been divided into a series of in-process phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment records, etc., are examined in order to assure that the study is performed in accordance with the U.S. EPA Good Laboratory Practice Regulations (40 CFR 792 and 40 CFR 160), and the OECD Principles of Good Laboratory Practice and to assure that the study is conducted according to the protocol and relevant Standard Operating Procedures.

The following are the inspection dates, phases inspected, and report dates of QA inspections of this study.

Inspect On	23-Mar-01 - 23-Mar-01 To Study Dir 23-Mar-01 To Mgmt 23-Mar-01
Phase	Protocol Review
Inspect On	09-Apr-01 - 09-Apr-01 To Study Dir 09-Apr-01 To Mgmt 13-Apr-01
Phase	Refeeding the test system
Inspect On	10-May-01 - 11-May-01 To Study Dir 11-May-01 To Mgmt 21-May-01
Phase	Draft Report
Inspect On	12-Jun-01 - 12-Jun-01 To Study Dir 12-Jun-01 To Mgmt 13-Jun-01
Phase	Draft to Final Report

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

[redacted] 13 Jun 2001

Jennifer Klopsis DATE
QUALITY ASSURANCE

CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Issued by Study Director: _____ 13 June 2001
Ramadevi Gudi
BioReliance Study Director
Date

Approved by Study Monitor: _____ 12 JUN 2001
Maria Donner, Ph.D.
Senior Research Scientist
Date

000078

STUDY INFORMATION

9th Collective Nomenclature: 1,3-Propanediol

Synonyms/Codes:
- 1,3-Dihydroxypropane
- 1,3-Propylene glycol
- 1,3-Propylenediol
- 2-Deoxyglycerol
- H-24783

Haskell Number: 24783

CAS Registry Number: 504-63-2

Composition: 1,3-Propanediol

Purity: 99.9%

Known Impurities: Not supplied by the sponsor

Physical Characteristics: Colorless liquid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Sponsor: E. I. du Pont de Nemours and Company
Wilmington, Delaware 19898
U.S.A.

Study Initiated/Completed: March 22, 2001 / (see report cover page)

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SUMMARY

The test substance, H-24783, was tested in the chromosome aberration assay using Chinese hamster V79 cells in both the absence and presence of an Aroclor-induced S9 activation system in order to evaluate the clastogenic potential of the test substance. The assay was conducted following the US EPA Health Effects Test Guidelines (OPPTS 870.5550, 1998), the Commission Directive 87/302/EEC, EEC Part B and the OECD Guideline for the Testing of Chemicals, Guideline 473 (1998).

Water was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The test substance was soluble in water at a concentration of 50 mg/mL, the maximum concentration tested.

In the chromosome aberration assay, the maximum dose tested was 5000 µg/mL. The cells were treated for 4 and 20 hours in the non-activated test system and for 4 hours in the S9 activated test system, and all cells were harvested at 20 hours after treatment initiation. The test substance was soluble in treatment medium at all dose levels in all treatment conditions.

Selection of dose levels for the cytogenetics test was based upon post-treatment cell growth inhibition relative to the solvent control and solubility of the test substance.

Treatment Time (hours)	Recovery Time (hours)	Harvest Time (hours)	S9	Cell growth inhibition at highest dose scored (µg/mL)	Mitotic Index Reduction*	LED (lowest effective dose) for Structural Aberrations	LED for Numerical Aberrations
4	16	20	-	None at 5000	15%	None	None
20	0	20	-	9% at 5000	9%	None	None
4	16	20	+	None at 5000	29%	None	None

* relative to solvent control at high dose evaluated for chromosome aberrations

The positive and solvent controls fulfilled the requirements for a valid test. Based on the findings of this study, H-24783 was concluded to be negative for the induction of structural and numerical chromosome aberrations in Chinese hamster V79 cells in the presence and absence of Aroclor-induced rat liver S9.

PURPOSE

The purpose of this study was to evaluate the clastogenic potential of a test substance based upon its ability to induce chromosome aberrations in Chinese hamster V79 cells, according to the guidelines set forth by the OECD Guideline for the Testing of Chemicals, Guideline 473 (1998), the US EPA Health Effects Test Guidelines, OPPTS 870.5550 (1998) and the Commission Directive 87/302/EEC, EEC Part B.

The test substance, H-24783, was received by BioReliance on 8 February 2001 and was assigned the code number AA41BC. The test substance was characterized by the Sponsor as a colorless liquid that should be stored at ambient temperature with an expiration date of 22 December 2003, provided by the Sponsor. Upon receipt, the test substance was described as a clear colorless liquid and was stored at room temperature, protected from exposure to light.

The solvent used to deliver H-24783 to the test system was sterile water (CAS 7732-18-5), from Life Technologies Company. Mitomycin C (MMC; CAS No.: 50-07-7), was obtained from Sigma Chemical Company, and was dissolved and diluted in sterile distilled water to stock concentrations of 1 and 2 µg/mL for use as the positive control in the non-activated test system. Cyclophosphamide (CP; CAS No.: 6055-19-2), was obtained from Sigma Chemical Company, and was dissolved and diluted in sterile distilled water to stock concentrations of 100 and 200 µg/mL for use as the positive control in the S9 activated test system. For each positive control one dose with sufficient scorable metaphase cells was selected for analysis. The solvent for the test substance was used as the solvent control at the same concentration as that found in the test substance-treated groups.

Test System

Chinese hamster V79 cells were obtained from Dr. George Krautter (cell freeze date: 29 November 1999), Macon, GA. In order to assure the karyotypic stability of the cell line, working cell stocks were not used beyond passage 20. The freeze lot of cells was tested using the Hoechst staining procedure and found to be free of mycoplasma contamination. The Chinese hamster V79 cells were grown in 9% Dulbecco's modified eagles medium (DMEM, Gibco BRL # 41965-039) containing sodium pyruvate, glucose and pyridoxine HCl. To 500 mL of culture medium 50 mL of heat - inactivated fetal calf serum, 2.6 mL penicillin/streptomycin (10,000 IU/mL 10,000 µg/mL, Gibco BRL # 35400-027) were added.

Metabolic Activation System

Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice. The S9 was batch prepared and stored at

≤-70°C until used. Each bulk preparation of S9 was assayed for sterility and its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(α)anthracene to forms mutagenic to *Salmonella typhimurium* TA100.

Immediately prior to use, the S9 was thawed and mixed with a cofactor pool to contain 2 mM magnesium chloride, 6 mM potassium chloride, 1 mM glucose-6-phosphate, 1 mM nicotinamide adenine dinucleotide phosphate (NADP) and 20 µL S9 per milliliter medium (serum-free DMEM supplemented with, 100 units penicillin and 100 µg streptomycin/mL, and 2 mM L-glutamine).

Solubility Test

A solubility test was conducted to select the solvent. The test was conducted using purified water. The test substance was tested to determine the solvent that permitted preparation of the highest soluble stock concentration, up to 50 mg/mL.

Chromosome Aberration Assay

Selection of dose levels for the cytogenetics test was based upon post-treatment cell growth inhibition relative to the solvent control and solubility of the test substance. Whenever possible, the high dose to evaluate chromosome aberrations was selected to give at least 50% reduction in cell growth. The chromosome aberration assay was performed using standard procedures (Evans, 1976), by exposing duplicate cultures of V79 cells to the test substance as well as positive and solvent controls. For the chromosome aberration assay, V79 cells were seeded at approximately 5 x 10^5 cells/25 cm^2 flask and were incubated at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air for 16-24 hours. Treatment was carried out by refeeding duplicate flasks with 4.5 mL complete medium (DMEM supplemented with 10% FBS, 100 units penicillin and 100 µg streptomycin/mL, and 2 mM L-glutamine) for the non-activated study or 4.5 mL S9 reaction mixture for the S9 activated study, to which was added 500 µL of dosing solution of test or control substance in solvent or solvent alone. The osmolality of the highest concentration of dosing solution in the treatment medium was measured. The pH of the highest concentration of dosing solution in the treatment medium was measured using test tape.

In the non-activated study, the cells were exposed to the test substance for 4 hours or continuously for 20 hours up to the cell harvest at 37±1°C in a humidified atmosphere of 5±1% CO_2 in air (Swierenga et al., 1991). In the 4 hour exposure group, after the exposure period, the treatment medium was removed, the cells washed with calcium and magnesium free phosphate buffered saline (CMF-PBS), refed with complete medium and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid® was added to duplicate flasks for each treatment condition at a final concentration of 0.1 µg/mL and the flasks returned to the incubator until cell collection.

In the S9 activated study, the cells were exposed for 4 hours at 37±1°C in a humidified

atmosphere of 5±1% CO_2 in air (Swierenga et al., 1991). After the exposure period, the treatment medium was removed, the cells washed with CMF-PBS, refed with complete medium and returned to the incubator. Two hours prior to the scheduled cell harvest, Colcemid® was added to duplicate flasks for each treatment condition at a final concentration of 0.1 µg/mL and the flasks were returned to the incubator until cell collection.

A concurrent toxicity test was conducted in both the non-activated and the S9 activated test systems. After cell harvest an aliquot of the cell suspension was removed from each culture and counted using a Coulter counter. The presence of test substance precipitate was assessed using the unaided eye. Cell viability was determined by trypan blue dye exclusion. The cell counts and percent viability were used to determine cell growth inhibition relative to the solvent control.

Collection of Metaphase Cells

Two hours after the addition of Colcemid®, metaphase cells were harvested for both the non-activated and S9 activated studies by trypsinization. Cells were collected approximately 20 hours after initiation of treatment (Galloway et al., 1994). The cells were collected by centrifugation at approximately 800 rpm for 5 minutes. The cell pellet was resuspended in 2-4 mL 0.075 M potassium chloride (KCl) and allowed to stand at room temperature for 4-8 minutes. The cells were collected by centrifugation, the supernatant aspirated and the cells fixed with two washes of approximately 2 mL Carnoy's fixative (methanol:glacial acetic acid, 3:1, v/v). The cells were stored overnight or longer in fixative at approximately 2-8°C.

Slide Preparation

To prepare slides, the fixed cells were centrifuged at approximately 800 rpm for 5 minutes, the supernatant was aspirated, and 1 mL fresh fixative was added. After additional centrifugation (at approximately 800 rpm for 5 minutes) the supernatant fluid was decanted and the cells resuspended to opalescence in fresh fixative. A sufficient amount of cell suspension was dropped onto the center of a glass slide and allowed to air dry. Slides were identified by the study number, date prepared and the treatment condition. The dried slides were stained with 5% Giemsa, air dried and permanently mounted.

Evaluation of Metaphase Cells

Slides were coded using random numbers by an individual not involved with the scoring process. To ensure that a sufficient number of metaphase cells were present on the slides, the percentage of cells in mitosis per 500 cells scored (mitotic index) was determined for each treatment group. Metaphase cells with 20±2 centromeres were examined under oil immersion without prior knowledge of treatment groups. Initially, the non-activated and S9 activated 4 hour exposure groups were evaluated for chromosome aberrations and if a positive result was

obtained in the non-activated 4 hour exposure group, the non-activated 20 hour continuous exposure group was not evaluated for chromosome aberrations. A minimum of 200 metaphase spreads (100 per duplicate flask) were examined and scored for chromatid-type and chromosome-type aberrations (Scott et al., 1990). The number of metaphase spreads that were examined and scored per duplicate flask was reduced when the percentage of aberrant cells reached a statistically significant level before 100 cells are scored. Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure were scored as a break (chromatid or chromosome). Fragments observed with an exchange figure were not scored as an aberration but instead were considered part of the incomplete exchange. Pulverized chromosome(s), pulverized cells and severely damaged cells (≥10 aberrations) were also recorded. Chromatid and isochromatid gaps were recorded but not included in the analysis. The XY coordinates for each cell with chromosomal aberrations were recorded using a calibrated microscope stage. Polyploid and endoreduplicated cells were evaluated from each treatment flask per 100 metaphase cells scored.

Controls

Mitomycin C was used as the positive control in the non-activated study at final concentrations of 0.1 and 0.2 µg/mL. Cyclophosphamide was used as the positive control in the S9 activated study at final concentrations of 10 and 20 µg/mL. For both positive controls the dose level exhibiting a sufficient number of scorable metaphase cells was selected for analysis. The solvent vehicle for the test substance was used as the solvent control at the same concentration as that found in the test substance-treated groups.

Evaluation of Test Results

The toxic effects of treatment were based upon cell growth inhibition relative to the solvent-treated control and are presented for the aberration studies. The number and types of aberrations found, the percentage of structurally and numerically damaged cells (percent aberrant cells) in the total population of cells examined, and the mean aberrations per cell was calculated and reported for each group. Chromatid and isochromatid gaps are presented in the data but are not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations per cell.

Statistical analysis of the percent aberrant cells was performed using the Fisher's exact test. Fisher's test was used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's test at any test substance dose level, the Cochran-Armitage test was used to measure dose-responsiveness.

All conclusions were based on sound scientific basis; however as a guide to interpretation of the data, the test substance is considered to induce a positive response when the percentage of cells with aberrations was increased in a dose-responsive manner with one or more concentrations being statistically significant ($p \leq 0.05$). If the test substance did not demonstrate a statistically significant increase in aberrations it was concluded to be negative.

Criteria for a Valid Test

The use of the V79 cell line was based on the Sponsor's request and this cell line is not commonly used in chromosome aberration studies. The frequency of cells with structural chromosome aberrations in the solvent control must be $\leq 6\%$. The percentage of cells with chromosome aberrations in the positive control must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to the solvent control.

Deviations

No known deviations from the protocol or assay method SOPs occurred during the conduct of this study.

Archives

All raw data, the protocol, all reports, and stained and coded slides will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RAQA unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850. Paper records will be retained for at least three years after which time the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials returned to the Sponsor or destroyed will first be copied and the copy will be retained in the BioReliance archives for a minimum of 10 years.

RESULTS AND DISCUSSION

Solubility

Water was determined to be the solvent of choice based on the solubility of the test substance and compatibility with the target cells. The test substance was soluble in water at a concentration of 50 mg/mL, the maximum concentration tested.

Preliminary Toxicity Assay

In the absence of test substance precipitation and toxicity, 5000 µg/mL was the highest dose selected for analysis. Two additional dose levels were also included. V79 cells were exposed to solvent alone and to nine concentrations of test substance ranging from 0.5 µg/mL to 5000

µg/mL in the absence and presence of an S9 reaction mixture. The results of the evaluation of cell growth inhibition are presented in Tables 1, 2 and 3. The test substance was soluble in treatment medium at all dose levels tested in all treatment conditions. Based upon the results of the toxicity study, the dose levels selected for evaluation in the chromosome aberration assay were as follows:

Treatment Condition	Treatment Time (hours)	Recovery Time (hours)	Dose levels (µg/mL)
-S9 non-activated	4	16	625, 1250, 2500, 5000
-S9 non-activated	20	0	625, 1250, 2500, 5000
+S9 activated	4	16	625, 1250, 2500, 5000

Chromosome Aberration Assay

In the non-activated chromosome aberration assays the osmolality in treatment medium of the highest concentration tested, 5000 µg/mL, was 323 mmol/kg. The osmolality of the solvent (water) in treatment medium was 299 mmol/kg. The pH of the highest concentration of test substance in treatment medium was approximately 7. Changes of higher than 50 mmol are not acceptable.

No toxicity of H-24783 (cell growth inhibition, relative to the solvent control) in V79 cells when treated for 4 hours in the absence of S9 activation was observed at 5000 µg/mL, the highest test concentration evaluated for chromosome aberrations (Table 4). The activity of H-24783 in the induction of chromosome aberrations is presented by treatment flask in Table 5 and summarized by group in Table 10. The mitotic index at the highest dose level evaluated for chromosome aberrations, 5000 µg/mL, was 15% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 1250, 2500 and 5000 µg/mL. The percentage of cells with structural and numerical aberrations in the test substance-treated groups was not significantly increased above that of the solvent control ($p>0.05$, Fisher's exact test). The percentage of cells with structurally damaged chromosomes in the MMC (positive control) treatment group (11.5%) was found to be statistically significant.

No toxicity of H-24783 (cell growth inhibition relative to the solvent control) in V79 cells when treated for 4 hours in the presence of S9 activation was observed at 5000 µg/mL, the highest test concentration evaluated for chromosome aberrations (Table 6). The activity of H-24783 in the induction of chromosome aberrations is presented by treatment flask in Table 7 and summarized by group in Table 10. The mitotic index at the highest dose level evaluated for chromosome aberrations, 5000 µg/mL, was 29% reduced relative to the solvent control. The

dose levels selected for microscopic analysis were 1250, 2500 and 5000 µg/mL. The percentage of cells with structural and numerical aberrations in the test substance-treated groups was not statistically increased above that of the solvent control ($p>0.05$, Fisher's exact test). The percentage of cells with structurally damaged chromosomes in the CP (positive control) treatment group (21.0%) was found to be statistically significant.

In the absence of a positive response in the non-activated 4 hour exposure group, slides from the non-activated 20 hour exposure group were evaluated for chromosome aberrations. Toxicity of H-24783 (cell growth inhibition relative to the solvent control) was 9% at 5000 µg/mL, the highest test concentration evaluated for chromosome aberrations in the non-activated 20 hour continuous exposure group (Table 8). The activity of H-24783 in the induction of chromosome aberrations is presented by treatment flask in Table 9 and summarized by group in Table 10. The mitotic index at the highest dose level evaluated for chromosome aberrations, 5000 µg/mL, was 9% reduced relative to the solvent control. The dose levels selected for microscopic analysis were 1250, 2500 and 5000 µg/mL. The percentage of cells with structural or numerical aberrations in the test substance-treated groups was not significantly increased above that of the solvent control ($p>0.05$). The percentage of cells with structurally damaged chromosomes in the MMC (positive control) treatment group (29.0%) was found to be statistically significant.

CONCLUSION

The positive and solvent controls fulfilled the requirements for a valid test.

Based on the findings of this study, H-24783 was concluded to be negative for the induction of structural and numerical chromosome aberrations in Chinese hamster V79 cells in the presence and absence of Aroclor-induced rat liver S9.

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TABLE 1
PRELIMINARY TOXICITY TEST IN V79 CELLS TREATED WITH H24783 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD

Treatment µg/mL	Cell Count ($x10^6$)	Cell Viability (%)	Viable Cells/ Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	0.84	98%	0.82	100%	
H-24783					
0.5	1.03	99%	1.02	124%	-24%
1.5	1.12	100%	1.12	136%	-36%
5	0.95	98%	0.93	113%	-13%
15	1.18	99%	1.17	142%	-42%
50	1.11	100%	1.11	135%	-35%
150	0.94	100%	0.94	114%	-14%
500	0.98	98%	0.96	118%	-18%
1500	0.83	97%	0.81	98%	2%
5000	0.96	96%	0.92	113%	-13%

Treatment: V79 cells were treated in the absence of an exogenous source of metabolic activation for 4 hours at 37±1°C.
Cell Viability: determined by trypan blue dye exclusion.
Viable cells/flask = cell count x % viable cells
Cell Growth Index = (cells per flask treated group/cells per flask control group), expressed as a percentage.
Cell growth inhibition = 100% - % cell growth index; not calculated for negative controls.

TABLE 2
PRELIMINARY TOXICITY TEST IN V79 CELLS TREATED WITH H24783 IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD

Treatment µg/mL	Cell Count ($x10^6$)	Cell Viability (%)	Viable Cells/ Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	0.88	98%	0.86	100%	
H-24783					
0.5	0.95	94%	0.89	104%	-4%
1.5	1.16	98%	1.14	132%	-32%
5	0.97	98%	0.96	111%	-11%
15	0.88	97%	0.86	100%	0%
50	0.91	98%	0.89	104%	-4%
150	0.91	99%	0.90	105%	-5%
500	0.91	99%	0.91	105%	-5%
1500	0.95	100%	0.95	110%	-10%
5000	0.98	98%	0.96	112%	-12%

Treatment: V79 cells were treated in the presence of an exogenous source of metabolic activation for 4 hours at 37±1°C.
Cell Viability: determined by trypan blue dye exclusion.
Viable cells/flask = cell count x % viable cells
Cell Growth Index = (cells per flask treated group/cells per flask control group), expressed as a percentage.
Cell growth inhibition = 100% - % cell growth index; not calculated for negative controls.

TABLE 3
PRELIMINARY TOXICITY TEST IN V79 CELLS TREATED WITH H24783 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

20 HOUR CONTINUOUS TREATMENT

Treatment µg/mL	Cell Count ($x10^6$)	Cell Viability (%)	Viable Cells/ Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	0.89	99%	0.89	100%	
H-24783 0.5 ug/mL	0.86	98%	0.85	96%	4%
1.5 ug/mL	1.00	97%	0.97	110%	-10%
5 ug/mL	0.99	98%	0.97	110%	-10%
15 ug/mL	0.98	99%	0.97	110%	-10%
50 ug/mL	1.13	98%	1.11	125%	-25%
150 ug/mL	0.91	97%	0.88	100%	0%
500 ug/mL	0.92	99%	0.92	103%	-3%
1500 ug/mL	0.97	98%	0.95	107%	-7%
5000 ug/mL	1.21	96%	1.16	131%	-31%

Treatment: V79 cells were treated in the absence of an exogenous source of metabolic activation for 20 hours at 37±1°C.
Cell Viability: determined by trypan blue dye exclusion.
Viable cells/flask = cell count x % viable cells
Cell Growth Index = (cells per flask treated group/cells per flask control group), expressed as a percentage.
Cell growth inhibition = 100% - % cell growth index; not calculated for negative controls.

TABLE 4
CONCURRENT TOXICITY TEST IN V79 CELLS TREATED WITH H-24783 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD

Treatment µg/mL	Flask	Cell Count Average ($x10^6$)	Cell Viability (%)	Mean Cells per Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	A	2.07	98%			
	B	2.24	94%	2.07	100%	
H-24783						
625	A	2.45	95%			
	B	2.36	96%	2.29	111%	-11%
1250	A	2.48	97%			
	B	2.45	96%	2.38	115%	-15%
2500	A	2.64	98%			
	B	2.16	99%	2.36	114%	-14%
5000	A	2.44	97%			
	B	2.36	98%	2.34	113%	-13%
MMC	A	1.85	98%			
0.1	B	2.11	98%	1.94	94%	6%
MMC	A	2.03	97%			
0.2	B	2.05	96%	1.97	95%	5%

Treatment: V79 cells were treated in the absence of an exogenous source of metabolic activation for 4 hours at 37±1°C.
Cell Viability: determined by trypan blue dye exclusion.
Viable Cells/Flask = cell count x % viable cells, reported as mean of Flasks A and B.
Cell Growth Index = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage.
Cell Growth Inhibition = 100% - % cell growth index; not calculated for negative controls.

TABLE 5
CYTOGENETIC ANALYSIS OF V79 CELLS TREATED WITH H-24783 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD

Treatment	Flask	Mitotic Index (%)	Cells Scored	Cells with Aberrations: Numerical (%)	Cells with Aberrations: Structural (%)	Total Number of Structural Aberrations: Gaps	Chromatid: Br	Chromatid: Ex	Chromosome: Br	Chromosome: Dic	Chromosome: Ring	Severely Damaged Cells	Average Aberrations Per Cell
Water	A	16.8	100	2	0	0	0	0	0	0	0	0	0.000
	B	16.0	100	1	0	0	0	0	0	0	0	0	0.000
H-24783													
1250	A	15.2	100	3	0	0	0	0	0	0	0	0	0.000
	B	15.0	100	2	1	1	1	0	0	0	0	0	0.010
2500	A	14.8	100	1	0	0	0	0	0	0	0	0	0.000
	B	15.0	100	0	0	0	0	0	0	0	0	0	0.000
5000	A	14.2	100	2	1	0	0	0	0	1	0	0	0.010
	B	13.8	100	2	1	0	1	0	0	0	0	0	0.010
MMC	A	11.8	100	1	12	0	5	4	0	3	0	0	0.120
0.2	B	12.4	100	2	11	0	5	5	0	2	0	0	0.120

Treatment: V79 cells were treated for 4 hours at 37±1°C in the absence of an exogenous source of metabolic activation. An additional dose level of 625 µg/mL was tested as a safeguard against excessive toxicity at higher dose levels but was not required for microscopic examination.
Mitotic Index = number mitotic figures x 100/500 cells counted.
%Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.
Chromatid Breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.
Chromosome Breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.
Severely Damaged Cells includes cells with one or more pulverized chromosome and cells with 10 or more structural aberrations.
Average Aberrations Per Cell: severely damaged cells and pulverizations were counted as 10 aberrations.

TABLE 6
CONCURRENT TOXICITY TEST IN V79 CELLS TREATED WITH H-24783IN
THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD

Treatment µg/mL	Flask	Cell Count Average ($x10^6$)	Cell Viability (%)	Mean Cells per Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	A	2.07	99%			
	B	2.23	99%	2.13	100%	
H-24783						
625	A	2.37	100%			
	B	2.42	98%	2.37	112%	-12%
1250	A	2.49	97%			
	B	2.23	96%	2.28	107%	-7%
2500	A	2.37	98%			
	B	2.26	99%	2.28	107%	-7%
5000	A	2.45	94%			
	B	2.59	96%	2.39	112%	-12%
CP	A	1.95	99%			
10	B	2.41	98%	2.15	101%	-1%
CP	A	1.74	97%			
20	B	1.94	98%	1.79	84%	16%

Treatment: V79 cells were treated in the presence of an exogenous source of metabolic activation for 4 hours at 37±1°C.
Cell Viability: determined by trypan blue dye exclusion.
Viable Cells/Flask = cell count x % viable cells, reported as mean of Flasks A and B.
Cell Growth Index = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage.
Cell Growth Inhibition = 100% - % cell growth index; not calculated for negative controls.

TABLE 7
CYTOGENETIC ANALYSIS OF V79 CELLSTREATED WITH H-24783 IN THE PRESENCE OF EXOGENOUS METABOLIC ACTIVATION

4 HOUR TREATMENT, 16 HOUR RECOVERY PERIOD

Treatment µg/mL	Flask	Mitotic Index (%)	Cells Scored	Cells with Aberrations Numerical (%)	Cells with Aberrations Structural (%)	Total Number of Structural Aberrations: Gaps	Chromatid Br	Chromatid Ex	Chromosome Br	Chromosome Dic	Chromosome Ring	Severely Damaged Cells	Average Aberrations Per Cell
Water	A	11.2	100	1	0	0	0	0	0	0	0	0	0.000
	B	10.6	100	1	0	0	0	0	0	0	0	0	0.000
H-24783													
1250	A	13.6	100	1	0	0	0	0	0	0	0	0	0.000
	B	13.0	100	0	0	0	0	0	0	0	0	0	0.000
2500	A	9.0	100	2	0	0	0	0	0	0	0	0	0.000
	B	10.6	100	1	0	0	0	0	0	0	0	0	0.000
5000	A	7.6	100	2	0	0	0	0	0	0	0	0	0.000
	B	7.8	100	1	0	0	0	0	0	0	0	0	0.000
CP	A	7.0	50	2	22	0	25	1	3	0	0	0	0.580
10	B	6.2	50	1	20	0	17	1	3	0	0	1	0.620

Treatment: V79 cells were treated for 4 hours at 37±1°C in the presence of an exogenous source of metabolic activation. An additional dose level of 625 µg/mL was tested as a safeguard against excessive toxicity at higher dose levels but was not required for microscopic examination.
Mitotic Index = number mitotic figures x 100/500 cells counted.
%Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.
Chromatid Breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.
Chromosome Breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.
Severely Damaged Cells includes cells with one or more pulverized chromosome and cells with 10 or more structural aberrations.
Average Aberrations Per Cell: severely damaged cells and pulverizations were counted as 10 aberrations.

TABLE 8
CONCURRENT TOXICITY TEST IN V79 CELLS TREATED WITH H-24783IN
THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

20 HOUR CONTINUOUS TREATMENT

Treatment µg/mL	Flask	Cell Count Average ($x10^6$)	Cell Viability (%)	Mean Cells per Flask ($x10^6$)	Cell Growth Index (%)	Cell Growth Inhibition (%)
Water	A	2.51	97%			
	B	2.87	96%	2.59	100%	
H-24783						
625	A	2.45	98%			
	B	2.06	99%	2.22	86%	14%
1250	A	2.47	98%			
	B	2.23	97%	2.29	88%	12%
2500	A	2.42	97%			
	B	2.24	95%	2.24	86%	14%
5000	A	2.54	95%			
	B	2.37	96%	2.35	91%	9%
MMC	A	2.82	98%			
0.1	B	2.37	97%	2.53	98%	2%
MMC	A	2.06	95%			
0.2	B	2.25	98%	2.08	80%	20%

Treatment: V79 cells were treated in the absence of an exogenous source of metabolic activation for 20 hours at 37±1°C.
Cell Viability: determined by trypan blue dye exclusion.
Viable Cells/Flask = cell count x % viable cells, reported as mean of Flasks A and B.
Cell Growth Index = (mean cells per flask treated group/mean cells per flask control group), expressed as a percentage.
Cell Growth Inhibition = 100% - % cell growth index; not calculated for negative controls.

TABLE 9
CYTOGENETIC ANALYSIS OF V79 CELLSTREATED WITH H-24783 IN THE ABSENCE OF EXOGENOUS METABOLIC ACTIVATION

Treatment µg/mL	Flask	Mitotic Index (%)	Cells Scored	Cells with Aberrations: Numerical (%)	Cells with Aberrations: Structural (%)	Total Number of Structural Aberrations: Gaps	Chromatid: Br	Chromatid: Ex	Chromosome: Br	Chromosome: Dic	Chromosome: Ring	Severely Damaged Cells	Average Aberrations Per Cell
Water	A	16.8	100	1	0	0	0	0	0	0	0	0	0.000
	B	17.4	100	2	1	0	1	0	0	0	0	0	0.010
H-24783 1250	A	16.0	100	1	0	0	0	0	0	0	0	0	0.000
	B	16.8	100	1	0	0	0	0	0	0	0	0	0.000
2500	A	16.8	100	1	0	0	0	0	0	0	0	0	0.000
	B	17.8	100	2	0	0	0	0	0	0	0	0	0.000
5000	A	15.4	100	2	0	0	0	0	0	0	0	0	0.000
	B	15.8	100	2	1	0	1	0	0	0	0	0	0.010
MMC 0.1	A	6.4	50	2	32	3	17	3	4	0	0	2	0.880
	B	5.8	50	1	26	2	12	4	5	0	0	0	0.420

Treatment: V79 cells were treated for 20 hours at 37±1°C in the absence of an exogenous source of metabolic activation. An additional dose level of 625 µg/mL was tested as a safeguard against excessive toxicity at higher dose levels but was not required for microscopic examination.
Mitotic Index = number mitotic figures x 100/500 cells counted.
%Aberrant Cells: numerical cells include polyploid and endoreduplicated cells; structural cells exclude cells with only gaps.
Chromatid Breaks (Br) include chromatid and isochromatid breaks and fragments; chromatid exchange figures (Ex) include quadriradials, triradials and complex rearrangements.
Chromosome Breaks (Br) include breaks and acentric fragments; Dic, dicentric chromosome.
Severely Damaged Cells includes cells with one or more pulverized chromosome and cells with 10 or more structural aberrations.
Average Aberrations Per Cell: severely damaged cells and pulverizations were counted as 10 aberrations.

TABLE 10

SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time (hours)	Mean Mitotic Index (%)	Cells Scored	Aberrations Per Cell (Mean +/- SD)	Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
Water	-	4	16.4	200	0.000 ±0.000	1.5	0.0
H-24783							
1250	-	4	15.1	200	0.005 ±0.071	2.5	0.5
2500	-	4	14.9	200	0.000 ±0.000	0.5	0.0
5000	-	4	14.0	200	0.010 ±0.100	2.0	1.0
MMC 0.2	-	4	12.1	200	0.120 ±0.341	1.5	11.5**
Water	+	4	10.9	200	0.000 ±0.000	1.0	0.0
H-24783							
1250	+	4	13.3	200	0.000 ±0.000	0.5	0.0
2500	+	4	9.8	200	0.000 ±0.000	1.5	0.0
5000	+	4	7.7	200	0.000 ±0.000	1.5	0.0
CP 10	+	4	6.6	100	0.600 ±1.524	1.5	21.0**
Water	-	20	17.1	200	0.005 ±0.071	1.5	0.5
H-24783							
1250	-	20	16.4	200	0.000 ±0.000	1.0	0.0
2500	-	20	17.3	200	0.000 ±0.000	1.5	0.0
5000	-	20	15.6	200	0.005 ±0.071	2.0	0.5
MMC 0.1	-	20	6.1	100	0.650 ±1.629	1.5	29.0**

Treatment: Cells from all treatment conditions were harvested at 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: **, $p \leq 0.01$; using Fisher's exact test.

APPENDIX A

Study Protocol

QA ES 4/17/01 APPROVED

PROTOCOL AMENDMENT 1

Sponsor: **E.I. du Pont de Nemours and Company**

Test Substance: **1,3-Propanediol**

BioReliance Study No.: **AA41BC.391.BTL**

Sponsor Project No.: **DuPont-5683**

Protocol Title: **H-24783: *In Vitro* Mammalian Chromosome Aberration Test**

1. **LOCATION:** Page 5 of 10, § 7.5.2; Positive Controls, first and second sentences

AMENDMENT: Amend the section to, "Mitomycin C will be used at a concentration within 0.05-0.3 µg/ml as the positive control in the non-activated study. Cyclophosphamide will be used at a concentration within 10-50 µg/ml as the positive control in the S9-activated study."

REASON FOR THE AMENDMENT: To correct the concentration ranges of mitomycin C and cyclophosphamide used in the study.

APPROVALS:

[redacted]

BIORELIANCE STUDY DIRECTOR — 16 April 2001 **DATE**

[redacted]

BIORELIANCE STUDY MANAGEMENT — 16 Apr 2001 **DATE**

[redacted]

SPONSOR REPRESENTATIVE — 26 Apr 2001 **DATE**

BIORELIANCE Formerly Microbiological Associates

Received by RA/OA 22 Mar 2001

QA 3/23/01 APPROVED

Sponsor Project Number: DuPont –5683

BioReliance Study Number: AA41BC.391.BTL

H-24783: *In Vitro* Mammalian Chromosome Aberration Test

1.0 PURPOSE

The purpose of this study is to evaluate the clastogenic potential of a test substance based upon its ability to induce chromosome aberrations in Chinese hamster V79 cells.

2.0 SPONSOR

2.1	Name:	E.I. du Pont de Nemours and Company
2.2	Address:	Stine Haskell Research Center DuPont Haskell Laboratory P.O. Box 50, 1090 Elkton Road Newark, DE 19714-0050
2.3	Representative :	Maria Donner, Ph.D. Phone: 302-366-5251 Fax: 302-366-5207 Email: maria.donner@usa.dupont.com
2.4	Sponsor Project #:	DuPont-5683
2.5	WR #:	13615
2.6	Haskell #:	H-24783
2.7	Service Code:	531

3.0 IDENTIFICATION OF TEST AND CONTROL SUBSTANCES

3.1	Test Substance:		1,3-Propanediol
3.2	Test Substance I.D.:		H-24783 (to be used in the report text)
3.3	Controls:	Solvent:	Test Substance Solvent (or Vehicle)
		Positive:	Mitomycin C (MMC) Cyclophosphamide (CP)

3.4 Test Substance Characterization

Unless alternate arrangements are made, the testing facility at BioReliance will not perform analysis of the dosing solutions. The Sponsor will be directly responsible for determination and documentation of the analytical purity and composition of the

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test substance, and the stability and strength of the test substance in the solvent (or vehicle).

3.5 Test Substance Retention Sample

The retention of a reserve sample of the test substance will be the responsibility of the Sponsor.

4.0 TESTING FACILITY AND KEY PERSONNEL

4.1	Name:	Toxicology Testing Facility BioReliance
4.2	Address:	9630 Medical Center Drive Rockville, MD 20850
4.3	Study Director:	Ramadevi Gudi, Ph.D. Phone: (301) 610-2169 Fax: (301) 738-2362 E-mail: rgudi@bioreliance.com

5.0 PROPOSED TEST SCHEDULE

5.1	Experimental Start Date:	April 3, 2001
5.2	Experimental Termination Date:	May 4, 2001
5.3	Draft Report Date:	May 18, 2001
5.4	Final Report Date:	2 weeks after Sponsor approves draft

6.0 TEST SYSTEM

The Chinese hamster V79 cells are grown in 9% Dulbecco's modified eagles medium (Gibco BRL # 41965-039) containing sodium pyruvate, glucose and pyridoxine HCl. To 500 mL of culture medium 50 mL of heat - inactivated fetal calf serum, 2.6 mL penicillin/streptomycin (10,000IU/mL 10,000UG/mL, Gibco BRL # 35400-027) are added. V79 cells double every 12-16 hours with a high cloning efficiency, both necessary for the appropriate performance of the study. These cells have a stable karyotype with a modal chromosome number of 22 ± 1.

7.0 EXPERIMENTAL DESIGN AND METHODOLOGY

The chromosome aberration assay will be conducted using standard procedures (Evans, 1976; Bradley et. al., 1981), by exposing cultures of V79 cells to a minimum of four concentrations of the test substance as well as to positive and solvent controls. In the non-

activated test system, treatment will be for 4 hours and for 20 hours; in the S9 activated test system, treatment will be for 4 hours (Swierenga et al., 1991). To ensure evaluation of first division metaphase cells the dividing cells will be arrested in metaphase and harvested for microscopic evaluation of chromosome aberrations at approximately 20 hours (1.5 normal cell cycles) after the initiation of treatment (Galloway et al., 1994). The clastogenic potential of the test substance will be measured by its ability to increase structural chromosome aberrations in a dose-responsive manner when compared to the solvent control group. In the event of a positive response in the 4 hour non-activated study, the prolonged treatment non-activated study may not be scored. The test substance will also be assessed for its ability to induce numerical chromosome aberrations.

7.1 Solubility Determination

Unless the Sponsor has indicated the test substance solvent, a solubility determination will be conducted to determine the solvent and the maximum soluble concentration up to a maximum of 500 mg/ml. Solvents compatible with this test system, in order of preference, include but are not limited to sterile water (CAS 7732-18-5), dimethylsulfoxide (CAS 67-68-5), ethanol (CAS 64-17-5), and acetone (CAS 67-64-1). The solvent will be the test substance solvent, selected in order of preference, that permits preparation of the highest soluble stock concentration, up to 500 mg/ml.

7.2 Preliminary Toxicity Test for Selection of Dose Levels

Selection of the dose levels for the cytogenetics assay will be based upon post-treatment toxicity (cell growth inhibition relative to the solvent control) and solubility of the test substance. V79 cells will be exposed to solvent alone and to at least nine concentrations of test substance. The highest concentration tested will be 5 mg/ml whichever is lower for freely soluble test substances, or the maximum concentration resulting in a workable suspension for poorly soluble test substances not to exceed 5 mg/ml. The pH will be measured at the highest test substance treatment condition and will be adjusted, if necessary, in order to maintain a neutral pH in the treatment medium. The osmolality of the highest dose level, lowest precipitating dose level (where applicable) and the highest soluble dose level (where applicable) in treatment medium will also be measured. Cells seeded 16-24 hours earlier will be exposed for 4 hours in the absence and presence of S9 and for 20 hours in the absence of S9. Just prior to trypsinization the cell cultures will be visually inspected for the extent of monolayer confluency relative to the solvent control. Twenty hours after treatment initiation the cells will be harvested by trypsinization and counted using an automatic cell counter and the cell viability will be assessed using trypan blue dye exclusion. The cell counts and percent viability will be used to determine cell growth inhibition relative to the solvent control.

Whenever possible, the high dose to evaluate chromosome aberrations will be selected to give at least 50% toxicity (cell growth inhibition relative to the solvent

control) irrespective of solubility but not to exceed 5 mg/ml. At least two additional dose levels, demonstrating minimal or no toxicity will be included. In the event the test substance cannot be dissolved at a high enough concentration in an appropriate solvent to be toxic, then the highest dose to be tested in the chromosome aberration assay will be the concentration resulting in minimum precipitation in test medium. Precipitation will be determined by direct visual inspection. In the event the test substance demonstrates a dose-responsive increase in toxicity at concentrations that exceed solubility in treatment medium, then the highest dose to be tested will be the maximum concentration that results in at least 50% toxicity. In the event that neither cytotoxicity nor insolubility is observed in the preliminary test, the highest dose in the chromosome aberration assay will be 5 mg/ml whichever is lower. If excessive precipitation of the test substance-solvent solution occurs upon addition to treatment medium, or if the osmolality of the treatment medium is considered excessive, the Sponsor will be consulted.

7.3 Frequency and Route of Administration

Target cells will be treated for 4 hours in the absence and presence of S9 and for 20 hours in the absence of S9, by incorporation of the test substance-solvent mixture into the treatment medium. This technique has been demonstrated to be an effective method of detection of chemical clastogens in this test system (Evans, 1976).

If the Sponsor is aware of specific metabolic requirements, then this information will be utilized in the preparation of the study design. Verification of a clear positive response is not required. Negative results will not be confirmed when justification can be provided. Equivocal results may be confirmed, upon consultation with the Sponsor, and may employ a modification of the study design. This guidance is based on the OECD Guideline 473 (1997) and ICH Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (1996).

7.4 Activation System

Aroclor 1254-induced rat liver S9 will be used as the metabolic activation system. The S9 will be prepared from male Sprague-Dawley rats induced with a single intraperitoneal injection of Aroclor 1254, 500 mg/kg, five days prior to sacrifice. The S9 will be batch prepared and stored frozen at approximately -70°C until used. Each batch preparation of S9 will be assayed for sterility and its ability to metabolize 2-aminoanthracene and 7,12-dimethylbenz(α)anthracene to forms mutagenic to *Salmonella typhimurium* TA100.

Immediately prior to use, the S9 will be thawed and mixed with cofactors to contain 2 mM magnesium chloride ($MgCl_2$,) 6 mM potassium chloride (KCl), 1mM glucose-6-phosphate, 1 mM nicotinamide adenine dinucleotide phosphate (NADP) and 20 µl S9 per ml serum free medium DMEM (serum-free medium

supplemented with, 100 units penicillin and 100 µg streptomycin/mL, and 2 mM L-glutamine).

7.5 Controls

7.5.1 Solvent (or Vehicle) Control

The solvent for the test substance will be used as the solvent control. For solvents other than water, physiological buffer, or medium, the final concentration in treatment medium will not exceed 1%.

7.5.2 Positive Controls

Mitomycin C will be used at a concentration within 2-4 µg/ml as the positive control in the non-activated study. Cyclophosphamide will be used at a concentration within 2-10 µg/ml as the positive control in the S9-activated study.

7.6 Preparation of Target Cells

Exponentially growing V79 cells will be seeded in complete medium (Dulbecco's modified eagles medium (Gibco BRL # 41965-039) containing sodium pyruvate, glucose and pyridoxine HCl and 50 mL of heat inactivated fetal calf serum, 2.6 mL of Pencillin/streptomycin (10,000 IU/mL 10,000µg/mL,)) for each treatment condition at approximately 5 x 10^5 cells/25 cm^2 flask. The flasks will be incubated at 37 ± 1°C in a humidified atmosphere of 5 ± 1% CO_2 in air for 16-24 hours.

7.7 Identification of Test System

Using a permanent marking pen, the treatment flasks will be identified by the BioReliance study number and a code system to designate the treatment condition and test phase.

7.8 Treatment of Target Cells

Treatment will be carried out in duplicate by refeeding the flasks with 5 ml complete medium for the non-activated treatment or 5 ml S9 reaction mixture for the S9-activated treatment, to which will be added 50 µl of dosing solution of test or control article in solvent or solvent alone. Larger volumes of dosing solution may be used if water, physiological buffer, or medium is used as the solvent.

In the non-activated study, the cells will be treated for 4 hours and for 20 hours; in the S9-activated study the cells will be treated for 4 hours. Treatment will be carried out at 37 ± 1°C in a humidified atmosphere of 5 ± 1% CO_2 in air. After the 4 hour

treatment period in the non-activated and the S9-activated studies, the treatment medium will be aspirated, the cells washed with phosphate buffered saline, refed with complete medium and returned to the incubator.

A concurrent toxicity test to determine cell growth inhibition relative to the solvent control will be conducted in both the non-activated and the S9-activated studies.

7.9 Collection of Metaphase Cells

Cells will be collected approximately 20 hours after initiation of treatment. This post-treatment harvest time represents approximately 1.5 normal cell cycles and was selected to ensure that the cells are analyzed in the first division metaphase after initiation of treatment. Two hours prior to cell harvest, Colcemid® will be added to the cultures at a final concentration of 0.1 µg/ml.

Cells will be harvested by trypsinization, collected by centrifugation and an aliquot will be removed for counting using an automatic cell counter and trypan blue dye exclusion. The remainder of the cells will be swollen with 0.075M KCl, washed with two consecutive changes of fixative (methanol:glacial acetic acid, 3:1 v/v), capped and stored overnight or longer at approximately 2-8°C. The cell counts and percent viability will be used to determine cell growth inhibition relative to the solvent control (% toxicity). To prepare slides, the cells will be collected by centrifugation and resuspended in fresh fixative. The suspension of fixed cells will be applied to glass microscope slides and air-dried. The slides will be identified by the experiment number, treatment condition and date. The slides will be stained with Giemsa and permanently mounted.

7.10 Scoring for Metaphase Aberrations

To ensure that a sufficient number of metaphase cells are present on the slides, the percentage of cells in mitosis per 500 cells scored (mitotic index) will be determined and recorded for each coded treatment group selected for scoring chromosome aberrations. Slides will be coded using random numbers by an individual not involved with the scoring process. In the event of a positive response in the 4 hour non-activated study, the prolonged treatment non-activated study may not be scored. Metaphase cells with 22 ± 1 centromeres will be examined under oil immersion without prior knowledge of treatment groups. Whenever possible, a minimum of 200 metaphase spreads from each dose level (100 per duplicate flask) will be examined and scored for chromatid-type and chromosome-type aberrations (Scott et al., 1990). The number of metaphase spreads that will be examined and scored per duplicate flask may be reduced if the percentage of aberrant cells reaches a statistically significant level before 100 cells are scored. Chromatid-type aberrations include chromatid and isochromatid breaks and exchange figures such as quadriradials (symmetrical and asymmetrical interchanges), triradials, and complex rearrangements. Chromosome-type aberrations include chromosome breaks and

exchange figures such as dicentrics and rings. Fragments (chromatid or acentric) observed in the absence of any exchange figure will be scored as a break (chromatid or chromosome). Fragments observed with an exchange figure will not be scored as an aberration but will be considered part of the incomplete exchange. Pulverized chromosome(s), pulverized cells and severely damaged cells ($\geq$ 10 aberrations) will also be recorded. Chromatid and isochromatid gaps will be recorded but not included in the analysis. The XY coordinates for each cell with a structural aberration will be recorded using a calibrated microscope stage. The percent polyploid and endoreduplicated cells will be evaluated per 100 cells for each dose level analyzed for structural aberrations.

8.0 CRITERIA FOR DETERMINATION OF A VALID TEST

8.1 Solvent Control

The frequency of cells with structural chromosome aberrations in the solvent control must be $\leq$ 6%.

8.2 Positive Control

The percentage of cells with aberrations must be statistically increased ($p \leq 0.05$, Fisher's exact test) relative to the solvent control.

9.0 EVALUATION OF TEST RESULTS

The toxic effects of treatment are based upon cell growth inhibition relative to the solvent control and will be presented for the toxicity and aberration studies. The number and types of aberrations found, the percentage of structurally and numerically damaged cells (percent aberrant cells) in the total population of cells examined, and the mean aberrations per cell will be calculated and reported for each treatment group. Chromatid and isochromatid gaps are presented in the data but are not included in the total percentage of cells with one or more aberrations or in the frequency of structural aberrations per cell. Statistical analysis of the percentage of aberrant cells will be performed using the Fisher's exact test. The Fisher's test will be used to compare pairwise the percent aberrant cells of each treatment group with that of the solvent control. In the event of a positive Fisher's exact test at any test substance dose level, the Cochran-Armitage test will be used to measure dose-responsiveness. All conclusions will be based on sound scientific basis; however, as a guide to interpretation of the data, the test substance will be considered to induce a positive response when the percentage of cells with aberrations is increased in a dose-responsive manner with one or more concentrations being statistically significant ($p \leq 0.05$). However, values that are statistically significant but do not exceed the range of historic negative or solvent controls may be judged as not biologically significant. Test substances not demonstrating a statistically significant increase in aberrations will be concluded to be negative.

Sponsor Project Number: DuPont –5683

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10.0 REPORT

A report of the results of this study will be prepared by BioReliance and will accurately describe all methods used for generation and analysis of the data.

Results presented will include, but not be limited to:

• Test substance: identification and CAS no., if known; physical nature and purity, if known; physicochemical properties relevant to the conduct of the study, if known; stability of test substance, if known.

• Solvent/Vehicle: justification for choice of vehicle; solubility and stability of test substance in solvent/vehicle, if known.

• Source of cells, karyotype features (modal chromosome number) and suitability of the cell type used, absence of mycoplasma, cell cycle length, passage number.

• Test conditions: composition of medium; CO_2 concentration; incubation time; cell seeding density; solvent and solvent selection rationale; concentration of test substance and concentration selection rationale; composition and acceptability criteria for the metabolic activation (S9) system; duration of treatment; duration of treatment with and concentration of Colcemid®; type of metabolic activation system used; positive and solvent controls; methods of slide preparation; number of cell cultures; criteria for scoring aberrations and criteria for considering studies positive, negative.

• Results: description of precipitation; pH and osmolality of the treatment medium; cell growth inhibition relative to the solvent control; mitotic index and number of metaphases analyzed; type and number of aberration (structural and numerical) given separately for each treated and control culture; concentration-response relationship; statistical analysis; historical control data.

11.0 RECORDS AND ARCHIVES

All raw data, the protocol and all reports will be maintained according to Standard Operating Procedure OPQP3040 by the BioReliance RAQA unit headquartered at: BioReliance, 14920 Broschart Road, Rockville, MD 20850. Per this SOP, paper records will be retained for at least three years after which time the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials returned to the Sponsor or destroyed will first be copied and the copy will be retained in the BioReliance archives for a minimum of 10 years.

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12.0 REGULATORY REQUIREMENTS/GOOD LABORATORY PRACTICE

This protocol has been written to comply with OECD Guideline 473 (*In Vitro* Mammalian Chromosome Aberration Test), July 1997 and with the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (1996 and 1997).

This study will be performed in compliance with the provisions of the Good Laboratory Practice Regulations for Non-clinical Laboratory Studies.

Will this study be submitted to a regulatory agency ? Yes
If so, to which agency or agencies? OECD, TSCA

Unless arrangements are made to the contrary, unused dosing solutions will be disposed of following administration to the test system and all residual test substance will be disposed of following finalization of the report.

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Sponsor Project Number: DuPont –5683

BioReliance Study Number: AA41BC.391.BTL

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14.0 APPROVAL

[redacted]

SPONSOR REPRESENTATIVE — 15 MAR 2001 DATE

[redacted]

(Print or Type Name)

[redacted]

BIORELIANCE STUDY DIRECTOR — 22 Mar 2001 DATE

[redacted]

BIORELIANCE STUDY MANAGEMENT — 22 Mar 2001 DATE

MALONALDEHYDE (MALONDIALDEHYDE)

Data were last reviewed in IARC (1985) and the compound was classified in *IARC Monographs* Supplement 7 (1987).

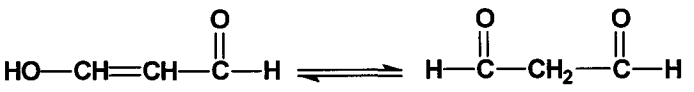
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 542-78-9
Chem. Abstr. Name: Propanedial
IUPAC Systematic Name: Malonaldehyde
Synonym: Malondialdehyde

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_3H_4O_2$ Relative molecular mass: 72.07

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Solid (needles) (United States National Library of Medicine, 1997)
(*b*) *Melting-point*: 72–74°C (IARC, 1985)
(*c*) *Stability*: Highly pure malonaldehyde is quite stable under neutral conditions but not under acidic conditions such as those used to prepare it by hydrolysis of its bis(dialkyl)acetal. Since malonaldehyde has $pK_a = 4.46$, it exists under physiological conditions as its conjugate base (⁻O–CH=CH–CHO), which is relatively stable to self-condensation (IARC, 1985).
(*d*) *Conversion factor*: mg/m³ = 2.95 × ppm

1.2 Production and use

Malonaldehyde is produced and used in small quantities, principally for research purposes (United States National Library of Medicine, 1997).

1.3 Occurrence

1.3.1 *Occupational exposure*

Exposure to malonaldehyde may occur in research laboratories.

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1.3.2 *Environmental occurrence*

Malonaldehyde has been detected in the leaves of pea and cotton plants. It is found in many foodstuffs and can be present at high levels in rancid foods. It has been detected in fish meat, fish oil, rancid salmon oil, rancid nuts, rancid flour, orange juice essence, vegetable oils, fats, fresh frozen green beans, milk, milk fat, rye bread and in raw, cured and cooked meats (United States National Library of Medicine, 1997).

1.3.3 *Human tissues and secretions*

Malonaldehyde is found in human and animal tissue as an end-product of lipid peroxidation. It is also a side-product of prostaglandin and thromboxane biosynthesis. Malonaldehyde is present in blood platelets and in serum (IARC, 1985).

1.4 Regulations and guidelines

The American Conference of Governmental Industrial Hygienists (ACGIH) (1997) has not proposed any occupational exposure limit for malonaldehyde in workplace air.

No international guideline for malonaldehyde in drinking-water has been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Malonaldehyde and its bis(dimethylacetal) and sodium salt were tested for carcinogenicity in mice by skin application. Its bis(dimethylacetal) and sodium salt were tested in mice by oral administration in drinking-water. The two studies by oral administration were inadequate for evaluation. After topical application, no increase in the incidence of skin tumours was observed in one study. In one two-stage mouse-skin assay, a high dose of malonaldehyde (possibly containing impurities) showed initiating activity. In two other two-stage assays using lower doses, no initiating or promoting activity was observed (IARC, 1985).

3.1 Oral administration

3.1.1 *Mouse*

Three groups of 50 male and 50 female $B6C3F_1$ mice, eight weeks of age, were administered 0 (vehicle control), 60 or 120 mg/kg bw malonaldehyde sodium salt (purity, 63–79% malonaldehyde sodium salt; 22–38% water; and 1% impurities as chloride and acetone) in distilled water by oral gavage on five days per week for up to 105 weeks. Survival rates at termination for males were 23/50 control, 19/50 low-dose and 14/50

high-dose ($p < 0.02$, Cox's method) and for females were 41/50 control, 38/50 low-dose and 30/50 high-dose. Body weight was slightly (less than 10%) lower than that of the vehicle controls in high-dose males during the second half of the study. Body weight was slightly (approximately 10%) higher than that of the vehicle controls in high-dose females during most of the study. No tumour type was increased in incidence in malonaldehyde-exposed mice compared with the vehicle controls (United States National Toxicology Program, 1988). [The Working Group noted the high mortality in high-dose males.]

3.1.2 *Rat*

Three groups of 50 male and 50 female Fischer 344/N rats, seven weeks of age, were administered 0 (vehicle controls), 50 or 100 mg/kg bw malonaldehyde sodium salt (purity, 63–79% malonaldehyde sodium salt; 22–38% water; and 1% impurities as chloride and acetone) in distilled water by oral gavage on five days per week for up to 105 weeks, at which time the surviving animals were killed. Survival rates at termination for males were 37/50 control, 33/50 low-dose and 15/50 high-dose and for females were 37/50 control, 37/50 low-dose and 14/50 high-dose. The survival of the high-dose groups was significantly lower than that of the vehicle controls ($p < 0.001$). Mean body weights of high-dose male rats were 10–20% lower than those of the vehicle controls from week 33 to week 72 and 20–26% lower from week 72 to the end of the study. Mean body weights of low-dose male rats were 3–7% lower than those of vehicle controls from week 67 to the end of the study. Mean body weights of high-dose females were 10–20% lower than those of the vehicle controls from week 54 to week 72 and 21–36% lower from week 72 to the end of the study. The incidences of thyroid follicular-cell adenomas were 3/50 control males, 3/49 low-dose males and 9/50 high-dose males ($p < 0.05$) and 2/50 control females, 0/50 low-dose females and 5/50 high-dose females ($p < 0.05$); the incidences of follicular-cell carcinomas were 1/50 control males, 5/49 low-dose males and 5/50 high-dose males ($p < 0.05$) and 0/50 control females, 1/50 low-dose females and 2/50 high-dose females. Overall rates of thyroid follicular-cell tumours were 4/50 control males, 8/49 low-dose males and 13/50 high-dose males ($p = 0.015$) and 2/50 control females, 1/50 low-dose females and 7/50 high-dose females ($p = 0.03$). The incidences of pancreatic islet-cell adenomas in males were 0/49, 9/50 ($p < 0.002$) and 1/49 in the control, low- and high-dose groups, respectively (United States National Toxicology Program, 1988). [The Working Group noted the high mortality and the strong reduction in body weights in the high-dose males and females, indicating that the maximum tolerated dose was exceeded and also that the increased incidences of pancreatic islet-cell adenomas in males were not dose-related.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

No data were available to the Working Group.

4.1.2 *Experimental systems*

Two aldehyde dehydrogenases in the rat-liver cytosol fraction account for virtually all of the metabolizing activity for malonaldehyde (IARC, 1985).

Twelve hours after oral intubation of [1,3-^{14}C]malonaldehyde to rats, 60–70%, 5–15% and 9–17% of radioactivity was recovered in expired CO_2, faeces and urine, respectively (Siu & Draper, 1982).

After oral administration of malonaldehyde (158 mg/kg bw) to rats, increased quantities of formaldehyde, acetaldehyde, acetone and malonaldehyde itself were found in the urine. Additionally, methyl ethyl ketone, not found in control rats, was present in the urine of the animals that had received malonaldehyde (Akubue *et al.*, 1994).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

Malonaldehyde, given in the drinking-water, induced morphological changes in the liver and mild dysplasia at all doses tested (2–500 mg/kg bw per day) in female ICR mice; pancreatic damage was observed at doses of 500 mg/kg bw per day (IARC, 1985). Histological changes were observed in the liver only of female ICR Swiss mice given 10–1000 μg/kg bw malonaldehyde in the drinking-water for one year, but no dose–response relationship was observed (Bird *et al.*, 1982a).

4.3 Reproductive and developmental effects

No data were available to the Working Group.

4.4 Genetic and related effects

4.4.1 *Humans*

Malonaldehyde reacts with DNA to form an adduct with 2′-deoxyguanosine which has been characterized as 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrimido[1,2-α]purin-10(3H)-one (M_1G-dR) (Marnett, 1994). This adduct is present at quantifiable levels in DNA from many human tissues.

Chaudhary *et al.* (1994) found M_1G-dR in DNA from human liver samples (four males, two females) at levels ranging from 5 to 11 adducts/10^7 bases, using gas chromatography/electron capture negative chemical ionization mass spectrometry.

Leuratti *et al.* (1997) found M_1G-dR in DNA from human gastric biopsy samples at levels ranging from 1 to 9 adducts/10^8 normal nucleotides, using a high-performance liquid chromatography/^{32}P-postlabelling method (results presented as an abstract).

Fang *et al.* (1996) found that levels of M_1G-dR were influenced by dietary fats, with men and women consuming unsaturated fats having higher levels of the adduct in DNA from peripheral white blood cells than those who consumed saturated fats.

4.4.2 *Experimental systems* (see Table 1 for references)

Malonaldehyde induced mutation in bacteria that were either DNA repair competent or were sensitive to oxidative DNA damage. It had been suggested previously that all or part of the activity might be attributable to impurities, which occur as a result of malonaldehyde instability. However, specially purified or specially synthesized malonaldehyde continued to show mutagenic activity in *S. typhimurium his* D3052 (Basu & Marnett, 1983).

In one study in *Drosophila melanogaster*, malonaldehyde induced somatic mutations but not sex-linked recessive lethal mutations.

Malonaldehyde formed adducts with purified rat liver DNA, dAMP and dGMP *in vitro*; these adducts were observed with the ^{32}P-postlabelling technique (Wang & Liehr, 1995).

Malonaldehyde induced dose-dependent increases in sister chromatid exchanges but did not produce chromosomal aberrations in Chinese hamster ovary cells, but did induce chromosomal aberrations and micronuclei in rat primary skin fibroblasts.

At concentrations of malonaldehyde which produce reversion to histidine prototrophy in *Salmonella typhimurium* (TA100), characteristic adducts were found in bacterial DNA. The adducts were formed in a dose-dependent manner.

Injection of [^{14}C]malonaldehyde into male C57BL/6 mice resulted in covalent binding to liver DNA and haemoglobin.

Agarwal and Draper (1992) found background levels of malonaldehyde–guanine adduct in rat liver DNA, using high-performance liquid chromatography–fluorescence. Similar results were found by Vaca *et al.* (1992) and Wang and Liehr (1995) using ^{32}P-postlabelling. Chaudhary *et al.* (1994) used gas chromatography/electron capture negative chemical ionization mass spectrometry to measure background levels in rat liver. The levels were significantly elevated following administration of carbon tetrachloride (0.1 mL/kg bw), which induced lipid peroxidation (Chaudhary *et al.*, 1994; Wang & Liehr, 1995).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Malonaldehyde is found in many foodstuffs and can be present at high levels in rancid foods. It is present as a lipid metabolite in human and animal tissues. It is probably used only as a research chemical.

Table 1. Genetic and related effects of malonaldehyde

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
ERD, *Escherichia coli rec* strains, differential toxicity	+	NT	72	Yonei & Furui (1981)
SA0, *Salmonella typhimurium* TA100, reverse mutation	–	NT	NG	Marnett & Tuttle (1980)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	NT	1000	Levin *et al.* (1982)
SA2, *Salmonella typhimurium* TA102, reverse mutation	+	NT	900	Marnett *et al.* (1985)
SA4, *Salmonella typhimurium* TA104, reverse mutation	+	NT	2520	Marnett *et al.* (1985)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	NT	NG[d]	Mukai & Goldstein (1976)
SA5, *Salmonella typhimurium* TA1535, reverse mutation	–	NT	NG	Marnett & Tuttle (1980)
SA7, *Salmonella typhimurium* TA1537, reverse mutation	–	NT	NG[d]	Mukai & Goldstein (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	NG[d]	Mukai & Goldstein (1976)
SA8, *Salmonella typhimurium* TA1538, reverse mutation	–	NT	NG	Marnett & Tuttle (1980)
SA9, *Salmonella typhimurium* TA98, reverse mutation	–	NT	NG	Marnett & Tuttle (1980)
SAS, *Salmonella typhimurium his* G46, reverse mutation	–	NT	NG[d]	Mukai & Goldstein (1976)
SAS, *Salmonella typhimurium his* C3076, reverse mutation	+	NT	NG[d]	Mukai & Goldstein (1976)
SAS, *Salmonella typhimurium his* D3052, reverse mutation	+	NT	NG[d]	Mukai & Goldstein (1976)
SAS, *Salmonella typhimurium* TA1975, reverse mutation	+	NT	500	Shamberger *et al.* (1979)
SAS, *Salmonella typhimurium* TA1977, reverse mutation	+	NT	500	Shamberger *et al.* (1979)
SAS, *Salmonella typhimurium* TA1978, reverse mutation	+	NT	500	Shamberger *et al.* (1979)

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Table 1 (contd)

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
SAS, *Salmonella typhimurium his* C207, reverse mutation	+	NT	500	Shamberger *et al.* (1979)
SAS, *Salmonella typhimurium his* C3076, reverse mutation	+	NT	500	Shamberger *et al.* (1979)
SAS, *Salmonella typhimurium his* D3052, reverse mutation	+	NT	500	Shamberger *et al* (1979)
SAS, *Salmonella typhimurium his* G46, reverse mutation	–	NT	4000	Shamberger *et al.* (1979)
SAS, *Salmonella typhimurium his* D3052, reverse mutation	+	NT	94	Marnett & Tuttle (1980)
SAS, *Salmonella typhimurium his* D3052, reverse mutation	+[c]	NT	72	Basu & Marnett (1983)
ECF, *Escherichia coli* H/r30 (uvr^+rec^+), forward mutation, streptomycin resistance	+	NT	144	Yonei & Furui (1981)
ECR, *Escherichia coli* H/r30 (uvr^+rec^+), forward mutation, arginine prototrophy	+	NT	144	Yonei & Furui (1981)
DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations	–		6125 feed	Szabad *et al.* (1983)
DMM, *Drosophila melanogaster*, somatic mutations	+		6125 feed	Szabad *et al.* (1983)
G5T, Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	+	NT	2 9	Yau (1979)
G51, Gene mutation, mouse lymphoma L5178Y cells, methotrexate resistance *in vitro*	+	NT	3.6	Yau (1979)
SIC, Sister chromatid exchange, Chinese hamster ovary CHO cells *in vitro*	+	+	15	Anderson *et al.* (1990)
MIA, Micronucleus test, Sprague-Dawley rat primary skin fibroblasts *in vitro*	+	NT	7.2	Bird *et al.* (1982)
CIC, Chromosomal aberrations, Chinese hamster ovary CHO cells *in vitro*	–	–	3270	Anderson *et al* (1990)
CIR, Chromosomal aberrations, Sprague-Dawley rat primary skin fibroblasts *in vitro*	+	NT	7.2	Bird *et al.* (1982)
BID, Binding (covalent) to calf thymus DNA *in vitro*	+	NT	7200	Vaca *et al.* (1992)
BID, Binding (covalent) to DNA from *Salmonella typhimurium* TA100 *in vitro*	+	NT	721	Sevilla *et al.* (1997)

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Table 1 (contd)

Test system	Results[a]		Dose (LED or HID)[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
BVD, Binding (covalent) to liver DNA, male C57BL/6 mice *in vivo*	+		22.5 ip × 1	Kautiainen *et al.* (1993)
BVP, Binding (covalent) to haemoglobin, male C57BL/6 mice *in vivo*	+		22.5 ip × 1	Kautiainen *et al.* (1993)
BVD, Binding (covalent) to DNA, C57/BL6 mouse liver *in vivo*	+		1.3 ip × 1	Vaca *et al.* (1992)

[a] +, positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw/day; ip, intraperitoneal injection
[c] Highly purified malonaldehyde
[d] Confusion over actual dose applied

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5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Malonaldehyde sodium salt was tested for carcinogenicity in one experiment in mice and in one experiment in rats by oral administration. No increase in tumour incidence was found in mice. In rats, the incidence of follicular-cell tumours of the thyroid was increased in both sexes at the high dose and the incidence of pancreatic islet-cell adenomas was increased in low-dose males.

Malonaldehyde, its bis(dimethylacetal) and its sodium salts were tested for carcinogenicity in mice by skin application; no carcinogenic activity was observed.

5.4 Other relevant data

Background exposures to malonaldehyde occur in experimental animals and humans, as determined by the presence of specific DNA adducts in blood and other tissues. It is mutagenic to bacteria.

5.5 Evaluation

No epidemiological data relevant to the carcinogenicity of malonaldehyde were available.

There is *limited evidence* in experimental animals for the carcinogenicity of malonaldehyde.

Overall evaluation

Malonaldehyde is *not classifiable as to its carcinogenicity to humans (Group 3).*

6. References

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LABORATORY OF PHARMACOLOGY
AND TOXICOLOGY

LPT

LPT Report No. 7413/92
Degussa AG-US-IT-No. 92-0063-
DGR IUA-No. 5025

EXAMINATION OF THE INFLUENCE OF 1,3-PROPANEDIOL, POLYMER GRADE ON THE PREGNANT RAT AND THE FETUS BY ORAL ADMINISTRATION

(according to OECD method no. 414)

T-1226
DN 2/2/18

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92-0063 DGR

December 21st, 1992

This report consists of 180 pages
and 5 pages of GLP-Certificates

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LABORATORY OF PHARMACOLOGY
AND TOXICOLOGY

LPT

LPT Report No. 7413/92
Degussa AG-US-IT No. 92-0063-
DGC IUA No. 5025

MANAGEMENT STATEMENT

EXAMINATION OF THE INFLUENCE OF
1,3-PROPANEDIOL, POLYMER GRADE
ON THE PREGNANT RAT AND THE FETUS BY ORAL ADMINISTRATION

(according to OECD method no. 414)

The study was performed according to

- 'Good Laboratory Practice' Regulations of the EEC enacted in Germany in the 'Chemikaliengesetz' dated March 14th, 1990, BGBL. I, pp. 521, 1990;
- 'Nonclinical Laboratory Studies. Good Laboratory Practice Regulations' of the FDA (USA); Federal Register, 43, No. 247, dated December 22nd, 1978, Federal Register, 52, No. 172, dated September 4th, 1987;
- 'Good Laboratory Practice' Regulations of the OECD, dated February 4th, 1983;
- 'Good Laboratory Practice' Standards for Safety Studies on Drugs of Japan, Notification No. 313 of the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, dated March 31st, 1982 and subsequent amendment dated October 5th, 1988.

The management hereby certifies that in the course of controls conducted by QAU on quality, integrity and conformity to protocol, no findings were revealed which are considered to have an impact on the validity of the data or on the interpretation of the results in this report.

[redacted]

3.2.1993

Prof.Dr.med.F.Leuschner
(Management)

Date

[redacted]

3.2.93

Dr.rer.nat.K.-E.Mitterer
(Study Director)

Date

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LABORATORY OF PHARMACOLOGY
AND TOXICOLOGY

LPT

LPT Report No. 7413/92
Degussa AG-US-IT No. 92-0063-
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QUALITY ASSURANCE STATEMENT

Based on a quality assurance review, it was concluded that this report accurately reflects the data for the

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- 'Good Laboratory Practice' Standards for Safety Studies on Drugs of Japan, Notification No. 313 of the Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, dated March 31st, 1982 and subsequent amendment dated October 5th, 1988.

Protocol dated June 9th, 1992 and 4 amendments.

Controls on accuracy and correspondence with the protocol were performed on 17.09.1992, 28.09.1992, 13.10.1992, 10.11.1992 and 03.02.1993. The results were reported to the study director and the management on the same or the next following working day.

Approved and submitted by: [redacted] 9.2.93

F. Hübscher
Director of Quality
Assurance Unit (QAU)

Date

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EXAMINATION OF THE INFLUENCE OF
1,3-PROPANEDIOL, POLYMER GRADE
ON THE PREGNANT RAT AND THE FETUS BY ORAL ADMINISTRATION

(according to OECD method no. 414)

Signature list of scientists and further professionals involved in this study/date:

Study Director: [redacted] 21.12.92
Dr.rer.nat.K.-E.Mitterer

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Prof.Dr.med.F.Leuschner

Veterinarian: [redacted] 21.12.92
Dipl.vet.med.A.Dörr

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W.Hartmann

Pathology: [redacted] 21.12.92
Dr.rer.nat.K.-E.Mitterer

Statistics: [redacted] 21.12.92
Dr.rer.nat.A.Leuschner

EXPLANATIONS AND ABBREVIATIONS USED IN THIS REPORT

1. **Abbreviations and symbols**

b.w.	=	body weight
m	=	male
f	=	female
M	=	mean value
SD	=	standard deviation
#	=	reference to foot-notes in the tables
[1] - n	=	reference to foot-notes in the text

2. **Table 7** (pages 40 - 43)

(1) = to reach a total of 20 pregnant rats/group, 25 animals/group were mated

(2) = 20 pregnant rats/group were evaluated (cf study protocol, dated June 9th, 1992);

3. **Some units of measurement**

absolute food consumption in grams

relative food consumption in grams/kg b.w./day

body weight in grams

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1. GENERAL INFORMATION

1.1.	Aim of experiment	examination of the influence of 1,3-Propanediol, Polymer Grade, administered orally during the 'critical' phase of organogenesis on the pregnant rat and the fetus
1.2.	Route of administration	oral, by gavage
1.3.	Selection of route of administration	the oral route is a likely route for human exposure to the test material
1.4.	Test substance	

Test compound:	designation	:	1,3-Propanediol, Polymer Grade (CAS 504-63-2) abbreviated to '1,3-Propanediol' in this report
	batch no.	:	22, control no. 2712 (April 1991)
	characteristics	:	liquid, colourless, odourless
	storage conditions	:	in a closed container
	stability	:	stable until June 1997
	strength	:	99.8%

see appendix 1 for certificate of analysis

Sample of the test substance for retention:	stored at LPT Laboratory of Pharmacology and Toxicology Prof.Dr.med.F.Leuschner Redderweg 8 D-2104 Hamburg 92 (after 1-2 years at branch station Mienenbüttel)
Vehicle:	0.8% aqueous hydroxypropyl-methylcellulose gel[1] batch no. MM90100512E
Dose-levels:	250 mg and 1000 mg/kg b.w.
Control group:	10 ml vehicle/kg b.w.
Administration volume:	10 ml/kg b.w.

[1] METHOCEL E 4 M, supplier: SYNOPHARM Apotheker Fritz Zimmermann D-2000 Barsbüttel b. Hamburg

1.5. Experimental animals

Species:	rat
Strain:	Sprague-Dawley
Stock:	Hag: SD
Breeder:	Lippische Versuchstierzucht HAGEMANN GmbH D-4923 Extertal 1
Selection of species:	the rat is a commonly used rodent species for such type of studies
Number of animals:	75 females (incl. 15 reserve animals), sufficient number of animals to grant at least 60 pregnant rats
Groups:	1 control : 20 pregnant females 2 250 mg/kg b.w. : 20 pregnant females 3 1000 mg/kg b.w. : 20 pregnant females
Course of treatment:	from the 6th to 15th day of pregnancy

1.6. Sponsor/Testing facility/Responsible personnel

Sponsor:	Initiative Umweltrelevante Altstoffe e.V. Kennedyallee 93 D-6000 Frankfurt 70
Testing facility:	CHR.FRED LEUSCHNER & Co. Laboratory for Toxicological and Pharmacological Examinations D-2355 Löhndorf/Post Wankendorf
Study director:	Dr.rer.nat.K.-E.Mitterer CHR.FRED LEUSCHNER & Co. Laboratory for Toxicological and Pharmacological Examinations D-2355 Löhndorf/Post Wankendorf
Management:	Prof.Dr.med.F.Leuschner
Veterinarian:	Dipl.vet.med.A.Dörr
Animal husbandry:	W.Hartmann
Pathology:	Dr.rer.nat.K.-E.Mitterer

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Statistics:	Dr.rer.nat.A.Leuschner
Quality Assurance Unit (QAU):	F.Hübscher

The study was performed according to the OECD method no. 414, the EEC guidelines and in compliance with the principles of 'Good Laboratory Practice' Regulations (cf Management- and QAU-Statement as well as the enclosed 5 pages of GLP-certificates for details).

Archives of data and biological material:	all specimens, raw data and other documents generated at LPT during the course of this study, together with the copy of the final report, were lodged in the LPT archives; duration of storage according to the periods laid down in the ChemG
during the study:	in archives 8 and 9 CHR.FRED LEUSCHNER & Co. Laboratory for Toxicological and Pharmacological Examinations D-2355 Löhndorf/Post Wankendorf
after reporting:	written raw data and biological material and the copy of the final report in the archive Löhndorf D-2355 Löhndorf/Post Wankendorf
Standard Operation Procedure (SOP):	all works were carried out according to standard operation procedures and during all stages of the study; they may be expected in those divisions which were engaged in the study and in the Quality Assurance Unit (QAU)

1.7. Study dates

Start of study (first day of conception):	September 22nd, 1992
1st administration:	September 28th, 1992
Termination of the experimental phase:	November 16th, 1992
Date of final report:	December 21st, 1992
Date of protocol:	June 9th, 1992
Amendment:	No. 1, dated August 24th, 1992 No. 2, dated September 22nd, 1992 No. 3, dated November 4th, 1992 No. 4, dated November 16th, 1992

2. SUMMARY

2.1. Conduct of study:

Test substance:	1,3-Propanediol, Polymer Grade (CAS 504-63-2), batch no. 22, control no. 2712 - abbreviated to '1,3-Propanediol' in this report.
Test species:	Rat/Sprague-Dawley Hag: SD/Lippische Versuchstierzucht HAGEMANN GmbH, D-4923 Extertal 1. Three groups of 20 female rats each (plus 5 female rats/group as reserve).
Route of administration:	Oral, by gavage.
Dose-levels:	Group 1: Control (vehicle) Group 2: 250 mg 1,3-Propanediol/kg b.w. Group 3: 1000 mg 1,3-Propanediol/kg b.w. (treatment from day 6 to 15 of gestation).
Vehicle:	0.8% aqueous hydroxypropyl-methylcellulose gel.
Volume of administration:	10 ml/kg b.w.

2.2. Findings:

Influence on the dam

Mortality:	No substance-related mortality was observed.
Clinical symptoms:	No clinical signs were observed in the low- and the high-dosed dams.
Body weight, body weight change:	Body weights remained within the normal range at 250 mg and 1000 mg 1,3-Propanediol/kg b.w., by gavage, body weight gain showed no distinct influence of the test compound at 250 mg/kg b.w., also. Body weight gain was slightly (-32%) but not significantly (at $p \leq 0.01$) inhibited between gestation days 6 and 9 in the high-dosed dams (1000 mg/kg b.w.).
Food consumption, drinking-water consumption:	The food consumption was not impaired at 1,3-Propanediol doses of 250 mg and 1000 mg/kg b.w., by gavage. Treatment did not influence drinking-water consumption.
Autopsy findings:	No substance-related pathological changes were detected at autopsy.

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Influence on the fetus:

No distinct influence on the prenatal development was detected.
The fetal incidence of all retardations was slightly and significantly increased (FISHER's exact test, $p \leq 0.05$) at the skeletal examination (staining of the skeleton according to DAWSON's method) for the high dose-level group, in particular the skull (retarded ossification): 250 mg/kg: $p \leq 0.05$, 1000 mg/kg: $p \leq 0.01$. However, the significance observed is due to the low incidence of retarded skull ossification found in the control group in this study which is much below the background incidence. The incidences found for the dose-level groups are within the range of the background hence no biological relevance was attached to this finding. All other fetal parameters were within the normal range of the control group.
External macroscopic inspection and examination of soft tissue (WILSON's method) revealed no substance-related variations and/or retardations.

No malformed fetuses were detected in the substance-treated groups (control: one fetus with shortened hindlimbs and missing toes at the right foot).
No dead fetuses occurred in the substance treated and control dams.

2.3. Conclusion:

Under the present test conditions, the no-observed-effect-level (NOEL) for the dams and for the fetal organism was 250 mg 1,3-Propanediol/kg b.w., by gavage (treatment from the 6th to 15th day of pregnancy). 1000 mg/kg b.w. was within the beginning maternal and fetotoxic range.

1,3-Propanediol did not possess teratogenic properties.

3. INTRODUCTION

The present paper reports on the results of an experiment in which the influence of 1,3-Propanediol, Polymer Grade, batch no. 22, control no. 2712, abbreviated to '1,3-Propanediol', on the pregnant rat and the fetus was examined by oral administration.
1,3-Propanediol was administered to pregnant rats during the 'critical' phase of organogenesis. Particular attention was paid to possible teratogenic properties. The oral route of administration was chosen according to the potential exposure to man.

The study was performed according to the OECD method no. 414, the EEC guidelines and in compliance with the principles of 'Good Laboratory Practice' Regulations (cf Management-Statement, QAU-Statement and the enclosed 5 pages - incl. 4 GLP-certificates of LPT for details).

4. IDENTIFICATION OF THE TEST SUBSTANCE

After receipt at LPT the test compound was inspected. Batch number(s), amount and characteristics (i.e. colour, consistency, form and size of particles) were recorded and compared with information given by the sponsor. An identification sheet is filed with the raw data.

5. CHOICE OF DOSE-LEVELS

During a pilot study, using 2 pregnant rats/group from the same strain as selected for the main experiment, 1,3-Propanediol dose-levels of 62.5 mg, 125.0 mg, 250.0 mg, 500.0 mg and 1000.0 mg/kg b.w., by gavage, were administered from day 6 to 15 of pregnancy. In addition, a control group was treated with 0.8% hydroxypropyl-methylcellulose gel for control purposes.
1000.0 mg/kg b.w. is the highest requested dose according to the OECD method 414 (limit test for embryotoxicity).
Except for a slight transient toxic effect after the first dosing (very slightly reduced food consumption), all dose-levels up to 1000.0 mg/kg b.w. were well-tolerated by the dams. None of the doses influenced the prenatal development (cf separate LPT report dated August 24th, 1992).

Based on the afore-described results, 250.0 mg and 1000.0 mg 1,3-Propanediol/kg b.w./day, by gavage, treatment from the 6th to 15th day of pregnancy were selected by the sponsor as doses for the main experiment.

6. STABILITY OF TEST COMPOUND/VEHICLE PREPARATIONS

The test substance is stable until June 1997 according to written information given by the sponsor on June 2nd, 1992.
The substance-vehicle suspensions were freshly prepared each day immediately before dosing.

For the determination of the concentration and homogeneity in the substance-vehicle preparations the following samples were taken at the time-points stated below and were stored at -20°C or colder until analysis by LPT. The results are reported in appendix 4 to this report.

start of treatment

Samples of approx. 10 ml were taken for each dose group immediately after preparation of the test suspensions as well as 8 and 24 hours after storage at room temperature (3 samples/dose-level). These samples were needed for determination of the concentration.

termination of treatment

Samples of approx. 10 ml were taken for each dose group during treatment with the test suspension always before administration to the last animal/dose-level (1 sample/dose-level) for determination of the concentration.

7. METHODS

7.1. Principle

Pregnant rats are treated with the test substance during the 'critical' phase of organogenesis. Their development during the gestation period is observed. On day 20 of pregnancy the animals are laparotomised and examined macroscopically for implantation sites, resorptions in the uterus and for condition of the fetuses.

7.2. Animals

For this experiment sexually mature, female Sprague-Dawley rats, Hag: SD, a strain bred by Lippische Versuchstierzucht HAGEMANN GmbH, D-4923 Extertal 1, were used. The animals had an adaptation period of 7 days. The body weight of the animals was 193 g to 242 g at the start of study (day 0 of pregnancy), at this time-point the age of the animals was approx. 56 days.

For identification, each rat received a continuous number between 1 and 75. A pattern of points was set on paws and/or tail by tattoo. Additionally, the animal cages were numbered.

Fertile ('proved') 4 - 12 months old male rats of the same breed served as partners. They were repeatedly employed, at the earliest three days after successful copulation. The female breeding partners were randomly chosen.
Matings were monogamous.

7.3. Dose-levels and administration

Three groups of pregnant rats were established each obtained from matings which were carried out on a daily basis. The rats were assigned to their respective group according to their mating day i.e. in a cyclic way following the listing of positive findings in the vaginal smear. Day 0 of pregnancy was the day on which sperm was found in the vaginal smears.

Group	1,3-Propanediol dose in mg/kg b.w./day by gavage	Number of female rats used	Rat Nos.
1 female animals	control: 10 ml 0.8% aqueous hydroxypropyl-methylcellulose gel/ kg b.w./day by gavage	25	1 - 25
2 female animals	250	25	26 - 50
3 female animals	1000	25	51 - 75

In order to obtain 20 pregnant animals/group more animals (25/group) were used at the start of the study.

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In all, 40 animals showing signs of pregnancy and treated with 1,3-Propanediol were compared with 20 pregnant control rats, employing always the first 20 surviving pregnant animals per group.

0.8% aqueous hydroxypropyl-methylcellulose gel served as the vehicle for the test substance. Administration by gavage was carried out from the 6th to the 15th day of pregnancy (constantly administered volume: 10 ml/kg b.w.). The control animals received 10 ml of the vehicle/kg b.w./day in the same manner. The amount of test compound was adjusted daily to the actual body weight.

The male rats remained untreated.

7.4. Food, water and accommodation

ALTROMIN 1314 (supplied by: ALTROMIN GmbH, P.O.Box 285, D-4937 Lage/Lippe, composition: see 7.4.1.) served as food. This food and tap water (in bottles) were offered daily ad lib. Periodic analyses of food and bedding material (granulated wood, type A2, supplier: J.Rettenmaier & Söhne GmbH & Co., D-7092 Ellwangen-Holzmühle) for contaminants are conducted at least twice a year by the Landwirtschaftliche Untersuchungs- und Forschungsanstalt Kiel, Gutenbergstr. 77, D-2300 Kiel 1.

All analyses were based on EPA/USA, Proposed Health Effects Test Standards for Toxic Substances Control Act Test Rules, Federal Register 44, 27334 - 27375, May 1979. The results were within the admissible limits (limitations for contaminants in the diet and the bedding material are given at 7.4.2. and 7.4.3.).

Samples of drinking-water are taken twice a year by Wasserwerk Wankendorf and periodic analyses are performed by the Institut für Tiergesundheit und Lebensmittelqualität der Landwirtschaftskammer Schleswig-Holstein, Gutenbergstraße 77, D-2300 Kiel 1.

In addition, the analyses are performed once a year by the Institut für Tiergesundheit und Lebensmittelqualität (ITL) der Landwirtschaftskammer Schleswig-Holstein according to EPA/USA, Proposed Health Effects Test Standards for Toxic Substances Control Act Test Rules, Federal Register 44, 27334 - 27375, May 1979. The results were within the admissible limits.

Limitation of contaminants in the drinking-water see 7.4.4.

The dams were kept singly in MAKROLON cages type III. The room temperature was 22°C ± 2°C (maximum range) and the relative humidity 50% ± 15% (maximum range). Deviations from the maximum range caused for example during the cleaning procedure, are dealt with in SOPs. Rooms were lit (150 lux at 1.50 m room height) in a 12-hour light/12-hour dark cycle.

The cages were cleaned and changed once a week.

7.4.1. Composition of the diet

Standard Diet for Rats and Mice

ALTROMIN 1314

(ALTROMIN GmbH, D-4937 Lage/Lippe)

Ingredients (average % content in the diet)	
crude protein	22.5
crude fat	5.0
crude fibres	4.5
ash	6.5
moisture	13.5
nitrogen-free extract	48.0

Metabolizable Energy	
kcal/kg	3000.0
MJ/kg	12.5

Amino Acids (average % content in the diet)	
lysine	1.20
methionine	0.40
cystine	0.30
phenylalanine	1.00
tyrosine	0.70
arginine	1.40
histidine	0.50
tryptophane	0.20
threonine	0.80
isoleucine	1.00
leucine	1.80
valine	1.10

Minerals (average % content in the diet)	
calcium	0.9
phosphorus	0.7
magnesium	0.2
sodium	0.2
potassium	1.0

Trace Elements (average content in mg per 1000 g of diet)	
manganese	45.0
iron	160.0
copper	13.0
zinc	60.0
iodine	1.0
fluorine	15.0

Vitamins
(additive per 1000 g of diet)

vitamin A	15000.0	IU
vitamin D_3	600.0	IU
vitamin E	75.0	mg
vitamin K_3	3.0	mg
vitamin B_1	18.0	mg
vitamin B_2	12.0	mg
vitamin B_6	9.0	mg
vitamin B_{12}	24.0	µg
nicotinic acid	36.0	mg
pantothenic acid	21.0	mg
folic acid	2.0	mg
biotin	60.0	µg
choline	600.0	mg
vitamin C	36.0	mg

7.4.2. Limitation of contaminants in the diet

	min.	max.
Aflatoxin (B_1, B_2, G_1, G_2), ppb total		5.0
Lindane, ppb		20.0
Heptachlor, ppb		20.0
Malathion, ppm		2.5
DDT (total), ppb		100.0
Dieldrin, ppb		20.0
Cadmium, ppb		160.0
Arsenic, ppm		1.0
Lead, ppm		1.5
Mercury, ppb		100.0
Selenium, ppm	0.1	0.6
PCB, ppb		50.0

7.4.3. Limitation of contaminants in the bedding material (in mg/kg)

	max.
Aflatoxin (B_1)	0.01
Chlordane	0.05
Endrine	0.02
Fluorine	150.00
Lindane	0.10
Heptachlor and epoxide	0.03
DDT, DDE, DDD	0.05
Dieldrine and aldrine	0.02
Arsenic	2.00
Lead	5.00
Mercury	0.10
Nitrite (Na-Nitrite)	15.00
HCB	0.03

7.4.4. Limitation of contaminants in the drinking-water

	min.	max.
Aflatoxin (B_1, B_2, G_1, G_2), ppb total		5.0
Estrogenic Activity, ppb (DES eq.)		1.0
Lindane, ppb		20.0
Heptachlor, ppb		20.0
Malathion, ppm		2.5
DDT (total), ppb		100.0
Dieldrin, ppb		20.0
Cadmium, ppb		160.0
Arsenic, ppm		1.0
Lead. ppm		1.5
Mercury, ppb		100.0
Selenium, ppm	0.1	0.6
PCB, ppb		50.0
Nitrosamine, ppb		10.0

7.5. Breeding test

Mating was monogamous: 1 male and 1 female animal were placed in one cage during the dark period. Each morning a vaginal smear was taken to check for the presence of sperm. If the smear was negative, mating was repeated. The day on which sperm was found was considered the day of conception (day 0 of pregnancy).
This procedure was repeated until enough pregnant dams were available for the groups. Rats which did not become pregnant were excluded from the analysis of the results and replaced by other animals.

7.6. **Examinations**

Dams

7.6.1. Daily checks (once in the morning and once as late on the day as practicable) were performed on behaviour, external appearance, mortality and faeces.

7.6.2. Body weight was ascertained daily - always at the same time in the morning - and these measurements were also used for calculating the daily amount of test compound to be administered.
Body weight change: The difference between the body weights for the periods 0-3, 3-6, 6-9, 9-12, 12-15, 15-18, 18-20 were calculated.

7.6.3. Food consumption was determined daily by weighing the residue. Intake of drinking-water was observed daily.

7.6.4. Dissection technique and evaluation:
On the 20th day of gestation the surviving rats were laparotomised under ether narcosis. The ovaries and uteri were removed and examined. To check for possible drug effects a dissection was carried out which included macroscopic examination of internal organs. A full macroscopic inspection was carried out in the prematurely deceased dams as soon as possible after their exitus.

Fetuses were removed and the following examinations performed:

A count was taken of fetuses and placentae.

Sex and viability of fetuses were determined. Animals are said to be viable when they are found alive (spontaneous breathing, spontaneous movement).

Number and size of resorptions were determined.

Corpora lutea in the ovaries, implantations and location of fetuses in the uterus were determined.

The gravid uterus weight was determined.

Weights and length of fetuses and weight of the placentae were determined (fetuses were considered as runts if their weight was less than 70% of the mean litter weight).

Fetuses were inspected externally for damages, especially for malformations.

Fetuses were dissected and the number and type of possible variations (incl. retardations) or malformations[2] was determined macroscopically:

In 50% of the number of fetuses/litter thorax and peritoneal cavity (without damage to ribs and sternum) were opened: location, size and condition of the internal organs were determined. Then the skeletons were stained with Alizarin according to DAWSON, the skeletal system was examined. Determination of the number and an examination of retardations, variations as well as malformations was carried out.

In 50% of the number of fetuses/litter body sections were made and examined for variations according to WILSON.

Evaluation/parameters

Corpora lutea per dam

Implantations per dam

Fetuses (alive and dead) per dam

Resorptions per dam

early resorptions: < 2 mm
late resorptions: > 2 mm

$$\text{resorption rate in \%} = \frac{\text{resorptions}}{\text{implantations}} \times 100$$

Runts per dam

Malformed fetuses per dam

$$\text{malformation rate in \%} = \frac{\text{malformed fetuses}}{\text{fetuses}} \times 100$$

Variations (incl. retardations)

$$\text{variation rate in \%} = \frac{\text{fetuses with variations}}{\text{fetuses}} \times 100$$

$$\text{Pre-implantation loss in \%} = \frac{\text{corpora lutea-implantations}}{\text{corpora lutea}} \times 100$$

$$\text{Post-implantation loss in \%} = \frac{\text{implantations-living fetuses}}{\text{implantations}} \times 100$$

7.7. Statistical evaluation

The following statistical methods were used:

After randomisation of the animals an analysis of variance was carried out.

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2 for definition of 'malformations' and 'variations/ retardations' see appendix 2

Homogeneity of variances was tested for mean values by using the Bartlett chi-square test. When the variances are homogeneous, a one-way analysis of variance was applied. When the results indicate a significant difference among groups, the DUNNETT test ($p \leq 0.01$) was used to compare the experimental groups with the control group.

In case of heterogeneity of variances, Student's t-test was carried out, limit of significance was $p \leq 0.01$. All data were evaluated statistically in this manner. In tables in which the individual results differ significantly from those of the control group, these data are stated.

For the comparison of malformation-, resorption-, retardation- and variation rates the

Exact test of R.A.FISHER's ($p \leq 0.05$) or chi^2-test

were employed.

7.8. Experimental period

This experiment was carried out from September 22nd, 1992 (first day of conception) to November 16th, 1992 (termination of the experimental phase).

8. RESULTS

8.1. Examination of the dams:

The results of this part of the study can be found in the summary tables 1 (maternal clinical symptoms), 2 (maternal body weights), 3 (mean maternal body weight change), 4 (mean gravid uterine weights and net maternal body weight change), 5 (maternal food consumption), 6 (maternal necropsy observations) and 7 (summary of reproduction data).
The relevant individual values are to be found in tables A 1 - A 7.

Synopsis of Animals examined/evaluated

Dosage	Number of rats mated	Animal nos. of pregnant rats	Deceased dams (nos.)	Not pregnant dams[3] (nos.)	Reserve animals (not examined)
Control	1 - 25	1-3, 5-15, 17-22 (n = 20)	0	4, 16	23, 24, 25
250 mg/kg	26 - 50	26-27, 29-35, 37-47 (n = 20)	0	28, 36	48, 49, 50
1000 mg/kg	51 - 75	53 - 72 (n = 20)	0	51, 52	73, 74, 75

8.1.1. Behaviour, external appearance, faeces, mortality

In the dams treated with 250 mg and 1000 mg 1,3-Propanediol/kg b.w., by gavage (treatment from the 6th to 15th day of gestation) no substance-related influence was found on behaviour, external appearance and faeces.

No dam died prematurely.

[3] not pregnant = negative according to SALEWSKI, E.: Naunyn-Schmiedebergs arch. exp. pathol. and pharmacol., 247, 367 (1964)

8.1.2. Food and drinking-water consumption

Absolute and relative food consumption remained unchanged at 250 mg and 1000 mg 1,3-Propanediol/kg b.w., by gavage, treatment from the 6th to 15th day of gestation.

Drinking-water consumption showed no substance-related changes.

8.1.3. Body weight and body weight change

Body weight and body weight gain were within the normal range of the controls at 250 mg 1,3-Propanediol/kg b.w., by gavage, treatment from the 6th to 15th day of gestation.

This is also true for body weights of the high-dosed dams (1000 mg 1,3-Propanediol/kg b.w., by gavage, treatment from the 6th to 15th day of gestation). However, there was a tendency towards an inhibition of body weight gain between the 6th and 9th day of pregnancy (-32%). The difference from the controls was not significant at $p \leq 0.01$.

8.1.4. Examinations of the dams at termination

8.1.4.1. Necropsy findings

There were no substance-related observations at necropsy in any of the dams.

The low-dosed dam no. 46 (250 mg 1,3-Propanediol/kg b.w./day, by gavage, treatment from the 6th to 15th day of pregnancy) showed a spontaneous induration of the right lobe of the lungs, in addition, the pericardium of this rat was adhered to the lungs and the thymus.

8.1.4.2. Uterus weight

The gravid uterus weights of the animals treated with 250 mg and 1000 mg 1,3-Propanediol/kg b.w., by gavage, treatment from the 6th to 15th day of gestation were not influenced by the exposure to the test compound.

8.1.4.3. Reproduction data of dams

The fertility rate was 90.9% for all three groups.

There were no substance-related and/or statistically significant differences between the groups (control, 250 mg and 1000 mg 1,3-Propanediol/kg b.w., by gavage) in conception rate, in the mean number of corpora lutea and implantation sites or in the values calculated for the pre-implantation loss and the number of viable fetuses. The number of resorptions and consequently the post-implantation loss were not increased at 250 mg and 1000 mg/kg b.w. (resorption rate low dose: 5.7%, high dose: 7.6% and post-implantation

loss low dose: 5.8%, high dose: 7.6%; resorption rate and post-implantation loss of control dams: 10.8% and 10.8%, respectively).

8.2. Examination of the fetuses:

Results of this part can be found in the summary tables 8 (mean placental weights and fetal body weights), 9 (mean fetal lengths), 10 (summary of all classified fetal external observations), 11 (summary of fetal external malformations), 12 (summary of fetal external variations), 13 (summary of fetal skeletal retardations and/or variations), and 14 (summary of fetal soft tissue variations).
The relevant individual values or findings are to be found in tables B 1 - B 7.

8.2.1. Sex distribution of fetuses

The sex distribution of the fetuses in test groups 2 and 3 (250 mg and 1000 mg 1,3-Propanediol/kg b.w., by gavage, treatment from the 6th to 15th day of gestation) was comparable with the control fetuses. The differences observed in comparison to the control are without any biological relevance.

8.2.2. Weight of placentae

The mean placental weights in test groups 2 and 3 (250 mg and 1000 mg 1,3-Propanediol/kg b.w., by gavage) were not influenced by the administration of the test compound to the dams. The differences observed in comparison to the control are without any biological relevance.

8.2.3. Weight and length of fetuses

The mean fetal weights and length were not influenced by the test substance exposure. All values were within the range of biological variation.

8.2.4. External examination of the fetuses

The external examination of the fetuses revealed no malformations at the two tested dose-levels of 250 mg and 1000 mg 1,3-Propanediol/kg b.w., by gavage, treatment from the 6th to 15th day of gestation.
One control fetus from the dam no. 5 showed shortened hindlimbs (bilateral), toes of the right foot were missing.

No macroscopically visible variation was found at 250 mg and 1000 mg 1,3-Propanediol/kg b.w., by gavage, treatment from the 6th to 15th day of gestation.
One variation (haemorrhage behind the lens of the right eye) was detected in one control fetus of dam no. 1.

Dead fetuses did not occur in the substance treated and control dams.

These changes belong to the spontaneous range as to their type and the number of affected fetuses.

8.2.5. Skeletal examination of the fetuses

No malformations were observed during skeletal examinations (according to the method of DAWSON) in fetuses from dams treated with 250 mg and 1000 mg 1,3-Propanediol/kg b.w., by gavage (treatment from the 6th to 15th day of pregnancy).
One malformed control fetus is described in section 8.2.4. 'External examination of the fetuses'.

The variations elicited were related to the ribs (accessory 14th ribs, wavy ribs) and the sternum (sternebra(e) bipartite or misaligned) and were found in all groups - including the controls - to about the same extent.

In all groups signs of retardations (incomplete or missing ossification of hyoid, skull, 5th metacarpalia, vertebral bodies and/or sternebra(e)) were found without any clear differences of biological relevance between the groups (incl. the controls). The fetal incidence of all retardations was slightly and significantly increased (FISHER's exact test, $p \leq 0.05$) at the highest tested dose (1000 mg 1,3-Propanediol/kg b.w., by gavage). A significantly retarded ossification of the skull was seen at 250 mg 1,3-Propanediol/kg b.w., by gavage (FISHER's exact test, $p \leq 0.05$) and at 1000 mg 1,3-Propanediol/kg b.w., by gavage (FISHER's exact test, $p \leq 0.01$). The significance is due to the low incidence in the control group in this study which is much below the background incidence. The incidences found in the dose-level groups are within the range of the background data (45.7% ± 17.4%) and hence the significance is not considered to be substance-related.

8.2.6 Soft tissue examination of the fetuses

The examination of the organs of the fetuses (method according to WILSON) revealed no malformations in test groups 2 and 3 (250 mg and 1000 mg 1,3-Propanediol/kg b.w., by gavage).

Variations were detected in all groups. The very common findings (uni- or bilateral dilated renal pelvis, haemorrhages of the liver, 4th cerebral ventricle enlarged) in the rat strain used in this study occurred without any dose-response relationship.
The type and number of variations found in the 1,3-Propanediol treated groups were similar to those found in the control group.

For overall assessment of the above-mentioned findings see 8.2.7.

8.2.7. Abstract and assessment of fetal external, soft tissue and skeletal observations

As can be seen from tables 10-14 there are no statistically significant differences between the different test groups at all concerning summarised external, soft tissue, skeletal and overall **malformations** and **variations**. Skeletal **retardations** (fetal incidence) were slightly and significantly increased (FISHER's exact test, $p \leq 0.05$) at the highest test dose of 1000 mg/kg b.w.. No external findings were made in the low- and high-dosed fetuses (250 mg and 1000 mg 1,3-Propanediol/kg b.w., by gavage).

All other differences between the control and the 250 mg and 1000 mg/kg b.w. fetuses are without any biological relevance, because they were found without any dose-response relationship.

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE 1 Summary of Maternal Clinical Symptoms

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Dams evaluated	n =	20	20	20
No remarkable observations	n =	20	20	20
	%	100.0	100.0	100.0

explanations and abbreviations see preface of the report

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1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

TABLE 2 Mean Maternal Body Weights during Gestation - Grams

Time-Point		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Day 0	M	209.3	210.5	214.9
	SD	9.2	11.1	9.7
	n =	20	20	20
Day 3	M	221.7	221.4	226.5
	SD	9.4	10.9	10.8
	n =	20	20	20
Day 6	M	235.5	236.0	239.7
	SD	10.9	13.0	11.2
	n =	20	20	20
Day 9	M	246.6	245.2	247.1
	SD	12.4	14.5	14.7
	n =	20	20	20
Day 12	M	261.1	260.6	262.4
	SD	13.6	16.5	15.2
	n =	20	20	20

continued on next page

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1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE 2 Mean Maternal Body Weights during Gestation - Grams

Time-Point		Test Group 1 Control 10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Day 15	M	277.0	275.4	278.3
	SD	16.4	18.4	15.6
	n =	20	20	20
Day 18	M	312.0	311.6	310.1
	SD	20.9	22.7	19.6
	n =	20	20	20
Day 20	M	339.9	342.1	341.1
	SD	24.5	25.2	19.6
	n =	20	20	20

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explanations and abbreviations see preface of the report

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1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Mean Maternal Body Weight Change during Gestation - Grams

TABLE 3

Time-Period		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Days 0 to 3	M	12.4	11.0	11.6
	SD	3.5	4.9	4.2
	n =	20	20	20
Days 3 to 6	M	13.8	14.6	13.2
	SD	4.9	4.6	3.4
	n =	20	20	20
Days 6 to 9	M	11.1	9.2	7.5 ? very slight
	SD	5.1	8.2	8.0
	n =	20	20	20
Days 9 to 12	M	14.5	15.4	15.3
	SD	4.5	6.2	4.3
	n =	20	20	20
Days 12 to 15	M	15.9	14.8	15.9
	SD	5.1	6.8	4.5
	n =	20	20	20

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explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE 3 Mean Maternal Body Weight Change during Gestation - Grams

Time-Period		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Days 15 to 18	M	35.1	36.2	31.9
	SD	7.3	6.4	11.7
	n =	20	20	20
Days 18 to 20	M	27.9	30.5	31.0
	SD	5.3	4.7	10.5
	n =	20	20	20

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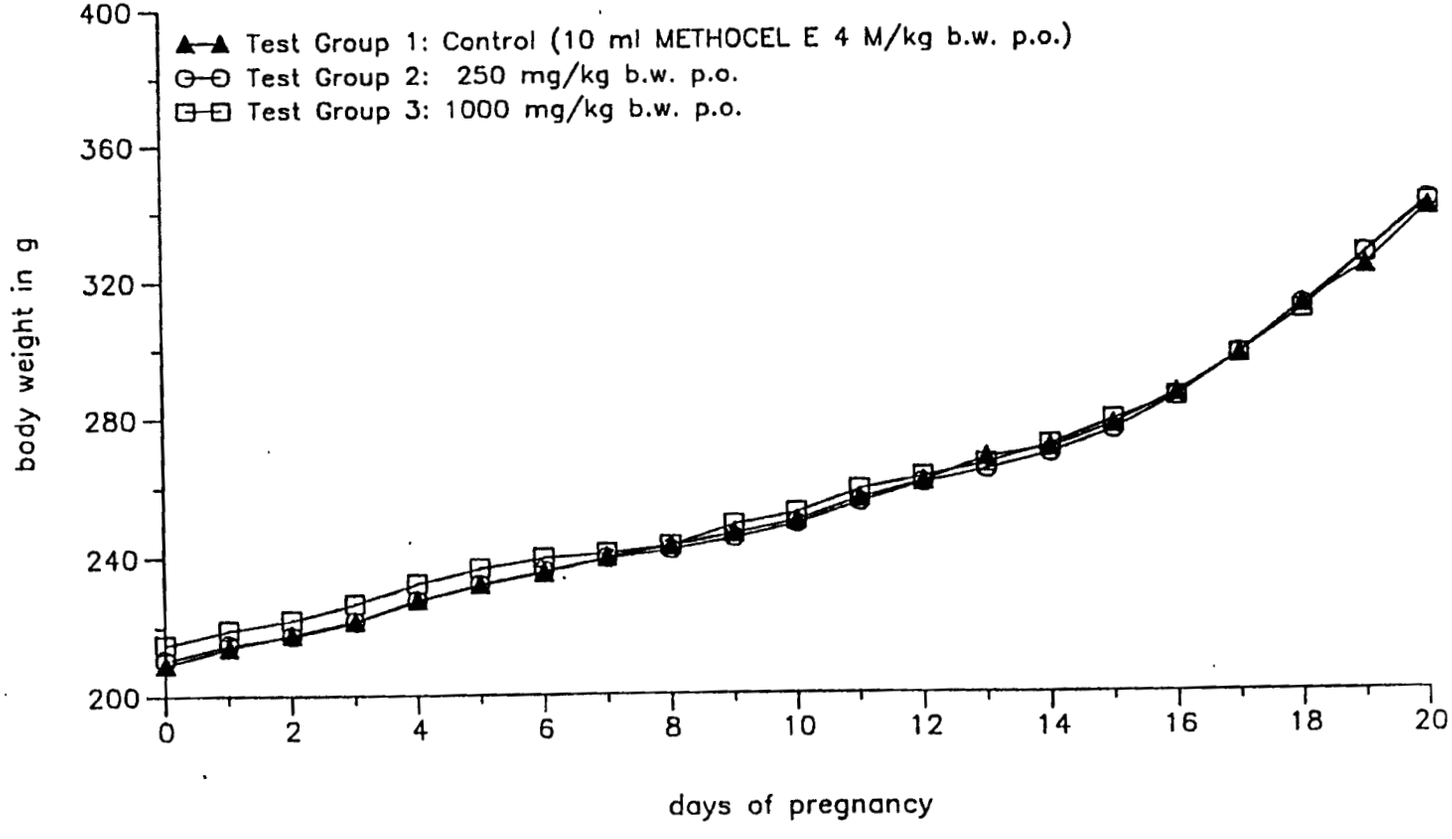
FIGURE 1 EXAMINATION OF THE INFLUENCE OF 1,3-PROPANEDIOL ON THE PREGNANT RAT AND THE FETUS BY ORAL ADMINISTRATION

body weight of female animals

daily mean value of 20 animals each

treatment from 6th – 15th day of pregnancy



1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

TABLE 4 Mean Gravid Uterine Weights and Net Maternal Body Weight Change - Grams

Criteria		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Gravid Uterus	M	73.9	77.4	73.7
	SD	14.0	11.6	9.7
	n =	20	20	20
Carcass	M	266.0	264.7	267.4
	SD	16.5	17.8	16.7
	n =	20	20	20
Net Body Weight Change from Day 6	M	30.5	28.7	27.7
	SD	9.4	9.0	9.3
	n =	20	20	20

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1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE 5 Maternal Absolute Food Consumption during Gestation - Grams/Day

Time-Period		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Days 0 to 3	M	21.1	20.9	22.4
	SD	1.9	1.7	1.8
	n =	20	20	20
Days 3 to 6	M	22.8	22.6	23.2
	SD	1.7	2.3	2.0
	n =	20	20	20
Days 6 to 9	M	23.1	23.1	22.4
	SD	2.5	3.7	4.7
	n =	20	20	20
Days 9 to 12	M	23.9	23.5	23.7
	SD	2.8	3.0	2.4
	n =	20	20	20
Days 12 to 15	M	24.3	23.3	23.6
	SD	2.2	3.9	2.6
	n =	20	20	20

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1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE 5 Maternal Absolute Food Consumption during Gestation - Grams/Day

Time-Period		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Days 15 to 18	M	25.7	25.7	25.7
	SD	2.7	2.8	3.1
	n =	20	20	20
Days 18 to 20	M	25.0	25.0	24.9
	SD	2.6	2.9	2.6
	n =	20	20	20

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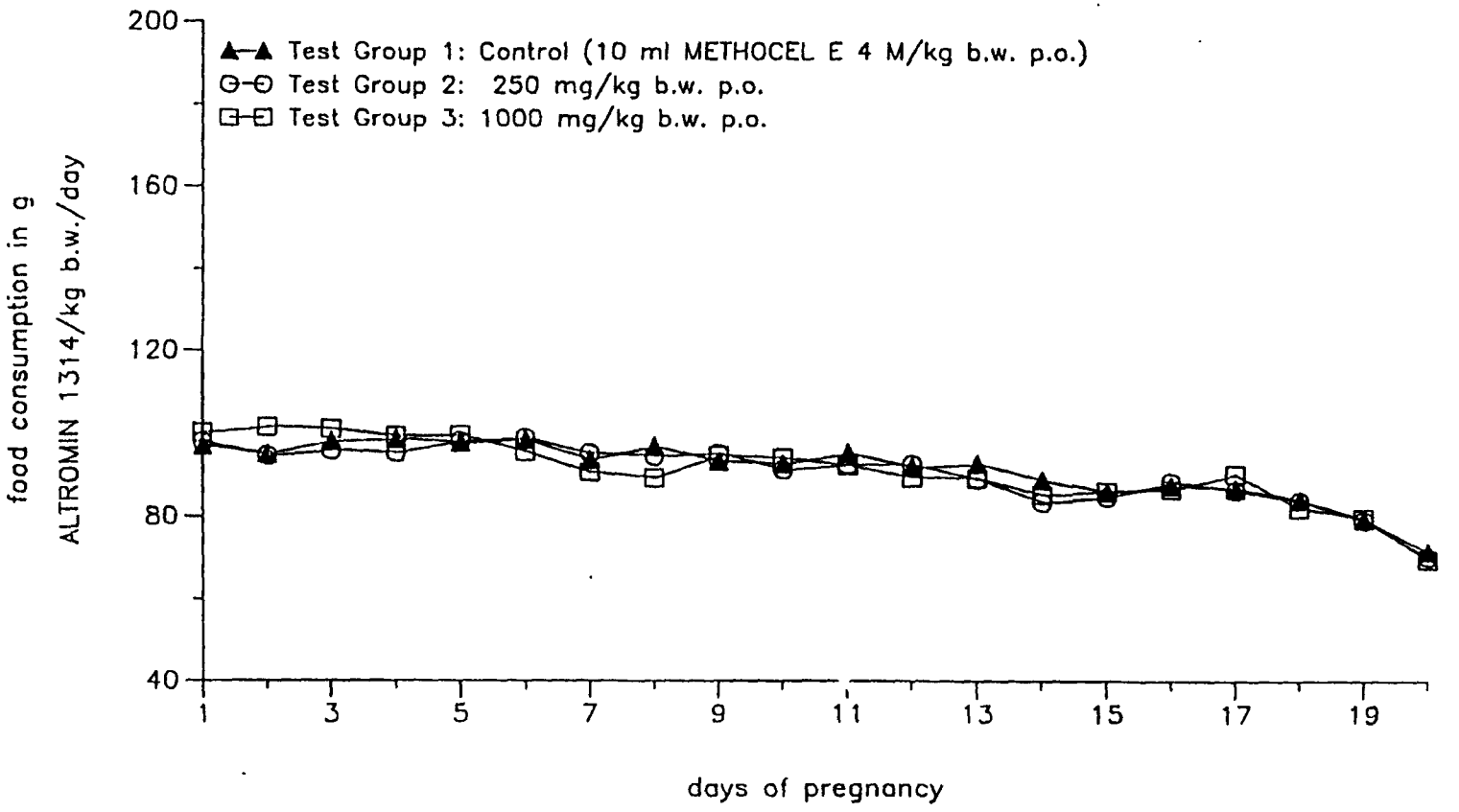
FIGURE 2

EXAMINATION OF THE INFLUENCE OF 1,3-PROPANEDIOL ON THE PREGNANT RAT AND THE FETUS BY ORAL ADMINISTRATION

food consumption of female animals

daily mean value of 20 animals each

treatment from 6th – 15th day of pregnancy



1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE 6 Summary of Maternal Necropsy Observations

Criteria		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Dams examined	n =	20	20	20
No remarkable observations	n =	20	19	20
	%	100.0	95.0	100.0
Lungs: right lobe indurated; pericardium: adhered to lungs and thymus	n =	0	1	0
	%	0.0	5.0	0.0

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explanations and abbreviations see preface of the report

TABLE 7

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of Reproduction Data

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Females mated (1)	n =	25	25	25
surplus, not evaluated	n =	3	3	3
pregnant (2)	n =	20/22	20/22	20/22
	%	90.9	90.9	90.9
aborted	n =	0	0	0
premature births	n =	0	0	0
Dams with viable fetuses	n =	20	20	20
Dams with complete resorptions	n =	0	0	0
Female mortality	n =	0	0	0
	%	0.0	0.0	0.0
Pregnant at Caesarean-section	n =	20	20	20
	%	100.0	100.0	100.0

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1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE 7 Summary of Reproduction Data

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Pregnant at Caesarean-section	n =	20	20	20
Corpora lutea	M	13.8	13.8	13.9
	SD	2.2	1.8	1.4
	total	276	276	277
Implantation sites	M	13.4	13.4	13.3
	SD	1.9	2.0	1.7
	total	267	268	266
Pre-implantation loss	mean %	2.8	3.0	3.8
	SD	6.6	6.1	9.6
Post-implantation loss	mean %	10.8	5.8	7.6
	SD	12.6	6.3	8.4

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TABLE 7

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of Reproduction Data

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Pregnant at Caesarean-section	n =	20	20	20
Resorptions:				
total	M	1.5	0.8	0.9
	SD	1.8	0.9	0.9
	total	30	16	18
	mean %	10.8	5.7	7.6
	SD	12.6	6.3	8.4
early	total	24	15	15
	mean %	8.7	5.4	6.2
	SD	8.8	5.5	6.1
late	total	6	1	3
	mean %	2.1	0.4	1.4
	SD	6.6	1.6	5.1
Dead fetuses	n =	0	0	0

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explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of Reproduction Data

TABLE 7

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Dams with viable fetuses	n =	20	20	20
Live fetuses	M	11.9	12.6	12.4
	SD	2.0	1.8	1.7
	total	237	252	248
	mean %	89.2	94.3	92.4
	SD	12.6	6.3	8.4
	left % #	47.3	47.6	42.3
	right % #	52.7	52.4	57.7
Females	total	111	119	115
	mean %	47.4	47.4	46.6
	SD	15.7	15.9	11.1
Males	total	126	133	133
	mean %	52.6	52.6	53.4
	SD	15.7	15.9	11.1

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distribution in the uterine horns

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TABLE 7

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of Reproduction Data
(Summary of all classified fetal external, soft tissue, and skeletal observations)

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Litters evaluated	n =	20	20	20
Fetuses evaluated	n =	237	252	248
live	n =	237	252	248
dead	n =	0	0	0
Total malformations				
fetal incidence	n =	1	0	0
	%	0.4	0.0	0.0
litter incidence	n =	1	0	0
	%	5.0	0.0	0.0
Total variations (external)				
fetal incidence	n =	1	0	0
	%	0.4	0.0	0.0
litter incidence	n =	1	0	0
	%	5.0	0.0	0.0

continued on next page

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of Reproduction Data
(Summary of all classified fetal external, soft tissue, and skeletal observations)

TABLE 7

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Total variations (soft tissue)				
Fetuses evaluated	n =	118	126	124
fetal incidence	n =	31	25	28
	%	26.3	19.8	22.6
litter incidence	n =	16	15	15
	%	80.0	75.0	75.0
Total variations (skeletal observations)				
Fetuses evaluated	n =	119	126	124
fetal incidence	n =	37	29	36
	%	31.1	23.0	29.0
litter incidence	n =	15	11	13
	%	75.0	55.0	65.0

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explanations and abbreviations see preface of the report

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TABLE 7

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of Reproduction Data
(Summary of all classified fetal external, soft tissue, and skeletal observations)

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Total retardations (skeletal observations)				
Fetuses evaluated	n =	119	126	124
fetal incidence	n =	76	80	96
	%	63.9	63.5	77.4//
litter incidence	n =	18	19	20
	%	90.0	95.0	100.0

// significantly different from the control ($p \leq 0.05$)

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE 8 Mean Placental and Fetal Body Weights

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Placental Weights	Units: Grams			
of male fetuses	M	0.56	0.55	0.58
	SD	0.08	0.07	0.07
	n =	20	20	20
of female fetuses	M	0.54	0.54	0.56
	SD	0.07	0.07	0.07
	n =	20	20	20
Fetal weights	Units: Grams			
of male fetuses	M	3.82	3.87	3.76
	SD	0.44	0.31	0.22
	n =	20	20	20
of female fetuses	M	3.66	3.68	3.57
	SD	0.43	0.34	0.21
	n =	20	20	20

explanations and abbreviations see preface of the report

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TABLE 9

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Mean Fetal Lengths

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Fetal lengths male + female fetuses	Units: mm			
	M	34.9	35.0	35.2
	SD	2.0	1.0	1.1
	n =	20	20	20

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of all Classified Fetal External Observations

TABLE 10

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Litters evaluated	n =	20	20	20
Fetuses evaluated	n =	237	252	248
live	n =	237	252	248
dead	n =	0	0	0
Total malformations				
fetal incidence	n =	1	0	0
	%	0.4	0.0	0.0
litter incidence	n =	1	0	0
	%	5.0	0.0	0.0
Total variations				
fetal incidence	n =	1	0	0
	%	0.4	0.0	0.0
litter incidence	n =	1	0	0
	%	5.0	0.0	0.0

explanations and abbreviations see preface of the report

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TABLE 11

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of Fetal External Malformations

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Litters evaluated	n =	20	20	20
Fetuses evaluated	n =	237	252	248
live	n =	237	252	248
dead	n =	0	0	0
Both hindlimbs shortened; right foot: toes missing				
fetal incidence	n =	1	0	0
	%	0.4	0.0	0.0
litter incidence	n =	1	0	0
	%	5.0	0.0	0.0

explanations and abbreviations see preface of the report

TABLE 12

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of Fetal External Variations

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Litters evaluated	n =	20	20	20
Fetuses evaluated	n =	237	252	248
live	n =	237	252	248
dead	n =	0	0	0
Right eye: haemorrhage behind the lens				
fetal incidence	n =	1	0	0
	%	0.4	0.0	0.0
litter incidence	n =	1	0	0
	%	5.0	0.0	0.0

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explanations and abbreviations see preface of the report

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TABLE 13

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of all Classified Fetal Skeletal Observations

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Litters evaluated	n =	20	20	20
Fetuses evaluated	n =	119	126	124
live	n =	119	126	124
dead	n =	0	0	0
Total malformations				
fetal incidence	n =	0#	0	0
	%	0.0	0.0	0.0
litter incidence	n =	0	0	0
	%	0.0	0.0	0.0
Total variations				
fetal incidence	n =	37	29	36
	%	31.1	23.0	29.0
litter incidence	n =	15	11	14
	%	75.0	55.0	70.0

but see table 11

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE 13 Summary of all Classified Fetal Skeletal Observations

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Total retardations				
fetal incidence	n =	76	80	96#
	%	63.9	63.5	77.4
litter incidence	n =	18	19	20
	%	90.0	95.0	100.0

significantly different from control ($p \leq 0.05$)

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1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of Fetal Skeletal Retardations

TABLE 13

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Litters evaluated	n =	20	20	20
Fetuses evaluated	n =	119	126	124
live	n =	119	126	124
dead	n =	0	0	0
Skull incompletely ossified				
fetal incidence	n =	32	52#	52##
	%	26.9	41.3	41.9
litter incidence	n =	15	17	16
	%	75.0	85.0	80.0
Hyoid bone incompletely ossified				
fetal incidence	n =	9	17	13
	%	7.6	13.5	10.5
litter incidence	n =	6	6	7
	%	30.0	30.0	35.0

significantly different from the control ($p \leq 0.05$)
significantly different from the control ($p \leq 0.01$)

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explanations and abbreviations see preface of the report

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1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

TABLE 13 Summary of Fetal Skeletal Retardations

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Sternebra(e) not or incompletely ossified or reduced in size				
fetal incidence	n =	55	49	58
	%	46.2	38.9	46.8
litter incidence	n =	17	16	20
	%	85.0	80.0	100.0
Thoracic vertebral body/bodies dumb-bell-shaped, bipartite or incompletely ossified				
fetal incidence	n =	17	20	18
	%	14.3	15.9	14.5
litter incidence	n =	9	12	11
	%	45.0	60.0	55.0
Lumbar vertebral body/bodies dumb-bell-shaped				
fetal incidence	n =	1	0	2
	%	0.8	0.0	1.6
litter incidence	n =	1	0	1
	%	5.0	0.0	5.0
Metacarpalia (bilateral) not ossified				
fetal incidence	n =	2	1	1
	%	1.7	0.8	0.8
litter incidence	n =	1	1	1
	%	5.0	5.0	5.0

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explanations and abbreviations see preface of the report

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TABLE 13

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of Fetal Skeletal Variations

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Litters evaluated	n =	20	20	20
Fetuses evaluated	n =	119	126	124
live	n =	119	126	124
dead	n =	0	0	0
Sternebra(e) misaligned				
fetal incidence	n =	13	7	10
	%	10.9	5.6	8.1
litter incidence	n =	8	6	7
	%	40.0	30.0	35.0
Accessory 14th rib(s)				
fetal incidence	n =	11	10	13
	%	9.2	7.9	10.5
litter incidence	n =	8	6	6
	%	40.0	30.0	30.0
Rib(s) wavy				
fetal incidence	n =	15	13	17
	%	12.6	10.3	13.7
litter incidence	n =	7	6	7
	%	35.0	30.0	35.0

explanations and abbreviations see preface of the report

TABLE 14

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of all Classified Fetal Soft Tissue Observations

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Litters evaluated	n =	20	20	20
Fetuses evaluated	n =	118	126	124
live	n =	118	126	124
dead	n =	0	0	0
Total malformations				
fetal incidence	n =	0	0	0
	%	0.0	0.0	0.0
litter incidence	n =	0	0	0
	%	0.0	0.0	0.0
Total variations				
fetal incidence	n =	31	25	28
	%	26.3	19.8	22.6
litter incidence	n =	16	15	15
	%	80.0	75.0	75.0

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explanations and abbreviations see preface of the report

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TABLE 14

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of Fetal Soft Tissue Variations

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Litters evaluated	n =	20	20	20
Fetuses evaluated	n =	118	126	124
live	n =	118	126	124
dead	n =	0	0	0
Unilateral dilation of renal pelvis				
fetal incidence	n =	8	6	12
	%	6.8	4.8	9.7
litter incidence	n =	7	5	11
	%	35.0	25.0	55.0
Bilateral dilation of renal pelvis				
fetal incidence	n =	9	9	11
	%	7.6	7.1	8.9
litter incidence	n =	7	7	7
	%	35.0	35.0	35.0

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explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE 14 Summary of Fetal Soft Tissue Variations

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Haemorrhages on the liver surface				
fetal incidence	n =	6	7	5
	%	5.1	5.6	4.0
litter incidence	n =	6	7	4
	%	30.0	35.0	20.0
4th cerebral ventricle enlarged				
fetal incidence	n =	8	1	5
	%	6.8	0.8	4.0
litter incidence	n =	6	1	5
	%	30.0	5.0	25.0
Unilateral hydro-ureter				
fetal incidence	n =	1	3	1
	%	0.9	2.4	0.8
litter incidence	n =	1	3	1
	%	5.0	15.0	5.0

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explanations and abbreviations see preface of the report

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TABLE 14

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Summary of Fetal Soft Tissue Variations

		Test Group 1 Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	Test Group 2 250 mg 1,3-Propanediol/kg b.w. p.o.	Test Group 3 1000 mg 1,3-Propanediol/kg b.w. p.o.
Bilateral hydro-ureter				
fetal incidence	n =	1	0	0
	%	0.9	0.0	0.0
litter incidence	n =	1	0	0
	%	5.0	0.0	0.0
Slightly dislocated kidney (unilateral)				
fetal incidence	n =	3	0	1
	%	2.5	0.0	0.8
litter incidence	n =	3	0	1
	%	15.0	0.0	5.0

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE A 1 Individual Maternal Clinical Symptoms

Dam No.	Observations
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)
1	No remarkable observations
2	No remarkable observations
3	No remarkable observations
4	Not pregnant
5	No remarkable observations
6	No remarkable observations
7	No remarkable observations
8	No remarkable observations
9	No remarkable observations
10	No remarkable observations
11	No remarkable observations
12	No remarkable observations
13	No remarkable observations
14	No remarkable observations
15	No remarkable observations
16	Not pregnant
17	No remarkable observations
18	No remarkable observations

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TABLE A 1

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Clinical Symptoms

Dam No.	Observations
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)
19	No remarkable observations
20	No remarkable observations
21	No remarkable observations
22	No remarkable observations
23	Not examined
24	Not examined
25	Not examined

explanations and abbreviations see preface of the report

1,3 Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE A 1 Individual Maternal Clinical Symptoms

Dam No.	Observations
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.
26	No remarkable observations
27	No remarkable observations
28	Not pregnant
29	No remarkable observations
30	No remarkable observations
31	No remarkable observations
32	No remarkable observations
33	No remarkable observations
34	No remarkable observations
35	No remarkable observations
36	Not pregnant
37	No remarkable observations
38	No remarkable observations
39	No remarkable observations
40	No remarkable observations
41	No remarkable observations

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TABLE A 1

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Clinical Symptoms

Dam No.	Observations
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.
42	No remarkable observations
43	No remarkable observations
44	No remarkable observations
45	No remarkable observations
46	No remarkable observations
47	No remarkable observations
48	Not examined
49	Not examined
50	Not examined

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Clinical Symptoms

TABLE A 1

Dam No.	Observations
	Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.
51	Not pregnant
52	Not pregnant
53	No remarkable observations
54	No remarkable observations
55	No remarkable observations
56	No remarkable observations
57	No remarkable observations
58	No remarkable observations
59	No remarkable observations
60	No remarkable observations
61	No remarkable observations
62	No remarkable observations
63	No remarkable observations
64	No remarkable observations
65	No remarkable observations

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explanations and abbreviations see preface of the report

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TABLE A 1

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Clinical Symptoms

Dam No.	Observations
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.
66	No remarkable observations
67	no remarkable observa :ions
68	No remarkable observations
69	No remarkable observations
70	No remarkable observations
71	No remarkable observations
72	No remarkable observations
73	Not examined
74	Not examined
75	Not examined

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE A 2 Individual Maternal Body Weights during Gestation - Grams

Dam No.	Days of Gestation 0	3	6	9	12	15	18	20
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w.p.o.)							
1	216	230	240	252	263	288	328	358
2	207	223	232	252	266	287	324	360
3	198	210	218	228	236	247	277	306
4	not pregnant							
5	208	217	227	239	253	267	294	315
6	215	226	243	259	281	300	342	372
7	202	219	246	259	278	290	329	356
8	204	212	224	237	257	264	309	338
9	194	212	229	246	251	266	300	327
10	223	230	245	262	277	304	345	380
11	213	223	247	258	276	295	334	370
12	212	220	233	251	261	276	300	326
13	233	246	257	267	278	288	327	347
14	207	217	226	236	252	265	300	328
15	206	222	232	237	252	271	300	330
16	not pregnant							
17	213	230	246	256	266	286	320	351
18	209	221	235	245	258	275	292	316

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TABLE A 2

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Body Weights during Gestation - Grams

Dam No.	Days of Gestation 0	3	6	9	12	15	18	20
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)							
19	194	202	213	219	235	244	273	291
20	216	232	244	253	275	290	335	370
21	208	222	239	241	257	272	314	338
22	208	220	234	235	250	264	297	319
23	not examined							
24	not examined							
25	not examined							
M	209.3	221.7	235.5	246.6	261.1	277.0	312.0	339.9
SD	9.2	9.4	10.9	12.4	13.6	16.4	20.9	24.5

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Body Weights during Gestation - Grams

TABLE A 2

Dam No.	Days of Gestation 0	3	6	9	12	15	18	20
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.							
26	201	222	229	235	244	248	282	316
27	201	205	219	229	235	257	286	313
28	not pregnant							
29	210	220	235	252	254	275	313	343
30	204	217	233	249	269	281	311	333
31	200	211	221	239	249	265	294	327
32	218	231	248	259	273	289	326	360
33	198	210	231	238	256	276	317	344
34	230	240	254	267	286	295	336	374
35	207	222	239	251	264	286	323	357
36	not pregnant							
37	229	242	261	270	287	309	348	376
38	193	207	214	226	235	244	276	304
39	210	223	243	269	281	304	345	380
40	212	220	239	240	259	269	296	319
41	205	219	227	233	250	268	301	337

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explanations and abbreviations see preface of the report

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TABLE A 2

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Body Weights during Gestation - Grams

Dam No.	Days of Gestation 0	3	6	9	12	15	18	20
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.							
42	210	219	237	248	262	272	311	340
43	204	218	235	246	271	294	332	367
44	209	207	214	215	231	244	269	291
45	225	230	246	251	275	285	337	370
46	232	239	251	236	260	277	316	345
47	211	226	244	251	271	270	312	345
48	not examined							
49	not examined							
50	not examined							
M	210.5	221.4	236.0	245.2	260.6	275.4	311.6	342.1
SD	11.1	10.9	13.0	14.5	16.5	18.4	22.7	25.2

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Body Weights during Gestation - Grams

TABLE A 2

Dam No.	Days of Gestation 0	3	6	9	12	15	18	20
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.							
51	not pregnant							
52	not pregnant							
53	213	230	248	255	270	288	313	331
54	218	223	240	252	264	283	276	346
55	214	223	241	244	261	278	316	342
56	199	201	217	226	239	256	299	327
57	228	237	249	261	278	295	330	365
58	216	225	233	244	250	266	284	304
59	212	222	236	249	262	271	313	345
60	230	240	256	262	285	299	341	371
61	227	247	264	280	295	314	349	372
62	215	227	235	240	249	264	293	320
63	210	225	239	244	261	283	315	350
64	205	218	234	240	262	272	309	342
65	201	214	229	240	254	266	302	338
66	219	229	239	239	253	265	282	310
67	226	235	247	258	272	292	322	355

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TABLE A 2

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Body Weights during Gestation - Grams

Dam No.	Days of Gestation 0	3	6	9	12	15	18	20
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.							
68	210	227	239	217	240	268	299	330
69	222	238	250	265	283	296	331	367
70	210	222	230	237	254	268	309	335
71	225	235	246	258	270	285	326	352
72	197	211	221	231	245	256	293	319
73	not examined							
74	not examined							
75	not examined							
M	214.9	226.5	239.7	247.1	262.4	278.3	310.1	341.1
SD	9.7	10.8	11.2	14.7	15.2	15.6	19.6	19.6

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Body Weight Change during Gestation - Grams

TABLE A 3

Dam No.	Days of Gestation 0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18	18 - 20
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)						
1	14	10	12	11	25	40	30
2	16	9	20	14	21	37	36
3	12	8	10	8	11	30	29
4	not pregnant						
5	9	10	12	14	14	27	21
6	11	17	16	22	19	42	30
7	17	27	13	19	12	39	27
8	8	12	13	20	7	45	29
9	18	17	17	5	15	34	27
10	7	15	17	15	27	41	35
11	10	24	11	18	19	39	36
12	8	13	18	10	15	24	26
13	13	11	10	11	10	39	20
14	10	9	10	16	13	35	28
15	16	10	5	15	19	29	30
16	not pregnant						
17	17	16	10	10	20	34	31
18	12	14	10	13	17	17	24

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TABLE A 3

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Body Weight Change during Gestation - Grams

Dam No.	Days of Gestation 0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18	18 - 20
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)						
19	8	11	6	16	9	29	18
20	16	12	9	22	15	45	35
21	14	17	2	16	15	42	24
22	12	14	1	15	14	33	22
23	not examined						
24	not examined						
25	not examined						
M	12.4	13.8	11.1	14.5	15.9	35.1	27.9
SD	3.5	4.9	5.1	4.5	5.1	7.3	5.3

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Body Weight Change during Gestation - Grams

TABLE A 3

Dam No.	Days of Gestation 0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18	18 - 20
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.						
26	21	7	6	9	4	34	34
27	4	14	10	6	22	29	27
28	not pregnant						
29	10	15	17	2	21	38	30
30	13	16	16	20	12	30	22
31	11	10	18	10	16	29	33
32	13	17	11	14	16	37	34
33	12	21	7	18	20	41	27
34	10	14	13	19	9	41	38
35	15	17	12	13	22	37	34
36	not pregnant						
37	13	19	9	17	22	39	28
38	14	7	12	9	9	32	28
39	13	20	26	12	23	41	35
40	8	19	1	19	10	27	23
41	14	8	6	17	18	33	36

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explanations and abbreviations see preface of the report

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TABLE A 3

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Body Weight Change during Gestation - Grams

Dam No.	Days of Gestation 0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18	18 - 20
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.						
42	9	18	11	14	10	39	29
43	14	17	11	25	23	38	35
44	- 2	7	1	16	13	25	22
45	5	16	5	24	10	52	33
46	7	12	-15	24	17	39	29
47	15	18	7	20	- 1	42	33
48	not examined						
49	not examined						
50	not examined						
M	11.0	14.6	9.2	15.4	14.8	36.2	30.5
SD	4.9	4.6	8.2	6.2	6.8	6.4	4.7

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE A 3 Individual Maternal Body Weight Change during Gestation - Grams

Dam No.	Days of Gestation 0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18	18 - 20
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.						
51	not pregnant						
52	not pregnant						
53	17	18	7	15	18	25	18
54	5	17	12	12	19	- 7	70
55	9	18	3	17	17	38	26
56	2	16	9	13	17	43	28
57	9	12	12	17	17	35	35
58	9	8	11	6	16	18	20
59	10	14	13	13	9	42	32
60	10	16	6	23	14	42	30
61	20	17	16	15	19	35	23
62	12	8	5	9	15	29	27
63	15	14	5	17	22	32	35
64	13	16	6	22	10	37	33
65	13	15	11	14	12	36	36
66	10	10	0	14	12	17	28
67	9	12	11	14	20	30	33

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explanations and abbreviations see preface of the report

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TABLE A 3

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Body Weight Change during Gestation - Grams

Dam No.	Days of Gestation 0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18	18 - 20
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.						
68	17	12	-22	23	28	31	31
69	16	12	15	18	13	35	36
70	12	8	7	17	14	41	26
71	10	11	12	12	15	41	26
72	14	10	10	14	11	37	26
73	not examined						
74	not examined						
75	not examined						
M	11.6	13.2	7.5	15.3	15.9	31.9	31.0
SD	4.2	3.4	8.0	4.3	4.5	11.7	10.5

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE A 4 Individual Uterine Weights and Net Maternal Body Weight Change - Grams

Dam No.	Uterus Weight (gravid)	Carcass Weight	Net Body Weight Change from Day 6
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
1	87.8	270.2	30.2
2	76.0	284.0	52.0
3	66.7	239.3	21.3
4	not pregnant		
5	61.7	253.3	26.3
6	82.8	289.2	46.2
7	77.0	279.0	33.0
8	91.3	246.7	22.7
9	71.6	255.4	26.4
10	98.4	281.6	36.6
11	92.5	277.5	30.5
12	52.4	273.6	40.6
13	67.5	279.5	22.5
14	74.5	253.5	27.5
15	77.1	252.9	20.9
16	not pregnant		
17	74.7	276.3	30.3
18	41.9	274.1	39.1

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explanations and abbreviations see preface of the report

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TABLE A 4

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Uterine Weights and Net Maternal Body Weight Change - Grams

Dam No.	Uterus Weight (gravid)	Carcass Weight	Net Body Weight Change from Day 6
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
19	58.7	232.3	19.3
20	86.8	283.2	39.2
21	70.9	267.1	28.1
22	68.0	251.0	17.0
23	not examined		
24	not examined		
25	not examined		
M	73.9	266.0	30.5
SD	14.0	16.5	9.4

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE A 4 Individual Uterine Weights and Net Maternal Body Weight Change - Grams

Dam No.	Uterus Weight (gravid)	Carcass Weight	Net Body Weight Change from Day 6
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
26	75.3	240.7	11.7
27	68.1	244.9	25.9
28	not pregnant		
29	83.1	259.9	24.9
30	65.4	267.6	34.6
31	73.7	253.3	32.3
32	75.2	284.8	36.8
33	83.6	260.4	29.4
34	98.4	275.6	21.6
35	93.8	263.2	24.2
36	not pregnant		
37	80.4	295.6	34.6
38	71.2	232.8	18.8
39	94.6	285.4	42.4
40	55.5	263.5	24.5
41	78.2	258.8	31.8

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explanations and abbreviations see preface of the report

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TABLE A 4

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Uterine Weights and Net Maternal Body Weight Change - Grams

Dam No.	Uterus Weight (gravid)	Carcass Weight	Net Body Weight Change from Day 6
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
42	76.3	263.7	26.7
43	80.4	286.6	51.6
44	59.5	231.5	17.5
45	94.0	276.0	30.0
46	70.2	274.8	23.8
47	70.9	274.1	30.1
48	not examined		
49	not examined		
50	not examined		
M	77.4	264.7	28.7
SD	11.6	17.8	9.0

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE A 4 Individual Uterine Weights and Net Maternal Body Weight Change - Grams

Dam No.	Uterus Weight (gravid)	Carcass Weight	Net Body Weight Change from Day 6
	Test Group 3: 1000 mg 1,3 Propanediol/kg b.w. p.o.		
51	not pregnant		
52	not pregnant		
53	59.3	271.7	23.7
54	79.1	266.9	26.9
55	57.7	284.3	43.3
56	83.9	243.1	26.1
57	80.2	284.8	35.8
58	63.6	240.4	7.4
59	82.7	262.3	26.3
60	82.9	288.1	32.1
61	70.1	301.9	37.9
62	66.6	253.4	18.4
63	71.6	278.4	39.4
64	78.9	263.1	29.1
65	79.7	258.3	29.3
66	60.0	250.0	11.0
67	89.5	265.5	18.5

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explanations and abbreviations see preface of the report

TABLE A 4

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Uterine Weights and Net Maternal Body Weight Change - Grams

Dam No.	Uterus Weight (gravid)	Carcass Weight	Net Body Weight Change from Day 6
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
68	60.3	269.7	30.7
69	76.7	290.3	40.3
70	78.1	256.9	26.9
71	83.0	269.0	23.0
72	70.1	248.9	27.9
73	not examined		
74	not examined		
75	not examined		
M	73.7	267.4	27.7
SD	9.7	16.7	9.3

explanations and abbreviations see preface of the report

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TABLE A 5

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Absolute Food Consumption during Gestation - Grams/Day

Dam No.	Days of Gestation 0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18	18 - 20
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)						
1	23.3	23.3	23.7	24.3	26.0	27.3	26.0
2	23.7	25.0	26.7	28.0	26.7	30.0	29.0
3	18.7	20.3	19.0	19.3	19.7	21.3	21.5
4	not pregnant						
5	19.0	23.3	23.0	23.3	23.3	23.3	25.5
6	23.0	24.0	26.0	29.7	27.3	28.3	28.0
7	18.7	23.3	24.7	26.0	25.0	26.0	27.0
8	19.7	21.0	21.7	24.3	22.7	24.7	25.5
9	18.7	20.0	21.7	20.0	23.7	19.0	24.0
10	20.7	21.3	22.7	25.0	25.3	26.7	26.5
11	23.3	24.3	24.7	27.7	26.7	27.0	28.5
12	16.0	23.0	24.7	25.7	25.3	26.3	26.5
13	21.7	23.0	24.7	25.0	24.0	27.3	24.5
14	20.0	21.7	22.7	21.7	23.3	24.3	23.0
15	18.3	20.3	20.0	20.7	21.3	23.3	23.0
16	not pregnant						
17	24.0	25.7	27.3	25.0	25.7	28.3	28.0
18	21.3	23.7	24.0	22.7	22.7	26.3	24.0

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explanations and abbreviations see preface of the report

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TABLE A 5

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Absolute Food Consumption during Gestation - Grams/Day

Dam No.	Days of Gestation 0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18	18 - 20
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)						
19	21.0	21.3	21.7	20.7	20.7	23.3	21.0
20	22.7	24.3	22.3	23.7	27.3	29.0	25.5
21	22.7	24.3	23.3	25.0	25.7	26.7	22.5
22	21.7	22.3	17.0	21.0	23.0	25.3	20.0
23	not examined						
24	not examined						
25	not examined						
M	21.1	22.8	23.1	23.9	24.3	25.7	25.0
SD	1.9	1.7	2.5	2.8	2.2	2.7	2.6

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Absolute Food Consumption during Gestation - Grams/Day

TABLE A 5

Dam No.	Days of Gestation 0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18	18 - 20
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.						
26	20.0	20.7	20.0	21.0	13.0	21.7	23.0
27	17.7	19.0	19.3	20.3	20.0	23.3	22.0
28	not pregnant						
29	21.0	22.3	24.0	23.0	24.0	26.0	20.0
30	21.0	21.0	23.7	25.3	23.0	25.3	23.5
31	18.3	20.3	22.7	21.3	21.3	24.0	24.0
32	22.0	24.3	24.7	25.7	28.3	21.3	27.0
33	22.0	23.3	23.0	24.3	26.3	29.0	25.0
34	21.0	20.0	21.7	23.3	21.7	25.3	24.5
35	23.7	25.7	26.0	26.3	24.7	28.0	26.0
36	not pregnant						
37	24.0	24.0	27.3	25.3	26.3	27.3	26.5
38	20.7	19.3	21.0	19.0	19.7	22.7	21.5
39	18.7	25.0	28.0	27.0	27.7	29.0	28.0
40	20.3	22.0	22.3	21.0	22.0	23.0	22.5
41	21.0	21.3	22.0	22.3	22.7	25.3	24.5

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TABLE A 5

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Absolute Food Consumption during Gestation - Grams/Day

Dam No.	Days of Gestation 0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18	18 - 20
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.						
42	19.3	24.3	25.3	26.0	24.0	27.7	28.0
43	22.7	25.7	26.0	29.7	31.0	31.7	28.0
44	20.0	19.3	20.3	20.0	21.0	21.7	20.5
45	21.7	26.0	27.0	27.0	26.3	28.3	29.5
46	20.3	24.0	11.7	19.7	22.3	25.7	30.0
47	23.0	24.0	25.0	23.0	20.3	26.7	25.0
48	not examined						
49	not examined						
50	not examined						
M	20.9	22.6	23.1	23.5	23.3	25.7	25.0
SD	1.7	2.3	3.7	3.0	3.9	2.8	2.9

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Foetus by Oral Administration
TABLE A 5 Individual Absolute Food Consumption during Gestation - Grams/Day

Dam No.	Days of Gestation 0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18	18 - 20
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.						
51	not pregnant						
52	not pregnant						
53	22.0	23.0	24.3	25.3	23.3	27.0	23.5
54	21.0	21.0	24.3	22.3	24.3	19.7	27.0
55	22.7	23.0	22.7	26.3	26.3	30.0	29.5
56	19.0	20.7	20.7	22.7	22.0	26.3	23.5
57	22.7	24.7	24.7	28.0	26.3	29.0	26.5
58	20.0	19.3	20.3	20.3	24.0	21.7	19.5
59	24.0	22.3	23.7	25.0	21.3	25.7	23.0
60	23.0	23.3	21.7	25.7	24.7	28.3	27.0
61	26.7	26.3	28.0	26.3	28.3	28.3	26.0
62	20.7	22.7	19.3	20.0	20.7	23.0	21.5
63	22.7	24.7	25.0	24.0	26.0	27.7	28.0
64	23.0	25.0	26.7	25.0	20.3	27.0	27.5
65	22.7	23.7	25.0	23.7	21.7	26.7	25.0
66	21.7	21.7	20.0	19.0	20.0	18.7	23.5
67	22.7	23.3	24.0	23.3	22.3	24.3	24.5

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TABLE A 5

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Absolute Food Consumption during Gestation - Grams/Day

Dam No.	Days of Gestation 0 - 3	3 - 6	6 - 9	9 - 12	12 - 15	15 - 18	18 - 20
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.						
68	25.3	27.3	5.3	22.0	27.7	29.0	28.0
69	24.7	25.0	26.3	27.3	21.0	26.7	26.0
70	21.3	23.0	22.3	23.3	26.0	26.7	22.5
71	22.3	23.0	23.0	23.7	24.7	25.3	23.0
72	20.3	20.3	20.0	21.7	21.7	22.3	22.5
73	not examined						
74	not examined						
75	not examined						
M	22.4	23.2	22.4	23.7	23.6	25.7	24.9
SD	1.8	2.0	4.7	2.4	2.6	3.1	2.6

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Necropsy Observations

TABLE A 6

Dam No.	Observations
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)
1	No remarkable observations
2	No remarkable observations
3	No remarkable observations
4	Not pregnant
5	No remarkable observations
6	No remarkable observations
7	No remarkable observations
8	No remarkable observations
9	No remarkable observations
10	No remarkable observations
11	No remarkable observations
12	No remarkable observations
13	No remarkable observations
14	No remarkable observations
15	No remarkable observations
16	Not pregnant
17	No remarkable observations
18	No remarkable observations

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TABLE A 6

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Necropsy Observations

Dam No.	Observations
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)
19	No remarkable observations
20	No remarkable observations
21	No remarkable observations
22	No remarkable observations
23	Not examined
24	Not examined
25	Not examined

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Necropsy Observations

TABLE A 6

Dam No.	Observations
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.
26	No remarkable observations
27	No remarkable observations
28	Not pregnant
29	No remarkable observations
30	No remarkable observations
31	No remarkable observations
32	No remarkable observations
33	No remarkable observations
34	No remarkable observations
35	No remarkable observations
36	Not pregnant
37	No remarkable observations
38	No remarkable observations
39	No remarkable observations
40	No remarkable observations
41	No remarkable observations

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TABLE A 6

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Necropsy Observations

Dam No.	Observations
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.
42	No remarkable observations
43	No remarkable observations
44	No remarkable observations
45	No remarkable observations
46	Lungs: right lobe indurated; Pericardium: partly adhered to lungs and thymus
47	No remarkable observations
48	Not examined
49	Not examined
50	Not examined

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Necropsy Observations

TABLE A 6

Dam No.	Observations
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.
51	Not pregnant
52	Not pregnant
53	No remarkable observations
54	No remarkable observations
55	No remarkable observations
56	No remarkable observations
57	No remarkable observations
58	No remarkable observations
59	No remarkable observations
60	No remarkable observations
61	No remarkable observations
62	No remarkable observations
63	No remarkable observations
64	No remarkable observations
65	No remarkable observations

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explanations and abbreviations see preface of the report

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TABLE A 6

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Maternal Necropsy Observations

Dam No.	Observations
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.
66	No remarkable observations
67	No remarkable observations
68	No remarkable observations
69	No remarkable observations
70	No remarkable observations
71	No remarkable observations
72	No remarkable observations
73	Not examined
74	Not examined
75	Not examined

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE A 7 Individual Reproduction Data

Dam No.	Corpora lutea	Implant sites	Resorptions early	Resorptions late	Resorptions total	Fetuses dead	Fetuses live	Sex male	Sex female	Average fetal body weight males	Average fetal body weight females
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)										
1	19	19	4	0	4	0	15	9	6	3.58	3.53
2	14	14	3	0	3	0	11	7	4	4.23	4.23
3	12	12	0	0	0	0	12	2	10	3.55	3.32
4	not pregnant										
5	12	12	2	0	2	0	10	5	5	3.72	3.82
6	13	13	0	0	0	0	13	9	4	3.82	3.60
7	15	15	3	1	4	0	11	7	4	4.20	4.10
8	16	16	1	0	1	0	15	6	9	3.98	3.62
9	13	13	1	0	1	0	12	7	5	3.90	3.80
10	13	13	0	0	0	0	13	6	7	5.15	4.86
11	14	14	1	0	1	0	13	10	3	4.41	4.00
12	10	10	2	0	2	0	8	3	5	3.73	3.70
13	12	11	0	0	0	0	11	7	4	3.77	3.55
14	15	13	0	0	0	0	13	5	8	3.64	3.71
15	14	14	0	1	1	0	13	8	5	3.68	3.42
16	not pregnant										
17	13	13	1	0	1	0	12	4	8	3.83	3.60
18	19	14	3	4	7	0	7	3	4	3.03	2.78

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TABLE A 7

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Reproduction Data

Dam No.	Corpora lutea	Implant sites	Resorptions early	Resorptions late	Resorptions total	Fetuses dead	Fetuses live	Sex male	Sex female	Average fetal body weight males	Average fetal body weight females
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)											
19	12	12	1	0	1	0	11	4	7	3.45	3.49
20	14	14	0	0	0	0	14	9	5	3.64	3.44
21	12	12	0	0	0	0	12	9	3	3.31	3.07
22	14	13	2	0	2	0	11	6	5	3.78	3.48
23	not examined										
24	not examined										
25	not examined										
n =	276	267	24	6	30	0	237	126	111	-	-
M	13.8	13.4			1.5		11.9	6.3	5.6	3.82	3.66
SD	2.2	1.9			1.8		2.0	2.3	2.0	0.44	0.43

explanations and abbreviations see preface of the report

TABLE A 7

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Reproduction Data

Dam No.	Corpora lutea	Implant sites	Resorptions early	Resorptions late	Resorptions total	Fetuses dead	Fetuses live	Sex male	Sex female	Average fetal body weight males	Average fetal body weight females
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.										
26	13	13	0	0	0	0	13	4	9	3.65	3.58
27	11	11	0	0	0	0	11	6	5	4.00	3.56
28	not pregnant										
29	17	17	2	0	2	0	15	6	9	3.57	3.27
30	13	10	0	0	0	0	10	2	8	4.15	4.09
31	12	12	0	0	0	0	12	7	5	3.76	3.60
32	13	13	1	0	1	0	12	6	6	3.97	3.67
33	14	13	1	0	1	0	12	7	5	4.40	4.30
34	15	15	0	0	0	0	15	6	9	4.25	3.87
35	15	15	0	0	0	0	15	11	4	4.25	4.28
36	not pregnant										
37	15	15	1	0	1	0	14	9	5	3.36	3.20
38	14	14	2	1	3	0	11	4	7	4.23	3.94
39	15	15	0	0	0	0	15	12	3	3.86	3.73
40	10	10	1	0	1	0	9	7	2	3.67	3.50
41	15	14	1	0	1	0	13	8	5	3.85	3.66

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explanations and abbreviations see preface of the report

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TABLE A 7

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Reproduction Data

Dam No.	Corpora lutea	Implant sites	Resorptions early	Resorptions late	Resorptions total	Fetuses dead	Fetuses live	Sex male	Sex female	Average fetal body weight males	Average fetal body weight females
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.										
42	15	13	0	0	0	0	13	5	8	3.98	3.76
43	14	14	1	0	1	0	13	7	6	3.89	3.78
44	11	10	0	0	0	0	10	6	4	3.97	4.08
45	16	16	1	0	1	0	15	7	8	3.84	3.36
46	14	14	2	0	2	0	12	5	7	3.30	3.19
47	14	14	2	0	2	0	12	8	4	3.35	3.23
48	not examined										
49	not examined										
50	not examined										
n =	276	268	15	1	16	0	252	133	119	-	-
M	13.8	13.4			0.8		12.6	6.7	6.0	3.87	3.68
SD	1.8	2.0			0.9		1.8	2.3	2.1	0.31	0.34

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Reproduction Data

TABLE A 7

Dam No.	Corpora lutea	Implant sites	Resorptions early	Resorptions late	Resorptions total	Fetuses dead	Fetuses live	Sex male	Sex female	Average fetal body weight males	Average fetal body weight females
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.										
51	not pregnant										
52	not pregnant										
53	12	12	1	2	3	0	9	3	6	3.93	3.55
54	13	13	0	0	0	0	13	7	6	3.97	3.52
55	13	9	0	0	0	0	9	5	4	3.98	3.73
56	14	14	0	0	0	0	14	5	9	3.80	3.78
57	14	14	1	0	1	0	13	10	3	3.89	3.57
58	12	12	1	0	1	0	11	7	4	3.61	3.70
59	14	14	1	0	1	0	13	7	6	4.14	3.75
60	14	14	1	0	1	0	13	6	7	4.00	3.93
61	17	12	0	0	0	0	12	7	5	3.51	3.42
62	12	12	0	0	0	0	12	6	6	3.65	3.32
63	13	11	0	0	0	0	11	7	4	3.91	3.85
64	15	15	2	0	2	0	13	7	6	3.80	3.60
65	14	14	0	0	0	0	14	8	6	3.79	3.62
66	13	13	2	0	2	0	11	5	6	3.58	3.40
67	16	16	1	0	1	0	15	9	6	3.96	3.62

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explanations and abbreviations see preface of the report

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TABLE A 7

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Reproduction Data

Dam No.	Corpora lutea	Implant sites	Resorptions early	Resorptions late	Resorptions total	Fetuses dead	Fetuses live	Sex male	Sex female	Average fetal body weight males	Average fetal body weight females
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.										
68	14	14	2	0	2	0	12	6	6	3.22	3.17
69	16	16	0	1	1	0	15	9	6	3.78	3.65
70	13	13	1	0	1	0	12	8	4	3.65	3.65
71	15	15	1	0	1	0	14	7	7	3.60	3.33
72	13	13	1	0	1	0	12	4	8	3.48	3.20
73	not examined										
74	not examined										
75	not examined										
n =	277	266	15	3	18	0	248	133	115	-	-
M	13.9	13.3			0.9		12.4	6.7	5.8	3.76	3.57
SD	1.4	1.7			0.9		1.7	1.7	1.4	0.22	0.21

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
TABLE B 1 Individual Fetal Status and Uterine Location

Dam No.	Implant No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)																			
1	MA	FA	FA	FA	FA	MA	E	E	E	E	MA	MA	MA	MA	FA	FA	MA	MA	MA
2	MA	MA	MA	MA	E	MA	MA	FA	MA	FA	E	E	FA	FA					
3	FA	FA	FA	MA	FA	FA	FA	FA	FA	MA	FA	FA							
4	not pregnant																		
5	MA	FA	FA	E	FA	MA	MA	FA	E	MA	MA	FA							
6	MA	MA	MA	MA	FA	FA	MA	MA	FA	MA	FA	MA	MA						
7	MA	E	MA	FA	E	L	MA	E	FA	MA	MA	MA	MA	FA	FA				
8	FA	FA	FA	FA	MA	MA	E	FA	FA	FA	MA	MA	FA	FA	MA	MA			
9	MA	MA	FA	FA	MA	FA	MA	FA	FA	MA	MA	MA							
10	FA	FA	MA	MA	MA	MA	FA	MA	FA	FA	MA	FA	FA						
11	MA	MA	MA	MA	MA	MA	MA	FA	MA	MA	FA	E	MA	FA					
12	FA	MA	FA	E	MA	FA	FA	FA	E	MA									
13	FA	FA	MA	MA	MA	MA	FA	MA	MA	FA	MA								
14	FA	FA	FA	MA	FA	FA	MA	FA	FA	MA	MA	MA	FA						
15	FA	MA	MA	L	MA	FA	FA	FA	FA	MA	MA	MA	MA	MA					
16	not pregnant																		
17	FA	FA	FA	MA	FA	MA	MA	FA	FA	MA	E	FA	FA						
18	MA	E	FA	FA	L	FA	L	MA	E	FA	E	L	MA	L					

M = male A = alive L = late resorptions
F = female E = early resorptions

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explanations and abbreviations see preface of the report

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TABLE B 1

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Status and Uterine Location

Dam No.	Implant No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)																			
19	MA	FA	MA	MA	FA	FA	MA	FA	FA	E	FA	FA							
20	MA	MA	MA	MA	FA	MA	FA	MA	MA	FA	FA	MA	MA	FA					
21	MA	FA	MA	MA	MA	MA	MA	FA	MA	MA	MA	FA							
22	E	FA	MA	MA	MA	FA	E	FA	FA	MA	MA	MA	FA						
23	not examined																		
24	not examined																		
25	not examined																		

M = male A = alive L = late resorptions
F = female E = early resorptions

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Status and Uterine Location

TABLE B 1

Dam No.	Implant No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.																			
26	MA	FA	FA	FA	FA	FA	FA	FA	FA	FA	MA	MA	MA						
27	MA	MA	FA	FA	FA	MA	MA	MA	MA	FA	FA								
28	not pregnant																		
29	MA	E	MA	FA	MA	FA	FA	FA	FA	MA	MA	FA	FA	FA	FA	MA	E		
30	FA	MA	FA	FA	FA	MA	FA	FA	FA	FA									
31	MA	MA	FA	MA	FA	MA	FA	FA	MA	FA	MA	MA							
32	E	FA	MA	MA	MA	FA	FA	FA	MA	MA	MA	FA	FA						
33	MA	FA	FA	MA	FA	MA	MA	MA	E	MA	FA	MA	FA						
34	MA	FA	FA	FA	FA	FA	MA	MA	MA	MA	MA	FA	FA	FA	FA				
35	MA	MA	MA	MA	FA	MA	MA	MA	FA	FA	MA	MA	FA	MA	MA				
36	not pregnant																		
37	FA	FA	MA	FA	FA	MA	MA	MA	E	FA	MA	MA	MA	MA	MA				
38	MA	FA	FA	E	E	MA	FA	FA	MA	FA	MA	FA	L	FA					
39	MA	FA	MA	MA	MA	MA	MA	MA	MA	MA	FA	FA	MA	MA	MA				
40	FA	E	MA	MA	MA	MA	MA	MA	MA	FA									
41	MA	FA	MA	MA	E	FA	MA	FA	MA	MA	FA	MA	FA	MA					

M = male A = alive L = late resorptions
F = female E = early resorptions

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explanations and abbreviations see preface of the report

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TABLE B 1

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Status and Uterine Location

Dam No.	Implant No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.																			
42	FA	MA	MA	FA	FA	FA	MA	FA	FA	FA	MA	FA	MA						
43	MA	FA	MA	E	FA	FA	MA	FA	MA	FA	MA	MA	MA	FA					
44	FA	MA	FA	MA	MA	MA	MA	FA	MA	FA									
45	MA	MA	FA	MA	MA	FA	E	FA	FA	MA	MA	FA	FA	FA	FA	MA			
46	E	MA	FA	FA	FA	MA	MA	FA	MA	FA	MA	FA	FA	E					
47	FA	MA	MA	MA	E	MA	MA	MA	E	FA	MA	FA	FA	MA					
48	not examined																		
49	not examined																		
50	not examined																		

M = male A = alive L = late resorptions
F = female E = early resorptions

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Status and Uterine Location

TABLE B 1

Dam No.	Implant No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.																			
51	not pregnant																		
52	not pregnant																		
53	L	E	FA	FA	FA	MA	MA	L	FA	MA	FA	FA							
54	FA	MA	MA	FA	FA	MA	FA	MA	MA	MA	MA	FA	FA						
55	MA	MA	FA	MA	FA	FA	MA	FA	MA										
56	MA	FA	FA	FA	FA	FA	FA	MA	FA	MA	MA	FA	MA	FA					
57	MA	MA	MA	FA	FA	MA	MA	MA	E	MA	MA	MA	MA	FA					
58	MA	FA	MA	MA	FA	FA	FA	MA	MA	MA	MA	E							
59	MA	FA	MA	E	FA	MA	MA	MA	FA	FA	FA	MA	FA	MA					
60	FA	FA	MA	MA	MA	FA	FA	MA	FA	FA	MA	FA	MA	E					
61	MA	FA	MA	MA	MA	MA	FA	FA	MA	MA	FA	FA							
62	FA	MA	FA	MA	FA	MA	MA	MA	FA	MA	FA	FA							
63	MA	MA	FA	MA	FA	MA	FA	MA	FA	MA	MA								
64	E	MA	MA	FA	MA	FA	FA	MA	MA	FA	MA	FA	E	MA	FA				
65	FA	FA	MA	FA	FA	MA	FA	MA	MA	MA	FA	MA	MA	MA					
66	E	FA	FA	FA	E	MA	MA	FA	FA	MA	FA	MA	MA						
67	FA	MA	FA	MA	MA	FA	MA	E	FA	MA	MA	MA	FA	FA	MA	MA			

M = male A = alive L = late resorptions
F = female E = early resorptions

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explanations and abbreviations see preface of the report

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TABLE B 1

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Status and Uterine Location

Dam No.	Implant No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.																			
68	E	MA	MA	FA	FA	FA	E	MA	FA	MA	FA	MA	FA	MA					
69	L	MA	MA	FA	FA	MA	FA	FA	MA	MA	FA	MA	FA	MA	MA	MA			
70	MA	MA	MA	MA	FA	FA	FA	MA	MA	FA	MA	MA	E						
71	MA	FA	MA	MA	MA	MA	FA	FA	MA	FA	MA	FA	FA	FA	E				
72	MA	FA	FA	MA	E	MA	FA	FA	FA	FA	FA	FA	MA						
73	not examined																		
74	not examined																		
75	not examined																		

M = male A = alive L = late resorptions
F = female E = early resorptions

explanations and abbreviations see preface of the report

TABLE B 2

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Placental Weights - Grams

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)																				
1	0.50	0.49	0.54	0.51	0.60	0.44	0.54	E	E	E	E	0.59	0.54	0.42	0.50	0.48	0.55	0.47	0.44	0.56
2	0.62	0.57	0.52	0.65	0.79	E	0.69	0.51	0.52	0.58	0.60	E	E	0.71	0.63					
3	0.59	0.68	0.53	0.57	0.60	0.50	0.66	0.64	0.62	0.60	0.57	0.59	0.55							
4	not pregnant																			
5	0.47	0.49	0.51	0.49	E	0.51	0.45	0.43	0.49	E	0.45	0.48	0.41							
6	0.54	0.48	0.49	0.52	0.51	0.51	0.52	0.79	0.52	0.45	0.60	0.58	0.52	0.50						
7	0.62	0.60	E	0.80	0.63	E	L	0.74	E	0.49	0.52	0.65	0.69	0.60	0.53	0.54				
8	0.48	0.49	0.48	0.50	0.40	0.59	0.49	E	0.48	0.42	0.50	0.62	0.48	0.52	0.40	0.41	0.49			
9	0.50	0.60	0.59	0.59	0.40	0.50	0.55	0.45	0.50	0.47	0.41	0.43	0.46							
10	0.48	0.47	0.50	0.42	0.50	0.40	0.46	0.51	0.49	0.47	0.51	0.50	0.48	0.48						
11	0.73	0.70	0.87	0.75	0.89	0.78	0.68	0.82	0.57	0.75	0.79	0.69	E	0.82	0.80					
12	0.65	0.72	0.68	0.71	E	0.71	0.52	0.60	0.62	E	0.67									
13	0.59	0.61	0.58	0.55	0.59	0.55	0.53	0.61	0.59	0.71	0.62	0.60								
14	0.49	0.54	0.46	0.42	0.52	0.50	0.54	0.48	0.43	0.47	0.57	0.49	0.49	0.41						
15	0.50	0.54	0.47	0.59	L	0.57	0.60	0.43	0.45	0.48	0.48	0.41	0.50	0.44	0.53					
16	not pregnant																			
17	0.54	0.47	0.49	0.48	0.52	0.53	0.58	0.66	0.56	0.48	0.58	E	0.58	0.55						
18	0.71	0.60	E	0.75	0.80	L	0.56	L	0.59	E	0.82	E	L	0.86	L					

E = early resorptions
L = late resorptions

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explanations and abbreviations see preface of the report

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1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Placental Weights - Grams

TABLE B 2

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)																			
19	0.45	0.48	0.49	0.45	0.49	0.49	0.41	0.44	0.40	0.42	E	0.44	0.39							
20	0.55	0.58	0.48	0.49	0.55	0.61	0.54	0.61	0.67	0.51	0.50	0.56	0.60	0.50	0.48					
21	0.53	0.60	0.62	0.63	0.49	0.50	0.50	0.55	0.51	0.51	0.50	0.49	0.51							
22	0.53	E	0.48	0.40	0.57	0.50	0.56	E	0.47	0.60	0.70	0.58	0.49	0.50						
23	not examined																			
24	not examined																			
25	not examined																			
n =	20																			
M	0.55																			
SD	0.08																			

E = early resorptions
L = late resorptions

explanations and abbreviations see preface of the report

TABLE B 2

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Placental Weights - Grams

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.																			
26	0.46	0.46	0.44	0.46	0.44	0.39	0.53	0.46	0.46	0.40	0.51	0.48	0.44	0.47						
27	0.52	0.71	0.40	0.59	0.53	0.50	0.43	0.52	0.51	0.62	0.40	0.47								
28	not pregnant																			
29	0.48	0.48	E	0.45	0.43	0.49	0.32	0.49	0.70	0.45	0.53	0.45	0.45	0.41	0.79	0.45	0.43	E		
30	0.58	0.59	0.58	0.52	0.70	0.52	0.49	0.61	0.60	0.60	0.56									
31	0.44	0.60	0.52	0.51	0.41	0.50	0.35	0.38	0.40	0.42	0.40	0.43	0.36							
32	0.52	E	0.59	0.45	0.62	0.50	0.41	0.60	0.42	0.56	0.57	0.55	0.52	0.45						
33	0.60	0.78	0.67	0.52	0.46	0.60	0.60	0.52	0.69	E	0.61	0.50	0.59	0.69						
34	0.67	0.63	0.54	0.78	0.62	0.68	0.70	0.65	0.71	0.63	0.65	0.65	0.80	0.69	0.68	0.69				
35	0.54	0.55	0.68	0.47	0.52	0.51	0.52	0.57	0.59	0.53	0.50	0.55	0.59	0.52	0.50	0.43				
36	not pregnant																			
37	0.59	0.58	0.67	0.79	0.59	0.50	0.67	0.50	0.70	E	0.45	0.56	0.52	0.59	0.59	0.58				
38	0.59	0.53	0.52	0.51	E	E	0.69	0.68	0.52	0.65	0.60	0.60	0.65	L	0.55					
39	0.48	0.43	0.54	0.33	0.60	0.43	0.43	0.35	0.55	0.46	0.62	0.60	0.40	0.51	0.62	0.34				
40	0.51	0.47	E	0.52	0.56	0.43	0.53	0.53	0.56	0.45	0.52									
41	0.47	0.49	0.40	0.46	0.56	E	0.26	0.46	0.45	0.46	0.50	0.50	0.51	0.50	0.51					

E = early resorptions
L = late resorptions

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TABLE B 2

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Placental Weights - Grams

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.																			
42	0.58	0.63	0.66	0.57	0.66	0.85	0.52	0.55	0.55	0.53	0.48	0.55	0.52	0.42						
43	0.58	0.48	0.50	0.54	E	0.62	0.64	0.52	0.59	0.62	0.75	0.62	0.56	0.59	0.53					
44	0.56	0.59	0.53	0.52	0.62	0.55	0.55	0.54	0.48	0.63	0.58									
45	0.68	0.68	0.83	0.60	0.74	0.61	0.69	E	0.82	0.77	0.72	0.80	0.59	0.58	0.63	0.61	0.56			
46	0.56	E	0.60	0.60	0.61	0.54	0.66	0.59	0.50	0.60	0.56	0.54	0.55	0.60	E					
47	0.58	0.69	0.50	0.59	0.51	E	0.72	0.60	0.58	E	0.58	0.59	0.57	0.51	0.48					
48	not examined																			
49	not examined																			
50	not examined																			
n =	20																			
M	0.55																			
SD	0.07																			

E = early resorptions
L = late resorptions

explanations and abbreviations see preface of the report

TABLE B 2

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Placental Weights - Grams

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.																			
51	not pregnant																			
52	not pregnant																			
53	0.56	L	E	0.55	0.51	0.61	0.52	0.77	L	0.56	0.53	0.51	0.50							
54	0.61	0.58	0.58	0.70	0.65	0.69	0.67	0.52	0.60	0.50	0.70	0.60	0.55	0.59						
55	0.56	0.75	0.57	0.49	0.51	0.59	0.52	0.49	0.49	0.59										
56	0.61	0.55	0.69	0.63	0.62	0.66	0.55	0.61	0.64	0.61	0.62	0.49	0.69	0.51	0.71					
57	0.52	0.60	0.50	0.51	0.43	0.72	0.52	0.51	0.38	E	0.65	0.50	0.51	0.50	0.42					
58	0.56	0.53	0.51	0.53	0.52	0.50	0.53	0.56	0.59	0.68	0.62	0.60	E							
59	0.68	0.67	0.62	0.58	E	0.91	0.75	0.78	0.75	0.65	0.61	0.72	0.65	0.60	0.55					
60	0.63	0.65	0.65	0.51	0.62	0.48	0.69	0.92	0.55	0.68	0.70	0.65	0.50	0.64	E					
61	0.55	0.50	0.62	0.72	0.51	0.59	0.45	0.45	0.58	0.45	0.49	0.67	0.59							
62	0.42	0.42	0.45	0.46	0.40	0.39	0.51	0.45	0.35	0.40	0.40	0.40	0.37							
63	0.65	0.54	0.56	0.72	0.62	0.57	0.62	0.59	0.88	0.58	0.61	0.65								
64	0.46	E	0.47	0.61	0.46	0.47	0.47	0.36	0.41	0.36	0.35	0.49	0.52	E	0.48	0.47				
65	0.52	0.50	0.49	0.45	0.53	0.55	0.65	0.50	0.50	0.48	0.63	0.54	0.52	0.48	0.50					
66	0.51	E	0.45	0.45	0.55	E	0.49	0.56	0.56	0.46	0.52	0.58	0.56	0.46						
67	0.58	0.51	0.56	0.56	0.55	0.62	0.58	0.52	E	0.66	0.49	0.60	0.66	0.59	0.48	0.72	0.59			

E = early resorptions
L = late resorptions

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1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Placental Weights - Grams

TABLE B 2

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.																			
68	0.59	E	0.55	0.80	0.50	0.55	0.61	E	0.70	0.62	0.51	0.78	0.60	0.40	0.42					
69	0.54	L	0.63	0.61	0.62	0.61	0.45	0.49	0.47	0.53	0.60	0.51	0.50	0.50	0.48	0.48	0.59			
70	0.63	1.09	0.62	0.68	0.64	0.50	0.56	0.61	0.70	0.61	0.72	0.71	0.52	E						
71	0.55	0.60	0.52	0.59	0.70	0.49	0.46	0.60	0.50	0.62	0.43	0.60	0.50	0.50	0.59	E				
72	0.56	0.50	0.54	0.50	0.59	E	0.70	0.62	0.50	0.51	0.51	0.50	0.60	0.68						
73	not examined																			
74	not examined																			
75	not examined																			
n =	20																			
M	0.56																			
SD	0.06																			

E = early resorptions
L = late resorptions

explanations and abbreviations see preface of the report

TABLE B 3

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Body Weights - Grams

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)																				
1	3.56	4.0	3.2	3.8	3.7	3.6	3.7	E	E	E	E	4.0	3.9	3.7	3.5	3.3	3.6	3.2	3.0	3.2
2	4.23	4.0	4.1	4.5	4.5	E	4.0	4.2	4.2	4.3	4.3	E	E	4.2	4.2					
3	3.36	3.5	3.3	3.8	3.7	3.3	3.2	3.2	3.2	3.5	3.4	3.3	2.9							
4	not pregnant																			
5	3.77	3.9	3.9	4.0	E	4.0	4.0	4.0	3.5	E	3.4	3.3	3.7							
6	3.75	3.8	4.0	3.6	3.6	3.6	3.7	4.1	4.1	3.6	3.9	3.5	3.7	3.6						
7	4.16	4.2	E	4.0	4.1	E	L	4.2	E	4.1	4.2	4.3	4.2	4.3	4.4	3.8				
8	3.77	3.8	3.6	3.8	3.5	3.9	4.1	E	3.4	3.3	4.0	4.1	3.9	3.6	3.6	3.9	4.0			
9	3.86	3.8	4.5	3.9	3.9	4.0	3.8	3.9	3.5	3.9	3.8	3.6	3.7							
10	4.99	6.0	4.6	5.0	4.6	5.0	5.3	4.3	5.0	4.9	4.9	6.0	4.7	4.6						
11	4.32	4.1	4.8	4.2	4.3	4.6	4.2	4.4	3.8	4.6	4.5	4.1	E	4.4	4.1					
12	3.71	3.5	3.7	3.7	E	4.0	3.7	3.7	3.9	E	3.5									
13	3.69	3.4	3.6	3.9	3.9	3.7	3.8	3.7	4.0	3.9	3.5	3.2								
14	3.68	3.6	3.7	3.7	3.8	3.7	3.6	3.6	4.0	3.4	3.7	3.5	3.6	4.0						
15	3.58	3.4	3.7	4.0	L	4.0	3.6	3.1	3.7	3.3	3.7	3.6	3.6	3.4	3.4					
16	not pregnant																			
17	3.68	3.3	3.6	3.6	3.7	3.7	3.6	3.9	3.9	3.5	4.1	E	3.6	3.6						
18	2.89	3.6	E	2.9	2.0	L	2.9	L	2.8	E	3.3	E	L	2.7	L					

E = early resorptions
L = late resorptions

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TABLE B 3

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Body Weights - Grams

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)																				
19	3.47	3.4	3.2	3.4	3.6	3.4	3.6	3.4	3.6	3.5	E	3.7	3.4							
20	3.57	3.8	3.5	3.5	3.3	3.7	3.9	3.6	3.8	3.6	3.4	3.3	3.8	3.6	3.2					
21	3.25	3.4	3.1	3.3	3.4	3.3	3.3	3.2	3.2	3.5	3.1	3.3	2.9							
22	3.65	E	3.6	3.3	4.1	3.8	3.3	E	3.3	3.7	4.0	3.8	3.7	3.5						
23	not examined																			
24	not examined																			
25	not examined																			
n =	20																			
M	3.75																			
SD	0.44																			

E = early resorptions
L = late resorptions

explanations and abbreviations see preface of the report

TABLE B 3

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Body Weights - Grams

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.																			
26	3.60	3.7	3.6	3.4	3.6	3.7	3.9	3.4	3.5	3.4	3.7	3.4	3.8	3.7						
27	3.80	4.1	3.9	3.8	3.6	3.5	4.0	4.0	4.0	4.0	3.4	3.5								
28	not pregnant																			
29	3.39	3.6	E	4.0	3.7	3.7	3.7	3.2	2.8	2.9	3.4	3.7	3.5	3.2	2.9	3.5	3.0	E		
30	4.10	3.9	4.0	3.8	4.4	3.9	4.3	4.2	4.0	4.3	4.2									
31	3.69	3.9	4.2	3.7	3.5	3.5	3.9	3.5	3.6	3.8	3.7	3.4	3.6							
32	3.82	E	3.8	3.7	4.1	3.9	3.2	3.9	3.6	3.9	4.1	4.1	3.9	3.6						
33	4.36	4.5	4.5	4.2	4.4	4.3	4.4	4.4	4.5	E	4.5	4.2	4.1	4.3						
34	4.02	4.6	3.6	4.0	3.9	3.9	4.0	4.3	4.2	4.2	4.0	4.2	3.9	4.2	3.7	3.6				
35	4.26	4.3	4.4	4.1	4.2	4.3	4.7	4.1	4.2	4.5	4.2	4.6	4.1	4.1	4.3	3.8				
36	not pregnant																			
37	3.30	3.1	3.2	3.3	3.4	3.2	3.6	3.6	3.2	E	3.1	3.5	3.5	3.3	3.1	3.1				
38	4.05	4.1	4.1	3.9	E	E	4.2	3.9	3.9	4.1	3.8	4.5	3.9	L	4.1					
39	3.83	4.0	3.6	4.1	3.9	4.3	4.0	3.8	3.5	3.8	4.0	3.9	3.7	3.8	3.7	3.4				
40	3.63	3.4	E	3.8	3.8	3.4	3.5	3.7	3.6	3.9	3.6									
41	3.78	3.8	3.6	3.6	3.8	E	3.6	3.9	3.6	4.1	3.9	3.8	3.8	3.7	3.9					

E = early resorptions
L = late resorptions

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TABLE B 3

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Body Weights - Grams

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.																			
42	3.85	3.8	3.9	4.3	3.9	3.6	3.6	4.1	4.0	3.3	3.9	3.9	4.0	3.7						
43	3.78	3.8	3.9	4.1	E	4.2	3.8	4.0	3.4	3.9	3.9	3.8	3.6	4.0	3.5					
44	4.01	5.7	4.2	3.5	3.9	3.9	3.9	4.0	3.6	3.9	3.5									
45	3.59	3.7	3.7	3.5	4.4	4.0	3.7	E	4.0	2.2	3.7	3.8	3.5	3.6	2.8	3.6	3.6			
46	3.23	E	3.5	3.5	3.3	3.2	3.4	3.2	2.9	3.3	3.1	3.1	3.2	3.1	E					
47	3.31	3.1	3.2	3.4	2.9	E	3.5	3.6	3.5	E	3.3	3.5	3.4	3.1	3.2					
48	not examined																			
49	not examined																			
50	not examined																			
n =	20																			
M	3.77																			
SD	0.31																			

E = early resorptions
L = late resorptions

explanations and abbreviations see preface of the report

TABLE B 3

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Body Weights - Grams

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.																			
51	not pregnant																			
52	not pregnant																			
53	3.68	L	E	3.5	3.6	3.6	3.9	3.9	L	3.4	4.0	3.6	3.6							
54	3.76	3.7	3.9	4.2	3.7	3.7	4.2	2.9	4.0	3.8	3.9	3.8	3.5	3.6						
55	3.87	4.3	3.9	3.7	3.8	3.8	3.8	4.0	3.6	3.9										
56	3.79	3.6	3.9	3.8	3.6	4.1	3.7	4.2	4.2	3.7	4.4	3.3	3.4	3.5	3.6					
57	3.82	3.8	4.1	3.9	3.4	3.6	3.8	4.0	3.7	E	4.4	3.9	3.8	3.6	3.7					
58	3.65	3.5	3.5	3.5	3.5	3.9	3.7	3.7	3.7	3.6	3.8	3.7	E							
59	3.96	4.1	3.8	4.2	E	3.8	4.0	4.0	4.3	3.8	3.8	3.8	4.2	3.5	4.2					
60	3.96	3.8	4.0	4.1	4.1	3.9	4.0	4.0	4.2	4.0	3.9	4.0	3.8	3.7	E					
61	3.48	3.6	3.7	3.6	3.8	3.5	3.3	3.3	3.6	3.3	3.5	3.3	3.2							
62	3.48	3.2	3.8	3.4	3.6	3.5	3.6	3.9	3.5	3.3	3.5	3.3	3.2							
63	3.89	3.8	3.9	3.7	3.9	3.9	3.8	3.9	4.0	3.9	4.0	4.0								
64	3.71	E	4.1	4.2	3.5	3.7	3.3	3.8	3.6	3.6	3.8	3.4	3.4	E	4.0	3.8				
65	3.71	3.6	3.6	3.7	3.6	3.9	4.0	3.4	4.1	3.7	3.9	3.6	3.6	3.7	3.6					
66	3.48	E	3.4	3.3	3.4	E	3.8	3.3	3.4	3.5	3.7	3.4	3.7	3.4						
67	3.82	3.4	4.0	3.4	3.7	4.4	3.9	3.9	E	3.8	4.0	3.8	4.2	3.8	3.4	3.7	3.9			

E = early resorptions
L = late resorptions

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TABLE B 3

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Body Weights - Grams

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.																			
68	3.20	E	3.4	3.3	3.2	3.5	3.1	E	2.4	3.1	3.2	3.6	3.6	2.5	3.4					
69	3.73	L	3.8	3.6	3.7	3.7	3.9	3.8	3.5	3.7	4.0	3.6	4.0	3.6	3.1	4.0	3.9			
70	3.65	3.5	3.8	3.7	3.8	3.6	3.6	3.7	3.6	3.8	3.7	3.3	3.7	E						
71	3.46	3.4	3.3	3.5	3.7	3.4	3.6	3.2	3.3	3.9	3.2	3.7	3.6	3.5	3.2	E				
72	3.29	3.2	3.0	2.6	3.5	E	3.6	3.3	3.2	3.2	3.3	3.3	3.7	3.6						
73	not examined																			
74	not examined																			
75	not examined																			
n =	20																			
M	3.67																			
SD	0.21																			

E = early resorptions
L = late resorptions

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TABLE B 4

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Lengths - mm

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)																			
1	34.0	35	30	31	35	34	35	E	E	E	E	36	35	36	34	35	35	35	30	33
2	36.9	38	36	37	39	E	40	35	37	35	36	E	E	37	38					
3	33.6	34	32	38	35	34	34	31	32	35	36	32	30							
4	not pregnant																			
5	36.5	36	35	360	E	39	38	37	36	E	36	35	37							
6	36.2	39	37	35	35	36	35	36	36	36	36	35	38	37						
7	36.3	36	E	37	38	E	L	35	E	37	37	35	39	35	34	36				
8	35.0	34	36	35	36	37	35	35	33	35	35	34	35	33	35	37				
9	33.3	30	32	30	35	32	34	34	34	34	33	35	36							
10	38.7	39	42	38	38	43	42	41	40	38	36	36	36	34						
11	36.9	35	37	37	40	35	37	37	37	41	37	36	E	35	36					
12	35.9	34	37	38	E	38	35	35	37	E	33									
13	34.2	35	36	34	35	34	33	35	33	36	33	32								
14	31.7	32	30	33	30	31	28	25	34	34	35	34	32	34						
15	31.8	36	34	36	L	32	35	30	32	32	32	32	28	27	27					
16	not pregnant																			
17	34.5	36	35	32	31	34	32	36	35	36	36	E	35	36						
18	31.4	34	E	33	25	L	30	L	32	E	33	E	L	33	L					

E = early resorptions
L = late resorptions

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explanations and abbreviations see preface of the report

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TABLE B 4

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Lengths - mm

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)																				
19	32.9	32	31	32	34	29	32	34	35	34	E	35	34							
20	35.8	37	36	38	37	35	38	34	37	37	34	32	35	37	35					
21	35.8	34	36	38	35	35	37	33	36	36	39	37	34							
22	36.7	E	35	38	39	36	35	E	35	35	38	37	38	38						
23	not examined																			
24	not examined																			
25	not examined																			
n =	20																			
M	34.9																			
SD	2.0																			

E = early resorptions
L = late resorptions

explanations and abbreviations see preface of the report

TABLE B 4

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Lengths - mm

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.																			
26	34.4	37	34	34	35	35	36	34	35	33	32	33	34	357						
27	36.1	36	34	35	32	36	36	39	37	40	37	35								
28	not pregnant																			
29	35.3	35	E	36	37	36	38	37	35	32	35	34	35	33	37	36	34	E		
30	35.8	35	38	36	37	36	37	37	35	34	33									
31	32.8	34	35	30	31	35	34	35	30	34	30	30	36							
32	34.2	E	36	35	36	39	32	38	32	32	34	33	31	32						
33	35.9	32	33	32	32	34	34	42	38	E	40	39	37	38						
34	34.5	39	38	37	36	39	33	36	33	32	33	32	33	30	34	33				
35	37.7	35	37	40	38	38	35	39	38	38	39	39	36	38	38	37				
36	not pregnant																			
37	35.2	35	35	34	33	35	36	36	37	E	38	37	33	37	32	35				
38	34.4	38	37	33	E	E	36	32	33	37	35	36	30	L	31					
39	35.0	33	35	35	34	33	38	35	35	35	36	37	36	35	33					
40	35.1	33	E	34	36	35	37	35	35	35	36									
41	35.2	34	33	37	36	E	37	30	36	35	35	35	37	36	37					

E = early resorptions
L = late resorptions

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explanations and abbreviations see preface of the report

TABLE B 4

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Lengths - mm

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.																				
42	33.6	30	35	35	33	35	32	33	35	32	36	33	35	33						
43	33.8	35	34	33	E	36	33	32	34	37	35	33	32	35	31					
44	35.6	35	36	35	36	37	37	35	35	36	34									
45	35.5	38	37	33	37	38	36	E	38	28	36	40	35	35	30	32	40			
46	35.1	E	35	35	36	35	35	36	34	36	35	32	37	35	E					
47	35.7	30	37	36	32	E	35	38	40	E	37	35	36	36	36					
48	not examined																			
49	not examined																			
50	not examined																			
n =	20																			
M	35.0																			
SD	1.0																			

E = early resorptions
L = late resorptions

explanations and abbreviations see preface of the report

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TABLE B 4

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Lengths - mm

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.																			
51	not pregnant																			
52	not pregnant																			
53	34.8	L	E	34	35	34	35	36	L	34	36	35	34							
54	36.0	36	37	38	34	34	37	34	37	39	38	36	33	35						
55	35.8	38	35	37	37	33	34	37	35	36										
56	34.7	35	34	35	30	32	36	35	38	33	37	34	36	36	35					
57	36.2	368	39	37	34	36	36	37	37	E	37	36	37	34	34					
58	34.0	35	32	33	34	32	32	35	37	32	37	35	E							
59	37.8	40	36	36	E	37	39	39	39	38	37	38	38	38	36					
60	36.0	35	35	36	37	36	35	35	40	37	37	35	34	36	E					
61	35.9	36	36	36	37	36	36	35	34	37	34	37	37							
62	34.7	37	36	31	37	34	35	36	34	33	34	34	35							
63	35.5	36	37	35	34	35	33	37	35	36	37	35								
64	35.6	E	35	37	35	37	34	37	35	36	37	33	31	E	38	37				
65	34.7	32	31	35	34	36	37	31	35	34	37	35	38	36	35					
66	33.1	E	32	33	35	E	33	32	33	34	35	35	31	31						
67	35.0	36	35	35	34	36	35	36	E	37	34	32	34	35	36	35	35			

E = early resorptions
L = late resorptions

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TABLE B 4

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal Lengths - mm

Dam No.	Mean	Fetus No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
	Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.																			
68	33.1	E	35	34	35	31	32	E	27	31	35	34	36	31	36					
69	34.6	L	36	36	35	35	34	36	35	37	34	33	34	35	301	33	36			
70	36.4	35	40	36	35	36	38	36	35	36	39	36	35	E						
71	34.9	35	35	34	35	34	37	34	34	36	33	36	34	35	37	E				
72	34.4	33	35	32	35	E	36	38	31	35	30	35	36	37						
73	not examined																			
74	not examined																			
75	not examined																			
n =	20																			
M	35.2																			
SD	1.1																			

E = early resorptions
L = late resorptions

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal External Observations

TABLE B 5

Dam No.	Number of fetuses	Observations
		Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)
1	15	fetus no. 2, right eye: haemorrhage behind the lens, otherwise no remarkable observations
2	11	No remarkable observations
3	12	No remarkable observations
4	0	not pregnant
5	10	fetus no. 11: both hindlimbs shortened, right foot: toes missing, otherwise no remarkable observations
6	13	No remarkable observations
7	11	No remarkable observations
8	15	No remarkable observations
9	12	No remarkable observations
10	13	No remarkable observations
11	13	No remarkable observations
12	8	No remarkable observations
13	11	No remarkable observations
14	13	No remarkable observations
15	13	No remarkable observations
16	0	Not pregnant
17	12	No remarkable observations
18	7	No remarkable observations

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TABLE B 5

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal External Observations

Dam No.	Number of fetuses	Observations
	Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)	
19	11	No remarkable observations
20	14	No remarkable observations
21	12	No remarkable observations
22	11	No remarkable observations
23	-	Not examined
24	-	Not examined
25	-	Not examined

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal External Observations

TABLE B 5

Dam No.	Number of fetuses	Observations
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.	
26	13	No remarkable observations
27	11	No remarkable observations
28	0	Not pregnant
29	15	No remarkable observations
30	10	No remarkable observations
31	12	No remarkable observations
32	12	No remarkable observations
33	12	No remarkable observations
34	15	No remarkable observations
35	15	No remarkable observations
36	0	Not pregnant
37	14	No remarkable observations
38	11	No remarkable observations
39	15	No remarkable observations
40	9	No remarkable observations
41	13	No remarkable observations

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Degussa AG-US-IT-No. 92-0063-
DGC IUA-No. 5025

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explanations and abbreviations see preface of the report

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TABLE B 5

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal External Observations

Dam No.	Number of fetuses	Observations
	Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.	
42	13	No remarkable observations
43	13	No remarkable observations
44	10	No remarkable observations
45	15	No remarkable observations
46	12	No remarkable observations
47	12	No remarkable observations
48	-	Not examined
49	-	Not examined
50	-	Not examined

explanations and abbreviations see preface of the report

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal External Observations

TABLE B 5

Dam No.	Number of fetuses	Observations
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.	
51	0	Not pregnant
52	0	Not pregnant
53	9	No remarkable observations
54	13	No remarkable observations
55	9	No remarkable observations
56	14	No remarkable observations
57	13	No remarkable observations
58	11	No remarkable observations
59	13	No remarkable observations
60	13	No remarkable observations
61	12	No remarkable observations
62	12	No remarkable observations
63	11	No remarkable observations
64	13	No remarkable observations
65	14	No remarkable observations

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explanations and abbreviations see preface of the report

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TABLE B 5

1,3-Propanediol
Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration
Individual Fetal External Observations

Dam No.	Number of fetuses	Observations
	Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.	
66	11	No remarkable observations
67	15	No remarkable observations
68	12	No remarkable observations
69	15	No remarkable observations
70	12	No remarkable observations
71	14	No remarkable observations
72	12	No remarkable observations
73	-	Not examined
74	-	Not examined
75	-	Not examined

explanations and abbreviations see preface of the report

TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
1	1	Skull (parietal, interparietal): retarded ossification; Sternebra(e) not ossified, 5th or reduced in size, 6th
	3	Skull (interparietal): retarded ossification; Sternebra(e) reduced in size, 6th; additional 14th rib
	5	5th - 11th rib (bilateral) wavy; 10th thoracic vertebra centre dumb-bell-shaped
	11	Skull (interparietal): retarded ossification; Sternebra(e) reduced in size, 6th
	13	Sternebra(e) incompletely ossified, 5th or reduced in size, 6th; 3rd - 11th rib (bilateral) wavy
	15	11th thoracic vertebra centre dumb-bell-shaped
	17	Skull (parietal, interparietal, supra-occipital): retarded ossification; Hyoid not ossified; Sternebra(e) incompletely ossified, 5th; 11th rib (right) wavy; 12th thoracic vetebra centre dumb-bell-shaped
	19	Sternebra(e) incompletely ossified, 5th or reduced in size, 6th
2	2	No remarkable observations
	4	14 pairs of ribs
	7	No remarkable observations
	9	No remarkable observations
	13	14 pairs of ribs
3	1	Sternebra(e) not ossified, 5th
	3	Sternebra(e) not ossified, 5th or misaligned, 3rd, 4th
	5	Sternebra(e) not ossified, 5th; 4th - 11th rib (bilateral) wavy
	7	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) incompletely ossified, 5th; 4th - 11th rib (bilateral) wavy
	9	Sternebra(e) incompletely ossified, 5th or reduced in size, 6th

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
3 (continued)		
	11	Sternebra(e) not ossified, 5th or reduced in size, 6th
4	not pregnant	
5	1	Skull (interparietal, supraoccipital): retarded ossification; Sternebra(e) not ossified, 6th; 12th and 13th thoracic and 1st lumbar vertebra centre dumb-bell-shaped
	3	Sternebra(e) incompletely ossified, 5th or reduced in size, 6th; 6th - 10th rib (right) wavy
	6	14 pairs of ribs
	8	Skull (parietal, supraoccipital): retarded ossification; 7th - 11th rib (right) wavy; 10th - 13th thoracic vertebra centre dumb-bell-shaped
	11#	14 pairs of ribs; 11th and 12th thoracic vertebra centre dumb-bell-shaped
6	1	Skull (parietal): retarded ossification; Sternebra(e) reduced in size, 6th
	3	Sternebra(e) incompletely ossified, 5th or misaligned, 3rd - 5th
	5	Skull (parietal): retarded ossification; Hyoid not ossified; Sternebra(e) not, 6th or incompletely ossified, 5th
	7	Skull (parietal, interparietal): retarded ossification; Sternebra(e) misaligned, 4th, 5th
	9	Hyoid not ossified; Sternebra(e) not ossified, 6th
	11	Skull (parietal, interparietal, supra-occipital): retarded ossification; Hyoid not ossified; Sternebra(e) not ossified, 6th
	13	Sternebra(e) not ossified, 2nd, 6th

Malformed foetus (shortened pelvic extremities, right phalanges missing)

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
7	3	Sternebra(e) incompletely ossified, 5th
	7	No remarkable observations
	10	Skull (parietal): retarded ossification; Sternebra(e) incompletely ossified, 2nd
	12	No remarkable observations
	14	Sternebra(e) incompletely ossified, 5th
8	1	10th thoracic vertebra centre dumb-bell-shaped
	3	No remarkable observations
	5	Skull (parietal): retarded ossification; Sternebra(e) reduced in size, 6th
	8	Skull (parietal, interparietal): retarded ossification; Sternebra(e) incompletely ossified, 5th
	10	Skull (parietal, supraoccipital): retarded ossification
	12	Sternebra(e) incompletely ossified, 2nd, 5th or misaligned, 2nd - 4th
	14	No remarkable observations
	16	Sternebra(e) incompletely ossified, 2nd or misaligned, 3rd - 5th
9	2	No remarkable observations
	4	Sternebra(e) incompletely ossified, 5th
	6	No remarkable observations
	8	Sternebra(e) incompletely ossified, 5th
	10	No remarkable observations
	12	No remarkable observations
10	2	Sternebra(e) misaligned, 5th
	4	Sternebra(e) incompletely ossified, 5th or misaligned, 3rd, 4th
	6	No remarkable observations
	8	No remarkable observations
	10	No remarkable observations
	12	No remarkable observations

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
11	1	additional 14th rib
	3	No remarkable observations
	5	No remarkable observations
	7	Sternebra(e) misaligned, 3rd, 4th
	9	No remarkable observations
	11	No remarkable observations
	14	No remarkable observations
12	2	Sternebra(e) incompletely ossified, 2nd or reduced in size, 6th or misaligned, 3rd, 4th; 4th - 13th rib (bilateral) wavy; 10th and 12th thoracic vertebra centre dumb-bell-shaped
	5	Skull (frontal, parietal, interparietal, supraoccipital): retarded ossification; 5th - 12th rib (bilateral) wavy; 9th, 10th, 12th and 13th thoracic vertebra centre dumb-bell-shaped
	7	Skull (parietal, interparietal, supra-occipital): retarded ossification; 5th - 12th rib (bilateral) wavy; 8th, 9th and 11th thoracic vertebra centre dumb-bell-shaped
	10	Skull (parietal): retarded ossification; 9th, 11th and 12th thoracic vertebra centre dumb-bell-shaped
13	2	Skull (parietal): retarded ossification; Hyoid not ossified; Sternebra(e) incompletely ossified, 5th
	4	14 pairs of ribs; 6th - 9th and 11th - 13th rib (left) and 5th - 12th rib (right) wavy
	6	14 pairs of ribs
	8	Skull (frontal, parietal, interparietal, supraoccipital): retarded ossification; Hyoid not ossified; Sternebra(e) not ossified, 5th or reduced in size, 6th; 5th - 13th rib (bilateral) wavy
	10	Sternebra(e) incompletely ossified, 5th

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
14	1	13th thoracic vertebra centre bipartite
	3	Sternebra(e) misaligned, 4th, 5th
	5	Sternebra(e) incompletely ossified, 5th or reduced in size, 6th
	7	Sternebra(e) not ossified, 2nd or misaligned, 3rd - 5th
	9	Sternebra(e) reduced in size, 6th; additional 14th rib
	11	No remarkable observations
	13	No remarkable observations
15	2	Sternebra(e) incompletely ossified, 5th
	5	Skull (parietal, interparietal): retarded ossification
	7	Sternebra(e) not ossified, 5th, 6th
	9	Hyoid not ossified; 14 pairs of ribs; 10th thoracic vertebra centre dumb-bell-shaped
	11	No remarkable observations
	13	Skull (interparietal): retarded ossification
16	not pregnant	
17	1	Sternebra(e) incompletely ossified, 5th
	3	No remarkable observations
	5	Sternebra(e) not ossified, 2nd, 5th or misaligned, 3rd, 4th
	7	No remarkable observations
	9	Sternebra(e) incompletely ossified, 5th
	12	Skull (parietal): retarded ossification; Sternebra(e) not ossified, 5th or misaligned, 3rd, 4th; 13th thoracic vertebra centre dumb-bell-shaped
18	1	Skull (parietal, interparietal): retarded ossification; Sternebra(e) incompletely ossified, 5th; 8th - 10th rib (right) wavy; 10th thoracic vertebra centre dumb-bell-shaped

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
18 (continued)		
	4	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) not ossified, 2nd, 4th - 6th or reduced in size, 3rd; 9th and 10th thoracic vertebra centre dumb-bell-shaped; 5th metacarpalia (bilateral) not ossified
	8	Skull (parietal): retarded ossification; Sternebra(e) not ossified, 5th or reduced in size, 6th
	13	Hyoid not ossified; Sternebra(e) not ossified, 5th, 6th; 6th - 11th rib (bilateral) wavy; 5th metacarpalia (bilateral) not ossified
19	2	Skull (interparietal): retarded ossification
	4	No remarkable observations
	6	Sternebra(e) incompletely ossified, 5th
	8	No remarkable observations
	11	Sternebra(e) incompletely ossified, 5th
20	1	Skull (parietal): retarded ossification
	3	No remarkable observations
	5	No remarkable observations
	7	No remarkable observations
	9	Sternebra(e) incompletely ossified, 5th
	11	Skull (parietal, interparietal, supra-occipital): retarded ossification
	13	Skull (parietal, interparietal): retarded ossification
21	1	No remarkable observations
	3	12th thoracic vertebra centre dumb-bell-shaped
	5	Skull (parietal, interparietal, supra-occipital): retarded ossification; Hyoid not ossified; 11th rib (right) wavy
	7	No remarkable observations
	9	14 pairs of ribs
	11	No remarkable observations

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
22	2	Sternebra(e) not ossified, 5th
	4	Skull (parietal): retarded ossification
	6	Sternebra(e) not ossified, 5th
	9	Sternebra(e) not ossified, 5th
	11	Sternebra(e) not ossified, 5th
	13	Sternebra(e) incompletely ossified, 5th or reduced in size, 6th
23	not examined	
24	not examined	
25	not examined	

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FREIE UND HANSESTADT HAMBURG

BEHÖRDE FÜR ARBEIT, GESUNDHEIT UND SOZIALES

GLP - Bescheinigung

gem. Anhang 2 des Chemikaliengesetzes
in der Bekanntmachung der Neufassung
vom 14.3.1990 (BGBl. I S. 521)

Bescheinigung

Hiermit wird bestätigt, daß die Prüfungseinrichtung(en)

Certificate

It is hereby certified that the test facility(ies)

in Redderweg 8, 2104 Hamburg 92
(Ort, Anschrift) / (location, address)

der / of Praxis Prof. Dr. med. F. Leuschner/Laboratory of Pharmakology and Toxikology (LPT)
(Firma) / (company name)

am/on 15.1.1992, 29.1.1992, 24.3.1992, 7.5.1992
(Datum) / (date)

von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist (sind).

was (were) inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

Pharmakologisch-toxikologische Prüfungen
auf Herz-Kreislauf-Parameter an Hunden zur Bestimmung der Sicherheitspharmakologie in toxisch relevanten Dosen.

Pharmakologisch-toxikologische Prüfungen
in vitro an Zellsystemen zur Erfassung des mutagenen Potentials mit Hilfe
- des Ames-testes (Prokaryonten)
- des Genmutationstestes an Eukaryonten,
- des Säuger zytogenetischen Testes (z.B. Humanlymphozyten) sowie

Pharmakologisch-toxikologische Prüfungen mit Hilfe
des Knochenmarks von Nagern zur Feststellung von Chromosomenaberrationen.

Hamburg, den 10.7.1992

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[redacted]

Lettau

TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
26	1	Sternebra(e) incompletely ossified, 2nd, 5th or misaligned, 3rd, 4th
	3	Sternebra(e) incompletely ossified, 5th
	5	Skull (parietal): retarded ossification; Sternebra(e) not ossified, 5th
	7	No remarkable observations
	9	No remarkable observations
	11	Skull (parietal): retarded ossification; Sternebra(e) incompletely ossified, 5th
	13	Sternebra(e) incompletely ossified, 5th or Sternebra(e) misaligned, 3rd, 4th
27	2	Sternebra(e) incompletely ossified, 2nd or reduced in size, 6th
	4	Skull (parietal): retarded ossification; Sternebra(e) incompletely ossified, 5th or reduced in size, 6th
	6	No remarkable observations
	8	13th thoracic vertebra centre bipartite
	10	13th thoracic vertebra centre bipartite
28	not pregnant	
29	1	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) not ossified, 6th; 11th rib (bilateral) wavy
	4	Skull (parietal, supraoccipital): retarded ossification; Sternebra(e) not, 6th or incompletely ossified, 5th
	6	Skull (parietal, supraoccipital): retarded ossification; Sternebra(e) not ossified, 5th, 6th
	8	Sternebra(e) not ossified, 5th; 10th thoracic vertebra centre dumb-bell-shaped
	10	Sternebra(e) not ossified, 5th
	12	Skull (interparietal, supraoccipital): retarded ossification; Sternebra(e) not ossified, 5th

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
29 (continued)		
	14	Skull (interparietal, supraoccipital): retarded ossification; Sternebra(e) incompletely ossified, 5th or reduced in size, 6th
	16	additional 14th rib
30	2	No remarkable observations
	4	12th thoracic vertebra centre dumb-bell-shaped
	6	10th thoracic vertebra centre dumb-bell-shaped
	8	No remarkable observations
	10	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) not ossified, 5th
31	2	Skull (parietal): retarded ossification
	4	Skull (parietal): retarded ossification; Sternebra(e) not ossified, 5th
	6	Hyoid not ossified
	8	Skull (parietal, supraoccipital): retarded ossification
	10	Skull (parietal, supraoccipital): retarded ossification; Sternebra(e) reduced in size, 6th
	12	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) reduced in size, 6th
32	3	Sternebra(e) misaligned, 5th
	5	No remarkable observations
	7	12th thoracic vertebra centre incompletely ossified
	9	No remarkable observations
	11	Sternebra(e) incompletely ossified, 5th
	13	No remarkable observations
33	2	No remarkable observations
	4	No remarkable observations

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
33 (continued)		
	6	Skull (parietal): retarded ossification
	8	No remarkable observations
	11	No remarkable observations
	13	No remarkable observations
34	2	No remarkable observations
	4	No remarkable observations
	6	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) not ossified, 5th
	8	Skull (parietal, supraoccipital): retarded ossification; Sternebra(e) reduced in size, 6th
	10	Skull (parietal, interparietal, supra-occipital): retarded ossification; 12th thoracic vertebra centre dumb-bell-shaped
	12	Skull (parietal, interparietal, supra-occipital): retarded ossification
	14	No remarkable observations
35	1	11th thoracic vertebra centre dumb-bell-shaped, 12th thoracic vertebra centre bipartite
	3	No remarkable observations
	5	Skull (parietal): retarded ossification; Sternebra(e) not ossified, 5th
	7	No remarkable observations
	9	Sternebra(e) incompletely ossified, 5th
	11	No remarkable observations
	13	Sternebra(e) incompletely ossified, 5th or misaligned, 3rd, 4th
	15	additional 14th rib
36	not pregnant	

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
37	2	Skull (parietal, interparietal, supra-occipital): retarded ossification; Hyoid not ossified; 10th and 11th rib (right) wavy
	4	Skull (supraoccipital): retarded ossification; Sternebra(e) not, 2nd or incompletely ossified, 5th or reduced in size, 6th; 4th - 12th rib (bilateral) wavy
	6	Skull (parietal, interparietal, supra-occipital): retarded ossification; Hyoid not ossified; Sternebra(e) incompletely ossified, 5th or reduced in size, 6th; 4th - 12th rib (bilateral) wavy; 10th thoracic vertebra centre dumb-bell-shaped
	8	Skull (parietal, interparietal): retarded ossification; Sternebra(e) incompletely ossified, 5th or reduced in size, 6th; 4th - 10th rib (bilateral) wavy
	11	Skull (parietal, interparietal, supra-occipital): retarded ossification; Hyoid not ossified; Sternebra(e) incompletely ossified, 5th
	13	Skull (parietal, interparietal, supra-occipital): retarded ossification; Hyoid not ossified; Sternebra(e) not ossified, 2nd, 3rd, 5th or reduced in size, 6th; 4th - 11th rib (bilateral) wavy
	15	Skull (parietal, interparietal, supra-occipital): retarded ossification; Hyoid not ossified; Sternebra(e) not ossified, 2nd, 5th, 6th; 4th - 11th rib (bilateral) wavy; 10th and 13th thoracic vertebra centre dumb-bell-shaped
38	2	No remarkable observations
	6	6th - 10th rib (right) wavy

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
38 (continued)		
	8	6th - 10th rib (right) wavy
	10	14 pairs of ribs
	12	Sternebra(e) incompletely ossified, 5th
39	1	Skull (parietal, interparietal, supra-occipital): retarded ossification; 14 pairs of ribs
	3	14 pairs of ribs
	5	Skull (parietal): retarded ossification; Hyoid not ossified; Sternebra(e) reduced in size, 6th
	7	Skull (parietal): retarded ossification; 14 pairs of ribs
	9	No remarkable observations
	11	Skull (parietal, interparietal, supra-occipital): retarded ossification; 5th - 13th rib (bilateral) wavy
	13	Skull (parietal, interparietal, supra-occipital): retarded ossification; 5th - 13th rib (bilateral) wavy
	15	Skull (parietal): retarded ossification; Hyoid not ossified; Sternebra(e) reduced in size, 6th; 12th thoracic vertebra centre dumb-bell-shaped
40	3	Skull (parietal, interparietal, supra-occipital): retarded ossification; Hyoid not ossified
	5	11th and 12th thoracic vertebra centre dumb-bell-shaped; additional 14th rib
	7	Sternebra(e) incompletely ossified, 5th or misaligned, 5th
	9	14 pairs of ribs
41	1	Skull (parietal): retarded ossification; Sternebra(e) incompletely ossified, 5th; 4th - 8th rib (bilateral) wavy

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
41 (continued)		
	3	Skull (parietal, supraoccipital): retarded ossification; 14 pairs of ribs
	6	Skull (interparietal): retarded ossification; Sternebra(e) not ossified, 5th
	8	Skull (parietal, interparietal): retarded ossification; Hyoid not ossified; Sternebra(e) incompletely ossified; 2nd, 5th
	10	No remarkable observations
	12	Sternebra(e) incompletely ossified and misaligned, 5th; 14 pairs of ribs
	14	No remarkable observations
42	2	No remarkable observations
	4	Skull (parietal, interparietal, supra-occipital): retarded ossification; 4th thoracic vertebra centre bipartite; 10th, 12th, 13th thoracic vertebra centre dumb-bell-shaped
	6	No remarkable observations
	8	No remarkable observations
	10	No remarkable observations
	12	12th thoracic vertebra centre dumb-bell-shaped
43	1	No remarkable observations
	3	No remarkable observations
	6	No remarkable observations
	8	No remarkable observations
	10	No remarkable observations
	12	No remarkable observations
	14	No remarkable observations
44	2	12th thoracic vertebra centre dumb-bell-shaped
	4	Skull (interparietal, supraoccipital): retarded ossification; 12th and 13th thoracic vertebra centre dumb-bell-shaped

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
44 (continued)		
	6	Skull (parietal, supraoccipital): retarded ossification; 4th - 12th rib (bilateral) wavy
	8	No remarkable observations
	10	Skull (parietal, interparietal, supra-occipital): retarded ossification
45	2	10th and 12th thoracic vertebra centre dumb-bell-shaped
	4	Skull (interparietal, supraoccipital): retarded ossification
	6	No remarkable observations
	9	Sternebra(e) not ossified, 2nd, 3rd, 6th; 11th and 13th thoracic vertebra centre dumb-bell-shaped; 5th metacarpalia (bilateral) not ossified
	11	Skull (interparietal, supraoccipital): retarded ossification; Sternebra(e) in-completely ossified, 5th; 11th and 13th thoracic vertebra centre dumb-bell-shaped
	13	No remarkable observations
	15	No remarkable observations
46	2	Skull (parietal, supraoccipital): retarded ossification
	4	Skull (parietal): retarded ossification; Sternebra(e) not ossified, 2nd
	6	Skull (parietal): retarded ossification
	8	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) not ossified, 5th or reduced in size, 6th
	10	Skull (interparietal): retarded ossification; Sternebra(e) not, 2nd, 6th or incompletely ossified, 5th
	12	Sternebra(e) not, 2nd or incompletely ossified, 5th or reduced in size, 6th

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
47	1	Skull (parietal, interparietal, supra-occipital): retarded ossification; Hyoid not ossified; Sternebra(e) incompletely ossified, 5th or reduced in size, 6th
	3	Hyoid not ossified; Sternebra(e) incompletely ossified, 2nd, 5th
	6	Hyoid not ossified; Sternebra(e) incompletely ossified, 5th
	8	Hyoid not ossified
	11	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) incompletely ossified, 5th misaligned, 4th, 3rd
	13	Skull (interparietal): retarded ossification; Sternebra(e) incompletely ossified, 5th
48	not examined	
49	not examined	
50	not examined	

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
51	not pregnant	
52	not pregnant	
53	3	Skull (parietal, supraoccipital): retarded ossification
	5	Skull (parietal, supraoccipital): retarded ossification; Sternebra(e) incompletely ossified, 5th
	7	Skull (parietal, supraoccipital): retarded ossification; 9th - 11th rib (right) wavy
	10	Skull (supraoccipital): retarded ossification; 11th and 12th rib (left) and 5th - 12th rib (right) wavy
	12	No remarkable observations
54	2	14 pairs of ribs
	4	Skull (parietal, interparietal): retarded ossification; 12th thoracic vertebra centre dumb-bell-shaped; additional 14th rib
	6	Skull (parietal, supraoccipital): retarded ossification; Hyoid not ossified; Sternebra(e) incompletely ossified, 2nd, 5th
	8	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) incompletely ossified, 5th or reduced in size, 6th
	10	Skull (parietal): retarded ossification
	12	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) not ossified, 5th
55	1	Skull (parietal, supraoccipital): retarded ossification; Hyoid not ossified; 8th - 11th rib (right) wavy; 11th thoracic and 1st lumbar vertebra centre dumb-bell-shaped
	3	Skull (parietal, interparietal): retarded ossification

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
55 (continued)		
	5	Skull (parietal, supraoccipital): retarded ossification; Sternebra(e) incompletely ossified, 5th; 9th - 11th thoracic and 1st and 2nd lumbar vertebra centre dumb-bell-shaped
	7	Sternebra(e) incompletely ossified, 5th or misaligned, 3rd, 4th
	9	Skull (parietal, interparietal, supra-occipital): retarded ossification; 5th - 13th rib (bilateral) wavy; 10th and 12th thoracic vertebra centre dumb-bell-shaped
56	2	14 pairs of ribs
	4	14 pairs of ribs
	6	No remarkable observations
	8	14 pairs of ribs
	10	Skull (interparietal): retarded ossification
	12	12th thoracic vertebra centre dumb-bell-shaped
	14	Sternebra(e) incompletely ossified, 2nd or reduced in size, 6th
57	2	Skull (parietal, interparietal, supra-occipital): retarded ossification
	4	Sternebra(e) incompletely ossified, 5th
	6	10th thoracic vertebra centre dumb-bell-shaped
	8	No remarkable observations
	11	Sternebra(e) not ossified, 5th or reduced in size, 6th
	13	Sternebra(e) reduced in size, 6th
58	1	Skull (parietal, interparietal, supra-occipital): retarded ossification
	3	Skull (supraoccipital): retarded ossification; Sternebra(e) incompletely ossified, 5th
	5	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) incompletely ossified, 5th

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
58 (continued)		
	7	Skull (parietal, interparietal): retarded ossification; 6th - 12th rib (bilateral) wavy; 11th thoracic vertebra centre dumb-bell-shaped
	9	Skull (parietal, interparietal): retarded ossification; 8th - 11th rib (right) wavy
	11	Skull (parietal, interparietal, supra-occipital): retarded ossification; 5th - 11th rib (bilateral) wavy; 12th thoracic vertebra centre dumb-bell-shaped
59	2	Skull (parietal, interparietal, supra-occipital): retarded ossification
	5	13th thoracic vertebra centre dumb-bell-shaped
	7	No remarkable observations
	9	No remarkable observations
	11	Sternebra(e) incompletely ossified, 5th
	13	Sternebra(e) not ossified, 5th
60	1	11th thoracic vertebra centre dumb-bell-shaped
	3	Sternebra(e) incompletely ossified, 5th
	5	Sternebra(e) incompletely ossified, 5th or misaligned, 3rd, 4th
	7	Sternebra(e) misaligned, 4th, 5th
	9	No remarkable observations
	11	Skull (interparietal, supraoccipital): retarded ossification; Hyoid not ossified
	13	Hyoid not ossified; Sternebra(e) incompletely ossified, 5th
61	2	Skull (parietal, interparietal): retarded ossification; Sternebra(e) incompletely ossified, 5th or reduced in size, 6th; 11th thoracic vertebra centre dumb-bell-shaped

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
61 (continued)		
	4	Skull (parietal, interparietal): retarded ossification
	6	Skull (parietal): retarded ossification; Sternebra(e) misaligned, 3rd - 5th
	8	Sternebra(e) not ossified, 2nd or reduced in size, 6th; 14 pairs of ribs
	10	Skull (parietal, interparietal): retarded ossification; Sternebra(e) reduced in size, 6th; 11th rib (bilateral) wavy
	12	Skull (parietal): retarded ossification; Sternebra(e) incompletely ossified, 5th or reduced in size, 6th; 14 pairs of ribs
62	2	Skull (interparietal, supraoccipital): retarded ossification; Hyoid not ossified; additional 14th rib
	4	Hyoid not ossified; 14 pairs of ribs
	6	Sternebra(e) not ossified, 2nd
	8	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) incompletely ossified, 2nd or misaligned, 3rd, 4th; 14 pairs of ribs; 5th - 11th rib (bilateral) wavy
	10	Skull (parietal, supraoccipital): retarded ossification; Sternebra(e) incompletely ossified, 2nd
	12	No remarkable observations
63	2	4th - 11th rib (bilateral) wavy; 10th and 13th thoracic vertebra centre dumb-bell-shaped
	4	6th - 10th rib (right) wavy; 12th thoracic vertebra centre dumb-bell-shaped
	6	Skull (interparietal): retarded ossification
	8	No remarkable observations
	10	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) incompletely ossified, 5th or misaligned, 3rd, 4th; 11th rib (right) wavy

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
64	2	No remarkable observations
	4	No remarkable observations
	6	Sternebra(e) reduced in size, 6th
	8	No remarkable observations
	10	Sternebra(e) not ossified, 5th
	12	Sternebra(e) incompletely ossified, 5th
	15	Sternebra(e) incompletely ossified, 5th or reduced in size, 6th
65	2	Skull (parietal): retarded ossification; Sternebra(e) not ossified, 5th or reduced in size, 6th
	4	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) reduced in size, 6th
	6	Skull (parietal, interparietal, supra-occipital): retarded ossification; Sternebra(e) not ossified, 5th, 6th
	8	Skull (parietal): retarded ossification
	10	Skull (parietal): retarded ossification
	12	Sternebra(e) incompletely ossified, 5th or reduced in size, 6th
	14	No remarkable observations
66	3	No remarkable observations
	6	No remarkable observations
	8	No remarkable observations
	10	Sternebra(e) incompletely ossified, 5th
	12	Sternebra(e) not ossified, 5th
67	1	No remarkable observations
	3	Sternebra(e) not ossified, 5th or reduced in size, 6th
	5	Sternebra(e) incompletely ossified, 5th
	7	Sternebra(e) incompletely ossified, 5th or misaligned, 3rd, 4th
	10	Sternebra(e) not, 5th or incompletely ossified, 2nd or misaligned, 3rd, 4th
	12	Hyoid not ossified
	14	Hyoid not ossified; Sternebra(e) not ossified, 5th
	16	Sternebra(e) incompletely ossified, 5th

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
68	3	Sternebra(e) incompletely ossified, 5th or reduced in size, 6th
	5	Sternebra(e) not ossified, 5th or reduced in size, 6th
	8	Sternebra(e) not ossified, 5th
	10	Hyoid not ossified; Sternebra(e) reduced in size, 5th, 6th; 14 pairs of ribs
	12	Skull (parietal, supraoccipital): retarded ossification; Hyoid not ossified; Sternebra(e) not ossified, 5th, 6th; 5th metacarpalia (bilateral) not ossified
	14	Skull (parietal, interparietal): retarded ossification; Hyoid not ossified; Sternebra(e) incompletely ossified, 5th or reduced in size, 6th; additional 14th rib
69	3	Sternebra(e) incompletely ossified, 2nd
	5	Sternebra(e) incompletely ossified, 5th; 11th thoracic vertebra centre dumb-bell-shaped
	7	No remarkable observations
	9	No remarkable observations
	11	Sternebra(e) incompletely ossified, 5th
	13	No remarkable observations
	15	Sternebra(e) incompletely ossified, 5th
70	1	No remarkable observations
	3	Sternebra(e) not ossified, 2nd
	5	Sternebra(e) misaligned, 4th, 5th
	7	No remarkable observations
	9	No remarkable observations
	11	Skull (interparietal, supraoccipital): retarded ossification; Sternebra(e) misaligned, 5th
71	1	Skull (parietal, supraoccipital): retarded ossification; 11th rib (left) and 11th and 12th rib (right) wavy
	3	Skull (parietal, interparietal, supra-occipital): retarded ossification; 10th rib (right) wavy

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
71 (continued)		
	5	Skull (parietal, interparietal, supra-occipital): retarded ossification; Hyoid not ossified; 10th - 12th rib (right) wavy
	7	Skull (interparietal): retarded ossification; Sternebra(e) incompletely ossified, 5th or reduced in size, 6th
	9	Skull (parietal, interparietal, supra-occipital): retarded ossification
	11	Skull (parietal, interparietal, supra-occipital): retarded ossification; 11th and 12th rib (left) and 5th - 12th rib (right) wavy
	13	Skull (parietal): retarded ossification; Sternebra(e) incompletely ossified, 5th; 11th rib (right) wavy
72	1	Sternebra(e) incompletely ossified, 5th
	3	Skull (parietal, supraoccipital): retarded ossification; Sternebra(e) incompletely ossified, 5th or reduced in size, 6th; 12th thoracic vertebra centre bipartite
	6	Skull (parietal): retarded ossification; 11th thoracic vertebra centre dumb-bell-shaped
	8	Sternebra(e) incompletely ossified, 2nd, 5th
	10	Skull (parietal, supraoccipital): retarded ossification; Sternebra(e) incompletely ossified, 2nd; 14 pairs of ribs; 10th and 11th thoracic vertebra centre dumb-bell-shaped
	12	Skull (parietal): retarded ossification; 10th thoracic vertebra centre dumb-bell-shaped

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TABLE B 6 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Skeletal Observations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
73		not examined
74		not examined
75		not examined

000280

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
1	2	no remarkable observations
	4	no remarkable observations
	6	bilateral dilation of renal pelvis
	12	no remarkable observations
	14	slightly dislocated kidney, left (up to 1.5 mm) with hydro-ureter
	16	no remarkable observations
	18	no remarkable observations
2	1	no remarkable observations
	3	no remarkable observations
	6	no remarkable observations
	8	no remarkable observations
	10	no remarkable observations
	14	bilateral dilation of renal pelvis
3	2	bilateral dilation of renal pelvis
	4	4th cerebral ventricle enlarged
	6	bilateral dilation of renal pelvis
	8	no remarkable observations
	10	no remarkable observations
	12	no remarkable observations
4	not pregnant	
5	2	no remarkable observations
	5	no remarkable observations
	7	no remarkable observations
	10	no remarkable observations
	12	4th cerebral ventricle enlarged
6	2	no remarkable observations
	4	no remarkable observations
	6	3 haemorrhages on liver surface (Ø 1 mm, 2 mm); slightly dislocated kidney, left (up to 2 mm)
	8	dilation of left renal pelvis
	10	no remarkable observations
	12	no remarkable observations

000281

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
7	1	no remarkable observations
	4	no remarkable observations
	9	no remarkable observations
	11	haemorrhage on liver surface (3x2x2 mm)
	13	no remarkable observations
	15	no remarkable observations
8	2	no remarkable observations
	4	bilateral dilation of renal pelvis
	6	no remarkable observations
	9	no remarkable observations
	11	dilation of left renal pelvis
	13	no remarkable observations
	15	no remarkable observations
9	1	no remarkable observations
	3	no remarkable observations
	5	no remarkable observations.
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations
10	1	no remarkable observations
	3	no remarkable observations
	5	no remarkable observations
	7	no remarkable observations
	9	bilateral dilation of renal pelvis; haemorrhage on liver surface (Ø 2 mm)
	11	dilation of right renal pelvis
	13	bilateral dilation of renal pelvis
11	2	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations
	8	no remarkable observations
	10	no remarkable observations
	13	no remarkable observations

000282

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
12	1	no remarkable observations
	3	no remarkable observations
	6	no remarkable observations
	8	no remarkable observations
13	1	no remarkable observations
	3	haemorrhage on liver surface (2x1x1 mm)
	5	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
	11	bilateral dilation of renal pelvis
14	2	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations
	8	no remarkable observations
	10	no remarkable observations
	12	no remarkable observations
15	1	no remarkable observations
	3	no remarkable observations
	6	no remarkable observations
	8	no remarkable observations
	10	no remarkable observations
	12	no remarkable observations
	14	4th cerebral ventricle enlarged; slightly dislocated kidney, left (up to 2 mm)
16	not pregnant	
17	2	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations
	8	4th cerebral ventricle enlarged
	10	no remarkable observations
	13	no remarkable observations
18	3	4th cerebral ventricle enlarged
	6	4th cerebral ventricle enlarged
	10	dilation of right renal pelvis

000283

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 1: Control (10 ml METHOCEL E 4 M/kg b.w. p.o.)		
19	1	haemorrhage on liver surface (3x2x1 mm)
	3	no remarkable observations
	5	no remarkable observations
	7	dilation of left renal pelvis
	9	4th cerebral ventricle enlarged
	12	4th cerebral ventricle enlarged
20	2	bilateral dilation of renal pelvis; bilateral hydro-ureter
	4	no remarkable observations
	6	no remarkable observations
	8	haemorrhage on liver surface (4x2x1 mm)
	10	no remarkable observations
	12	no remarkable observations
	14	no remarkable observations
21	2	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations
	9	dilation of left renal pelvis
	10	no remarkable observations
	12	dilation of right renal pelvis
22	3	no remarkable observations
	5	dilation of left renal pelvis
	8	no remarkable observations
	10	no remarkable observations
	12	no remarkable observations
23	not examined	
24	not examined	
25	not examined	

000284

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
26	2	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations
	8	no remarkable observations
	10	no remarkable observations
	12	haemorrhage on liver surface (3x1x1 mm)
27	1	no remarkable observations
	3	no remarkable observations
	5	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations
28	not pregnant	
29	3	no remarkable observations
	5	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations
	13	haemorrhage on liver surface (2x1x1 mm)
	15	no remarkable observations
30	1	no remarkable observations
	3	no remarkable observations
	5	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
31	1	no remarkable observations
	3	no remarkable observations
	5	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations

000285

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
32	2	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations
	8	bilateral dilation of renal pelvis
	10	no remarkable observations
	12	bilateral dilation of renal pelvis
33	1	no remarkable observations
	3	no remarkable observations
	5	bilateral dilation of renal pelvis
	7	dilation of left renal pelvis
	10	no remarkable observations
	12	no remarkable observations
34	1	no remarkable observations
	3	no remarkable observations
	5	bilateral dilation of renal pelvis; haemorrhage on liver surface (3x1x1 mm)
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations
	13	no remarkable observations
	15	4th cerebral ventricle enlarged
35	2	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations
	8	dilation of left renal pelvis
	10	bilateral dilation of renal pelvis
	12	hydro-ureter, left
	14	no remarkable observations
36	not pregnant	
37	1	no remarkable observations
	3	no remarkable observations
	5	no remarkable observations
	7	no remarkable observations
	10	no remarkable observations
	12	dilation of left renal pelvis
	14	no remarkable observations

000286

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
38	1	no remarkable observations
	3	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations
	14	no remarkable observations
39	2	no remarkable observations
	4	no remarkable observations
	6	bilateral dilation of renal pelvis
	8	no remarkable observations
	10	no remarkable observations
	12	no remarkable observations
	14	no remarkable observations
40	1	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations.
	8	no remarkable observations
	10	hydro-ureter, left
41	2	no remarkable observations
	4	dilation of right renal pelvis
	7	no remarkable observations
	9	dilation of right renal pelvis
	11	hydro-ureter, left
	13	no remarkable observations
42	1	no remarkable observations
	3	no remarkable observations
	5	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations
	13	no remarkable observations

000287

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 2: 250 mg 1,3-Propanediol/kg b.w. p.o.		
43	2	no remarkable observations
	5	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations
	13	haemorrhage on liver surface (3x2x1 mm)
44	1	haemorrhage on liver surface (Ø 3 mm)
	3	no remarkable observations
	5	dilation of left renal pelvis
	7	no remarkable observations
	9	no remarkable observations
45	1	no remarkable observations
	3	no remarkable observations
	5	no remarkable observations
	8	no remarkable observations
	10	no remarkable observations
	12	bilateral dilation of renal pelvis
	14	bilateral dilation of renal pelvis
	16	no remarkable observations
46	3	bilateral dilation of renal pelvis
	5	no remarkable observations
	7	haemorrhage on liver surface (3x2x2 mm)
	9	no remarkable observations
	11	no remarkable observations
	13	no remarkable observations
47	2	no remarkable observations
	4	no remarkable observations
	7	no remarkable observations
	10	no remarkable observations
	12	haemorrhage on liver surface (Ø 2 mm)
	14	no remarkable observations
48	not examined	
49	not examined	
50	not examined	

000288

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
51	not pregnant	
52	not pregnant	
53	4	no remarkable observations
	6	no remarkable observations
	9	no remarkable observations
	11	dilation of left renal pelvis
54	1	no remarkable observations
	3	bilateral dilation of renal pelvis
	5	bilateral dilation of renal pelvis
	7	bilateral dilation of renal pelvis; 3 haemorrhages on liver surface (Ø 1 mm, Ø 2 mm)
	9	no remarkable observations
	11	no remarkable observations
	13	dilation of right renal pelvis; haemorrhage on liver surface (4x2x2 mm)
55	2	bilateral dilation of renal pelvis
	4	no remarkable observations
	6	no remarkable observations
	8	no remarkable observations
56	1	no remarkable observations
	3	bilateral dilation of renal pelvis
	5	bilateral dilation of renal pelvis
	7	4th cerebral ventricle enlarged
	9	no remarkable observations
	11	no remarkable observations
	13	no remarkable observations
57	1	no remarkable observations
	3	no remarkable observations
	5	no remarkable observations
	7	no remarkable observations
	10	no remarkable observations
	12	bilateral dilation of renal pelvis
	14	no remarkable observations

000289

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
58	2	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations
	8	no remarkable observations
	10	no remarkable observations
59	1	4th cerebral ventricle enlarged
	3	no remarkable observations
	6	no remarkable observations
	8	no remarkable observations
	10	no remarkable observations
	12	dilation of right renal pelvis
	14	no remarkable observations
60	2	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations
	8	no remarkable observations
	10	no remarkable observations
	12	dilation of left renal pelvis; hydro-ureter, left
61	1	dilation of right renal pelvis
	3	4th cerebral ventricle enlarged; dilation of left renal pelvis
	5	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations
62	1	no remarkable observations
	3	dilation of right renal pelvis
	5	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations

000290

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
63	1	no remarkable observations
	3	no remarkable observations
	5	slightly dislocated kidney, left (up to 2 mm); 2 haemorrhages on liver surface (Ø 1 mm, 3x2x2 mm)
	7	no remarkable observations
	9	no remarkable observations
	11	dilation of right renal pelvis
64	3	no remarkable observations
	5	no remarkable observations
	7	4th cerebral ventricle enlarged
	9	no remarkable observations
	11	dilation of left renal pelvis
	14	bilateral dilation of renal pelvis
65	1	no remarkable observations
	3	no remarkable observations
	5	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations
	13	no remarkable observations
66	2	no remarkable observations
	4	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations
	13	no remarkable observations
67	2	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations
	9	no remarkable observations
	11	haemorrhage on liver surface (4x2x1 mm)
	13	no remarkable observations
	15	no remarkable observations

000291

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
68	2	no remarkable observations
	4	no remarkable observations
	6	dilation of right renal pelvis
	9	no remarkable observations
	11	4th cerebral ventricle enlarged; bilateral dilation of renal pelvis
	13	no remarkable observations
69	2	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations
	8	no remarkable observations
	10	no remarkable observations
	12	no remarkable observations
	14	no remarkable observations
	16	no remarkable observations
70	2	no remarkable observations
	4	no remarkable observations
	6	no remarkable observations
	8	no remarkable observations
	10	no remarkable observations
	12	no remarkable observations
71	2	no remarkable observations
	4	dilation of right renal pelvis
	6	2 haemorrhages on liver surface (Ø 2 mm); bilateral dilation of renal pelvis
	8	bilateral dilation of renal pelvis
	10	no remarkable observations
	12	no remarkable observations
	14	no remarkable observations
72	2	no remarkable observations
	4	no remarkable observations
	7	no remarkable observations
	9	no remarkable observations
	11	no remarkable observations
	13	dilation of left renal pelvis

000292

TABLE B 7 1,3-Propanediol - Examination of the Influence on the Pregnant Rat and the Fetus by Oral Administration

Individual Fetal Soft Tissue Variations

Dam No.	Fetus No.	Observation
Test Group 3: 1000 mg 1,3-Propanediol/kg b.w. p.o.		
73		not examined
74		not examined
75		not examined

000293

The following pages 169 - 170 (incl. this page) contain

APPENDIX 1

to the study

EXAMINATION OF THE INFLUENCE OF 1,3-PROPANEDIOL, POLYMER GRADE ON THE PREGNANT RAT AND THE FETUS BY ORAL ADMINISTRATION

(according to OECD method no. 414)

Certificate of Analysis

of the Test Substance

000294

Data on Test Substance No. _____ Study No. _____

Supplier : Degussa AG

Test Substance/
Trade Name/Identity : 1,3 - Propane diol

Synonym : Trimethylene glycol
1,3 - Dihydroxy propane

CAS No. : 504-63-2

Batch No./Date of
Production : Charge 22, Kontrollnr. 2712, (April 1991)

Physical Appearance : liquid, colorless, odorless

Purity (Method)/
Known Impurities : Content 1,3 Propane diol: 99.8 % (GC, area%)
Water 0.13 % (K.F.)

Solubility : Completely miscible with water

Density : 1.052 g/cm³

pH-Value : 4.5 - 7.0 (100 g / 1000 g H_2O)

Storage : in closed container, due to hygroscopicity

Stability : Provided the a.m. storage conditions the test substance is stable until 06 / 1997

Hanau, 02. Juni 1992
Place, Date

Signature

The following pages 171 - 173 (incl. this page) contain

APPENDIX 2

to the study

EXAMINATION OF THE INFLUENCE OF 1,3-PROPANEDIOL, POLYMER GRADE ON THE PREGNANT RAT AND THE FETUS BY ORAL ADMINISTRATION

(according to OECD method no. 414)

Differentiation of Malformations and Variations

000296

DIFFERENTIATION OF MALFORMATIONS AND VARIATIONS

The relationship between 'variations and retardations' and 'malformations' should be seen under the aspect of cumulative historic data.

1. Variations and retardations

1.1. are alterations which are close to the normal anatomical and physiological findings in embryos as well as fetuses and newborns;
1.2. are alterations which - if occurring in men - do not cause social disadvantage;
1.3. are alterations which occur also without substance-treatment
1.4. and are no distinct symptoms of malformations.

Variations and retardations mostly occur in the areas of:

skull	(e.g. enlarged fontanelle)
brain	slight ventricular enlargement
sternebrae	(e.g. unequal development of ossification centres, crippled sternal centre)
ribs and vertebral body	(e.g. additional ribs, cervical ribs, single wavy ribs, unilateral defective vertebral body and furcal ribs)
extremities	(e.g. slight abnormal position)
situs	(e.g. dislocation of testis)
kidneys	dilatation of renal pelvis
varying regions	haemorrhages (old and new)

2. Malformations

are morphological and/or functional damages which - if occurring in men - would endanger the existence or social position.

Typical classic malformations are:

hydrocephalus	
exencephaly	
schistasis	(e.g. cleft palate, cleft jaw, cleft lip, omphalocele)
micro- and macrophthalmia	
opaque lens	

- continued-

Malformations continued

defective position of spinal cord	(e.g. short tail, spina bifida, defective cervical vertebrae, scoliosis, kyphosis, lordosis)
shifted and fused dorsal, lumbar and coccygeal vertebrae	
aplasia/hypoplasia of extremities	
hypoplasia of kidney	
generalised oedema	
monster	
crossed legs	
Trousseau's phenomenon	
hernia	(e.g. diaphragm)
heterotaxia	(e.g. heart, stomach, liver)

000298

The following pages 174 - 178 (incl. this page) contain

APPENDIX 3

to the study

EXAMINATION OF THE INFLUENCE OF 1,3-PROPANEDIOL, POLYMER GRADE ON THE PREGNANT RAT AND THE FETUS BY ORAL ADMINISTRATION

(according to OECD method no. 414)

BACKGROUND DATA

Summarized results of teratology studies in Sprague-Dawley rats

BACKGROUND DATA

Summarized results of teratology studies in Sprague-Dawley rats
from 1988 to 1991

1. General Reproductive Indices

Indices		Control animals	Animals not significantly influenced by any test compound
No. of groups examined		25	39
No. of pregnant animals examined		580	911
No. of living and dead fetuses			
absolute		7340	11517
per dam		12.7 ± 0.6	12.6 ± 0.5
No. of dead fetuses at laparotomy			
absolute		2	5
at laparotomy		0.027 ± 0.136	0.069 ± 0.246
No. of resorptions per dam		0.87 ± 0.23	0.89 ± 0.23
Rate of early resorptions in %		89.0 ± 19.7	79.6 ± 12.7
No. of implantations per dam		13.5 ± 0.6	13.5 ± 0.5
No. of corpora lutea per dam		13.9 ± 0.6	13.8 ± 0.5
No. of malformations	total	36	42
	in %	0.490	0.365
	per dam	0.064 ± 0.089	0.057 ± 0.082
No. of runts	total	26	26
	per dam	0.044 ± 0.053	0.028 ± 0.030
Fetuses with variations of the skeleton	mean in %	13.4 ± 6.3	13.1 ± 5.7
Foetuses with retardations of the skeleton	mean in %	76.3 ± 14.2	76.2 ± 15.6
Fetuses with visceral variations	mean in %	12.9 ± 5.3	13.7 ± 4.8
No. of twins	total	3	8
Sex ratio (m:f)		1.032 ± 0.135	1.009 ± 0.126
Mean weight of fetuses in g		3.61 ± 0.09	3.63 ± 0.10
Mean weight of placentae in g		0.566 ± 0.018	0.562 ± 0.016

000300

BACKGROUND DATA

Summarized results of teratology studies in Sprague-Dawley rats from 1988 to 1991

2. Skeletal Retardations and Variations

Indices	Control animals		Animals not significantly influenced by any test compound	
No. of groups examined	25		39	
No. of fetuses examined	3677		5759	
No. of litters examined	580		911	
	total no.	mean in %	total no.	mean in %
R - Skull, retarded ossification	1667	45.7 ± 17.4	2562	44.7 ± 15.5
Sternebrae - one or more				
R - no or retarded ossification	2608	70.9 ± 13.0	3851	66.9 ± 13.7
V - one or more misaligned	215	5.77 ± 3.33	310	5.36 ± 2.75
V - one or more bipartite	18	0.480 ± 0.517	22	0.380 ± 0.588
V - one or more bipartite and misaligned	10	0.267 ± 0.500	16	0.272 ± 0.423
V - on or more misaligned and partially fused	1	0.026 ± 0.128	3	0.051 ± 0.180
Ribs				
V - uni- or bilateral absent 13th	34	0.886 ± 1.967	49	0.823 ± 1.630
V - uni- or bilateral additional 14th	69	1.88 ± 2.33	96	1.66 ± 1.96
V - one or more wavy ribs	330	8.96 ± 5.93	472	8.11 ± 4.39
Vertebrae				
- thoracic centra				
R - one or more not ossified	1	0.033 ± 0.164	0	-
R - one or more bipartite	114	3.09 ± 2.06	182	3.18 ± 2.07
R - one or more retarded ossification	51	1.37 ± 2.15	90	1.58 ± 2.13
- sacral centra				
R - one or more bipartite	9	0.249 ± 0.967	19	0.333 ± 1.408
R - one or more retarded ossification	3	0.077 ± 0.287	4	0.069 ± 0.299
- caudal centra				
R - not ossified	2	0.065 ± 0.224	3	0.054 ± 0.190
Extremities				
R - one or both metacarpalia no. V not ossified	215	6.37 ± 10.58	267	4.41 ± 7.02

R = retardation
V = variation

000301

BACKGROUND DATA

Summarized results of teratology studies in Sprague-Dawley rats
from 1988 to 1991

3. External/visceral Variations

Indices	Control animals		Animals not significantly influenced by any test compound	
No. of groups examined	25		39	
No. of fetuses examined	3663		5737	
No. of litters examined	580		911	
	total no.	mean in %	total no.	mean in %
uni- or bilateral increased renal pelvic cavitation	266	7.13 ± 4.78	453	7.73 ± 4.97
4th brain ventricle dilated	81	2.24 ± 2.56	140	2.44 ± 2.53
unilateral misplaced kidney	11	0.324 ± 0.856	6	0.115 ± 0.367
haemorrhagic liver	133	3.69 ± 2.00	244	4.19 ± 2.08
haemorrhagic kidney	0	-	1	0.018 ± 0.110
haemorrhagic adrenal	2	0.067 ± 0.234	0	-
haemorrhagic cerebellum	1	0.026 ± 0.130	1	0.016 ± 0.099
haemorrhagic lung	1	0.027 ± 0.133	1	0.018 ± 0.110
ventricular haematoma (4th brain ventricle)	1	0.027 ± 0.133	1	0.018 ± 0.110
thoracic haematoma	2	0.064 ± 0.221	1	0.021 ± 0.132
abdominal haematoma	0	-	1	0.017 ± 0.105

000302

BACKGROUND DATA

Summarized results of teratology studies in Sprague-Dawley rats
from 1988 to 1991

4. Malformations

Indices	Control animals		Animals not significantly influenced by any test compound	
No. of groups examined	25		39	
No. of fetuses examined	7340		11517	
No. of litters examined	580		911	
	total no.	mean in %	total no.	mean in %
Acephalia, spina bifida, omphalocele, short extremities	1	0.016 ± 0.080	-	-
Microcephaly	-	-	1	0.008 ± 0.053
Encephalocele or preliminary ence-phalocele	2	0.029 ± 0.101	1	0.008 ± 0.052
Encephalocele, spina bifida, cleft palate	1	0.013 ± 0.066	0	-
Open eyelid	0	-	2	0.021 ± 0.132
Brachygnathia	0	-	1	0.009 ± 0.054
Thoracic situs inversus#	1	0.036 ± 0.179	1	0.021 ± 0.132
Omphalocele	0	-	1	0.008 ± 0.050
Short abdomen, short tail	20	0.196 ± 0.430	16	0.144 ± 0.375
Short abdomen, short tail, uni- or bilateral tailor's posture	10	0.145 ± 0.505	23	0.196 ± 0.472
Short abdomen, short tail, cleft palate	0	-	1	0.008 ± 0.052
Short tail	2	0.029 ± 0.101	3	0.025 ± 0.088
Tailor's posture	0	-	1	0.008 ± 0.049

examined in half the number of fetuses

000303

The following pages 179 - 180 (incl. this page) contain

APPENDIX 4

to the study

EXAMINATION OF THE INFLUENCE OF
1,3-PROPANEDIOL
ON THE PREGNANT RAT AND THE FETUS BY ORAL ADMINISTRATION
(according to OECD method no. 414)

Determination of the test substance concentration

000304

Concentration of the test substance in the test substance-vehicle suspensions:

Dose-level in mg/kg b.w.	Nominal Concentration in mg/ml	Actual Concentration in mg/ml M	Yield in mean %
at the start of treatment (6th day of pregnancy of the first mated females):			
immediately after preparation of the suspension:			
250	25	24.27	97.08
1000	100	100.92	100.92
8 hours after storage of the suspension at room temperature:			
250	25	24.98	99.90
1000	100	101.35	101.35
24 hours after storage of the suspension at room temperature:			
250	25	22.85	91.38
1000	100	103.10	103.10
at the end of treatment (15th day of pregnancy of the last mated females):			
before administration to the last animal:			
250	25	23.65	94.60
1000	100	96.47	96.47

The analyses were performed by means of GC:

GC-System	:	HEWLETT PACKARD System 5890 Series II
Integrator	:	HEWLETT PACKARD 3396 Series II
Column	:	Chrompack 50 m x 0.25 mm
Coating	:	CP-Wax-52 CB, 0.2 µm
Temperature	:	250°C (FID)
Flow rate	:	Helium 0.9 ml/min
Injection volume	:	2 µl
Run length	:	20 min
Evaluation	:	External standard calibration

Analyses were carried out by LPT. The analytical raw data are filed with the project raw data.

000305

GLP-Confirmation in accordance with Section 19b of the Chemicals Act

Since 1979, LPT has been inspected 4 times by the FDA, with regard to GLP standards, to their satisfaction, the latest inspection having been carried out in 1988 (24.10.-11.11.1988).

The certificates of the GLP inspection of the central institute in D-2104 Hamburg-Neugraben and of the branches Mienenbüttel and Löhndorf in accordance with Section 19b of the Chemicals Act are attached.

Redderweg 8, D-2104 Hamburg 92, Germany
Telephone: (040) 701 50 21-23, Telefax: (040) 701 50 26
Directors: Prof. F. Leuschner M.D., J. Leuschner D. Phil., F. Hübscher (QAU)

GLP - Bescheinigung

gem. Anhang 2 des Chemikaliengesetzes
in der Bekanntmachung der Neufassung
vom 14.3.1990 (BGBl. I S. 521)

Bescheinigung

Hiermit wird bestätigt, daß die Prüfungseinrichtungen(en)

in **Redderweg 8, 2104 Hamburg 92**
(Ort, Anschrift) / (location, address)

der / of **Praxis Prof. Dr. med. F. Leuschner/Laboratory of Pharmakology and Toxikology (LPT)**
(Firma) / (company name)

am/on **5.11.1990, 28.2.1991, 13.3.1991, 22.5.1991, 24.7.1991**
(Datum) / (date)

von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist (sind).

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.

Certificate

It is hereby certified that the test facility(ies)

was (were) inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

- Überprüfungen pharmakologisch-toxikologischer Eigenschaften von zertifizierten, chemischen Stoffen auf physiologische bzw. anatomisch-pathologische Veränderungen nach Applikation an Nagetieren und Kaninchen,
- Gewinnung von Gewebeproben zur histologischen Untersuchung nach der Empfehlung 83/571/EWG
- Prüfungen in vitro am Darm im Organbad

zur Gewinnung von Daten im Rahmen von Prüfungen auf akute, subchronische und chronische Toxizität.

Hamburg, den 25.7.1991

Lettau

NIEDERSÄCHSISCHES UMWELTMINISTERIUM

Niedersächsisches Umweltministerium · Postfach 4107 · 3000 Hannover 1

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Laboratory of pharmacology
and toxicology
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12. Juni 1991
Erl.....

Ihr Zeichen, Ihre Nachricht vom	(Bitte bei Antwort angeben) Mein Zeichen	☎ (05 11) 104-	Hannover
	306 - 45003/01/7	3450	11.06.1991

Betr.: Gute Laborpraxis (GLP)
hier: GLP-Inspektion Ihrer Betriebsstätte Mienenbüttel

Bezug: Ihre telefonische Anfrage vom 07.06.91

Sehr geehrte Damen und Herren,

auf Ihre o.a. Anfrage teile ich Ihnen folgendes mit:

Die Niedersächsische GLP-Kommission, die auf Ihren Antrag hin am 30.01., 24.04. und 25.04.91 in Ihrer in Niedersachsen gelegenen Betriebsstätte Mienenbüttel eine Inspektion auf Einhaltung der Grundsätze der GLP nach Anhang 1 zum Chemikaliengesetz durchgeführt hat, ist zu dem Ergebnis gekommen, daß in dieser Betriebsstätte GLP-gerecht gearbeitet wird.

Mit freundlichen Grüßen
Im Auftrage

(Dr. Kuhrt)

022 024 002
10 03

000308

Dienstgebäude Archenstraße 2 Hannover | Telefon (05 11) 104-0 | Telefax (05 11) 104-33 99 | Telex 5118280 - nuhan | Postanschrift Archenstraße 2 3000 Hannover 1

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Konto-Nr. 101 259 27 Hannover Landesamt Hannover (BLZ 250 5 ...

GLP-Bescheinigung

Bescheinigung	Certificate
Hiermit wird bestätigt, daß die Prüfungseinrichtung	It is hereby certified that the test facility

Laboratorium für Pharmakologie und Toxikologie (LPT)

D - 2355 Löhndorf
(Ort,Anschrift/location,address)

der/of Laboratory of Pharmacology and Toxicology;Prof.Dr.F.Leuschner
(Firma/company name)

am/on 05. September 1991
(Datum/date)

von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist. Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.	was inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice. It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

- Reproduktionstoxizität (Tierversuche)
- Fertilitätstoxizität, Teratogenität, Peri- und Postnataltoxizität
- Kanzerogenität (Kurzzeit- und Langzeitprüfung)
- Ökotoxizität (Ökotoxikologische Untersuchungen nach Sektion 2 und 3 der OECD
- Inhalationstoxizität (Hund, Kaninchen, Nagetiere)

Kiel, 26.9.91
Ort/Datum

Im Auftrage
[redacted]
Dr. Steinmann

FREIE UND HANSESTADT HAMBURG

BEHÖRDE FÜR ARBEIT, GESUNDHEIT UND SOZIALES

GLP - Bescheinigung

gem. Anhang 2 des Chemikaliengesetzes
in der Bekanntmachung der Neufassung
vom 14.3.1990 (BGBl. I S. 521)

Bescheinigung

Hiermit wird bestätigt, daß die Prüfungseinrichtung(en)

Certificate

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in Redderweg 8, 2104 Hamburg 92
(Ort, Anschrift) / (location, address)

der / of Praxis Prof. Dr. med. F. Leuschner/Laboratory of Pharmakology and Toxikology (LPT)
(Firma) / (company name)

am/on 15.1.1992, 29.1.1992, 24.3.1992, 7.5.1992
(Datum) / (date)

von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist (sind).

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Pharmakologisch-toxikologische Prüfungen
auf Herz-Kreislauf-Parameter an Hunden zur Bestimmung der Sicherheitspharmakologie
in toxisch relevanten Dosen.
Pharmakologisch-toxikologische Prüfungen
in vitro an Zellsystemen zur Erfassung des mutagenen Potentials mit Hilfe
- des Ames-testes (Prokaryonten)
- des Genmutationstestes an Eukaryonten,
- des Säuger zytogenetischen Testes
(z.B. Humanlymphozyten) sowie

Pharmakologisch-toxikologische Prüfungen mit Hilfe
des Knochenmarks von Nagern zur Feststellung von Chromosomenaberrationen.

Hamburg, den 10.7.1992

[redacted]

Lettau

000310

National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation Of Risks To Human Reproduction

NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Propylene Glycol

000311

March 2004 NIH Publication No. 04-4482

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Preface

The National Toxicology Program (NTP) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in 1998. The CERHR is a publicly accessible resource for information about adverse reproductive and/or developmental health effects associated with exposure to environmental and/or occupational chemicals. The CERHR is located at the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health and Dr. Michael Shelby is the director.[1]

The CERHR broadly solicits nominations of chemicals for evaluation from the public and private sectors. The CERHR follows a formal process for review and evaluation of nominated chemicals that includes multiple opportunities for public comment. Chemicals are selected for evaluation based upon several factors including the following:

- potential for human exposure from use and occurrence in the environment
- extent of public concern
- production volume
- availability of scientific evidence for reproductive and/or developmental toxicity.

The CERHR convenes a scientific expert panel that meets in a public forum to review, discuss, and evaluate the scientific literature on the selected chemical. Public comment is invited prior to and during the meeting. The expert panel produces a report on the chemical's reproductive and developmental toxicities and provides its opinion of the degree to which exposure to the chemical is hazardous to humans. The panel also identifies areas of uncertainty and where additional data are needed. The CERHR expert panels use explicit guidelines to evaluate the scientific literature and prepare the expert panel reports. Expert panel reports are made public and comments are solicited.

Next, the CERHR prepares the NTP-CERHR monograph. The NTP-CERHR monograph includes the NTP brief on the chemical evaluated, the expert panel report, and all public comments. The goal of the NTP brief is to provide the public, as well as government health, regulatory, and research agencies, with the NTP's interpretation of the potential for the chemical to adversely affect human reproductive health or children's health. The NTP-CERHR monograph is made publicly available electronically on the CERHR web site and in hard copy or CD-ROM from the CERHR.

---

[1]Information about the CERHR is available on the web at *<http://cerhr.niehs.nih.gov>* or by contacting the director:

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Information about the NTP is available on the web at *<http://ntp-server.niehs.nih.gov>* or by contacting the NTP Office of Liaison and Scientific Review at the NIEHS:

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Introduction

In 1999, the CERHR Core Committee, an advisory committee composed of representatives from NTP member agencies, recommended propylene glycol and ethylene glycol for expert panel review. Propylene glycol was selected because it is a high production volume chemical and there is widespread human exposure. Ethylene glycol is the subject of a separate monograph.

Propylene glycol is used as a chemical intermediate in the manufacture of unsaturated polyester resins, and is found in cosmetics, personal care products, pharmaceuticals, food, liquid detergents, deicing fluids, antifreeze/engine coolant, paints, coatings, and tobacco products. Propylene glycol also is used in the production of plasticizers, 2-methylpiperazine, 1,2-propylene diamine, hydroxylated polyester, polyester-type fluorescent resin matrix, and polyether polyols.

As part of the evaluation of propylene glycol, the CERHR convened a panel of scientific experts (Appendix I) to review, discuss, and evaluate the scientific evidence on the potential reproductive and developmental toxicities of the chemical. There was a public meeting of the CERHR Ethylene Glycol/Propylene Glycol (EG/PG) Expert Panel on February 11-13, 2003. The CERHR received public comments throughout the evaluation process.

The NTP has prepared an NTP-CERHR monograph for propylene glycol. This monograph includes the NTP brief on propylene glycol, a list of the expert panel members (Appendix I), the expert panel's report on propylene glycol (Appendix II), and all public comments received on the expert panel's report on propylene glycol (Appendix III). The NTP-CERHR monograph is intended to serve as a single, collective source of information on the potential for propylene glycol to adversely affect human reproduction or development. Those interested in reading this monograph may include individuals, members of public interest groups, and staff of health and regulatory agencies.

The NTP brief included within this monograph presents the NTP's interpretation of the potential for exposure to propylene glycol to cause adverse reproductive or developmental effects in people. It is based on information about propylene glycol provided in the expert panel report, the public comments, and additional scientific information available since the expert panel meetings. The NTP brief is intended to provide clear, balanced, scientifically sound information on the potential for propylene glycol exposures to result in adverse health effects on development and reproduction.

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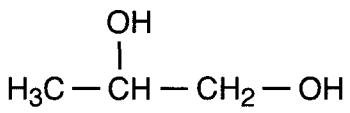
NTP Brief on Propylene Glycol

NTP Brief

What is Propylene Glycol?

Propylene Glycol (PG) is a small, hydroxy-substituted hydrocarbon with the chemical formula $C_3H_8O_2$ and the structure shown in Figure 1.

Figure 1. Chemical structure of PG



PG is used as a chemical intermediate in the production of unsaturated polyester resins. PG is used in liquid detergents, deicing fluids, antifreeze/engine coolant, paints and coatings. PG is one of the chemicals 'generally recognized as safe' (GRAS) by the U.S. Food and Drug Administration and is used in foods, cosmetics, pharmaceuticals and tobacco products.

Commercial PG is manufactured by direct hydrolysis of propylene oxide by water. In 1999, 1,083 million pounds of PG were produced in the U.S. with apparent consumption of 854 million pounds.

PG can be released into the environment from industrial disposal and PG-containing consumer products. PG is water-soluble and has the potential to leach into groundwater, but is rapidly degraded. The half-life of PG in water is estimated to be 1 to 4 days under aerobic conditions and 3 to 5 days under anaerobic conditions.

Are People Exposed to PG?*

Yes. The general public is exposed to PG by dermal contact with or ingestion of PG-containing products. Inhalation of PG vapors from such products may also occur. Dermal exposure can result from contact with PG-containing products such as cosmetics, antifreeze solutions, coolants, windshield deicers, or pharmaceutical creams. Oral exposure to PG can occur through its use in food and tobacco products and in prescription and over-the-counter medicines. PG is rapidly degraded in water; no information was located on PG levels in drinking water.

There is limited information on average U.S. exposure levels and no information on exposure levels due to dermal contact was noted. The average U.S. daily intake of PG from food products is estimated at 34 mg/kg bw/day for a 70 kg person. *[NOTE: mg/kg bw/day=milligrams per kilogram of body weight per day.]* Since PG has GRAS status and may not be listed as a specific ingredient in some foods, dietary intake based upon product labeling could result in an underestimation of intake. PG is also an ingredient in both over-the-counter and prescription pharmaceuticals. In adult humans, the mean serum half-life of PG is approximately 2 to 4 hours.

Occupational exposure to PG may occur through dermal contact or inhalation. Exposure studies indicate that exposure levels vary depending on protective gear worn, route of exposure, and length of exposure. A threshold limit value has not been defined for PG. However, the American Industrial Hygiene Association, Workplace Environmental Exposure Level recommended guide is "50 ppm as an eight-hour time-weighted average (TWA8) for total vapor and aerosol, and 10 mg/m^3 as a TWA8 for aerosol alone."

Can PG Affect Human Development or Reproduction?

Probably Not. There are no studies available on the effect of PG on human reproduction or development. Laboratory animal studies reviewed by the expert panel showed no effect on develop-

* Answers to this and subsequent questions may be: *Yes, Probably, Possibly, Probably Not, No or Unknown*

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ment and/or reproduction at the highest doses tested (Figure 2).

Scientific decisions concerning health risks are generally based on what is known as a "weight-of-evidence" approach. In this case, recognizing the lack of human data and lack of adverse effects in laboratory animals after exposure to high doses (Figure 2), the NTP judges the scientific evidence sufficient to conclude that PG probably does not adversely affect human development or reproduction.

Supporting Evidence

As presented in the Expert Panel Report on PG (see report for details and literature citations), the panel concluded that PG does not produce developmental toxicity in offspring of laboratory animals treated with the highest oral doses tested, i.e., 1,230 mg/kg bw/day in rabbits; 10,400 mg/kg bw/day in mice; 1,600 mg/kg bw/day in rats; 1,550 mg/kg bw/day in hamsters.

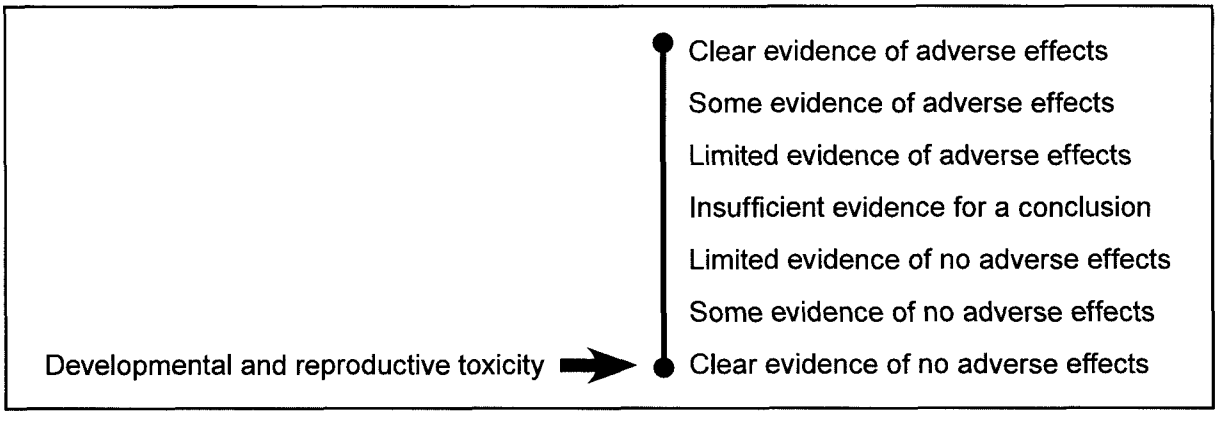
In an NTP continuous breeding study, no effects on fertility were observed in male or female mice that received PG in drinking water at doses up to 10,100 mg/kg bw/day. No effects on fertility were seen in either the first or second generation of treated mice.

The expert panel noted that the pharmacokinetics of PG are reasonably well understood in animals and humans. Information on the absorption, distribution, metabolism, and excretion of PG indicates that the absence of adverse effects in laboratory animals is likely to be relevant to humans. The rate-limiting step in PG metabolism is its conversion to lactaldehyde by alcohol dehydrogenase. Studies indicate that this reaction saturates in humans at doses that are 8-10 fold lower than needed to saturate the same step in laboratory animals. Saturation of this metabolic step is thought to be protective since PG has lower general toxicity than its metabolites.

Are Current Exposures to PG High Enough to Cause Concern?

Probably Not. Metabolism studies indicate that PG has a short half-life in humans. These data, combined with evidence that saturation of human metabolism occurs at doses 8-10 fold lower than observed in laboratory animals, suggest that human exposure levels are not high enough to cause concern. While there are no data on the PG exposures of the general U.S. population, it has been estimated that adults are exposed to approximately 34 mg/kg bw/day through food products. Limited data suggest that occupational exposures are not

Figure 2. *The weight of evidence that PG causes adverse developmental or reproductive effects in laboratory animals*



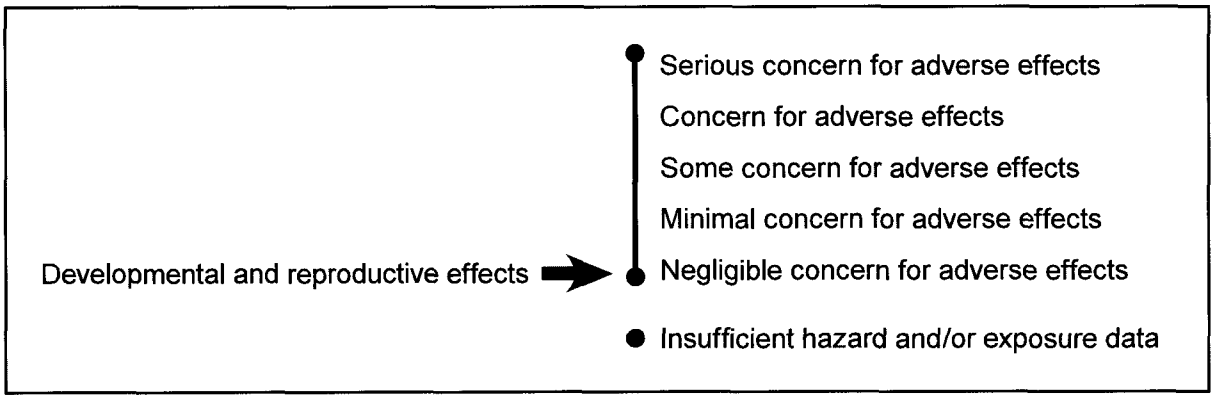
excessive. Based on the limited exposure data, pharmacokinetic studies, and laboratory animal studies the NTP offers the following conclusion (Figure 3):

The NTP concurs with the CERHR EG/PG Expert Panel that there is negligible concern for adverse developmental or reproductive toxicity from PG exposures in humans.

Studies evaluated by the expert panel indicate that high oral doses of PG produced no adverse developmental or reproductive effects in multiple laboratory animal species.

These conclusions are based on the information available at the time this brief was prepared. As new information on toxicity and exposure accumulate, it may form the basis for either lowering or raising the levels of concern expressed in the conclusions.

Figure 3. NTP conclusions regarding the possibilities that human development or reproduction might be adversely affected by exposure to PG



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References

No additional references found.

Appendix I. NTP-CERHR Ethylene Glycol/Propylene Glycol Expert Panel

A 9-member panel of scientists covering disciplines such as toxicology, epidemiology, biostatistics and industrial hygeine was recommended by the Core Committee, a federal oversight committee for CERHR, and approved by the Director of the National Toxicology Program. The panel critically reviewed documents and identified key studies and issues for plenary discussions. At a public meeting held February 11-13, 2003, the expert panel discussed these studies, the adequacy of available data, and identified data needed to improve future assessments. The expert panel reached conclusions on whether estimated exposures may result in adverse effects on human reproduction or development. Panel assessments were based on the scientific evidence available at the time of the public meeting. The expert panel report was made available for public comment on May 15, 2003, and the deadline for public comments was July 14, 2003 (*Federal Register* 68:94 [15 May 2003] pp. 26325-26326). The Expert Panel Report on PG is provided in Appendix II and the public comments received on the report are in Appendix III. Input from the public and interested groups throughout the panel's deliberations was invaluable in helping to assure completeness and accuracy of the reports. The Expert Panel Report on PG is also available on the CERHR website <*http://cerhr.niehs.nih.gov*>.

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Appendix I. NTP-CERHR Ethylene Glycol/Propylene Glycol Expert Panel

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National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation Of Risks To Human Reproduction

Appendix II

NTP-CERHR EXPERT PANEL REPORT ON THE REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF PROPYLENE GLYCOL

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May 2003 NTP-CERHR-PG-03

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ABBREVIATIONS

ACC	American Chemistry Council
ADH	alcohol dehydrogenase
ALDH	aldehyde dehydrogenase
ANOVA	analysis of variance
ATP	adenosine triphosphate
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the concentration versus time curve
Avg	average
BIBRA	British Industrial Biological Research Association
bw	body weight
C	Celsius
cc	cubic centimeters
cm^2	centimeters squared
C_{max}	peak concentration
CAS RN	Chemical Abstracts Service Registry Number
CERHR	Center for the Evaluation of Risks to Human Reproduction
CNS	central nervous system
CSF	cerebral spinal fluid
d	day
dL	deciliter
DMBA	dimethylbenzanthracene
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
e.g.	*exempli gratia*; "for example"
EPA	Environmental Protection Agency
f	female
FAO/WHO	Food and Agriculture Organization/World Health Organization
FDA	Food and Drug Administration
fl oz	fluid ounces
g	gram
GC	gas chromatography
gd	gestation day
GRAS	generally recognized as safe
GSH	glutathione
hr	hour
HCG	human chorionic gonadotrophin
Hg	mercury
HPLC	high pressure liquid chromatography
HSDB	Hazardous Substances Data Bank
IM	intramuscular
IP	intraperitoneal
IPCS	International Programme on Chemical Safety
IU	international units

IV	intravenous
k_{abs}	absorption coefficient
kg	kilogram
K_m	Michaelis constant
K_{ow}	octanol-water partition coefficient
K_S	solubility constant
L	liter
lb	pound
LD_{50}	lethal dose, 50% mortality
LOAEL	lowest observed adverse effect level
m	male
M	molar
max	maximum
m^3	meters cubed
m^2	meters squared
meq	milliequivalents
mg	milligram
min	minute
mL	milliliter
mM	millimolar
mmol	millimole
MS	mass spectroscopy
mw	molecular weight
n, no., #	number
NAD	nicotinamide adenine dinucleotide
NZW	New Zealand White
ng	nanogram
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute of Occupational Safety and Health
nmol	nanomole
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
OSHA	Occupational Safety and Health Administration
Osm	osmolal
PBPK	physiologically based pharmacokinetic
PG	propylene glycol
PMSG	pregnant mare serum gonadotrophin
pnd	postnatal day
ppm	parts per million
RBC	red blood cell
RIA	radioimmunoassay
SCE	sister chromatid exchange
SD	standard deviation
SEM	standard error of the mean

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SIDS	screening information data set
SPF	specific pathogen free
TLV	threshold limit value
T_{max}	maximum time
USDA	United States Department of Agriculture
USP	United States Pharmacopeia
v	volume
V_{max}	maximal velocity of metabolism
V_d	volume of distribution
VOC	volatile organic compound
wt	weight
wk	week
wt%	weight percentage
μg	microgram
μmol	micromole

Appendix

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PREFACE

The National Toxicology Program (NTP) and the National Institute of Environmental Health Sciences (NIEHS) established the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR) in June, 1998. The purpose of the Center is to provide timely, unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed.

Propylene glycol was selected for evaluation by the CERHR based on its high production and widespread public exposure due to its use as an antifreeze and deicing agent, as well as its use in paints, coatings, foods, drugs, and cosmetics.

This evaluation results from the efforts of a nine-member panel of government and non-government scientists that culminated in a public expert panel meeting held February 11-13, 2003. This report has been reviewed by CERHR staff scientists and by members of the Ethylene Glycol/Propylene Glycol Expert Panel. Copies have been provided to the CERHR Core Committee, which is made up of representatives of NTP-participating agencies. This report is a product of the expert panel and is intended to (1) interpret the strength of scientific evidence that propylene glycol is a reproductive or developmental toxicant based on data from in vitro, animal, or human studies, (2) assess the extent of human exposures to include exposures of the general public, occupational groups, and other sub-populations, (3) provide objective and scientifically thorough assessments of the scientific evidence that adverse reproductive/developmental health effects may be associated with such exposures, and (4) identify knowledge gaps to help establish research and testing priorities to reduce uncertainties and increase confidence in future assessments of risk.

The Expert Panel Report on Propylene Glycol will be a central part of the subsequent NTP CERHR Monograph. The monograph will include the NTP CERHR Brief, the expert panel report, and all public comments on the expert panel report. The NTP CERHR Monograph will be made publicly available and transmitted to appropriate health and regulatory agencies.

The NTP-CERHR is headquartered at NIEHS, Research Triangle Park, NC and is staffed and administered by scientists and support personnel at NIEHS and at Sciences International, Inc., Alexandria, Virginia.

Reports can be obtained from the website <*http://cerhr.niehs.nih.gov/*> or from:

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A REPORT OF THE CERHR ETHYLENE GLYCOL AND PROPYLENE GLYCOL EXPERT PANEL:

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Note to Reader:

This report is prepared according to the Guidelines for CERHR Panel Members established by NTP/NIEHS. The guidelines are available from the CERHR web site <*http://cerhr.niehs.nih.gov/*>. The format for Expert Panel Reports includes synopses of studies reviewed, followed by an evaluation of the Strengths/Weaknesses and Utility (Adequacy) of the study for a CERHR evaluation. Statements and conclusions made under Strengths/Weaknesses and Utility evaluations are those of the Expert Panel and are prepared according to the NTP/NIEHS guidelines. In addition, the Panel often makes comments or notes limitations in the synopses of the study. **Bold, square brackets** are used to enclose such statements. As discussed in the guidelines, square brackets are used to enclose key items of information not provided in a publication, limitations noted in the study, conclusions that differ from authors, and conversions or analyses of data conducted by the panel.

1.0 CHEMISTRY, USAGE, AND EXPOSURE

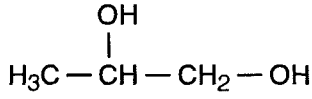
1.1 Chemistry

1.1.1 Nomenclature

The Chemical Abstracts Service Registry Number (CAS RN) for propylene glycol is 57-55-6. Synonyms or trade names for propylene glycol include: 1,2-propanediol; 1,2-dihydroxypropane; methylethylene glycol; trimethyl glycol; 1,2-propylene glycol; monopropylene glycol; propane-1,2-diol; alpha-propylene glycol; Dowfrost; PG 12; Sirlene; Solar Winter Ban; propanediol *(1)*; 2-dihydroxypropanol; methylethyl glycol; methyl glycol; 2,3 propanediol; and alpha propylene glycol *(2)*. The American Chemistry Council (ACC) *(3)* stated that the name Sirlene is no longer used.

1.1.2 Formula and Molecular Weight

Figure 1-1: *Chemical Structure of Propylene Glycol*



Chemical Formula: $C_3H_8O_2$
Molecular Weight: 76.095

1.1.3 Chemical and Physical Properties

Viscous, colorless, odorless hydroscopic liquid with a low vapor pressure. Physicochemical properties are listed in Table 1-1.

Table 1-1: Physicochemical Properties of Propylene Glycol[a]

Property	*Value*
Vapor Pressure	0.07 mm Hg at 20°C[b]
Melting Point	<-60°C
Boiling Point	188.2°C
Density	1.0361 g/cc at 20 °C[b]
Solubility in Water	Soluble
Log K_{ow}	-0.912[b]
Stability	Stable
Reactivity	Can react with oxidizing agents

[a]HSDB *(2)*, [b]ATSDR *(4)*

1.1.4 Technical Products and Impurities

According to the ACC *(3)*, impurities of propylene glycol include chlorides (1 ppm max), iron (1.0 ppm max), water (0.2 wt% max), and dipropylene glycol (<0.2%).

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Manufacturers of propylene glycol are The Dow Chemical Company, Freeport, TX and Plaquemine, LA; Lyondell Chemical Company in Pasadena, TX; Huntsman Corporation in Port Neches, TX; and Arch Chemicals, Inc., in Brandenburg, KY *(3)*.

1.2 Use and Human Exposure

1.2.1 Production

Commercial propylene glycol is manufactured by direct hydrolysis of propylene oxide by water *(5)*. Propylene oxide is made using the chlorohydrin process where the propylene oxide is recovered as a pure product before conversion to the glycol. In 1999, 1,083 million pounds of propylene glycol were produced in the U.S. with apparent consumption of 854 million pounds *(5)*.

1.2.2 Use

Of the 854 million pounds of propylene glycol consumed in the U.S., uses included (in million pounds and % wt) as a chemical intermediate in the manufacture of unsaturated polyester resins (228, 26.7%), cosmetics and personal care products, pharmaceuticals, and human food (170, 19.9%), liquid detergents (135, 15.8%), deicing fluids (85, 10%), antifreeze/engine coolant (55, 6.4%), paints and coatings (40, 4.7%), tobacco humectant (25, 2.9%), other fluids (32, 3.8%), and other applications (84, 9.8%) *(5)*. Propylene glycol is also used in the production of plasticizers (e.g., polypropylene adipate), 2-methylpiperazine, 1,2-propylene diamine, hydroxylated polyester, polyester-type fluorescent resin matrix, and polyether polyols *(2)*.

The following summary obtained from the Agency for Toxic Substances and Disease Registry (ATSDR) *(4)* and the Hazardous Substances Data Bank (HSDB) *(2)* provides information about propylene glycol uses and exposures:

> Propylene glycol is a colorless, odorless, water-soluble liquid considered safe for use in commercial formulations of foods, drugs, and cosmetics. Propylene glycol has been approved as safe in various food colors, flavorings, drugs, cosmetics, and as a direct additive to food. It is used as a humectant in tobacco, pet food, and in dentifrices; in veterinary medicine it is used as a glycogenic in ruminants. Propylene glycol is commonly used in the pharmaceutical industry as a solvent for drugs, as a stabilizer for vitamins, and in ointments for medicinal applications. It is used as a lubricant or heat transfer fluid in situations where leakage could lead to contact with food. It is used as an antifreeze, deicing solution, and as an additive to latex paints and coatings to improve freeze-thaw capability. Propylene glycol is also used in the generation of artificial mists and fogs used in fire safety training, and theatrical and stage productions. This widespread use of propylene glycol stems from its low level of toxicity.

Propylene glycol is used as a softener for cellulose films in the United Kingdom *(2, 6)*.

Propylene glycol is Food and Drug Administration (FDA) approved for use in food, tobacco, and pharmaceutical products as an inert ingredient *(7)*. It is considered to be generally recognized as safe (GRAS) for direct addition to foods *(7)*. GRAS substances, such as propylene glycol, are also permitted in packaging materials as long as the substances "are used in amounts not to exceed that required to accomplish their intended physical or technical effect" *(7)*. Inert ingredients are required

to be listed in over-the-counter drugs *(8)*.

Propylene glycol is a humectant in pet food products, but not in cat foods. Because of the sensitivity of the cat erythrocyte to Heinz body formation (denatured proteins, primarily hemoglobin) by propylene glycol and the possibility of inducing anemia in cats, propylene glycol was removed from cat food products (semi-moist cat food) by the FDA in 1996 *(9)*.

1.2.3 Occurrence

Propylene glycol is released into the environment from industrial disposal and from consumer products containing this chemical. Airports are required by the Environmental Protection Agency (EPA) *(10)* to monitor storm water runoff and to recycle deicing solutions. Propylene glycol is water-soluble and has the potential to leach into groundwater, but is rapidly degraded. The half-life of propylene glycol in water is estimated to be 1−4 days under aerobic and 3−5 days under anaerobic conditions *(4)*. No information was found on this compound in any environmental medium. Propylene glycol was not listed as an organic wastewater contaminant in a recent report by Kolpin et al. *(11)*.

1.2.4 Human Exposure

1.2.4.1 General Population Exposure

The general population can be exposed to propylene glycol through dermal contact with consumer products such as cosmetic products, antifreeze solutions, coolants, windshield deicers, or pharmaceutical creams. Oral exposure to propylene glycol can occur through its use in food and tobacco products and as a solvent for pharmaceutical products *(2)*. In Japan, average daily intake of propylene glycol as a food additive has been reported to be 43.0 mg/person **[43 mg/60 kg=0.71 mg/kg bw/day]** (Louekari et al. *(12)* [from Market Basket Study, Japan 1982]).

Data for per capita daily intake of propylene glycol in food products have been estimated for the United States in a recent report by the United Nations Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives *(13)*. In reviewing the annual volume of production of 31 flavoring agents, propylene glycol per capita consumption was estimated at 2,400,000 μg/day **[34.28 mg/kg bw/day for a 70 kg person]**. (This value was based upon the 1995 update of data collected since 1972 by the Flavor and Extract Manufacturers' Association.)

In a review by the Cosmetic Ingredient Review Expert Panel *(14)*, data on the percent concentration and use of propylene glycol in cosmetics was summarized; these data are presented in Table 1-2. These data were based upon information provided to the FDA in 1984 on propylene glycol use in cosmetic formulations and consisted of a total of 5,676 cosmetic products in 74 categories, with 2,597 product formulations containing between 1 and 10% propylene glycol.

Table 1-2. Product Formulation Data for Propylene Glycol
(Adapted from Cosmetic Ingredient Review (14))

Product Category	Number of product formulations within each concentration range					
	>50%	50–10%	10–1%	1–0.1%	Unknown	Total
Baby shampoos			3	1	3	7
Baby lotions/oils/powders/creams			6		2	8
Other baby products			2	1		3
Bath oils/tablets/salts		6	2	18	3	29
Bubble baths		1	21	64	37	123
Other bath preparations		3	15	25	5	48
Eyebrow pencil			1			1
Eyeliner		2	49	4		55
Eye shadow		3	89	44	39	175
Eye lotion			3	1		4
Eye makeup remover		4	9	4		17
Mascara		4	36	6	14	60
Other eye makeup preparations			27	7	9	43
Colognes/toilet waters		5	35	56		96
Perfumes		1	7	20		28
Powders dusting/talcum, aftershave talc			1	8	1	10
Sachets		10	14	4		28
Other fragrance preparations	1	4	28	10		43
Hair conditioners			24	20	14	58
Hair sprays (aerosol fixatives)			1	8	1	10
Hair straighteners			22			22
Permanent waves		3	2	27	11	43
Rinses (noncoloring)			4	6	3	13
Shampoos (noncoloring)		4	70	83	54	211
Tonics/dressings/other hair grooming aids		2	18	8	3	31
Wave sets		1	6	11		18
Other hair preparations		2	6	7	1	16
Hair dyes/colors (requiring caution statement)		60	222	1	5	288
Hair rinses (coloring)			22	7		29
Hair shampoos (coloring)			1	2		3
Hair lighteners with color			1			1
Hair bleaches			6			6
Other hair coloring preparations		1	3		1	5
Blushers (all types)	1	3	45	19	17	85
Face powders			16	13		29
Foundations		45	150	11	56	262
Leg and body paints			3			3
Lipstick			4	633	544	1181
Makeup bases		12	261	9	52	334

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Table 1-2 (continued)

Product Category	Number of product formulations within each concentration range					
	>50%	50–10%	10–1%	1–0.1%	Unknown	Total
Rouges		2	11	9	8	30
Makeup fixatives				1	3	4
Other makeup preparations		4	25	31	41	131
Cuticle softeners			9	3		12
Nail creams/lotions			6	1		7
Nail polish and enamel removers				2		2
Other manicuring preparations		3	3			6
Dentifrices (aerosol/liquid/paste/powder)		1	1			2
Mouthwashes/breath fresheners			3			3
Other oral hygiene products				1		1
Bath soaps/detergents			11	28		39
Deodorants (underarm)	19	13	71	12	9	124
Douches		5	1	1		7
Feminine hygiene products			1	1		2
Other personal cleanliness products		3	33	17		53
Aftershave lotions		1	54	36	6	97
Beard softeners		2			1	3
Preshave lotions			1	3	4	8
Shaving cream (aerosol brushless lather)		2	18	9	5	34
Other shaving preparations		1	5	5	2	13
Skin cleansing products (cold creams/lotions/liquids/pads)		17	195	35	29	276
Depilatories		2	2	2		6
Face/hand/body (excl. shaving preparations)		15	168	79	55	417
Foot powders/sprays			1			1
Hormone products		1	3	1		5
Moisturizing products		7	269	58	24	358
Night preparations		5	59	9	10	83
Paste masks (mud packs)		2	15		2	19
Skin lighteners		1	66	32	37	136
Skin fresheners		1	8	4	1	14
Wrinkle-smoothing products (removers)		1	8	4	1	14
Other skin care preparations		5	76	32	32	149
Suntan gels/creams/liquids		2	34	15	15	76
Indoor tanning preparations			10		2	12
Other suntan preparations		1	9	1	4	15
Ingredient Total	***21***	***279***	***2,597***	***1,579***	***1,200***	***5,676***

Propylene glycol is rapidly degraded in water and CERHR was unable to locate any information on propylene glycol in drinking water.

Propylene glycol may be released by some carpeting *(2)*. In a technical study by Hodgson et al. *(15)*, emissions of volatile organic compounds (VOC) from four different types of new carpets were measured. Exposure chamber air samples were collected onto multisorbent samplers packed with Tenax-TA, Ambersorb XE-340, and activated charcoal, in series. The chemicals were thermally desorbed from the sampler, concentrated, and injected into a capillary gas chromatograph with a mass spectrometer used as a detector. One carpet with a polyvinyl chloride backing emitted propylene glycol, vinyl acetate, formaldehyde, isooctane, and 2-ethyl-1-hexanol. Propylene glycol and vinyl acetate had the highest concentrations and emission rates for this carpet. The estimated emission rates ranged from 690 $\mu g/m^2/hr$ 24 hours after installation to 193 $\mu g/m^2/hr$ at 168 hours after installation. The other three carpet types did not emit propylene glycol.

The FDA estimated that the human daily dietary intake of propylene glycol to be a 'few mg per kg **[body weight]** per day' *(16)*. **[No details were given on how exposures were estimated.]** In a 2002 report by the United Nations Joint FAO/WHO Expert Committee on Food Additives *(13)*, per capita consumption of propylene glycol in the United States was estimated at 2,400,000 μg/day **[34.28 mg/kg bw/day for a 70 kg person]**. The average daily dietary intake of propylene glycol in Japan was estimated to be 43 mg/person **[0.7 mg/kg bw/day based on a 60 kg person]** *(12)*. The WHO food additive series *(17)* lists the acceptable human daily intake of propylene glycol at <25 mg/kg bw/day.

Appendix

1.2.4.2 Medical Exposure

Propylene glycol is used in some pharmaceuticals that are administered intravenously (see Table 2-8). This represents a unique exposure route for certain subpopulations.

1.2.4.3 Occupational Exposure

Occupational exposure to propylene glycol may occur through direct dermal contact while handling products containing this compound or through inhalation of airborne propylene glycol resulting from heating or spraying processes *(2)*.

Neither the Occupational Safety and Health Administration (OSHA) nor the American Conference of Governmental Industrial Hygienists (ACGIH) has established exposure limits for propylene glycol vapors. No Threshold Limit Value (TLV) has been defined for propylene glycol, but an American Industrial Hygiene Association (AIHA) Workplace Environmental Exposure Level (WEEL) guide of 50 ppm (total exposure) and inhalation aerosol exposure of 10 mg/m^3 has been determined *(18)*.

A 1981–1983 National Occupational Exposure Survey (NOES) of U.S. workers led NIOSH to estimate that 1,748,454 people were potentially exposed to propylene glycol at the workplace *(2)*. Ninety-eight percent of exposures are with trade name products containing propylene glycol, rather than in the production of propylene glycol itself *(2)*.

Norbäck et al. *(19)* studied the exposure of Swedish painters to VOCs from indoor application of water-based paints. VOCs were sampled on different sorbents within the personal breathing zone of the painter and analyzed by gas chromatography (GC)/mass spectroscopy (MS). Propylene glycol was

one of the VOC constituents measured. Exposure measurements for propylene glycol were taken over a 1-hour period of water-based paint application for 20 batches of paint from 5 different manufacturers. Propylene glycol was detected in 12 of the 20 samples. Personal exposure to propylene glycol during application of water-based paints yielded a geometric mean of 350 $\mu g/m^3$ with a maximum value of 12,700 $\mu g/m^3$.

Laitinen et al. *(20)* examined exposure to ethylene and propylene glycol in Finnish motor servicing workers. Ten male mechanics from five different garages participated in the study. The only protective equipment used by some workers was leather gloves. Ten age-matched male office workers served as controls. Differences between groups were evaluated by Student's t-test. Air concentrations of ethylene glycol and propylene glycol were measured during the entire shift. Neither ethylene glycol nor propylene glycol vapors were detected in the breathing zones of workers; detection limits for each compound were given as 1.9 cm^3/m^3 and 3.2 cm^3/m^3, respectively. Urine samples were collected after the work shift and analyzed for ethylene glycol, oxalic acid, and propylene glycol **[method of urine collection, storage, and extraction and quality control not reported]**. There were no differences found between controls and propylene glycol-exposed mechanics.

Deicing fluids are low viscosity glycols used to remove ice or snow that would increase drag on the aircraft. The antifreeze components in a deicing solution vary with the manufacturer, usage, and environmental conditions. Commercial Type I fluid is applied hot as a mixture of fluid and hot water to deice the exterior of aircraft. Type IV fluids are usually applied after the aircraft is deiced to keep ice from reforming. Approximately 90% of Type I fluids and 50% of Type IV fluids are propylene-glycol based *(3, 5)*. Performance criteria for deicing fluids are governed by specifications of the Aerospace Division of the Society of Automotive Engineers (SAE) *(21)*. Both inhalation and dermal exposures to workers using deicing solutions can occur.

The levels of propylene glycol in aircraft deicing workers (n=7, age 31–52 years, sex not given) using either undiluted or water-diluted propylene glycol heated to 60°C was measured in urine samples collected pre- and post-shift *(22)*. Workers were wearing coats, rubber gloves, and masks. The detection limit for the method used to measure propylene glycol in urine was 20 $\mu g/L$. Urine samples were also collected from a comparison group of non-exposed persons (n=16, sex and age not given). For the exposed workers, the median pre-shift urine level was 1.49 mg/L (range 0.72–13.44 mg/L) and 1.67 mg/g creatinine (range 0.41–10.58 mg/g creatinine) and the median post-shift urine level was 2.07 mg/L (range 0.77–9.04 mg/L) and 2.46 mg/g creatinine (range 1.22–10.27 mg/g creatinine). Propylene glycol concentrations in the post-shift worker urine samples were only slightly higher than those of the unexposed comparison group.

In a study simulating concentrations of propylene glycol mist used in aviation emergency training, Wieslander et al. *(23)* concluded that short (1 minute), high exposure (geometric mean concentration of 309 mg/m^3, range 176–851 mg/m^3) to propylene glycol mist may cause acute ocular and upper airway irritation. The duration of these effects was not measured, as measurements were taken within 15 minutes of exposure.

A Health Hazard Evaluation (HHE) on occupational exposure to propylene glycol during aircraft deicing operations was conducted by NIOSH *(24)*. Evaluation of deicing procedures was conducted at

the Denver International Airport (DIA) in March 1996. At DIA, United Airlines uses a 50% solution of propylene glycol in water, heated to 180° F for deicing aircraft. Trucks with dual 800-gallon tanks, spray hoses, and booms are used. The amount of fluid used for deicing each plane ranges from 50 to 200 gallons. Personal breathing-zone air samples were collected from six ground sprayers, one basket man, and one truck driver. Air samples were collected on XAD-7 OVS tubes at a flow rate of 0.5 L/min for 6 hours and analyzed by GC/MS for propylene glycol according to NIOSH Method 5523. Seven workers (Table 1-3) had a range of exposures from 10 to 21 mg/m^3 with a mean of 15 mg/m^3, based on a 6-hour collection.

Table 1-3. Exposure to Airborne Propylene Glycol HETA 95-0069 (24)

Job	*Concentration (mg/m^3)*
Ground Sprayer	14
Ground Sprayer	10
Ground Sprayer	16
Ground Sprayer	11
Ground Sprayer	17
Ground Sprayer	94*
Truck Driver	19
Basket Man	21

*Air sample was visibly contaminated with liquid propylene glycol. This was caused by a worker being accidentally sprayed with the deicing fluid during sampling.

The author concluded that "there was no hazard from overexposure to deicing fluid. ...Airborne exposure to propylene glycol was low and propylene glycol has low toxicity."

Propylene glycol does not bioaccumulate in organisms and rapidly biodegrades in the soil and in water *(25)*. However, this process is oxygen-demanding and can deplete dissolved oxygen levels in water *(26)*. The Clean Water Act requires airports to implement plans for deicer management to control storm water contamination. Therefore, airports must monitor propylene glycol storm water runoff and scavenge and recycle deicing solutions *(10)*.

1.3 Utility of Data

Limited human exposure data for propylene glycol were available for Expert Panel review. The utility of the occupational exposure data available is limited by either the small sample size or a high proportion of non-detected values. Estimates of propylene glycol workplace exposures are based on a 1981–1983 NOES of U.S. workers and may not reflect current occupational exposure. These data are insufficient to evaluate occupational exposure to propylene glycol.

An estimate of U.S. consumer exposure was available from a 2002 report by the United Nations Joint FAO/WHO Expert Committee on Food Additives *(13)*. In reviewing the annual production volume of 31 flavoring agents, per capita consumption of propylene glycol was estimated at 2,400,000 µg/day **[34.28 mg/kg bw/day for a 70 kg person]**. This value exceeded the estimated per capita

consumption in Japan (1982) by approximately 50-fold **[43 mg/60 kg=0.71 mg/kg bw/day]**. These estimates of human exposure are for food products and do not include exposure from pharmaceutical products or exposure through inhalation. Propylene glycol is found in many pharmaceuticals that are administered intravenously. There are limited data on the effects and exposure levels of chronic (intravenous) administration of propylene glycol in infants and children and no information was found on chronic exposure in pregnant women.

1.4 Summary of Human Exposure Data

In 1999, 1,083 million pounds of propylene glycol were produced in the U.S. with apparent consumption of 854 million pounds *(5)*. Of the apparent amount consumed, uses included, in million pounds and percentages, unsaturated polyester resins (228, 26.7%); cosmetics and personal care products, pharmaceuticals, and human food (170, 19.9%); liquid detergents (135, 15.8%); deicing fluids (85, 10%); antifreeze/engine coolant (55, 6.4%); paints and coatings (40, 4.7%); tobacco humectant (25, 2.9%); other fluids (32, 3.8%); and other applications (84, 9.8%) *(5)*. Propylene glycol is approved by the FDA for use in food, tobacco, and pharmaceutical products and has GRAS status for direct addition to foods.

The general population is exposed to propylene glycol by oral intake, dermal contact, and inhalation. The average daily intake of propylene glycol from food products in the United States has been estimated at 2,400 mg/day **[34 mg/kg bw/day for a 70 kg person]** *(13)*. In Japan, the estimated average daily intake of propylene glycol as a food additive was reported to be 43 mg per person **[43 mg/60 kg=0.71 mg/kg bw/day]** (Louekari et al. *(12)* [from Market Basket Study, Japan 1982]). The Joint FAO/WHO Expert Committee on Food Additives *(13)* concluded that "the safety of these substances [propylene glycol and propylene glycol stearate] would ... not be expected to be of concern." Since propylene glycol has GRAS status and may not be listed as a specific ingredient in some foods, dietary intake based upon product labeling would result in an underestimation of intake. Propylene glycol is an inert ingredient in some pharmaceutical preparations and is also found in many pharmaceuticals that are administered intravenously, which represents a unique exposure route for certain subpopulations.

Occupational exposure to propylene glycol may occur through dermal contact or through inhalation of airborne propylene glycol from heating or spraying processes. No TLV has been defined for propylene glycol, but an AIHA WEEL guide of 50 ppm (total exposure) and an inhalation aerosol exposure of 10 mg/m^3 have been determined. NIOSH estimated that 1,748,454 people (1981–1983 NOES survey as cited in NIOSH report, 1983 *(2)*) are potentially exposed to propylene glycol in the workplace, primarily through contact with trade name products containing propylene glycol.

Several small occupational exposure studies measuring propylene glycol were located. In a study by Laitinen et al. *(20)*, motor-servicing worker exposure to propylene glycol and ethylene glycol was measured. Propylene glycol was below the detection level in air and levels in the urine of exposed workers did not differ from urinary levels in unexposed controls. As dermal exposure to workers was not measured, it was not possible to determine whether urinary levels of propylene glycol found in the workers were due to low exposure or to low dermal absorption.

Norbäck et al. *(19)* measured airborne propylene glycol exposure of Swedish painters during indoor application of water-based paints. Propylene glycol was detected in 12 of 20 samples with a geometric

mean of 350 μg/m^3 and a maximum value of 12,700 μg/m^3.

The levels of propylene glycol in aircraft deicing workers (n=7, age 31–52 years, sex not given) using either undiluted or water-diluted propylene glycol heated to 60°C was measured in urine samples collected pre- and post-shift *(22)*. Urine samples were also collected from a comparison group of non-exposed persons (n=16, sex and age not given). For the exposed workers, the median pre-shift urine level was 1.49 mg/L (range 0.72–13.44 mg/L) and 1.67 mg/g creatinine (range 0.41–10.58 mg/g creatinine). For the exposed workers, the median post-shift urine level was 2.07 mg/L (range 0.77–9.04 mg/L) and 2.46 mg/g creatinine (range 1.22–10.27 mg/g creatinine). For the unexposed comparison group, the median urine level was 1.35 mg/L (range 0.29–10.7 mg/L) and 1.18 mg/g creatinine (range 0.46–18.77 mg/g creatinine).

In a Health Hazard Evaluation (HHE) conducted by NIOSH on workers (n=8) using propylene glycol during aircraft deicing operations *(24)*, personal breathing-zone air samples over a 6-hour period were collected. Seven workers had exposures ranging from 10 to 21 mg/m^3 with a mean of 15 mg/m^3 (1 worker sample excluded due to a suspect high value).

2.0 GENERAL TOXICOLOGICAL AND BIOLOGICAL PARAMETERS

2.1 Toxicokinetics and Metabolism

The toxicokinetics and metabolism data for propylene glycol were initially examined by consulting authoritative reviews *(4, 27)* and an independent review *(28)*. The toxicokinetics sections in those reviews were somewhat brief, and a decision was made by CERHR to review relevant original studies in humans and studies in animals pertinent to reproductive and developmental toxicity.

2.1.1 Absorption

2.1.1.1 Human

Studies of the pharmacokinetics of propylene glycol in humans have been conducted primarily in conjunction with on-going patient therapy where propylene glycol was administered as a vehicle for medications.

Oral

Yu et al. *(29)* examined the pharmacokinetic profile of propylene glycol during multiple oral-dosing regimens. The 22 subjects were outpatients who participated in a phenytoin bioavailability study where propylene glycol was used as a solvent. In one study, 16 adults received a 20.7 g/dose 3 times daily for a minimum of 3 days. In another study, 6 individuals received a 41.4 g/dose twice daily for a period of 3 days. These oral doses were given in conjunction with 100 mg phenytoin in 7.25 mL of alcohol USP, 6 µL of Peach Flavor, 5 mL of glycerin USP, and 8 mL of 70% (w/w) fructose. Propylene glycol was rapidly absorbed from the gastrointestinal tract with maximum plasma concentrations obtained within 1 hour of dosing. The average serum half-life of propylene glycol for the study with 16 and 6 individuals was determined by the authors to be 3.8 and 4.1 hours, respectively. The average total body clearance was determined by the authors to be approximately 0.1 L/kg/hr, although there was significant variability in clearance rate among individuals. The apparent volume of distribution was determined by the authors to be approximately 0.5 L/kg, which approximates the volume of distribution of total body water *(29)*.

Strength/Weaknesses: This study by Yu et al. *(29)* provides data on the oral absorption of propylene glycol as well as on serum half-life, and apparent volume of distribution and total body clearance after repeated oral doses of either 20.7 g 3 times daily or 41.4 g 2 times daily, for a minimum of 3 days. The results are in agreement with expectations for a highly water-soluble, small molecule: rapid absorption, distribution into total body water, relatively short half-life, and rapid total body clearance. One study limitation is the study subjects' concomitant exposure to ethanol; propylene glycol and ethanol are substrates that compete for alcohol dehydrogenase in the initial step of metabolism. While the doses of propylene glycol were high, the data do indicate ready bioavailability of the chemical. The half-life estimates are generally consistent with the results of Speth et al. *(30)* to be discussed later.

Utility (Adequacy) for CERHR Evaluation Process: Data in the Yu et al. *(29)* study are generally adequate to estimate kinetic parameters, but inadequate for quantitative determination of bioavailability.

Rectal

In a study using human volunteers, Kollöffel et al. *(31)* studied rectal absorption and other kinetic

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parameters in children and adults. Propylene glycol and water (1:1) were used as solvents in the formulation of a rectal solution of paracetamol. Absorption of propylene glycol through the rectum was rapid with peak concentrations obtained at 1 ± 0.6 hour (average $\pm$SD) in children (5–12 years old) and 1.5 ± 0.3 hours in adults. Peak plasma concentrations were measured at 171 mg/L **[2.2 mM]** in 4 children dosed with 0.173 g/kg bw propylene glycol and 119 mg/L **[1.6 mM]** in 10 adults dosed with 8.64 g propylene glycol **[123 mg/kg bw assuming a 70 kg bw]**. The serum half-life was determined to be 2.8 ± 0.7 hours in adults and 2.6 ± 0.3 hours in children. The apparent volume of distribution was 0.79 ± 0.30 L/kg in adults and 0.77 ± 0.17 L/kg in children *(31)*.

Strength/Weaknesses: Kolloffel et al. *(31)* determined C_{max} and T_{max} and then used a linear curve-fitting program to recalculate C_{max} and T_{max}, values as well as half-life, apparent volume of distribution, and clearance after different doses of propylene glycol were administered per rectum to adults and children. The small number of children (n=4) and the age range (5–12 years) does not permit a judgment as to whether bioavailability may differ as a function of age within childhood or between children and adults. The values reported are in the expected range providing confirmatory evidence for the reliability of kinetic parameters determined by Speth et al. *(30)*. Plasma levels in children (age 5–12 years) were only slightly higher than in adults. The half-life was virtually the same in children as in adults, which is in agreement with alcohol dehydrogenase activity reaching adult levels by the age of 5 years *(32)*. The extent of oral absorption cannot be judged from these data but a visual inspection of plasma concentrations after intravenous (IV) infusion *(30)* and rectal administration *(31)* indicate very high bioavailability. Thus, oral bioavailability will also be very high. Although it appears that children absorb propylene glycol significantly faster and attain higher peak plasma concentration than adults, the differences are modest and of doubtful toxicological significance.

Utility (Adequacy) for CERHR Evaluation Process: The study by Kolloffel et al. *(31)* is useful to indirectly assess bioavailability.

Dermal

There is limited information on the absorption of propylene glycol through intact human skin. In a study of human skin biopsy specimens from adults 19–50 years of age, MacKee *(33)* found no penetration of radioactive tracer materials after up to 1 hour permeation time using propylene glycol alone as a vehicle **[visual evidence of tracer uptake into biopsied skin, but no analytical confirmation provided]**. Enhancers, such as surfactants, increased absorption.

Three studies are described briefly below that involved patients with significant medical complications. In 45 patients (0.5–87 years old) with second- and third-degree burns on 21–95% of their body, propylene glycol was absorbed through skin following dermal treatment with sulfadiazine in a propylene glycol vehicle; serum levels of propylene glycol in those patients ranged from 0 to 0.98 g/dL **[0 to 129 mM]** *(4, 34)*. In an 8-month-old infant with second- and third-degree burns and complicating toxic epidermal necrolysis over 78% of his body, dermal treatment with silver sulfadiazine in propylene glycol resulted in a peak propylene glycol blood level of 1.059 g/dL **[139 mM]** *(35)*. A blood propylene glycol level of 0.070 g/dL **[9.2 mM]** in an infant was attributed to Mycostatin cream usage for diaper rash *(36)*.

Strengths/Weaknesses: The MacKee study *(33)* showed what is expected of a highly water-soluble substance: that dermal absorption of propylene glycol through the intact skin is very limited.

Weaknesses of this study are the insensitive, non-quantitative method for assessing chemical uptake and the extensive manipulation of the skin following the permeation period (excision which apparently produced bleeding), which may have lead to losses of both skin and permeated chemical from handling the tissue. The three clinical studies *(34-36)* present evidence of propylene glycol bioavailability in circumstances that preclude confident extrapolation to a healthy general population. They do indicate that once the stratum corneum is impaired (removed such as in burns or irritated), dermal absorption may become a significant source of exposure.

Utility (Adequacy) for CERHR Evaluation Process: The MacKee *(33)* study has minimal utility for drawing conclusions regarding propylene glycol penetration across healthy human skin. However, when combined with the rat dermal penetration *in vitro* study *(37)* also showing no uptake, and given the difficulty water soluble molecules generally have penetrating the stratum corneum, the Panel concluded that the dermal absorption rate across intact skin is likely to be slow. Therefore, it can also be expected that any dermal exposure to propylene glycol will result in systemic levels far below saturation of metabolic clearance.

Inhalation

Bau et al. *(38)* **[as reported in HSDB *(2)*]** reported that less than 5% of a technetium-labeled aerosol containing 10% propylene glycol **[propylene glycol not directly measured]** in deionized water was taken up by humans after inhalation for 1 hour in a mist tent. The authors measured the aerosol mass median diameter to be 4.8–5.4 microns, a size small enough to have enabled penetration to the deep lung. Ninety percent of the dose was found in the nasopharynx and it rapidly entered the stomach with very little entering the lungs. Propylene glycol was not measured. The low vapor pressure (0.07 mmHg, approx equal to ~90 ppm or ~270 mg/m^3) of propylene glycol in combination with the short half-life before saturation of metabolism does not allow the build up of toxicologically relevant doses.

Strength/Weaknesses: Since propylene glycol was not directly measured by Bau et al. *(38)*, absorption through the nasal mucosa cannot be determined. However, the low dose rate from inhalation exposure and the small surface area would not lead to significant absorption of propylene glycol.

Utility (Adequacy) for CERHR Evaluation Process: Since inhalation of chemicals is kinetically related to IV infusion, it is of interest to know if propylene glycol is efficiently absorbed from the lungs. As a small, water soluble molecule, it is reasonable to predict that propylene glycol would be absorbed by the lungs. However, with a low vapor pressure (0.07 mm Hg), inhalation of toxicologically relevant doses of propylene glycol is not possible unless heated to higher temperatures. Therefore, the remaining question is whether propylene glycol in a carrier medium could lead to significant exposure by inhalation. Bau et al. *(38)* provides a quantitative answer. Of an average of 263 mL of nebulized aerosol, 8.1 mL containing 10% propylene glycol was retained per hour, corresponding to about 0.8 g of compound, which in turn amounts to 0.09 g/kg per 8 hours. Therefore, it can be concluded that under normal conditions of exposure, propylene glycol via inhalation is of limited toxicological relevance.

2.1.1.2 Animals

Oral

Animal studies demonstrate that propylene glycol is rapidly absorbed following oral exposure. ATSDR *(4)* reports the findings of a study by Christopher et al. *(39)* in which plasma levels of propylene glycol were measured at 19.1 and 8.4 mM in 2 cats fed a diet with 12% propylene glycol [1.60 g/kg bw/day] for 5 weeks. Morshed et al. *(40)* found that propylene glycol blood concentration (41.04 mM) reached its maximum level 1 hour after 4 New Zealand White (NZW) rabbits were administered 38.66 mmol/kg bw [2.942 g/kg bw] as a 28.4% aqueous solution by gavage. Morshed et al. *(41)* orally administered an aqueous solution of propylene glycol at 4.83–77.28 mmol/kg bw [0.368–5.881 g/kg bw] to 6 male Wistar rats/group and found that absorption occurred by a first order process; time to peak absorption was related to dose and ranged from roughly 10 minutes at the low dose to 2 hours at the high dose. An older study by Lehman and Newman *(42)* demonstrated peak blood levels of propylene glycol approximately 2–3 hours after oral dosing in dogs.

Strength/Weaknesses: The Christopher et al. *(39)* study provides very limited data (one time point only) on plasma concentration of propylene glycol after repeated administration of one of two dose rates administered in the diet. It is impossible to derive any kinetic information from such a study other than the qualitative statement that propylene glycol is absorbed to some extent by the cat from the diet.

In contrast, Morshed et al. *(41)* provided a more complete set of data indicating dose-dependent T_{max} for propylene glycol in the dose range of 0.4–5.9 g/kg. The authors did not calculate absorption half-lives or determine the extent of absorption. They concluded that gastrointestinal absorption occurred by a first order process because of the linear rise of plasma concentration at each of the five doses. **[This is an improper conclusion. Data are plotted on an arithmetic scale from which calculation of kinetic rate constants is not possible. There is no indication of curve stripping to calculate k_{abs}. The fact that elimination appears linear on an arithmetic scale indicates a zero order process. If absorption were first order, the absorption rate should increase with increasing concentration in the gastrointestinal tract. The fact that absorption rate did not increase in this manner suggests some limitation with higher bolus doses – e.g., possible delayed gastric emptying. In any case, more complete information is needed to assess bioavailability from the oral route (e.g., Vd, AUC, total body clearance rate, or a comparison IV study in rats).]** The other Morshed et al. *(40, 43)* papers and the Lehman and Newman *(42)* paper also do not provide data suitable for quantitative evaluation. There are reliable quantitative data for the gastrointestinal absorption of diethylene glycol in the rat *(44)* with absorption half-lives ranging from 5 to 40 min (average 16 min) amounting to 80–100% of the dose. Since diethylene glycol has a higher molecular weight but comparable hydrophilicity, it is likely that very rapid gastrointestinal absorption occurs also for propylene glycol. This is also the case for ethylene glycol as indicated by rapid urinary excretion *(45)*.

Utility (Adequacy) for CERHR Evaluation Process: Available animal data are not well suited for quantitative estimation of gastrointestinal absorption of propylene glycol. Nevertheless, all data including structure-activity relationships point toward very rapid and complete absorption. This is plausible for a highly water-soluble small molecule which will cross membranes with bulk flow of water across aqueous pores.

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Dermal

Information on *in vivo* dermal absorption of propylene glycol in animals was not located. ATSDR notes that "*In vitro* studies of the penetration of propylene glycol through the rat abdominal stratum corneum have been conducted" *(4)*. Fresh abdominal skin from male Wistar rats was used in experiments in which propylene glycol, or a mixture of propylene glycol and oleic acid, were evaluated for absorption properties *(46)*. When propylene glycol was applied for up to 2 hours, no compound was detected in the dermis. However, when 0.15 M oleic acid was added to the propylene glycol, it was detected in the dermis after 30 minutes of exposure, but not after 5 or 15 minutes *(46)*. ATSDR *(4)* reported that hairless mouse skin overestimates absorption of propylene glycol by human skin while shed snake skin underestimates absorption. Therefore, the authors concluded that human skin should be used for absorption studies if possible.

2.1.2 Distribution

Speth et al. *(30)* reported on the pharmacokinetics of IV administration of propylene glycol involving six cancer patients who were sufficiently healthy to care for themselves and had normal liver and kidney function. They reported that clearance decreased as dose increased over a dose-range of 3–15 mg/m^2. There was a first order elimination with an average terminal half-life of 2.3 ± 0.7 hours. Considerable interpatient variation was noted. The apparent volume of distribution ranged from ~0.55–0.94 L/kg. In other studies with oral or rectal exposure, apparent volumes of distribution ranged from ~0.52–0.79 L/kg *(29, 31)*.

Strength/Weaknesses: This study *(30)* provides sound pharmacokinetic data from a limited number of individuals who were exposed intravenously to propylene glycol. However, the Speth et al. conclusion that clearance of propylene glycol in humans occurs by a first order process is questionable, as is the calculation of an average half-life of 2.3 ± 0.7 hours.

Utility (Adequacy) for CERHR Evaluation Process: This human study with IV exposure and those with oral and rectal exposure indicate that propylene glycol is uniformly distributed in total body water without a significant distribution to specific tissues. It can be predicted with certainty that propylene glycol will distribute into the water compartment of the placenta and fetus.

2.1.3 Metabolism

In what is considered to be the main pathway of propylene glycol metabolism in mammals *(4, 39)*, propylene glycol is oxidized by alcohol dehydrogenase to lactaldehyde, then to lactate by aldehyde dehydrogenase. The lactate is further metabolized to pyruvate, carbon dioxide, and water. Lactate also contributes to glucose formation through gluconeogenic pathways *(39)*. Lactate, via phosphoenol pyruvate, can be detoxified into glucose and stored as glycogen, as has been demonstrated by Wittman et al. *(47)* for propylene glycol in rats. Excess production of lactic acid resulting from very large exposures to propylene glycol can produce a metabolic anion gap **[anion gap=(Na^+)–(Cl^-+total CO_2)]** and metabolic acidosis *(4)*. Serum levels of >180 mg/L **[2.37mM]** can result in toxicity *(48)*.

In most mammals, part of the absorbed propylene glycol is eliminated unchanged by the kidney, while another portion is excreted by the kidneys as a glucuronic acid conjugate *(2, 28)*. The amount of propylene glycol eliminated by the kidneys has been estimated for humans at 45% *(48)*, for dogs at 55–88% *(49)*, and for rabbits at 24–14.2% *(50)*. Cats do not have the ability to produce the

glucuronidated metabolite *(28)*. Alternate stereo-specific reaction pathways have been described for the metabolism of propylene glycol and are described below.

In adult humans, the mean serum half-life of propylene glycol is approximately 2–4 hours *(30)*. Kelner and Bailey *(51)* studied the pharmacokinetics of propylene glycol in humans in conjunction with the IV administration of medications. Propylene glycol concentrations were measured in sera and cerebral spinal fluids (CSFs) from five patients receiving medication containing propylene glycol as a vehicle; lactate and pyruvate concentrations were also measured. The authors stated that all patients had normal hepatic and renal function based upon laboratory tests. The authors found a significant ($p < 0.01$) correlation of lactate concentrations in the serum and CSF to the corresponding propylene glycol concentrations in these fluids. The authors concluded that although the increase in serum lactate could be due to the patients' clinical conditions, it was unlikely in light of the correlation between propylene glycol and lactate concentrations. For two patients, the authors had propylene glycol/creatinine clearance ratios and were able to calculate the serum half-life of propylene glycol. The authors estimated this to be 4.7 and 5.6 hours, respectively, for these patients. **[The dose was not stated, but because of the severe lactic acidosis, the results suggest that it must have been higher than the 2x41.4g dose/day for 3 days administered by Yu and Sawchuck *(50)*, which did not cause lactic acidosis.]**

While it is clear that total body clearance of propylene glycol occurs by metabolism and by renal excretion of the parent compound, there are no data in humans from which to assess the percentage fate of propylene glycol by these mechanisms. In the rabbit, Yu and Sawchuck *(50)* observed that metabolic clearance accounts for 85.8–97.6% of total clearance at lower doses. Morshed et al. *(41)* provided evidence that the rate-determining step in the metabolic clearance of propylene glycol in the rat is the NAD-dependent alcohol dehydrogenase. Using the dehydrogenase inhibitor pyrazole, there was a dose-dependent inhibition of the dehydrogenase leading to a dose-dependent increase in urinary excretion of propylene glycol. They found that the maximum metabolizing capacity in the rat was 8.33 mmole of propylene glycol/kg bw/hour, which they stated would extrapolate to 1.06 kg bw/day for a 70 kg human.

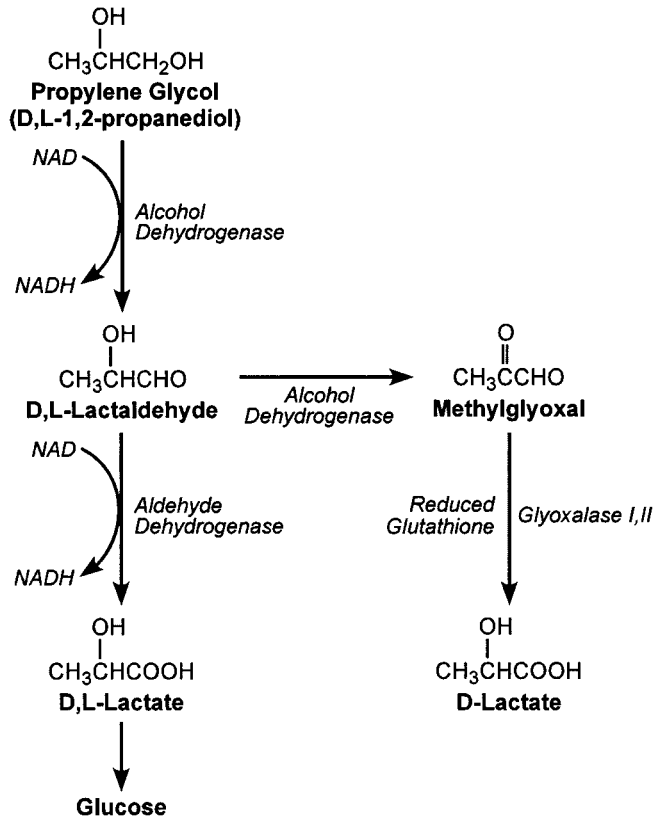
The Expert Panel believes that Speth et al. *(30)* supports the conclusion that humans clear propylene glycol similarly to rats and rabbits. However, saturation of metabolic clearance seems to occur at lower doses in humans than in rats and rabbits. Speth et al. (Table 2 of the study) *(30)* indicates that saturation of metabolic clearance seems to occur at a dose of about 7 g/day in some patients but not in others. Metabolic clearance does not seem to be affected at ~5 g/day (although no lower dose was used to prove it conclusively) and is uniformly decreased above 12.6 g/day. Speth et al. *(30)* provide evidence of metabolic saturation in propylene glycol metabolism at doses of approximately 7 g/day as seen by lengthening half-life and nonlinear increases in AUC and C_{max}. When this dose is converted to mmol/kg based upon the body weights reported for the three subjects receiving this dose, the value is 1.6 mmole/kg, which is considerably lower than the K_m reported by Morshed et al. in rats. Therefore, the half-life of propylene glycol before saturation of metabolic clearance when it would occur by a first order process is 1.6 ± 0.2 ($\pm$SD) hours. This increased to above 3 hours after metabolic saturation of doses above 12 g/day, when metabolic clearance occurs by a zero order process. This is confirmed by Yu et al. *(29)* who found a "terminal elimination" half-life of ~4 hours in patients administered even higher doses (3 x 20.7 and 2 x 41.4 g/day) of propylene glycol. Unlike the half-life

of a compound cleared by a first order process, which is constant, the half-life of a chemical cleared by a zero order process is dose-dependent as is amply documented for propylene glycol.

2.1.3.1 Metabolism and Stereospecificity

Synthesis of propylene glycol results in a 1:1 ratio of D and L stereoisomer forms. There is some, although incomplete, information in the literature about stereospecificity of the enzymes in the propylene glycol metabolic pathways (Figure 2-1).

Figure 2-1. Propylene Glycol Metabolism in Mammals



From Christopher et al. (*39*)

In the main metabolic pathway, D and L forms of lactaldehyde and lactate are formed *(4, 39)*. In the horse and rabbit, ADH will oxidize the L form of propylene glycol and lactaldehyde more efficiently than the D form *(52)*. L-lactic acidosis has been observed in both humans and animals following exposure to propylene glycol *(39, 40)*.

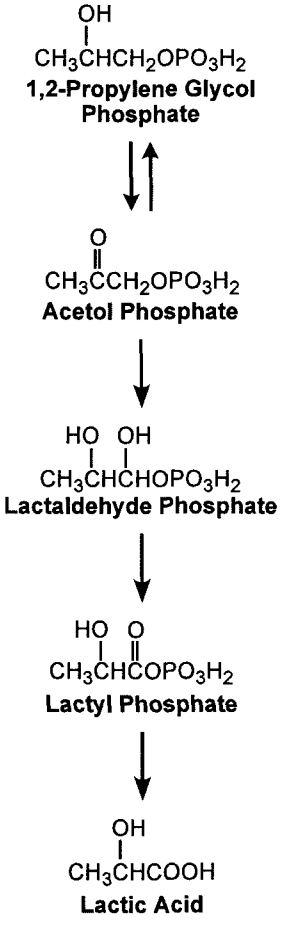
The conversion of lactaldehyde to methylglyoxal by ADH and then to D-lactate by glyoxalase and reduced glutathione is thought to be an alternate route of metabolism (Figure 2-1). D-lactate is cleared more slowly than L-lactate and is considered a poor substrate for gluconeogenesis.

Methylglyoxal synthetase can convert the substrate, dihydroxyacetone phosphate, to methylglyoxal. However, in conditions where ketone levels are high, such as diabetes or starvation, methylglyoxal

synthetase activity is increased, producing more methylglyoxal and D-lactate. Excessive production of D-lactate may result in its accumulation, especially in the brain, which has a low level of catabolizing enzymes *(39)*. Therefore, in cases of ketosis, excess levels of D-lactate may be exacerbated by propylene glycol.

In a third possible metabolic pathway, propylene glycol can be phosphorylated, converted to acetol phosphate, lactaldehyde phosphate, lactyl phosphate, and lactic acid (see Figure 2-2) *(49)*. Metabolism of D and L forms of propylene glycol in this pathway is species-specific. The rabbit converts the L-form of phosphorylated propylene glycol to lactic acid, whereas the rat and mouse can convert both forms *(52, 53)*.

Figure 2-2. Phosphorylated Propylene Glycol Metabolism in Mammals



From Ruddick *(49)*.

A limited number of studies were summarized in detail since they demonstrate evidence of *in vitro* stereospecificity of ADH *(52)*, L-lactatemia in rabbits *(40)*, and increased D-lactate formation in cats *(39)*.

Stereospecificity of ADH was studied by Huff *(52)*. *In vitro* rabbit liver ADH K_s values were obtained

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for ethanol, L-propylene glycol, and D-propylene glycol substrates and were 0.63, 3.6, and 33.3 μmoles/mL, respectively. K_s values obtained for acetaldehyde, L-lactaldehyde, and D-lactaldehyde were 3.6, 1.4, and 3.7 μmole/mL, respectively. A similar trend in values was observed with horse liver ADH. Therefore, ADH from horse and rabbit liver exhibited stereospecific preference for L-propylene glycol and L-lactaldehyde.

Strength/Weaknesses: Stereospecificity of metabolism should be considered because technical grade propylene glycol contains the stereoisomers in a 1:1 ratio. Huff *(52)* determined the K_m values for oxidation of the D- and L-forms by alcohol dehydrogenase and found that L-propylene glycol is 5–9 times more readily metabolized to L-lactaldehyde by rabbit and horse alcohol dehydrogenase than is the D-form. Therefore, it is plausible that D-propylene glycol will be cleared more slowly since this is the rate-determining step in the metabolic clearance of these compounds. Moreover, accumulation of D-lactate has been documented in cats *(39)* and humans *(54)*, which was partially attributed to D-lactate being a poor substrate for gluconeogenesis, a detoxification pathway for L-lactate. In addition, D, L-lactaldehydes are oxidized to methyl glyoxal with loss of the chirality center, which glyoxylase with GSH as co-substrate converts stereospecifically to D-lactate.

Another pathway occurs by phosphorylation of propylene glycol followed by oxidation steps without loss of the chirality center. Here, species differences were found; rabbits converted the L-form more readily to lactic acid, but rats and mice did it equally well with both forms *(52, 53)*. Due to incomplete time-point sampling and a lack of quantitative numbers regarding fluxes through the different pathways, it is not possible to put together a complete picture of stereospecific metabolism of D, L-propylene glycol.

It is of no toxicological consequence whether L- or D-lactatemia develops because both can contribute to the development of lactic acidosis. The longer half-life of D-lactate can be easily factored in via the Michaelis-Menten equation into a physiologically based pharmacokinetic (PBPK) model. The weakness of this approach is that D-lactate was shown to be efficiently utilized in man *(54)*, but its tubular reabsorption was shown to be retarded, particularly at higher concentrations (>3 meq/L). Since chirality is lost during oxidation of D, L-lactate, the preferential use of L-lactate must be due to a lower K_m of lactate dehydrogenase for L- than for D-lactate. In any event, reduced tubular reabsorption enhances overall clearance of D-lactate, whereas reduced utilization for gluconeogenesis runs counter to this effect, apparently outweighing both its reduced tubular reabsorption and its utilization in the Krebs cycle that produces CO_2.

The overall conclusion from all data is that acute exposure to D, L-propylene glycol can cause L-lactic acidosis (if the dose is very high) due to the more rapid biotransformation (alcohol dehydrogenase being the rate-determining step) of L-propylene glycol to L-lactate, whereas subchronic/chronic exposure leads to D-lactic acidosis due to accumulation of D-lactate derived from the glyoxylase/ GSH pathway and from being a poor substrate for gluconeogenesis.

Utility (Adequacy) for CERHR Evaluation Process: The database is sufficient to understand and predict metabolic clearance of D, L-propylene glycol in man.

The role of propylene glycol metabolism in lactatemia in the rabbit was investigated by Morshed et al.

(40). Propylene glycol was administered to NZW rabbits by gavage in a single dose of 38.66 mmol/kg **[2.942 g/kg]** (1 mL 28.4% (v/v)) aqueous solution per 100 g bw. Whole blood was withdrawn from the marginal ear vein after a 24 hour fast and at 0.25, 1, and 3 hours after administration of propylene glycol. Blood pH and the levels of propylene glycol and D- and L-lactate and pyruvate were determined. The level of propylene glycol was estimated colorimetrically and the levels of lactate and pyruvate were estimated enzymatically. Data were evaluated by analysis of variance for repeated measures and were expressed as mean ± SD; a value of $P < 0.05$ was statistically significant. As noted in Table 2-1, blood propylene glycol concentrations were at a maximum 1 hour post-dosing.

Table 2-1. Levels of Propylene Glycol and its Metabolites in New Zealand White Rabbits after Oral Propylene Glycol (From Morshed et al. (40))

Parameter	*Fast*	*0.25 h*	*1 h*	*3 h*
Propylene Glycol	0 (0)	30.23 ± 12.45*** (0)	41.04 ± 9.98*** (0)	36.55 ± 8.0*** (0)
L-Lactate	1.04 ± 0.22 (1.08 ± 0.25)	2.55 ± 0.62** (1.12 ± 0.19)	2.03 ± 0.48** (1.0 ± 0.25)	1.77 ± 0.36** (1.07 ± 0.18)
D-Lactate	0.005 ± 0.005 (0.004 ± 0.003)	0.025 ± 0.004*** (0.005 ± 0.005)	0.10 ± 0.02*** (0.006 ± 0.004)	0.15 ± 0.03*** (0.10 ± 0.01)
Pyruvate	0.54 ± 0.10 (0.51 ± 0.08)	0.60 ± 0.14 (0.57 ± 0.10)	0.63 ± 0.13 (0.55 ± 0.12)	0.58 ± 0.10 (0.50 ± 0.14)
Lactate/pyruvate	1.92 ± 0.07 (2.12 ± 0.10)	4.27 ± 0.18*** (1.96 ± 0.09)	3.22 ± 0.05*** (1.82 ± 0.12)	3.05 ± 0.10*** (2.14 ± 0.08)

Note: Values are means ± SD obtained from four propylene glycol treated rabbits and are expressed as mmol/liter except the lactate/pyruvate, which is a ratio. This ratio was calculated using the data in this table and considering L-lactate as the total body lactate. Data in the parentheses indicate the values obtained from saline-administered control rabbits (n=4); ** $p<0.01$; *** $p<0.001$.

Appendix

Treatment with propylene glycol significantly ($P < 0.01$) increased the concentration of L-lactate, which reached a plateau at 0.25 hours following exposure. D-lactate levels were significantly increased and reached maximum concentration at 3 hours after administration of oral propylene glycol. Although significant, the authors considered the increase in D-lactate to be negligible and noted that L-lactate levels were similar to total lactate levels. Levels of pyruvate remained unaffected before and after administration of propylene glycol. Blood pH was not significantly altered when compared to control values. The authors note that these findings are different than the results from oral administration of propylene glycol to the rat *(55)*.

Strength/Weaknesses: The Morshed et al. *(40)* paper provides some useful information about the early phase of metabolism of propylene glycol in rabbits; its usefulness for propylene glycol kinetics is limited because of poor sampling intervals. Blood levels of propylene glycol dropped from a maximum of 41.0 mM at 1 hour after dosing to 36.6 mM at 3 hours after dosing. A very rough estimate under the assumption of the first order one compartment model would indicate a half-life of about 12 hours in the rabbit. It must be emphasized that neither assumption may be correct, because the high dose and the very slow flux of L-lactate indicates that the system operated according to a zero order process. **[In any event, neither Morshed et al. *(40, 55)* paper is properly interpreted.]**

The study in rats *(55)* did not determine blood levels of propylene glycol although it used many doses and a sufficient number of time points. Lactate levels are plotted on an arithmetic scale, which allows half-life estimates by a visual inspection but no exact calculation. The statement "The elimination time ranged from 1.40 to 5.82 hour which followed apparent first order kinetics" is contradictory. The half-life of first order processes is a constant and independent of dose. Except for the two lower doses (0.4 and 0.8 mg/kg), which were below saturation of metabolic clearance, the higher doses (1.6, 3.2, and 6.0 ml/kg) were above saturation of metabolic clearance and therefore the metabolite (lactate) reflected the kinetics of the parent compound (saturation of alcohol dehydrogenase being the rate-determining step) with dose-dependent increase in its half-life.

The time course evaluated for propylene glycol-induced lactatemia in rabbits was too short to allow for any conclusions regarding D- or L-lactate half-life in the study of Morshed et al. *(40)*. That study also contains contradictory data in that blood L-lactate concentrations peaked at the earliest time point (0.25 hours) and declined thereafter (see Table 2-1 above). However, the propylene glycol concentration peaked at 1 hour and fell only slightly by 3 hours. This irregular decline of primary metabolite in the face of increasing parent compound concentrations is not readily interpretable. One might conclude from this paper that L-lactate is orders of magnitude more important as a metabolite of propylene glycol than is D-lactate. However, it should be made clear that this may only be true for the rabbit, as Morshed et al. point out that rat ADH is more efficient in metabolizing D-propylene glycol than is rabbit ADH, which leads to slightly greater overall lactate levels from propylene glycol metabolism in rats than in rabbits. The lack of information of D- vs. L-lactate formation in humans makes it unclear whether humans are more like the rat or rabbit.

Utility (Adequacy) for CERHR Evaluation Process: The usefulness of the Morshed et al. *(40, 55)* data is limited for reproductive and developmental considerations. It is clear from these papers that high doses of propylene glycol will result in sustained hyperlactemia, probably without lactic acidosis, because of the efficient removal of lactate via gluconeogenesis.

In a study examining clinical chemistry abnormalities, 5 or 6 cats of each sex were fed a diet containing 12% propylene glycol (low dose, 1.60 g/kg bw/day) for 5 weeks (a dose equivalent to that found in commercial soft-moist cat foods), or a high dose diet containing 41% propylene glycol (8.00 g/kg bw/day) for 22 days *(39)*. Propylene glycol (99.7% purity) was a racemic mixture of D- and L-isomers. Predosing observations were made such that each group of cats served as its own control. Clinical chemistry analyses were conducted on serum samples. L- (+) lactate was determined enzymatically using L-lactate dehydrogenase and D- (-) lactate was determined on days 0, 10, and 24 of the low-dose diet and days 0, 6, 10, and 24 of the high-dose diet. Data were analyzed by ANOVA and significance was at the $p<0.05$ level. Plasma levels of propylene glycol were measured in two of the low-dose cats. Propylene glycol levels on day 24 of dosing were 19.1 and 8.4 mM and propylene glycol was not detected in the control plasma. The authors reported a linear correlation between increases in anion gap **[anion gap=(Na^+)-Cl^-+total CO_2)]** and D-lactate in cats fed the low dose. Serum levels of D-lactate increased with days of propylene glycol ingestion and levels of L-lactate decreased in low-dose cats (Table 2-2). The authors noted previous observations where propylene glycol was found to produce L-lactic acidosis in humans and animals including cats shortly after exposure. Because their study first measured lactic acid exposure at 1 week following exposure, it is unknown if acute increases in L-lactate concentration occurred in the cats.

Table 2-2. Serum Lactate Levels in Cats Ingesting 1.6 g or 8.0 g Propylene Glycol/kg bw/day*

	0 days ingestion	*10 days ingestion*	*24 days ingestion*
D-lactate (1.6 g/kg)	0.08 ± 0.03 mmol/L	1.90 ± 0.80 mmol/L	1.96 ± 0.75 mmol/L
L-lactate (1.6 g/kg)	1.02 ± 0.18 mmol/L		0.60 (approx)*
D-lactate (8.0 g/kg)		4.21 ± 1.95 mmol/L	7.12 ± 0.14 mmol/L

* Value taken from graph; 0.32 ± 0.10 mmol/L lactate at 35 days ingestion.
** Christopher *(39)*.

Strength/Weaknesses: The Christopher et al. *(39)* paper is important because it links the anion gap with D-lactate levels in plasma in cats after repeated doses of propylene glycol. Plasma levels of propylene glycol were determined in two low dose (1.6 g/kg bw/day) cats, which in itself is not suitable for any kind of kinetic modeling. Nevertheless these data (19.1 and 8.4 mmol/L) are in agreement with the Morshed et al. *(41)* results, which showed that administration of a single dose (1.6 g/kg) of propylene glycol resulted in peak plasma concentration in the same concentration range (about 8 mmol/L). Thus, it appears that the half-life of propylene glycol is short in cats as well since there seems to be no accumulation of it after repeated administration.

Utility (Adequacy) for CERHR Evaluation Process: Christopher et al. *(39)* is a useful study linking human data *(54)* with animal data regarding D-lactatemia.

Appendi

2.1.3.2 Overall Summary of Metabolism

It appears that high, acute doses of propylene glycol can lead to lactic acidosis. Unless the dose is very high, L-lactate is efficiently converted (detoxified) to glucose. However, D-lactate is not readily converted in the gluconeogenic pathway and therefore tends to accumulate after subacute/chronic dosing leading to D-lactic acidosis. Logically, lactate dehydrogenase must have a much higher affinity for L-lactate than for D-lactate because chirality is lost at the level of pyruvate and D- and L-lactate derived intermediates become indistinguishable upstream of pyruvate.

It may be more likely that at high propylene glycol doses and plasma lactate loads, lactate clearance via utilization in intermediary metabolism is saturated. Limited evidence for this is suggested in the D, L-lactate dosing study of Oh et al. *(54)*. Ten male volunteers received one of two different infusion rates (n = 5 per group) of D, L-lactate in which a doubling in the D-lactate blood level yielded only a 1.5-fold increase in D-lactate utilization rate but a 3.5-fold increase in D-lactate urinary excretion. The levels of D-lactate in this study were in the same range as those reported for total lactate at the high doses in rats *(55)*. The rate of L-lactate excretion and utilization were not reported in the human study *(54)*.

2.1.3.3 Developmental and Species Specific Variations in Metabolism and Enzyme Activities

Activities of enzymes such as ADH and ALDH can affect how fast propylene glycol is cleared from the body, thus affecting potential toxicity. A number of studies examined both the activities of these enzymes in human placenta and the age-related activity of the enzymes. Although most studies focused on ethanol metabolism, they are still relevant to propylene glycol metabolism, since ADH and ALDH activities are investigated. Therefore, CERHR conducted a brief review of the data.

Placental Metabolic Capacity
Studies in humans and rodents suggest that the placenta has extremely limited capacity to metabolize propylene glycol. Pares et al. *(56)* isolated Class III ADH from full term human placenta and found it had low activity for ethanol and a K_m value for octanol that was 100-times higher than the Class I ADH enzyme found in human liver. Zorzano and Herrera *(57)* found that ALDH from full-term human placentas had a lower activity and V_{max}, and a higher K_m value than ALDH isoenzymes from liver.

In rats, placenta was found to have no ADH activity and ALDH activity in placenta was found to be 4–7% of liver activity *(58)*.

Developmental Aspects of Metabolic Capacity
Activity of ADH and ALDH was found to vary with developmental stage.

Sjoblom et al. *(58)* found that in Wistar rats ADH activity in liver was low before birth, being 5 and 16 % of adult activity on gd 15 and 20, respectively. There was a rapid increase at birth: 53% of adult levels on postnatal (pnd) 1 with a continued gradual increase with age to 82% of adult activity on pnd 47. Similar developmental patterns were noted for ALDH in rat liver.

Pikkarainen and Raiha *(32)* measured *in vitro* ADH activity in the livers of human fetuses, children, and adults (n=1–3/age group) using ethanol as a substrate. The ADH activity in 2-month-old fetal livers was about 3–4% that of adults. In 4–5-month-old fetuses, ADH activity was roughly 10% that of adults, and in infancy, activity was about 20% that of adults. ADH activity increased in children with age, and at 5 years of age, activity reached a level within ranges noted for adults. Great variation was noted in adult ADH activity.

Somewhat different results were reported subsequently by Smith et al. *(59)* who examined human liver ADH activity using ethanol as a substrate and also examined the ontogeny of individual ADH class I isoforms. They reported total ADH activity in 9–22-week-old fetal liver that was 30% of adult values, and in premature infants and children less than 1 year old, activity was 50% of adult values. Individual enzyme activity was determined using starch gel electrophoresis with an *in situ* assay. A total of 222 liver samples were assayed, 56 from fetuses (9–22 weeks gestation), 37 from premature infants and infants less than 1 year of age, and 129 from adults greater than 20 years of age. In fetal liver samples with a mean gestational age of 11 weeks, only the ADH1A enzyme was detectable. By 17 weeks, both ADH1A and ADH1B were measurable, although ADH1A predominated. By 19 weeks, products from all three loci were observed, with ADH1A greater than ADH1B, and ADH1B greater than ADH1C. At 30 weeks, ADH1A and ADH1B levels were equivalent, but still greater than ADH1C, but by 36 weeks, ADH1B expression dominated. In the adult, hepatic ADH1A expression was nondetectable, whereas expression from the ADH1B and ADH1C loci were equivalent. Interestingly, this progressive change in expression was tissue-specific. In lung, there were no observed differences between the fetal and adult samples and only ADH1C was detectable. ADH expression in the intestine and kidney was low and did not change appreciably with age.

Thus, it would appear that human liver ADH is expressed early in development and may well contribute to propylene glycol metabolic disposition. However, given the paucity of knowledge regarding isoform specificity towards propylene glycol, it is uncertain how these data on ethanol metabolism might be

extrapolated. Assuming the enzyme most active in ethanol metabolism, ADH1B, is also most active in propylene glycol metabolism, the significant fetal metabolism is not predicted to occur until later in gestational development (20–36 weeks).

Strength/Weaknesses: There are consistent data in both animals and humans showing that alcohol dehydrogenase is much lower prenatally. In humans, adult levels were reached by the age of 5 years and in rats on day 47 after parturition.

Utility (Adequacy) for CERHR Evaluation Process: D, L-lactate, metabolites of D, L-propylene glycols, are postulated to be associated with toxicity in mammalian species. Therefore a lack of *in situ* conversion in the fetus would seem to decrease the toxicity of propylene glycol. Since lactate distributes into total body water, the fetus will share the mother's metabolic load and associated acidosis, if present. The lower metabolism capability in newborns and infants, however, may partially protect them from metabolic acidosis after ingestion of propylene glycol.

Hepatic Metabolic Capacity in Humans Versus Rats

Zorzano and Herrera *(60, 61)* found different ADH isoenzymes in liver homogenates from humans (class I ADH) and rats (ADH-3), which differed greatly in kinetic properties. Using ethanol as a substrate at a pH of 10.5, activity, K_m, and V_{max} in humans was measured at 6.24 Units/g tissue, 2.10 mM, and 7.70 Units/g tissue, respectively, while activity, K_m, and V_{max} in rats was measured at 2.72 Units/g tissue, 1.02 mM, and 2.96 Units/g tissue, respectively. Two different low K_m ALDH isoenzymes were found in humans and rats but they had similar activities using acetaldehyde as the substrate at pH 8.8 (humans: K_m=9 μM and V_{max}=0.85 Units/g tissue; rats: K_m=10 μM and V_{max}=0.87 Units/g tissue).

Inter-individual Variability Due to Generic Polymorphisms

Reviews by Agarwal *(62)*, Bosron and Li *(63)*, Pietruszko *(64)*, and Burnell et al. *(65)* discussed genetic polymorphisms for ADH and ALDH in humans. Class I ADH, the primary ADH in human liver, is a dimer composed of randomly associated polypeptide units encoded by three loci (ADH1A, ADH1B, and ADH1C). Polymorphisms resulting in altered phenotypes are observed at the ADH1B and ADH1C loci.

There are two primary ALDH isoenzymes in human liver, ALDH2 (also referred to as E_2, ALDHI, or $ALDH_2$) and ALDH1 (also referred to as an E_1, ALDHII, or $ALDH_1$) *(62-64)*. About 50% of Japanese and Chinese carry a phenotypically null variant of the ALDH2 enzyme.

2.1.4 Elimination

In mammals, part of the propylene glycol dose is eliminated unchanged by the kidney and part is metabolized by the liver to lactic acid and further metabolized to pyruvic acid; in mammals, with the exception of cats, the remainder is conjugated with glucuronic acid *(2)* and eliminated in the urine. The amount of propylene glycol eliminated by the kidneys has been estimated for humans at 45% *(48)*, for dogs at 55–88% *(49)*, and for rabbits at 2.4–14.2% *(50)*. Morshed et al. *(41)* provided evidence in the rat that increasing doses of propylene glycol increased elimination by the kidneys. Dosages of 19, 38, and 77 mmole/kg bw resulted in 2.3, 7, and 17% renal excretion of propylene glycol. Maximum urinary excretion of propylene glycol was determined using pyrazole (1.0 mmole/

kg bw), a competitive inhibitor of propylene glycol. High urinary clearance was observed with 75% excretion of the ingested dose within 24 hours.

2.1.4.1 Humans

In human adults receiving 20.7 or 41.4 g propylene glycol 2–3 times daily for a minimum of 3 days, the total body clearance was dependent on serum concentration and was approximately 0.1 L/kg bw/hour; elimination half-life in those same subjects was about 4 hours *(29)*. **[The influence of ethyl alcohol administration must be considered when interpreting results since it will compete with propylene glycol for the dehydrogenase enzymes.]** In a study where adults and children were rectally exposed once to ~123–173 mg/kg bw propylene glycol **[blood levels 1.6–2.2 mM],** the clearance rate was 0.2 L/hour/kg and half-life was 2.6–2.8 hours *(31)*. In 6 adults receiving propylene glycol intravenously, blood levels of propylene glycol were measured at 48–425 μg/mL **[0.63–5.6 mM]** and an average half-life of 2.3 hours was estimated *(30)*.

A small number of studies suggest that elimination of propylene glycol in infants is slower than in adults. In an 8-month-old infant exposed to propylene glycol through medication applied to burns, the propylene glycol blood level was 1.059 g/dL **[139 mM]** and the elimination half-life was measured at 16.9 hours *(35)*. Ten infants exposed to 10 mL **[10.36 g]** propylene glycol in a parenteral vitamin solution daily for 5 days had propylene glycol blood levels of ~65–950 mg/dL **[8.5–125mM]** and elimination half-lives of 10.8–30.5 hours, with a mean of 19.3 hours *(36)*.

Excretion of propylene glycol has been studied in patients with second and third degree burns over more than 20% of their total body surface *(34)*. According to ATSDR *(4)*, "Sulfadiazine preparations containing propylene glycol were applied dermally over a period of 3–7 days after admission to the hospital. Serum and urinary levels of propylene glycol were measured. Propylene glycol was detected in the serum of 24 of 45 patients and in the urine of 40 of 45 patients. Average urinary levels were 1.3 mg/mL with a range of 0–17.9 mg/mL for patients who lived, and 2.9 mg/mL with a range of 0–23 mg/mL for patients who died. Propylene glycol levels correlated with total burn surface area and total third degree burn surface area."

Strength/Weaknesses: Elimination kinetics of propylene glycol are well understood. Speth et al. *(30)* provides the major kinetic parameters needed for calculations. The saturation of metabolic clearance occurs in humans at about 7 g, which is somewhat lower than in animals. Kollöffel et al. *(31)* provide data in 10 adults which indicate that at a dose of 8.64 g, elimination of propylene glycol was zero order because it was nearly linear on an arithmetic scale. At a dose of 5.1 g/day the half-life of propylene glycol was 1.6±0.20 hours, at doses of 7.2 to 7.7 g/day it was 1.9±0.15 hours, and at doses of 12.6 to 21.0 g/day it was 3.2±0.12 hours *(30)*. The data of Kollöffel et al. *(31)* provide 2.6±0.2 hours as half-life in adults at a dose of 8.64 g/day. At a dose of 3 x 20.7 to 2 x 41.4 g/day, Yu et al. *(29)* estimated an elimination half-life of about 4 hours. Thus, the half-life of propylene glycol increased from 1.6 to 4 hours as the dose increased from 5.1 to 2 x 41.4 g/day. The half-life of chemicals eliminated by first order processes is independent of dose. Therefore, it is certain that in humans propylene glycol is eliminated by zero order kinetics at or above a dose of 5.1 g/day. Clearance data and AUCs verify this conclusion.

The infant studies suggest prolonged half-lives of propylene glycol *(35, 36)* in the range of 10.8–30.5

hours in infants receiving a dose of about 3 g propylene glycol. While such data are consistent with very low alcohol dehydrogenase activity perinatally *(32)*, they cannot be considered definitive due to confounding effect(s) associated with the disease treatment and the drugs associated with such therapy. In addition, the Kulick et al. *(34)* paper is not suitable for determination of elimination kinetics because only one time point was measured. Prolonged half-life of propylene glycol in infants is also supported by a recent report showing that it accumulated to very high levels (up to 2,000 μg/mL) in serum of these children *(66)*.

The infant studies suggest prolonged half-lives of propylene glycol *(35, 36)* in the range of 10.8–30.5 hours in infants receiving a dose of about 3 g propylene glycol. While such data are consistent with very low alcohol dehydrogenase activity perinatally *(32)*, they cannot be considered definitive due to confounding effect(s) associated with the disease treatment and the drugs associated with such therapy. In addition, the Kulick et al. *(34)* paper is not suitable for determination of elimination kinetics because only one time point was measured. Prolonged half-life of propylene glycol in infants is also supported by a recent report showing that it accumulated to very high levels (up to 2,000 μg/mL) in serum of these children *(66)*.

Utility (Adequacy) for CERHR Evaluation Process: There are sufficient data available on the elimination kinetics of propylene glycol in humans to model elimination in adults; data in infants and in the fetus are less certain.

Appendix

2.1.4.2 Animals

"Dose-dependent elimination of propylene glycol is seen in rats, with saturation of the pathways at doses above 5.88 g/kg. An apparent maximum elimination rate of 8.3 mmol/kg/hour (0.63 g/kg/hour) was observed" *(4)*.

Yu and Sawchuk *(50)* studied the metabolism and elimination of propylene glycol after acute or chronic IV administration to NZW male rabbits. Rabbits were exposed acutely by IV injection to either 0.5, 1.0, or 2.0 g/kg bw (three rabbits per dose group). There was evidence of a saturation of propylene glycol metabolism at the 2.0 g/kg bw acute dose, as evidenced by the decreased metabolic clearance. The half-life and the terminal elimination phase rate constant was not significantly affected over this dose range. An additional few rabbits were exposed by continuous IV infusion to propylene glycol delivered at various rates (2.8–6.3 mg/min/kg bw) over the course of 51–52 hours. Both V_{max} and K_m were lower in the case of prolonged exposure, but the V_{max}/K_m ratio was approximately 3-fold greater than under acute dosing. Plots of metabolic clearance from single rabbits dosed acutely versus continuously indicate higher metabolic clearance rates from continuous exposure. **[This raises the possibility of the induction of a second, low K_m form of ADH during the 51–52 hours of infusion.]** The authors concluded that metabolism of propylene glycol was the dominant disposition pathway with a concentration-dependent metabolic clearance; renal excretion of propylene glycol was only 2.4–14.2% of the total dose after acute administration, most likely due to kidney reabsorption. Authors also concluded that for both acute and chronic administration of propylene glycol, the clearance of propylene glycol is lower at higher plasma concentrations and the rate of elimination of propylene glycol was dependent upon urine flow.

Ruddick *(49)* cited an earlier study by Lehman and Newman *(42)* where dogs were force fed 8 mL/kg

and 12 mL/kg of a 50% aqueous solution of propylene glycol. Blood concentrations were 1.3 g/dL **[171 mM]** 2 hours after dosing and 0.9 g/dL **[118 mM]** 4 hours after dosing. Recovery of 12–45% of the unchanged administered dose in the urine led the authors to conclude that the compound was eliminated by the kidney and a large portion of unexcreted chemical was metabolized.

Strength/Weaknesses: Animal data are consistent with human data regarding the elimination kinetics (practically the same elimination half-life before saturation of metabolic clearance) of propylene glycol, although minor species differences may be present. Saturation of metabolic clearance occurs at somewhat higher doses in animals; therefore, the half-life of elimination becomes dose-dependent (zero order) at higher doses.

Utility (Adequacy) for CERHR Evaluation Process: It is useful to have mechanistic insight into the process of elimination of propylene glycol as represented by the Yu and Sawchuk *(50)* paper on the urinary flow dependence of elimination as well as on the dose-dependence of metabolic clearance.

The Ruddick *(49)* and Lehman and Newman *(42)* papers are not suitable for quantitative kinetic evaluation.

2.2 General Toxicity

The majority of information in this section is summarized from the reviews by ATSDR *(4)* and LaKind et al. *(28)* and from the SIDS Initial Assessment Report for 11th SIAM *(27)* and the EPA Health and Environmental Effects Document on Propylene Glycol *(67)*. No toxicity studies have been located on propylene glycol subsequent to the 2001 SIDS Initial Assessment Report. A very limited number of toxicity studies included an examination of the reproductive organs and those studies are discussed in detail.

Propylene glycol has very low systemic toxicity in experimental animals and very high doses are used in most acute studies to determine a toxic level. It is primarily metabolized to lactic acid and pyruvic acid, both of which are normal constituents of the citric acid cycle. CNS, hematologic, hyperosmotic, and cardiovascular effects have been noted in humans and animals and high serum concentrations of propylene glycol may result in lactic acidosis and hyperosmotic changes in the blood *(4, 27, 49)*. Symptoms of acute propylene glycol intoxication in animals include CNS depression and narcosis. Individuals with compromised hepatic or renal function would be less apt to clear propylene glycol, and hence would be more susceptible to toxicity due to high blood levels *(2, 4, 68)*. No system or organ has been established as a target for the acute oral lethal effects of propylene glycol *(69)*.

Lactate can be detoxified into glucose and stored as glycogen as has been demonstrated by Wittman et al. *(47)* in propylene glycol-exposed rats. Doses of 0.5–2.0 g/kg of propylene glycol were administered to female rats and liver glycogen content and blood glucose were determined 90 minutes after dosing. Liver glycogen content nearly doubled and fasting blood glucose increased from 88 to about 140 mg%. Lactic acidosis was not reported. **[Lactic acidosis is not expected at these relatively low doses of propylene glycol. However, lactic acidosis can develop if these two detoxification pathways cannot remove excess lactic acid sufficiently.]**

ATSDR *(4)* stated that "The mechanism of action of propylene glycol is not well understood". **[In**

fact, much is known about the mechanism of action.] Lactatemia has been well documented in animals and there are supporting human data. Cats administered 12 (1.6 g/kg bw/day) or 41% (8.0 g/kg bw/day) propylene glycol in the diet (dry weight) for 22 days, showed a time-dependent increase in plasma lactate and in anion gap *(39)*. Morshed et al. *(40, 43)* produced more data on the dose-dependence of blood lactate and/or pyruvate in rats and rabbits given propylene glycol orally. Finally, a human case report *(48)* demonstrated that repeated infusions of lorazepam dissolved in propylene glycol can lead to lactic acidosis with increased osmolar gap (21 mOsm/L). Furthermore, increased blood glucose (296 mg/dL) and elevated pyruvate level (1.01 mg/dL) indicate that the same metabolic pathways of detoxification occur in humans as in animals. Glasgow et al. *(36)* reported a good correlation between osmolality gap and serum propylene glycol concentrations in ten infants. The half-life was reported as 19.3 hours (range 10.8–30.5 hours), which is about 10 times longer than in adults. Alcohol dehydrogenase activity is up to 10 times lower in infants *(32)* than in adults providing an explanation for the prolonged half-life in the latter and at the same time further evidence that this enzyme is the rate-determining enzyme in the clearance of propylene glycol. Other endpoints of toxicity are anesthesia, probably by the same mechanism as other alcohols, and hemolysis, which may be due to the osmolality gap.

Strength/Weaknesses: There is an adequate database to assess the toxicity of propylene glycol *(4, 27, 28, 67)*. Very high doses of propylene glycol cause CNS, hematologic/hyperosmotic, and perhaps cardiovascular effects, as well as lactic acidosis. Animals lethally intoxicated undergo CNS depression, narcosis, and eventual respiratory arrest.

Utility (Adequacy) for CERHR Evaluation Process: There are sufficient reliable reviews to obtain any information needed for informed toxicological judgment.

Appendix

2.2.1 Humans

2.2.1.1 Oral Exposure

A lethal oral dose of propylene glycol has not been reported for humans *(28)*, but it is estimated that the human lethal oral dose is >15 g/kg or >32 fl oz for a 150 lb person *(2)*. In adults, serum levels of >180 mg/L **[2.37 mM]** have resulted in toxicity *(48)*. In one case, an 11-year-old child receiving oral doses of 2–4 mL per day for 13 months as a component of a vitamin D preparation (estimated dose 4–8 g/kg bw/day) resulted in seizures and CNS depression *(28)*. In acutely ill infants, death has occurred after repeated exposure to propylene glycol in medication; CNS depression and seizures have been reported after multiple oral doses *(36, 70)* **[see Section 2.5 Potentially Sensitive Subpopulations]**. According to HSDB *(2)*, the acceptable daily intake of propylene glycol as a food additive is 25 mg/kg body weight.

2.2.1.2 Dermal Exposure

Contact dermatitis has been reported from propylene glycol exposure in a wide variety of topical preparations *(28)* and ingestion of propylene glycol in sensitized individuals has produced flares of dermatitis *(28)*. Skin irritation resulting from topical exposure is manifest as erythematous reactions restricted to sites of exposure. The irritation potential is enhanced after prolonged dermal exposure, under dermal occlusion, and in combination with triethanolamine-stearate, a cosmetic emulsifier *(71, 72)*. The nature of the skin reaction of propylene glycol-sensitive patients has been a matter of controversy *(73, 74)*. In a study by Hannuksela and Forstrom *(73)*, primary irritant reactions to the

skin and type IV delayed hypersensitivity reactions were observed following oral ingestion or topical application of propylene glycol. However, in most cases, the skin reaction was due to a primary irritation, not to an allergic reaction *(72)*.

2.2.1.3 Inhalation Exposure

Propylene glycol is a component of theatrical fog and is used for special effects. The Actors' Equity Association and the League of American Theaters and Producers sponsored a study which included an examination of the health effects of theatrical fog in response to actors' concerns about exposure *(75)*. The health endpoints selected for investigation were irritant effects to the respiratory tract and eyes. This study was conducted over 2 years with 439 actors from 16 musicals, and consisted of a baseline questionnaire, daily checklists, and medical evaluation. There was no clinically significant adverse impact on pulmonary function or in rates of asthma associated with exposure to propylene glycol. However, "peak exposures to elevated localized air concentrations following release of glycol smoke are associated with increased reporting of respiratory, throat, and nasal symptoms, and findings of vocal cord inflammation." The study authors recommended that exposures to propylene glycol by actors not exceed peak or ceiling concentrations of 40 mg/m^3.

NIOSH conducted a study in 1990 on the use of theatrical fog in Broadway theaters *(76)*. Personal breathing zone and general area air sampling and a questionnaire on irritant effects (130 questionnaires from productions with theatrical smoke, 90 questionnaires from productions without theatrical smoke) were collected from personnel from four productions using theatrical smoke and five productions without theatrical smoke. Air samples collected yielded propylene glycol concentrations <2.1 mg/m^3. However, there was a significant (.05) increase in the reporting of respiratory irritant symptoms such as runny nose, stuffy nose, and sneezing by personnel from productions using theatrical smoke.

In a study by Cohen and Crandall *(77)* [reviewed by LaKind et al. *(28)*], propylene glycol was recommended as a vehicle for administration of bronchodilator drugs. No adverse clinical effects were observed after subjects were exposed to an inhalant mist of isoproterenol-HCl containing 40% propylene glycol for 15 minutes at a temperature of 115–124° F.

Wieslander, Norbäck, and Lindgren *(23)* examined experimental exposure of volunteers to propylene glycol mist simulating concentrations routinely used in aviation emergency training. Twenty-seven non-asthmatic volunteers (22 males, 5 females) were exposed in an aircraft simulator to propylene glycol mist over a 1-minute period (average concentration 360 mg/m^3; range 176–851 mg/m^3). Average age was 44 ± 11 years. None of the subjects had previous occupational exposure to propylene glycol. Medical examinations were performed both within 15 minutes before and after the exposure. Exams included an estimate of tear film stability breakup time, nasal patency by acoustic rhinometry, lung function by dynamic spirometry, and a self-rated symptom questionnaire. After 1 minute of exposure there was a statistically significant difference when compared to pre-exposure levels in tear film stability (decreased; $p=0.02$) and ocular and throat irritation ratings (both increased; $P<0.001$) **[P values determined by Student's t test for paired comparisons]**. The forced expiratory volume in 1 second over the forced vital capacity was slightly reduced and the self-rating of severity of dyspnea increased. There were no apparent changes in nasal patency, vital capacity, forced vital capacity, nasal symptoms, dermal symptoms, smell of solvents, or any other systemic symptoms. The authors concluded that short exposure to propylene glycol mist from artificial smoke generators may

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cause acute ocular and upper airway irritation.

2.2.1.4 Parenteral Exposure

Hemolysis, CNS depression, hyperosmolality, and lactic acidosis have been reported after IV administration of propylene glycol *(68)*. Rapid IV infusion of concentrated propylene glycol-containing drugs has been associated with respiratory depression, arrhythmias, hypotension, and seizures. Propylene glycol is used as a vehicle for IV administration of drugs such as lorazepam, etomidate, phenytoin, diazepam, digoxin, hydralazine, esmolol, chlordiazepoxide, multivitamins, nitroglycerin, pentobarbital sodium, phenobarbital sodium, and trimethoprim-sulfamethoxazole. Therefore, patients, especially children and infants, receiving IV drugs can be at risk for propylene glycol toxicity *(28)* **[see Section 2.5 Potentially Sensitive Subpopulations]**.

Information on the dose of propylene glycol necessary to induce toxicity is limited. Some reports describing the dose of propylene glycol given and the serum concentration measured in cases of toxicity in humans are contained in Table 2-8 in Section 2.5, Potentially Sensitive Subpopulations.

2.2.2 Experimental Animal Data

General toxicity studies in animals are discussed in the sections below and summarized in Table 2-4 on page II-35.

2.2.2.1 Oral Exposure

LD_{50} oral toxicity values are listed in Table 2-3.

Table 2-3. Propylene Glycol Oral Toxicity Values

Species	*LD50 (g/kg)*	*Reference*
Rat	8–46	ATSDR (4)
Mouse	25–32	ATSDR (4)
Rabbit	18–20	ATSDR (4)
Dog	19	HSDB (2)
Guinea Pig	18–20	ATSDR (4)
Human	>15 (estimated)	HSDB (2)

A wide range of LD_{50} values has been reported for the rat. In a study by Morshed et al. *(43)*, 6 male Wistar rats were dosed by gavage with saline or 2.942 g/kg bw/day propylene glycol in water for 10, 20, or 30 days. No deaths occurred over any of the time intervals. However, a 41% reduction in body weight was noted at 10 days and an increase in body weight was noted at 20 and 30 days in treated animals as compared to respective saline controls.

Strength/Weaknesses: This study by Morshed et al. *(43)* does not have strengths, only weaknesses. Controls gained 16.9 g during the first 10 days (1.69 g/day on average), 23.3 g after 20 days (1.17 g/day on average), and 40.15 g after 30 days (1.34 g/day on average). Well-maintained rats do not display such weight gain variability.

Utility (Adequacy) for CERHR Evaluation Process: None.

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In a study by Weatherby and Haag *(78)* [reviewed by OECD *(27)*] in rats, only minimal kidney changes were observed and the LD_{50} value was determined to be 33.5 g/kg.

Strength/Weaknesses: This is an older study *(78)* which characterized acute toxicity of propylene glycol in rats and rabbits by various routes of administration. As expected, propylene glycol was most toxic when administered IV. Toxicity decreased IV > IM > subcutaneous > oral. There was no apparent species difference. Information provided on the chronic administration of propylene glycol is sparse but the hemolysis experiment with human blood *in vitro* demonstrates conclusively the hemolytic potential above 0.111 M.

Utility (Adequacy) for CERHR Evaluation Process: This study by Weatherby and Haag *(78)* is useful for the characterization of acute toxicity, but is less useful for chronic toxicity.

Acute oral toxicity in rabbits was studied by administering a 20% aqueous solution of propylene glycol by stomach tube over a 1-hour period (15.75–21.00 g/kg) *(79)* [reviewed in LaKind et al. *(28)*; OECD *(27)*]. Animals exhibited an increased respiratory rate, loss of equilibrium, depression, analgesia, coma, and died by 36 hours post dosing. The minimum fatal dose was determined to be 18.9 g/kg (3 of 9 deaths), with 100% mortality at a dose of 21 g/kg (4 of 4 deaths).

Strength/Weaknesses: The Braun and Cartland *(79)* paper predates the Weatherby and Haag *(78)* publication and represents a less extensive but nevertheless reliable documentation of the acute toxicity of propylene glycol administered IM and subcutaneously to rats and orally to rabbits. Results of the two studies are very similar. Data on chronic toxicity are scant.

Utility (Adequacy) for CERHR Evaluation Process: This report is useful for the characterization of acute, but not chronic, toxicity.

Chronic toxicity studies reflect that propylene glycol has a very low order of toxicity. In the following toxicity studies by Morris et al. *(80)* and Gaunt et al. *(81)*, reproductive tissues were examined.

Albino rats (inbred strain, male and female, 20 rats/group) were administered 0, 2.45, and 4.9% of propylene glycol in the diet (0, 1.23, and 2.45 g/kg bw/day, respectively) for 2 years. Other glycol chemicals were also part of this chronic study. Body weights and food consumption were determined at weekly intervals. No changes were noted when compared to control animals for growth rate, food and water consumption, and animal survival. There were no differences between control and propylene glycol groups in gross and microscopic lesions in the lung, heart, liver, spleen, kidney, adrenal glands, and testes **[individual data or summary tables not reported]**. The authors noted that there were no bladder stones or signs of chronic kidney damage and no change in the gross morphology of the testes when compared to control animals. "Slight liver damage" **[authors' words]** was observed in the propylene glycol exposed group *(80)* [reviewed in LaKind et al. *(28)*; OECD *(27)*]. **[No statistical analyses were performed and the histopathology of the liver is not described.]**

Strength/Weaknesses: The Morris et al. *(80)* paper predates standardized chronic toxicity test protocols and some may view it as poorly controlled. However, the experiment is well-described including the limitations. Therefore, it appears reasonable to accept that daily doses of 4.9% propylene glycol

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in the diet (~3 g/kg) caused centrilobular atrophy, bile duct proliferation, and fatty degeneration in the liver even though it is not stated in the paper at which dose slight liver damage was observed. The highest doses (1.7 to 2.1g/kg) used by Gaunt et al. *(81)* were close to the lower dose in this study and no liver effect was reported by Gaunt et al. Therefore, the lower dose probably did not cause liver damage. Failure to conduct statistical analyses weakens this study.

Utility (Adequacy) for CERHR Evaluation Process: The Morris et al. *(80)* study can only serve as a modest indicator that 3 g/kg propylene glycol chronically might cause slight liver injury.

In 2-year and 15-week toxicity studies in rats given propylene glycol in the diet *(81)*, body weight, renal concentration tests, organ weights, histology, and incidence of neoplasms were described. Necropsy at the end of the study included gross and microscopic examination of the male and female reproductive tracts. Charles River CD rats from a Specific Pathogen Free (SPF) breeding colony were used in this study. At the start of the study, the weight range of the males was 120–150 g and of the females was 120–140 g. **[Statistical methods were not described and standard errors for treatment groups were not presented.]** The studies were run concurrently.

For the short-term study, groups of 15 male and 15 female rats were fed diets containing 0, or 50,000 ppm propylene glycol [Shell Co. Ltd., >99% purity] for 15 weeks. Body weights and food consumption were not recorded. During the last week of treatment, renal concentration tests were estimated over a 6-hour water deprivation period. At necropsy, blood was collected for hematology and blood concentrations of urea, glutamic-oxalacetic, and glutamic-pyruvic transaminases were determined. At necropsy, brain, heart, liver, spleen, kidneys, adrenals, gonads, and pituitary were weighed. In the short-term study, the authors reported no differences between the control rats and those fed the 50,000 ppm diet for the parameters measured, including the urine and serum analyses, blood chemistry, and organ weights **[data not reported].**

In the long-term study, groups of 30 male and 30 female rats were fed diets containing either 0, 6,250, 12,500, 25,000, or 50,000 ppm propylene glycol for 2 years. Animals and food consumption were monitored daily and body weights recorded at 2 week intervals. Blood was collected from the tail vein of 8 male and 8 female rats in the 0, 25,000, and 50,000 ppm dose groups at 13, 21, 52, and 80 weeks of the study; and in the 0, 6,250, and 12,500 ppm groups at week 54 of the study. A urinary concentration test was done on selected rats from the 0, 25,000, and 50,000 ppm dose groups. Measurements were made of both specific gravity and urine volume over a 6-hour water deprivation period, during a 2-hour period after a 25 mL/kg water load, and then during a 4-hour period beginning 16 hours after the water load. At necropsy, brain, heart, liver, spleen, kidneys, adrenals, gonads, stomach, small intestine, and cecum were weighed. Samples of these organs, the following organs, and any tissue that appeared abnormal were preserved in 10% buffered formalin: salivary gland, trachea, aorta, thymus, lymph nodes, pituitary, urinary bladder, colon, rectum, pancreas, uterus, and muscle.

In the 2-year study, the mean daily intakes of propylene glycol were approximately 0, 0.2, 0.4, 0.9, and 1.7 g/kg in males and 0, 0.3, 0.5, 1.0, and 2.1 g/kg in females for the 0, 6,250, 12,500, 25,000, or 50,000 ppm propylene glycol dose groups, respectively. **[The authors did not provide daily food consumption or bi-monthly animal weight data.]** No abnormalities were observed among groups in deaths, behavior, or food consumption. The authors reported no significant differences between the

control and treated groups with respect to blood chemistry or renal concentration tests. Organ weights (including gonads) and organ weights relative to terminal body weight were similar between control and treated groups. Incidences of histological findings and the incidence of neoplasms in various tissues were presented, but the tabulated data did not include reproductive organs. Abnormalities cited were similar for the control and treated groups. The authors noted that the changes observed were consistent with those of aging rats and concluded that a "no-untoward-effect level" found in this study was 2.1 g/kg for male rats and 1.7 g/kg for female rats **[highest dose used]**.

Strength/Weaknesses: Gaunt et al. *(81)* is a well-conducted carcinogenicity bioassay which clearly demonstrates that an average daily dose of 1.7 g/kg in male rats and an average daily dose of 2.1g/kg in female rats had no adverse effect (NOAEL) on body weight gain, mortality, hematology, urinary cell excretion, renal function, serum chemistry, or absolute and relative organ weights. The histopathological changes were consistent with those expected in aging rats. No malignancy could be attributed to treatment. Although reference is made in the text to "no statistically significant differences," it is not stated what statistical methods were used. However, the reputation of the British Industrial Biological Research Association (BIBRA) and of the authors of this paper lend credibility to the statement. It is unfortunate that a higher dose was not used, because as conducted, the Panel did not learn anything about the chronic toxicity in rats, only about propylene glycol's safety. Up to 78 weeks there is no discernible effect on body weight but thereafter, there might have been a slight body weight effect. Unfortunately, no standard error is given and mortality was high in all groups, which was at least partially due to a high rate of pulmonary infection.

Utility (Adequacy) for CERHR Evaluation Process: This study by Gaunt et al. *(81)* establishes a highly credible NOAEL for propylene glycol in terms of chronic toxicity in both male and female rats. This information could be very useful when evaluating reproductive/developmental toxicity (i.e., a maternal NOAEL).

Propylene glycol administered in the drinking water of rats at doses >13.2 g/kg bw/day for 140 days resulted in CNS depression and minor liver injury (reviewed by Mortensen *(72)* and LaKind et al. *(28)*). In a 2-year drinking water study in rats (dosed up to1.834 g/kg bw/day), no renal pathology and very slight liver damage was found *(28)*.

The Seidenfeld and Hanzlik *(82)* paper predates all other publications thus far evaluated. It includes detailed observation of the animals. A mix of acute and subchronic studies was conducted in rats and rabbits. Acute studies provided the dose ranges for the later, more detailed experiments of Braun and Cartland *(79)* and Weatherby and Haag *(78)*. **[The Panel notes that even though the style of the Seidenfeld and Hanzlik publication may appear outdated, the data seem reliable. In fact, the dose x time product for slight vacuolization of the liver is 1,862g x day in this study and 2,160g x day in the Morris et al. *(80)* report. Thus, it can be concluded that slight hepatic injury could be expected in rats at a daily intake of 2 g/kg bw of propylene glycol. The study by Seidenfeld and Hanzlik is useful because now the Morris et al. *(80)* report can be viewed as confirmatory evidence for the slight liver damage as a high dose effect.]**

Utility (Adequacy) for CERHR Evaluation Process: This study is useful because now the Morris et al. *(80)* report can be viewed as confirmatory evidence for the slight liver damage as a high dose effect.

Propylene glycol was fed to dogs as a carbohydrate source in the diet at a concentration of 8% (2 g/kg bw/day) and 20% (5 g/kg bw/day) for 2 years; a control group was fed an equal caloric amount of dextrose and a second control group did not receive the dextrose. No adverse effects were observed in the low-dose group. In the high-dose group, there was evidence of RBC destruction (packed cell volume and hemoglobin values were lower and reticulocytes were higher than control values). There were no differences in kidney weights compared to the control group and no other indications of toxicity *(67, 83)*.

Strength/Weaknesses: Weil et al. *(83)* studied the toxicity of propylene glycol in beagle dogs fed in the diet at 2 and 5 g/kg bw/day for 2 years. A roughly isocaloric diet to the propylene glycol containing dextrose was fed to a positive control group. After appropriate statistical evaluation, the authors concluded that 5 g/kg bw/day of propylene glycol in the diet resulted in enhanced erythrocyte destruction with signs of increased erythropoiesis. Use of a positive control group was useful to identify this effect as caused by propylene glycol. The NOAEL for chronic toxicity in dogs (2 g/kg bw/day) was essentially identical to the rat NOAEL.

Utility (Adequacy) for CERHR Evaluation Process: This paper is very useful because it has a dose that was actually toxic, which allows judgement of the ratio between LOAEL and NOAEL.

No effects were found on the kidneys in studies by VanWinkle and Newman *(84)* in dogs. Female dogs were administered 5% propylene glycol in drinking water two times a day for up to 9 months; male dogs were allowed to drink 600 mL of 10% propylene glycol daily. Kidney function was measured by phenosulfonphthalein excretion and liver function by rose bengal in the blood and galactose and uric acid in the urine. No pathological changes were found in these organs *(28)*.

Strength/Weaknesses: In these experiments *(84)*, liver and kidney function of dogs provided drinking water containing 5% propylene glycol (5 .1 cm^3=5.3 g/kg body weight) were determined and found not to be effected. However, dogs given water with 10% propylene glycol died and those provided with 10% propylene glycol containing water in the morning and clean water in the evening showed impaired renal function as indicated by increased blood urea. Authors stated that control values ranged from 14 to 24 mg% and after drinking the glycol for 6 months the range was 12–33 mg%. Statistical analysis was not performed and if it had been, it certainly would have shown no difference. There are no hematology measurements.

Utility (Adequacy) for CERHR Evaluation Process: The studies of Van Winkle and Newman *(84)* may be considered inadequate by today's standards, but they still provide useful data as confirmatory evidence for the NOAEL of 2 g/kg bw/day established by Weil et al. *(83)* in dogs.

Table 2-4. Summary of Toxicity of Propylene Glycol in Experimental Animals (data from OECD (27) and ATSDR (4))

Species	*Route*	*Dose/Duration*	*Findings (g/kg bw/day)*	*Study*
Rat	Oral	1%–50% in drinking water for 140 d	NOAEL 13.2 (equiv to 10% in water)	Seidenfeld and Hanzlik (*82*)
	Oral	0.625%–5% in feed for 103 wk	NOAEL 1.70 (m) NOAEL 2.10 (f) (equiv to 5% in feed)	Gaunt et al. (*81*)
	Inhalation	321 ppm for 90 d	Enlarged goblet cells/ thickened tracheal epithelium	Suber et al. (*85*)
	Inhalation	0.17–0.35 mg/L for 18 months continuous exposure	LOAEL 112 ppm (50% increase in body weight)	Robertson (*86*)
Rabbit	Dermal	0.52 g/one time (~0.17 g/kg bw)	Neat material not irritating	Clark et al. (*87*)
	Inhalation	10% for 20 min or 120 min	Increased degenerated goblet cells @ 20 min and 120 min	Konradova et al. (*88*)
Monkey	Inhalation	32–112 ppm 13 months	LOAEL 112 ppm	Robertson (*86*)
Cat	Oral	0.080–4.24 g/kg bw/day in feed for 2–3 months	LOAEL 0.424 NOAEL 0.080 (Heinz body formation)	Reviewed by OECD (*27*)
	Oral	6 or 12% in feed for 117 d	LOAEL 0.741–1.60 (Heinz body formation) NOAEL < 0.741–1.60	Bauer et al. (*89*)
	Oral	1.6 g/kg bw/day for 5 wks or 8.0 g/kg bw/day for 22 d	Low dose, anion gap; high dose polyuria/polydipsia, ataxia, depression	Christopher et al. (*39*)
Dog	Oral	8 or 20% in feed for 104 wks	LOAEL 5.00 (equiv 20% feed) (anemia) NOAEL 2.00 (equiv 8% feed)	Weil et al. (*83*)

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2.2.2.2 Dermal Exposure

Propylene glycol was tested on the clipped skin of NZW rabbits according to three protocols (the cosmetic protocol, the Association Francaise de Normalization protocol, and the OECD protocol); in all three tests, propylene glycol was classified as a nonirritant *(28)*.

Strength/Weaknesses: Irritation potential of propylene glycol, although minimal, has been established in man.

Utility (Adequacy) for CERHR Evaluation Process: None.

2.2.2.3 Inhalation Exposure

The ATSDR review *(4)* states that studies available on inhalation exposure of animals to propylene glycol are inconclusive. An acute inhalation study with 10% propylene glycol **[mg/L not stated]** for 20 or 120 minutes in rabbits resulted in degenerated goblet cells in the trachea *(88)*. However, a subchronic exposure study in rats *(85)* did not support these findings. Rats exposed to 321 ppm over 90 days had thickened respiratory epithelium and enlarged goblet cells *(85)*. Monkeys (n=29) and rats **[number not specified]** were continuously exposed to propylene glycol vapor at doses of 32–113 ppm for 13 months. At 113 ppm, hemoglobin levels were slightly increased; there were no adverse effects noted on body weight or on the renal, respiratory, gastrointestinal, hepatic, or endocrine systems *(4)*.

Strength/Weaknesses: Konradova et al. *(88)* demonstrated that a 10% propylene glycol mist inhaled by rabbits resulted in enhanced mucolytic activity (+69%) of respiratory goblet cells. This is not surprising from a surface tension lowering agent. In fact, the effect of pure propylene glycol was less pronounced than that of clinically used mucolytics (Broncholysin, Histabron). Other conclusions regarding ciliated cells are difficult to assess because of the smallness of the effect. Moreover, a much more thorough study of inhalation of a propylene glycol aerosol did not confirm these findings *(85)*.

Utility (Adequacy) for CERHR Evaluation Process: None

The Suber et al. *(85)* paper appears to be a well-conducted subchronic, nose-only inhalation study by a contract laboratory. Nominal doses were 0.0, 0.16, 1.0, and 2.2 mg/L of propylene glycol with an air flow rate of 1.0–1.5 L/min to each animal. Absorption was not determined, but system toxicity could not be expected even if 100% of the highest dose had been absorbed. As is clear in Bau et al. *(38)*, only a fraction of inhaled propylene glycol will be absorbed into the systemic circulation through the lungs. Nasal hemorrhage is compatible with the known irritation potential of propylene glycol. Goblet cell score was significantly increased in the nasal turbinates, which is plausible for a surface-active agent facilitating the discharge of mucous from the swollen goblet cells.

Utility (Adequacy) for CERHR Evaluation Process: This is a useful study *(85)* that confirms the view arrived at for kinetic reasons that exposure by inhalation to propylene glycol does not pose a significant toxicological problem.

Robertson et al. *(86)* examined the chronic toxicity of propylene glycol by inhalation in Rhesus monkeys and rats. This is a very interesting study because both rats and monkeys were exposed

continuously to saturated/supersaturated air of propylene glycol (55–113 ppm) for up to 1 year. At the highest dose, hemoglobin levels seemed to have increased. However, since no standard error is given and no statistical analysis was performed, it is uncertain whether this is a real effect. Otherwise, no adverse effects were found in spite of extensive gross and histopathologic examination. In fact, both rats and monkeys inhaling propylene glycol gained more weight than the controls. The health status of monkeys was poor, which was not uncommon in 1947. Assuming Rhesus monkeys inhale about 2 m^3 of air per day, the data indicate that primates may safely inhale about 1 g of propylene glycol per day. Although this paper uses unusual reporting methods by today's conventions, it certainly appears reliable and interpretable.

Utility (Adequacy) for CERHR Evaluation Process: Continuous exposure to propylene glycol vapor (without vehicle) in a primate species provides important evidence.

2.2.2.4 Hematological effects

Results from animal studies indicate that intermediate and chronic exposure to propylene glycol may lead to hemolysis of RBCs. After a 90-day inhalation exposure to 321 ppm of propylene glycol, female rats had decreased white blood cell count, while exposure to 707 ppm of propylene glycol decreased hemoglobin concentrations. No dose-related changes in RBCs were observed in male rats *(85)*. After exposure of rats to 5% propylene glycol in the diet for 2 years, there were no hematological effects noted *(81)*. However, Saini et al. *(90)* [reviewed by OECD *(27)*] found that a single oral dose of either 0.73 or 2.94 g/kg bw given to female Wistar rats, produced a reversible, statistically significant decrease in hemoglobin, packed cell volume, and RBC counts for 2 days. Electron microscopy revealed a rough RBC surface. However, in an early study by Robertson et al. *(86)*, Rhesus monkeys continuously exposed to concentrations of propylene glycol in air up to 112 ppm for 13 months had a slightly greater increase **[statistical analyses not reported]** in RBCs and hemoglobin content than the control animals.

Cats exposed to oral administration of propylene glycol developed Heinz bodies in RBCs and experienced decreased RBC survival *(89, 91)*. Heinz bodies are composed of denatured proteins, primarily hemoglobin. Cats exposed orally to 1.2, 1.6, 2.4, and 3.6 g/kg bw/day of propylene glycol for 2, 5, or 17 weeks developed increased numbers of RBCs with Heinz bodies. The cat is very sensitive to propylene glycol toxicity, with a 0.44 mg/kg bw/day dose reported to result in Heinz body formation in erythrocytes (reviewed by OECD *(27)*). This sensitivity occurs at concentrations that were present in soft moist cat foods and lead the FDA to remove propylene glycol from cat foods in 1996 *(9)*.

In a study by Weil et al. *(83)* dogs were fed propylene glycol at 2 and 5 g/kg bw/day through the diet. Significant hematological changes were noted in the high dose group after two years; hemoglobin, hematocrit, and total erythrocyte counts were lower, whereas, poikilocytes and reticulocytes were increased.

Strength/Weaknesses: There are few and inconsistent changes in hematologic parameters in the Suber et al. *(85)* study. No inferences can be made for erythropoiesis.

Utility (Adequacy) for CERHR Evaluation Process: None

Strength/Weaknesses: Saini et al. *(90)* reported hematologic effects of propylene glycol in rats

administered single doses of 0.7 or 3 g/kg bw by gavage. There is sufficient experimental detail given to deem the results reliable. However, Gaunt et al. *(81)* did not find any hematologic effect after feeding about 2 g/kg bw/day for 2 years. It is very likely that the acute changes seen by Saini et al. *(90)* have been overcome by 2 years due to adaptation.

Utility (Adequacy) for CERHR Evaluation Process: This is a useful report *(90)* that confirms that the hematopoietic system is also a target of propylene glycol in rats, albeit at higher chronic doses than in cats, dogs, and probably monkeys.
Strength/Weaknesses: The Robertson et al. *(86)* study has a very large uncertainty attached to it, as discussed earlier, and provides marginal evidence of a hematologic effect in non-human primates.

Utility (Adequacy) for CERHR Evaluation Process: The hemolytic capability of propylene glycol has been demonstrated *in vitro* in human erythrocytes *(78)*. However, the primate data presented by Robertson et al. *(86)* do not provide evidence of a hematological effect of propylene glycol on primates.

Strength/Weaknesses: Christopher et al. *(91)* reported D-lactic acidosis and Heinz body formation in cats administered daily 1.6 or 8 g/kg propylene glycol for up to 35 days. Authors conclusively demonstrated a dose-dependent reduction of erythrocyte survival. Bauer et al. *(89)* confirms in essence the findings of Christopher et al. *(91)* and refines the dose response on Heinz body formation and erythrocyte survival.

Utility (Adequacy) for CERHR Evaluation Process: Christopher et al. *(91)* provide an excellent study that establishes a plausible mechanism for propylene glycol-induced hemolysis and the Bauer et al. *(89)* study provides important confirmatory evidence for the impairment of hematopoiesis by propylene glycol. Thus, the hemolysis potential of high doses of propylene glycol, which is a plausible effect, is firmly established in two species (cat and dog) and reasonably well substantiated in other species including man.

2.3 Genetic Toxicity

2.3.1 Humans

No studies were located regarding *in vivo* genotoxic effects in humans or animals.

2.3.2 Experimental systems

2.3.2.1 In Vitro

ATSDR *(4)* states that "Propylene glycol was not mutagenic in *S. typhimurium* strains TA 98, TA100, TA1535, TA1537, and TA1538 with and without metabolic activation. Propylene glycol was negative for sister chromatid exchange and changes in alkaline elution rate using Chinese hamster cells or human fibroblasts" (Table 2-5).

Table 2-5. Genotoxicity of Propylene Glycol In Vitro (from ATSDR (4))

Species (test system)	*Endpoint*	*Results with activation*	*Results without activation*	*Reference*
Prokaryotic organisms:				
S. typhimurium	Gene mutation	Negative	Negative	Clark et al. (87)
S. typhimurium	Gene mutation	Negative	Negative	Pfeiffer and Dunkelberg (92)
Mammalian cells:				
Human fibroblasts	Chromosome aberrations	Negative	Negative	Tucker et al. (93)
Chinese hamster cells	Chromosome aberrations	Negative	Negative	Tucker et al. (93)
Chinese hamster lung cells	DNA damage	Negative	Negative	Swenberg et al. (94)

Propylene glycol was one of a number of chemicals evaluated for mutagenicity in a study of chemicals used and formed after the fumigation of foodstuffs *(92)*. A modified Ames test used histidine-dependent *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537. Propylene glycol (98 % purity, diluted in water, test volume, 0.1 mL) was added to 2 mL distilled water and 0.1 mL (10^8) bacteria. This mixture was added to 2 mL Topagar and poured into a Petri dish containing histidine-free agar, incubated for 48 hours at 37°C, and revertant colonies counted. Liver microsomes were not incorporated into the test mixture. The authors concluded that propylene glycol, as well as ethylene glycol and diethylene glycol, showed no mutagenic activity with any of the four *Salmonella* strains **[data not shown by authors]**. All experiments were performed 6–10 times **[controls and statistics are not described].**

Strength/Weaknesses: Pfeiffer and Dunkelberg *(92)* studied mutagenicity of ethylene oxides, propylene oxide, various halo-alcohols, and several glycols. The test systems used were those normally used for *S. typhimurium* strains TA98, T4100, TA1535, and TA1537 without metabolic activation. The reaction mixture was modified to accommodate the low water solubility of ethylene oxide and propylene oxide. As expected, the epoxides gave strong positive results, the halo-alcohols variable responses, and the glycols were uniformly negative. There are no weaknesses apparent in these experiments.

Utility (Adequacy) for CERHR Evaluation Process: This study *(92)* provides experimental confirmation of the expected and the plausible.

Propylene glycol was one of the chemicals evaluated by Swenberg et al. *(94)* using an *in vitro* assay to assess DNA damage and predict carcinogenic potential. Chinese hamster lung fibroblast (V79) cells were grown in tissue culture to which radioactive thymidine was added for 20–24 hours, then the radioactivity was removed and the cells were incubated for 4–20 hours in a non-radioactive medium. Cells were then exposed to test chemicals for up to 4 hours with or without the presence of a liver microsomal enzyme activation system (S-9). Cell viability was assessed by measurement of cellular ATP levels. DNA damage was measured by an increase in elution rate under alkaline conditions of single-stranded fibroblast DNA from polyvinyl filters. Propylene glycol exposure for 1, 2, or 4 hours

with or without a rat microsomal activation system did not cause a significant increase in the elution rate from that of non-treated cells **[statistical method not described or referenced]**.

Strength/Weaknesses: Clastogenicity of a large number of compounds was tested by an *in vitro/* alkaline DNA elution assay *(94)*. The complete lack of experimental detail regarding propylene glycol diminishes its value.

Utility (Adequacy) for CERHR Evaluation Process: The study *(94)* is of very little use to CERHR, although it confirms the expected and the plausible.

Propylene glycol is listed as a chemical giving negative results in the sister chromatid exchange assay using normal human fibroblast cells. The highest concentration tested was 0.1 M *(93)*. **[Details of this assay were not given.]**

Strength/Weaknesses: Sister chromatid exchange was tested with a high number of chemicals as reviewed by Tucker et al. *(93)*. Propylene glycol was found to be negative in this test system.

Utility (Adequacy) for CERHR Evaluation Process: It is helpful to know that propylene glycol was negative in still another chromosomal test.

Propylene glycol was included in the primary mutagenicity screening of food additives used in Japan *(95)*. *Salmonella/*microsome tests (Ames tests) and chromosomal aberration tests using a Chinese hamster fibroblast cell line were performed. Propylene glycol (99% purity) was negative in the Ames test (dimethyl sulfoxide [DMSO] solvent, 32 mg/mL maximum non-cytotoxic dose) and positive in the chromosomal aberration test (maximum dose 32 mg/mL). A chemical is positive in the chromosomal aberration test if the total incidence of cells with aberrations is 10% or higher. For propylene glycol in saline, 38% of cells had aberrations after 48 hours and the incidence of polyploid cells was reported to be 1%. These results were not discussed further by the authors.

Strength/Weaknesses: A high number of food additives was screened for mutagenicity and clastogenicity *(95)*. The Ames test was conducted in the usual *S. typhimurium* strains and chromosomal aberrations were tested in a Chinese hamster fibroblast cell line. There is sufficient experimental detail to deem the results reliable. Once again, propylene glycol was negative in the Ames test but positive in the clastogenicity test.

Utility (Adequacy) for CERHR Evaluation Process: This study *(95)* is not useful because the biological significance of these *in vitro* data are unclear.

The FDA *(96)* submitted propylene glycol for mutagenic evaluation **[discussed in the *In Vivo* Section 2.3.2.2]**. Along with the *in vivo* assays, one *in vitro* cytogenetics study was performed. WI-38 cells (human embryonic lung cells) were exposed to concentrations of propylene glycol at 0.001, 0.01, and 0.1 μg/mL. Concentrations of 0.1 μg/mL resulted in complete destruction of the cells. A negative control of saline and a positive control of 0.1 μg/mL triethylene melamine were used. The authors concluded that propylene glycol produced no significant aberrations in the anaphase **[sic]** chromosomes of the cells at the dosage levels employed in this study.

Strength/Weaknesses: This is a comprehensive evaluation of the mutagenicity of propylene glycol *in vitro* and *in vivo (96)*. There is sufficient experimental detail to satisfy doubts that propylene glycol is neither mutagenic nor clastogenic.

Utility (Adequacy) for CERHR Evaluation Process: The study *(96)* confirms the expected and is plausible.

2.3.2.2 In Vivo

Propylene glycol was tested using the mouse micronucleus test with 38 other food additives *(97)*. The micronucleus test was conducted in 8-week-old ddY mice (6/dose group). Animals were dosed by intraperitoneal (IP) injection, once/day for 5 days with propylene glycol. Femoral marrow cells were flushed with fetal bovine serum. Slides were fixed in methanol and stained with Giemsa. Preparations were coded so that the scorer was not aware of the treatment. One thousand polychromatic erythrocytes (PCE) per mouse were scored under 100x power and the number of micronucleated polychromatic erythrocytes (MNPCE) was recorded. Results were compared with control groups and historical negative control groups. The frequency of MNPCEs in each treatment group was compared with the binomial distribution specified by historical control data from that laboratory. Dose-response relationships were tested by the Cochran-Armitage trend test. A positive result was recorded when one or more treatment groups showed a statistically significant difference ($P < 0.01$). Dose groups and results with propylene glycol are given in Table 2-6 below. Test results were negative.

Table 2-6. Results of the Micronucleus Test Using Mouse Bone Marrow Cells (97)

Propylene glycol, saline, IP	*MNPCEs (%)*	*PCEs (%)*	*Mortality*	*Trend Test*
0 mg/kg bw	0.20±0.19	43.9±12.2	0/6	NS*
2,500	0.20±0.18	53.6±9.2	0/6	
5,000	0.17±0.10	52.8±6.3	0/6	
10,000	Mortality	Mortality	6/6	

*NS: non-significant

Strength/Weaknesses: Propylene glycol was negative in the micronucleus test *(97)*. A wide dose range (2.5–15.0 g/kg bw) was used, which covered the whole spectrum of effects including 50% mortality at the highest dose. The study was conducted blind and analyzed by appropriate statistics. Chemicals expected to have a positive response did indeed show a statistically significant increase in micronuclei. There are no apparent weaknesses to this study.

Utility (Adequacy) for CERHR Evaluation Process: This study *(97)* provides *in vivo* confirmation for the lack of clastogenicity of propylene glycol.

The FDA submitted propylene glycol for mutagenic evaluation *(96)* in three genotoxicity test systems: host mediated assay, dominant lethal assay, and *in vivo* cytogenetic studies. The three *in vivo* assays are discussed below (Table 2-7) and the *in vitro* cytogenetics study is discussed in the *In Vitro* Section 2.3.2.1.

In the host-mediated assay (*in vivo*, mice), doses of propylene glycol at 30, 2,500, and 5,000 mg/kg bw

and negative control of saline, and positive controls of 350 mg/kg bw ethyl methane sulfonate and 100 mg/kg bw dimethyl nitrosamine were tested. Acute studies (1 dose by gavage of chemical, followed by IP injection with *S. typhimurium* 30 min after dosing) produced no significant increases in mutation frequencies with *Salmonella* TA1530 and with all levels of *Salmonella* G46, except the 5,000 mg/kg bw level, which produced a weak questionable positive response. *Saccharomyces* D3 showed increased recombinant frequencies at all levels except the acute high dose. Subacute studies (dosing once/day by gavage for 5 days, inoculating IP 30 minutes after last dose) produced increased recombinant frequencies at all levels. While some statistically-significant differences were noted in the mid- and high-dose animals from both phases of the investigation, comparison with historic data demonstrated that this was a consequence of unrepresentative low control data rather than a substance-specific effect. Therefore the authors concluded that propylene glycol has no capacity to induce mutations.

For the dominant lethal assay (*in vivo*, rats), propylene glycol was administered by gavage at 30, 2,500, and 5,000 mg/kg bw and a negative control of saline and a positive control of 0.3 mg/kg bw triethylene melamine were tested. Propylene glycol was considered non-mutagenic in rats in this assay at these doses.

For cytogenetics studies (*in vivo*, rats), propylene glycol was administered by gavage at 30, 2,500, and 5,000 mg/kg bw, and a negative control of saline and a positive control of 0.3 mg/kg bw triethylene melamine were tested. Propylene glycol produced no significant increases in aberrations of bone marrow cells when administered orally at these doses.

Appendix

Table 2-7. In Vivo Genotoxicity Results (96)

Assay	*Dose of Propylene Glycol*	*Endpoint*	*Result*
Host Mediated Assay, mice	30, 2,500, 5,000 mg/kg bw	Increase in mutation frequencies: *Salmonella* TA1530 and G46 *Saccharomyces* D3	Negative
Dominant Lethal Assay, male rats treated	30, 2,500, 5,000 mg/kg bw	Increase in % dead implants in pregnant, untreated female	Negative
Cytogenetics studies, rats	30, 2,500, 5,000 mg/kg bw	Chromosome aberrations (bone marrow)	Negative

Strength/Weaknesses: The Litton Bionetics, Inc. *(96)* report is a detailed and comprehensive *in vitro* and *in vivo* evaluation of propylene glycol for genotoxicity. There are no apparent weaknesses in this report.

Utility (Adequacy) for CERHR Evaluation Process: The data in this report *(96)* demonstrate propylene glycol's lack of genotoxicity.

2.4 Carcinogenicity

2.4.1 Human Data

No data on carcinogenicity in humans were identified

2.4.2 Experimental Animal Data

2.4.2.1 Oral Exposure

In a long-term dietary toxicity study in rats by Gaunt et al. *(81)* **[see Section 2.2.2.1, General Toxicity]**, rats were fed propylene glycol up to 5% (2,500 mg/kg bw/day) in their diet for 103 weeks. Death rate, body weight gain, food consumption, hematology, and renal clearance were monitored. No significant differences were noted between control and treated rats for the parameters examined. There were no treatment-related increases in neoplasms.

Charles River CD rats from a SPF breeding colony were used in this study. At the start of the study, the weight range of males was 120–150 g and of females was 120–140 g. **[Statistical methods were not described and standard errors for treatment groups were not presented.]** In a 2-year study, the mean daily intakes of propylene glycol were approximately 0, 0.2, 0.4, 0.9, and 1.7 g/kg bw in males and 0, 0.3, 0.5, 1.0, and 2.1 g/kg bw in females for the 0, 6,250, 12,500, 25,000, and 50,000 ppm propylene glycol dose groups, respectively. **[The authors did not provide daily food consumption or bi-monthly animal weight data.]** No abnormalities were observed among groups in deaths, behavior, or food consumption. The authors reported no significant differences between the control and treated groups with respect to blood chemistry or renal concentration tests. Organ weights (including gonads) and organ weights relative to terminal body weight were similar between control and treated groups. Necropsy at the end of the study included gross and microscopic examination of the male and female reproductive tracts. Incidences of histological findings and the incidence of neoplasms in various tissues were presented, but the tabulated data did not include reproductive organs. Abnormalities cited were similar for the control and treated groups. The authors noted that the changes observed were consistent with those of aging rats and concluded that a "no-untoward-effect level" found in this study was 2.1 g/kg bw for male rats and 1.7 g/kg bw for female rats **[highest dose used].**

Strength/Weaknesses: Gaunt et al. *(81)* reported on a state-of-the-art carcinogenicity bioassay (four different doses) with propylene glycol. Average body weights of males were about 12% and those of females about 10% below controls, in the highest dose groups, although there is no statistical analysis of the data to know for sure if these are real differences. There were no treatment-related malignancies.

Utility (Adequacy) for CERHR Evaluation Process: It is clear that propylene glycol does not cause cancer at or near a toxic level administered in the diet.

2.4.2.2 Dermal Exposure

In skin-painting studies, Stenback and Shubik *(98)* examined the potential carcinogenicity and toxicity of several commonly used cutaneous agents including propylene glycol. Seven-week-old female Swiss mice (50/concentration) were treated with 10, 50, and 100% propylene glycol in acetone over the lifetime of the animal. Propylene glycol (0.02 mL) was dropped onto the shaved dorsum (1-inch square area) twice a week. Animals were allowed to die naturally or were sacrificed moribund. Complete necropsies were performed and all tumors were examined histologically. The skin tumor incidence seen in the treated animals (2–4%) was comparable to the values obtained with acetone controls (50 animals) and with untreated animals (135 animals). DMBA (10 μg 2 times/wk) treatment (positive control, 50 animals) resulted in a 78% skin tumor incidence. The method of statistical evaluation was cited but not

described in the text by the authors. The authors concluded that there was no increase in dermal tumors or change in longevity in female Swiss mice after chronic treatment with propylene glycol.

Strength/Weaknesses: Stenback and Shubik *(98)* conducted a skin-painting experiment with, among other chemicals, propylene glycol. A uniform protocol was followed, which is problematic for compounds as different in their kinetics and dynamics as propylene glycol and DMBA. The dose was 0.02 mL pure propylene glycol or 50 and 10% solutions in acetone twice a week. It is in agreement with propylene glycol's low irritation potential that there were no skin tumors in treated mice, although this strain of mice (Swiss females) is exquisitely sensitive to the induction of skin tumors. The highest dose translates to approximately 0.8 g/kg bw twice a week. Systemic effects would not be expected from this dose rate even if absorption was 100%.

Utility (Adequacy) for CERHR Evaluation Process: This is a useful study *(98)*, with highly predictable outcomes.

2.5 Potentially Sensitive Subpopulations

A few case reports have been published suggesting exacerbation of clinical signs in hospitalized individuals as a result of propylene glycol. These cases are primarily associated with individuals with compromised liver or kidney function, with burn patients, and with overdosage of premature infants. As discussed in Section 2.1.4, after absorption, the kidneys eliminate 45% of propylene glycol with the remainder metabolized by the liver to lactic acid, pyruvic acid, or acetone. Therefore, patients with impaired liver or kidney function would be at increased risk for developing propylene glycol toxicity *(48)*. In patients with renal insufficiency, high propylene glycol levels have been associated with lactic acidosis (hyperlactemia) *(99, 100)*. Propylene glycol has been found in the blood of alcoholics with cirrhosis of the liver without detectable measurable blood alcohol levels *(101)*.

Propylene glycol toxicity has been suspected in patients with an abnormal serum osmolal gap[1]. In some cases, a review of the patient's medication history identified propylene glycol as a vehicle in the medications administered. The following information has been taken primarily from data presented in complex clinical case studies the cause of medical symptoms observed undetermined. Some examples of clinical cases of suspected propylene glycol toxicity are summarized in Table 2-8 below.

2.5.1 Oral and Intravenous Use

Oral or IV administration of propylene glycol may exacerbate dermatitis in some individuals *(102)*.

Propylene glycol is used as a vehicle for IV administration of drugs such as lorazepam, etomidate, phenytoin, diazepam, digoxin, hydralazine, esmolol, chlordiazepoxide, nitroglycerin, pentobarbital sodium, phenobarbital sodium, and trimethoprim-sulfamethoxazole and is a vehicle for some IV vitamin preparations. Serum concentrations of propylene glycol received through IV medications have been shown to correlate with serum lactate concentrations in patients with normal renal and hepatic function *(51)*. In children, seizures and respiratory depression have occurred after taking liquid medications containing propylene glycol *(103, 104)*.

[1] osmolal gap = measured serum osmolality − calculated serum osmolality; normal gap < 10; calculated osmolality = $2[Na^+]$ + glucose/20 + BUN/3 ; an increased osmolal gap can be indicative of increased solute in the blood.

Table 2-8. Some Clinical Complications Associated with Propylene Glycol (PG) Use

Patient; Reference	*Route*	*Findings*
8-month-old male infant; Fligner (*35*)	Dermal; silver sulfadiazine therapy in propylene glycol for burns, 78% surface area, 10.6 g/L **[139.5 mM]** PG serum level	Cardiopulmonary arrest, respiratory acidosis, increased osmolal gap
3.4 kg infant, cardiac surgery, heart failure; Huggon (*70*)	IV, PG vehicle in enoximone and glyceryl trinitrate infusions	Hyperosmolality
Premature infants; MacDonald (*106*)	IV, propylene glycol as part of a daily multivitamin preparation, 3g/ day PG (alternative product delivering 0.3g/day PG had no effect on other premature infants)	Seizures
Premature infant, 27 wk gestation; Glasgow (*36*)	IV, propylene glycol as part of a daily multivitamin preparation, 9.3 g/L **[122.4 mM]** PG serum level	Serum hyperosmolality, acute renal failure
11-year-old boy, candidiasis-endocrinopathy syndrome with hypoparathyroidism; Arulanantham (*104*)	Oral, PG vehicle in dihydrotachysterol	Seizures
16-year-old boy, onset of seizures; Yorgin et al. (*107*)	IV, PG vehicle in pentobarbital and phenobarbital	Exacerbation of seizures, reversible acute renal failure
39-year-old woman, history of seizures; Lolin (*108*)	Most likely ingestion, 4 g/L **[52.6 mM]** PG serum level	Status epilepticus, metabolic acidosis, plasma hyperosmolality, respiratory depression
45-year-old man, respiratory distress, on ventilator; Arbour (*109*)	IV, PG vehicle in lorazepam, 1.7 g/L **[22.4 mM]** PG serum level	Hyperosmolality, metabolic acidosis
58-year-old man, renal disease, chronic schizophrenia; Cate (*68*)	Most likely ingestion, 0.7 g/L **[9.21 mM]** PG serum level	Unconscious, lactic acidosis, azotemia
60-year-old man, respiratory distress, on ventilator; Arbour (*48*)	IV, PG vehicle in lorazepam, infused for 5 d at 2.5 g PG/hr	Hyperosmolality
70-year-old woman, complications with surgery; Bedichek & Kirschbaum (*110*)	IV, 479 g PG administered with etomidate and other medications over a 24 hr period	Seizures, status epilepticus

2.5.2 Infants

The decreased size of premature infants and an increased serum half-life **[see Section 2.1.4.1]** for propylene glycol in premature infants *(35, 36)* predispose them to a greater probability of toxic effects from over administration of propylene glycol. There is particular concern for very small infants and those receiving multiple IV medications containing propylene glycol. Absorption of propylene glycol from ointments applied to burns and injection of multivitamin products in infants has resulted in

serum hyperosmolality *(36, 70)*, which was associated with cardiorespiratory arrest in one case *(70)*.

In one report, propylene glycol was shown to have a longer (16.9 h) half-life in a premature infant when compared with the half-life in adults (5 h) *(35)*. Glasgow *(36)* measured the serum half-life in infants. Ten infants received 10 mL IV of daily multivitamin preparation (containing 30% propylene glycol) once a day for at least 5 days. Four infants had a serum level >3.0 g/L **[39.5mM]** propylene glycol. The range of serum propylene glycol values was 0.65–9.5 g/L **[8.55–125mM]**. In the control group, propylene glycol was not detected in six infants; two other infants had propylene glycol serum levels of 0.7g/L **[9.21mM]**. The propylene glycol levels in the serum of the control infants were attributed to Mycostatin cream usage for diaper rash and phenobarbital therapy. Thirty-six hours later, serum levels were taken. The mean half-life in these infants was calculated to be 19.3 hours with a range of 10.8–30.5 hours.

Propylene glycol serum concentration, serum lactate, and osmolar gap were measured in 11 intubated pediatric intensive care patients [1–15-month-old, 6 females] on continuous lorazepam infusion *(66)*. Differences in propylene glycol concentration, serum lactate concentration, and osmolar gap at the beginning of therapy, 48 hours into therapy, and at the end of therapy were compared using repeated measures analysis of variance. Lorazepam infusion rates ranged from 0.1 to 0.33 mg/kg bw/hour and lasted 3–14 days. All patients in this study had normal renal function. At the end of therapy, serum levels of propylene glycol ranged from approximately 0.2 to 2 mg/mL. A significant correlation between the cumulative dose of lorazepam and propylene glycol serum concentration at the end of therapy was demonstrated ($p<0.005$). However, propylene glycol accumulation was not associated with an elevation in serum lactate concentrations or osmolar gap. The authors caution that although "continuous lorazepam infusion seems to be a safe option for sedating patients with normal renal function in the pediatric intensive care unit, it would be prudent to monitor for lactic acidosis and hyperosmolality... ".

Propylene glycol is commonly used as a vehicle in topical, oral, or injectable medications *(16)*. The American Academy of Pediatrics recommends mandatory labeling of inactive ingredients **[classified by the FDA as pharmaceutical excipients]** for all prescription and over-the-counter products *(105)*. At present, labeling is voluntary for prescription drugs, and since 1998, is required for over-the-counter drugs. This requirement was a result of the FDA Modernization Act of 1997 *(8)*.

2.6 Summary

2.6.1 Toxicokinetics and Metabolism

The absorption, distribution, metabolism, and excretion of propylene glycol have been studied in humans, cats, rats, mice, and rabbits. The studies reviewed by the Panel identified no major differences between humans and animals in the toxicity of propylene glycol. Toxic effects of propylene glycol occur only at very high doses. The domestic cat is the most sensitive species to propylene glycol toxicity, producing Heinz body anemia in response to propylene glycol as an additive (at 6% w/w or above) to its diet. The toxicokinetic properties are very similar across species studied. A consideration in the selection of experimental species is the metabolism of D- and L-optical isomers. Commercial propylene glycol is a 1:1 D, L mixture of both stereoisomers, and species differences in the rate of metabolism and excretion of D- and L- forms of propylene glycol are noted by the Panel. However, due to incomplete time point sampling and a lack of quantitative numbers regarding fluxes through

the different pathways, it was not possible for the Panel to provide a complete description of the stereospecific metabolism of D, L propylene glycol in different species. However, there are sufficient data in humans to conclude that acute exposure to D-, L-propylene glycol can cause L-lactic acidosis (if the dose is very high) due to the more rapid biotransformation (ADH being the rate determining step) of L-propylene glycol to L-lactate. However, with subchronic/chronic exposure to propylene glycol, D-lactic acidosis occurs due to the accumulation of D-lactate. D-lactate is derived from the glyoxylase/GSH pathway and since it is a poor substrate for gluconeogenesis, there would be a greater accumulation of the D-lactate than L-lactate with chronic exposures.

Dermal absorption studies in humans have shown that absorption of propylene glycol through intact skin is very limited. However, once the dermal layers are disturbed (such as with burns or irritation), dermal absorption can be a significant source of exposure.

In humans, absorption of propylene glycol after oral exposure reached maximum plasma concentrations within 1 hour of dosing and the average serum half-life was estimated to be from 1 to 4 hours. From rectal absorption studies, the half-life of propylene glycol was determined to be 2.8 0.7 hours in adults and 2.6 ± 0.3 hours in children (5–12 years) *(31)*. The similarity in the half-life for adults and children in this age range is in agreement with alcohol dehydrogenase reaching adult levels by 5 years of age *(32)*. Glasgow et al. *(36)* reported an average half-life in 10 infants of 19.3 hours (range 10.8–30.5 hours), which is about 10 times longer than in adults. Alcohol dehydrogenase activity is up to ten times lower in infants *(32)* than in adults, providing an explanation for the prolonged half-life of propylene glycol in infants.

There are excellent data on the determination of the apparent volume of propylene glycol distribution in humans and animals; these data demonstrate that it distributes into total body water. In human studies, volumes of distribution were measured at 0.52 L/kg with oral dosing *(29)*, 0.77–0.79 L/kg with rectal exposure *(31)*, and approximately 0.55–0.94 L/kg with IV exposure *(30)*. Therefore, it can be concluded that propylene glycol will distribute into the water compartment of the placenta and fetus.

Since lactate distributes into total body water, the fetus will also experience the mother's metabolic acidosis if present and lactate would be present in breast milk. However, newborns and infants may be protected from metabolic acidosis after ingestion of propylene glycol due to a slower metabolic conversion to lactate.

Except for the amount entering the nasopharynx and being swallowed, under normal exposure conditions propylene glycol exposure by inhalation is not toxicologically relevant due to its low vapor pressure (0.07 mm Hg).

Total body clearance occurs by metabolic clearance and by renal excretion. Morshed et al. *(41)* provide evidence in the rat that the rate-determining step in the metabolic clearance of propylene glycol is NAD-dependent alcohol dehydrogenase. The Panel concludes from the data of Speth et al. *(30)* that humans clear propylene glycol similarly to rats and rabbits, but saturation of metabolic clearance occurs at lower doses in humans than in rats and rabbits. From the data of Speth et al. *(30)* and Yu et al. *(29)* the Panel determined that metabolic clearance follows a first-order process (up to doses of approximately 12 g/day) with a constant half-life of 1.6 ± 0.2 h (± SD). Beyond this dose, the

serum half-life becomes dose dependent (zero order process) with a serum half-life above 3 hours. Propylene glycol is converted to lactic acid by ADH and further to pyruvate, which provides energy through the Krebs cycle; lactate can be detoxified into glucose and stored as glycogen, providing other sources of energy *(47)*.

The Panel concluded that the toxicokinetic data for propylene glycol are sufficient for evaluating the potential for propylene glycol to pose a risk to human reproduction.

2.6.2 General Toxicity

Propylene glycol has very low systemic toxicity in experimental animals and very high doses are required to determine a toxic level *(4, 27, 28)*. CNS, hematologic, hyperosmotic, and cardiovascular effects have been noted in humans and animals and high serum concentrations of propylene glycol may result in lactic acidosis and hyperosmotic changes in the blood. Animals lethally intoxicated undergo CNS depression, narcosis, and respiratory arrest. In humans, a lethal oral dose has been estimated to be >15 g/kg for an adult *(2)*. Mortality has occurred in hospitalized infants after repeated exposure to propylene glycol in medication **(see Potentially Sensitive Subpopulations)**.

Acute oral toxicity has been well characterized in the rat, mouse, rabbit, dog, and guinea pig with LD_{50} values, 8–46 g/kg bw (See Table 2-3), reported at very high oral doses.

In a 2-year study by Gaunt et al. *(81)*, an average daily dose of 1.7 g/kg bw in male rats and 2.1 g/kg bw in female rats had no adverse effect on body weight gain, mortality, hematology, urinary cell excretion, renal function, serum chemistry, or absolute and relative organ weights. Weil et al. *(83)* studied the toxicity of propylene glycol fed in the diet to dogs at 2 and 5 g/kg bw/day for 2 years. No adverse effect was noted in the low-dose group; there was evidence of RBC destruction in the high-dose group. The Panel concluded that in assessing toxicity from chronic exposure, 2 g/kg bw/day is a NOAEL for dogs and rats; 5g/kg bw/day is a LOAEL for dogs.

In a continuous inhalation study, Robertson et al. *(86)* examined chronic toxicity of propylene glycol (55–113 ppm) in Rhesus monkeys and rats for up to 1 year. Both rats and monkeys inhaling propylene glycol gained more weight than the control group; no adverse effects were noted. The Panel estimates that the monkeys inhaled approximately 1 g of propylene glycol per day.

Results from animal studies indicate that intermediate and chronic exposure to propylene glycol may lead to changes in hematological parameters and hemolysis of RBCs. Cats exposed to oral administration of propylene glycol developed Heinz bodies in RBCs and decreased RBC survival. Doses as low as 0.424 g/kg bw/day have resulted in Heinz body formation in cat erythrocytes *(27)*. In a study in dogs fed 5 g/kg bw/day for 2 years *(83)*, evidence of RBC destruction was noted. The Panel concluded that there is sufficient data on the hemolytic potential of high doses of propylene glycol in the cat and dog, and limited substantiated data in other species, including humans.

The Panel concluded that there are sufficient data to characterize the acute and chronic toxicity of propylene glycol in laboratory animals, including non-human primates. In humans, information on toxicity is limited to medical case studies. However, because of the similarities in the toxicokinetic profile of propylene glycol across species, the toxicity data from the animal studies can be extrapolated

to human exposures.

2.6.3 Genetic Toxicity

No studies were located regarding *in vivo* genotoxic effects in humans. Propylene glycol was consistently negative in *in vitro* and *in vivo* animal tests.

2.6.4 Carcinogenicity

No data on carcinogenicity in humans were identified.

Gaunt et al. *(81)* reported a 2-year bioassay where rats were fed up to 5% (2,500 mg/kg bw/day) propylene glycol in their diet. No treatment-related neoplasms were noted. The Panel concluded that dietary administration of propylene glycol does not cause cancer at or near a toxic level.

2.6.5 Potentially Sensitive Subpopulations

There have been reports of propylene glycol toxicity in individuals with compromised liver or kidney function and in infants who have inadvertently received an overdose of propylene glycol in conjunction with drug therapies. Serum half-life of propylene glycol in infants is longer than in adults. Fligner et al. *(35)* reported a half-life of 16 hours for a premature infant as compared to 5 hours in adults. Glasgow *(36)* measured serum half-life in ten infants. The range of serum values was 0.65–9.5 g/L **[8.55–125mM]**. Mean half-life of propylene glycol was calculated to be 19.3 hours with a range of 10.8–30.5 hours which is about 10 times longer than in adults. Alcohol dehydrogenase can be up to ten times lower in infants, which would account for the prolonged half-life in infants.

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3.0 DEVELOPMENTAL TOXICITY DATA

3.1 Human Data

No human developmental toxicity data were identified.

3.2 Experimental Animal Data

3.2.1 Oral Exposure

3.2.1.1 Prenatal and Perinatal Toxicity Studies

A prenatal oral developmental toxicity study of propylene glycol was performed by Bushy Run Research Center *(111)*. The results are summarized in Table 3-2 (pg. 52). This study was conducted in compliance with Good Laboratory Practices. Propylene glycol was administered by gavage to Charles River CD-1 mice from gd 6 to 15 *(111)*. Pregnant CD-1 mice (4 dose groups, 30 mice/dose group, pregnancy determined by presence of copulation plug [gd 0]) received undiluted propylene glycol (99.9% purity) once a day in the following dosage volumes: 0.5, 5.0, or 10.0 mL/kg bw/day [0.52, 5.2, or 10.4 g/kg bw day, respectively]. A control group of 30 pregnant females received 10.0 mL/kg bw/day of Milli-Q™ water. Females were approximately 46 days old and males were 49 days old upon receipt. Animals were acclimated for approximately 2 weeks before cohabitation. The mice were maintained on a 12-hour, light-dark cycle and food and water were available throughout the study. Pregnancy rate ranged from 93.3 to 100% and there were no unscheduled deaths during the study. Maternal weight and food and water consumption were monitored at 3 day intervals. After initiation of treatment, all animals were observed twice daily for morbidity and mortality.

At scheduled necropsy on gd 18, maternal body weight, liver and kidney weights, gravid uterine weight, number of corpora lutea, and number of implantation sites/resorptions were noted. All live and dead fetuses were sexed, weighed, and examined for external malformations; all live fetuses were examined for visceral malformations using a modification of methods described by Staples; 1/2 of live fetuses in each litter were decapitated and the heads were examined for craniofacial malformations using sectioning methods modified from Wilson; all live fetuses (1/2 intact and 1/2 decapitated) were stained with Alizarin Red S and examined for skeletal malformations **[cartilage not stained]**.

Maternal Parameters

Pregnancy rate ranged from 93.3 to 100% and there were no unscheduled deaths during the study. No treatment-related clinical signs were noted in any dose group; there were no treatment-related effects on maternal body weights or food consumption throughout gestation. Water consumption from gd 6 to 15 showed a significant ($p<0.01$) increase over control values in the 10 mL/kg bw/day dose groups and for gd 15-18 in the 5 mL/kg bw/day ($p<0.05$) and 10.0 mL/kg bw/day dose groups ($p<0.05$). Water consumption was also significantly higher in the high-dose group from gd 6 to 9 ($p<0.05$) and gd 6 to 15 ($p<0.01$) (See Table 3-2 for water consumption data on gd 0-6, 6-15, 15-18). There were no treatment-related necropsy findings in the dams; no effects on body weight, gravid uterine weight, corrected body weight, corrected weight change, or relative and absolute liver and kidney weights. No effect was noted on the number of corpora lutea, resorptions, dead fetuses, or sex ratio. The percentages of preimplantation loss and live fetuses were similar across treatment groups.

Table 3-1. Summary of Gestational Water Consumption (g/animal/day) (111)

Group	Doses (mL/kg bw/day) [mean ±SD]			
	0	0.5	5.0	10.0
Gd 0–6 (pre-treatment)	10.90±4.298	10.91±4.952	11.13±2.207	11.08±2.075
Gd 6–15 (treatment period)	12.83±3.675	12.14±2.167	13.80±2.253	15.35±2.925**
Gd 15–18 (post-treatment)	11.41±1.574	11.46±1.897	13.02±4.503*	13.06±2.149*

*p<0.05; **p<0.01

Fetal Parameters

A statistically significant (p<0.05) decrease (3%) in fetal body weight in the high-dose group was not considered biologically relevant due to the magnitude of the change and the lack of a dose-related trend. No differences were noted in malformations by category (external, visceral, skeletal) or in total malformations among all treatment groups. An increase in unossified cervical centra was noted in the high-dose group (p<0.05, 9/29 litters vs 2/28 in control group). However, this was not considered biologically relevant by the authors, as this finding was similar to historical control values in this laboratory. Significant increases in fetal atelectasis, poorly ossified supraoccipital bone, and a decrease in the extra ossification site in the nasal fontanel in the low-dose group were not considered to be biologically relevant due to the lack of a dose-effect relationship.

Statistical analyses used the pregnant dam or the litter as the unit of comparison. The authors of the Bushy Run Research Center Report noted that "the data for a quantitative continuous variables were inter-compared for the three treatment groups and the control group by use of Levene's test for equality of variances, analysis of variance (ANOVA), and t-tests. The t-tests were used when the F value from the ANOVA was significant. When Levene's test indicated equal variances, and the ANOVA was significant, a pooled t-test was used for pairwise comparisons. When Levene's test indicated heterogeneous variances, all groups were compared by an ANOVA for unequal variances followed, when necessary, by the separate variance t-test or pairwise comparisons. Nonparametric data were statistically evaluated using the Kruskal-Wallis test, followed by the Mann-Whitney U test when appropriate. Incidence data were compared using the Fisher's Exact Test. For all statistical tests, the probability value of <0.05 (two-tailed) was used as the critical level of significance."

The authors concluded that ". . . dosages up to 10.0 mL/kg bw/day were not associated with any treatment-related effects on endpoints such as clinical signs, body weight, body weight gain, food consumption, or pregnancy outcome. Increases in water consumption were observed in dams from the 5.0 and 10.0 mL/kg bw/day groups and were probably a physiologic response to the high dosages given by gavage. There was no evidence of treatment-related effects on developmental parameters. Therefore, in this study, the "no-observed-effect level" (NOEL) **[NOAEL[2]]** for maternal effects was 0.5 mL/kg bw/day. The NOEL **[NOAEL]** for developmental toxicity was at least 10.0 mL/kg bw/day."

[2] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL or LOAEL will be used throughout this document.

Table 3-2. Summary of Developmental Toxicity Study of Propylene Glycol Given by Gavage to CD-1 Mice on GD 6–15 (111)

Effect	*Doses (mL/kg bw/day) [mean ±SD]*			
	0	*0.5*	*5.0*	*10.0*
Maternal corrected body weight change (body weight at sacrifice minus gravid uterine weight, g)	6.21±1.87	6.46±2.05	6.89±1.57	6.67±2.35
Maternal liver weight (% of corrected body weight)	7.585±0.5711	7.595±0.7563	7.540±0.6111	7.681±0.7679
Maternal kidney weight (% of corrected body weight)	1.217±0.1047	1.202±0.1248	1.202±0.0838	1.261±0.1235
% Live fetuses/litter	93.5±8.08	91.5±18.76	94.3±6.73	89.6±19.45
Fetal body weight/litter (g)	1.351±0.0734	1.315±0.0799	1.361±0.0947	1.306±0.0733*
Total # litters with live malformed fetuses/# examined	9/28	8/28	10/28	13/29

Dams were sacrificed on gd 18 and fetuses from 28–30 litters/group were evaluated for prenatal developmental toxicity.
*$p < 0.05$

Strengths/Weaknesses: The Driscoll et al. *(111)* study is GLP-compliant with adequate numbers of animals per group and follows a design that permits evaluation of dose-response relationships. The Panel concurs that developmental and maternal NOAELs were determined under the conditions used in the study.

Utility (Adequacy) for CERHR Evaluation Process: This study *(111)* is useful for risk extrapolation purposes, with the caveat that exposures were delivered as bolus doses by means of gavage administration, which is unlikely to mirror expected human exposure. The study design limits developmental toxicity conclusions to exposure during the prenatal period.

The FDA *(112)* sponsored a "Teratologic evaluation of FDA 71–56 (Propylene Glycol) in mice, rats, hamsters and rabbits." These prenatal studies were conducted under contract for FDA by the Food and Drug Research Laboratories, Inc. in East Orange, NJ. **[This NTIS available report does not give detailed experimental protocol information (such as chemical purity, stability, or dose analysis). Protocol details such as gross necropsy and examination of uterine contents methods are not given.]**

Mice

Timed-mated outbred CD-1 albino mice (25/group) were dosed by oral intubation with propylene glycol as a water solution from gd 6 to 15. Observation of the vaginal sperm plug occurred on gd 0. Dose groups were 0, 16, 74.3, 345, and 1,600 mg/kg bw/day. Aspirin at a dose of 150 mg/kg bw was used as a positive control. Body weights of the dams were recorded on gd 0, 6, 11, 15, and 17. Food consumption and clinical signs were also monitored **[stated in text, but data not reported]**. All but one pregnant dam in the 74.3 mg/kg bw/day dose group survived to term. **[No maternal deaths were reported in the other dose groups.]** On gd 17 all dams were anesthetized and a Cesarean section performed. There were no apparent treatment-related differences in the number of implantation sites, resorptions,

fetal body weight, and viability among the dose groups. All fetuses were examined for external abnormalities, 1/3 of the fetuses from each litter were Wilson sectioned for visceral examination, the remaining 2/3 of each litter were examined for skeletal defects by clearing the tissue with potassium hydroxide and staining the bone with Alizarin Red S dye. **[Cartilage was not stained.]**

The following conclusion was reported for mice by the study authors *(112)*:

> "The administration of up to 1,600 mg/kg body weight of the test material to pregnant mice for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Results in mice are listed in Tables 3-3 and 3-4.

Table 3-3. Mouse Maternal and Fetal Toxicity Data for PG [no statistical analyses reported] (112)

	Sham	***Aspirin (mg/kg bw)***	***PG (mg/kg bw)***			
		150	***16.0***	***74.3***	***345.0***	***1,600.0***
Pregnancies						
Total #	22	23	22	22	20	23
Died/aborted (before gd 17)	0	0	0	1	0	0
To term (on gd 17)	22	23	22	21	20	23
Live Litters						
Total #	22	22	22	21	20	21
Implantation Sites						
Avg/dam	11.8	12.5	11.8	11.8	11.3	11.0
Resorptions						
% dams with partial resorptions	45.5	34.8	31.8	14.3	50.0	17.4
% dams with complete resorptions	–	4.35	–	–	–	4.35
Live Fetuses						
Avg/dam	10.4	11.5	11.4	11.4	10.5	10.2
Sex ratio (M/F)	0.78	0.74	0.80	0.79	0.86	0.86
Avg Fetus wt, in grams	0.90	0.84	0.88	0.90	0.91	0.96
Dead Fetuses						
% litters with dead fetuses	31.8	–	9.09	19.1	20.0	4.35
% litters with all dead fetuses	–	–	–	–	–	4.35

–: No data presented in FDRL report. The reason for the lack of data was not specified.

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Table 3-4. Summary of Mouse Fetal Skeletal and Soft Tissue Findings for PG (112)*

	Sham	Aspirin (mg/kg bw)	PG (mg/kg bw)			
		150	16.0	74.3	345.0	1,600.0
Live Fetuses Examined	161/22	185/22	173/22	170/21	145/20	165/21
Sternebrae						
Incomplete ossification	66/16	34/15	62/18	75/16	39/11	28/12
Bipartite	–	9/7	2/2	–	6/4	3/3
Extra	–	–	3/2	–	–	–
Missing	22/10	26/11	14/7	11/7	33/10	13/6
Ribs						
Incomplete ossification	–	1/1	–	–	–	1/1
Fused/split	–	–	–	1/1	–	–
More than 13	37/13	41/18	30/16	34/16	24/13	38/18
Vertebrae						
Incomplete ossification	3/2	8/6	2/1	1/1	10/4	9/4
Skull						
Incomplete closure	3/3	–	–	–	–	1/1
Extremities						
Incomplete ossification	–	7/6	–	–	7/3	3/2
Other						
Hyoid, missing	23/10	37/15	37/12	20/11	35/13	17/10
Hyoid, reduced	19/10	11/7	19/11	27/13	27/11	16/12
Soft Tissue						
Gastroschisis	1/1	–	–	–	1/1	–
Meningo-encephalocele	–	1/1	–	–	–	–

* Number of fetuses affected/Number of litters affected

–: No data presented in FDRL report. The reason for the lack of data was not specified.

Rats

Timed-mated Wistar albino rats (25/group) were dosed by oral intubation with propylene glycol as a water solution from gd 6 to 15. Observation of the vaginal sperm plug was gd 0. Dose groups were 0, 16, 74.3, 345, and 1,600 mg/kg bw/day. Aspirin at a dose of 250 mg/kg bw was used as a positive control. Body weights of the dams were recorded on gd 0, 6, 11, 15, and 20. Food consumption and clinical signs were also monitored **[stated in text, but data not reported]**. All dams survived to term. On gd 20 all dams were anesthetized and a Cesarean section performed. There were no apparent treatment-related differences in the number of implantation sites, resorptions, fetal body weight, and viability among the dose groups. All fetuses were examined for external abnormalities, 1/3 of the fetuses from each litter were Wilson sectioned for visceral examination, the remaining 2/3 of each litter were examined for skeletal defects by clearing the tissue with potassium hydroxide and staining

the bone with Alizarin Red S dye. **[Cartilage was not stained.]**

The following conclusion was reported for rats by the study authors *(112)*:

> "The administration of up to 1600 mg/kg/ (body weight) of the test material to pregnant rats for 10 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Results in rats are listed in Tables 3-5 and 3-6.

Table 3-5. Rat Maternal and Fetal Toxicity Data for PG [no statistical analyses reported] (112)

	Sham	***Aspirin (mg/kg bw)***	***PG (mg/kg bw)***			
		250	***16.0***	***74.3***	***345.0***	***1,600.0***
Pregnancies						
Total #	22	21	23	22	20	24
Died/aborted (before gd 20)	0	0	0	0	0	0
To term (on gd 20)	22	21	23	22	20	24
Live Litters						
Total #	22	20	23	22	20	24
Implantation Sites						
Avg/dam	11.4	10.7	11.2	11.1	12.3	10.7
Resorptions						
% dams with partial resorptions	18.2	42.9	17.4	4.55	10.0	–
% dams with complete resorptions	–	4.76	–	–	–	–
Live Fetuses						
Avg/dam	11.1	9.43	11.0	11.0	12.1	10.7
Sex ratio (M/F)	0.90	1.06	1.02	1.05	0.83	0.98
Avg fetus wt, in grams	3.39	2.68	3.91	3.73	3.91	3.75
Dead Fetuses						
Total	–	–	–	–	–	–

–: No data presented in FDRL report. The reason for the lack of data was not specified.

Table 3-6. Summary of Rat Fetal Skeletal and Soft Tissue Findings for PG (112)*

	Sham	Aspirin (mg/kg bw)	PG (mg/kg bw)			
		250	16.0	74.3	345.0	1,600.0
Live Fetuses Examined	173/22	137/20	179/23	169/22	167/20	180/24
Sternebrae						
Incomplete ossification	82/20	91/20	92/19	64/18	35/11	31/12
Bipartite	3/3	5/4	–	2/1	–	1/1
Missing	2/2	86/19	13/5	5/5	–	8/5
Ribs						
Incomplete ossification	–	1/1	–	–	–	–
Fused/split	–	1/1	–	–	–	–
Wavy	1/1	46/16	23/9	27/11	11/5	15/8
Less than 12	2/2	2/1	–	–	–	–
More than 13	7/3	91/19	3/1	1/1	6/4	3/3
Vertebrae						
Scoliosis	1/1	–	–	–	–	–
Incomplete ossification	–	101/19	1/1	13/7	3/3	18/9
Skull						
Incomplete closure	26/14	47/16	27/15	23/11	22/11	25/13
Missing	–	6/2	–	–	–	–
Extremities						
Incomplete ossification	–	3/1	–	–	–	–
Other						
Hyoid, missing	15/8	65/18	19/10	16/8	13/9	15/7
Hyoid, reduced	20/9	19/10	17/9	9/6	16/8	15/8
Soft Tissue						
Gastroschisis	–	1/1	–	–	–	–
Exophthalmos	–	2/1	–	–	–	–
Encephalo-myelocele	–	8/3	–	–	–	–
Meningo-encephalocele	–	4/2	–	–	–	–
Hydrocephalus	–	1/1	–	–	–	–

* Number of fetuses affected/Number of litters affected
–: No data presented in FDRL report. The reason for the lack of data was not specified.

Hamsters

Timed-mated outbred Golden hamsters (25/group) were dosed by oral intubation with propylene glycol from gd 6 to 10. Observation of motile sperm in the vaginal smear was gd 0. Dose groups were 0, 15.5, 72, 334.5, and 1,550 mg/kg bw/day. Aspirin at a dose of 250 mg/kg bw/day was used as

a positive control. On gd 14, a Cesarean section was performed. There were no apparent treatment-related differences in the number of implantation sites, resorptions, fetal body weight, or viability among the dose groups. All fetuses were examined for external abnormalities, 1/3 of the fetuses from each litter were Wilson sectioned for visceral examination, the remaining 2/3 of each litter were examined for skeletal defects by clearing the tissue with potassium hydroxide and staining the bone with Alizarin Red S dye. **[Cartilage was not stained.]**

The following conclusion was reported for hamsters by the study authors *(112)*:

> "The administration of up to 1550 mg/kg/ (body weight) of the test material to pregnant hamsters for 5 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Results in hamsters are listed in Tables 3-7 and 3-8.

Table 3-7. Hamster Maternal and Fetal Toxicity Data for PG [no statistical analyses reported] (112)

	Sham	*Aspirin (mg/kg bw)*	*PG (mg/kg bw)*			
		250	*15.5*	*72.0*	*334.4*	*1,550.0*
Pregnancies						
Total #	21	21	24	25	22	22
Died/aborted (before gd 14)	0	2	0	0	0	1
To term (on gd 14)	21	19	24	25	22	21
Live Litters						
Total #	21	19	24	25	22	21
Implantation Sites						
Avg/dam	14.3	15.2	13.8	13.8	14.2	13.7
Resorptions						
% dams with partial resorptions	4.76	21.1	12.5	20.0	4.55	28.6
% dams with complete resorptions	–	–	–	–	–	–
Live Fetuses						
Avg/dam	14.2	14.6	13.5	13.5	14.1	12.4
Sex ratio (M/F)	0.95	0.79	1.12	1.07	0.91	0.94
Avg fetus wt, in grams	1.74	1.78	1.79	1.80	1.84	1.79
Dead Fetuses						
% litters with dead fetuses	4.76	10.5	8.33	8.00	4.55	14.3
% litters with all dead fetuses	–	–	–	–	–	–

–: No data presented in FDRL report. The reason for the lack of data was not specified.

Table 3-8. Summary of Hamster Fetal Skeletal and Soft Tissue Findings for PG (112)*

	Sham	*Aspirin (mg/kg bw)*	*PG (mg/kg bw)*			
		250	*15.5*	*72.0*	*334.4*	*1,550.0*
Live Fetuses Examined	207/21	193/19	228/24	233/25	214/22	184/21
Sternebrae						
Incomplete ossification	67/18	167/19	51/17	58/19	63/16	57/15
Bipartite	23/14	26/14	23/15	15/10	30/15	17/11
Extra	1/1	1/1	1/1		1/1	6/4
Missing	37/13	45/15	47/17	20/11	24/10	27/12
Ribs						
Fused/split						1/1
More than 13	41/17	30/14	37/14	47/21	63/19	31/13
Vertebrae						
Scoliosis	1/1	–	–	–	–	–
Incomplete ossification	4/3	5/3	4/2	3/2	2/2	1/1
Skull						
Incomplete closure	–	2/2	–	–	–	–
Extremities						
Incomplete ossification	–	1/1	2/2	4/4	3/2	1/1
Other						
Hyoid, missing	4/4	2/2	5/5	2/2	1/1	–
Hyoid, reduced	9/6	25/10	7/5	1/1	5/3	–
Soft Tissue						
Hydrocephalus	1/1	–	–	–	–	–
Atelocardia		1/1	–	–	–	–
Fetal monster	–	–	–	1/1	–	–
Umbilical Hernia	2/2	–	–	–	–	–
Dephallia	1/1	–	–	–	–	–
Meningo-encephalocele	–	–	2/1	1/1	–	–

* Number of fetuses affected/Number of litters affected
–: No data presented in FDRL report. The reason for the lack of data was not specified.

Rabbits

Dutch-belted female rabbits were dosed by oral intubation with propylene glycol from gd 6 to 18. Dose groups were 0, 12.3, 57.1, 267, and 1,230 mg/kg bw/day. 6-Aminonicotinamide (2.5 mg/kg) dosed on gd 9 was a positive control. On gd 0, each doe received an injection of human chorionic gonadotropin (400 IU) and 3 hours later was artificially inseminated with diluted donor buck semen. On gd 29 a Cesarean section was performed. There were no apparent treatment-related differences in the number of corpora

lutea, implantation sites, resorptions, fetal body weight, and viability among dose groups. All fetuses were examined for external abnormalities. The live fetuses from each litter were placed in an incubator for 24 hours for evaluation of neonatal survival. All surviving pups were sacrificed at the end of that time and examined by dissection for visceral abnormalities. All fetuses were cleared with potassium hydroxide and stained with Alizarin Red S dye and examined for skeletal defects. **[Cartilage was not stained.]**

The following conclusion was reported for rabbits by the study authors *(112)*:

> "The administration of up to 1230 mg/kg/ (body weight) of the test material to pregnant rats **[sic]** for 13 consecutive days had no clearly discernible effect on nidation or on maternal or fetal survival. The number of abnormalities seen in either soft or skeletal tissues of the test groups did not differ from the number occurring spontaneously in the sham-treated controls."

Results in rabbits are listed in Tables 3-9 and 3-10.

Table 3-9. Rabbit Maternal and Fetal Toxicity Data [no statistical analyses reported] (112)

	Sham	*6 AN* (mg/kg bw)*	*PG (mg/kg bw)*			
		2.5	*12.3*	*57.1*	*267.0*	*1,230.0*
Pregnancies						
Total #	11	10	11	12	14	13
Died/ aborted (before gd 29)	0	0	2	1	2	0
To term (on gd 29)	11	10	9	11	12	13
Corpora Lutea						
Total #	156	176	182	190	198	199
Avg/dam	11.1	11.7	10.1	13.6	10.4	13.3
Live Litters						
Total #	11	10	9	11	12	13
Implantation Sites						
Avg/dam	6.36	6.90	7.67	6.36	5.25	7.54
Resorptions						
% dams with partial resorptions	45.5	50.0	22.2	45.5	16.7	15.4
% dams with complete resorptions	–	–	–	–	–	–
Live Fetuses						
Avg/dam	5.91	5.00	7.33	5.00	5.08	7.31
Sex ratio (M/F)	0.81	0.79	1.13	1.29	0.69	1.11
Avg fetus wt, in grams	42.3	32.5	36.4	39.9	42.9	39.0
Dead Fetuses						
Total	–	–	–	–	–	–

* 6-Aminonicotinamide, positive control

–: No data presented in FDRL report. The reason for the lack of data was not specified.

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*Table 3-10. Summary of Rabbit Fetal Skeletal and Soft Tissue Findings for PG** (112)*

	Sham	*6AN* (mg/kg bw)*	*PG (mg/kg bw)*			
		2.5	*12.3*	*57.1*	*267.0*	*1,230.0*
Live Fetuses Examined	65/11	50/10	66/9	55/11	61/12	95/13
Sternebrae						
Incomplete ossification	1/1	5/2	1/1	–	2/2	10/6
Bipartite	–	–	1/1	–	2/2	–
Fused	–	6/36	–	–	–	–
Extra	1/1	1/1	2/2	3/3	1/1	
Missing	–	3/2	1/1	–	–	11/3
Ribs						
Incomplete ossification	–	–	–	–	–	–
Fused/split	–	14/7	–	–	–	–
Vertebrae						
Fused	–	1/1	–	–	–	–
Scoliosis	–	10/4	–	–	–	–
Tail Defects	–	48/9	–	–	–	–
Scrambled	–	22/6	–	–	–	–
Soft Tissue						
Anopia, short tail	–	1/1	–	–	–	–
Encephalocele	–	7/1	–	–	–	–
Med Rotation of Hindlimbs	–	17/6	–	–	–	–
Umbilical Hernia	–	1/1	–	–	–	–
Scoliosis	–	1/1	–	–	–	–
Harelip	–	2/2	–	–	–	–

* 6-Aminonicotinamide, positive control
** Number of fetuses affected/Number of litters affected
–: No data presented in FDRL report. The reason for the lack of data was not specified.

Based upon the conclusions of the study authors, the NOAELs for maternal and fetal toxicity of propylene glycol were at the highest dose tested are summarized in Table 3-11.

Table 3-11. NOAEL Levels for Maternal and Fetal Toxicity of PG (summarized from (112))

Species	*NOAEL (mg/kg bw/day) (at highest dose tested)*
Mice	≥1,600
Rats	≥1,600
Hamsters	≥1,550
Rabbits	≥1,230

Strengths/Weaknesses: In general, adequate numbers of animals (25 dams per treatment group) were employed in these studies *(112)*. In most cases, average and percent summaries were provided without associated standard errors, which prevented an assessment of the statistical significance of differences reported. Differences between the negative control and dose groups were small and not likely to be statistically different, but there were a few cases where formal analysis would have been helpful. The report provided detailed information only on fetal weights and resorptions but no corresponding information on malformations, nor was detailed information on maternal body weights over the course of the study presented. No historical control data were presented to allow assessment of the importance of observance of specific malformations. A variety of endpoints were assessed, including both maternal and fetal endpoints. Multiple doses of test compound were used in each species, so dose-response relationships could be assessed.

Aspirin was used as the positive control treatment for mice, rats, and hamsters and 6-aminonicotinamide was used for rabbits. Results indicate that aspirin is only mildly teratogenic for mice and hamsters but is strongly teratogenic for rats. 6-aminonicotinamide is clearly teratogenic for rabbits. The use of a weak positive control makes clear conclusions for mice and hamsters more difficult.

The major limitation in the study is in the presentation. Very few experimental details were presented, and it is not clear if any formal statistical analysis was performed. For example, the rationale for the selection of the positive control and the doses used is not given. The sequence for necropsy of the dose groups is not known. Whether the necropsy was done on an entire dose group within the same time period or over the entire necropsy period can affect the findings of minor developmental delay (such as delayed ossification and wavy ribs). Such findings can be apparent in the first groups sacrificed, but not as apparent in later groups. Detailed necropsy information such as the number of unossified vertebrae is not reported. In some cases, it is not possible to reconstruct litter incidences of effects from the data presented. The same endpoints were not collected across all species; for example, the number of corpora lutea were apparently only recorded for rabbits and not for the other three species. While the average numbers of implantation sites across test groups in mice, rats, and hamsters suggest that propylene glycol did not have a large impact on pre-implantation loss, it would have increased confidence in the data if corpora lutea had also been counted.

At the highest dose tested, propylene glycol did not seem to affect mice or hamsters in the parameters examined (maternal weight, number of implants per litter, fetal weight, death and resorptions, and malformations). A large number of malformations were observed in mice across all treatments, including positive and negative controls, causing concern about the validity of the whole study. Similar concerns are not present for hamsters. With rats, higher numbers of wavy ribs and incomplete ossification of the vertebrae were observed at the same level as the positive control, suggesting a propylene glycol effect. The incidences of these defects did not appear to be dose-related. No propylene glycol effects were observed in rabbits.

Utility (Adequacy) for CERHR Evaluation Process: These data *(112)* would appear to be of limited use for the CERHR evaluative process. The lack of detail presented in the report as well as the lack of statistical analysis makes it difficult to form solid conclusions. The lack of formal statistical analysis suggests that these data might be more useful to help confirm results demonstrated in other studies. In two of the four studies, the choice of the positive control compound does not appear to be

appropriate. Generally, propylene glycol did not appear to have had major adverse effects in any of the four species tested and, when effects were present, they did not appear to be dose-responsive. The study suggests that the NOAEL level for mice, hamsters, and rabbits is at least 1,600, 1,550, 1,230 mg/kg bw/day, respectively; the levels are given in Table 3-11. The appropriateness of the NOAEL level for rats (1,600 mg/kg bw/day) given in Table 3-11 depends on the importance attributed to the rib and vertebrae malformations observed. The general lack of effect gives some measure of comfort, but important observations may not have been made. The Panel judges the data in this report insufficient to predict human health effects.

Kavlock et al. *(113)* employed an *in vivo* teratology screening procedure to evaluate propylene glycol along with 45 other chemicals. Timed-pregnant CD-1 mice (approx. 60-days-old) were dosed with propylene glycol in water **[% purity not stated]** by oral gavage on gd 8–12 at a dose of 10,000 mg/kg bw/day. In this assay, pregnant females were dosed at a level predicted to induce a mild degree of maternal toxicity or at a level stated in the literature to be teratogenic. In the propylene glycol experimental block, a control group was dosed with water (40 mice) and groups of 30 mice were exposed to propylene glycol or another substance (sucrose). Maternal toxicity endpoints examined were number pregnant, mortality, and number of animals with resorptions. For fetal toxicity, the number of live pups and their weights on pnd 1 and 3 were recorded. Data analysis was performed using the General Linear Models procedure on SAS. When a significant effect of treatment was detected by ANOVA analysis, individual group means were compared with a Student's t-test on least-squares means.

For propylene glycol, maternal and fetal parameters were not significantly different from values of control animals. Out of 30 animals dosed with propylene glycol, 83% were pregnant; no dams died and there were no resorptions. For 40 control animals dosed with vehicle, 68% were pregnant; no dams died and there were no resorptions. Neonatal values for pup survival and weight are included in Table 3-12.

Table 3-12. Pup Survival and Weight after Treatment of Pregnant CD-1 Mice by Gavage with Propylene Glycol (10 g/kg bw/day) from gd 8 to 12 (113)

Compound	*PND 1*		*PND 3*	
	# live	*wt (g)*	*# live*	*wt (g)*
Control (water)	*10.08±0.46	1.59±0.02	10.00±0.45	1.88±0.04
Propylene Glycol (in water)	10.60±0.44	1.53±0.03	10.52±0.44	1.84±0.03

*mean ± standard error of the mean

Strengths/Weaknesses: An adequate number of mice were used in this study *(113)* in the group exposed to propylene glycol. Only a single dose of propylene glycol was used, and the endpoints evaluated and the dosing period used are not those commonly evaluated in a comprehensive developmental toxicity study.

Utility (Adequacy) for CERHR Evaluation Process: These data appear to be of limited value for the CERHR evaluative process. A high dose of propylene glycol was used with no apparent adverse effects on the offspring, which is reassuring. However, the lack of a dose-response, as well as the differences in measured endpoints, make these data less convincing.

3.2.2 Injection

3.2.2.1 Prenatal toxicity

Chick eggs

In an early study by Gebhardt *(114)*, propylene glycol was found to be teratogenic when injected into chick eggs. Eggs (avg wt 59 g) from White Leghorn chickens were used in this study. Propylene glycol **[0.05 mL, >99% purity]** was injected into the air chamber or yolk sac of the egg. Control eggs had the same size needle inserted into the egg for 2 seconds, but were not injected. Eggs (18–30) were injected on one of incubation days 0 through 7 with either propylene glycol or sham treatment. Eggs were rotated hourly and incubated at 38°C and 55% relative humidity. Candling was done on the fourth and sixth days of incubation and all unfertilized eggs and eggs with dead embryos were recorded and removed. Gross morphology was studied on the 15th day of egg incubation by clearing the skeleton and staining with Alizarin Red S. **[Statistical methods were not reported.]** The number of embryos that died within the first 15 days of development were recorded and malformations in the surviving embryos were determined. The authors noted that the embryos were most sensitive to propylene glycol injection into the air chamber on day 4 of development, when 90% of the embryos died within 2 hours and 20% of the surviving embryos had asymmetric malformations of the limbs **[time/percent mortality graph provided, no other data provided]**. In a second experiment, propylene glycol or propylene glycol diluted 1:1 and 1:2 in water was injected into the air chamber of day 4 chick embryos **[see Table 3-13 below, controls were not described by the authors]**. The authors speculated that the apparent toxic effect of propylene glycol on day 4 may be due to disruption of the embryo vasculature.

Table 3-13. Teratogenic Effect of Propylene Glycol Injected into the Air Chamber of 4-Day-Old Chick Embryos (114)

Dilution	*# of Eggs*	*% Mortality*	*% Malformed Surviving Embryos*
Undiluted	227	90	21
Diluted 1:1	165	82	27
Diluted 1:2	144	57	8

Strengths/Weaknesses: The study *(114)* was performed in a non-mammalian species. An adequate number of embryos were evaluated in each group.

Utility (Adequacy) for CERHR Evaluation Process: These data appear to be of little use in the CERHR evaluative process. Experiments performed in chick embryos are not relevant to assessing risks to humans. Additionally, the data in this study conflict with those reported by Landauer and Salam *(115)*, further weakening their relevance.

Propylene glycol and dimethyl sulfoxide were compared with water as solvents for teratogens in chick embryos *(115)*. Chick embryos (White Leghorn chicken eggs) were injected (0.2 mL) into the yolk sac with teratogen on day 4 of incubation and fetuses examined on day 19. Teratogens tested were: bidrin, 6-aminonicotinamide, 3-acetylpyridine, sulfanilamide, 3-amino-1,2,4 triazole, physostigmine sulfate, and nicotine sulfate. The authors found less teratogenicity of known human teratogens when

the solvent was either dimethyl sulfoxide or propylene glycol as compared to water. Although the data for solvent injection alone are not presented in this paper, the authors stated that they did not find propylene glycol toxic to day 4 chick embryos as Gebhardt *(114)* previously reported.

Strengths/Weaknesses: The study *(114)* was performed in a non-mammalian species.

Utility (Adequacy) for CERHR Evaluation Process: These data appear to be of little use in the CERHR evaluative process. Experiments performed in chick embryos are not relevant to assessing risks to humans.

3.2.3 Mechanistic and In Vitro Studies

3.2.3.1 Embryo culture

Kowalczyk et al. *(116)* examined by *in vitro* culture the effects of propylene glycol, glycerol, and several alcohols on mouse preimplantation development. Random-bred mice (Harlan Sprague-Dawley) were superovulated (5 IU PMSG IP followed in 48 hours with 5 IU HCG) and paired with B6SJL/J males. Female mice were sacrificed on gd 2 (day of vaginal plug=gd 1) for collection of two-cell embryos or on gd 3 for collection of eight-cell morulae. Oviducts were flushed with M2 medium and embryos were cultured in Ham's F-10 media. Embryos at the two-cell stage were washed 3x in Ham's F-10 and cultured 24 hours in medium containing 6–131 mM propylene glycol (0, 0.05, 0.1, 0.2, or 1.0%). Embryos were then washed in Hams F-10 (three times) and cultured in propylene glycol-free medium for 5 days to observe development to the blastocyst stage. Embryos collected at the morulae stage were exposed to propylene glycol for 24 hours. The percentage of embryos cavitating and the blastocoel volume was recorded at 0, 4, 8, and 24 hours after removal of the propylene glycol from the medium. All experiments were repeated at least 3 times (43 embryos/treatment group, avg.). Differences in the control and treatment groups were tested for significance ($p<0.01$) using Chi-Squared analysis. Embryos exposed to propylene glycol or glycerol exhibited development to the blastocyst stage that was comparable with controls. Morulae cultured 24 hours in medium with 0, 0.05, 0.1, 0.2, or 1.0% propylene glycol or glycerol cavitated at a rate that was comparable with stage-matched controls **[data not shown]**. Blastocoel volume expansion was unaffected **[method referenced, but not described]**. The authors concluded that the progression of preimplantation embryo development to the blastocyst stage is not affected by propylene glycol at 0.05, 0.1, 0.2, or 1.0%. The authors found that ethanol stimulated embryo development and cavitation whereas the other alcohols tested (methanol, 2-propanol, 1-propanol, and 1-butanol) were toxic to blastocyst formation.

Strengths/Weaknesses: Several doses of propylene glycol were tested for their effects on blastocyst formation and cavitation rate using a mammalian species.

Utility (Adequacy) for CERHR Evaluation Process: Although reassuring in that the doses of propylene glycol used in the study had little effect on murine preimplantation development, these data appear to be of little use in the CERHR evaluative process.

3.2.3.2 Cryoprotectant

Propylene glycol is a permeating cryoprotectant used to depress the temperature at which intracellular ice forms and to stabilize the plasma membrane. It is routinely used as a cryoprotectant in the cryo-

preservation of human oocytes. In an effort to optimize cryopreservation of oocytes, a number of studies examined methods to improve cryopreservation techniques *(117-124)* **[not reviewed in this report]**.

Studies by Damien et al. *(125)* evaluated the usage of propylene glycol with faster ultra rapid embryo freezing protocols. The purpose of this study was to identify the maximal concentration of propylene glycol and sucrose that will not adversely alter the development of the mouse pronuclear stage embryo and to determine the mechanism by which propylene glycol mediates embryotoxicity. Pronuclear mouse zygotes from superovulated B6D2Fi mice were evaluated. Each series of experiments was replicated 3–5 times. In both the control and 1.5 M propylene glycol-treated group, 78% of the zygotes developed into 2-cell embryos. With 3 M propylene glycol, 7% of the zygotes developed into 2-cell embryos (ANOVA, $P<0.05$). The zygotes were observed over a 20-minute period at 22°C under phase optics. In a second series of experiments, pronuclear mouse zygotes were incubated in either fluorescein diacetate or Acridine Orange and then transferred to either phosphate buffered saline or propylene glycol in water **[% purity not reported]**. Fluorescence is maintained as long as the cell membrane is not damaged and was retained in 98% of the zygotes exposed to 1.5 M propylene glycol, 81% (Chi-Squared test, $P<0.05$) exposed to 3.0 M propylene glycol, and 5% (Chi-Squared test, $P<0.05$) exposed to 6.0 M propylene glycol (Table 3-14). A shift in fluorescent wavelength at 3.0 M propylene glycol also indicated that the pH of the embryos had decreased. The authors concluded that a 20-minute exposure to 1.5 M propylene glycol did not affect embryonic development, while concentrations greater than or equal to 3.0 M inhibited embryonic development through cell membrane damage and pH changes.

Table 3-14. The Effect of a 20-Minute Exposure of Propylene Glycol (PG) on the Percentage of Zygotes Showing Fluorescein Diacetate and Acridine Orange Fluorescence (125)

DYE	*0M PG*	*1.5M PG*	*3.0M PG*	*6.0M PG*
Fluorescein diacetate	100(52)	98(50)	81(53)*	5(64)*
Acridine orange	95(56)	95(40)	7(46)*	0(32)*

The total number of embryos is given in parentheses.
*The percentage of embryos that maintained fluorescence was significantly reduced ($P<0.05$).

Strengths/Weaknesses: The strengths of this study *(125)* are that 3 or 4 concentrations of propylene glycol covering a 12-fold concentration range were used *in vitro* to determine the effect of this compound on early embryonic development. Also, the authors carefully monitored volume changes and fluorescence. However, various concentrations of sucrose were also added to the zygotes. To determine the percentage of zygotes that developed into 2-cell embryos, the number of zygotes were pooled across sucrose concentrations, thereby ignoring any effect of sucrose. In addition, widely different numbers of zygotes were used in the experiments, from 66 in the 1.5 M propylene glycol group to 155 in the 0 M propylene glycol group.

Utility (Adequacy) for CERHR Evaluation Process: These data are of little use to the Panel in the CERHR evaluative process.

3.2.3.3 Hydra Screening Assay

In an evaluation of the utility of the hydra prescreening developmental assay to predict experimental

findings in laboratory animals *(126)*, propylene glycol was one of 14 glycols and glycol ethers evaluated and compared to published animal data. Adult polyps of *Hydra attenuata* are grown under conditions **[not specified]** such that they will reproduce by asexual budding. For each assay, approximately 700–1,000 adult hydra are dissociated mechanically into component cells and randomly re-associated into small pellets (~20 pellets) by gentle centrifugation. After 92 hours of incubation **[conditions not specified]**, approximately 10–20 adult hydra will form from each pellet and form free-standing polyps. By incubation of adult hydra or pellets in the presence of test chemical at log increment dilutions, the minimum effective concentrations (MEC) of the test substance capable of producing adult (A) and developmental (D) toxicity can be determined. The A/D ratio will increase in size as embryo toxicity increases over adult toxicity **[controls or further experimental details were not reported, no statistical methods were reported]**. The A/D ratio reported by the authors for propylene glycol was 1.3. **[Results for propylene glycol and how toxicity detected or measured were not discussed by the authors.]** Although no animal data or rank order are given, the authors conclude, "The results of these hydra assays of glycols and glycol ethers typify results to be expected in mammals" (Table 3-15).

Table 3-15. Developmental Toxicity of Glycols and Glycol Ethers in Hydra (126)

Test Chemical	*A=MEC (adult) mL/L*	*D=MEC('embryo') mL/L*	*A/D*
Ethylene glycol (EG)	50	30	1.7
Propylene glycol	40	30	1.3
Hexylene glycol	20	6	3.3
EG monomethyl ether	40	30	1.3
EG monoethyl ether	30	6	5
EG monobutyl ether	4	0.9	4.4
EG monophenyl ether	1	0.3	3.3
EG monomethyl ether monoacetate	0.7	0.7	1.0
EG monoethyl ether monoacetate	0.6	0.6	1.0
EG diacetate	0.2	0.2	1.0
Diethylene glycol	30	30	1.0
Diethylene glycol monomethyl ether	30	20	1.5
Diethylene glycol dibutyl ether	0.9	0.4	2.2

Strengths/Weaknesses: A major weakness in the Johnson et al. *(126)* study is the use of an invertebrate animal.

Utility (Adequacy) for CERHR Evaluation Process: These data *(126)* are of little use in the CERHR evaluative process. These experiments were performed in artificial 'embryos' created from dissociated marine invertebrates. Data from this assay are not relevant to assessing risks to humans.

3.3 Utility of Data

No human data on developmental toxicity in humans were identified. Based upon the Driscoll et al. *(111)* study, the Panel concluded that the available data are sufficient to evaluate prenatal developmental

toxicity by the oral route in mice. Although data for other experimental animal species by the same route are inadequately presented, the finding of a lack of an effect is consistent with results of the Driscoll study. There were no acceptable postnatal developmental studies identified by the Panel. However, the lack of an effect on postnatal survival and reproductive performance after oral exposure of the pregnant mouse was determined for propylene glycol in a continuous breeding study addressed in Section 4.2 *(127)*. These data are sufficient to assess prenatal, but insufficient to assess postnatal, developmental toxicity in humans.

3.4 Summary

3.4.1 Human Data

No human data on developmental toxicity were identified.

3.4.2 Experimental Animal Data

A prenatal developmental toxicity study was conducted in mice orally exposed to propylene glycol at the Bushy Run Research Center *(111)*. Under the conditions used in this study, which was performed in compliance with EPA GLP regulations, a developmental toxicity NOEL **[NOAEL[3]]** for fetuses of 10.0 g/kg bw/day was reported; this was the highest dose tested. The maternal NOEL **[NOAEL]** was 0.50 g/kg bw/day based on increases in water consumption observed at 5.0 and 10.0 g/kg bw/day. It is reasonable to speculate that this effect was a physiological response to the high doses of propylene glycol administered.

Prenatal developmental toxicity studies were conducted in mice, rats, hamsters, and rabbits orally exposed to propylene glycol at the Food & Drug Research Laboratories, Inc. *(112)* under contract for the FDA. NOAEL levels determined for maternal and fetal toxicity were at the maximum doses used: 1.6 g/kg bw/day for rats and mice, 1.55 g/kg bw/day for hamsters, and 1.23 g/kg bw/day for rabbits. Propylene glycol did not appear to have any major adverse effects in any of the four species tested. Unfortunately, detailed information on study design is not presented in this report, and no statistical information is presented. Although propylene glycol is apparently without detrimental effect to the fetus, the Panel concluded that these data, as presented, are inadequate to be used as the sole study to interpret developmental toxicity.

Propylene glycol was also tested in a CD-1 mouse screening assay by Kavlock et al. *(113)*. Timed-pregnant CD-1 mice were dosed with propylene glycol in water by oral gavage on gd 8–12 at a dose of 10 g/kg bw/day. Endpoints examined were number of dams pregnant, mortality, and the number of dams with resorptions; the number of live pups and their weights on pnd 1 and pnd 3 were recorded. No significant adverse effects were noted for the maternal and fetal parameters evaluated. The Panel concluded that although an adequate number of animals were used, the endpoints evaluated and the dosing period used were not adequate for a comprehensive developmental toxicity study.

The Panel concluded that the available data are sufficient to evaluate the developmental toxicity of propylene glycol in mice. Data from the Driscoll et al. *(111)* study indicate that oral prenatal exposure

[3] Since the Expert Panel is considering only adverse effects in the selection of effect levels, the terminology of NOAEL or LOAEL will be used throughout this document.

to propylene glycol is not a developmental toxicant at doses of up to 10 g/kg bw/day in mice. Data in several other species, while inadequately presented, are consistent with the findings in the mouse. The only data available to evaluate postnatal effects of propylene glycol are those from the continuous breeding study in mice conducted for an assessment of possible fertility effects *(128)*. When mice were exposed throughout gestation and lactation and to 34 weeks of age with estimated doses as high as 10 g/kg bw/day, no adverse effects were observed on fertility indices. These data suggest that development was not significantly impaired.

4.0 REPRODUCTIVE TOXICITY DATA

4.1 Human Data

No human reproductive toxicity studies were identified.

4.2 Experimental Animal Data

An early report examined the toxicology and reproductive performance of rats **[strain not specified]** fed propylene glycol **[purity not specified]** or glycerol in the diet *(129)*. Minimal experimental information is reported. However, some data are provided from this multigeneration reproductive study in which the animal diets were formulated so that an isocaloric amount of propylene glycol (from 0–30% (w/w)) replaced cornstarch in the feed. Animals were monitored and continued on the diet through three successive generations. Animals were fed *ad libitum* and body weights were measured weekly. Six dose groups and one control group (three males and six females per group) were used. Two females were housed with one male **[length of time not reported]**; a weekly record was made of the average amount of diet consumed. At 70–80 days of age, females were monitored for pregnancy and removed to individual cages before litter delivery. The number, date, and average weight of the young were recorded. Less thrifty pups were culled if the number exceeded six pups per litter. Litters were weighed at weekly intervals until weaning. Three males and six females were chosen per dose group from the first litter animals and retained on the same diet. The study was continued through three successive generations. The authors provide a summary table of "Composite responses of three generations of female rats produced on each of several diets" **[food consumption data are not provided]** (see Table 4-1).

Table 4-1. Composite Responses of Three Generations of Female Rats Produced on PG in the Diet (129)

% PG (w/w)	*# females*	*# females with litters*	*# litters born*	*# pups born*	*Avg wt of pups (g)*	*# of pups weaned*	*Avg # of pups/ dam*	*Avg # of pups/ litter*
0	36	36	91	689	6.0	422	19.1	7.4
2.5	18	16	38	260	5.5	147	16.3	6.8
5.0	18	18	40	315	5.4	193	17.5	7.8
7.5	18	18	40	229	5.8	144	12.7	5.7
10.0	18	16	46	280	5.8	158	17.5	6.1
20.0	18	16	38	204	6.0	120	12.7	5.4
30.0	18	9	18	113	6.0	77	12.5	6.3

This data table shows that the percentage of females reproducing ranged from 88–100% for the 0-20% propylene glycol dose groups and 50% for the 30% propylene glycol dose group, and the average number of young born per litter ranged from 5.4 to 7.8 pups for the 0–30% propylene glycol dose groups. The authors noted that in the 30% propylene glycol dose group, 18 females had 11 litters born from the first generation females, 6 litters from the second, and one litter from the third generation, and that "Rats receiving the 30% propylene glycol diet failed to produce the third generation of

young." The authors conclude that "In view of the limited data available, it is difficult to state with any degree of certainty what effect the composition of the diet had on the ability of the females to reproduce." **[This report does not identify the specific statistical methods used.]**

Following this study, some of the progeny from the third generation (9 males and 18 females each from the 10 and 20% propylene glycol dose groups) were continued through three additional generations. The animals from each of these groups were subdivided into three subgroups containing three males and six females. The animals of one subgroup were continued on the original diet of either 10 or 20% propylene glycol; the animals of the second subgroup were changed to control diet (0% propylene glycol); the animals of the third subgroup were changed to a corresponding dose of glycerol. Data reported for subgroups one and two are presented below (see Table 4-2).

Table 4-2. Composite Responses of Three Generations of Female Rats Produced on PG in the Diet (129)

%PG[a]/%PG[b]	*# females*	*# females with litters*	*# litters born*	*# pups born*	*Avg wt of pups (g)*	*# of pups weaned*	*Avg # of pups/dam*	*Avg # of pups/litter*
10/0	14	14	32	226	5.5	158	16.1	7.1
10/10	16	16	35	223	5.4	158	14.0	6.4
20/0	18	18	39	361	5.6	197	20.6	9.3
20/20	18	18	35	237	5.6	154	13.2	6.8

[a] Previously fed diet for three generations
[b] Diet during three-generation test period

These data show that the percentage of females reproducing was 100% for the 0–20% propylene glycol dose groups and the average number of young born per litter ranged from 6.4 to 9.3 pups for the 0–20% propylene glycol dose groups. **[The authors did not comment on these data and failed to provide information on their statistical analyses.]**

Strengths/Weaknesses: The rat study cited above *(86, 129)* was conducted more than 50 years ago, prior to GLP. Many experimental details (e.g., animal strain, statistics, and even some reproductive data) were not provided.

Utility (Adequacy) for CERHR Evaluation Process: Since many experimental details are not provided, this study *(129)* is not useful in assessing reproductive hazard.

There has been one other multigeneration reproductive study on propylene glycol *(127)*.

NTP tested propylene glycol for reproductive/developmental toxicity in conjunction with testing of glycol ethers in order to examine structure-activity correlations. Using the reproductive assessment by continuous breeding (RACB) protocol, Lamb *(127)* investigated the reproductive function of male and female mice (COBS crl:CD-1 (ICR) BR outbred albino) exposed to propylene glycol in drinking water. A quality assurance audit was done on all study records. Propylene glycol (>99% purity) was chemically characterized. Stability studies and mixing studies were performed; aliquots

of all formulations were analyzed. Concentrations were within 5% of the nominal value. Standard statistical analyses were done on the reproductive and fertility data. Statistical significance was at the P=0.05 level. Reproductive data were evaluated by the Cochran-Armitage test for dose related trends in fertility and mating indices; pairwise comparisons between the control and dose groups were made using Fisher's Exact test. Pup and litter data were evaluated by the Kruskal-Wallis test and Jonckheere's test. Pairwise comparisons were made with Wilcoxon's rank-sum test. All analyses were performed on males, females, and both sexes combined; to remove any potential effect of number of pups in litter on pup weight, an analysis of covariance was performed.

A dose range-finding study (Task 1) was done with mice exposed to propylene glycol in drinking water for 14 days. Dose groups (8 male and 8 female mice/group; 2 mice of the same sex housed per cage) were 0, 0.5, 1.0, 2.5, 5.0, and 10.0% (w/v) propylene glycol. During the testing period, there was no mortality in any of the dose groups. However, in the high-dose group, males and females gained weight over control animals (2 and 7% heavier, respectively) and animals in the 10% dose group drank more water than the control group (60% more for males and 58% more for females). **[Food consumption not reported; caloric intake among dose groups not standardized.]**

Task 2 is designed to determine the effect of the chemical on fertility and reproduction. Animals were exposed to propylene glycol (>99% purity) in drinking water for a total of 18 weeks: one week prior to cohabitation, 14 weeks during cohabitation, and 3 weeks after cohabitation. A vehicle control group (40 males/40 females) and 3 dose groups of 20 males and 20 females per dose group were used. Based upon the results of Task 1, Task 2 drinking water concentrations were set at 0, 1, 2.5, 5% (w/v) propylene glycol. Chemical consumption estimates in this study were 0, 1.82, 4.80, and 10.1 g/kg bw/day for each of the respective dose groups; body weights of F_0 parents were monitored on study days 0, 7, 28, 56, 84, and 112. Live litters born during the cohabitation phase were weighed, sexed, and examined for external abnormalities and then sacrificed. Approximate delivery time and number of dead and cannibalized pups were noted. Offspring from the last litter (5th litter) of the control and high-dose groups were allowed to mature and reproductive performance was evaluated (Task 4). During the cohabitation phase, no chemical-related deaths and no significant chemical-related clinical signs of toxicity were noted. Propylene glycol had no significant effect on any of the following reproductive parameters in F_0 animals: number of litters per pair, number of live pups per litter, sex ratio, pup weights, number of days to litter, and dam weights at delivery. F_0 parents were not necropsied.

F_1 pup survival and body weights through pnd 14 were monitored in the control (34/39 litters/breeding pairs) and the high dose groups (19/20 litters/breeding pairs) from the final litter (5th litter). Propylene glycol had no effect on F_1 pup survival or body weight gain **[note that dams were still being exposed to propylene glycol from the drinking water during the preweaning period].**

A Task 3 crossover study is done to determine the affected sex. Since there was no effect of propylene glycol on fertility, this study was not conducted.

Task 4 was designed to evaluate the reproductive performance of the last litter (5th litter) from Task 2. F_1 males and females (20 each/dose group) were randomly selected from the control and high-dose groups (5% propylene glycol in drinking water) in Task 2 and mated on pnd 64–84 to animals from

the same dose group. Breeding pairs were separated after 7 days of cohabitation or after detection of a copulatory plug; the male and female were then housed singly. F_1 animals were weighed at weaning, first day of cohabitation, and then weekly. Water consumption was monitored weekly starting the first week after cohabitation. The high-dose group animals received exposure to propylene glycol throughout Task 2: from their dosed dam and then continuous exposure from drinking water (author-estimated daily dose of propylene glycol, 14.4 g/kg bw/day). There were no differences between the control and high-dose groups with respect to body weights or water consumption. The mating index for control and treated groups was 85%; the fertility index was 75% for control and 80% treated groups (nonsignificant). There were no significant differences in F_2 litter size, number of live pups, sex ratio, or pup weights. After delivery of the F_2 pups, the F_1 adults were necropsied. Sperm morphology and vaginal cytology evaluations **[on females that did not have pups]** were conducted. There were no significant differences in body or kidney and liver weights or serum calcium concentrations (both sexes). In males, there were no significant differences in the average weights of seminal vesicles, right cauda, prostate, right testis, and right epididymis. Sperm motility, sperm counts, or incidence of abnormal sperm did not significantly differ from control animals. In females, there was no difference in estrual cyclicity when compared to control animals. No organs were examined histologically. **[Note that for Task 2 and Task 4 food consumption not reported; caloric intake among dose groups was not standardized.]**

From the NTP studies, the authors concluded that propylene glycol administered in the drinking water at up to the 5.0% dose level had "no effect on the fertility and reproduction in adult or second generation CD-1 mice. Furthermore, there was no apparent effect with respect to body and organ weights (both absolute and adjusted), sperm motility, sperm counts per g caudal tissue, incidence of abnormal sperm, estrual cyclicity, and calcium levels in blood-serum of second generation mice."

The results of this NTP study are briefly summarized and compared to 47 other continuous breeding studies in a publication by Morrissey et al. *(128)*.

Bolon et al. *(130)* assessed differential follicle counts in mouse ovaries (ten mice/group) in animals that had been exposed to propylene glycol using the NTP continuous breeding protocol and reported that it had no effect on follicular counts.

Strengths/Weaknesses: The NTP multi-generational study *(127)* provided an acceptable toxicological protocol, and found that propylene glycol administered in the drinking water at up to a 5% dose level had no effect on fertility and reproduction in adult and second generation mice. Only the mouse and the rat have been studied, and findings from the two rat studies were inconclusive. The NTP study using mice reported no reproductive toxicity.

Utility (Adequacy) for CERHR Evaluation Process: This GLP study *(127)* is adequate for assessing reproductive hazard.

4.3 Utility of Data

There are no available data on the reproductive toxicity of propylene glycol in humans. An NTP multigeneration study *(127)* in mice concluded that propylene glycol administered in concentrations up to 5% (w/v) in the drinking water of mice did not cause reproductive toxicity in males or females

or their progeny. These data were judged by the Panel to be relevant to consideration of human risk.

4.4 Summary

4.4.1 Human Data

No human reproductive toxicity studies were identified.

4.4.2 Experimental Animal Data

The study by Guerrant et al. *(129)* in rats were conducted more than 50 years ago, prior to GLP protocols. Many experimental details were not provided and the results are judged inconclusive by the Panel.

In the NTP multi-generation study *(127)*, propylene glycol was administered in the drinking water to mice at 0, 1, 2.5, and 5% (w/v) dose level; there was no effect on fertility or reproduction in the first and second generation mice. There was no apparent effect with respect to body, kidney, and liver weights, pup survival, sperm motility, sperm counts, incidence of abnormal sperm, or estrual cyclicity. During the cohabitation phase, no chemical-related deaths and no significant chemical-related clinical signs of toxicity were noted. Propylene glycol had no significant effect on any of the following reproductive parameters in F_0 animals: number of litters per pair; number of live pups per litter; sex ratio; pup weights; number of days to litter; and dam weights at delivery.

The Panel concluded that there is adequate evidence in mice that propylene glycol does not cause reproductive toxicity in males and females when exposure is up to 5% propylene glycol in drinking water over an 18-week exposure period (1 week prior to cohabitation, 14 weeks during cohabitation, and 3 weeks after cohabitation) or in their progeny. The Panel judged these data relevant for assessing human risk.

5.0 SUMMARIES, CONCLUSIONS AND CRITICAL DATA NEEDS

5.1 Summary and Conclusions of Reproductive and Developmental Hazards

5.1.1 Developmental Toxicity

Prenatal developmental toxicity was assessed in CD-1 mice (gd 6–15), Wistar rats (gd 6–15), Golden hamsters (gd 6–10), and Dutch-belted rabbits (gd 6–18) by daily oral intubation. Neither developmental nor maternal toxicity was detected at the highest dose used in each of these studies (mice – 1.6 g/kg bw/day or 10.4 g/kg bw/day; rats – 1.6 g/kg bw/day; hamsters – 1.55 g/kg bw/day; rabbits – 1.23 g/kg bw/day). These data are sufficient to conclude that propylene glycol is not a developmental toxicant in these species under these treatment conditions. These data are assumed relevant for assessing human hazard. No human developmental toxicity data were identified.

5.1.2 Kinetics

The pharmacokinetics of propylene glycol are reasonably well understood in humans as well as animals. Data indicate rapid and extensive absorption followed by rapid distribution into total body water. The rate-determining step in its metabolism is alcohol dehydrogenase which, when saturated, switches from a first order process into a zero order process. Saturation of metabolism appears to occur in rats and rabbits at a dose of about 1.6 to 2 g/kg bw, whereas in humans this seems to happen at a dose of about 0.2 g/kg bw. Since alcohol dehydrogenase activity is not fully developed in infants, saturation of metabolism occurs at lower doses. In accordance with a zero order process, the half-life of propylene glycol in humans and rats increases from about 1.5 hours to more than 5 hours with increasing doses above metabolic saturation. By a NAD-dependent reaction, alcohol dehydrogenase converts propylene glycol to lactaldehyde, which is further metabolized to lactate. Since propylene glycol has a chiral center, technical grade propylene glycol results in the formation of 50/50 D, L-lactate. L-lactate is indistinguishable from endogenous lactate, which is a good substrate for gluconeogenesis. D-lactate is less readily converted to glucose than L-lactate, which prolongs its half-life leading, under conditions of prolonged exposure (e.g., IV infusion), to D-lactic acidosis. It is difficult to cause L-lactic acidosis even with very high doses of propylene glycol because of its efficient detoxification via gluconeogenesis.

The second reason for lack of development of L-lactic acidosis is the saturation of alcohol dehydrogenase, which results in a constant rate of lactate production. Due to removal of L-lactate by gluconeogenesis, a further increase in lactate levels is not possible after saturation of metabolism.

The excretion of propylene glycol is species-dependent. Humans clear about 45% of propylene glycol via kidney, and in dogs, up to 88%. In rats and rabbits, very little of the parent compound is excreted by the kidney until saturation of metabolism occurs. Inhibition of alcohol dehydrogenase by pyrazole increases urinary excretion of propylene glycol to 75% in rats, as expected.

Since propylene glycol has very low intrinsic toxicity, saturation of metabolism plays a protective role in its toxicity since the conversion of propylene glycol to the more toxic lactate (particularly D-lactate) is slowed. Because of low alcohol dehydrogenase activity in infants and children, this protective effect is more pronounced in infants and up to 5 years of age.

Appendix

There are few uncertainties in the kinetics of propylene glycol. They all relate to the expression of various isoforms of alcohol dehydrogenase in various species and in different tissues. An investigation of the above question almost certainly will provide the answer for the 8–10 times lower dose required for saturation of metabolism in humans compared to rats and rabbits.

5.1.3 Reproductive Toxicity

In an NTP continuous breeding study *(127)*, propylene glycol was administered to mice in the drinking water at up to 5% (w/v) **[10.1 g/kg bw/day].** This dose had no effect on fertility of either males or females in either the first or second generation. These data are sufficient to conclude that propylene glycol is not a reproductive toxicant in males or females or in their progeny under the conditions of this study. These data are assumed relevant for assessing human hazard. No data on human reproductive toxicity were found.

5.2 Summary of Human Exposure

In 1999, 1,083 million pounds of propylene glycol were produced in the U.S. with apparent consumption of 854 million pounds *(5)*. Of the apparent amount consumed, uses included, in million pounds and percentages: unsaturated polyester resins (228, 26.7%); cosmetics and personal care products; pharmaceuticals, and human food (170, 19.9%); liquid detergents (135, 15.8%); deicing fluids (85, 10%); antifreeze/engine coolant (55, 6.4%); paints and coatings (40, 4.7%); tobacco humectant (25, 2.9%); other fluids (32, 3.8%); and other applications (84, 9.8%) *(5)*.

The general population is exposed to propylene glycol by oral intake, dermal contact, and inhalation. The average daily intake of propylene glycol from food products in the United States has been estimated at 2,400 mg/day **[34 mg/kg bw/day for a 70 kg person]** *(13)*. Propylene glycol is an inert ingredient in some pharmaceutical preparations. Propylene glycol is also found in many pharmaceuticals that are administered intravenously, which represents a unique exposure route for certain subpopulations.

Occupational exposure to propylene glycol may occur through dermal contact or through inhalation of airborne propylene glycol from heating or spraying processes. An AIHA WEEL Guide of 50 ppm (total exposure) and an inhalation aerosol exposure of 10 mg/m^3, each an 8-hour TWA, have been recommended *(18)*. Propylene glycol occupational exposure data are limited to several small studies. Laitinen et al. *(20)* measured propylene glycol exposure in motor servicing workers. Propylene glycol was not detected in air, and urinary propylene glycol levels did not differ between exposed workers and unexposed controls. Norbäck et al. *(19)* measured airborne propylene glycol exposure (geometric mean 350 μg/m^3, maximum 12,700 μg/m^3) among Swedish painters during indoor application of water-based paints. Propylene glycol levels were measured in urine samples collected pre- and post-shift from aircraft deicing workers (range: 0.72–13.44 mg/L; 0.41–10.58 mg/g creatinine); and in urine samples from a comparison group (range 0.29–10.7 mg/L, 1.18 mg/g creatinine) *(22)*. In a NIOSH HHE of aircraft deicing workers, personal breathing zone air samples for propylene glycol over a 6-hour period ranged from 10 to 21 mg/m^3, with a mean of 15 mg/m^3 *(24)*.

5.3 Overall Conclusions

No human data on reproductive or developmental toxicity are available. Although the serum half-life of propylene glycol is greater in infants and children than in adults, the concern for postnatal developmental toxicity in infants and children younger than 5 years of age is diminished by low levels

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of alcohol dehydrogenase. Furthermore, published data documenting high blood levels of propylene glycol during continuous therapeutic infusion in pediatric intensive care patients 15 months of age and younger were not associated with any acute toxicity *(66)*. The knowledge that human metabolism of propylene glycol saturates at an 8–10 times lower dose than in rats or rabbits provides further confidence that human developmental or reproductive risks are of negligible concern.

Available data are sufficient to conclude that this compound is not a reproductive or developmental toxicant in mice, rats, hamsters, or rabbits. The oral dose levels identified from animal studies are:

- NOAEL ≥ 10 g/kg bw/d in mice, highest dose tested
- NOAEL ≥ 1.6 g/kg bw/d in rats, highest dose tested
- NOAEL ≥ 1.55 g/kg bw/d in hamsters, highest dose tested
- NOAEL ≥ 1.23 g/kg bw/d in rabbits, highest dose tested

There are no major differences in general toxicity between humans and animals (except the cat), and toxicity only occurs at very high doses (LD_{50} values of 8–46 g/kg in rats, and is estimated to be >15 g/kg bw in humans).

Based on these findings, the Panel concludes that current estimated exposures to propylene glycol are of negligible concern for reproductive or developmental toxicity in humans.

5.4 Critical Data Needs

Although the Panel has only negligible concern for developmental and reproductive effects, it suggests that there is a critical data need for long-term follow up of children and pregnant women exposed to high dose propylene glycol from continuous IV infusion.

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National Toxicology Program
U.S. Department of Health and Human Services

Center For The Evaluation Of Risks To Human Reproduction

PUBLIC COMMENTS ON THE EXPERT PANEL REPORT ON PROPYLENE GLYCOL

Appendix III

000418

COURTNEY M. PRICE
VICE PRESIDENT
CHEMSTAR

American Chemistry Council

July 10, 2003

via US Mail and e-mail

RECEIVED
JUL 14 2003
CERHR

Dr. Michael Shelby
CERHR
P.O. Box 12233
MD EC-32
Research Triangle Park, NC 27709

Re: Comments of the Propylene Oxide/Propylene Glycol Panel on the NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Propylene Glycol (NTP-CERHR-PG-03, May 2003) 68 Federal Register:26325 (May 15, 2003).

Dear Dr. Shelby:

The Propylene Oxide/Propylene Glycol Panel (PO/PG Panel) of the American Chemistry Council submits the attached comments in response to the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) *Expert panel Report on the Reproductive and Developmental Toxicity of Propylene Glycol* (Report) and the May 15, 2003 Federal Register Announcemnt. The member companies of the PO/PG Panel comprise the major domestic producers of propylene glycol in the United States.[1]

The PO/PG Panel commends the CERHR and expert panel participants for conducting an open and collegial review meeting, focused on the robust and voluminous scientific literature relevant to evaluation of the reproductive and developmental toxicity of the glycols. The PO/PG Panel agrees with the expert panel's conclusion that "*current estimated exposures to propylene glycol are of negligible concern for reproductive or developmental toxicity in humans*" (p. 76). While agreeing with the overall conclusions, the PO/PG Panel also notes that many of the deficiencies of the December 2002 draft Report, especially those in Chapter 2 ("General Toxicology and Biological Effects") remain unchanged in the May 2003 Report. As a result of the failure to heed the earlier comments, the expert panel's conclusions are not as strongly supported as the data demonstrate.

Appendix III

[1] Members of the Propylene Oxide/Propylene Glycol Panel are The Dow Chemical Company, Huntsman Corporation, and Lyondell Chemical Company.

1300 Wilson Boulevard, Arlington, VA 22209 • Tel 703-741-5600 • Fax 703-741-6091 • http://www.americanchemistry.com

In lieu of reiterating previously submitted comments, the PO/PG Panel incorporates the comments of January 23, 2003 into these comments by reference, with a request that special attention be paid to the review by Dr. Mark Udden, a clinical hematologist at Baylor College of Medicine, of the failure to heed our earlier comments.

The comments included in the following submission expand on three significant deficiencies in the May 2003 Report that remain little changed from the December 2002 draft:

- Analysis of the data relating to wavy ribs and incomplete ossification of the vertebrae noted in the older FDA developmental toxicity study conducted in rats indicates that this common finding is not attributable to propylene glycol.
- Interpretation of information on the hematological effects of propylene glycol is inconsistent. Overall, evidence that propylene glycol adversely affects red blood cells in rats and humans is lacking.
- Evidence for the existence of potentially sensitive sub-populations is inconsistent, and the CERHR conclusion that such sub-populations exist is not justified.

Unfortunately, the CERHR does not contemplate further revision of the expert panel's Report. The PO/PG Panel requests therefore, that the deficiencies of the Report be addressed in the *NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Propylene Glycol* that will be prepared to complete the CERHR review process.

If you or your staff has any questions, please contact the PO/PG Panel Manager, Dr. Anne P. LeHuray at (703) 741-5630 or *anne_lehuray@americanchemistry.com.*

Sincerely yours,

Courtney M. Price
Vice President, CHEMSTAR

Enclosures

Appendix III

000420

U.S. NATIONAL TOXICOLOGY PROGRAM (NTP)
CENTER FOR THE EVALUATION OF RISKS TO HUMAN REPRODUCTION (CERHR)

NTP-CERHR EXPERT PANEL REPORT
ON THE
REPRODUCTIVE AND DEVELOPMENTAL TOXICITY OF PROPYLENE GLYCOL
MAY 2003
NTP-CENTER-PG-03

COMMENTS
OF THE
PROPYLENE OXIDE/PROPYLENE GLYCOL PANEL
OF THE
AMERICAN CHEMISTRY COUNCIL

COMMENTS SUBMITTED: JULY 10, 2003

Appendix III

000421

INTRODUCTION

The Propylene Oxide/Propylene Glycol Panel (PO/PG Panel) of the American Chemistry Council (ACC) submits these comments in response to the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR) notice (68 Federal Register:26325, May 15, 2003) indicating that CERHR released the *Expert panel Report on the Reproductive and Developmental Toxicity of Propylene Glycol* (Report). As described in the Federal Register notice, the Report is intended to reflect "*the summaries and conclusions of the expert panel's evaluation of the scientific data for potential reproductive and/or developmental hazards associated with exposure to ... propylene glycol.*"

The expert panel met in February 2003 to review and evaluate reproductive and developmental toxicities of propylene glycol. In response to the draft Report released in preparation for the February meeting (67 Federal Register:236, Dec. 9, 2002), the PO/PG Panel submitted detailed comments on the draft Report on January 23, 2003. CERHR posted these comments on its web site (http://cerhr.niehs.nih.gov/news/egpg/pg_pubcomm.html) prior to the expert panel meeting.

The PO/PG Panel commends the CERHR and expert panel participants for conducting an open and collegial review meeting, focused on the robust and voluminous scientific literature relevant to evaluation of the reproductive and developmental toxicity of the glycols. The PO/PG Panel agrees with the expert panel's conclusion that "*current estimated exposures to propylene glycol are of negligible concern for reproductive or developmental toxicity in humans*" (p. 76). While agreeing with the overall conclusions, the PO/PG Panel also notes that many of the deficiencies of the December 2002 draft Report, especially those in Chapter 2 ("General Toxicology and Biological Effects") remain unchanged in the May 2003 Report. As a result of the failure to heed the earlier comments, the expert panel's conclusions are not as strongly supported as the data demonstrates.

In lieu of reiterating previously submitted comments, the PO/PG Panel incorporates the comments of January 23, 2003 into these comments by reference, with a request that special attention be paid to the review by Dr. Mark Udden, a clinical hematologist at Baylor College of Medicine, of the failure to heed our earlier comments.

This set of comments expands on three significant deficiencies in the May 2003 Report that remain little changed from the December 2002 draft:

- Analysis of the data relating to wavy ribs and incomplete ossification of the vertebrae noted in the older FDA developmental toxicity study conducted in rats indicates that this common finding is not attributable to propylene glycol.
- Interpretation of information on the hematological effects of propylene glycol is inconsistent. Overall, evidence that propylene glycol adversely affects red blood cells in rats and humans in lacking.
- Evidence for the existence of potentially sensitive sub-populations is inconsistent, and the CERHR conclusion that such sub-populations exist is not justified.

Unfortunately, the CERHR does not contemplate further revision of the expert panel's Report. The PO/PG requests therefore, that the deficiencies of the Report be addressed in the NTP-CERHR Monograph on the Potential Human Reproductive and Developmental Effects of Propylene Glycol that will be prepared to complete the CERHR review process.

Appendix III

COMMENTS

1. Analysis of the data relating to wavy ribs and incomplete ossification of the vertebrae noted in the older FDA developmental toxicity study conducted in rats indicates that this common finding is not attributable to propylene glycol.

In two instances, the Report contains discussion of a purported effect of propylene glycol on wavy ribs and incomplete ossification of the vertebrae in the older FDA developmental toxicity study conducted in rats:

Page 61:

> With rats, higher numbers of wavy ribs and incomplete ossification of the vertebrae were observed at the same level as the positive control, suggesting a propylene glycol effect. The incidences of these defects did not appear to be dose-related.

Page 62:

> The appropriateness of the NOAEL level for rats (1,600 mg/kg bw/day) given in Table 3-11 depends on the importance attributed to the rib and vertebrae malformations observed.

Table 3-6 of the Report contains the data used to support these statements. Using the data in Table 3-6, the incidence and percentages for wavy ribs and incomplete ossification of the vertebrae have been calculated. The data is presented in the table below.

Wavy ribs						
pup incidence	1/173	46/137	23/179	27/169	11/167	15/180
litter incidence	1/22	16/20	9/23	11/22	5/20	8/24
% pup	0.60%	34%	13%	16%	7%	8%
% litter	5%	80%	39%	50%	25%	33%
Vertebrae – incomplete ossification						
pup incidence	0/0	101/137	1/179	13/169	3/167	18/180
litter incidence	0/0	19/20	1/23	7/22	3/20	9/24
% pup	0%	74%	0.50%	8%	2%	10%
% litter	0%	95%	4%	32%	15%	38%

As demonstrated in the table, the data do not support the statement that "*With rats, higher numbers of wavy ribs and incomplete ossification of the vertebrae were observed at the same level as the positive control....*" Clearly the positive control values (80%, 95%) are more than twice as high as those reported even in the 1,600 mg/kg high dose group (33%, 38%).

One may question whether there was an effect in these endpoints based upon comparison with the negative control ("Control") group. Given the differences in the strains of rats used in the 1973 FDA study and those currently available, it would be inappropriate to conduct a quantitative comparison of the incidence of wavy ribs and incomplete ossification of the vertebrae from historical databases currently available in testing laboratories. However, as a qualitative comparison, it is important to note that historically, wavy ribs and incomplete ossification of the vertebrae are very common findings in developmental toxicity studies conducted with rats. The most striking finding, evident in the data in the table above, is the unusually low incidence of these lesions in the negative control population.

Additional support for discounting the significance of these findings is the lack of any dose-response relationships for these endpoints. For example, in the case of the wavy ribs, the lowest dose level tested (16 mg/kg; 100-fold lower than the high dose group) had a higher incidence that the highest dose level tested (1,600 mg/kg).

2. Interpretation of information on the hematological effects of propylene glycol is inconsistent. Overall, evidence that propylene glycol adversely affects red blood cells in rats and humans in lacking.

The PO/PG Panel previously (January 23, 2003) submitted extensive comments to CERHR on the interpretation of hematological data for propylene glycol presented in the December 2002 draft Report. The previous submission included an expert evaluation of publications cited in the draft Report, prepared by Dr. Mark Udden, a clinical hematologist at Baylor College of Medicine. The May 2003 Report contains little evidence that Dr. Udden's review has been taken into account. Rather than repeat these comments (which remain valid and are presented again to CERHR for consideration), the focus here is instead on the inconsistencies in the Report text noted in the paragraphs that follow.

Section 2.2.2.4, page 38, fourth paragraph

> Christopher et al. (*91*)[1] provide an excellent study that establishes a plausible mechanism for propylene-induced hemolysis and the Bauer et al. (89) study provides important confirmatory evidence for the impairment of hematopoiesis by propylene glycol. Thus the hemolysis potential of high doses of propylene glycol, which is a plausible effect, is firmly established in two species (cat and dog) and reasonably well substantiated in other species including man.

This statement is an over-generalization of the available scientific data, and ignores the several established metabolic and physiological factors that predispose red blood cells from the cat to propylene glycol-induced toxicity. These are well known to the expert panel, because they are discussed at length by Christopher *et al.* (91) and also summarized by Dr. Udden. It is therefore difficult to understand why the expert panel Report appears to conclude that results obtained in the cat are indicative of a potential for propylene glycol to cause adverse hematological effects in humans: overall scientific evidence to support this is lacking. The work of Christopher, Bauer and co-workers focused on the susceptibility of the cat to propylene glycol-induced Heinz body formation with no mention of 'hemolysis' (which appears to be the

[1] Numbers in parentheses refer to the reference numbers of citations in the Report.

main end-point of interest to the expert panel). There also does not appear to be any evidence from either study for any inhibition of hematopoiesis (cited by the expert panel Report as a toxic response to propylene glycol). The PO/PG Panel strongly recommends that CERHR reconsider the basis for these conclusions, which are at variance with the published data.

Indeed, the only evidence presented by the expert panel in support of the "hemolytic capability" of propylene glycol in humans is a 65-year old *in vitro* study by Braun and Cartland (79). The expert panel is referred to Dr. Udden's report which includes several more up-to-date references and which also serves to put these *in vitro* findings into context: the findings are an osmotic, not a toxiciological, phenomenon seen at propylene glycol concentrations of 30% or greater. This contrasts with human *in vivo* data cited elsewhere by the expert panel (*e.g.*, Chicella *et al.*; (66)), which indicates that the maximum concentration of propylene glycol in serum from patients undergoing intravenous (IV) drug therapy is 763±660 mg/l (*i.e.*, approximately 0.08-0.14%), which is several hundred fold lower than that shown to cause hemoloysis *in vitro*. This data suggest that red blood cell hemolysis in patients exposed to propylene glycol by IV infusion is unlikely to be an issue.

With regard to the expert panel's view that the information from Saini *et al.* (90) "*confirms that the haemotopoietic system is also a target of propylene glycol in rats*," the PO/PG Panel again questions the scientific reliability of hematological data obtained from animals subjected to four retro-orbital bleeds within two days with <u>no concurrent control group</u>. Certainly there was a clear effect on some parameters that were measured, but with no controls it cannot be determined if this was due to propylene glycol or day-to-day experimental variability.

Overall, the expert panel's conclusions on the potential hematotoxicity of propylene glycol in humans is unsupported.

3. Evidence for the existence of potentially sensitive sub-populations is inconsistent, and the CERHR conclusion that such sub-populations exist is not justified.

The PO/PG Panel previously submitted (January 23, 2003) extensive comments on the expert panel's view that individuals with compromised liver or kidney function, burn patients or premature infants may be "at increased risk for developing propylene glycol toxicity." There is little evidence that the previous comments have been taken into account in revising the Report, apart from inclusion of some moderating words (*i.e.*, "overdosage of premature infants"; "complex clinical case studies") in the introductory paragraph of Section 2.5 (page 44). Rather than repeat the previously submitted comments (which remain valid) the focus here instead is on inconsistencies in the report text described in paragraphs that follow.

Section 2.5, paragraph 1, line 6 (page 44)

> Patients with impaired liver or kidney function would be at increased risk for developing propylene glycol toxicity (*48*).

This is a logical statement given the importance of hepatic metabolism and renal excretion in overall clearance of propylene glycol from the body. It also seems self-evident that the toxicokinetics and toxicodynamics of propylene glycol will be altered in individuals with pre-existing liver or kidney disease or who develop hepatic or renal insufficiency as a result of clinical treatment. Against this background, interpretation of case reports of lactic acid acidosis,

increased osmolality and other clinical sequelae requires careful objective assessment: while propylene glycol may have been present in the circulation, there is only anecdotal evidence that propylene glycol was responsible for the effects reported in these individuals. This should be made clear in the Report, and the individual references cited by the expert panel should be assessed to determine if the references are scientifically reliable.

Section 2.5.1, page 44, final sentence

> Serum concentrations of propylene glycol received through IV medications have been shown to correlate with serum lactate concentrations in patients with normal renal and hepatic function (*51*).

The expert panel cites Kelner and Bailey (51), who reported serum propylene glycol and lactate concentrations in 5 subjects (two middle aged adults, three infants aged 4 months or younger) with 'normal' renal and liver function and who concluded that "propylene glycol administration may be an important cause of lactic acid acidosis" as support for this statement. In reaching this conclusion, Kelner and Bailey developed a regression model for relating serum concentrations of propylene glycol and lactate in their patients *i.e.*,

$$Y = 0.034X + 2.97$$

where Y is the lactate concentration in serum (mEq/l) at a propylene glycol concentration of X (mg/l). This suggests there is a clear (numerically positive) relationship between propylene glycol and lactate levels in serum.

However, other data cited in the Report, namely the publication by Chicella *et al.* (66; not present in the earlier version of the Report) lead to a very different conclusion. In Chicella *et al.* (66), no correlation was observed between serum propylene glycol and lactate in 11 patients (aged 1-15 months) receiving IV medication containing propylene glycol. Since Chicella *et al.* was a prospective study designed specifically to address issues of interest to the CERHR, it is unclear why its findings have been overlooked in favor of other less reliable data.

As part of their investigation, Chicella *et al.* recorded serum propylene glycol concentrations in their subjects at various time points leading to mean values of 85, 519 and 763 mg/l at (roughly) the start, middle and end of treatment. These are consistent with measured serum propylene glycol concentrations reported by Kelner and Bailey (roughly in a range of 50-400 mg/l). Based on the data and modeling of Kelner and Bailey, equivalent mean serum lactate values of 5.9, 21 and 29 mEq/l would be predicted for the Chicella subjects. In fact Chicella *et al.* reported very modest measured lactate values of 1.9, 1.7 and 1.7 mmol/l, respectively (consistent with their conclusion that no lactic acid acidemia was present in these patients).

The expert panel should consider the data of Chicella *et al.*, rather than those of Kelner and Bailey, to support the conclusions of section 2.5: that propylene glycol is a low concern for lactic acid acidemia.

Section 2.5.2, first line, page 45

> The decreased size of premature infants, and an increased serum half-life for propylene glycol in premature infants (*35, 36*), predispose them to a greater probability of toxic effects from over administration of propylene glycol.

Studies reported by Fligner *et al.* (35), Glasgow *et al.* (36) and Huggon *et al.* (70) are used to support this and related statements present in this section of the Report. As discussed in

the PO/PG Panel's January 23, 2003 submission, patients included in the cited case reports were already suffering from pre-exisiting clinical conditions that likely had a fundamental impact on the disposition of propylene glycol in the body, or they were administered powerful, potentially toxic pharmaceutical treatments as part of their therapy, or a combination of the two. This presumably explains in part the expert panel's observation (page 13, line 7) that data from Fligner *et al.* and Glasgow *et al.* reflect "circumstances that preclude confident extrapolation to a healthy general population." Huggon *et al.* also add a warning that impaired renal failure may have contributed to the increased osmolality recorded in their study. Given the unreliability of this information, the expert panel should re-evaluate whether these studies support the conclusion that premature infants are at greater risk of propylene glycol toxicity.

It is also clear from data presented in Chicella *et al.* (66) that no adverse responses were reported in slightly older infants (age 1-15 months) following IV drug therapy that resulted in a mean serum propylene glycol concentration as high as 763 mg/ml. Therefore, the basis of the expert panel's decision (Section 2.5.2, paragraph 3, final sentence) to "caution" that it may be necessary to monitor lactic acidosis and hyperosmolality in pediatric patients given IV infusions containing propylene glycol is unknown.

Given the considerable uncertainties in these studies (detailed in the PO/PG Panel's January 23, 2003 submission) the conclusion of the May 2003 Report, that the studies provide evidence of a sub-population with an enhanced sensitivity to propylene glycol related toxicity is questionable.

Section 2.5.2, first paragraph, last line (page 45):

> .....propylene glycol.....was associated with cardiopulmonary arrest in one case (*70*).

This statement, citing Huggon *et al.* (70) is factually incorrect: circulatory problems were present in this individual before any exposure to propylene glycol. There is no mention of propylene glycol-induced "cardiopulmonary arrest" in the publication.

Appendix III

NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 331

TOXICOLOGY AND CARCINOGENESIS STUDIES OF MALONALDEHYDE, SODIUM SALT (3-HYDROXY-2-PROPENAL, SODIUM SALT)

(CAS NO. 24382-04-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NATIONAL TOXICOLOGY PROGRAM

The National Toxicology Program (NTP), established in 1978, develops and evaluates scientific information about potentially toxic and hazardous chemicals. This knowledge can be used for protecting the health of the American people and for the primary prevention of disease. By bringing together the relevant programs, staff, and resources from the U.S. Public Health Service, DHHS, the National Toxicology Program has centralized and strengthened activities relating to toxicology research, testing and test development/validation efforts, and the dissemination of toxicological information to the public and scientific communities and to the research and regulatory agencies.

The NTP is made up of four charter DHHS agencies: the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF MALONALDEHYDE, SODIUM SALT (3-HYDROXY-2-PROPENAL, SODIUM SALT)

(CAS NO. 24382-04-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

J.W. Spalding, Ph.D., Chemical Manager

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

November 1988

NTP TR 331

NIH Publication No. 89-2587

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NOTE TO THE READER

This study was performed under the direction of the National Institute of Environmental Health Sciences as a function of the National Toxicology Program. The studies described in this Technical Report have been conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. Animal care and use were in accordance with the U.S. Public Health Service Policy on Humane Care and Use of Animals. All NTP toxicology and carcinogenesis studies are subjected to a data audit before being presented for public peer review.

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to identify any mistakes so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP. Comments and questions about the National Toxicology Program Technical Reports on Toxicology and Carcinogenesis Studies should be directed to Dr. J.E. Huff, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3780).

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge (and while supplies last) from the NTP Public Information Office, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709.

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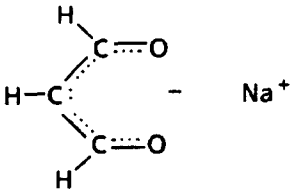
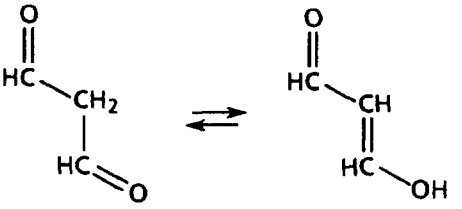


MALONALDEHYDE, SODIUM SALT

CAS No. 24382-04-5

$C_3H_3O_2Na$ Molecular weight 94.04

Synonyms: malonaldehyde, enol, sodium salt; propanedial, sodium; 3-hydroxy-2-propenal, sodium salt; sodium β-oxyacrolein



Malonaldehyde **Enol tautomer**

CAS No. 542-78-9

$C_3H_4O_2$ Molecular weight 72.06

ABSTRACT

Malonaldehyde occurs as a natural metabolic byproduct of prostaglandin biosynthesis and as an end product of polyunsaturated lipid peroxidation. Toxicology and carcinogenesis studies of malonaldehyde were conducted by administering the chemical as malonaldehyde, sodium salt, a stabilized form of malonaldehyde, in distilled water by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex for 14 days, 13 weeks, and 2 years. The study material was 63%-79% malonaldehyde, sodium salt, 22%-38% water, and 1% or less other impurities. The water content was taken into account when the dose mixtures were prepared.

Fourteen-Day and Thirteen-Week Studies: In the 14-day studies, groups of five rats and five mice of each sex were dosed with 250, 500, 750, 1,000, or 1,500 mg/kg malonaldehyde, sodium salt. Controls were untreated. Rats and mice that received 1,500 mg/kg malonaldehyde, sodium salt, did not survive to the end of the 14-day studies. No compound-related gross lesions were seen in the dosed animals.

In the 13-week studies, groups of 10 males and 10 females of each species were administered 0, 30, 60, 125, 250, or 500 mg/kg malonaldehyde, sodium salt. Nine of 10 male rats, 10/10 female rats, 3/10 male mice, and 1/10 female mice that received 500 mg/kg malonaldehyde, sodium salt, died before the

end of the studies. Body weights were reduced by more than 15% in rats receiving 250 or 500 mg/kg and in mice receiving 500 mg/kg.

Compound-related nonneoplastic lesions were present in the stomach, testis, and kidney of rats and in the pancreas, stomach, and testis of mice. Focal and multifocal erosive lesions were observed in the gastric mucosa of the glandular stomach in the 500 mg/kg groups of male and female rats. Dilatation of the gastric glands of the stomach mucosa occurred in the 500 mg/kg male mice. Lesions of the kidney included membranous glomerular nephropathy in the 250 and 500 mg/kg male rats and the 125, 250, and 500 mg/kg female rats and mineralization in the 250 and 500 mg/kg male rats and the 60, 125, 250, and 500 mg/kg female rats. Degeneration of the testicular germinal epithelium was observed in male rats and male mice receiving 250 and 500 mg/kg. Atrophy of the exocrine pancreas was seen in the 125, 250, and 500 mg/kg male and the 250 and 500 mg/kg female mice.

Based on these results, 2-year studies of malonaldehyde, sodium salt, were conducted by exposing groups of 50 F344/N rats of each sex at doses of 0, 50, or 100 mg/kg, administered 5 days per week for 103 weeks. Doses of 0, 60, or 120 mg/kg were administered on the same schedule to groups of 50 male and 50 female $B6C3F_1$ mice.

Body Weight and Survival in the Two-Year Studies: Final mean body weights at the end of the study were reduced by 26% and 36% for high dose male and female rats compared with those for the vehicle controls. The final mean body weight of high dose male mice was 92% that of the vehicle controls. The final mean body weights of low dose male mice, low dose rats, and all groups of female mice were comparable to those of the vehicle controls.

The survival of high dose male and female rats was significantly lower than that of the vehicle controls, with survival declining rapidly after week 76 for high dose males and after week 59 for high dose females (survival--male: vehicle control, 37/50; low dose, 33/50; high dose, 15/50; female: 37/50; 37/50; 14/50). Survival of all groups of male mice was low (male: 24/50; 20/50; 14/50; female: 41/50; 38/50; 30/50). Survival of the high dose group of male mice was significantly lower than that of the vehicle controls; no other significant differences in survival were observed between any groups of mice.

Nonneoplastic and Neoplastic Effects in the Two-Year Studies: The incidences of a variety of nonneoplastic lesions were increased in dosed rats of each sex, primarily in the high dose male and female rat groups. These lesions were ulceration and inflammation of the glandular stomach; epithelial hyperplasia of the forestomach; inflammation of the cornea, retinal atrophy, and cataracts of the crystalline lens; focal lipoid degeneration of the adrenal cortex; and diffuse pancreatic atrophy. Cytoplasmic vacuolization and cystic degeneration in the liver occurred at increased incidences in the high dose rat groups; in addition, the incidences of bile duct hyperplasia and bile duct fibrosis were increased in the high dose female and male rat groups, respectively. Bone marrow hematopoietic hyperplasia, hematopoiesis of the spleen, and ultimobranchial cysts of the thyroid gland occurred with increased incidences in high dose female rats.

The incidences of thyroid gland follicular cell adenomas or carcinomas (combined) were significantly increased in high dose male (vehicle control, 4/50; low dose, 8/49; high dose, 13/50) and female (2/50; 1/50; 7/50) rats. Follicular cell hyperplasia of the thyroid gland also occurred at an increased incidence in high dose female rats (10/50; 10/50; 26/50) but not in male rats (9/50; 7/49; 7/50). The incidence of pancreatic islet cell adenomas was increased in low dose male rats (0/49; 9/50; 1/49). Adenomas and adenomas or carcinomas (combined) of the anterior pituitary gland occurred at significantly lower incidences in high dose rats than those in vehicle controls (combined incidence--male: 20/47; 14/49; 8/49; female: 18/49; 10/49; 2/48).

Nonneoplastic lesions that occurred at increased incidences in dosed mice included atrophy of the pancreatic acinus and dilatation of the uterus. Depigmentation of hair shafts and change of coat color from agouti to gray were observed in high dose mice. No compound-related neoplasms were observed in dosed mice.

Genetic Toxicology: Malonaldehyde, sodium salt, was not mutagenic in the *Salmonella typhimurium*/microsome assay when tested at doses of up to 10,000 µg/plate in a preincubation protocol using the excision-repair deficient strains TA98, TA100, TA1535, and TA1537 with or without S9 metabolic activation. The chemical induced forward mutations in mouse L5178Y lymphoma cells in the absence of S9; it was not tested with S9. Malonaldehyde, sodium salt, was not mutagenic in the *Drosophila melanogaster* sex-linked recessive lethal mutagenicity test in which adult male flies were exposed either by feeding or by abdominal injection. In cytogenetic assays with cultured Chinese hamster ovary (CHO) cells, malonaldehyde, sodium salt, produced a dose-related increase in the frequency of sister chromatid exchanges both in the presence and absence of rat liver S9; no increase in the number of chromosomal aberrations was observed in CHO cells in the absence or presence of S9.

Audit: The data, documents, and pathology materials from the 2-year studies of malonaldehyde, sodium salt, have been audited. The audit found no special circumstances or significant deficiencies in the conduct or documentation of the studies which needed to be taken into consideration for reporting purposes.

Conclusions: Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity** for male and female F344/N rats administered malonaldehyde, sodium salt, as shown by the increased incidences of follicular cell adenomas or carcinomas (combined) of the thyroid gland. Pancreatic islet cell adenomas were also observed at an increased incidence in low dose male rats. There was *no evidence of carcinogenic activity* for $B6C3F_1$ mice administered 60 or 120 mg/kg malonaldehyde, sodium salt, in distilled water by gavage 5 days per week for 2 years.

Chemically related increased incidences of nonneoplastic lesions included ulcers and inflammation of the glandular stomach and epithelial hyperplasia of the forestomach; corneal inflammation, retinal atrophy, and cataracts of the crystalline lens; and cystic degeneration of the liver, bile duct fibrosis, and bile duct hyperplasia in rats. Most of these nonneoplastic lesions as well as the thyroid gland follicular cell neoplasms occurred primarily in the high dose rat groups, in which survival and final body weights were reduced in high dose male and female rats. Increased incidences of atrophy of the pancreatic acinus and pigmentation loss in hair shafts were seen in high dose mice.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 9.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 12-13.

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SUMMARY OF THE TWO-YEAR GAVAGE AND GENETIC TOXICOLOGY STUDIES OF MALONALDEHYDE, SODIUM SALT

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses (mg/kg)			
0, 50, or 100 in distilled water, 5 d/wk	0, 50, or 100 in distilled water, 5 d/wk	0, 60, or 120 in distilled water, 5 d/wk	0, 60, or 120 in distilled water, 5 d/wk
Survival rates in the 2-year study			
37/50; 33/50; 15/50	37/50; 37/50; 14/50	24/50; 20/50; 14/50	41/50; 38/50; 30/50
Nonneoplastic effects			
Ulcers and inflammation of the glandular stomach; epithelial hyperplasia of the forestomach; corneal inflammation, retinal atrophy, cataracts of the crystalline lens; cystic degeneration of the liver; bile duct fibrosis; bile duct hyperplasia	Ulcers and inflammation of the glandular stomach; epithelial hyperplasia of the forestomach; corneal inflammation, retinal atrophy, cataracts of the crystalline lens; cystic degeneration of the liver; bile duct hyperplasia	Atrophy of the pancreatic acinus; pigmentation loss in hair shafts	Atrophy of the pancreatic acinus; pigmentation loss in hair shafts; dilatation of the uterus
Neoplastic effects			
Thyroid gland follicular cell adenomas or carcinomas (combined) (4/50; 8/49; 13/50); pancreatic islet cell adenomas (0/49; 9/50; 1/49)	Thyroid gland follicular cell adenomas or carcinomas (combined) (2/50; 1/50; 7/50)	None	None
Other considerations			
Decrease in incidence of anterior pituitary gland adenomas or carcinomas (combined) (20/47;14/49; 8/49)	Decrease in incidence of anterior pituitary gland adenomas or carcinomas (combined) (18/49;10/49; 2/48)		
Level of evidence of carcinogenic activity			
Clear evidence	Clear evidence	No evidence	No evidence

Genetic toxicology

Salmonella (gene mutation)	Mouse L5178Y/TK$^{+/-}$ (gene mutation)	CHO Cells in Vitro: SCE	CHO Cells in Vitro: Aberration	Drosophila Sex-linked Rec. Lethals
Negative with and without S9	Positive without S9; no test with S9	Positive with and without S9	Negative with and without S9	Negative

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EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential.

Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans.

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence including: animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results ("Clear Evidence" and "Some Evidence"); one category for uncertain findings ("Equivocal Evidence"); one category for no observable effects ("No Evidence"); and one category for experiments that because of major flaws cannot be evaluated ("Inadequate Study"). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Reports series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following quintet is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemically related.
- **No Evidence of Carcinogenic Activity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate Study of Carcinogenic Activity** is demonstrated by studies that because of major qualitative or quantitative limitations cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. This should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- The adequacy of the experimental design and conduct;
- Occurrence of common versus uncommon neoplasia;
- Progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic lesions;
- Some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- Combining benign and malignant tumor incidences known or thought to represent stages of progression in the same organ or tissue;
- Latency in tumor induction;
- Multiplicity in site-specific neoplasia;
- Metastases;
- Supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- The presence or absence of dose relationships;
- The statistical significance of the observed tumor increase;
- The concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- Survival-adjusted analyses and false positive or false negative concerns;
- Structure-activity correlations; and
- In some cases, genetic toxicology.

These considerations together with the definitions as written should be used as composite guidelines for selecting one of the five categories. Additionally, the following concepts (as patterned from the International Agency for Research on Cancer Monographs) have been adopted by the NTP to give further clarification of these issues:

> The term *chemical carcinogenesis* generally means the induction by chemicals of neoplasms not usually observed, the induction by chemicals of more neoplasms than are generally found, or the earlier induction by chemicals of neoplasms that are commonly observed. Different mechanisms may be involved in these situations. Etymologically, the term *carcinogenesis* means induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign neoplasms. In the Technical Reports, the words *tumor* and *neoplasm* are used interchangeably.

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CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of Malonaldehyde, Sodium Salt, is based on the 13-week studies that began in October 1978 and ended in January 1979 and on the 2-year studies that began in February 1980 and ended in February 1982 at Battelle Columbus Laboratories (Columbus, Ohio).

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PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft Technical Report on malonaldehyde, sodium salt, on March 4, 1987, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF MALONALDEHYDE, SODIUM SALT

On March 4, 1987, the draft Technical Report on the toxicology and carcinogenesis studies of malonaldehyde, sodium salt, received peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Dr. J.W. Spalding, NIEHS, began the discussion by reviewing the experimental design, results, and proposed conclusions (clear evidence of carcinogenic activity for male or female rats, no evidence of carcinogenic activity for male or female mice).

Dr. Hughes, a principal reviewer, agreed with the conclusions for male and female mice. He proposed that the conclusions for male and female rats be changed to some evidence of carcinogenic activity because he felt that the maximum tolerated dose was exceeded in rats, possibly perturbing the endocrine axis and perhaps leading to endocrine tumor response through an indirect mechanism, because the total incidence of adenomas and carcinomas of the thyroid gland was low, because both short-term studies and initiation/promotion studies yielded mixed results, and because there was not a dose response for pancreatic islet cell tumors in male rats. Dr. Spalding pointed out that thyroid gland neoplasms are uncommon and the incidences in male and female rats at the top dose were well above the historical control range. Commenting on the inconsistencies in the short-term studies and initiation/promotion data cited, Dr. Spalding said that pre-1980 studies used mixtures of malonaldehyde and intermediates in its synthesis which had mutational activity. Dr. J. Huff, NIEHS, commented that the conclusions for rats were based on the thyroid gland neoplasia, not on the low dose effect for pancreatic tumors.

As a second principal reviewer, Dr. Popp agreed with the conclusions. He agreed with Dr. Hughes that the maximum tolerated dose had been exceeded for high dose male and female rats but felt that the implications were unclear. He suggested that the lower incidence of rats with pituitary gland neoplasms in high dose groups was probably due to reduced survival and was not a primary effect of the chemical, whereas the nonneoplastic eye lesions probably were chemically related. Dr. Spalding agreed that the eye lesions were chemically related and said that the discussion would be expanded.

As a third principal reviewer, Dr. Gallo agreed with the conclusions but noted that when the maximum tolerated dose is exceeded, interpretation of either positive or negative findings is sometimes difficult. He added, however, that based on the 13-week studies, the doses selected for the 2-year studies were appropriate. Since the chemical is an intermediate in the biosynthesis of prostaglandins, he suggested that the toxicity may override control mechanisms in the synthetic pathway. Dr. Gallo thought that the rationale for deciding to study malonaldehyde was weak.

In other discussion, Dr. Sivak proposed that a statement be included for the rat studies which indicates reduced survival and body weight gain in top dose groups. Dr. Hooper requested that all the genetic toxicology data be organized into a summary table to help the reader draw conclusions about the mutagenic activity of the malonaldehyde salt (see page 8 and Appendix E).

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SUMMARY OF PEER REVIEW COMMENTS (Continued)

Dr. Hughes moved that the Technical Report on malonaldehyde, sodium salt, be accepted with the conclusions as written for mice, no evidence of carcinogenic activity, but with the conclusions for rats changed to some evidence of carcinogenic activity, along with a statement that the maximum tolerated dose had been exceeded. Dr. Sivak asked that the statement be amended to replace the expression maximum tolerated dose with a description of the biologic alterations themselves, i.e., that there was decreased survival and a greater than 10% decrease in body weight gain in high dose groups. Dr. Hughes agreed. Dr. Gallo seconded the amended motion, and after considerable discussion, it was defeated by six votes to one (Dr. Hughes). Dr. Gallo moved that the Technical Report be accepted with the conclusions as written for mice, no evidence of carcinogenic activity, and for rats, clear evidence of carcinogenic activity, with Dr. Sivak's amendment. Dr. Sivak seconded the amended motion, and it was approved by six votes to one (Dr. Hughes).

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I. INTRODUCTION

Occurrence
Toxicity and Metabolism
Carcinogenicity Studies
Genetic Toxicology
Study Rationale

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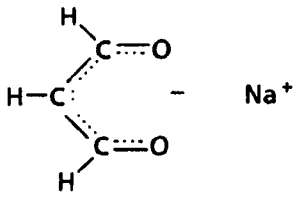
I. INTRODUCTION

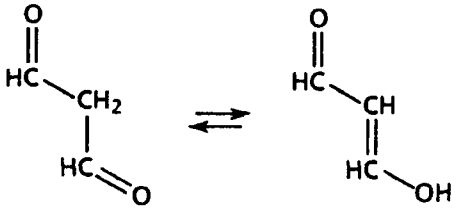


MALONALDEHYDE, SODIUM SALT

CAS No. 24382-04-5

$C_3H_3O_2Na$ Molecular weight 94.04

Synonyms: malonaldehyde, enol, sodium salt; propanedial, sodium; 3-hydroxy-2-propenal, sodium salt; sodium β-oxyacrolein



Malonaldehyde **Enol tautomer**

CAS No. 542-78-9

$C_3H_4O_2$ Molecular weight 72.06

Malonaldehyde is a three-carbon dialdehyde that in dilute aqueous solution exists in equilibrium with its enol form (Mashio and Kimura, 1960). Malonaldehyde exists in stable form as the enolate sodium salt (Huttel, 1941) and can be prepared from either 1,1,3,3-tetraethoxypropane or 1,1,3,3-tetramethoxypropane by acid hydrolysis.

Occurrence

Malonaldehyde occurs as a natural byproduct of the cyclooxygenase reaction in prostaglandin biosynthesis (Draper et al., 1986) and is formed as an end product of polyunsaturated lipid peroxidation (Bernheim et al., 1948; Hamberg and Samuelsson, 1967; Diczfalusy et al., 1977). The relative content of malonaldehyde in foods has been associated with oxidative rancidity; detection of malonaldehyde at low or minimal levels has been used for many years as a measure of the wholesomeness and freshness of foods. The reaction of malonaldehyde with 2-thiobarbituric acid to form a chromogen of extremely high absorptivity is the basis of a sensitive method for the qualitative measurement of malonaldehyde in food and food products (Crawford et al., 1965).

Two surveys have been conducted in North America to measure the malonaldehyde content of food items. In a study of 96 samples of fresh meat and fish obtained from supermarkets in Ontario, Canada, Siu and Draper (1978) found that the malonaldehyde content ranged from 0.14 μg/g in cooked ham to 10.05 μg/g in cooked chicken. The levels of malonaldehyde found by the Canadian study were somewhat lower than those described in a study by Shamberger et al. (1977), who reported that malonaldehyde levels ranged from 1 to 14 μg/g of tissue in food items

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obtained from Cleveland, Ohio, supermarkets and that cooking of the meat resulted in small decreases or twofold to fivefold increases in malonaldehyde content. Assuming that the average U.S. citizen consumes 120 g of meat daily (NAS, 1981), and based on the malonaldehyde content of cooked meat reported by Shamberger et al. (1974), it is estimated that the amount of malonaldehyde ingested per person per day through meat consumption alone could range from 240 to 1,200 µg from beef or turkey to 3,600 µg from chicken.

The impact, if any, of malonaldehyde on human health at these concentrations is not known. However, reports on the potential mutagenic and carcinogenic character (Shamberger et al., 1974) of this chemical have suggested the desirability of minimizing its occurrence during storage, marketing, and preparation of food.

Malonaldehyde in the presence of nitrite has been reported to facilitate the formation of nitrosamines from dimethylamine, diethylamine, piperidine, pyrrolidine, and morpholine (Kikugawa et al., 1980), although it is not clear whether malonaldehyde's stimulation of nitrosamine formation is much greater than that observed for the unsaturated fatty acids from which malonaldehyde is derived (Goutefongea et al., 1977).

Toxicity and Metabolism

The mean LD_{50} value for malonaldehyde, sodium salt, administered by gavage was determined to be 632 mg/kg body weight in male Wistar rats (Crawford et al., 1965). The mean LD_{50} value for malonaldehyde administered by gavage to female Swiss mice was 606 mg/kg body weight (Apaja, 1980).

After intubation of two male Wistar rats with [1,3-^{14}C]malonaldehyde (approximately 27.5 µg/kg), 84%-96% of the radiolabel was eliminated in 12 hours (Siu and Draper, 1982). Between 60% and 70% of the total dose was expired as [^{14}C]carbon dioxide, 9%-17% was recovered in the urine, and 5%-15%, in the feces. In vitro studies with [1,3-^{14}C]malonaldehyde demonstrated that malonaldehyde is metabolized primarily in the mitochondria. The probable pathway of malonaldehyde metabolism involves oxidation by mitochondrial aldehyde dehydrogenase followed by decarboxylation to produce carbon dioxide and acetate.

Malonaldehyde reacts readily with sulfhydryl and amino groups of proteins (Shin et al., 1972). It produces intramolecular and intermolecular linkages that result in the inactivation and polymerization of enzymes (Chio and Tappel, 1969). It can react with the nitrogenous bases of DNA (Brooks and Klamerth, 1968; Reiss et al., 1972) and thus inhibit DNA, RNA, and protein synthesis (Bird and Draper, 1980).

Carcinogenicity Studies

Groups of 50 female ICR Swiss mice were exposed for 12 months to a preparation of malonaldehyde, sodium salt (greater than 98% pure), in drinking water (acidified to pH 4 to prevent malonaldehyde polymerization) at concentrations calculated to provide a daily dose of 0, 0.1, 1, or 10 mg/kg (Bird et al., 1982a). There was no significant increase in tumors observed at any site in the dosed animals. Compound-related lesions were confined to the liver, but the total liver lesions were not dose dependent. The liver lesions included anisokaryosis, changes in cytoplasmic volume of hepatocytes, hepatocellular nodular hyperplasia (control, 0/48; 0.1 mg/kg, 1/49; 1 mg/kg, 2/50; 10 mg/kg, 2/48), and hepatomas (0/48; 0/49; 2/50; 0/48).

A malonaldehyde preparation of undefined purity administered to 25 animals in each dose group for 100 weeks in drinking water at concentrations of 1,250, 2,500, or 5,000 ppm did not increase the tumor incidence in male or female Swiss mice (Apaja, 1980). More males than females died at all three concentrations. At the highest concentration, there were no surviving males at week 80, and only one female survived to the end of the study. In a two-stage dermal carcinogenesis study, application of a single dose of either 6 or 12 mg of malonaldehyde in acetone to the backs of female Swiss mice (30 mice per dose group) was followed 3 weeks later by the application (5 days per week) of 0.1% croton oil for 27 weeks (Shamberger et al., 1974). Fifty-two percent of the dosed mice developed keratoacanthomas; the first tumors were seen at 11 weeks.

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The interpretation of these results is confounded because the malonaldehyde preparation used in this study was of undefined purity and the doses of 6 and 12 mg are estimates based on the assumption that malonaldehyde was the only hydrolysis product of 1,1,3,3-tetramethoxypropane. Marnett and Tuttle (1980) subsequently demonstrated that the acid hydrolysis of 1,1,3,3-tetramethoxypropane also produces several reactive chemical intermediate species, such as β-methoxyacrolein and 3,3-dimethoxypropionaldehyde, which are 20-30 times more mutagenic than the pure form of malonaldehyde or malonaldehyde, sodium salt.

In a later two-stage dermal carcinogenicity study of 28-52 weeks' duration, purified malonaldehyde, sodium salt, in acetone:dimethyl sulfoxide (80:20) was found to be inactive as a tumor initiator, promoter, or complete carcinogen for male and female SENCAR mice (40 mice per dose group) (Fischer et al., 1983). Doses of malonaldehyde, sodium salt, ranging from 20 to 500 µg were administered as single applications in studies to detect activity as an initiator or a complete carcinogen, and the same doses were administered in repetitive applications in a study designed to detect promoter activity. Benzo[*a*]pyrene served as a positive control for tumor initiation and as the complete carcinogen in this study, whereas 12-*O*-tetradecanoyl phorbol-13-acetate was used as the positive control for promotion. However, the highest dose (500 µg) of malonaldehyde used in these studies was tenfold less than the lowest dose applied in the study reported by Shamberger et al. (1974). Malonaldehyde, sodium salt, was also inactive in the in vitro Chinese hamster V-79 cell metabolic cooperation assay for promoters (Fischer et al., 1983).

Genetic Toxicology

Malonaldehyde was first shown to be mutagenic in several excision-repair-competent frameshift mutant strains of Salmonella in the absence of exogenous metabolic activation (Mukai and Goldstein, 1976; Shamberger et al., 1979). The tester strain D3052 was the most sensitive to malonaldehyde activity in both studies. However, the purity of malonaldehyde in these studies was undefined. In the earlier study (Mukai and Goldstein, 1976), malonaldehyde was generated by the acid hydrolysis of 1,1,3,3-tetramethoxypropane, and the hydrolysate was used directly as the source of malonaldehyde; the quantification of malonaldehyde was based on the amount of starting material 1,1,3,3-tetramethoxypropane and on the assumption that the reaction went to completion with malonaldehyde as the only end product. In the Shamberger et al. study (1979), neither the source nor method of malonaldehyde preparation was given, although in an earlier in vivo study reported by the same laboratory (Shamberger et al., 1974), malonaldehyde was prepared from 1,1,3,3-tetramethoxypropane in the manner described above. Subsequent studies (Marnett and Tuttle, 1980; Basu and Marnett, 1983) demonstrated that the product of 1,1,3,3-tetramethoxypropane hydrolysis included substantial amounts of reactive intermediates such as β-methoxyacrolein and 3,3-dimethoxypropionaldehyde, which were 125-160 and 105-135 times more active, respectively, as Salmonella mutagens than was malonaldehyde. Therefore, the mutagenic activity attributed to malonaldehyde in the earlier investigations is suspect. The mutagenicity of a highly purified preparation of malonaldehyde, sodium salt, in Salmonella strain D3052 was confirmed by Marnett and Tuttle (1980), although the magnitude of response was much less than that reported by the earlier investigators. Malonaldehyde was also found to be inactive in Salmonella strains TA98, TA100, TA1535, and TA1538, which are deficient in excision-repair capability. These observations confirmed the earlier studies of Mukai and Goldstein (1976) and Shamberger et al. (1979).

In a later study, further confirmation of the mutagenicity of malonaldehyde, sodium salt, in Salmonella came from the results of plate incorporation assays (Basu and Marnett, 1983) which demonstrated a dose-dependent increase in revertant colonies of the excision-repair-competent frameshift mutant strain D3052 over a dose range of 0-20 µmol/plate. The mutation frequency was low, about 5 revertants/µmol of malonaldehyde. A similar mutation frequency in two recently developed excision-repair-competent *Salmonella typhimurium* strains TA102 and TA104, which are base-substitution mutants that are highly sensitive to carbonyl compounds,

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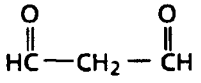
was observed after exposure to malonaldehyde, sodium salt, in a liquid preincubation procedure without S9 metabolic activation (Marnett et al., 1985). Malonaldehyde, sodium salt, was also reported to be an effective mutagen through base substitution in repair-competent *Escherichia coli* H/r30 but not in *E. coli* strains that were repair deficient (Yonei and Furui, 1981). The mutagenicity of malonaldehyde, sodium salt, was not observed by NTP in *S. typhimurium* assays with a preincubation protocol with the excision-repair-deficient strains TA98, TA100, TA1535, or TA1537 with or without Aroclor 1254-induced liver S9 from male Sprague Dawley rats or Syrian hamsters (Mortelmans et al., 1986; Table E1). These results reported by the NTP do confirm the earlier observations of Marnett and Tuttle (1980) that Salmonella tester strains that are deficient in excision-repair capability are not sensitive to malonaldehyde.

In NTP tests with mouse L5178Y lymphoma cells, malonaldehyde, sodium salt, induced forward mutations at the TK locus over a concentration range of 125-1,000 µg/ml in the absence of S9; it was not tested with S9 (Table E2). Yau (1979) also reported the induction of mutations in mouse L5178Y cells by malonaldehyde (undetermined purity and generated from the acid hydrolysis of tetramethoxypropane) within a concentration range of 10-100 µM and in the absence of exogenous metabolic activation. Feeding malonaldehyde (unspecified purity) to Drosophila larvae at a dose that resulted in 50% lethality induced a low frequency of somatic mosaicism that was detected in adult wing and eye tissue but did not induce sex-linked recessive lethal mutations (Szabad et al., 1983). Likewise, in NTP tests, no induction of sex-linked recessive lethal mutations was seen in Drosophila when malonaldehyde, sodium salt, was fed to adult males at a concentration of 25,000 ppm or injected as a 10,000-ppm solution (Woodruff et al., 1985; Table E5). Dose-related increases in micronuclei and chromosomal aberrations were observed in cultured rat skin fibroblast cells treated with 10^{-4} to 10^{-2} M concentrations of malonaldehyde, sodium salt, generated by acid hydrolysis of tetramethoxypropane and judged to be approximately 98% pure (Bird et al., 1982b). In NTP cytogenetic assays with cultured Chinese hamster ovary (CHO) cells, malonaldehyde, sodium salt, produced a clear dose-related increase in the frequency of sister chromatid exchanges both in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9. No increase in chromosomal aberrations was observed in CHO cells after treatment with up to 409 µg/ml of malonaldehyde, sodium salt, in the absence of S9 or up to 3,270 µg/ml in the presence of S9 (Tables E3 and E4).

Study Rationale

Malonaldehyde was nominated by the NCI for toxicology and carcinogenesis studies because of reports in the literature that this byproduct of lipid peroxidation of polyunsaturated fatty acids was mutagenic in bacteria (Mukai and Goldstein, 1976) and active as an initiator in a two-stage dermal carcinogenicity study in rodents (Shamberger et al., 1974). Further, malonaldehyde (I) has a structural resemblance to two known carcinogens, glycidaldehyde (II) and β-propiolactone (III) (Van Duuren et al , 1963). The oral route of administration was chosen for the present studies because human exposure to exogenous malonaldehyde is through the diet



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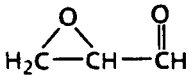

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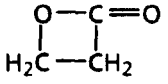

III

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II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF MALONALDEHYDE, SODIUM SALT

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

- Study Design
- Source and Specifications of Animals
- Animal Maintenance
- Clinical Examinations and Pathology
- Statistical Methods

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF MALONALDEHYDE, SODIUM SALT

3-Hydroxy-2-propenal, sodium salt (referred to in this report as malonaldehyde, sodium salt), was synthesized at the Midwest Research Institute (MRI) (Kansas City, Missouri) by the hydrolysis of 1,1,3,3-tetramethoxypropane. The starting material was obtained in 14 lots from Aldrich Chemical Company (Table 1). Each lot was converted to malonaldehyde, sodium salt, by mixing it with 1.0 N hydrochloric acid and stirring for 3 hours under nitrogen at 0° C. After storage for 48 hours under nitrogen at 5° C, sodium hydroxide was added to produce a reaction mixture with a pH of 10 which was maintained at 0° C. Malonaldehyde, sodium salt, was precipitated from the reaction mixture by the addition of acetone, separated by suction filtration, and dried in a desiccator over sodium hydroxide.

The study material was characterized by elemental analysis, ultraviolet/visible and nuclear magnetic resonance spectroscopy, and Karl Fischer water analysis. The nuclear magnetic resonance spectrum agreed with that expected for the structure of malonaldehyde enolate for all the batches prepared (Figure 1). The nuclear magnetic resonance spectra did not detect the presence of 3,3-dimethoxypropionaldehyde or methoxyacrolein, which are potential toxic impurities in the study material. Table 2 presents a summary of the analysis for each batch of study material. The analytical reports for the analyses performed in support of the malonaldehyde, sodium salt, studies are on file at NIEHS.

Stability studies of the bulk malonaldehyde, sodium salt, were run for 3 weeks at −20° C. Purity analysis by ultraviolet spectroscopy (266 nm) established that no degradation of the chemical occurred. Further confirmation of the stability of the bulk material during the toxicity studies (storage at −20° C) was obtained by Karl Fischer water analysis and ultraviolet spectroscopic analysis. No notable degradation was seen over the course of the studies.

TABLE 1. IDENTITY AND SOURCE OF 1,1,3,3-TETRAMETHOXYPROPANE LOTS USED IN THE PREPARATION OF MALONALDEHYDE, SODIUM SALT, FOR THE GAVAGE STUDIES

Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
Lot Number		
JHW11	JHW11, DE 112778, DE 112978	DE 122078, DC80-23, GB32880, GB80-80-2A, GB80-80-5G, GB80-80-5F, GB80-80-8, GB80-80-9-11, KK80-160-12, LK80-160-13, GO-80-160-20
Date of Initial Use		
Rats--8/24/78; mice--8/21/78	10/24/78, 12/19/78, 1/9/79	DE 122078: 3/17/80; DC80-23: 4/14/80; GB32880: 5/1/80; GB80-80-2A: 5/5/80; GB80-80-5G: 5/19/80; GB80-80-5F: 7/10/80; GB80-80-8: 8/6/80; GB80-80-9-11: rats--9/18/80, mice--10/2/80; KK80-160-12: rats--12/18/80, mice--1/22/81; LK80-160-13: rats--6/4/81, mice--5/6/81; GO-80-160-20: 9/21/81
Supplier		
Aldrich Chemical Co. (Milwaukee, WI)	Same as 14-d studies	Same as 14-d studies

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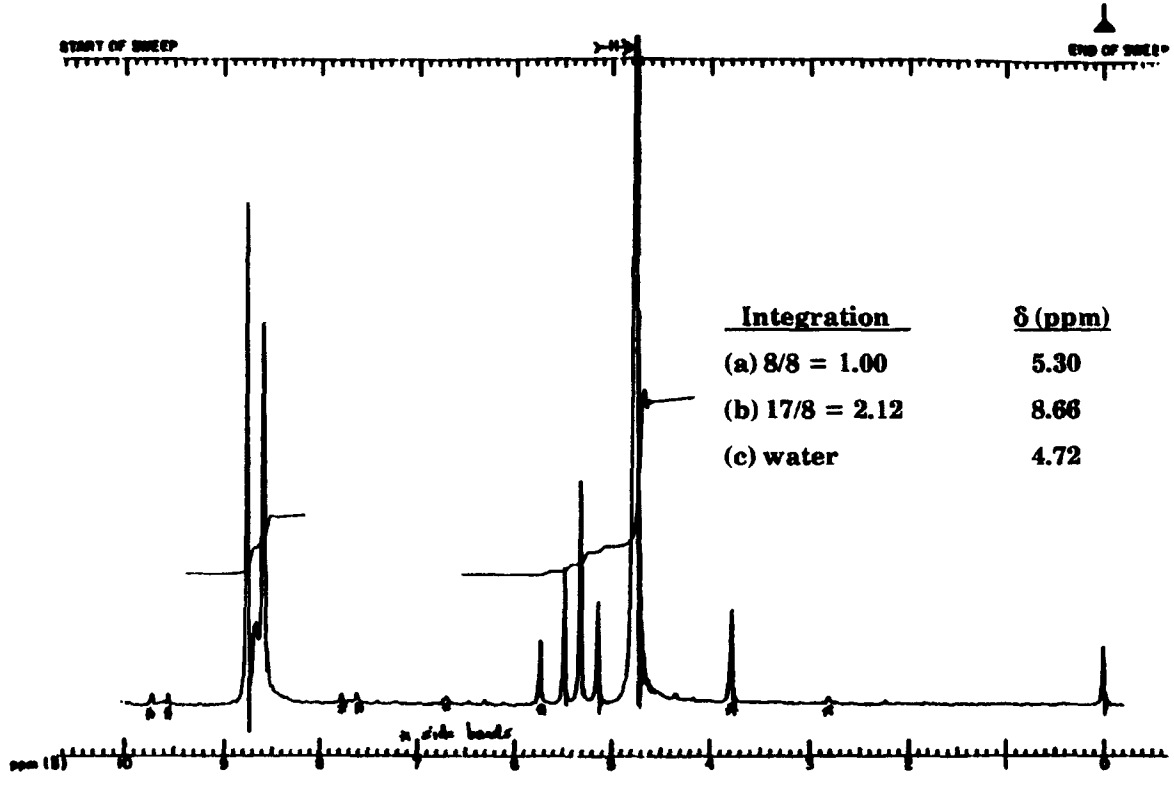

FIGURE 1. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF MALONALDEHYDE, SODIUM SALT (LOT NO. LK80-160-13)

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TABLE 2. SUMMARY OF RESULTS OF PURITY ANALYSIS OF THE LOTS USED IN THE GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Lot No. of Starting Material	Malonaldehyde, Sodium Salt (percent) (a)	Water (percent) (b)	Chloride (percent) (c)	Acetone (percent) (d)
JHW11	70.1 ± 1.4	30.0 ± 0.0	--	0.8
DE 112778	68.9 ± 0.5	30.6 ± 0.5	0.28	Trace
DE 112978	70.3 ± 0.3	31.5 ± 1.1	0.74	Trace
DE 122078	76.4 ± 0.9	24.4 ± 0.3	0.69	--
DC80-23	78.9 ± 0.4	21.9 ± 0.4	0.28	--
GB32880	66.6 ± 1.5	32.2 ± 0.6	1.0	Trace
GB80-80-2A	64.3 ± 2.2	35.2 ± 0.8	0.1	--
GB80-80-5G	64.4 ± 1.3	37.3 ± 0.8	0.5	--
GB80-80-5F	64.8 ± 0.5	37.8 ± 0.9	0.5	--
GB80-80-8	63.4 ± 0.4	36.0 ± 0.2	0.87	--
GB80-80-9-11	65.6 ± 0.3	33.1 ± 0.9	0.76	--
KK80-160-12	63.4 ± 1.1	35.2 ± 0.3	0.3	--
LK80-160-13	71.2 ± 0.5	29.6 ± 0.5	0.2	--
GO-80-160-20	67.8 ± 1.0	35.0 ± 1.5	0.6	--

(a) Based on ultraviolet spectroscopic analysis at 266 nm; mean ± standard deviation.
(b) Karl Fischer titration; mean ± standard deviation.
(c) By elemental analysis
(d) By nuclear magnetic resonance spectroscopy

PREPARATION AND CHARACTERIZATION OF DOSE MIXTURES

Dose mixtures were prepared by dissolving enough study material in distilled water to give the desired concentration (w/v) of anhydrous malonaldehyde, sodium salt (Table 3). Dose mixture stability studies by ultraviolet spectroscopy indicated that a solution of 1% malonaldehyde, sodium salt, in water was stable at −20° C for 7 days. Aliquots of formulated solutions of malonaldehyde, sodium salt, in water were stored at −20° C for no longer than 7 days in the 2-year studies.

Periodic analysis for malonaldehyde, sodium salt, in dose mixtures was performed by the study and analytical chemistry laboratories by the same spectrophotometric method to determine if the dose mixtures contained the correct concentrations of malonaldehyde, sodium salt. The results of analysis of one dose formulation (100 mg/ml) during the 13-week studies indicated that the concentration (99.6 mg/ml) was within specifications (±10% of the target concentration). During the 2-year studies, the dose mixtures were analyzed once every 2 months; concentrations varied from 53.5% to 127.1% of the target concentrations (Table 4). All dose mixtures except those mixed on 9/25/80 were within 91.0%-110.4% of the target concentrations. The dose mixtures prepared on 9/25/80 were outside of the specification limits and, therefore, not administered to the animals. The dose mixtures subsequently prepared on 9/26/80 were within specifications and were administered to the animals. Because 48/52 dose mixtures analyzed were within 10% of the target concentration, it is estimated that the dose mixtures were prepared within specifications 92% of the time. Results of periodic referee analysis performed by the analytical chemistry laboratory indicated variable agreement with the results from the study laboratory (Table 5).

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TABLE 3. PREPARATION AND STORAGE OF DOSE MIXTURES IN THE GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
Preparation A stock solution was prepared by placing a weighed portion of malonaldehyde, sodium salt, in a graduated cylinder, adding distilled water to the proper volume, and mechanically stirring for 40 min. Dose mixtures were prepared by serial dilution of the stock solution	Similar to 14-d studies	Similar to 14-d studies
Maximum Storage Time 17 d	7 d	7 d
Storage Conditions −20° C in the dark; vials of dose mixture kept at room temperature for 30-60 min before gavage administration	Same as 14-d studies	Similar to 14-d studies

TABLE 4. RESULTS OF ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Date Mixed	Concentration of Malonaldehyde, Sodium Salt, in Water for Target Concentration (mg/ml) (a)			
	10	12	20	24
02/21/80	10.6	12.5	19.85	25.15
04/10/80	9.5	12.15	19.85	24.0
06/05/80	9.75	13.25	20.5	24.35
08/07/80	10.0	11.9	19.8	25.2
09/25/80	(b) 6.1	(b) 8.4	(b) 10.7	(b) 30.5
09/26/80	(c) 10.1	(c) 12.5	(c) 19.9	(c) 24.8
11/20/80	10.7	12.5	18.6	24.2
01/29/81	9.71	11.39	19.96	24.66
03/12/81	10.3	12.9	20.5	26.2
05/14/81	9.8	13.0	20.4	22.8
07/09/81	9.8	12.6	19.2	24.3
09/17/81	9.7	12.0	20.3	24.6
11/06/81	9.8	11.8	19.6	24.0
01/08/82	9.1	11.4	18.8	24.2
Mean (mg/ml)	9.6	12.0	19.1	24.9
Standard deviation	1.14	1.22	2.59	1.85
Coefficient of variation (percent)	11.9	10.2	13.6	7.4
Range (mg/ml)	6.1-10.7	8.4-13.25	10.7-20.5	22.8-30.5
Number of samples	13	13	13	13

(a) Results of duplicate analysis
(b) Out of specifications, not administered to animals
(c) Remix, not included in the mean

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TABLE 5. RESULTS OF REFEREE ANALYSIS OF DOSE MIXTURES IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Date Mixed	Target Concentration (mg/ml)	Determined Concentration (mg/ml) Study Laboratory (a)	Referee Laboratory (b)
04/10/80	12	12.15	8.8
09/25/80	10	6.1	9.5
03/12/81	24	26.2	24.3
09/10/81	20	(c) 19.9	(c) 14.8
01/08/82	10	9.1	8.9

(a) Results of duplicate analysis
(b) Results of triplicate analysis
(c) Results from samples taken from animal room during dosing

FOURTEEN-DAY STUDIES

Male and female F344/N rats and $B6C3F_1$ mice were obtained from Charles River Breeding Laboratories and held for 14 days (rats) and 17 days (mice) before the studies began. Groups of five males and five females of each species were administered 250, 500, 750, 1,000, or 1,500 mg/kg malonaldehyde, sodium salt, in distilled water by gavage for 14 consecutive days. (Dose mixtures were adjusted for water content in the bulk chemical; animals were actually given 360, 720, 1,080, 1,440, or 2,160 mg/kg of the bulk malonaldehyde, sodium salt.) Controls were untreated.

Rats and mice were housed five per cage. Feed and water were available ad libitum. Details of animal maintenance are presented in Table 6. Rats and mice were observed two times per day and weighed initially and at the end of the studies. A necropsy was performed on all animals.

THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated administration of malonaldehyde, sodium salt, and to determine the doses to be used in the 2-year studies.

Four- to five-week-old male and female F344/N rats and $B6C3F_1$ mice were obtained from Charles River Breeding Laboratories, held for 17 days, and then assigned to cages according to a table of random numbers. Vehicle control and dosed groups were assigned to cages according to another table of random numbers.

Groups of 10 rats and 10 mice of each sex were administered 0, 30, 60, 125, 250, or 500 mg/kg malonaldehyde, sodium salt (anhydrous equivalent), in distilled water by gavage 5 days per week for 13 weeks.

Rats and mice were housed five per cage in polycarbonate cages. Feed and water were available ad libitum. Further experimental details are summarized in Table 6. Animals were checked two times per day; moribund animals were killed. Individual animal weights were recorded once per week.

At the end of the 13-week studies, survivors were killed. A necropsy was performed on all animals except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 6.

TWO-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats were administered malonaldehyde, sodium salt, at doses equivalent to 0, 50, or 100 mg/kg anhydrous malonaldehyde, sodium salt, in distilled water by gavage 5 days per week for 103 weeks. Groups of 50 male and 50 female mice were administered 0, 60, or 120 mg/kg on the same schedule.

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TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN		
Size of Study Groups		
5 males and 5 females of each species	10 males and 10 females of each species	50 males and 50 females of each species
Doses		
250, 500, 750, 1,000, or 1,500 mg/kg malonaldehyde, sodium salt, in distilled water by gavage; dose vol--10 ml/kg; controls were untreated	0, 30, 60, 125, 250, or 500 mg/kg malonaldehyde, sodium salt, in distilled water by gavage; dose vol -5 ml/kg	Rats--0, 50, or 100 mg/kg malonaldehyde, sodium salt, in distilled water by gavage; mice--0, 60, or 120 mg/kg; dose vol--5 ml/kg
Date of First Dose		
Rats--8/24/78; mice--8/21/78	10/24/78	Rats--2/18/80; mice--2/25/80
Date of Last Dose		
Rats--9/6/78, mice--9/3/78	1/22/79	Rats--2/5/82; mice--2/12/82
Duration of Dosing		
14 consecutive d	5 d/wk for 13 wk	5 d/wk for 102 wk
Type and Frequency of Observation		
Observed 2 × d; weighed initially and at the end of the studies	Observed 2 × d; weighed initially and 1 × wk thereafter	Observed 2 × d; weighed initially, 1 × wk for 13 wk, and 1 × mo thereafter
Necropsy and Histologic Examination		
Necropsy performed on all animals, histologic examination not performed	Necropsy performed on all animals; the following tissues examined histologically for vehicle control and high dose groups, for all animals dying before terminal kill, and for lower dose animals with compound-related lesions: adrenal glands, brain, colon, esophagus, femur, gallbladder (mice), heart, kidneys, liver, lungs and mainstem bronchi, mammary gland, mandibular lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, small intestine, stomach, thymus, thyroid gland, trachea, and urinary bladder	Necropsy and histologic examination performed on all animals; the following tissues were examined: adrenal glands, brain, cecum, colon, duodenum, esophagus, eyes, femur including marrow, gallbladder (mice), gross lesions, heart, kidneys, larynx, liver, lungs and mainstem bronchi, mammary gland, mandibular lymph nodes, pancreas, parathyroids, pituitary gland, prostate/testes or ovaries/uterus, salivary glands, skin, small intestine, spleen, stomach, thymus, thyroid gland, tissue masses, trachea, and urinary bladder
ANIMALS AND ANIMAL MAINTENANCE		
Strain and Species		
F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice	F344/N rats; B6C3F$_1$ mice
Animal Source		
Charles River Breeding Laboratories (Portage, MI)	Same as 14-d studies	Rats--Charles River Breeding Laboratories (Portage, MI); mice--Charles River Breeding Laboratories (Kingston, NY)
Study Laboratory		
Battelle Columbus Laboratories	Battelle Columbus Laboratories	Battelle Columbus Laboratories
Method of Animal Identification		
By cage	Toe clip	Toe clip and ear notch
Time Held Before Study		
Rats--14 d, mice--17 d	17 d	Rats--18 d; mice--20 d
Age When Placed on Study		
6 wk	7 wk	Rats--7 wk, mice--8 wk

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TABLE 6. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT (Continued)

Fourteen-Day Studies	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE (Continued)		
Age When Killed		
8 wk	20 wk	Rats--111-112 wk; mice--112-113 wk
Necropsy Dates		
Rats--9/8/78; mice--9/5/78	Rats--1/23/79; mice--1/24/79	Rats--2/16/82-2/19/82; mice--2/22/82-2/25/82
Method of Animal Distribution		
Distributed according to tables of random numbers	Same as 14-d studies	Animals distributed to weight classes and then assigned to cages according to one table of random numbers and to groups according to another table
Feed		
Purina Lab Chow® pellets (Ralston Purina Co., St. Louis, MO); available ad libitum	Same as 14-d studies	NIH 07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA) except for week of 4/27/81 when Purina Lab Chow® was used; available ad libitum
Bedding		
Absorb-Dri (Lab Products, Inc., Garfield, NJ)	Same as 14-d studies	Same as 14-d studies
Water		
Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as 14-d studies	Same as 14-d studies
Cages		
Polycarbonate (Lab Products, Inc., Garfield, NJ)	Same as 14-d studies	Same as 14-d studies
Cage Filters		
Reemay spun-bonded polyester filters (Snow Filtration, Cincinnati, OH)	Same as 14-d studies	Same as 14-d studies
Animals per Cage		
5	5	5
Other Chemicals on Study in the Same Room		
None	None	None
Animal Room Environment		
Temp--20°-23° C; humidity--40%-60%; fluorescent light 12 h/d; 15 room air changes/h	Same as 14-d studies	Temp--17°-26° C; humidity--15%-63%; fluorescent light 12 h/d; 15 room air changes/h

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Source and Specifications of Animals

The male and female F344/N rats and $B6C3F_1$ (C57BL/6N, female × C3H/HeN MTV^-, male) mice used in these studies were produced under strict barrier conditions at Charles River Breeding Laboratories under a contract to the Carcinogenesis Program. Breeding stock for the foundation colonies at the production facility originated at the National Institutes of Health Repository. Animals shipped for study were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Rats were shipped to the study laboratory at 4 weeks of age and mice at 5 weeks of age. The rats were quarantined at the study laboratory for 18 days and the mice for 20 days. Thereafter, a complete necropsy was performed on five animals of each sex and species to assess their health status. The rats were placed on study at 7 weeks of age and the mice at 8 weeks of age. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix F).

A quality control skin grafting program has been in effect since early 1978 to monitor the genetic integrity of the inbred mice used to produce the hybrid $B6C3F_1$ study animal. In mid-1981, data were obtained that showed incompatibility between the NIH C3H reference colony and the C3H colony from a Program supplier. In August 1981, inbred parental lines of mice were further tested for genetic integrity via isozyme and protein electrophoresis profiles that demonstrate phenotype expressions of known genetic loci.

The C57BL/6N mice were homogeneous at all loci tested. Eighty-five percent of the C3H mice monitored were variant at one to three loci, indicating some heterogeneity in the C3H line from this supplier. Nevertheless, the genome of this line is more homogeneous than that of randomly bred stocks.

Male mice from the C3H colony and female mice from the C57BL/6N colony were used as parents for the hybrid $B6C3F_1$ mice used in these studies. The influence of the potential genetic nonuniformity in the hybrid mice on these results is not known, but results of the studies are not affected because concurrent controls were included in each study.

Animal Maintenance

Rats and mice were housed five per cage. Feed and water were available ad libitum. Cages were not rotated during the studies (Figure 2). Details of animal maintenance are summarized in Table 6.

Clinical Examinations and Pathology

All animals were observed two times per day, and clinical signs were recorded once per day for the first 20 months and once per month thereafter. Body weights by cage were recorded once per week for the first 13 weeks of the studies and once per month thereafter. Mean body weights were calculated for each group. Animals found moribund and those surviving to the end of the studies were humanely killed. A necropsy was performed on all animals including those found dead, unless they were excessively autolyzed or cannibalized, missexed, or found missing. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study.

During necropsy, all organs and tissues were examined for grossly visible lesions. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissues examined microscopically are listed in Table 6.

When the pathology evaluation was completed, 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 quality assessment laboratory. The individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assessment pathologist. The

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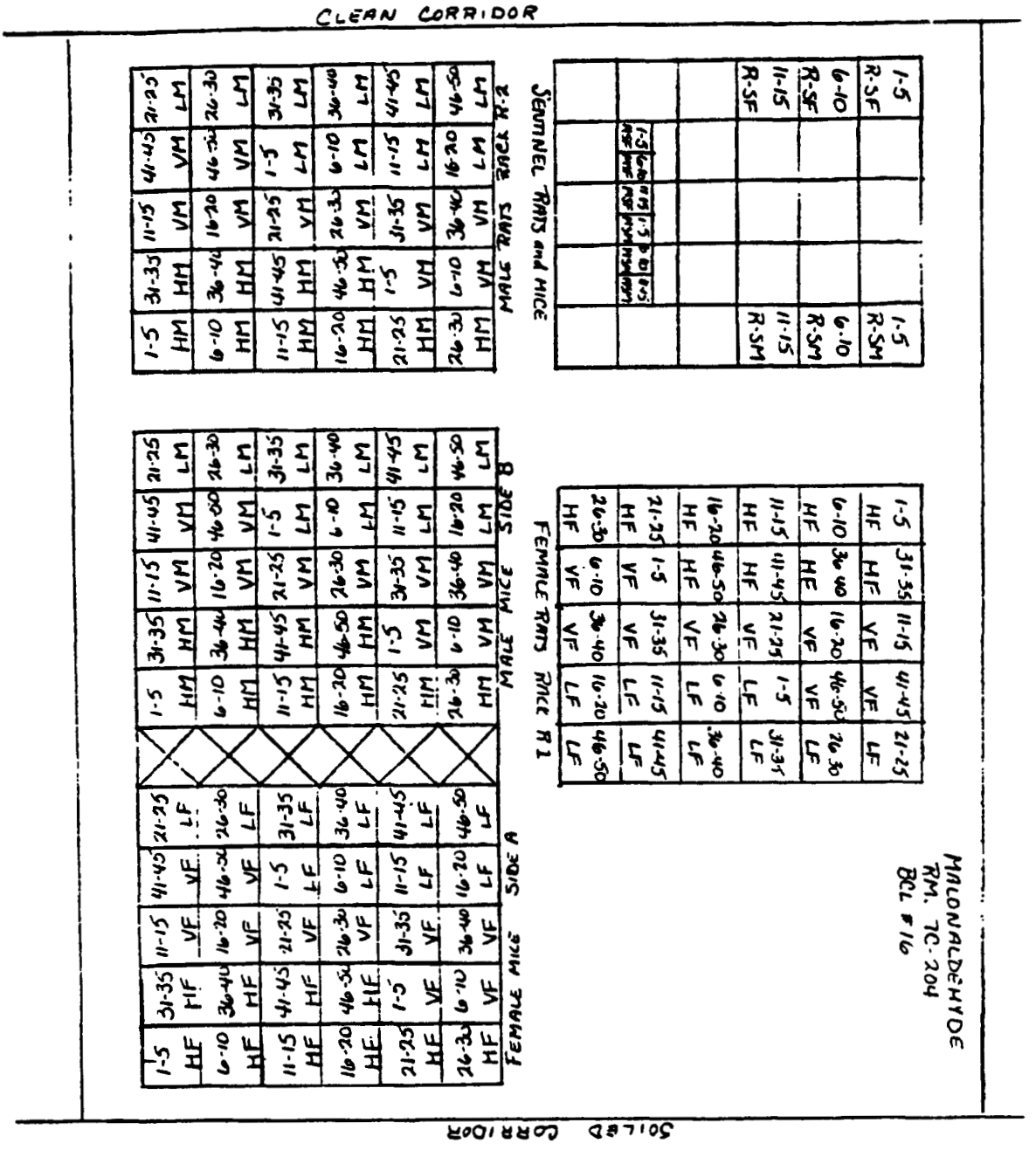

FIGURE 2. CAGE LOCATIONS FOR RATS AND MICE IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

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quality assessment report and slides were submitted to the Pathology Working Group (PWG) Chairperson, who reviewed all target tissues and those about which there was a disagreement between the laboratory and quality assessment pathologists.

Representative slides selected by the Chairperson were reviewed by the PWG without knowledge of previously rendered diagnoses. When the consensus diagnosis of the PWG differed from that of the laboratory pathologist, the laboratory pathologist was asked to reconsider the original diagnosis. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Group. For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are combined according to the guidelines of McConnell et al. (1986).

Slides/tissues are generally not evaluated in a blind fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle or unless there is an inconsistent diagnosis of lesions by the laboratory pathologist. Nonneoplastic lesions are not examined routinely by the quality assessment pathologist or PWG unless they are considered part of the toxic effect of the chemical.

Statistical Methods

Data Recording: Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, survival, body weight, and individual pathology results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Survival Analyses: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found to be missing or dead from other than natural causes; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) life table test for a dose-related trend. When significant survival differences were detected, additional analyses using these procedures were carried out to determine the time point at which significant differences in the survival curves were first detected. All reported P values for the survival analysis are two-sided.

Calculation of Incidence: The incidence of neoplastic or nonneoplastic lesions is given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence: Three statistical methods are used to analyze tumor incidence data. The two that adjust for intercurrent mortality employ the classical method for combining contingency tables developed by Mantel and Haenszel (1959). Tests of significance included pairwise comparisons of high dose and low dose groups with vehicle controls and tests for overall dose-response trends.

For studies in which compound administration has little effect on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death. Continuity-corrected tests are used in analysis of tumor incidence, and reported P values are one-sided. The procedures described below were also used to analyze the incidence of selected nonneoplastic lesions.

Life Table Analyses--The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the

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studies were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and vehicle control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the studies, were then combined by the Mantel-Haenszel method to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975). The underlying variable considered by this analysis is time to death due to tumor. If the tumor is rapidly lethal, then time to death due to tumor closely approximates time to tumor onset. In this case, the life table test also provides a comparison of the time-specific tumor incidences.

Incidental Tumor Analyses--The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the studies were "incidental"; i.e., they were merely observed at necropsy in animals dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and vehicle control groups were compared in each of five time intervals: weeks 0-52, weeks 53-78, weeks 79-92, week 93 to the week before the terminal-kill period, and the terminal-kill period. The denominators of these proportions were the number of animals actually examined for tumors during the time interval. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See Haseman, 1984, for the computational details of both methods.)

Unadjusted Analyses--Primarily, survival-adjusted methods are used to evaluate tumor incidence. In addition, the results of the Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage, 1971; Gart et al., 1979) are given in the appendixes containing the analyses of primary tumor incidence. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

Historical Control Data: Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control data base (Haseman et al., 1984, 1985) are included for those tumors appearing to show compound-related effects.

III. RESULTS

RATS

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

MICE

FOURTEEN-DAY STUDIES

THIRTEEN-WEEK STUDIES

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Survival

Pathology and Statistical Analyses of Results

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FOURTEEN-DAY STUDIES

All rats that received 1,500 mg/kg malonaldehyde, sodium salt, 2/5 males and 3/5 females that received 1,000 mg/kg, and 1/5 males that received 750 mg/kg died before the end of the studies (Table 7). Final mean body weights of rats that received 750 or 1,000 mg/kg malonaldehyde, sodium salt, were 15% or 7% lower than that of the controls for males and 24% or 25% lower for females. All dosed animals had rough hair coats. By day 4, the color of the urine of all dosed animals was a shade similar to that of the study material. By day 11, all surviving rats at 750 and 1,000 mg/kg exhibited generalized body weakness. The tissues from animals in the 14-day studies were not examined microscopically.

THIRTEEN-WEEK STUDIES

Nine of 10 male rats and 10/10 female rats that received 500 mg/kg malonaldehyde, sodium salt, died before the end of the studies (Table 8). Final mean body weights of males that received 125, 250, or 500 mg/kg were 5%, 16%, or 39% lower than that of the vehicle controls. Final mean body weights of females that received 125 or 250 mg/kg were 5% or 14% lower than that of the vehicle controls.

During the course of the 13-week studies, the clinical signs preceding the early deaths or moribund termination in the highest dosed animals were rough hair coats, red exudate around the eyes, and impairment of hindleg motor ability to the extent of complete immobilization in some animals.

TABLE 7. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE FOURTEEN-DAY GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent)
MALE					
(c) 0	5/5	117	177	+60	
250	5/5	115	165	+50	93.2
500	5/5	123	178	+55	100.6
750	(d) 4/5	107	151	+44	85.3
1,000	(e) 3/5	124	165	+41	93.2
1,500	(f) 0/5	109	(g)	(g)	(g)
FEMALE					
(c) 0	5/5	98	141	+43	
250	5/5	102	136	+34	96.5
500	5/5	102	129	+27	91.5
750	5/5	97	107	+10	75.9
1,000	(h) 2/5	103	106	+3	75.2
1,500	(i) 0/5	102	(g)	(g)	(g)

(a) Number surviving/number in group
(b) Mean body weight change of the survivors
(c) Controls were untreated.
(d) Day of death: 15 (1 day after last dose)
(e) Day of death: 4,7
(f) Day of death: 4,4,5,6,8
(g) No data are reported due to the 100% mortality in this group.
(h) Day of death: 9,11,14
(i) Day of death: 3,4,5,7,7

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TABLE 8. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	10/10	132 ± 4	331 ± 6	+199 ± 4	
30	10/10	137 ± 5	347 ± 2	+210 ± 5	105
60	10/10	139 ± 4	345 ± 10	+206 ± 8	104
125	10/10	142 ± 4	314 ± 6	+172 ± 5	95
250	10/10	139 ± 4	278 ± 8	+139 ± 9	84
500	(d) 1/10	138 ± 3	203 ± 0	+68 ± 0	61
FEMALE					
0	10/10	113 ± 1	195 ± 3	+82 ± 2	
30	10/10	107 ± 2	187 ± 4	+80 ± 2	96
60	10/10	105 ± 3	188 ± 3	+83 ± 1	96
125	10/10	110 ± 3	185 ± 6	+75 ± 3	95
250	10/10	112 ± 2	167 ± 4	+55 ± 4	86
500	(e) 0/10	110 ± 2	(f)	(f)	(f)

(a) Number surviving/number initially in group
(b) Initial mean group body weight ± standard error of the mean. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Week of death: 3,6,7,8,8,8,8,10,12
(e) Week of death: 5,5,7,7,7,7,7,7,8,8
(f) No data are reported due to the 100% mortality in this group.

Compound-related lesions were present in the stomach, kidney, and testis, primarily in the two highest dose groups (Table 9). Lesions in the kidney consisted of thickenings in the glomerular tuft, which appeared to involve basement membranes and mesangial areas. The designation "membranous glomerular nephropathy" was given to the lesion for which evidence of thickening of Bowman's capsule or proliferation of visceral or parietal epithelium was present without associated inflammation. These kidney lesions occurred in nine males and eight females at 500 mg/kg, one male and nine females at 250 mg/kg, and six females at 125 mg/kg. Mild mineralization of the kidney was present in male rats from the two highest dose groups and in female rats from the four highest dose groups. Renal tubular pigmentation and/or basophilia occurred in several rats from the 500 mg/kg group. Renal tubular pigmentation was observed in 10 females in the 250 mg/kg group. Focal and multifocal erosive lesions were observed in the gastric mucosa of the glandular stomach in 500 mg/kg male and female rats. A diffuse degeneration of virtually all the testicular germinal epithelium was observed in 3/10 males that received 250 mg/kg and in 9/10 males that received 500 mg/kg; the lesions in the 250 mg/kg group were less severe than those in the 500 mg/kg group. Lymphoid depletion in the spleen, thymus, and mandibular lymph nodes occurred in the two highest male and female dose groups, and increased splenic extramedullary hematopoiesis and bone marrow hyperplasia were observed in the 500 mg/kg groups.

Dose Selection Rationale: Because of reduced weight gain and compound-related lesions in the bone marrow, spleen, lymph nodes, thymus, stomach, kidney, and testis at the higher doses in the 13-week studies, doses selected for rats for the 2-year studies were 50 and 100 mg/kg malonaldehyde, sodium salt, administered in distilled water by gavage 5 days per week.

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TABLE 9. NUMBER OF RATS WITH LESIONS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Site/Lesion	Dose (mg/kg) 0	60	125	250	500
MALE					
Number of rats examined	10	10	10	10	10
Stomach, gastric mucosa					
Erosion	0	0	0	0	5
Kidney					
Membranous glomerular nephropathy	0	0	0	1	9
Mineralization	0	0	0	6	9
Tubular pigmentation	0	0	0	0	4
Tubular basophilia	0	0	0	0	3
Bone marrow					
Hyperplasia	0	0	0	0	5
Spleen					
Lymphoid depletion, B-cell area	0	0	0	9	6
Lymphoid depletion	0	0	0	0	4
Extramedullary hematopoiesis	0	0	0	0	7
Mandibular lymph node					
Lymphoid depletion	0	0	0	0	2
Thymus					
Lymphocytic depletion	0	0	0	0	3
Testis, seminiferous tubules					
Degeneration	0	0	0	3	9
FEMALE					
Number of rats examined	10	10	10	10	10
Stomach, gastric mucosa					
Erosion	0	0	0	0	4
Kidney					
Membranous glomerular nephropathy	0	0	6	10	8
Mineralization	0	2	6	7	5
Tubular pigmentation	0	0	0	10	1
Tubular basophilia	0	0	0	0	6
Bone marrow					
Hyperplasia	0	0	0	0	4
Spleen					
Lymphoid depletion, B-cell area	0	0	0	10	7
Lymphoid depletion	0	0	0	0	3
Extramedullary hematopoiesis	0	0	0	0	9
Mandibular lymph node					
Lymphoid depletion	0	0	0	0	3
Thymus					
Lymphocytic depletion	0	0	0	0	5

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of high dose male rats were 10%-20% lower than those of the vehicle controls from week 33 to week 72 and 20%-26% lower from week 72 to the end of the study (Table 10 and Figure 3). Mean body weights of low dose male rats were 3%-7% lower than those of the vehicle controls from week 67 to the end of the study. Mean body weights of high dose female rats were 10%-20% lower than those of the vehicle controls from week 54 to week 72 and 21%-36% lower from week 72 to the end of the study. Mean body weights of low dose and vehicle control female rats were similar throughout the study. No compound-related clinical signs were observed.

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TABLE 10. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Weeks on Study	Vehicle Control Av. Wt. (grams)	Vehicle Control No. of Survivors	50 mg/kg Av. Wt. (grams)	50 mg/kg Wt. (percent of veh. controls)	50 mg/kg No. of Survivors	100 mg/kg Av. Wt. (grams)	100 mg/kg Wt. (percent of veh. controls)	100 mg/kg No. of Survivors
MALE								
0	111	50	115	104	50	112	101	50
1	171	50	170	99	50	171	100	50
2	199	50	198	99	50	200	101	50
3	219	50	219	100	50	223	102	50
4	234	50	238	102	50	240	103	50
5	250	50	255	102	50	259	104	50
6	264	50	265	100	50	267	101	50
7	267	50	269	101	50	271	101	50
8	280	50	281	100	50	279	100	50
9	293	50	294	100	50	295	101	50
10	304	50	305	100	50	305	100	50
11	309	50	310	100	50	302	98	50
12	318	50	317	100	50	307	97	50
13	324	50	321	99	50	313	97	50
16	342	50	340	99	50	328	96	50
20	359	50	358	100	50	344	96	50
25	391	50	388	99	50	367	94	50
29	396	50	402	102	50	367	93	50
33	408	50	401	98	50	369	90	50
37	420	50	417	99	50	378	90	50
41	425	50	423	100	50	372	88	49
46	431	50	432	100	50	383	89	48
50	448	50	445	99	50	391	87	48
54	453	50	442	98	50	385	85	48
59	460	49	447	97	50	381	83	48
63	457	48	447	98	50	368	81	47
67	460	48	448	97	50	375	82	47
72	469	48	452	96	50	367	78	46
76	459	48	427	93	48	350	76	45
78	456	48	424	93	48	351	77	43
81	455	44	429	94	47	345	76	37
85	452	43	427	94	46	353	78	32
90	462	39	445	96	42	347	75	27
94	461	39	445	97	40	341	74	23
99	448	37	434	97	36	334	75	19
103	447	37	430	96	33	332	74	15
FEMALE								
0	98	50	102	104	50	101	103	50
1	128	50	130	103	50	127	101	50
2	137	50	139	101	50	136	99	50
3	143	50	146	102	50	144	101	50
4	152	50	156	103	50	152	100	50
5	160	50	161	101	50	159	99	50
6	164	50	166	101	50	164	100	50
7	164	50	166	101	50	163	99	50
8	167	50	169	101	50	166	99	50
9	171	50	173	101	50	169	99	50
10	174	50	175	101	50	173	99	50
11	174	50	175	101	50	171	98	50
12	178	50	179	101	49	173	97	50
13	181	50	183	101	49	177	98	50
16	189	50	187	99	49	184	97	50
20	190	50	192	101	49	190	100	50
25	205	49	207	101	49	203	99	50
29	207	49	208	100	49	202	98	50
33	208	49	211	101	49	203	98	50
37	213	48	215	101	49	205	96	50
41	216	48	220	102	49	207	96	50
46	223	48	228	102	48	215	96	50
50	234	48	243	104	47	214	91	50
54	240	48	247	103	47	214	89	49
59	245	48	251	102	47	206	84	43
63	253	47	256	101	47	214	85	39
67	260	47	262	101	46	215	83	39
72	274	47	276	101	45	217	79	37
76	269	47	272	101	45	211	78	35
78	271	46	268	99	45	207	76	34
81	272	46	267	98	45	215	79	31
85	280	44	275	98	43	213	76	26
90	286	42	280	98	42	207	72	25
94	282	42	280	99	42	199	71	23
99	286	39	286	100	39	203	71	16
103	290	37	289	100	37	187	64	14

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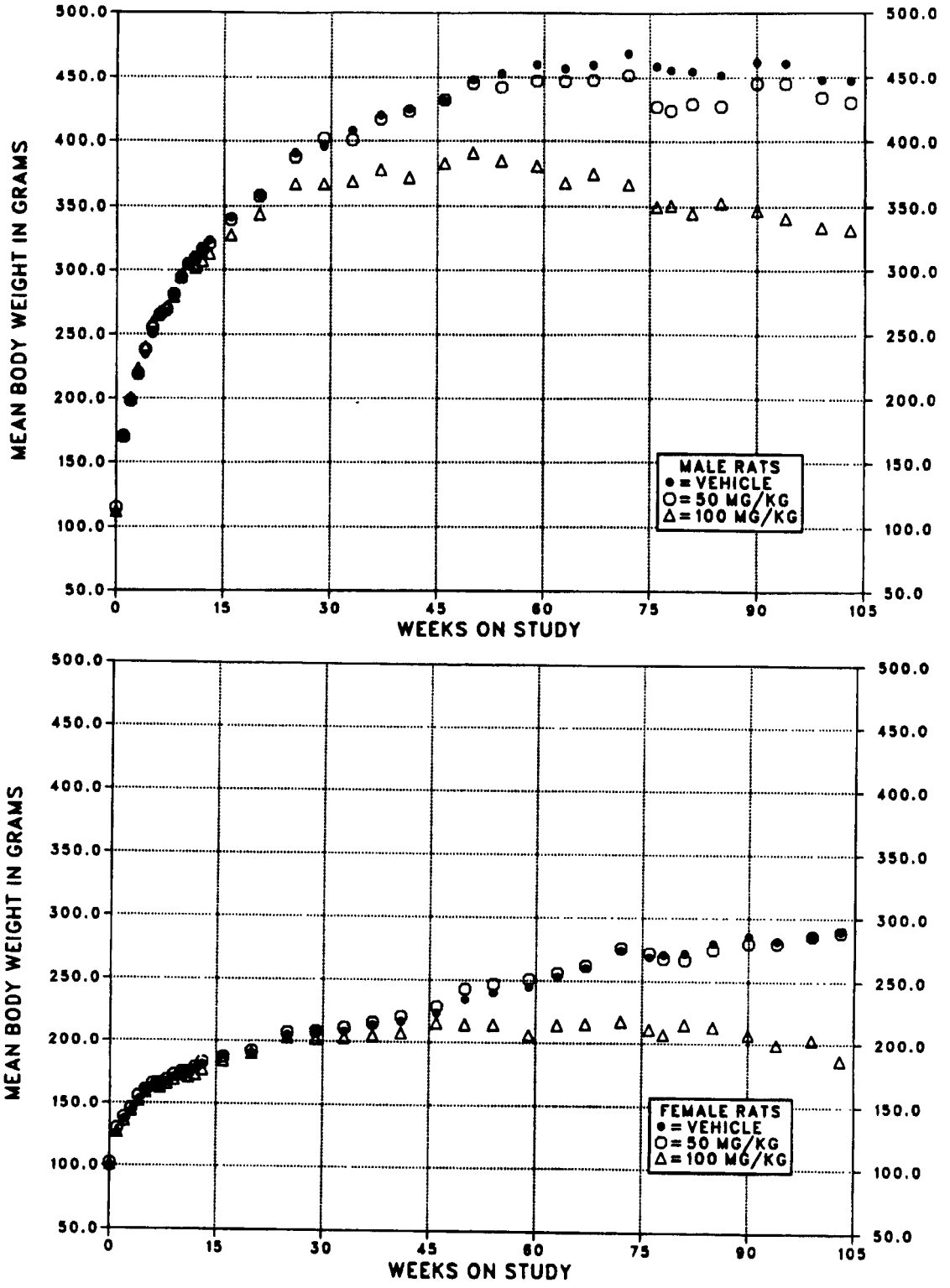

FIGURE 3. GROWTH CURVES FOR RATS ADMINISTERED MALONALDEHYDE, SODIUM SALT, IN WATER BY GAVAGE FOR TWO YEARS

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Survival

Estimates of the probabilities of survival for male and female rats administered malonaldehyde, sodium salt, at the doses used in these studies and for vehicle controls are shown in Table 11 and in the Kaplan and Meier curves in Figure 4. Survival of the high dose groups of both male (after week 88) and female (after week 68) rats was significantly lower than that of the vehicle controls.

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions of the thyroid gland, pancreatic islets, pancreas, subcutaneous tissue, hematopoietic system (bone marrow, spleen, or multiple organs), adrenal gland, anterior pituitary gland, liver, stomach, and eye.

Lesions in male rats are summarized in Appendix A. Histopathologic findings on neoplasms are summarized in Table A1. Table A2 gives the survival and tumor status for individual male rats. Table A3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table A3 (footnotes). Historical incidences of tumors in control male rats are listed in Table A4. Findings on nonneoplastic lesions are summarized in Table A5.

Lesions in female rats are summarized in Appendix B. Histopathologic findings on neoplasms are summarized in Table B1. Table B2 gives the survival and tumor status for individual female rats. Table B3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table B3 (footnotes). Historical incidences of tumors in control female rats are listed in Table B4. Findings on nonneoplastic lesions are summarized in Table B5.

TABLE 11. SURVIVAL OF RATS IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	50 mg/kg	100 mg/kg
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	13	17	34
Accidentally killed	0	0	1
Killed at termination	37	33	15
Survival P values (c)	<0.001	0.633	<0.001
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	13	12	34
Accidentally killed	0	1	2
Killed at termination	37	37	14
Survival P values (c)	<0.001	0.846	<0.001

(a) Terminal-kill period: male--week 104; female--weeks 104-105
(b) Includes animals killed in a moribund condition
(c) The result of the life table trend test is in the vehicle control column, and the results of the life table pairwise comparisons with the vehicle controls are in the dosed columns.

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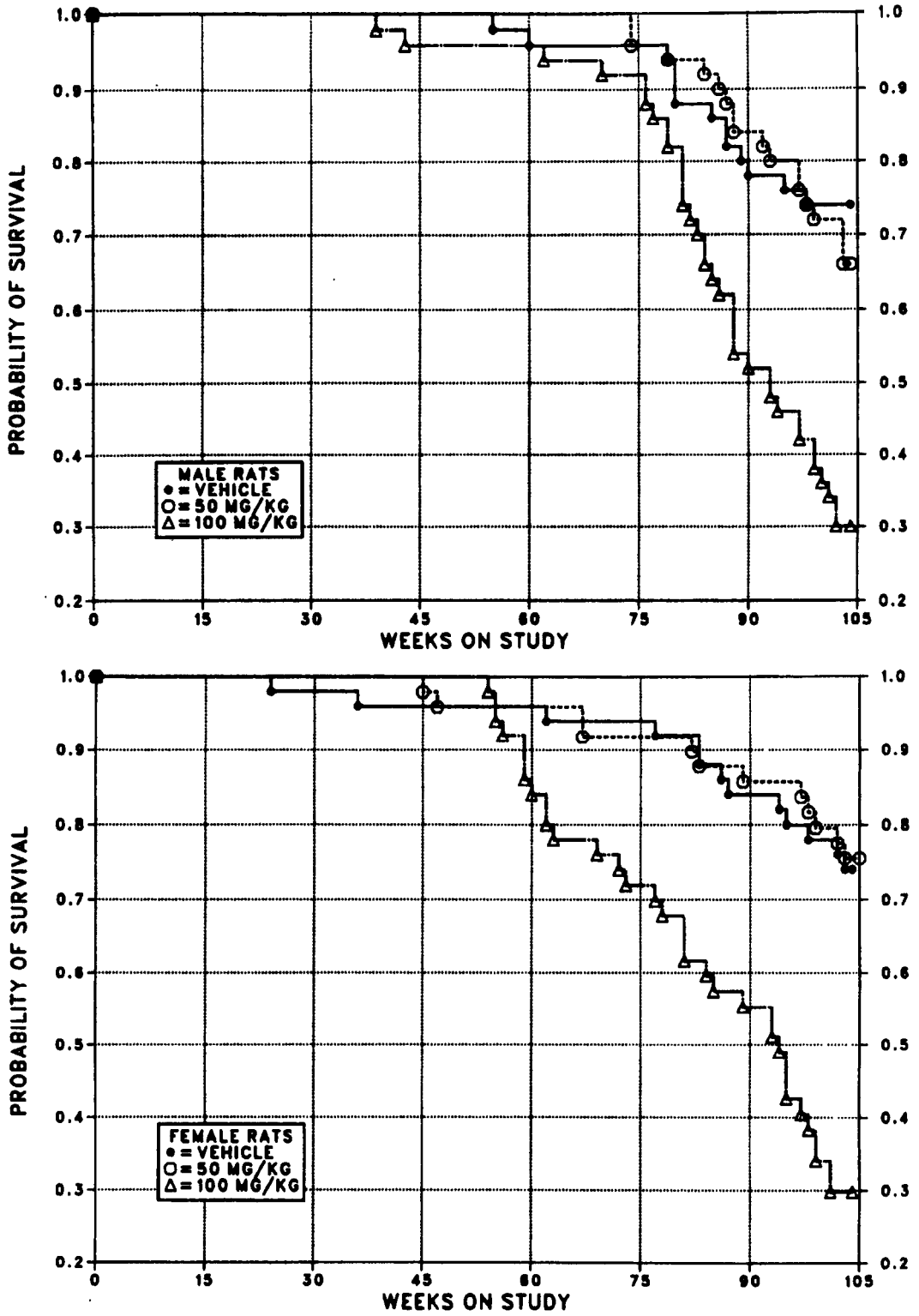

FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES FOR RATS ADMINISTERED MALONALDEHYDE, SODIUM SALT, IN WATER BY GAVAGE FOR TWO YEARS

Thyroid Gland: Follicular cell hyperplasia was observed at an increased incidence ($P<0.001$) in high dose female rats (Table 12). Hyperplasia was characterized by micropapillary folds of columnar cells or microfollicle formation on the wall of slightly enlarged follicles that did not compress adjacent structures.

Follicular cell adenomas, carcinomas, and adenomas or carcinomas (combined) in male and female rats occurred with positive trends (Table 12). The incidences of adenomas in high dose male and female rats and carcinomas in high dose male rats were significantly greater than those in the vehicle controls by the life table test; the incidences of follicular cell adenomas or carcinomas (combined) in high dose male and female rats were significantly ($P<0.05$) greater than those in the vehicle controls by the life table and incidental tumor tests. Two high dose male rats had bilateral follicular cell tumors: one had a bilateral carcinoma, and the second had an adenoma in one lobe and a carcinoma in the other lobe. A third high dose male had two adenomas in one lobe. Follicular cell adenomas were usually well-circumscribed masses consisting of variably sized follicles often with papillary fronds extending into the lumen. The follicular cell carcinomas (Figures 5-8) were more pleomorphic (Figure 7) and consisted of irregular follicular structures lined by anaplastic epithelial cells; a scirrhous response (Figure 7) was commonly seen with these tumors.

An increased incidence of thyroid gland follicular cell hyperplasia is often associated with an increased incidence of follicular cell adenomas and/or carcinomas. In this study, however, the increased incidence of follicular cell tumors in low and high dose male rats was not accompanied by increased follicular cell hyperplasia. However, in high dose female rats, a significant increase in follicular cell hyperplasia was associated with an increase in thyroid gland follicular cell tumors.

Ultimobranchial cysts occurred at an increased incidence ($P<0.001$) in high dose female rats (male: vehicle control, 0/50; low dose, 0/49; high dose, 2/50; female: 1/50; 0/50; 12/50). These embryologic remnants of the ultimobranchial bodies were unilocular or multilocular spaces lined by squamous epithelium.

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TABLE 12. ANALYSIS OF THYROID GLAND FOLLICULAR CELL LESIONS IN RATS IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT (a)

	Vehicle Control	50 mg/kg	100 mg/kg
MALE			
Hyperplasia			
Overall Rates	9/50 (18%)	7/49 (14%)	7/50 (14%)
Adenoma			
Overall Rates	3/50 (6%)	3/49 (6%)	9/50 (18%)
Adjusted Rates	7.3%	9.1%	34.4%
Terminal Rates	2/37 (5%)	3/33 (9%)	3/15 (20%)
Week of First Observation	55	104	81
Life Table Tests	P=0.003	P=0.618	P=0.007
Incidental Tumor Tests	P=0.026	P=0.618	P=0.068
Carcinoma			
Overall Rates	1/50 (2%)	5/49 (10%)	5/50 (10%)
Adjusted Rates	2.1%	13.3%	19.8%
Terminal Rates	0/37 (0%)	3/33 (9%)	2/15 (13%)
Week of First Observation	79	87	79
Life Table Tests	P=0.019	P=0.099	P=0.041
Incidental Tumor Tests	P=0.105	P=0.109	P=0.116
Adenoma or Carcinoma (b)			
Overall Rates	4/50 (8%)	8/49 (16%)	13/50 (26%)
Adjusted Rates	9.2%	22.0%	48.1%
Terminal Rates	2/37 (5%)	6/33 (18%)	5/15 (33%)
Week of First Observation	55	87	79
Life Table Tests	P<0.001	P=0.154	P<0.001
Incidental Tumor Tests	P=0.008	P=0.168	P=0.015
FEMALE			
Hyperplasia			
Overall Rates	10/50 (20%)	10/50 (20%)	26/50 (52%)
Adenoma			
Overall Rates	2/50 (4%)	0/50 (0%)	5/50 (10%)
Adjusted Rates	5.3%	0.0%	30.4%
Terminal Rates	1/37 (3%)	0/37 (0%)	4/14 (29%)
Week of First Observation	103		69
Life Table Tests	P=0.015	P=0.240N	P=0.020
Incidental Tumor Tests	P=0.069	P=0.240N	P=0.083
Carcinoma			
Overall Rates	0/50 (0%)	1/50 (2%)	2/50 (4%)
Adenoma or Carcinoma (c)			
Overall Rates	2/50 (4%)	1/50 (2%)	7/50 (14%)
Adjusted Rates	5.3%	2.7%	37.6%
Terminal Rates	1/37 (3%)	1/37 (3%)	4/14 (29%)
Week of First Observation	103	105	69
Life Table Tests	P=0.001	P=0.500N	P=0.003
Incidental Tumor Tests	P=0.026	P=0.500N	P=0.045

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table A3 (footnotes).
(b) Historical incidence in water gavage controls (mean ± SD): 2/144 (1% ± 1%); historical incidence in untreated controls (mean ± SD): 27/1,928 (1% ± 2%)
(c) Historical incidence in water gavage controls (mean ± SD): 4/146 (3% ± 3%); historical incidence in untreated controls (mean ± SD): 20/1,952 (1% ± 1%)

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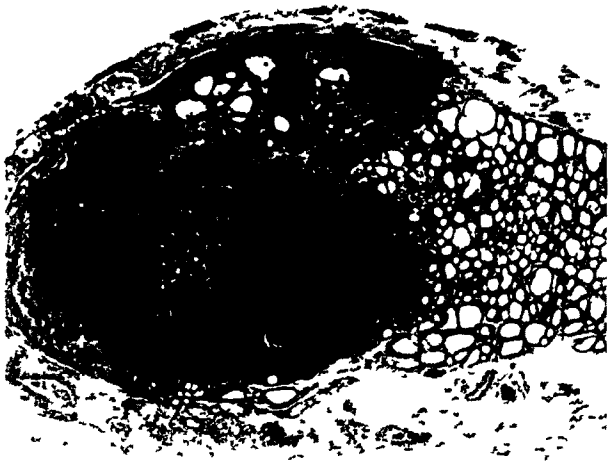

Figure 5 Photomicrograph of thyroid gland with a follicular cell carcinoma from a male rat given malonaldehyde, sodium salt Over half the normal tissue is replaced by neoplastic follicular epithelium Normal thyroid follicles containing colloid are present in the right hand portion of the gland H&E, magnification 9×

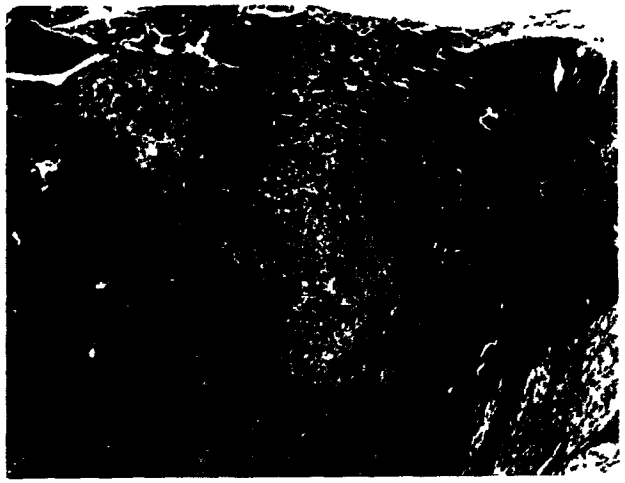

Figure 6 Photomicrograph of a thyroid follicular cell carcinoma that has totally obliterated the gland and invaded adjacent skeletal muscle H&E, magnification 9×

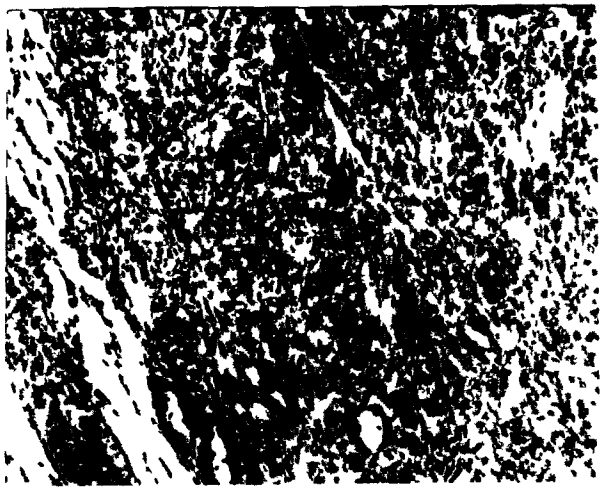

Figure 7 Photomicrograph of the thyroid follicular cell carcinoma shown in Figure 6 The neoplastic epithelium is arranged in pleomorphic tubular structures and is invading the adjacent muscle The neoplasm has elicited a scirrhous response characterized by the formation of collagenous connective tissue that is interspersed among the neoplastic follicular epithelium H&E, magnification 25×

Figure 8 Photomicrograph of a moderately well differentiated thyroid follicular cell carcinoma This neoplasm has not elicited a scirrhous response, and the follicular epithelium is arranged in tubular and follicle like structures H&E magnification 25×

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Pancreatic Islets: The incidences of islet cell adenomas and adenomas or carcinomas (combined) in low dose male rats were significantly greater than those in the vehicle controls (Table 13) and exceeded the range for historical control males (0/50-7/49).

Pancreas: An increased incidence and severity of diffuse atrophy of the acinar pancreas occurred in dosed male and female rats (male: vehicle control, 8/49; low dose, 26/50; high dose, 38/49; female: 5/50; 27/50; 42/50). This lesion was characterized by reduction in the number of secretory granules and reduction in the size of pancreatic acini and lobules. This lesion was distinct from the spontaneously occurring focal atrophy that was also diagnosed and characterized by fibrosis and atrophy of a single lobule.

Subcutaneous Tissue: Although the incidence of fibromas, fibrosarcomas, or myxosarcomas (combined) was slightly increased in low dose male rats, it was not significantly different from that in the vehicle controls (vehicle control, 2/50; low dose, 7/50; high dose, 3/50).

Hematopoietic System:

Bone Marrow--The incidences of hematopoietic hyperplasia in high dose female rats and reticulum cell hyperplasia in low dose female rats were greater ($P<0.01$) than those in the vehicle controls (hematopoietic hyperplasia--male: vehicle control, 5/50; low dose, 2/50; high dose, 5/50; female: 2/50; 4/50; 11/50; reticulum cell hyperplasia--male: 5/50; 3/50; 1/50; female: 6/50; 19/50; 7/50).

Spleen--Hematopoiesis was observed at an increased incidence ($P<0.01$) in high dose female rats (male: vehicle control, 3/50; low dose, 4/50; high dose, 1/50; female: 1/50; 2/50; 9/50).

TABLE 13. ANALYSIS OF PANCREATIC ISLET CELL TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	50 mg/kg	100 mg/kg
Adenoma			
Overall Rates	0/49 (0%)	9/50 (18%)	1/49 (2%)
Adjusted Rates	0.0%	26.1%	6.7%
Terminal Rates	0/37 (0%)	8/33 (24%)	1/15 (7%)
Week of First Observation		97	104
Life Table Tests	P=0.092	P=0.002	P=0.320
Incidental Tumor Tests	P=0.138	P=0.002	P=0.320
Carcinoma			
Overall Rates	1/49 (2%)	0/50 (0%)	0/49 (0%)
Adenoma or Carcinoma (a)			
Overall Rates	1/49 (2%)	9/50 (18%)	1/49 (2%)
Adjusted Rates	2.7%	26.1%	6.7%
Terminal Rates	1/37 (3%)	8/33 (24%)	1/15 (7%)
Week of First Observation	104	97	104
Life Table Tests	P=0.169	P=0.006	P=0.548
Incidental Tumor Tests	P=0.235	P=0.009	P=0.548

(a) Historical incidence in water gavage controls (mean ± SD): 12/147 (8% ± 4%); historical incidence in untreated controls (mean ± SD): 102/1,913 (5% ± 4%)

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Multiple Organs--Mononuclear cell leukemia in male rats occurred with a significant positive trend by life table analysis; the incidences in low and high dose males were not significantly different from that in vehicle controls and did not exceed the mean historical control incidence (Table 14).

Adrenal Gland: The incidence of lipoid degeneration of the adrenal gland cortex was significantly ($P<0.05$) increased in high dose male rats (vehicle control, 12/50; low dose, 13/50; high dose, 23/50) and in high dose female rats (19/50; 20/50; 30/50).

Pheochromocytomas in male rats occurred with a significant positive trend; the incidence in the high dose group was significantly greater than that in the vehicle controls by the life table test but not by the incidental tumor test (Table 15), which is the more appropriate test for this generally nonfatal tumor.

Anterior Pituitary Gland: Adenomas and adenomas or carcinomas (combined) in male and female rats occurred with significant negative trends, and the incidences in the high dose groups were significantly lower than those in the vehicle controls by the incidental tumor test (Table 16).

TABLE 14. ANALYSIS OF MONONUCLEAR CELL LEUKEMIA IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (a)

	Vehicle Control	50 mg/kg	100 mg/kg
Overall Rates	7/50 (14%)	10/50 (20%)	11/50 (22%)
Adjusted Rates	15.2%	23.7%	34.9%
Terminal Rates	1/37 (3%)	3/33 (9%)	0/15 (0%)
Week of First Observation	55	74	79
Life Table Tests	P=0.039	P=0.306	P=0.067
Incidental Tumor Tests	P=0.245N	P=0.503	P=0.272N

(a) Historical incidence of leukemia in water gavage controls (mean ± SD): 74/150 (49% ± 11%); historical incidence in untreated controls (mean ± SD): 583/1,977 (29% ± 12%)

TABLE 15. ANALYSIS OF ADRENAL GLAND LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	50 mg/kg	100 mg/kg
Medullary Focal Hyperplasia			
Overall Rates	18/50 (36%)	21/50 (42%)	16/50 (32%)
Pheochromocytoma (a)			
Overall Rates	5/50 (10%)	6/50 (12%)	8/50 (16%)
Adjusted Rates	13.5%	16.8%	40.0%
Terminal Rates	5/37 (14%)	5/33 (15%)	5/15 (33%)
Week of First Observation	104	74	76
Life Table Tests	P=0.014	P=0.430	P=0.016
Incidental Tumor Tests	P=0.048	P=0.427	P=0.055

(a) Historical incidence of pheochromocytomas or malignant pheochromocytomas (combined) in water gavage controls (mean ± SD): 63/149 (42% ± 4%); historical incidence in untreated controls: 452/1,950 (23% ± 12%)

TABLE 16. ANALYSIS OF ANTERIOR PITUITARY GLAND TUMORS IN RATS IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	50 mg/kg	100 mg/kg
MALE			
Adenoma			
Overall Rates	20/47 (43%)	14/49 (29%)	7/49 (14%)
Adjusted Rates	50.8%	38.3%	30.7%
Terminal Rates	16/35 (46%)	11/33 (33%)	3/15 (20%)
Week of First Observation	80	93	84
Life Table Tests	P=0.175N	P=0.189N	P=0.242N
Incidental Tumor Tests	P=0.010N	P=0.080N	P=0.016N
Carcinoma			
Overall Rates	0/47 (0%)	0/49 (0%)	1/49 (2%)
Adenoma or Carcinoma (a)			
Overall Rates	20/47 (43%)	14/49 (29%)	8/49 (16%)
Adjusted Rates	50.8%	38.3%	36.5%
Terminal Rates	16/35 (46%)	11/33 (33%)	4/15 (27%)
Week of First Observation	80	93	84
Life Table Tests	P=0.264N	P=0.189N	P=0.357N
Incidental Tumor Tests	P=0.022N	P=0.080N	P=0.039N
FEMALE			
Adenoma			
Overall Rates	16/49 (33%)	10/49 (20%)	2/48 (4%)
Adjusted Rates	39.8%	26.2%	14.3%
Terminal Rates	13/37 (35%)	8/36 (22%)	2/14 (14%)
Week of First Observation	83	102	104
Life Table Tests	P=0.032N	P=0.147N	P=0.060N
Incidental Tumor Tests	P=0.005N	P=0.146N	P=0.015N
Carcinoma			
Overall Rates	2/49 (4%)	0/49 (0%)	0/48 (0%)
Adenoma or Carcinoma (b)			
Overall Rates	18/49 (37%)	10/49 (20%)	2/48 (4%)
Adjusted Rates	43.7%	26.2%	14.3%
Terminal Rates	14/37 (38%)	8/36 (22%)	2/14 (14%)
Week of First Observation	83	102	104
Life Table Tests	P=0.013N	P=0.074N	P=0.034N
Incidental Tumor Tests	P=0.001N	P=0.068N	P=0.004N

(a) Historical incidence in water gavage controls (mean ± SD): 51/150 (34% ± 9%); historical incidence in untreated controls (mean ± SD): 428/1,861 (23% ± 11%)
(b) Historical incidence in water gavage controls (mean ± SD): 72/143 (50% ± 2%); historical incidence in untreated controls (mean ± SD): 931/1,952 (48% ± 11%)

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Liver: Cystic degeneration, cytoplasmic vacuolization, bile duct hyperplasia, and bile duct fibrosis occurred with increased incidences and/or severity in male and female rats (Table 17). Cystic degeneration (also referred to as spongiosis hepatis) consists of focal multilocular cystic formations containing granular material or, occasionally, erythrocytes. The cytoplasmic vacuolization affected randomly distributed clusters of hepatocytes and probably represents lipid accumulation within the cells. Bile duct hyperplasia and fibrosis occur spontaneously in aged rats, and the lesions in dosed rats were qualitatively similar. In affected rats, some of the portal triads in sections of the liver contained increased numbers of bile ducts or ductules surrounded by dense collagenous connective tissue.

Stomach: Acute and chronic inflammation, necrotizing inflammation characterized by epithelial necrosis and erosion of the glandular mucosa, and ulcers were observed at increased incidences in the glandular stomach of dosed male rats and high dose female rats (Table 18). Epithelial hyperplasia was observed at increased incidences in the forestomach near the junction with the glandular stomach in dosed male and high dose female rats.

Eye: Corneal inflammation in high dose male and female rats and retinal atrophy and cataracts of the crystalline lens were observed at increased incidences in dosed male and female rats (Table 19). The incidences of eye lesions in the dosed groups were not related to the rack position, e.g., top row and outside columns of rack versus inside (see Figure 2). Cages were not rotated on racks during the studies.

The increased incidences of stomach and eye lesions in dosed rats are compound related and appear to occur in response to the toxicity of the compound.

TABLE 17. NUMBER OF RATS WITH LESIONS OF THE LIVER IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Site/Lesion	Male			Female		
	Vehicle Control	50 mg/kg	100 mg/kg	Vehicle Control	50 mg/kg	100 mg/kg
Number of liver examined	50	50	50	50	50	50
Cytoplasmic vacuolization	7	7	12	6	6	18
Cystic degeneration	13	26	24	0	0	5
Bile duct hyperplasia (a)	50 (2.5)	45 (2.9)	50 (3.5)	17 (1.6)	15 (2.1)	35 (2.6)
Bile duct fibrosis (a)	4 (1.3)	8 (1.7)	28 (2.1)	1	0	1

(a) Mean grade of severity of hyperplasia or fibrosis for affected rats is given in parentheses: minimal = 1, mild = 2, moderate = 3, and marked = 4.

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TABLE 18. NUMBER OF RATS WITH LESIONS OF THE STOMACH IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Site/Lesion	Male			Female		
	Vehicle Control	50 mg/kg	100 mg/kg	Vehicle Control	50 mg/kg	100 mg/kg
Number of stomachs examined	50	49	50	50	50	50
Glandular stomach						
Inflammation	1	4	(a) 7	1	2	3
Inflammation, necrotizing	1	(a) 7	4	0	1	(b) 9
Ulcer	0	(a) 5	(b) 15	1	2	(b) 19
Forestomach						
Epithelial hyperplasia	3	8	(b) 18	4	5	(b) 18
Squamous cell papilloma	0	1	1	1	0	1

(a) $P<0.05$ vs. vehicle controls
(b) $P<0.01$ vs. vehicle controls

TABLE 19. NUMBER OF RATS WITH LESIONS OF THE EYE IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Site/Lesion	Male			Female		
	Vehicle Control	50 mg/kg	100 mg/kg	Vehicle Control	50 mg/kg	100 mg/kg
Number of rats examined	50	50	50	50	50	50
Corneal inflammation	2	2	(a) 19	2	2	(a) 25
Retinal atrophy	5	9	(a) 24	3	(a) 31	(a) 30
Crystalline lens cataract	4	(a) 14	(a) 19	4	(a) 26	(a) 31

(a) $P<0.01$ vs. vehicle controls

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FOURTEEN-DAY STUDIES

All mice that received 1,500 mg/kg malonaldehyde, sodium salt, 4/5 males that received 1,000 mg/kg, and 5/5 males that received 750 mg/kg died before the end of the studies (Table 20). The final mean body weights of males that received 500 or 1,000 mg/kg were 18% or 23% lower than that of the controls. The final mean body weight of females that received 1,000 mg/kg was 16% lower than that of the controls.

After 8 days, the urine of all dosed mice was nearly the same shade of yellow as the dose mixture. Male mice that received 750 mg/kg or more and female mice that received 1,500 mg/kg had rough hair coats and were inactive. No compound-related effects were observed at necropsy.

THIRTEEN-WEEK STUDIES

Three of 10 male mice and 1/10 female mice that received 500 mg/kg and 1/10 males and 1/10 females that received 125 mg/kg died before the end of the studies (Table 21). The final mean body weights of mice that received 500 mg/kg were 38% lower than that of the vehicle controls for males and 18% lower for females.

TABLE 20. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FOURTEEN-DAY GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Controls (percent)
MALE					
(d) 0	5/5	25.4	27.4	+2.0	
250	5/5	26.6	26.0	−0.6	94.9
500	5/5	27.8	22.4	−5.4	81.8
750	(e) 0/5	23.6	(f)	(f)	(f)
1,000	(g) 1/5	24.8	21.0	−3.8	76.6
1,500	(h) 0/5	24.8	(f)	(f)	(f)
FEMALE					
(d) 0	5/5	19.6	20.8	+1.2	
250	5/5	19.4	19.0	−0.4	91.3
500	5/5	20.0	19.8	−0.2	95.2
750	5/5	20.6	20.0	−0.6	96.2
1,000	5/5	18.6	17.4	−1.2	83.7
1,500	(i) 0/5	18.8	(f)	(f)	(f)

(a) Number surviving/number initially in the group
(b) Initial mean group body weight
(c) Mean body weight change of the survivors
(d) Controls were untreated.
(e) Day of death: 7,7,7,8,13
(f) No data are reported due to the 100% mortality in this group.
(g) Day of death: 10,10,11,11
(h) Day of death: 4,4,4,5,6
(i) Day of death: 7,7,7,8,8

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TABLE 21. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE THIRTEEN-WEEK GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial (b)	Final	Change (c)	Final Weight Relative to Vehicle Controls (percent)
MALE					
0	10/10	22.9 ± 0.5	34.1 ± 0.7	+11.2 ± 0.8	
30	10/10	24.4 ± 0.5	35.6 ± 0.7	+11.2 ± 0.4	104.4
60	10/10	23.7 ± 0.8	33.6 ± 0.8	+9.9 ± 0.8	98.5
125	(d) 9/10	25.3 ± 0.5	34.1 ± 0.9	+8.7 ± 0.7	100.0
250	10/10	23.9 ± 0.6	34.2 ± 1.0	+10.3 ± 1.0	100.3
500	(e) 7/10	23.4 ± 0.6	21.0 ± 1.0	−2.9 ± 1.3	61.6
FEMALE					
0	10/10	18.8 ± 0.4	26.0 ± 0.7	+7.2 ± 0.7	
30	10/10	19.1 ± 0.4	25.3 ± 0.5	+6.2 ± 0.5	97.3
60	10/10	19.1 ± 0.4	24.9 ± 0.6	+5.8 ± 0.4	95.8
125	(d) 9/10	18.9 ± 0.3	25.1 ± 0.6	+6.1 ± 0.6	96.5
250	10/10	19.1 ± 0.3	25.3 ± 1.0	+6.2 ± 0.7	97.3
500	(f) 9/10	18.8 ± 0.4	21.4 ± 0.5	+2.7 ± 0.4	82.3

(a) Number surviving/number initially in the group
(b) Initial mean group body weight ± standard error of the mean. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Week of death: 9
(e) Week of death: 7,11,13
(f) Week of death: 1

Compound-related lesions were observed in the pancreas, stomach, and testis (Table 22). Lesions in the pancreas diagnosed as exocrine atrophy were characterized by an accumulation of adipose tissue in the interstitium, with a reduced amount of normal-appearing pancreatic acinar tissue. This lesion was present in most mice at 250 and 500 mg/kg and in one male at 125 mg/kg. Mild dilatation of the gastric glands was observed in six males in the 500 mg/kg group. Degeneration of the germinal epithelium was observed in the testis of 2/10 males that received 500 mg/kg and 9/10 males that received 250 mg/kg. Lesions in lymphoid organs included lymphoid depletion. Increased splenic extramedullary hematopoiesis was present in three males in the 500 mg/kg group.

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TABLE 22. NUMBER OF MICE WITH LESIONS IN THE THIRTEEN-WEEK GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Site/Lesion	Dose (mg/kg) 0	60	125	250	500
MALE					
Number of mice examined	10	10	10	10	10
Pancreas					
Exocrine atrophy	0	0	1	10	8
Spleen					
Lymphoid depletion	0	0	0	0	6
Extramedullary hematopoiesis	0	0	0	0	3
Mandibular lymph node					
Lymphoid depletion	0	0	0	0	3
Stomach mucosa, gastric glands					
Dilatation	0	0	0	0	6
Testis, germinal epithelium					
Degeneration	0	0	0	9	2
FEMALE					
Number of mice examined	10	10	10	10	10
Pancreas					
Exocrine atrophy	0	0	0	8	9
Spleen					
Lymphoid depletion	0	0	0	0	1
Extramedullary hematopoiesis	0	0	0	0	0
Mandibular lymph node					
Lymphoid depletion	0	0	0	0	0
Stomach mucosa, gastric glands					
Dilatation	0	0	0	0	0

Dose Selection Rationale: Because of compound-related lesions in the pancreas, spleen, lymph nodes, stomach, and testis, doses selected for mice for the 2-year studies were 60 and 120 mg/kg malonaldehyde, sodium salt, administered in distilled water by gavage 5 days per week.

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of dosed and vehicle control male mice were comparable throughout most of the study (Table 23 and Figure 9). After week 93, mean body weights of the high dose male group were lower than those of the vehicle controls. Mean body weights of dosed female mice were greater than those of the vehicle controls throughout most of the study. Hair color of high dose mice changed from wild agouti to gray during the studies. Eczema, alopecia, rough hair coat, skin wounds, and genital inflammation or infection observed in all groups of male mice were considered to be a consequence of fighting.

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TABLE 23. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

Weeks on Study	Vehicle Control Av. Wt. (grams)	Vehicle Control No. of Survivors	60 mg/kg Av. Wt. (grams)	60 mg/kg Wt. (percent of veh. controls)	60 mg/kg No. of Survivors	120 mg/kg Av. Wt. (grams)	120 mg/kg Wt. (percent of veh. controls)	120 mg/kg No. of Survivors
MALE								
1	27 9	50	28 9	104	50	29 2	105	50
2	29 2	50	29 6	101	50	29 1	100	50
3	31 0	50	30 9	100	50	30 3	98	50
4	31 5	50	31 2	99	50	29 8	95	50
5	31 7	50	31 6	100	50	30 3	96	50
6	32 5	50	31 9	98	50	30 9	95	50
8	34 2	50	32 4	95	50	33 1	97	50
9	35 1	50	34 4	98	50	33 3	95	50
10	33 6	50	31 8	95	50	31 6	94	50
11	35 3	50	32 9	93	50	32 3	92	50
12	36 0	49	34 9	97	50	33 7	94	50
13	36 5	49	33 5	92	50	34 9	96	50
17	37 9	49	36 7	97	50	38 1	101	50
21	38 9	48	37 5	96	49	39 6	102	50
26	40 9	48	41 0	100	49	42 8	105	50
30	41 3	48	41 3	100	49	43 4	105	50
33	42 1	48	43 3	103	49	44 6	106	50
38	42 2	48	42 4	100	49	44 0	104	50
42	40 7	47	41 9	103	48	42 5	104	50
46	42 0	47	42 7	102	45	43 3	103	50
50	43 6	47	44 7	103	45	44 0	101	50
54	43 7	47	41 6	95	45	42 8	98	50
58	44 4	47	44 3	100	45	44 1	99	50
63	43 2	45	44 2	102	42	44 0	102	50
67	43 2	45	44 0	102	42	42 9	99	48
71	43 9	45	45 1	103	41	43 8	100	46
76	42 7	45	43 1	101	41	42 8	100	44
80	43 7	43	44 3	101	38	42 7	98	44
84	43 0	39	42 4	99	33	41 5	97	43
89	41 9	36	41 9	100	33	40 1	96	35
93	42 6	35	43 6	102	31	40 1	94	25
98	40 0	29	40 8	102	24	37 1	93	17
104	39 8	24	39 7	100	20	36 6	92	14
FEMALE								
1	20 8	50	20 7	100	50	20 4	98	50
2	20 7	50	20 8	100	50	21 5	104	50
3	21 9	50	22 6	103	50	22 2	101	50
4	22 4	50	22 4	100	50	22 4	100	50
5	22 6	50	22 6	100	50	23 0	102	50
6	23 1	50	22 9	99	50	23 6	102	50
8	23 3	50	24 6	106	50	23 2	100	50
9	23 8	50	24 6	103	50	24 6	103	50
10	23 8	50	22 8	96	50	23 8	100	50
11	25 0	50	23 5	94	50	24 8	99	50
12	25 5	50	24 4	96	50	24 3	95	50
13	25 9	50	25 1	97	50	25 4	98	50
17	25 7	50	26 3	102	50	26 9	105	50
21	26 8	50	26 6	99	50	27 5	103	50
26	29 4	50	29 6	101	50	30 0	102	50
30	29 0	50	29 5	102	50	30 9	107	50
33	30 2	50	31 9	106	50	33 7	112	50
38	30 9	50	31 7	103	49	33 6	109	50
42	29 2	50	30 5	104	49	32 6	112	50
46	30 4	50	32 3	106	49	35 3	116	50
50	31 7	49	34 2	108	49	36 9	116	50
54	32 8	49	33 5	102	49	37 3	114	49
58	33 4	49	35 8	107	49	37 7	113	49
63	33 8	49	35 7	106	49	39 1	116	49
67	34 4	49	36 7	107	48	40 2	117	49
71	36 8	49	39 5	107	48	41 7	113	49
76	36 2	48	39 3	109	47	41 0	113	48
80	36 6	47	38 9	106	46	41 8	114	46
84	37 0	46	39 1	106	44	40 8	110	42
89	37 7	46	41 5	110	44	40 6	108	41
93	40 4	43	43 0	106	44	37 8	94	38
98	36 6	42	38 5	105	42	35 6	97	32
104	35 9	41	37 5	104	38	35 7	99	30

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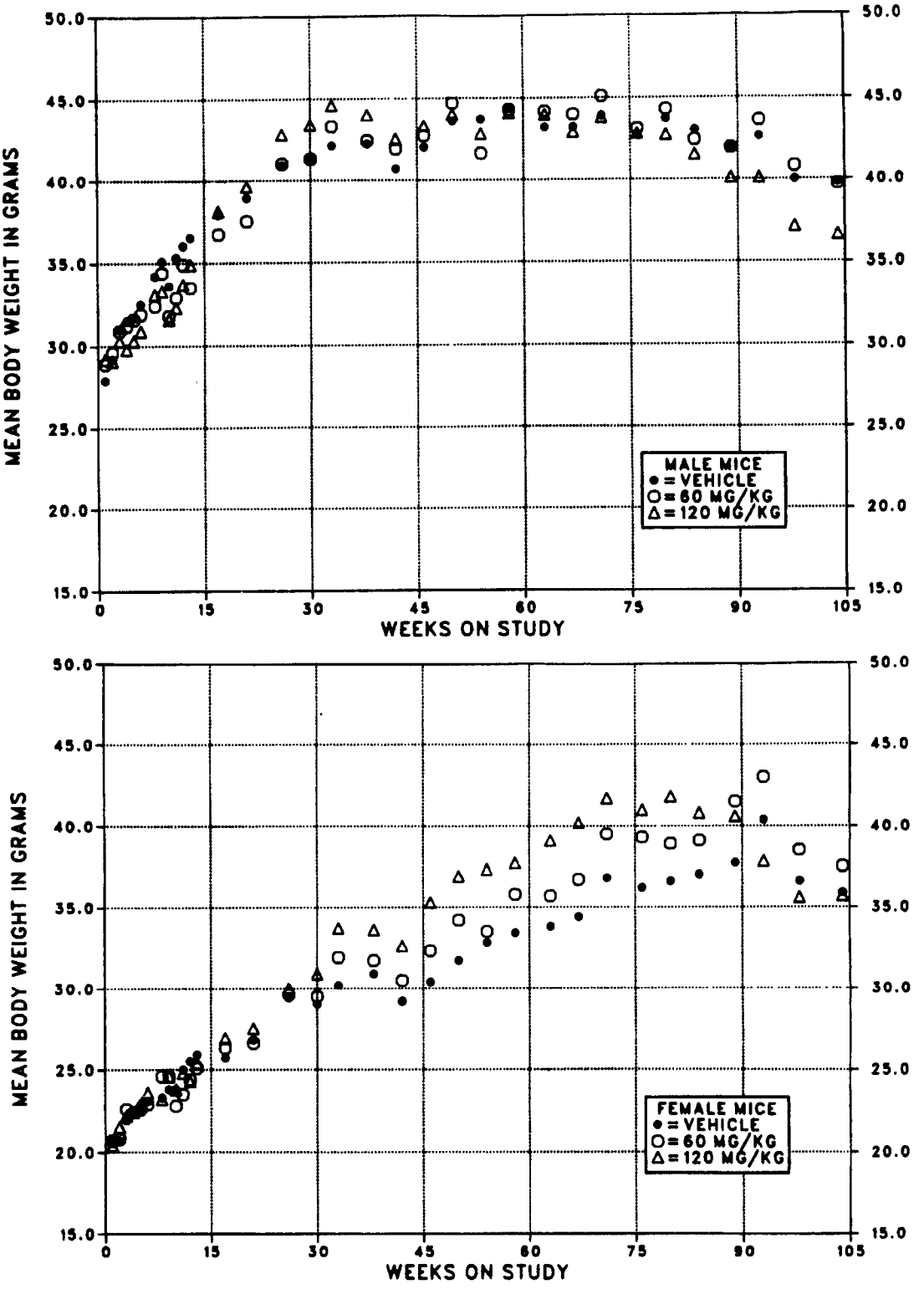

FIGURE 9. GROWTH CURVES FOR MICE ADMINISTERED MALONALDEHYDE, SODIUM SALT, IN WATER BY GAVAGE FOR TWO YEARS

III. RESULTS: MICE

Survival

Estimates of the probabilities of survival for male and female mice administered malonaldehyde, sodium salt, at the doses used in these studies and for vehicle controls are shown in Table 24 and in the Kaplan and Meier curves in Figure 10. The survival of the high dose group of male mice was significantly lower than that of the vehicle controls after week 92. No other significant differences in survival were observed between any groups of either sex.

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions of the skin, pancreatic acinus, and uterus.

Lesions in male mice are summarized in Appendix C. Histopathologic findings on neoplasms are summarized in Table C1. Table C2 gives the survival and tumor status for individual male mice. Table C3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table C3 (footnotes). Findings on nonneoplastic lesions are summarized in Table C4.

Lesions in female mice are summarized in Appendix D. Histopathologic findings on neoplasms are summarized in Table D1. Table D2 gives the survival and tumor status for individual female mice. Table D3 contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Table D3 (footnotes). Findings on nonneoplastic lesions are summarized in Table D4.

TABLE 24. SURVIVAL OF MICE IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	60 mg/kg	120 mg/kg
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	23	28	36
Accidentally killed	3	2	0
Killed at termination	23	19	14
Died during termination period	1	1	0
Survival P values (c)	0.014	0.333	0.017
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	9	11	13
Accidentally killed	0	1	(d) 7
Killed at termination	41	38	30
Survival P values (c)	0.297	0.787	0.368

(a) Terminal-kill period: male--week 104; female--weeks 104-105
(b) Includes animals killed in a moribund condition
(c) The result of the life table trend test is in the vehicle control column, and the results of the life table pairwise comparisons with the vehicle controls are in the dosed columns.
(d) During week 94, five high dose female mice in one cage died from drowning because of a malfunction of the water-dispensing system.

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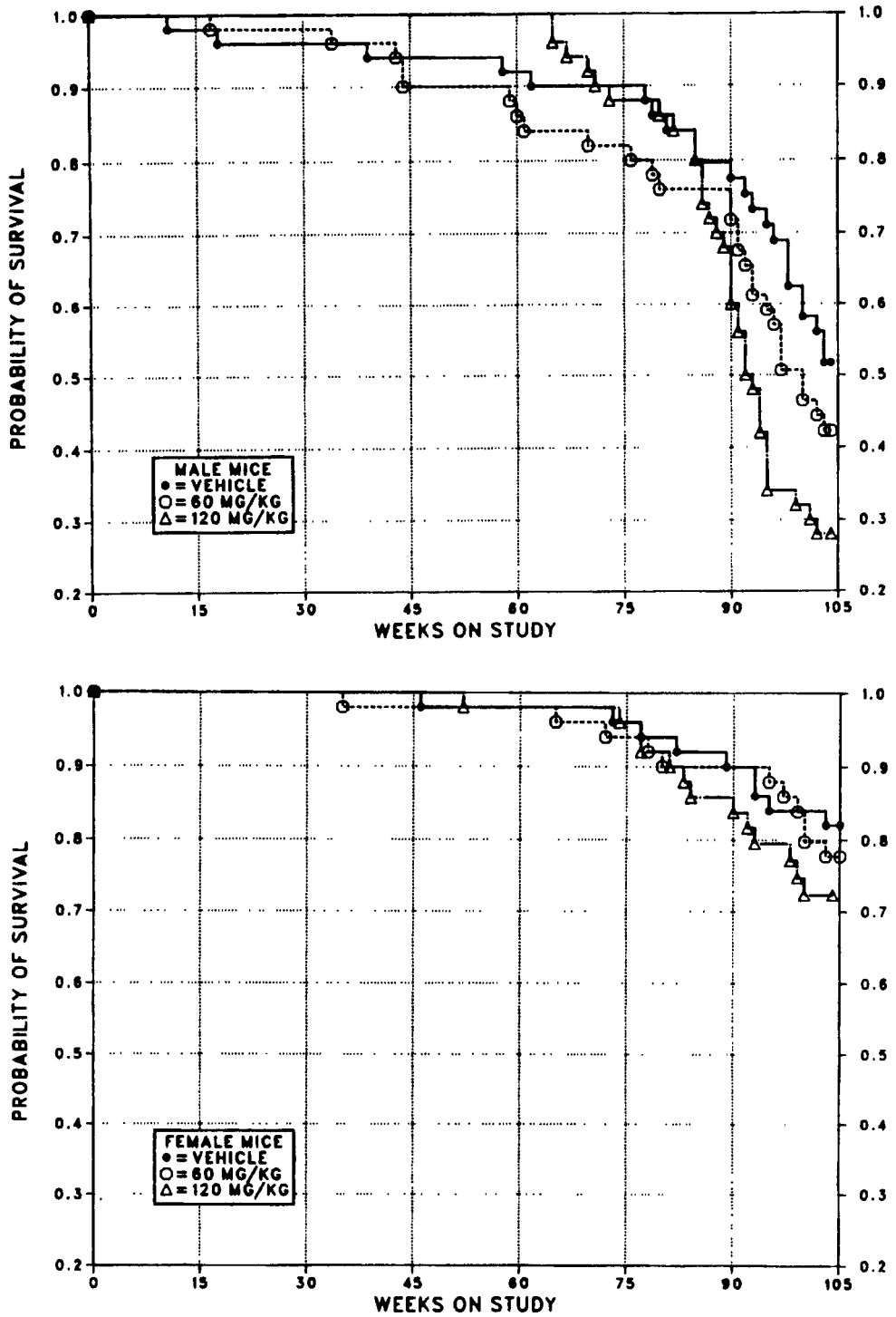

FIGURE 10. KAPLAN-MEIER SURVIVAL CURVES FOR MICE ADMINISTERED MALONALDEHYDE, SODIUM SALT, IN WATER BY GAVAGE FOR TWO YEARS

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At no site was an increased incidence of neoplasms observed in dosed mice. A significant increase in the incidence of nonneoplastic lesions occurred at the following sites:

Skin: An increased incidence ($P<0.001$) of pigmentation loss (listed as pigmentation, NOS, in Table C4) in hair shafts was observed in high dose mice (male: vehicle control, 0/50; low dose, 1/50; high dose, 31/50; female: 2/50; 0/50; 27/50).

Pancreatic Acinus: Atrophy was observed at increased incidences ($P<0.001$) in dosed mice (male: vehicle control, 5/43; low dose, 18/43; high dose, 31/45; female: 8/47; 26/50; 41/48).

Uterus: Dilatation was observed at increased incidences ($P<0.001$) in dosed female mice (vehicle control, 0/49; low dose, 12/48; high dose, 16/47.

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IV. DISCUSSION AND CONCLUSIONS

Results of Thirteen-Week Studies
Two-Year Studies in Rats
Two-Year Studies in Mice
Genetic Toxicology
Audit
Conclusions

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IV. DISCUSSION AND CONCLUSIONS

Malonaldehyde, sodium salt, was studied for potential toxicity and carcinogenicity in male and female F344/N rats and $B6C3F_1$ mice. The chemical was administered by gavage in distilled water. Since 1/5 male rats and 5/5 male mice that received 750 mg/kg malonaldehyde, sodium salt, did not survive to the end of the 14-day studies, doses for rats and mice in the 13-week studies were set at 0, 30, 60, 125, 250, and 500 mg/kg.

Results of Thirteen-Week Studies

Nine of 10 male rats and 10/10 female rats that received 500 mg/kg malonaldehyde, sodium salt, did not survive to the end of the 13-week studies. The final mean body weights of male and female rats that received 125 and 250 mg/kg were lower than those of vehicle controls at the end of 13 weeks. The final mean body weight of male rats administered 500 mg/kg was 61% that of vehicle controls. Both male and female rats had compound-related lesions in the stomach and kidney; these lesions occurred primarily in the two highest dose groups. Male rats at the two highest doses also had lesions of the testis. Although degeneration of the testicular germinal epithelium is frequently observed in moribund or debilitated rats, this lesion occurred in three male rats receiving 250 mg/kg, and all animals in this group survived until the end of the study. Thus, the testicular degeneration may have been compound related. The hyperplastic changes observed in the bone marrow and the spleen (hematopoiesis) may be secondary to the acute inflammatory changes associated with necrosis and erosion in the glandular stomach. Lymphoid depletion in the spleen, thymus, and mandibular lymph nodes is frequently observed in moribund or debilitated rats, and it may not be a primary, compound-related lesion.

Three of 10 male mice and 1/10 female mice that received 500 mg/kg died before the end of the 13-week studies. The final mean body weights of male and female mice that received 500 mg/kg were lower than those of the vehicle controls. Atrophy of the exocrine pancreas occurred in most mice in the two highest dose groups. Other lesions occurred in the stomach, spleen, and lymph nodes of male mice in the highest dose group and in the testis of the two highest dose groups. Based on these results, the doses of malonaldehyde, sodium salt, selected for the 2-year studies were 50 and 100 mg/kg for rats and 60 and 120 mg/kg for mice, administered in distilled water by gavage, 5 days per week.

Two-Year Studies in Rats

Survival and weight gains were poor in high dose rats. Survival of the high dose groups of male rats (after week 88) and female rats (after week 68) was significantly reduced compared with that of the vehicle controls. A 10% reduction in mean body weight was observed in the high dose groups as early as 33 and 54 weeks. Mean body weights of high dose males and females were 26% and 36% lower than those of vehicle controls at the end of the studies. The final mean body weight of low dose males was 4% lower than that of the vehicle controls, and the survival rate was 8% lower. Mean body weights and survival rates of low dose female rats were similar to those of vehicle controls throughout the study. No compound-related clinical signs were observed.

The incidences of thyroid gland follicular cell adenomas were increased in high dose rats (see Table 12). The occurrence of bilateral follicular cell neoplasms in two high dose male rats is an unusual finding and provides additional support for a compound-related effect. It should also be noted that the incidence of combined adenomas and carcinomas in the male rat vehicle controls (8%) was greater than the historical incidence (1.4%) in water gavage controls in three other studies at Battelle Columbus Laboratories and greater than the overall historical incidence (1.4%) in all untreated controls (Table A4). Follicular cell hyperplasia and ultimobranchial cysts occurred at increased incidences in high dose female rats; the incidences of these nonneoplastic lesions in dosed male rats were not markedly different from those in vehicle controls. Although follicular cell hyperplasia is a lesion that often precedes or is associated with the appearance of thyroid gland follicular cell neoplasms, analyses of thyroid tumor and follicular cell hyperplasia incidences in previous NTP/NCI studies indicate that there is no general pattern of correlation. Among four structurally related chemicals, 4,4'-thiodianiline (NCI, 1978),

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4,4'-methylenedianiline dihydrochloride (NTP, 1983), 4,4'-oxydianiline (NCI, 1980), and C.I. Basic Red 9 monohydrochloride (NTP, 1986), which all induced thyroid follicular cell tumors in both male and female rats, there was no consistent correlation between the induction of tumors and an increase in follicular cell hyperplasia. However, most of the positive correlations did occur at the highest doses.

The incidence of pancreatic islet cell adenomas or carcinomas (combined) in low dose male rats was significantly greater than that in vehicle controls (vehicle control, 1/49; low dose, 9/50; high dose, 1/49). Although the combined incidence of fibromas, fibrosarcomas, or myxosarcomas was slightly increased in low dose male rats (2/50; 7/50; 3/50), it was not significantly different from that in the vehicle controls. Since this increase was observed only at the low dose, with no significant dose-response trend evident, it was not considered to be related to the administration of malonaldehyde, sodium salt.

The incidences of bone marrow hematopoietic hyperplasia in high dose female rats (vehicle control, 2/50; low dose, 4/50; high dose, 11/50) and reticulum cell hyperplasia in low dose female rats (6/50; 19/50; 7/50) were greater than those in the vehicle controls. Hematopoiesis of the spleen occurred at an increased incidence in high dose female rats (1/50; 2/50; 9/50). This increased hematopoiesis in some animals may be related to the inflammation and erosion that occurred in the glandular stomach. Mononuclear cell leukemia in male rats (7/50; 10/50; 11/50) occurred with a significant positive trend by the life table test, but the incidence in dosed males was not significantly different from that in vehicle controls. The historical incidence of leukemia in male water gavage control rats at Battelle Columbus Laboratories and in all untreated controls is 49% and 29%, respectively; both of these values are greater than the 22% incidence for the high dose group in the present studies; therefore, this is probably not a chemically related effect.

Pheochromocytomas of the adrenal gland in male rats occurred with a significant positive trend; the incidence (16%) in the high dose group was significantly greater than that in the vehicle controls by the life table test but not by the more appropriate incidental tumor test. Adenomas or carcinomas (combined) of the anterior pituitary gland in male and female rats occurred with significant negative trends; the incidences in the male and female high dose groups, 16% and 4%, respectively, were significantly lower than those in the vehicle controls, 43% and 37%. The decrease in the incidences of anterior pituitary gland neoplasms in the high dose groups of male and female rats cannot be explained by reduced survival because the decrease is still observed when the incidences in animals of the same age are compared at the end of the studies. The conclusion is that the reduced incidence of anterior pituitary gland neoplasms is chemically related.

Compound-related nonneoplastic lesions were observed in the liver. Cystic degeneration was observed at increased incidences in dosed male rats and high dose female rats (male: vehicle control, 13/50; low dose, 26/50; high dose, 24/50; female: 0/50; 0/50; 5/50). Cytoplasmic vacuolization and bile duct hyperplasia were observed at increased incidences and/or severity in high dose female rats. Bile duct fibrosis was seen at an increased incidence and/or severity in dosed male rats (4/50; 8/50; 28/50).

The dose-related increase in diffuse pancreatic acinar atrophy in male and female rats may be secondary to debilitation, reduced feed consumption, or reduced weight gain in dosed animals.

Inflammation, necrotizing inflammation, and ulcers were observed at increased incidences in the glandular stomach of dosed male rats and high dose female rats. The presence of focal epithelial necrosis, erosion, and ulceration, fibroplasia, scarring, and chronic inflammation suggests a process of repeated injury and healing of the glandular mucosa. Since these lesions occur at the site of compound administration by water gavage, they are probably an indication of chronic malonaldehyde toxicity. Epithelial hyperplasia occurring near the junction of the glandular stomach and forestomach was observed at increased incidences in the forestomach of dosed male rats and high dose female rats. Squamous cell papillomas were observed in one animal in the low and high dose groups of male

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rats and in the vehicle control and high dose groups of female rats.

Corneal inflammation in high dose male and female rats and retinal atrophy and cataracts of the crystalline lens were observed at increased incidences in dosed male and female rats. These eye lesions occurred in 28%-48% of dosed male and 50%-62% of dosed female rats. Although the cage positions were not changed during the course of the study, the increased incidences of eye lesions in the dosed groups were not related to the rack position of the cages (top row and outside columns of rack vs. inside). When the cages and rack positions of the individual animals with eye lesions were identified (see Figure 2), it was clear that the eye lesions occurred at similar incidences whether the animals were located on the top and outside positions or on the interior positions of the rack; therefore, the increased incidences of eye lesions were considered to be chemically related.

The increased incidences of thyroid follicular cell tumors and of most of the nonneoplastic lesions were observed in high dose rats, groups in which malonaldehyde, sodium salt, toxicity was indicated by reduced survival rates of 59% and 62% and by a reduction of 26% and 36% of final body weights in the high dose male and female rat groups, respectively.

Two-Year Studies in Mice

Malonaldehyde, sodium salt, was administered to mice by gavage at 60 mg/kg and 120 mg/kg for 2 years. No significant differences in survival were observed between any groups of female mice. Survival in all male groups was similar at 89 weeks, but at the end of the study, high dose male mice showed reduced ($P=0.017$) survival relative to that of the vehicle controls. Mean body weights of dosed and vehicle control male mice were comparable throughout most of the study, but after week 93, the mean body weights of the high dose male group were 6%-8% lower than those of vehicle controls. Mean body weights of dosed female mice were greater than those of the vehicle controls throughout most of the study. The only compound-related clinical sign was the change in hair color from wild agouti to gray in high dose mice.

There was no increase in the incidences of neoplasms at any site in dosed mice. Several nonneoplastic lesions appeared to be compound related. The incidence of depigmentation of hair shafts was markedly increased in high dose male and female mice vs. that in vehicle controls. An increase in the incidences of pancreatic acinar cell atrophy was dose related in male and female mice. Dilatation of the uterus was observed at increased incidences in dosed female mice.

The increase in the number of natural deaths and of animals killed because they were moribund in high dose male mice and the increased incidences of nonneoplastic lesions in dosed mice are indicative of the chronic toxic effects produced by malonaldehyde, sodium salt, at the doses used in these studies. Even though there was poor survival in all groups of males at the end of the study (vehicle control, 24/50; low dose, 20/50; high dose, 14/50), the study was not considered to be inadequate because there was no evidence of even marginal neoplastic lesions seen in the dosed groups of either male or female mice; survival of the male mice was 50% or more in all groups (35/50; 31/50; 25/50) at week 93, after which the rate of deaths accelerated; the mice were housed five per cage, and clinical observations indicated fighting and bite wounds, a relatively common occurrence for group-housed $B6C3F_1$ mice. (Currently, all mice in NTP 2-year studies are housed individually.) Thus, this long-term study, although somewhat reduced in biologic and statistical sensitivity, is considered to be adequate for assessment of carcinogenicity.

Genetic Toxicology

Malonaldehyde, sodium salt, exhibited genetic toxicity in two of five assays sponsored by the NTP (Appendix E). The chemical induced forward mutations in mouse L5178Y lymphoma cells in the absence of an exogenous metabolic activation component; it was not tested with S9 in this assay. In the cytogenetic assays that detect chromosomal damage in cultured Chinese hamster ovary (CHO) cells, malonaldehyde, sodium salt, increased the frequency of sister chromatid exchanges, both in the presence and absence of rat liver S9; no increase in chromosomal aberrations was observed in CHO cells in the presence or absence of rat liver S9. No

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induction of sex-linked recessive lethal mutations was seen in Drosophila after exposure to malonaldehyde.

Malonaldehyde, sodium salt, was not mutagenic in any of the four *Salmonella typhimurium* strains (TA98, TA100, TA1535, or TA1538) used routinely in the NTP-sponsored Salmonella/microsome assay. These results confirmed earlier reports by Marnett and Tuttle (1980), who also observed that malonaldehyde, sodium salt, was inactive in these strains. This inactivity can be attributed to the fact that these mutant tester strains are all deficient in excision-repair capability, which has been shown to be a requirement for the expression of malonaldehyde-induced mutagenicity in Salmonella (Mukai and Goldstein, 1976; Marnett and Tuttle, 1980).

Independent studies of the genetic toxicity of malonaldehyde have yielded mixed results. Mukai and Goldstein (1976) were the first to report the mutagenicity of malonaldehyde preparations in Salmonella but only in those tester strains that retained excision-repair capability; the tester strain D3052 was the most sensitive to malonaldehyde. These results were later confirmed by Shamberger et al. (1979). In both of these studies, as in most investigations concerned with malonaldehyde toxicity conducted through 1980, the malonaldehyde preparations were of undefined purity and contained the more reactive intermediates, such as β-ethoxyacrolein, β-methoxyacrolein, and 3,3-dimethoxypropionaldehyde, which along with malonaldehyde are the products of the acid hydrolysis of the tetraalkoxypropanes (Marnett and Tuttle, 1980). The quantification of malonaldehyde in these hydrolysates was based on the amount of starting material and on the assumption that malonaldehyde was the only end product of the reaction. Thus, interpretation of these earlier studies is confounded because no consideration was given to the mutagenic activity contributed by the active intermediates and no further purification of malonaldehyde from the hydrolysis mixture was performed. Marnett and Tuttle (1980) were the first to report that the reaction intermediates produced by tetraalkoxypropane hydrolysis were more potent Salmonella mutagens than the pure malonaldehyde, sodium salt. These investigators also confirmed that malonaldehyde was mutagenic only in those Salmonella tester strains that retained excision-repair capability.

Audit

The experimental and tabulated data for the NTP Technical Report on malonaldehyde, sodium salt, were examined for accuracy, consistency, and compliance with Good Laboratory Practice requirements. As summarized in Appendix H, the audit revealed no major problems with the conduct of the studies or with collection and documentation of the experimental data. No discrepancies were found that influenced the final interpretation of the results of these studies.

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IV. DISCUSSION AND CONCLUSIONS

Conclusions

Under the conditions of these 2-year gavage studies, there was *clear evidence of carcinogenic activity** for male and female F344/N rats administered malonaldehyde, sodium salt, as shown by the increased incidences of follicular cell adenomas or carcinomas (combined) of the thyroid gland. Pancreatic islet cell adenomas were also observed at an increased incidence in low dose male rats. There was *no evidence of carcinogenic activity* for $B6C3F_1$ mice administered 60 or 120 mg/kg malonaldehyde, sodium salt, in distilled water by gavage 5 days per week for 2 years.

Chemically related increased incidences of nonneoplastic lesions included ulcers and inflammation of the glandular stomach and epithelial hyperplasia of the forestomach; corneal inflammation, retinal atrophy, and cataracts of the crystalline lens; and cystic degeneration of the liver, bile duct fibrosis, and bile duct hyperplasia in rats. Most of these nonneoplastic lesions as well as the thyroid gland follicular cell neoplasms occurred primarily in the high dose rat groups, in which survival and final body weights were reduced in high dose male and female rats. Increased incidences of atrophy of the pancreatic acinus and pigmentation loss in hair shafts were seen in high dose mice.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 9.
A summary of the Peer Review comments and the public discussion on this Technical Report appears on pages 12-13.

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V. REFERENCES

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V. REFERENCES

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APPENDIX A

SUMMARY OF LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

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TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Papilloma, NOS	1 (2%)		
Squamous cell papilloma	1 (2%)	1 (2%)	2 (4%)
Squamous cell carcinoma			1 (2%)
Sebaceous adenoma		1 (2%)	1 (2%)
Sebaceous adenocarcinoma	1 (2%)		
Keratoacanthoma	3 (6%)	3 (6%)	3 (6%)
*Subcutaneous tissue	(50)	(50)	(50)
Fibroma	1 (2%)	6 (12%)	2 (4%)
Fibrosarcoma		1 (2%)	1 (2%)
Myxosarcoma	1 (2%)		
RESPIRATORY SYSTEM			
#Lung	(50)	(50)	(50)
Squamous cell carcinoma, metastatic			1 (2%)
Alveolar/bronchiolar carcinoma		1 (2%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Malignant lymphoma, histiocytic type		1 (2%)	1 (2%)
Leukemia, mononuclear cell	7 (14%)	10 (20%)	11 (22%)
CIRCULATORY SYSTEM			
None			
DIGESTIVE SYSTEM			
*Tongue	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)
#Liver	(50)	(50)	(50)
Neoplastic nodule	1 (2%)	2 (4%)	1 (2%)
Hepatocellular carcinoma	2 (4%)		2 (4%)
#Pancreas	(49)	(50)	(49)
Acinar cell adenoma	1 (2%)		
#Forestomach	(50)	(49)	(50)
Squamous cell papilloma		1 (2%)	1 (2%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Tubular cell adenocarcinoma			1 (2%)
#Urinary bladder	(47)	(46)	(49)
Transitional cell papilloma	1 (2%)		
ENDOCRINE SYSTEM			
#Anterior pituitary	(47)	(49)	(49)
Carcinoma, NOS			1 (2%)
Adenoma, NOS	20 (43%)	14 (29%)	7 (14%)
#Adrenal	(50)	(50)	(50)
Cortical adenoma		1 (2%)	3 (6%)
#Adrenal medulla	(50)	(50)	(50)
Pheochromocytoma	5 (10%)	6 (12%)	8 (16%)

000500

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Thyroid	(50)	(49)	(50)
Follicular cell adenoma	3 (6%)	3 (6%)	9 (18%)
Follicular cell carcinoma	1 (2%)	5 (10%)	5 (10%)
C-cell adenoma	10 (20%)	11 (22%)	2 (4%)
C-cell carcinoma	2 (4%)	4 (8%)	
#Parathyroid	(42)	(37)	(42)
Adenoma, NOS	2 (5%)	4 (11%)	1 (2%)
#Pancreatic islets	(49)	(50)	(49)
Islet cell adenoma		9 (18%)	1 (2%)
Islet cell carcinoma	1 (2%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Fibroadenoma	2 (4%)	1 (2%)	
*Preputial gland	(50)	(50)	(50)
Adenoma, NOS	3 (6%)	1 (2%)	
Adenocarcinoma, NOS		1 (2%)	
Sebaceous adenoma	1 (2%)		
#Testis	(50)	(50)	(50)
Interstitial cell tumor	40 (80%)	45 (90%)	36 (72%)
*Epididymis	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)		
NERVOUS SYSTEM			
#Brain	(50)	(50)	(50)
Carcinoma, NOS, invasive			1 (2%)
Choroid plexus papilloma	1 (2%)		
SPECIAL SENSE ORGANS			
None			
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
*Tunica vaginalis	(50)	(50)	(50)
Mesothelioma, NOS	2 (4%)		2 (4%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Mesothelioma, NOS	2 (4%)		3 (6%)
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	2	10	19
Moribund sacrifice	11	7	15
Terminal sacrifice	37	33	15
Dosing accident			1

000501

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	49	49	47
Total primary tumors	116	132	108
Total animals with benign tumors	47	48	46
Total benign tumors	95	107	77
Total animals with malignant tumors	16	19	22
Total malignant tumors	16	23	25
Total animals with secondary tumors##			2
Total secondary tumors			2
Total animals with tumors uncertain benign or malignant	5	2	6
Total uncertain tumors	5	2	6

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically
** Primary tumors all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors metastatic tumors or tumors invasive into an adjacent organ

000502

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT: VEHICLE CONTROL

ANIMAL NUMBER	010	033	028	014	031	037	012	002	004	029	032	015	047	001	003	005	006	007	008	009	011	013	016	017	018
WEEKS ON STUDY	055	060	079	080	080	080	085	087	087	089	090	095	098	104	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	N	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma, NOS																									
Squamous cell papilloma																							X		
Sebaceous adenocarcinoma																									
Keratoacanthoma																		X							
Subcutaneous tissue	+	+	+	+	+	+	+	+	N	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
Myxosarcoma		X																							
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	−	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	−	+	−	−	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule															X										
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinar cell adenoma																									
Esophagus	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional cell papilloma																									
ENDOCRINE SYSTEM																									
Pituitary	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS				X						X	X		X			X	X		X		X	X			
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma									-									X							
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma	X													X											
Follicular cell carcinoma			X																						
C cell adenoma																X			X						
C cell carcinoma																	X								
Parathyroid	+	+	−	+	+	−	−	+	+	+	+	+	−	−	+	+	−	+	+	+	+	+	+	+	+
Adenoma, NOS																							X		
Pancreatic islets	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell carcinoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	+	N	+	+	N	N	N	+	N	+	N	N	N	N	N	N	N	N	N	N	+	N	+	N	+
Fibroadenoma																							X		
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor			X			X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X
Prostate	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS													X												
Sebaceous adenoma																						X			
Epididymis	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leiomyosarcoma																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Choroid plexus papilloma																									
BODY CAVITIES																									
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, NOS										X															
Leukemia, mononuclear cell	X				X		X	X	X		X										X				

+ Tissue examined microscopically
− Required tissue not examined microscopically
X Tumor incidence
N Necropsy, no autolysis, no microscopic examination
S Animal missexed

No tissue information submitted
C Necropsy, no histology due to protocol
A Autolysis
M Animal missing
B No necropsy performed

000503

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: VEHICLE CONTROL (Continued)

ANIMAL NUMBER	019	020	021	022	023	024	025	026	027	030	034	035	036	038	039	040	041	042	043	044	045	046	048	049	050	TOTAL TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Papilloma, NOS																	X									1
Squamous cell papilloma																										1
Sebaceous adenocarcinoma																							X			1
Keratoacanthoma					X					X																3
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma								X																		1
Myxosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Thymus	+	−	+	+	+	−	+	+	−	−	−	+	+	+	+	+	+	+	+	−	+	+	+	−	−	37
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule																										1
Hepatocellular carcinoma																X	X									2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinar cell adenoma																X										1
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	47
Transitional cell papilloma			X																							1
ENDOCRINE SYSTEM																										
Pituitary	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma, NOS	X			X	X						X	X	X	X				X		X			X	X		20
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma	X											X		X									X			5
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma												X														3
Follicular cell carcinoma																										1
C cell adenoma			X							X				X	X	X			X	X					X	10
C cell carcinoma																			X							2
Parathyroid	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Adenoma, NOS					X																					2
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islet cell carcinoma																					X					1
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	+	N	N	N	N	N	N	N	+	+	+	+	N	N	+	+	N	N	N	N	N	N	*50
Fibroadenoma															X											2
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X			X	40
Prostate	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	47
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS															X				X							3
Sebaceous adenoma																										1
Epididymis	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leiomyosarcoma	X																									1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Choroid plexus papilloma								X																		1
BODY CAVITIES																										
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Mesothelioma, NOS			X								X															2
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, NOS												X														2
Leukemia, mononuclear cell																										7

* Animals necropsied

000504

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT: LOW DOSE

ANIMAL NUMBER	023	028	016	045	010	002	032	038	009	003	021	033	043	022	025	029	039	001	004	005	006	007	008	011	012
WEEKS ON STUDY	074	074	079	084	086	087	088	088	092	093	097	097	098	099	103	103	103	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma								X																	
Sebaceous adenoma																									
Keratoacanthoma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma															X									X	
Fibrosarcoma							X																		
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																						X			
Trachea	+	−	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Thymus	+	−	+	+	−	+	+	+	+	+	+	−	+	+	−	+	−	+	+	−	−	+	−	+	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Small intestine	−	−	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS										X	X						X		X						
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma																			X						
Pheochromocytoma	X																			X				X	
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																			X						
Follicular cell carcinoma						X					X													X	
C cell adenoma												X							X	X					
C cell carcinoma													X												
Parathyroid	−	−	+	+	+	+	+	+	−	−	−	+	+	+	−	+	+	+	−	−	+	+	+	−	+
Adenoma, NOS			X		X																				
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma												X													
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	+	+	+	+	N	+	N	N	+	+	N	+	N	+	N	+	+	N	+	+	N	N
Fibroadenoma																									
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor	X	X	X	X	X	X	X		X		X	X	X		X	X	X	X	X	X		X	X	X	X
Prostate	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
Adenocarcinoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, histiocytic type						X																			
Leukemia, mononuclear cell		X	X								X	X	X	X	X					X					

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TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	013	014	015	017	018	019	020	024	026	027	030	031	034	035	036	037	040	041	042	044	046	047	048	049	050	TOTAL TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell papilloma																										1
Sebaceous adenoma					X																					1
Keratoacanthoma										X								X							X	3
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma	X				X						X											X				6
Fibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	45
Thymus	−	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	+	+	−	+	+	+	+	−	+	35
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule							X															X				2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell papilloma																			X							1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	46
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma, NOS					X		X	X		X			X					X				X	X	X	X	14
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cortical adenoma																										1
Pheochromocytoma		X								X			X													6
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell adenoma						X										X										3
Follicular cell carcinoma								X				X														5
C cell adenoma	X	X	X								X		X	X			X				X					11
C cell carcinoma											X					X	X									4
Parathyroid	−	+	+	+	+	+	−	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	37
Adenoma, NOS																			X						X	4
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma		X			X		X		X		X		X				X							X		9
REPRODUCTIVE SYSTEM																										
Mammary gland	+	N	+	+	+	N	N	+	N	+	+	N	+	N	N	+	+	+	+	N	N	+	N	+	N	*50
Fibroadenoma													X													1
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	45
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	48
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS	X																									1
Adenocarcinoma, NOS								X																		1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, histiocytic type																										1
Leukemia, mononuclear cell										X														X		10

* Animals necropsied

000506

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT: HIGH DOSE

ANIMAL NUMBER	004	034	007	010	041	048	012	032	038	006	008	045	047	025	021	014	035	019	005	016	017	039	043	028	015
WEEKS ON STUDY	039	043	062	070	076	076	077	079	079	081	081	081	081	082	083	084	084	085	086	088	088	088	088	090	093
INTEGUMENTARY SYSTEM																									
Skin	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N
Squamous cell papilloma																									
Squamous cell carcinoma				X																					
Sebaceous adenoma			X																						
Keratoacanthoma												X													
Subcutaneous tissue	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N
Fibroma																									
Fibrosarcoma												X													
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma, metastatic				X																					
Alveolar/bronchiolar carcinoma																									
Trachea	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	−	−	−	+	−	−	−	−	+	+	+	+	+	+	−	−	+	+	+	+	−	+	+	+	+
Thymus	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell papilloma																									
Salivary gland	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Hepatocellular carcinoma																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Small intestine	+	−	+	−	+	−	+	+	+	+	+	+	+	+	−	+	−	−	−	+	+	+	+	+	+
Large intestine	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenocarcinoma																									
Urinary bladder	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Adenoma, NOS																	X				X				X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma		X																	X						X
Pheochromocytoma					X											X									
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma											X		X		X			X	X						
Follicular cell carcinoma								X					X			X									
C cell adenoma																									
Parathyroid	+	+	+	+	−	+	+	−	+	−	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+
Adenoma, NOS																									
Pancreatic islets	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	+	N	N	N	N	N	N	+	N	N	+	N	+	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell tumor				X					X	X	X		X	X	X	X		X		X		X	X	X	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, invasive																									
BODY CAVITIES																									
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mesothelioma, NOS																								X	
Malignant lymphoma, histiocytic type												X													
Leukemia, mononuclear cell									X		X	X								X			X	X	

000507

TABLE A2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	050	003	013	042	027	036	030	026	020	029	001	002	009	011	018	022	023	024	031	033	037	040	044	046	049	TOTAL TISSUES TUMORS
WEEKS ON STUDY	093	094	097	097	099	099	100	101	102	102	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell papilloma														X		X										2
Squamous cell carcinoma																										1
Sebaceous adenoma																										1
Keratoacanthoma																			X					X		3
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																						X		X		2
Fibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma, metastatic																										1
Alveolar/bronchiolar carcinoma											X			X												2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	39
Thymus	+	−	+	+	−	−	+	−	−	+	+	−	+	−	+	+	−	−	−	+	−	+	+	+	−	32
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell papilloma																					X					1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule														X												1
Hepatocellular carcinoma													X			X										2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																				X						1
Small intestine	+	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenocarcinoma													X													1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS																						X				1
Adenoma, NOS		X																	X	X					X	7
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cortical adenoma																										3
Pheochromocytoma							X					X	X								X	X	X			8
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma								X					X						X					X		9
Follicular cell carcinoma																		X		X						5
C cell adenoma																					X	X				2
Parathyroid	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	42
Adenoma, NOS																			X							1
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islet cell adenoma																			X							1
REPRODUCTIVE SYSTEM																										
Mammary gland	N	+	+	+	N	+	N	+	+	N	N	N	+	+	N	+	N	N	N	+	N	N	+	+	+	*50
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell tumor	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		36
Prostate	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, invasive																						X				1
BODY CAVITIES																										
Tunica vaginalis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Mesothelioma, NOS								X							X											2
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mesothelioma, NOS												X					X									3
Malignant lymphoma, histiocytic type																										1
Leukemia, mononuclear cell	X	X		X					X	X																11

* Animals necropsied

000508

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	50 mg/kg	100 mg/kg
Skin: Keratoacanthoma			
Overall Rates (a)	3/50 (6%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	8 1%	9 1%	15 4%
Terminal Rates (c)	3/37 (8%)	3/33 (9%)	2/15 (13%)
Week of First Observation	104	104	81
Life Table Tests (d)	P=0 230	P=0 610	P=0 299
Incidental Tumor Tests (d)	P=0 271	P=0 610	P=0 355
Cochran Armitage Trend Test (d)	P=0 583		
Fisher Exact Test (d)		P=0 661	P=0 661
Skin: Squamous Cell Papilloma or Carcinoma			
Overall Rates (a)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted Rates (b)	2 7%	2 3%	15 2%
Terminal Rates (c)	1/37 (3%)	0/33 (0%)	2/15 (13%)
Week of First Observation	104	88	70
Life Table Tests (d)	P=0 081	P=0 756	P=0 107
Incidental Tumor Tests (d)	P=0 163	P=0 719	P=0 151
Cochran Armitage Trend Test (d)	P=0 202		
Fisher Exact Test (d)		P=0 753	P=0 309
Skin: Papilloma or Squamous Cell Papilloma or Carcinoma			
Overall Rates (a)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted Rates (b)	5 4%	2 3%	15 2%
Terminal Rates (c)	2/37 (5%)	0/33 (0%)	2/15 (13%)
Week of First Observation	104	88	70
Life Table Tests (d)	P=0 188	P=0 518N	P=0 200
Incidental Tumor Tests (d)	P=0 304	P=0 555N	P=0 261
Cochran Armitage Trend Test (d)	P=0 399		
Fisher Exact Test (d)		P=0 500N	P=0 500
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	1/50 (2%)	6/50 (12%)	2/50 (4%)
Adjusted Rates (b)	2 7%	17 5%	13 3%
Terminal Rates (c)	1/37 (3%)	5/33 (15%)	2/15 (13%)
Week of First Observation	104	103	104
Life Table Tests (d)	P=0 092	P=0 044	P=0 205
Incidental Tumor Tests (d)	P=0 154	P=0 066	P=0 205
Cochran Armitage Trend Test (d)	P=0 417		
Fisher Exact Test (d)		P=0 056	P=0 500
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	1/50 (2%)	7/50 (14%)	3/50 (6%)
Adjusted Rates (b)	2 7%	19 4%	15 4%
Terminal Rates (c)	1/37 (3%)	5/33 (15%)	2/15 (13%)
Week of First Observation	104	88	81
Life Table Tests (d)	P=0 051	P=0 026	P=0 105
Incidental Tumor Tests (d)	P=0 136	P=0 035	P=0 139
Cochran Armitage Trend Test (d)	P=0 283		
Fisher Exact Test (d)		P=0 030	P=0 309
Subcutaneous Tissue: Fibroma, Fibrosarcoma, or Myxosarcoma			
Overall Rates (a)	2/50 (4%)	7/50 (14%)	3/50 (6%)
Adjusted Rates (b)	4 7%	19 4%	15 4%
Terminal Rates (c)	1/37 (3%)	5/33 (15%)	2/15 (13%)
Week of First Observation	60	88	81
Life Table Tests (d)	P=0 124	P=0 070	P=0 265
Incidental Tumor Tests (d)	P=0 302	P=0 088	P=0 396
Cochran Armitage Trend Test (d)	P=0 427		
Fisher Exact Test (d)		P=0 080	P=0 500

000509

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	50 mg/kg	100 mg/kg
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	7/50 (14%)	10/50 (20%)	11/50 (22%)
Adjusted Rates (b)	15.2%	23.7%	34.9%
Terminal Rates (c)	1/37 (3%)	3/33 (9%)	0/15 (0%)
Week of First Observation	55	74	79
Life Table Tests (d)	P=0 039	P=0.306	P=0.067
Incidental Tumor Tests (d)	P=0.245N	P=0.503	P=0.272N
Cochran-Armitage Trend Test (d)	P=0.185		
Fisher Exact Test (d)		P=0.298	P=0.218
Liver: Neoplastic Nodule or Hepatocellular Carcinoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	8 1%	6.1%	20.0%
Terminal Rates (c)	3/37 (8%)	2/33 (6%)	3/15 (20%)
Week of First Observation	104	104	104
Life Table Tests (d)	P=0.216	P=0.552N	P=0.233
Incidental Tumor Tests (d)	P=0.216	P=0.552N	P=0.233
Cochran-Armitage Trend Test (d)	P=0.588		
Fisher Exact Test (d)		P=0.500N	P=0.661N
Pituitary Gland: Adenoma			
Overall Rates (a)	20/47 (43%)	14/49 (29%)	7/49 (14%)
Adjusted Rates (b)	50.8%	38.3%	30.7%
Terminal Rates (c)	16/35 (46%)	11/33 (33%)	3/15 (20%)
Week of First Observation	80	93	84
Life Table Tests (d)	P=0.175N	P=0.189N	P=0.242N
Incidental Tumor Tests (d)	P=0.010N	P=0.080N	P=0.016N
Cochran-Armitage Trend Test (d)	P=0 002N		
Fisher Exact Test (d)		P=0.112N	P=0.002N
Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (a)	20/47 (43%)	14/49 (29%)	8/49 (16%)
Adjusted Rates (b)	50.8%	38.3%	36.5%
Terminal Rates (c)	16/35 (46%)	11/33 (33%)	4/15 (27%)
Week of First Observation	80	93	84
Life Table Tests (d)	P=0.264N	P=0.189N	P=0.357N
Incidental Tumor Tests (d)	P=0 022N	P=0.080N	P=0.039N
Cochran-Armitage Trend Test (d)	P=0.003N		
Fisher Exact Test (d)		P=0.112N	P=0.005N
Adrenal Gland: Cortical Adenoma			
Overall Rates (a)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted Rates (b)	0 0%	3.0%	8.8%
Terminal Rates (c)	0/37 (0%)	1/33 (3%)	0/15 (0%)
Week of First Observation		104	43
Life Table Tests (d)	P=0 028	P=0.477	P=0.085
Incidental Tumor Tests (d)	P=0.203	P=0.477	P=0.500
Cochran-Armitage Trend Test (d)	P=0.060		
Fisher Exact Test (d)		P=0.500	P=0.121
Adrenal Gland: Pheochromocytoma			
Overall Rates (a)	5/50 (10%)	6/50 (12%)	8/50 (16%)
Adjusted Rates (b)	13.5%	16.8%	40.0%
Terminal Rates (c)	5/37 (14%)	5/33 (15%)	5/15 (33%)
Week of First Observation	104	74	76
Life Table Tests (d)	P=0.014	P=0.430	P=0.016
Incidental Tumor Tests (d)	P=0.048	P=0.427	P=0.055
Cochran-Armitage Trend Test (d)	P=0.226		
Fisher Exact Test (d)		P=0.500	P=0.277

000510

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	50 mg/kg	100 mg/kg
Thyroid Gland: Follicular Cell Adenoma			
Overall Rates (a)	3/50 (6%)	3/49 (6%)	9/50 (18%)
Adjusted Rates (b)	7 3%	9 1%	34 4%
Terminal Rates (c)	2/37 (5%)	3/33 (9%)	3/15 (20%)
Week of First Observation	55	104	81
Life Table Tests (d)	P=0 003	P=0 618	P=0 007
Incidental Tumor Tests (d)	P=0 026	P=0 618	P=0 068
Cochran Armitage Trend Test (d)	P=0 034		
Fisher Exact Test (d)		P=0 651	P=0 061
Thyroid Gland: Follicular Cell Carcinoma			
Overall Rates (a)	1/50 (2%)	5/49 (10%)	5/50 (10%)
Adjusted Rates (b)	2 1%	13 3%	19 8%
Terminal Rates (c)	0/37 (0%)	3/33 (9%)	2/15 (13%)
Week of First Observation	79	87	79
Life Table Tests (d)	P=0 019	P=0 099	P=0 041
Incidental Tumor Tests (d)	P=0 105	P=0 109	P=0 116
Cochran Armitage Trend Test (d)	P=0 090		
Fisher Exact Test (d)		P=0 098	P=0 102
Thyroid Gland: Follicular Cell Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	8/49 (16%)	13/50 (26%)
Adjusted Rates (b)	9 2%	22 0%	48 1%
Terminal Rates (c)	2/37 (5%)	6/33 (18%)	5/15 (33%)
Week of First Observation	55	87	79
Life Table Tests (d)	P<0 001	P=0 154	P<0 001
Incidental Tumor Tests (d)	P=0 008	P=0 168	P=0 015
Cochran Armitage Trend Test (d)	P=0 011		
Fisher Exact Test (d)		P=0 168	P=0 016
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	10/50 (20%)	11/49 (22%)	2/50 (4%)
Adjusted Rates (b)	27 0%	32 0%	13 3%
Terminal Rates (c)	10/37 (27%)	10/33 (30%)	2/15 (13%)
Week of First Observation	104	97	104
Life Table Tests (d)	P=0 296N	P=0 388	P=0 245N
Incidental Tumor Tests (d)	P=0 231N	P=0 440	P=0 245N
Cochran-Armitage Trend Test (d)	P=0 019N		
Fisher Exact Test (d)		P=0 479	P=0 014N
Thyroid Gland: C-Cell Carcinoma			
Overall Rates (a)	2/50 (4%)	4/49 (8%)	0/50 (0%)
Adjusted Rates (b)	5 4%	11 5%	0 0%
Terminal Rates (c)	2/37 (5%)	3/33 (9%)	0/15 (0%)
Week of First Observation	104	98	
Life Table Tests (d)	P=0 493N	P=0 295	P=0 452N
Incidental Tumor Tests (d)	P=0 353N	P=0 377	P=0 452N
Cochran Armitage Trend Test (d)	P=0 223N		
Fisher Exact Test (d)		P=0 329	P=0 247N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	11/50 (22%)	13/49 (27%)	2/50 (4%)
Adjusted Rates (b)	29 7%	36 7%	13 3%
Terminal Rates (c)	11/37 (30%)	11/33 (33%)	2/15 (13%)
Week of First Observation	104	97	104
Life Table Tests (d)	P=0 274N	P=0 296	P=0 191N
Incidental Tumor Tests (d)	P=0 161N	P=0 389	P=0 191N
Cochran Armitage Trend Test (d)	P=0 013N		
Fisher Exact Test (d)		P=0 385	P=0 007N

000511

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	50 mg/kg	100 mg/kg
Parathyroid: Adenoma			
Overall Rates (a)	2/42 (5%)	4/37 (11%)	1/42 (2%)
Adjusted Rates (b)	6 1%	11 6%	7 7%
Terminal Rates (c)	2/33 (6%)	2/26 (8%)	1/13 (8%)
Week of First Observation	104	79	104
Life Table Tests (d)	P=0 495	P=0 275	P=0 676
Incidental Tumor Tests (d)	P=0 566N	P=0 266	P=0 676
Cochran Armitage Trend Test (d)	P=0 408N		
Fisher Exact Test (d)		P=0 279	P=0 500N
Pancreatic Islets: Islet Cell Adenoma			
Overall Rates (a)	0/49 (0%)	9/50 (18%)	1/49 (2%)
Adjusted Rates (b)	0 0%	26 1%	6 7%
Terminal Rates (c)	0/37 (0%)	8/33 (24%)	1/15 (7%)
Week of First Observation		97	104
Life Table Tests (d)	P=0 092	P=0 002	P=0 320
Incidental Tumor Tests (d)	P=0 138	P=0 002	P=0 320
Cochran Armitage Trend Test (d)	P=0 420		
Fisher Exact Test (d)		P=0 001	P=0 500
Pancreatic Islets: Islet Cell Adenoma or Carcinoma			
Overall Rates (a)	1/49 (2%)	9/50 (18%)	1/49 (2%)
Adjusted Rates (b)	2 7%	26 1%	6 7%
Terminal Rates (c)	1/37 (3%)	8/33 (24%)	1/15 (7%)
Week of First Observation	104	97	104
Life Table Tests (d)	P=0 169	P=0 006	P=0 548
Incidental Tumor Tests (d)	P=0 235	P=0 009	P=0 548
Cochran Armitage Trend Test (d)	P=0 576		
Fisher Exact Test (d)		P=0 009	P=0 753
Preputial Gland: Adenoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted Rates (b)	7 9%	3 0%	0 0%
Terminal Rates (c)	2/37 (5%)	1/33 (3%)	0/15 (0%)
Week of First Observation	98	104	
Life Table Tests (d)	P=0 147N	P=0 337N	P=0 293N
Incidental Tumor Tests (d)	P=0 065N	P=0 224N	P=0 103N
Cochran Armitage Trend Test (d)	P=0 060N		
Fisher Exact Test (d)		P=0 309N	P=0 121N
Preputial Gland: Adenoma or Sebaceous Adenoma			
Overall Rates (a)	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted Rates (b)	10 5%	3 0%	0 0%
Terminal Rates (c)	3/37 (8%)	1/33 (3%)	0/15 (0%)
Week of First Observation	98	104	
Life Table Tests (d)	P=0 081N	P=0 210N	P=0 213N
Incidental Tumor Tests (d)	P=0 035N	P=0 131N	P=0 077N
Cochran Armitage Trend Test (d)	P=0 026N		
Fisher Exact Test (d)		P=0 181N	P=0 059N
Preputial Gland: Adenoma, Sebaceous Adenoma, or Adenocarcinoma			
Overall Rates (a)	4/50 (8%)	2/50 (4%)	0/50 (0%)
Adjusted Rates (b)	10 5%	6 1%	0 0%
Terminal Rates (c)	3/37 (8%)	2/33 (6%)	0/15 (0%)
Week of First Observation	98	104	
Life Table Tests (d)	P=0 127N	P=0 382N	P=0 213N
Incidental Tumor Tests (d)	P=0 065N	P=0 285N	P=0 077N
Cochran Armitage Trend Test (d)	P=0 037N		
Fisher Exact Test (d)		P=0 339N	P=0 059N

000512

TABLE A3. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	50 mg/kg	100 mg/kg
Testis: Interstitial Cell Tumor			
Overall Rates (a)	40/50 (80%)	45/50 (90%)	36/50 (72%)
Adjusted Rates (b)	90.8%	95.7%	97.1%
Terminal Rates (c)	33/37 (89%)	31/33 (94%)	14/15 (93%)
Week of First Observation	79	74	70
Life Table Tests (d)	P<0.001	P=0.076	P<0.001
Incidental Tumor Tests (d)	P=0.278	P=0.072	P=0.275
Cochran-Armitage Trend Test (d)	P=0.188N		
Fisher Exact Test (d)		P=0.131	P=0.242N
All Sites: Mesothelioma			
Overall Rates (a)	4/50 (8%)	0/50 (0%)	5/50 (10%)
Adjusted Rates (b)	10.3%	0.0%	27.2%
Terminal Rates (c)	3/37 (8%)	0/33 (0%)	3/15 (20%)
Week of First Observation	89		90
Life Table Tests (d)	P=0.127	P=0.075N	P=0.111
Incidental Tumor Tests (d)	P=0.304	P=0.085N	P=0.317
Cochran-Armitage Trend Test (d)	P=0.417		
Fisher Exact Test (d)		P=0.059N	P=0.500
All Sites: Benign Tumors			
Overall Rates (a)	47/50 (94%)	48/50 (96%)	46/50 (92%)
Adjusted Rates (b)	97.9%	97.9%	100.0%
Terminal Rates (c)	36/37 (97%)	32/33 (97%)	15/15 (100%)
Week of First Observation	55	74	43
Life Table Tests (d)	P<0.001	P=0.257	P<0.001
Incidental Tumor Tests (d)	P=0.335	P=0.433	P=0.408
Cochran-Armitage Trend Test (d)	P=0.417N		
Fisher Exact Test (d)		P=0.500	P=0.500N
All Sites: Malignant Tumors			
Overall Rates (a)	16/50 (32%)	19/50 (38%)	22/50 (44%)
Adjusted Rates (b)	34.5%	44.1%	68.6%
Terminal Rates (c)	8/37 (22%)	10/33 (30%)	7/15 (47%)
Week of First Observation	55	74	70
Life Table Tests (d)	P=0.003	P=0.305	P=0.005
Incidental Tumor Tests (d)	P=0.328	P=0.399	P=0.321
Cochran-Armitage Trend Test (d)	P=0.129		
Fisher Exact Test (d)		P=0.338	P=0.151
All Sites: All Tumors			
Overall Rates (a)	49/50 (98%)	49/50 (98%)	47/50 (94%)
Adjusted Rates (b)	98.0%	98.0%	100.0%
Terminal Rates (c)	36/37 (97%)	32/33 (97%)	15/15 (100%)
Week of First Observation	55	74	43
Life Table Tests (d)	P<0.001	P=0.329	P<0.001
Incidental Tumor Tests (d)	P=0.407N	P=0.736N	P=0.616N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Test (d)		P=0.753N	P=0.309N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

000513

TABLE A4a. HISTORICAL INCIDENCE OF THYROID GLAND FOLLICULAR CELL TUMORS IN MALE F344/N RATS (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence in Water Gavage Controls at Battelle Columbus Laboratories (b)			
Chlorpheniramine maleate	0/50	1/50	1/50
Tetrakis(hydroxymethyl)phosphonium chloride	0/47	0/47	0/47
Tetrakis(hydroxymethyl)phosphonium sulfate	0/47	1/47	1/47
TOTAL	0/144 (0.0%)	2/144 (1.4%)	2/144 (1.4%)
SD (c)	0.00%	1.19%	1.19%
Range (d)			
High	0/50	1/47	1/47
Low	0/50	0/47	0/47
Overall Historical Incidence in Untreated Controls			
TOTAL	(e) 16/1,928 (0.8%)	(f) 11/1,928 (0.6%)	(e,f) 27/1,928 (1.4%)
SD (c)	1.41%	0.91%	1.75%
Range (d)			
High	2/44	2/89	3/50
Low	0/50	0/50	0/50

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) No other water gavage studies are included in the historical data base.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Includes one cystadenoma and one papillary cystadenoma
(f) Includes one papillary adenocarcinoma

000514

TABLE A4b. HISTORICAL INCIDENCE OF ADRENAL GLAND CORTICAL TUMORS IN MALE F344/N RATS (a)

Study	Incidence in Controls	
	Adenoma	Adenoma or Carcinoma
Historical Incidence in Water Gavage Controls at Battelle Columbus Laboratories (b)		
Chlorpheniramine maleate	0/49	0/49
Tetrakis(hydroxymethyl)phosphonium chloride	0/50	0/50
Tetrakis(hydroxymethyl)phosphonium sulfate	3/50	3/50
TOTAL	3/149 (2 0%)	3/149 (2 0%)
SD (c)	3 46%	3 46%
Range (d)		
High	3/50	3/50
Low	0/50	0/50
Overall Historical Incidence in Untreated Controls		
TOTAL	28/1,950 (1 4%)	30/1,950 (1 5%)
SD (c)	1 81%	1 84%
Range (d)		
High	4/49	4/49
Low	0/50	0/50

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) No other water gavage studies are included in the historical data base
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals

000515

TABLE A4c. HISTORICAL INCIDENCE OF ADRENAL GLAND MEDULLARY TUMORS IN MALE F344/N RATS (a)

Study	Incidence in Controls		
	Pheochromocytoma	Malignant Pheochromocytoma	Pheochromocytoma or Malignant Pheochromocytoma
Historical Incidence in Water Gavage Controls at Battelle Columbus Laboratories (b)			
Chlorpheniramine maleate	21/49	0/49	21/49
Tetrakis(hydroxymethyl)phosphonium chloride	19/50	0/50	19/50
Tetrakis(hydroxymethyl)phosphonium sulfate	22/50	1/50	23/50
TOTAL	62/149 (41.6%)	1/149 (0.7%)	63/149 (42.3%)
SD (c)	3 19%	1.15%	4.03%
Range (d)			
High	22/50	1/50	23/50
Low	19/50	0/50	19/50
Overall Historical Incidence in Untreated Controls			
TOTAL	427/1,950 (21.9%)	30/1,950 (1.5%)	452/1,950 (23.2%)
SD (c)	12.41%	2.00%	12.39%
Range (d)			
High	31/49	4/49	32/49
Low	2/50	0/50	3/50

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) No other water gavage studies are included in the historical data base.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

000516

TABLE A4d. HISTORICAL INCIDENCE OF LEUKEMIA IN MALE F344/N RATS (a)

Study	Incidence in Controls
Historical Incidence in Water Gavage Controls at Battelle Columbus Laboratories (b)	
Chlorpheniramine maleate	25/50
Tetrakis(hydroxymethyl)phosphonium chloride	19/50
Tetrakis(hydroxymethyl)phosphonium sulfate	30/50
TOTAL	74/150 (49 3%)
SD (c)	11 02%
Range (d)	
High	30/50
Low	19/50
Overall Historical Incidence in Untreated Controls	
TOTAL	583/1,977 (29 5%)
SD (c)	11 59%
Range (d)	
High	30/50
Low	5/50

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) No other water gavage studies are included in the historical data base
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals

000517

TABLE A4e. HISTORICAL INCIDENCE OF PANCREATIC ISLET CELL TUMORS IN MALE F344/N RATS (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence in Water Gavage Controls at Battelle Columbus Laboratories (b)			
Chlorpheniramine maleate	3/50	2/50	5/50
Tetrakis(hydroxymethyl)phosphonium chloride	2/49	0/49	2/49
Tetrakis(hydroxymethyl)phosphonium sulfate	4/48	1/48	5/48
TOTAL	9/147 (6.1%)	3/147 (2.0%)	12/147 (8.2%)
SD (c)	2.13%	2.00%	3.54%
Range (d)			
High	4/48	2/50	5/48
Low	2/49	0/49	2/49
Overall Historical Incidence in Untreated Controls			
TOTAL	63/1,913 (3.3%)	40/1,913 (2.1%)	102/1,913 (5.3%)
SD (c)	3.35%	2.54%	3.58%
Range (d)			
High	6/49	4/49	7/49
Low	0/88	0/50	0/50

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) No other water gavage studies are included in the historical data base.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

000518

TABLE A4f. HISTORICAL INCIDENCE OF PITUITARY GLAND TUMORS IN MALE F344/N RATS (a)

Study	Incidence in Controls		
	Adenoma (b)	Carcinoma (c)	Adenoma or Carcinoma (b,c)
Historical Incidence in Water Gavage Controls at Battelle Columbus Laboratories (d)			
Chlorpheniramine maleate	12/50	0/50	12/50
Tetrakis(hydroxymethyl)phosphonium chloride	17/50	1/50	18/50
Tetrakis(hydroxymethyl)phosphonium sulfate	21/50	0/50	21/50
TOTAL	50/150 (33.3%)	1/150 (0.7%)	51/150 (34.0%)
SD (e)	9.02%	1.15%	9.17%
Range (f)			
High	21/50	1/50	21/50
Low	12/50	0/50	12/50
Overall Historical Incidence in Untreated Controls			
TOTAL	387/1,861 (20.8%)	41/1,861 (2.2%)	428/1,861 (23.0%)
SD (e)	11.25%	2.88%	11.10%
Range (f)			
High	24/46	5/45	25/46
Low	2/39	0/50	2/39

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) Includes adenoma, NOS, chromophobe adenoma, and acidophil adenoma.
(c) Includes carcinoma, NOS, and chromophobe carcinoma
(d) No other water gavage studies are included in the historical data base.
(e) Standard deviation
(f) Range and SD are presented for groups of 35 or more animals.

000519

TABLE A4g. HISTORICAL INCIDENCE OF INTEGUMENTARY SYSTEM TUMORS IN MALE F344/N RATS (a)

Study	Incidence in Controls		
	Fibroma (b)	Fibrosarcoma (c)	Fibroma or Fibrosarcoma (b,c)
Historical Incidence in Water Gavage Controls at Battelle Columbus Laboratories (d)			
Chlorpheniramine maleate	2/50	2/50	4/50
Tetrakis(hydroxymethyl)phosphonium chloride	0/50	1/50	1/50
Tetrakis(hydroxymethyl)phosphonium sulfate	0/50	3/50	3/50
TOTAL	2/150 (1.3%)	6/150 (4.0%)	8/150 (5.3%)
SD (e)	2.31%	2.00%	3.06%
Range (f)			
High	2/50	3/50	4/50
Low	0/50	1/50	1/50
Overall Historical Incidence in Untreated Controls			
TOTAL	110/1,977 (5.6%)	39/1,977 (2.0%)	148/1,977 (7.5%)
SD (e)	3.15%	2.72%	4.27%
Range (f)			
High	6/50	7/50	12/50
Low	0/50	0/50	0/49

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) Includes neurofibroma
(c) Includes sarcoma, NOS, and neurofibrosarcoma
(d) No other water gavage studies are included in the historical data base.
(e) Standard deviation
(f) Range and SD are presented for groups of 35 or more animals.

000520

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Inflammation, NOS	2 (4%)	5 (10%)	4 (8%)
Ulcer, NOS	1 (2%)		
Fibrosis	1 (2%)		
Hyperplasia, NOS	1 (2%)	1 (2%)	1 (2%)
*Subcutaneous tissue	(50)	(50)	(50)
Epidermal inclusion cyst			2 (4%)
RESPIRATORY SYSTEM			
#Trachea	(50)	(47)	(49)
Inflammation, acute	1 (2%)		
Inflammation, chronic	1 (2%)		
#Lung	(50)	(50)	(50)
Congestion, NOS			1 (2%)
Hemorrhage		2 (4%)	1 (2%)
Inflammation, NOS	6 (12%)	3 (6%)	5 (10%)
Foreign material, NOS			1 (2%)
Pigmentation, NOS			1 (2%)
Alveolar macrophages	1 (2%)		
Hyperplasia, alveolar epithelium	3 (6%)	3 (6%)	1 (2%)
Metaplasia, osseous			1 (2%)
HEMATOPOIETIC SYSTEM			
#Bone marrow	(50)	(50)	(50)
Inflammation, granulomatous focal	1 (2%)		
Atrophy, NOS		1 (2%)	
Hyperplasia, hematopoietic	5 (10%)	2 (4%)	5 (10%)
Hyperplasia, reticulum cell	5 (10%)	3 (6%)	1 (2%)
#Spleen	(50)	(50)	(50)
Collapse		1 (2%)	
Congestion, NOS	1 (2%)	1 (2%)	
Fibrosis	2 (4%)	2 (4%)	2 (4%)
Necrosis, coagulative	1 (2%)		
Hemosiderosis	1 (2%)	1 (2%)	
Depletion, lymphoid			1 (2%)
Hematopoiesis	3 (6%)	4 (8%)	1 (2%)
#Mandibular lymph node	(46)	(45)	(39)
Dilatation/sinus	1 (2%)	2 (4%)	1 (3%)
Epidermal inclusion cyst		1 (2%)	
Hemorrhage			1 (3%)
Plasmacytosis	1 (2%)	1 (2%)	
#Mediastinal lymph node	(46)	(45)	(39)
Hemorrhage			1 (3%)
Inflammation, chronic diffuse	1 (2%)		
Plasmacytosis		1 (2%)	
#Pancreatic lymph node	(46)	(45)	(39)
Inflammation, granulomatous focal		1 (2%)	
#Lumbar lymph node	(46)	(45)	(39)
Hemorrhage		1 (2%)	
#Mesenteric lymph node	(46)	(45)	(39)
Dilatation/sinus		3 (7%)	1 (3%)
Inflammation, granulomatous	1 (2%)		2 (5%)

000521

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Ileum	(49)	(46)	(40)
Hyperplasia, lymphoid		1 (2%)	
#Thymus	(37)	(35)	(32)
Cyst, NOS		1 (3%)	
CIRCULATORY SYSTEM			
#Brain	(50)	(50)	(50)
Thrombosis, NOS		1 (2%)	
#Heart	(50)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
#Heart/atrium	(50)	(50)	(50)
Thrombosis, NOS	1 (2%)		1 (2%)
#Heart/ventricle	(50)	(50)	(50)
Thrombosis, NOS			1 (2%)
#Myocardium	(50)	(50)	(50)
Degeneration, NOS	47 (94%)	46 (92%)	42 (84%)
#Cardiac valve	(50)	(50)	(50)
Inflammation, acute focal			1 (2%)
DIGESTIVE SYSTEM			
#Salivary gland	(49)	(47)	(49)
Dilatation/ducts			1 (2%)
Focal cellular change	4 (8%)	2 (4%)	
Atrophy, NOS		1 (2%)	4 (8%)
#Liver	(50)	(50)	(50)
Abnormal curvature		1 (2%)	
Inflammation, acute		1 (2%)	
Inflammation, granulomatous	1 (2%)	2 (4%)	1 (2%)
Fibrosis, condensation	1 (2%)		
Degeneration, cystic	13 (26%)	26 (52%)	24 (48%)
Necrosis, coagulative	5 (10%)	1 (2%)	2 (4%)
Hyperchromatism	1 (2%)		
Cytoplasmic vacuolization	7 (14%)	7 (14%)	12 (24%)
Basophilic cyto change	28 (56%)	23 (46%)	7 (14%)
Focal cellular change	27 (54%)	24 (48%)	10 (20%)
Hyperplasia, nodular	1 (2%)		
Angiectasis	2 (4%)	1 (2%)	1 (2%)
#Bile duct	(50)	(50)	(50)
Fibrosis	4 (8%)	8 (16%)	28 (56%)
Pigmentation, NOS			1 (2%)
Hyperplasia, NOS	50 (100%)	45 (90%)	50 (100%)
#Pancreas	(49)	(50)	(49)
Dilatation/ducts	2 (4%)		1 (2%)
Cyst, NOS		1 (2%)	
Inflammation, chronic	1 (2%)	1 (2%)	
#Pancreatic duct	(49)	(50)	(49)
Hyperplasia, NOS	4 (8%)		
#Pancreatic acinus	(49)	(50)	(49)
Necrosis, focal	1 (2%)		
Focal cellular change	2 (4%)	1 (2%)	1 (2%)
Atrophy, focal	23 (47%)	21 (42%)	25 (51%)
Atrophy, diffuse	8 (16%)	26 (52%)	38 (78%)
Hyperplasia, NOS	2 (4%)	3 (6%)	7 (14%)
#Esophagus	(49)	(50)	(49)
Inflammation, chronic		1 (2%)	1 (2%)
Scar			1 (2%)
#Gastric fundal gland	(50)	(49)	(50)
Dilatation, NOS			1 (2%)
Hyperplasia, NOS		2 (4%)	6 (12%)

000522

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Glandular stomach	(50)	(49)	(50)
Inflammation, NOS	1 (2%)	4 (8%)	7 (14%)
Ulcer, NOS		5 (10%)	15 (30%)
Inflammation, necrotizing	1 (2%)	7 (14%)	4 (8%)
#Forestomach	(50)	(49)	(50)
Inflammation, NOS		3 (6%)	1 (2%)
Ulcer, acute		3 (6%)	
Hyperplasia, epithelial	3 (6%)	8 (16%)	18 (36%)
#Jejunum	(49)	(46)	(40)
Inflammation, chronic	1 (2%)		
#Ileum	(49)	(46)	(40)
Inflammation, acute/chronic	1 (2%)		
Ulcer, healed	1 (2%)		
#Colon	(48)	(48)	(47)
Epidermal inclusion cyst	1 (2%)		
Parasitism	2 (4%)	3 (6%)	2 (4%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Cyst, NOS	2 (4%)		
Multiple cysts	1 (2%)		
Hemorrhagic cyst	1 (2%)		
Pyelonephritis, acute			1 (2%)
Nephropathy	50 (100%)	45 (90%)	47 (94%)
Nephrosis, NOS		4 (8%)	1 (2%)
Necrosis, coagulative		1 (2%)	
#Kidney/pelvis	(50)	(50)	(50)
Inflammation, acute			2 (4%)
#Urinary bladder	(47)	(46)	(49)
Calculus, gross observation only	1 (2%)	2 (4%)	
Mineralization		1 (2%)	
Hemorrhage	1 (2%)		
Inflammation, NOS		1 (2%)	4 (8%)
Hyperplasia, epithelial		2 (4%)	1 (2%)
ENDOCRINE SYSTEM			
#Anterior pituitary	(47)	(49)	(49)
Colloid cyst	4 (9%)	1 (2%)	
Congestion, NOS		1 (2%)	
Pigmentation, NOS		1 (2%)	
Clear cell change		1 (2%)	
Atrophy, diffuse		1 (2%)	
Hyperplasia, chromophobe cell	9 (19%)	12 (24%)	6 (12%)
Angiectasis	1 (2%)	1 (2%)	1 (2%)
#Adrenal	(50)	(50)	(50)
Necrosis, coagulative			3 (6%)
#Adrenal cortex	(50)	(50)	(50)
Degeneration, cystic			1 (2%)
Degeneration, lipoid	12 (24%)	13 (26%)	23 (46%)
Necrosis, coagulative	2 (4%)		1 (2%)
Cytoplasmic vacuolization	13 (26%)	9 (18%)	5 (10%)
Eosinophilic cyto change	7 (14%)	6 (12%)	5 (10%)
Clear cell change	1 (2%)		
Hypertrophy, focal	1 (2%)	3 (6%)	1 (2%)
Hyperplasia, focal	14 (28%)	14 (28%)	16 (32%)
#Adrenal medulla	(50)	(50)	(50)
Hyperplasia, focal	18 (36%)	21 (42%)	16 (32%)
#Thyroid	(50)	(49)	(50)
Ultimobranchial cyst			2 (4%)
Follicular cyst, NOS	4 (8%)	5 (10%)	3 (6%)

000523

TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM			
#Thyroid (Continued)	(50)	(49)	(50)
Focal cellular change			2 (4%)
Hyperplasia, C-cell	39 (78%)	39 (80%)	27 (54%)
Hyperplasia, follicular cell	9 (18%)	7 (14%)	7 (14%)
#Parathyroid	(42)	(37)	(42)
Hyperplasia, NOS	2 (5%)	3 (8%)	6 (14%)
#Pancreatic islets	(49)	(50)	(49)
Atrophy, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Dilatation/ducts	2 (4%)	2 (4%)	
Galactocele	1 (2%)		
Hyperplasia, NOS		4 (8%)	2 (4%)
Hyperplasia, cystic	1 (2%)	4 (8%)	
Lactation		1 (2%)	1 (2%)
*Mammary duct	(50)	(50)	(50)
Hyperplasia, epithelial		1 (2%)	
*Preputial gland	(50)	(50)	(50)
Impaction, NOS	1 (2%)	1 (2%)	1 (2%)
Inflammation, NOS	3 (6%)	2 (4%)	
#Prostate	(47)	(48)	(49)
Cyst, NOS		2 (4%)	3 (6%)
Inflammation, NOS	11 (23%)	15 (31%)	16 (33%)
Necrosis, NOS			1 (2%)
Pigmentation, NOS			2 (4%)
*Seminal vesicle	(50)	(50)	(50)
Dilatation, NOS	1 (2%)		1 (2%)
Inflammation, NOS	1 (2%)		1 (2%)
Atrophy, NOS	1 (2%)	3 (6%)	4 (8%)
#Testis	(50)	(50)	(50)
Mineralization			1 (2%)
Inflammation, NOS	2 (4%)		
Infarct, NOS			1 (2%)
Atrophy, NOS	5 (10%)	8 (16%)	17 (34%)
Hyperplasia, interstitial cell	24 (48%)	23 (46%)	21 (42%)
Angiectasis		1 (2%)	
#Testis/tubule	(50)	(50)	(50)
Necrosis, coagulative		1 (2%)	
*Epididymis	(50)	(50)	(50)
Inflammation, NOS		3 (6%)	2 (4%)
Fibrosis	1 (2%)		
NERVOUS SYSTEM			
#Brain	(50)	(50)	(50)
Hydrocephalus, NOS			1 (2%)
Cyst, NOS		1 (2%)	
Hemorrhage	2 (4%)	3 (6%)	2 (4%)
Inflammation, granulomatous focal			1 (2%)
Necrosis, ischemic			1 (2%)
#Brain/thalamus	(50)	(50)	(50)
Atrophy, pressure	1 (2%)		
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(50)
Inflammation, NOS			4 (8%)
Synechia, NOS			1 (2%)
*Eye/anterior chamber	(50)	(50)	(50)
Inflammation, chronic diffuse			1 (2%)

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TABLE A5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
SPECIAL SENSE ORGANS (Continued)			
*Eye/cornea	(50)	(50)	(50)
Inflammation, NOS	2 (4%)	2 (4%)	19 (38%)
Hyperplasia, epithelial			1 (2%)
*Eye/retina	(50)	(50)	(50)
Atrophy, NOS	5 (10%)	9 (18%)	24 (48%)
*Eye/crystalline lens	(50)	(50)	(50)
Cataract	4 (8%)	14 (28%)	19 (38%)
MUSCULOSKELETAL SYSTEM			
*Femur	(50)	(50)	(50)
Fibrous osteodystrophy	1 (2%)		2 (4%)
*Skeletal muscle	(50)	(50)	(50)
Degeneration, NOS			1 (2%)
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Vegetable foreign body			1 (2%)
Inflammation, granulomatous			1 (2%)
*Peritoneal cavity	(50)	(50)	(50)
Inflammation, acute fibrinous			1 (2%)
*Pleural cavity	(50)	(50)	(50)
Inflammation, acute fibrinous			1 (2%)
*Pleura	(50)	(50)	(50)
Inflammation, chronic	1 (2%)		
*Mesentery	(50)	(50)	(50)
Hemorrhage			1 (2%)
Inflammation, chronic		1 (2%)	1 (2%)
Inflammation, granulomatous		1 (2%)	
Necrosis, fat	1 (2%)	8 (16%)	2 (4%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Mineralization	1 (2%)		
Congestion, NOS	2 (4%)	3 (6%)	8 (16%)
Necrosis, coagulative		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
None			

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

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APPENDIX B

SUMMARY OF LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

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TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Squamous cell carcinoma		1 (2%)	
Basal cell tumor		1 (2%)	
*Subcutaneous tissue	(50)	(50)	(50)
Fibroma		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#Trachea	(48)	(49)	(49)
Follicular cell carcinoma, invasive			1 (2%)
#Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma			1 (2%)
C-cell carcinoma, metastatic		1 (2%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Leukemia, mononuclear cell	5 (10%)	12 (24%)	4 (8%)
#Renal lymph node	(43)	(43)	(39)
Pheochromocytoma, metastatic	1 (2%)		
CIRCULATORY SYSTEM			
None			
DIGESTIVE SYSTEM			
*Tongue	(50)	(50)	(50)
Squamous cell carcinoma	1 (2%)		
#Liver	(50)	(50)	(50)
Neoplastic nodule			1 (2%)
#Forestomach	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)		1 (2%)
#Jejunum	(50)	(48)	(44)
Leiomyoma	1 (2%)		
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Tubular cell adenoma		1 (2%)	
ENDOCRINE SYSTEM			
#Pituitary intermedia	(49)	(49)	(48)
Adenoma, NOS	1 (2%)		1 (2%)
#Anterior pituitary	(49)	(49)	(48)
Carcinoma, NOS	2 (4%)		
Adenoma, NOS	16 (33%)	10 (20%)	2 (4%)
#Adrenal	(50)	(50)	(50)
Cortical adenoma		1 (2%)	
#Adrenal medulla	(50)	(50)	(50)
Pheochromocytoma	4 (8%)	2 (4%)	1 (2%)
Pheochromocytoma, malignant	1 (2%)		

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TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Thyroid	(50)	(50)	(50)
Follicular cell adenoma	2 (4%)		5 (10%)
Follicular cell carcinoma		1 (2%)	2 (4%)
C-cell adenoma	9 (18%)	6 (12%)	1 (2%)
C-cell carcinoma		1 (2%)	
#Pancreatic islets	(50)	(50)	(50)
Islet cell adenoma		1 (2%)	
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Adenocarcinoma, NOS	1 (2%)		
Fibroadenoma	6 (12%)	10 (20%)	1 (2%)
*Clitoral gland	(50)	(50)	(50)
Adenoma, NOS	1 (2%)		
*Vagina	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)		
Sarcoma, NOS		1 (2%)	
#Uterus	(50)	(48)	(50)
Adenocarcinoma, NOS	1 (2%)		
Endometrial stromal polyp	9 (18%)	9 (19%)	5 (10%)
#Uterus/endometrium	(50)	(48)	(50)
Papilloma, NOS	1 (2%)		
NERVOUS SYSTEM			
#Brain	(50)	(50)	(50)
Carcinoma, NOS, invasive	2 (4%)		
Astrocytoma	1 (2%)		
SPECIAL SENSE ORGANS			
None			
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
None			
ALL OTHER SYSTEMS			
None			
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	4	5	18
Moribund sacrifice	9	7	16
Terminal sacrifice	37	37	14
Dosing accident		1	2

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TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	37	37	21
Total primary tumors	64	58	26
Total animals with benign tumors	31	28	17
Total benign tumors	52	42	19
Total animals with malignant tumors	12	16	6
Total malignant tumors	12	16	6
Total animals with secondary tumors##	3	1	1
Total secondary tumors	3	1	1
Total animals with tumors uncertain--benign or malignant			1
Total uncertain tumors			1

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

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TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT: VEHICLE CONTROL

ANIMAL NUMBER	037	045	030	006	004	041	016	032	001	009	050	002	044	003	005	007	008	010	011	012	013	014	015	017	018
WEEKS ON STUDY	024	036	062	077	083	083	086	087	094	095	098	102	103	104	104	104	104	104	104	104	104	104	104	104	104
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	+	+	+	+	−	+	+	−	+	+	+	−	+	+	+	+	−	+	+	+	+	+
Pheochromocytoma, metastatic																	X								
Thymus	−	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell carcinoma				X																					
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma					X																				
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma												X													
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS										X															
Adenoma, NOS					X						X		X										X	X	X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma					X									X											
Pheochromocytoma, malignant																	X								
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma													X								X				
C-cell adenoma										X				X					X				X		
Parathyroid	−	−	+	−	−	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	−	−	−
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS																									
Fibroadenoma																									
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
Vagina	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Squamous cell papilloma												X													
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma, NOS																									
Adenocarcinoma, NOS																								X	
Endometrial stromal polyp							X					X						X						X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS, invasive										X															
Astrocytoma		X																							
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell			X			X	X				X														

+ Tissue examined microscopically
− Required tissue not examined microscopically
X Tumor incidence
N Necropsy, no autolysis, no microscopic examination
S Animal missexed

No tissue information submitted
C Necropsy, no histology due to protocol
A. Autolysis
M Animal missing
B No necropsy performed

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TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: VEHICLE CONTROL (Continued)

ANIMAL NUMBER	019	020	021	022	023	024	025	026	027	028	029	031	033	034	035	036	038	039	040	042	043	046	047	048	049	TOTAL TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	43
Pheochromocytoma, metastatic																										1
Thymus	+	−	−	+	+	+	+	+	−	+	+	−	+	−	−	+	+	+	−	+	+	+	+	+	−	38
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Oral cavity	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell carcinoma																										1
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma																										1
Large intestine	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma, NOS																			X							2
Adenoma, NOS	X				X	X		X	X	X	X		X				X	X			X					17
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma	X				X																					4
Pheochromocytoma, malignant																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma																										2
C cell adenoma				X										X	X									X	X	9
Parathyroid	+	−	+	−	−	−	+	+	+	+	+	−	+	−	+	+	+	+	−	−	+	+	−	+	+	32
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	*50
Adenocarcinoma, NOS				X																						1
Fibroadenoma	X							X		X			X				X				X					6
Preputial/clitoral gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS	X																									1
Vagina	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Squamous cell papilloma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Papilloma, NOS																	X									1
Adenocarcinoma, NOS																										1
Endometrial stromal polyp															X					X			X	X	X	9
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, NOS, invasive																			X							2
Astrocytoma																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell														X												5

* Animals necropsied

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TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT: LOW DOSE

ANIMAL NUMBER	049	034	030	016	026	001	050	039	023	017	013	018	011	002	003	004	005	006	007	008	009	010	012	014	015
WEEKS ON STUDY	012	045	047	067	067	082	083	089	097	098	099	102	103	105	105	105	105	105	105	105	105	105	105	105	105
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																									
Basal cell tumor																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma				X																					
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C cell carcinoma, metastatic																						X			
Trachea	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	+	−	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−	−
Thymus	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tubular cell adenoma																									
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS												X	X								X		X		
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cortical adenoma															X										
Pheochromocytoma										X	X														
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma																							X		
C-cell adenoma													X							X			X		X
C-cell carcinoma																						X			
Parathyroid	+	+	−	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell adenoma																			X						
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma												X									X				X
Vagina	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS		X																							
Uterus	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
Endometrial stromal polyp															X				X	X			X		
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell					X			X	X	X	X		X							X				X	

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TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	019	020	021	022	024	025	027	028	029	031	032	033	035	036	037	038	040	041	042	043	044	045	046	047	048	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Squamous cell carcinoma																			X							1
Basal cell tumor																X										1
Subcutaneous tissue	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell carcinoma, metastatic																										1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	43
Thymus	−	+	−	+	+	+	+	+	+	+	−	+	−	+	+	−	+	−	+	+	+	+	+	+	+	41
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tubular cell adenoma			X																							1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	49
Adenoma, NOS	X	X								X													X	X	X	10
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cortical adenoma																										1
Pheochromocytoma																										2
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell carcinoma																X										1
C-cell adenoma	X							X																		6
C-cell carcinoma																										1
Parathyroid	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	42
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islet cell adenoma																										1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroadenoma	X								X		X	X			X		X	X								10
Vagina	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Sarcoma, NOS																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Endometrial stromal polyp					X					X					X			X		X						9
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell					X									X		X						X				12

* Animals necropsied

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TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT: HIGH DOSE

ANIMAL NUMBER	006	002	013	012	021	027	029	025	017	018	042	046	048	016	023	036	008	004	022	024	014	050	010	045	019
WEEKS ON STUDY	054	055	055	056	059	059	059	060	062	062	063	069	069	072	073	077	078	081	081	081	084	084	085	089	093
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell carcinoma, invasive																									
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	−	+	+	+	−	+	−	−	−	+	+	−	+	+	−	+	−	+	+	−	+	−	+	+	+
Thymus	−	−	−	+	+	+	+	+	+	+	+	−	−	+	−	−	+	+	−	−	−	+	+	−	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Neoplastic nodule																									
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma							X																		
Small intestine	+	+	+	+	+	+	+	−	+	+	−	+	+	−	+	+	+	+	+	+	+	−	−	+	+
Large intestine	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Adenoma, NOS																									
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																								X	
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma												X													
Follicular cell carcinoma																									
C-cell adenoma																									
Parathyroid	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	N	N	+	+	+	+	+	+	+
Fibroadenoma									X																
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp														X										X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leukemia, mononuclear cell																									

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TABLE B2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	040	026	007	033	041	009	003	001	020	028	031	005	011	015	030	032	034	035	037	038	039	043	044	047	049	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	093	094	095	095	095	097	098	099	099	101	101	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroma											X															1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																						X				1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	49
Follicular cell carcinoma, invasive											X															1
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	39
Thymus	+	+	+	+	+	+	−	−	−	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	34
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Neoplastic nodule						X																				1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Small intestine	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Large intestine	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
Pituitary	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma, NOS						X											X			X						3
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma																										1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell adenoma												X	X		X	X										5
Follicular cell carcinoma					X						X															2
C-cell adenoma																					X					1
Parathyroid	+	+	+	+	+	+	−	+	−	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	42
REPRODUCTIVE SYSTEM																										
Mammary gland	+	+	N	+	+	N	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibroadenoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endometrial stromal polyp												X							X				X			5
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leukemia, mononuclear cell	X	X										X		X												4

* Animals necropsied

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TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	50 mg/kg	100 mg/kg
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	5/50 (10%)	12/50 (24%)	4/50 (8%)
Adjusted Rates (b)	11.2%	27.6%	21.0%
Terminal Rates (c)	1/37 (3%)	6/37 (16%)	2/14 (14%)
Week of First Observation	62	67	93
Life Table Tests (d)	P=0.202	P=0.070	P=0.397
Incidental Tumor Tests (d)	P=0.221N	P=0.034	P=0.317N
Cochran-Armitage Trend Test (d)	P=0.443N		
Fisher Exact Test (d)		P=0.054	P=0.500N
Pituitary Gland: Adenoma			
Overall Rates (a)	16/49 (33%)	10/49 (20%)	2/48 (4%)
Adjusted Rates (b)	39.8%	26.2%	14.3%
Terminal Rates (c)	13/37 (35%)	8/36 (22%)	2/14 (14%)
Week of First Observation	83	102	104
Life Table Tests (d)	P=0.032N	P=0.147N	P=0.060N
Incidental Tumor Tests (d)	P=0.005N	P=0.146N	P=0.015N
Cochran-Armitage Trend Test (d)	P<0.001N		
Fisher Exact Test (d)		P=0.126N	P<0.001N
Pituitary Gland: Adenoma or Carcinoma			
Overall Rates (a)	18/49 (37%)	10/49 (20%)	2/48 (4%)
Adjusted Rates (b)	43.7%	26.2%	14.3%
Terminal Rates (c)	14/37 (38%)	8/36 (22%)	2/14 (14%)
Week of First Observation	83	102	104
Life Table Tests (d)	P=0.013N	P=0.074N	P=0.034N
Incidental Tumor Tests (d)	P=0.001N	P=0.068N	P=0.004N
Cochran-Armitage Trend Test (d)	P<0.001N		
Fisher Exact Test (d)		P=0.058N	P<0.001N
Adrenal Gland: Pheochromocytoma			
Overall Rates (a)	4/50 (8%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	10.1%	4.9%	3.7%
Terminal Rates (c)	3/37 (8%)	0/37 (0%)	0/14 (0%)
Week of First Observation	83	98	89
Life Table Tests (d)	P=0.300N	P=0.340N	P=0.457N
Incidental Tumor Tests (d)	P=0.086N	P=0.359N	P=0.275N
Cochran-Armitage Trend Test (d)	P=0.118N		
Fisher Exact Test (d)		P=0.339N	P=0.181N
Adrenal Gland: Pheochromocytoma or Malignant Pheochromocytoma			
Overall Rates (a)	5/50 (10%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	12.7%	4.9%	3.7%
Terminal Rates (c)	4/37 (11%)	0/37 (0%)	0/14 (0%)
Week of First Observation	83	98	89
Life Table Tests (d)	P=0.197N	P=0.222N	P=0.364N
Incidental Tumor Tests (d)	P=0.051N	P=0.235N	P=0.210N
Cochran-Armitage Trend Test (d)	P=0.060N		
Fisher Exact Test (d)		P=0.218N	P=0.102N
Thyroid Gland: Follicular Cell Adenoma			
Overall Rates (a)	2/50 (4%)	0/50 (0%)	5/50 (10%)
Adjusted Rates (b)	5.3%	0.0%	30.4%
Terminal Rates (c)	1/37 (3%)	0/37 (0%)	4/14 (29%)
Week of First Observation	103		69
Life Table Tests (d)	P=0.015	P=0.240N	P=0.020
Incidental Tumor Tests (d)	P=0.069	P=0.240N	P=0.083
Cochran-Armitage Trend Test (d)	P=0.118		
Fisher Exact Test (d)		P=0.247N	P=0.218

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TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	50 mg/kg	100 mg/kg
Thyroid Gland: Follicular Cell Adenoma or Carcinoma			
Overall Rates (a)	2/50 (4%)	1/50 (2%)	7/50 (14%)
Adjusted Rates (b)	5.3%	2.7%	37.6%
Terminal Rates (c)	1/37 (3%)	1/37 (3%)	4/14 (29%)
Week of First Observation	103	105	69
Life Table Tests (d)	P=0.001	P=0.500N	P=0.003
Incidental Tumor Tests (d)	P=0.026	P=0.500N	P=0.045
Cochran-Armitage Trend Test (d)	P=0.036		
Fisher Exact Test (d)		P=0.500N	P=0.080
Thyroid Gland: C-Cell Adenoma			
Overall Rates (a)	9/50 (18%)	6/50 (12%)	1/50 (2%)
Adjusted Rates (b)	23.5%	15.8%	7.1%
Terminal Rates (c)	8/37 (22%)	5/37 (14%)	1/14 (7%)
Week of First Observation	95	103	104
Life Table Tests (d)	P=0.102N	P=0.287N	P=0.156N
Incidental Tumor Tests (d)	P=0.050N	P=0.287N	P=0.100N
Cochran-Armitage Trend Test (d)	P=0.008N		
Fisher Exact Test (d)		P=0.288N	P=0.008N
Thyroid Gland: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	9/50 (18%)	7/50 (14%)	1/50 (2%)
Adjusted Rates (b)	23.5%	18.4%	7.1%
Terminal Rates (c)	8/37 (22%)	6/37 (16%)	1/14 (7%)
Week of First Observation	95	103	104
Life Table Tests (d)	P=0.126N	P=0.392N	P=0.156N
Incidental Tumor Tests (d)	P=0.065N	P=0.392N	P=0.100N
Cochran-Armitage Trend Test (d)	P=0.009N		
Fisher Exact Test (d)		P=0.393N	P=0.008N
Mammary Gland: Fibroadenoma			
Overall Rates (a)	6/50 (12%)	10/50 (20%)	1/50 (2%)
Adjusted Rates (b)	16.2%	26.3%	2.4%
Terminal Rates (c)	6/37 (16%)	9/37 (24%)	0/14 (0%)
Week of First Observation	104	102	62
Life Table Tests (d)	P=0.462N	P=0.204	P=0.297N
Incidental Tumor Tests (d)	P=0.278N	P=0.204	P=0.168N
Cochran-Armitage Trend Test (d)	P=0.078N		
Fisher Exact Test (d)		P=0.207	P=0.056N
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	9/50 (18%)	9/48 (19%)	5/50 (10%)
Adjusted Rates (b)	22.8%	25.0%	26.4%
Terminal Rates (c)	7/37 (19%)	9/36 (25%)	3/14 (21%)
Week of First Observation	86	105	72
Life Table Tests (d)	P=0.358	P=0.580	P=0.431
Incidental Tumor Tests (d)	P=0.508N	P=0.566	P=0.469N
Cochran-Armitage Trend Test (d)	P=0.167N		
Fisher Exact Test (d)		P=0.565	P=0.194N
All Sites: Benign Tumors			
Overall Rates (a)	31/50 (62%)	28/50 (56%)	17/50 (34%)
Adjusted Rates (b)	72.0%	66.6%	77.8%
Terminal Rates (c)	25/37 (68%)	23/37 (62%)	10/14 (71%)
Week of First Observation	83	67	59
Life Table Tests (d)	P=0.210	P=0.352N	P=0.186
Incidental Tumor Tests (d)	P=0.085N	P=0.356N	P=0.179N
Cochran-Armitage Trend Test (d)	P=0.003N		
Fisher Exact Test (d)		P=0.342N	P=0.005N

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TABLE B3. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	50 mg/kg	100 mg/kg
All Sites: Malignant Tumors			
Overall Rates (a)	12/50 (24%)	16/50 (32%)	6/50 (12%)
Adjusted Rates (b)	26.2%	35.9%	29.2%
Terminal Rates (c)	5/37 (14%)	9/37 (24%)	2/14 (14%)
Week of First Observation	36	45	93
Life Table Tests (d)	P=0.474	P=0.278	P=0.567N
Incidental Tumor Tests (d)	P=0.041N	P=0.241	P=0.048N
Cochran-Armitage Trend Test (d)	P=0.094N		
Fisher Exact Test (d)		P=0.252	P=0.097N
All Sites: All Tumors			
Overall Rates (a)	37/50 (74%)	37/50 (74%)	21/50 (42%)
Adjusted Rates (b)	78.6%	80.4%	85.3%
Terminal Rates (c)	27/37 (73%)	28/37 (76%)	11/14 (79%)
Week of First Observation	36	45	59
Life Table Tests (d)	P=0.168	P=0.568N	P=0.184
Incidental Tumor Tests (d)	P=0.018N	P=0.576	P=0.054N
Cochran-Armitage Trend Test (d)	P<0.001N		
Fisher Exact Test (d)		P=0.590N	P=0.002N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

000539

TABLE B4a. HISTORICAL INCIDENCE OF THYROID GLAND FOLLICULAR CELL TUMORS IN FEMALE F344/N RATS (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence in Water Gavage Controls at Battelle Columbus Laboratories (b)			
Chlorpheniramine maleate	0/47	0/47	0/47
Tetrakis(hydroxymethyl)phosphonium chloride	3/50	0/50	3/50
Tetrakis(hydroxymethyl)phosphonium sulfate	0/49	1/49	1/49
TOTAL	3/146 (2.1%)	1/146 (0.7%)	4/146 (2.7%)
SD (c)	3.46%	1.18%	3.05%
Range (d)			
High	3/50	1/49	3/50
Low	0/49	0/50	0/47
Overall Historical Incidence in Untreated Controls			
TOTAL	(e) 13/1,952 (0.7%)	(f) 7/1,952 (0.4%)	(e,f) 20/1,952 (1.0%)
SD (c)	1.11%	0.78%	1.34%
Range (d)			
High	2/42	1/47	2/42
Low	0/50	0/86	0/50

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) No other water gavage studies are included in the historical data base.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.
(e) Includes one papillary adenoma, one cystadenoma, and one papillary cystadenoma
(f) Includes one papillary carcinoma and one papillary cystadenocarcinoma

000540

TABLE B4b. HISTORICAL INCIDENCE OF LEUKEMIA IN FEMALE F344/N RATS (a)

Study	Incidence in Controls
Historical Incidence in Water Gavage Controls at Battelle Columbus Laboratories (b)	
Chlorpheniramine maleate	11/50
Tetrakis(hydroxymethyl)phosphonium chloride	4/50
Tetrakis(hydroxymethyl)phosphonium sulfate	23/49
TOTAL	38/149 (25.5%)
SD (c)	19.72%
Range (d)	
High	23/49
Low	4/50
Overall Historical Incidence in Untreated Controls	
TOTAL	375/2,021 (18.6%)
SD (c)	6.55%
Range (d)	
High	19/50
Low	3/50

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) No other water gavage studies are included in the historical data base.
(c) Standard deviation
(d) Range and SD are presented for groups of 35 or more animals.

000541

TABLE B4c. HISTORICAL INCIDENCE OF PITUITARY GLAND TUMORS IN FEMALE F344/N RATS (a)

Study	Incidence in Controls		
	Adenoma (b)	Carcinoma (c)	Adenoma or Carcinoma (b,c)
Historical Incidence in Water Gavage Controls at Battelle Columbus Laboratories (d)			
Chlorpheniramine maleate	24/48	1/48	25/48
Tetrakis(hydroxymethyl)phosphonium chloride	24/49	0/49	24/49
Tetrakis(hydroxymethyl)phosphonium sulfate	23/46	0/46	23/46
TOTAL	71/143 (49.7%)	1/143 (0.7%)	72/143 (50.3%)
SD (e)	0.59%	1.20%	1.58%
Range (f)			
High	24/48	1/48	25/48
Low	24/49	0/49	24/49
Overall Historical Incidence			
TOTAL	862/1,952 (44.2%)	71/1,952 (3.6%)	931/1,952 (47.7%)
SD (e)	11.56%	3.97%	11.02%
Range (f)			
High	33/47	8/49	33/47
Low	7/39	0/50	9/39

(a) Data as of August 30, 1985, for studies of at least 104 weeks
(b) Includes adenoma, NOS, chromophobe adenoma, and acidophil adenoma
(c) Includes carcinoma, NOS, and chromophobe carcinoma
(d) No other water gavage studies are included in the historical data base.
(e) Standard deviation
(f) Range and SD are presented for groups of 35 or more animals.

000542

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Inflammation, NOS	1 (2%)	†2 (4%)	1 (2%)
Hyperplasia, NOS	1 (2%)		
Acanthosis	1 (2%)	1 (2%)	1 (2%)
*Subcutaneous tissue	(50)	(50)	(50)
Epidermal inclusion cyst	2 (4%)		3 (6%)
Inflammation, acute/chronic			1 (2%)
Inflammation, chronic	1 (2%)		1 (2%)
RESPIRATORY SYSTEM			
#Trachea	(48)	(49)	(49)
Inflammation, acute	2 (4%)		
Inflammation, acute/chronic	1 (2%)		
#Lung	(50)	(50)	(50)
Vegetable foreign body			1 (2%)
Congestion, NOS		3 (6%)	4 (8%)
Hemorrhage			1 (2%)
Inflammation, NOS	6 (12%)	1 (2%)	7 (14%)
Pneumonia, aspiration			2 (4%)
Hyperplasia, alveolar epithelium	2 (4%)		1 (2%)
HEMATOPOIETIC SYSTEM			
#Bone marrow	(50)	(50)	(50)
Inflammation, granulomatous	1 (2%)		
Atrophy, NOS	3 (6%)		1 (2%)
Hyperplasia, hematopoietic	2 (4%)	4 (8%)	11 (22%)
Hyperplasia, granulocytic		1 (2%)	
Hyperplasia, reticulum cell	6 (12%)	19 (38%)	7 (14%)
#Spleen	(50)	(50)	(50)
Inflammation, granulomatous			1 (2%)
Hemosiderosis	7 (14%)	3 (6%)	1 (2%)
Depletion, lymphoid	2 (4%)		1 (2%)
Angiectasis		2 (4%)	
Hematopoiesis	1 (2%)	2 (4%)	9 (18%)
#Splenic capsule	(50)	(50)	(50)
Hematoma, NOS		2 (4%)	
#Mandibular lymph node	(43)	(43)	(39)
Inflammation, acute			2 (5%)
Inflammation, granulomatous		1 (2%)	
#Mediastinal lymph node	(43)	(43)	(39)
Inflammation, granulomatous		1 (2%)	
#Mesenteric lymph node	(43)	(43)	(39)
Dilatation/sinus	1 (2%)	3 (7%)	
Hemorrhage			1 (3%)
#Lung	(50)	(50)	(50)
Hematopoiesis		1 (2%)	
#Thymus	(38)	(41)	(34)
Ultimobranchial cyst	1 (3%)		
Cyst, NOS	2 (5%)	1 (2%)	
Hemorrhage		1 (2%)	
Inflammation, acute			1 (3%)
Fibrosis, focal	1 (3%)		
Depletion, lymphoid			1 (3%)
Hyperplasia, epithelial	1 (3%)		

000543

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
CIRCULATORY SYSTEM			
#Heart	(50)	(50)	(50)
Thrombosis, NOS	1 (2%)		
Inflammation, acute/chronic	1 (2%)		
#Heart/atrium	(50)	(50)	(50)
Thrombosis, NOS			1 (2%)
#Myocardium	(50)	(50)	(50)
Degeneration, NOS	41 (82%)	38 (76%)	26 (52%)
Necrosis, coagulative		1 (2%)	
#Jejunum	(50)	(48)	(44)
Lymphangiectasis			1 (2%)
DIGESTIVE SYSTEM			
#Salivary gland	(50)	(49)	(48)
Edema, NOS			1 (2%)
Inflammation, chronic focal	2 (4%)		
Focal cellular change	4 (8%)	3 (6%)	
Atrophy, NOS	1 (2%)		2 (4%)
Atrophy, focal	3 (6%)		
Hyperplasia, NOS			1 (2%)
#Liver	(50)	(50)	(50)
Abnormal curvature	2 (4%)	1 (2%)	2 (4%)
Hemorrhage, chronic		1 (2%)	
Inflammation, acute		1 (2%)	
Inflammation, acute/chronic	1 (2%)		
Inflammation, granulomatous	3 (6%)	3 (6%)	
Degeneration, cystic			5 (10%)
Degeneration, lipoid		1 (2%)	2 (4%)
Necrosis, coagulative	2 (4%)	2 (4%)	
Cytoplasmic vacuolization	6 (12%)	6 (12%)	18 (36%)
Basophilic cyto change	36 (72%)	40 (80%)	43 (86%)
Focal cellular change	8 (16%)	7 (14%)	10 (20%)
Hyperplasia, nodular		2 (4%)	1 (2%)
Angiectasis		2 (4%)	
#Bile duct	(50)	(50)	(50)
Fibrosis	1 (2%)		1 (2%)
Hyperplasia, NOS	17 (34%)	15 (30%)	35 (70%)
#Pancreas	(50)	(50)	(50)
Dilatation/ducts	2 (4%)		
Edema, NOS			2 (4%)
Inflammation, acute focal	1 (2%)		
#Pancreatic acinus	(50)	(50)	(50)
Edema, NOS			1 (2%)
Cytoplasmic vacuolization		1 (2%)	1 (2%)
Focal cellular change			2 (4%)
Eosinophilic cyto change			1 (2%)
Atrophy, NOS	3 (6%)	1 (2%)	
Atrophy, focal	8 (16%)	14 (28%)	12 (24%)
Atrophy, diffuse	5 (10%)	27 (54%)	42 (84%)
Hyperplasia, NOS	1 (2%)	1 (2%)	
*Pharynx	(50)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
#Esophagus	(50)	(50)	(50)
Dilatation, NOS			1 (2%)
Inflammation, acute necrotizing			1 (2%)
Inflammation, acute/chronic			1 (2%)
#Gastric fundal gland	(50)	(50)	(50)
Hyperplasia, NOS		1 (2%)	5 (10%)

000544

TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Glandular stomach	(50)	(50)	(50)
Mineralization		1 (2%)	
Inflammation, NOS	1 (2%)	2 (4%)	3 (6%)
Ulcer, NOS	1 (2%)	2 (4%)	19 (38%)
Inflammation, necrotizing		1 (2%)	9 (18%)
#Forestomach	(50)	(50)	(50)
Inflammation, NOS			2 (4%)
Hyperplasia, epithelial	4 (8%)	5 (10%)	18 (36%)
#Duodenum	(50)	(48)	(44)
Inflammation, acute/chronic	1 (2%)		
#Jejunum	(50)	(48)	(44)
Edema, NOS			1 (2%)
Inflammation, granulomatous	1 (2%)		
#Colon	(47)	(49)	(45)
Parasitism		1 (2%)	3 (7%)
#Cecum	(47)	(49)	(45)
Edema, NOS			1 (2%)
URINARY SYSTEM			
#Kidney	(50)	(50)	(50)
Pyelonephritis, acute			1 (2%)
Nephropathy	38 (76%)	38 (76%)	48 (96%)
#Urinary bladder	(49)	(48)	(49)
Edema, NOS		1 (2%)	
Inflammation, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#Pituitary intermedia	(49)	(49)	(48)
Cyst, NOS		1 (2%)	
#Anterior pituitary	(49)	(49)	(48)
Cyst, NOS	1 (2%)		
Colloid cyst	14 (29%)	7 (14%)	1 (2%)
Follicular cyst, NOS			1 (2%)
Clear cell change			1 (2%)
Hyperplasia, chromophobe cell	15 (31%)	16 (33%)	3 (6%)
Angiectasis	3 (6%)	5 (10%)	
#Adrenal	(50)	(50)	(50)
Congestion, NOS		1 (2%)	4 (8%)
Necrosis, coagulative			1 (2%)
#Adrenal cortex	(50)	(50)	(50)
Degeneration, lipoid	19 (38%)	20 (40%)	30 (60%)
Necrosis, NOS		1 (2%)	
Necrosis, coagulative		1 (2%)	3 (6%)
Cytoplasmic vacuolization	5 (10%)	2 (4%)	2 (4%)
Eosinophilic cyto change	5 (10%)	10 (20%)	
Hypertrophy, focal	5 (10%)	6 (12%)	1 (2%)
Hyperplasia, focal	12 (24%)	18 (36%)	14 (28%)
Angiectasis	1 (2%)	3 (6%)	4 (8%)
#Adrenal medulla	(50)	(50)	(50)
Hyperplasia, focal	10 (20%)	14 (28%)	4 (8%)
#Thyroid	(50)	(50)	(50)
Ultimobranchial cyst	1 (2%)		12 (24%)
Follicular cyst, NOS	4 (8%)	2 (4%)	2 (4%)
Focal cellular change			4 (8%)
Hyperplasia, C-cell	43 (86%)	44 (88%)	27 (54%)
Hyperplasia, follicular cell	10 (20%)	10 (20%)	26 (52%)
#Parathyroid	(32)	(42)	(42)
Hyperplasia, NOS		6 (14%)	1 (2%)

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TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM (Continued)			
#Pancreatic islets	(50)	(50)	(50)
Hyperplasia, focal		1 (2%)	
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Dilatation/ducts	1 (2%)	1 (2%)	1 (2%)
Hyperplasia, NOS	3 (6%)	3 (6%)	
Hyperplasia, focal	1 (2%)		
Hyperplasia, cystic	16 (32%)	24 (48%)	
*Nipple	(50)	(50)	(50)
Epidermal inclusion cyst		1 (2%)	3 (6%)
*Clitoral gland	(50)	(50)	(50)
Impaction, NOS		1 (2%)	
Inflammation, chronic		1 (2%)	
Hyperplasia, NOS		1 (2%)	
#Uterus	(50)	(48)	(50)
Dilatation, NOS	3 (6%)	6 (13%)	
#Uterus/endometrium	(50)	(48)	(50)
Cyst, NOS	4 (8%)	4 (8%)	11 (22%)
Inflammation, acute	1 (2%)	2 (4%)	
Inflammation, acute/chronic	1 (2%)		
Hyperplasia, cystic	2 (4%)		4 (8%)
#Ovary/parovarian	(50)	(50)	(50)
Inflammation, granulomatous		1 (2%)	
#Ovary	(50)	(50)	(50)
Cyst, NOS	7 (14%)	7 (14%)	5 (10%)
Inflammation, acute			1 (2%)
Fibrosis	1 (2%)		
NERVOUS SYSTEM			
#Brain/meninges	(50)	(50)	(50)
Inflammation, acute/chronic	1 (2%)		
#Brain	(50)	(50)	(50)
Hydrocephalus, NOS	1 (2%)		
Spongiosis			2 (4%)
Hemorrhage	2 (4%)	2 (4%)	
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(50)
Collapse		1 (2%)	1 (2%)
Inflammation, NOS	1 (2%)	1 (2%)	3 (6%)
*Eye/cornea	(50)	(50)	(50)
Inflammation, NOS	2 (4%)	2 (4%)	25 (50%)
*Eye/retina	(50)	(50)	(50)
Atrophy, NOS	3 (6%)	31 (62%)	30 (60%)
*Eye/crystalline lens	(50)	(50)	(50)
Cataract	4 (8%)	26 (52%)	31 (62%)
*Zymbal gland	(50)	(50)	(50)
Impaction, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*Femur	(50)	(50)	(50)
Fibrous osteodystrophy			1 (2%)
*Skeletal muscle	(50)	(50)	(50)
Inflammation, chronic focal	1 (2%)		

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TABLE B5. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Inflammation, acute/chronic		1 (2%)	
Inflammation, chronic	1 (2%)		
Abscess, chronic	1 (2%)		
Granuloma, NOS			1 (2%)
*Pleural cavity	(50)	(50)	(50)
Foreign material, NOS		1 (2%)	
*Pleura	(50)	(50)	(50)
Inflammation, acute fibrinous		1 (2%)	
*Pericardium	(50)	(50)	(50)
Hemorrhage	1 (2%)		
Inflammation, acute/chronic	1 (2%)		
Granuloma, foreign body			1 (2%)
*Mesentery	(50)	(50)	(50)
Inflammation, granulomatous			1 (2%)
Necrosis, fat	5 (10%)	5 (10%)	2 (4%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Congestion, NOS	2 (4%)	3 (6%)	4 (8%)
Hemorrhage			1 (2%)
Inflammation, fibrinous	1 (2%)		
SPECIAL MORPHOLOGY SUMMARY			
None			

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
† Multiple occurrence of morphology in the same organ; tissue is counted once only.
# Number of animals examined microscopically at this site

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APPENDIX C

SUMMARY OF LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

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TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Fibrosarcoma	1 (2%)		
*Subcutaneous tissue	(50)	(50)	(50)
Sarcoma, NOS	2 (4%)	4 (8%)	1 (2%)
Fibroma	2 (4%)	3 (6%)	
Fibrosarcoma	8 (16%)	6 (12%)	3 (6%)
Neurofibrosarcoma	1 (2%)		1 (2%)
RESPIRATORY SYSTEM			
#Lung	(47)	(50)	(49)
Hepatocellular carcinoma, metastatic	6 (13%)	2 (4%)	2 (4%)
Alveolar/bronchiolar adenoma	7 (15%)	5 (10%)	4 (8%)
Alveolar/bronchiolar carcinoma	5 (11%)	1 (2%)	4 (8%)
Fibrosarcoma, metastatic	2 (4%)		2 (4%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Malignant lymphoma, NOS		1 (2%)	1 (2%)
Malignant lymphoma, undiffer type	1 (2%)		1 (2%)
Malignant lymphoma, histiocytic type	2 (4%)	4 (8%)	1 (2%)
Malignant lymphoma, mixed type	1 (2%)	1 (2%)	
#Kidney	(50)	(47)	(49)
Malignant lymphoma, NOS			1 (2%)
CIRCULATORY SYSTEM			
*Multiple organs	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)
*Lower extremity	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)	
#Spleen	(47)	(48)	(50)
Hemangioma		1 (2%)	
Hemangiosarcoma, metastatic		1 (2%)	
#Heart	(47)	(50)	(50)
Hemangioma	1 (2%)		
#Liver	(50)	(49)	(49)
Hemangiosarcoma			1 (2%)
DIGESTIVE SYSTEM			
#Liver	(50)	(49)	(49)
Hepatocellular adenoma	4 (8%)	6 (12%)	5 (10%)
Hepatocellular carcinoma	14 (28%)	15 (31%)	14 (29%)
Mixed hepatocellular and bile duct carcinoma		1 (2%)	
Fibrosarcoma, metastatic			1 (2%)
#Pancreatic acinus	(43)	(43)	(45)
Adenocarcinoma, NOS	1 (2%)		
#Gastric fundal gland	(44)	(46)	(39)
Adenomatous polyp, NOS		1 (2%)	
#Forestomach	(44)	(46)	(39)
Squamous cell carcinoma		1 (2%)	

000550

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
URINARY SYSTEM			
None			
ENDOCRINE SYSTEM			
#Adrenal	(44)	(45)	(46)
Pheochromocytoma			1 (2%)
#Adrenal medulla	(44)	(45)	(46)
Pheochromocytoma	1 (2%)		
#Thyroid	(45)	(48)	(45)
Follicular cell adenoma		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
#Prostate	(42)	(44)	(47)
Adenoma, NOS	1 (2%)		
#Testis	(45)	(44)	(44)
Interstitial cell tumor	1 (2%)		
*Epididymis	(50)	(50)	(50)
Leiomyoma	1 (2%)		
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(50)	(50)	(50)
Adenoma, NOS	3 (6%)	2 (4%)	1 (2%)
Adenocarcinoma, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*Maxilla	(50)	(50)	(50)
Sarcoma, NOS	1 (2%)		
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Fibrosarcoma, metastatic			1 (2%)
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Mixed hepato and bile duct carcinoma, meta		1 (2%)	
Alveolar/bronchiolar carcinoma, metastatic	1 (2%)		1 (2%)
Sarcoma, NOS		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	16	22	25
Moribund sacrifice	8	7	11
Terminal sacrifice	23	19	14
Accidentally killed, nda	3	2	

000551

TABLE C1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	39	37	31
Total primary tumors	58	56	41
Total animals with benign tumors	19	16	11
Total benign tumors	21	19	12
Total animals with malignant tumors	30	31	25
Total malignant tumors	37	37	29
Total animals with secondary tumors##	9	4	5
Total secondary tumors	9	4	7

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

000552

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT: VEHICLE CONTROL

ANIMAL NUMBER	026	030	021	038	046	020	007	012	022	042	044	003	032	047	018	017	011	023	015	037	040	006	035	008	025
WEEKS ON STUDY	011	018	039	058	062	078	079	081	081	082	082	085	085	090	092	093	095	096	098	098	098	100	100	102	103
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS								X																	
Fibroma																									
Fibrosarcoma																X	X		X			X	X		X
Neurofibrosarcoma										X															
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic						X								X											X
Alveolar/bronchiolar adenoma				X																					
Alveolar/bronchiolar carcinoma							X											X							
Fibrosarcoma, metastatic																	X						X		
Trachea	−	+	+	+	+	+	−	−	+	+	+	−	+	−	−	+	+	−	−	+	+	+	+	+	−
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+
Lymph nodes	−	+	−	+	+	+	−	+	−	+	+	+	+	+	−	+	−	−	−	+	+	+	+	+	−
Thymus	−	+	−	+	+	−	−	−	−	−	+	−	+	+	−	−	−	−	−	−	+	−	+	−	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+
Hemangioma																									
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma							X																		
Hepatocellular carcinoma					X	X						X		X			X			X	X				X
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	N	+	N	N	N	+	N	N	N	N	N	N	N	+	N	N	N	N	+	+	N	N	N
Pancreas	+	+	−	−	+	−	−	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, NOS															X										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	−	−	+	+	−	+	−	−	+	+	+	−	+	+	+	+	+	+	+	+
Small intestine	+	+	−	+	−	−	−	−	+	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	−	+	+	−	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	−	+	+	−	+	−	−	+	−	+	−	+	+	+	+	+	+	−	+
ENDOCRINE SYSTEM																									
Pituitary	+	−	+	+	+	+	−	+	+	+	−	−	+	−	+	+	−	+	+	+	−	+	−	+	−
Adrenal	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma																									
Thyroid	−	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
Parathyroid	−	−	−	+	+	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	+	−	−	−	−
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−
Interstitial cell tumor																									
Prostate	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	−
Adenoma, NOS																X									
Epididymis	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Leiomyoma																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																						X			
MUSCULOSKELETAL SYSTEM																									
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Sarcoma, NOS							X																		
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Alveolar/bronchiolar carcinoma, metastatic							X																		
Malignant lymphoma, undiffer type																									
Malignant lymphoma, histiocytic type																		X							
Malignant lymphoma, mixed type													X												

+ Tissue examined microscopically
− Required tissue not examined microscopically
X. Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S. Animal missexed

No tissue information submitted
C Necropsy, no histology due to protocol
A: Autolysis
M: Animal missing
B. No necropsy performed

000553

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: VEHICLE CONTROL (Continued)

ANIMAL NUMBER	048	001	002	004	005	009	010	013	014	016	019	024	027	028	029	031	033	034	036	039	041	043	045	049	050	TOTAL TISSUES TUMORS
WEEKS ON STUDY	103	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Skin	N	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibrosarcoma						X																				1
Subcutaneous tissue	N	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS																							X			2
Fibroma					X			X																		2
Fibrosarcoma												X				X										6
Neurofibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	47
Hepatocellular carcinoma, metastatic				X	X																		X			6
Alveolar/bronchiolar adenoma								X										X	X		X	X			X	7
Alveolar/bronchiolar carcinoma				X															X						X	5
Fibrosarcoma, metastatic																										2
Trachea	−	−	−	+	−	−	−	−	+	−	−	−	−	−	+	+	+	+	−	−	+	+	+	+	+	27
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph nodes	+	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	−	+	37
Thymus	+	+	−	+	−	−	−	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	−	−	25
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	47
Hemangioma																								X		1
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma			X	X				X																		4
Hepatocellular carcinoma				X	X		X								X				X				X			14
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	N	N	N	+	+	N	+	+	+	+	+	N	N	N	+	+	+	+	+	N	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Adenocarcinoma, NOS																										1
Esophagus	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Small intestine	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	39
Large intestine	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	41
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	39
Adrenal	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	+	−	+	+	+	+	44
Pheochromocytoma																							X			1
Thyroid	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Parathyroid	−	−	−	+	−	−	−	−	+	−	−	−	−	+	+	−	−	+	−	−	−	+	+	−	+	13
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	45
Interstitial cell tumor																	X									1
Prostate	+	−	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	42
Adenoma, NOS																										1
Epididymis	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Leiomyoma															X											1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS												X	X													3
MUSCULOSKELETAL SYSTEM																										
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Sarcoma, NOS																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Alveolar/bronchiolar carcinoma, meta																										1
Malignant lymphoma, undiffer type										X																1
Malignant lymphoma, histiocytic type	X																									2
Malignant lymphoma, mixed type																										1

* Animals necropsied

000554

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDHYDE, SODIUM SALT: LOW DOSE

ANIMAL NUMBER	003	005	020	017	019	004	050	027	001	037	033	026	028	031	010	025	018	045	011	002	029	049	006	042	043
WEEKS ON STUDY	017	034	043	044	044	059	060	061	070	078	079	080	081	083	090	090	091	091	092	093	093	095	096	097	097
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	N	+	+	+	+	+
Sarcoma, NOS											X			X				X							
Fibroma																		X							
Fibrosarcoma								X																X	
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic												X													
Alveolar/bronchiolar adenoma													X												
Alveolar/bronchiolar carcinoma																									
Trachea	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+
Spleen	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Hemangiosarcoma, metastatic																									
Lymph nodes	+	+	−	−	+	−	+	+	+	−	−	+	+	−	+	−	+	−	−	+	−	+	+	+	−
Thymus	+	+	−	−	−	+	−	+	−	−	−	−	−	−	+	+	−	−	−	−	−	+	−	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma					X						X										X				
Hepatocellular carcinoma	X						X		X			X	X	X		X		X	X	X					
Mixed hepato/cholangiocarcinoma																							X		
Bile duct	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	N	N	N	N	N	N	+	N	N	N	N	+	N	+	+	+	N	N	+	N	N	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	−	−	+	+	+	−	−	+	−	−	+	+	+	+	+	−
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	−	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Squamous cell carcinoma																					X				
Adenomatous polyp, NOS																									
Small intestine	+	+	+	−	−	+	−	+	+	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+
Large intestine	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−
URINARY SYSTEM																									
Kidney	+	+	+	+	−	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	−	+	+	−	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	−	+	+	−	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+
Adrenal	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Thyroid	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Follicular cell adenoma																									
Parathyroid	−	−	−	−	+	−	+	−	+	+	+	−	+	+	−	+	−	−	−	+	+	+	−	+	−
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N
Testis	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
Adenocarcinoma, NOS																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mixed hepato/cholangio carcinoma, metastatic																							X		
Sarcoma, NOS									X																
Malignant lymphoma, NOS															X										
Malignant lymphoma, histiocytic type																									X
Malignant lymphoma, mixed type																									
Lower extremity, NOS																									
Hemangiosarcoma																									

000555

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	044	007	032	021	012	008	009	013	014	015	016	022	023	024	030	034	035	036	038	039	040	041	046	047	048	TOTAL TISSUES TUMORS
WEEKS ON STUDY	097	100	100	102	103	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS		X																								4
Fibroma																		X		X						3
Fibrosarcoma												X	X					X						X		6
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic							X																			2
Alveolar/bronchiolar adenoma		X						X												X	X					5
Alveolar/bronchiolar carcinoma																					X					1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangioma														X												1
Hemangiosarcoma, metastatic																	X									1
Lymph nodes	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	37
Thymus	+	−	−	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	30
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma					X	X																		X		6
Hepatocellular carcinoma							X			X					X		X					X				15
Mixed hepato/cholangiocarcinoma																										1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	N	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Squamous cell carcinoma																										1
Adenomatous polyp, NOS																									X	1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
ENDOCRINE SYSTEM																										
Pituitary	+	+	−	−	−	+	+	+	−	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	40
Adrenal	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	45
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell adenoma								X																		1
Parathyroid	−	+	+	−	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	31
REPRODUCTIVE SYSTEM																										
Mammary gland	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	−	+	+	+	+	+	+	+	44
Prostate	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	44
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS												X						X								2
Adenocarcinoma, NOS																									X	1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Mixed hepato/cholangio carcinoma, meta																										1
Sarcoma, NOS																										1
Malignant lymphoma, NOS																										1
Malignant lymphoma, histiocytic type											X		X			X										4
Malignant lymphoma, mixed type																									X	1
Lower extremity, NOS																										
Hemangiosarcoma																	X									1

* Animals necropsied

000556

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT: HIGH DOSE

ANIMAL NUMBER	008	047	012	005	049	024	039	014	011	022	013	016	026	050	002	001	009	037	041	046	023	025	003	031	032
WEEKS ON STUDY	065	065	067	070	071	073	080	082	085	085	086	086	086	087	088	089	090	090	090	090	091	091	092	092	092
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, NOS																									
Fibrosarcoma							X									X									
Neurofibrosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic						X									X										
Alveolar/bronchiolar adenoma				X																					
Alveolar/bronchiolar carcinoma																									
Fibrosarcoma, metastatic							X									X									
Trachea	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−	+	−	−	+	+	+	+	+	−
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph nodes	+	+	−	+	+	+	+	+	+	+	−	+	+	+	−	−	+	+	+	+	−	−	−	+	+
Thymus	+	+	−	+	+	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Hepatocellular carcinoma	X				X	X				X	X	X			X		X								
Fibrosarcoma, metastatic																X									
Hemangiosarcoma																									
Bile duct	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	+	+	N	N	N	N	+	N	N	+	N	+	+	N	+	+	+	+	+	+	+	+	N	+
Pancreas	+	+	−	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	−
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
Stomach	+	−	−	−	+	+	+	−	−	−	+	−	−	+	−	+	+	+	+	−	−	+	+	+	+
Small intestine	+	−	−	−	+	−	+	−	−	−	+	−	−	+	−	+	+	+	+	+	+	+	+	+	+
Large intestine	+	−	−	−	+	−	+	+	−	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, NOS															X										
Urinary bladder	+	−	+	−	+	−	+	+	−	−	+	−	−	+	−	−	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	−	−	+	+	−	+	+	−	+	−	+	−	+	−	+	+	+	+	+	+	+	+	+	+
Adrenal	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	−	+	+	−
Pheochromocytoma																									
Thyroid	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−
Follicular cell adenoma																									
Parathyroid	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	+	+	+	−
REPRODUCTIVE SYSTEM																									
Mammary gland	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Testis	+	+	+	+	+	−	+	−	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenoma, NOS																									
BODY CAVITIES																									
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Fibrosarcoma, metastatic							X																		
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Alveolar/bronchiolar carcinoma, metastatic																									
Hemangiosarcoma																									
Malignant lymphoma, NOS												X													
Malignant lymphoma, undifferentiated type																									
Malignant lymphoma, histiocytic type																									

000557

TABLE C2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	010	034	035	038	006	027	044	045	036	021	040	004	007	015	017	018	019	020	028	029	030	033	042	043	048	TOTAL: TISSUES TUMORS
WEEKS ON STUDY	093	094	094	094	095	095	095	095	099	101	102	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Sarcoma, NOS								X																		1
Fibrosarcoma																						X				3
Neurofibrosarcoma						X																				1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular carcinoma, metastatic																										2
Alveolar/bronchiolar adenoma				X									X						X							4
Alveolar/bronchiolar carcinoma					X				X														X		X	4
Fibrosarcoma, metastatic																										2
Trachea	+	−	+	+	+	−	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	40
HEMATOPOIETIC SYSTEM																										
Bone marrow	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	45
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph nodes	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Thymus	−	−	−	+	−	−	+	−	+	−	+	−	+	−	−	+	+	−	−	−	+	−	+	−	+	17
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma												X						X		X				X	X	5
Hepatocellular carcinoma		X											X						X		X		X		X	14
Fibrosarcoma, metastatic																										1
Hemangiosarcoma																			X							1
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder & common bile duct	N	+	+	+	+	+	+	N	+	+	N	+	+	+	+	N	+	+	N	N	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Esophagus	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	39
Small intestine	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	38
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	42
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Malignant lymphoma, NOS																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	−	+	+	+	−	−	+	+	+	−	+	+	+	+	+	+	+	+	39
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pheochromocytoma											X															1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	45
Follicular cell adenoma																X										1
Parathyroid	−	+	+	+	−	−	+	−	+	−	+	+	+	+	−	+	−	−	−	−	+	+	+	+	+	21
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Testis	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Prostate	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenoma, NOS																									X	1
BODY CAVITIES																										
Mediastinum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Fibrosarcoma, metastatic																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Alveolar/bronchiolar carcinoma, metast									X																	1
Hemangiosarcoma				X																						1
Malignant lymphoma, NOS																										1
Malignant lymphoma, undiffer type														X												1
Malignant lymphoma, histiocytic type	X																									1

* Animals necropsied

000558

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	60 mg/kg	120 mg/kg
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	8.3%	12.6%	0.0%
Terminal Rates (c)	2/24 (8%)	2/20 (10%)	0/14 (0%)
Week of First Observation	104	91	
Life Table Tests (d)	P=0.333N	P=0.428	P=0.362N
Incidental Tumor Tests (d)	P=0.268N	P=0.433	P=0.362N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Test (d)		P=0.500	P=0.247N
Subcutaneous Tissue: Fibrosarcoma			
Overall Rates (a)	8/50 (16%)	6/50 (12%)	3/50 (6%)
Adjusted Rates (b)	25.0%	24.8%	11.8%
Terminal Rates (c)	2/24 (8%)	4/20 (20%)	1/14 (7%)
Week of First Observation	93	61	80
Life Table Tests (d)	P=0.260N	P=0.527N	P=0.307N
Incidental Tumor Tests (d)	P=0.096N	P=0.417N	P=0.094N
Cochran-Armitage Trend Test (d)	P=0.078N		
Fisher Exact Test (d)		P=0.387N	P=0.100N
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	10/50 (20%)	8/50 (16%)	3/50 (6%)
Adjusted Rates (b)	31.9%	31.5%	11.8%
Terminal Rates (c)	4/24 (17%)	5/20 (25%)	1/14 (7%)
Week of First Observation	93	61	80
Life Table Tests (d)	P=0.160N	P=0.554N	P=0.184N
Incidental Tumor Tests (d)	P=0.044N	P=0.454N	P=0.047N
Cochran-Armitage Trend Test (d)	P=0.031N		
Fisher Exact Test (d)		P=0.398N	P=0.036N
Integumentary System: Fibrosarcoma			
Overall Rates (a)	9/50 (18%)	6/50 (12%)	3/50 (6%)
Adjusted Rates (b)	28.5%	24.8%	11.8%
Terminal Rates (c)	3/24 (13%)	4/20 (20%)	1/14 (7%)
Week of First Observation	93	61	80
Life Table Tests (d)	P=0.189N	P=0.429N	P=0.239N
Incidental Tumor Tests (d)	P=0.063N	P=0.320N	P=0.067N
Cochran-Armitage Trend Test (d)	P=0.045N		
Fisher Exact Test (d)		P=0.288N	P=0.061N
Integumentary System: Fibroma or Fibrosarcoma			
Overall Rates (a)	11/50 (22%)	8/50 (16%)	3/50 (6%)
Adjusted Rates (b)	35.3%	31.5%	11.8%
Terminal Rates (c)	5/24 (21%)	5/20 (25%)	1/14 (7%)
Week of First Observation	93	61	80
Life Table Tests (d)	P=0.113N	P=0.464N	P=0.139N
Incidental Tumor Tests (d)	P=0.028N	P=0.364N	P=0.033N
Cochran-Armitage Trend Test (d)	P=0.017N		
Fisher Exact Test (d)		P=0.306N	P=0.020N
Integumentary System: Sarcoma, Fibrosarcoma, or Neurofibrosarcoma			
Overall Rates (a)	12/50 (24%)	10/50 (20%)	5/50 (10%)
Adjusted Rates (b)	35.1%	34.9%	20.2%
Terminal Rates (c)	4/24 (17%)	4/20 (20%)	1/14 (7%)
Week of First Observation	81	61	80
Life Table Tests (d)	P=0.227N	P=0.569N	P=0.252N
Incidental Tumor Tests (d)	P=0.035N	P=0.449N	P=0.042N
Cochran-Armitage Trend Test (d)	P=0.045N		
Fisher Exact Test (d)		P=0.405N	P=0.054N

000559

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	60 mg/kg	120 mg/kg
Integumentary System: Fibroma, Sarcoma, Fibrosarcoma, or Neurofibrosarcoma			
Overall Rates (a)	14/50 (28%)	11/50 (22%)	5/50 (10%)
Adjusted Rates (b)	41.6%	38.9%	20.2%
Terminal Rates (c)	6/24 (25%)	5/20 (25%)	1/14 (7%)
Week of First Observation	81	61	80
Life Table Tests (d)	P=0.141N	P=0.504N	P=0.158N
Incidental Tumor Tests (d)	P=0.017N	P=0.385N	P=0.021N
Cochran-Armitage Trend Test (d)	P=0.017N		
Fisher Exact Test (d)		P=0.322N	P=0.020N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	7/47 (15%)	5/50 (10%)	4/49 (8%)
Adjusted Rates (b)	28.8%	20.7%	19.6%
Terminal Rates (c)	6/22 (27%)	3/20 (15%)	2/14 (14%)
Week of First Observation	58	81	70
Life Table Tests (d)	P=0.412N	P=0.451N	P=0.486N
Incidental Tumor Tests (d)	P=0.290N	P=0.393N	P=0.381N
Cochran-Armitage Trend Test (d)	P=0.187N		
Fisher Exact Test (d)		P=0.336N	P=0.238N
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	5/47 (11%)	1/50 (2%)	4/49 (8%)
Adjusted Rates (b)	18.2%	5.0%	23.4%
Terminal Rates (c)	3/22 (14%)	1/20 (5%)	2/14 (14%)
Week of First Observation	79	104	95
Life Table Tests (d)	P=0.516	P=0.131N	P=0.507
Incidental Tumor Tests (d)	P=0.514N	P=0.116N	P=0.607N
Cochran-Armitage Trend Test (d)	P=0.398N		
Fisher Exact Test (d)		P=0.088N	P=0.473N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	10/47 (21%)	5/50 (10%)	8/49 (16%)
Adjusted Rates (b)	36.8%	20.7%	40.1%
Terminal Rates (c)	7/22 (32%)	3/20 (15%)	4/14 (29%)
Week of First Observation	58	81	70
Life Table Tests (d)	P=0.465	P=0.184N	P=0.457
Incidental Tumor Tests (d)	P=0.446N	P=0.137N	P=0.546N
Cochran-Armitage Trend Test (d)	P=0.306N		
Fisher Exact Test (d)		P=0.105N	P=0.360N
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Overall Rates (a)	2/50 (4%)	4/50 (8%)	1/50 (2%)
Adjusted Rates (b)	6.8%	18.1%	4.0%
Terminal Rates (c)	0/24 (0%)	3/20 (15%)	0/14 (0%)
Week of First Observation	96	97	93
Life Table Tests (d)	P=0.553	P=0.261	P=0.681N
Incidental Tumor Tests (d)	P=0.503N	P=0.295	P=0.500N
Cochran-Armitage Trend Test (d)	P=0.406N		
Fisher Exact Test (d)		P=0.339	P=0.500N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	4/50 (8%)	6/50 (12%)	4/50 (8%)
Adjusted Rates (b)	12.9%	25.1%	15.5%
Terminal Rates (c)	1/24 (4%)	4/20 (20%)	1/14 (7%)
Week of First Observation	85	90	86
Life Table Tests (d)	P=0.338	P=0.281	P=0.464
Incidental Tumor Tests (d)	P=0.562N	P=0.315	P=0.569N
Cochran-Armitage Trend Test (d)	P=0.568		
Fisher Exact Test (d)		P=0.370	P=0.643

000560

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	60 mg/kg	120 mg/kg
Liver: Hepatocellular Adenoma			
Overall Rates (a)	4/50 (8%)	6/49 (12%)	5/49 (10%)
Adjusted Rates (b)	14.5%	20.9%	35.7%
Terminal Rates (c)	3/24 (13%)	2/20 (10%)	5/14 (36%)
Week of First Observation	79	44	104
Life Table Tests (d)	P=0.196	P=0.287	P=0.214
Incidental Tumor Tests (d)	P=0.225	P=0.333	P=0.250
Cochran-Armitage Trend Test (d)	P=0.421		
Fisher Exact Test (d)		P=0.357	P=0.487
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	14/50 (28%)	15/49 (31%)	14/49 (29%)
Adjusted Rates (b)	40.5%	42.7%	49.5%
Terminal Rates (c)	6/24 (25%)	5/20 (25%)	5/14 (36%)
Week of First Observation	62	17	65
Life Table Tests (d)	P=0.260	P=0.350	P=0.264
Incidental Tumor Tests (d)	P=0.404N	P=0.513	P=0.490N
Cochran-Armitage Trend Test (d)	P=0.518		
Fisher Exact Test (d)		P=0.474	P=0.563
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	17/50 (34%)	21/49 (43%)	17/49 (35%)
Adjusted Rates (b)	48.3%	56.4%	66.3%
Terminal Rates (c)	8/24 (33%)	7/20 (35%)	8/14 (57%)
Week of First Observation	62	17	65
Life Table Tests (d)	P=0.194	P=0.163	P=0.195
Incidental Tumor Tests (d)	P=0.506N	P=0.263	P=0.577N
Cochran-Armitage Trend Test (d)	P=0.511		
Fisher Exact Test (d)		P=0.242	P=0.555
Harderian Gland: Adenoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	11.5%	10.0%	7.1%
Terminal Rates (c)	2/24 (8%)	2/20 (10%)	1/14 (7%)
Week of First Observation	100	104	104
Life Table Tests (d)	P=0.405N	P=0.584N	P=0.517N
Incidental Tumor Tests (d)	P=0.368N	P=0.567N	P=0.456N
Cochran-Armitage Trend Test (d)	P=0.222N		
Fisher Exact Test (d)		P=0.500N	P=0.309N
Harderian Gland: Adenoma or Adenocarcinoma			
Overall Rates (a)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted Rates (b)	11.5%	15.0%	7.1%
Terminal Rates (c)	2/24 (8%)	3/20 (15%)	1/14 (7%)
Week of First Observation	100	104	104
Life Table Tests (d)	P=0.445N	P=0.576	P=0.517N
Incidental Tumor Tests (d)	P=0.409N	P=0.592	P=0.456N
Cochran-Armitage Trend Test (d)	P=0.238N		
Fisher Exact Test (d)		P=0.661	P=0.309N
All Sites: Benign Tumors			
Overall Rates (a)	19/50 (38%)	16/50 (32%)	11/50 (22%)
Adjusted Rates (b)	66.4%	56.2%	62.5%
Terminal Rates (c)	15/24 (63%)	9/20 (45%)	8/14 (57%)
Week of First Observation	58	44	70
Life Table Tests (d)	P=0.418N	P=0.568N	P=0.491N
Incidental Tumor Tests (d)	P=0.223N	P=0.472N	P=0.303N
Cochran-Armitage Trend Test (d)	P=0.052N		
Fisher Exact Test (d)		P=0.338N	P=0.063N

000561

TABLE C3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	60 mg/kg	120 mg/kg
All Sites: Malignant Tumors			
Overall Rates (a)	30/50 (60%)	31/50 (62%)	25/50 (50%)
Adjusted Rates (b)	69.2%	79.6%	76.3%
Terminal Rates (c)	11/24 (46%)	13/20 (65%)	8/14 (57%)
Week of First Observation	62	17	65
Life Table Tests (d)	P=0.279	P=0.256	P=0.312
Incidental Tumor Tests (d)	P=0.094N	P=0.441	P=0.112N
Cochran-Armitage Trend Test (d)	P=0.181N		
Fisher Exact Test (d)		P=0.500	P=0.211N
All Sites: All Tumors			
Overall Rates (a)	39/50 (78%)	37/50 (74%)	31/50 (62%)
Adjusted Rates (b)	88.4%	91.8%	92.8%
Terminal Rates (c)	19/24 (79%)	17/20 (85%)	12/14 (86%)
Week of First Observation	58	17	65
Life Table Tests (d)	P=0.273	P=0.344	P=0.289
Incidental Tumor Tests (d)	P=0.061N	P=0.527N	P=0.066N
Cochran-Armitage Trend Test (d)	P=0.049N		
Fisher Exact Test (d)		P=0.408N	P=0.063N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

000562

TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Mineralization		1 (2%)	
Dilatation, NOS		1 (2%)	
Ulcer, NOS			1 (2%)
Lymphocytic inflammatory infiltrate		1 (2%)	
Abscess, NOS	2 (4%)	1 (2%)	
Inflammation, acute/chronic	3 (6%)	3 (6%)	1 (2%)
Ulcer, chronic	1 (2%)	5 (10%)	3 (6%)
Inflammation, chronic focal			2 (4%)
Inflammation, chronic diffuse	1 (2%)		
Inflammation, chronic suppurative			2 (4%)
Abscess, chronic	1 (2%)	1 (2%)	2 (4%)
Inflammation with fibrosis		4 (8%)	
Fibrosis	1 (2%)		
Fibrosis, focal	4 (8%)	2 (4%)	1 (2%)
Fibrosis, diffuse	3 (6%)	6 (12%)	3 (6%)
Pigmentation, NOS		1 (2%)	31 (62%)
Atrophy, focal		1 (2%)	
Atrophy, diffuse			1 (2%)
Hyperplasia, focal	2 (4%)	1 (2%)	1 (2%)
Hyperplasia, adenomatous	1 (2%)		
Acanthosis	1 (2%)	2 (4%)	2 (4%)
Metaplasia, osseous	1 (2%)		
*Subcutaneous tissue	(50)	(50)	(50)
Foreign body, NOS	1 (2%)		
Inflammation, acute suppurative		1 (2%)	
Inflammation, acute/chronic		3 (6%)	
Inflammation, chronic suppurative	1 (2%)		
RESPIRATORY SYSTEM			
#Trachea	(27)	(48)	(40)
Inflammation, acute focal	1 (4%)		
#Tracheal gland	(27)	(48)	(40)
Hyperplasia, focal			1 (3%)
Dysplasia, NOS	1 (4%)		
#Lung/bronchiole	(47)	(50)	(49)
Vegetable foreign body		1 (2%)	
#Lung	(47)	(50)	(49)
Aspiration, foreign body	1 (2%)		
Atelectasis	1 (2%)	1 (2%)	
Congestion, acute	6 (13%)	13 (26%)	9 (18%)
Hemorrhage	4 (9%)	7 (14%)	4 (8%)
Inflammation, interstitial	1 (2%)	4 (8%)	2 (4%)
Inflammation, acute focal			1 (2%)
Inflammation, acute/chronic		1 (2%)	
Fibrosis, focal			1 (2%)
Foreign material, NOS		1 (2%)	
Alveolar macrophages			1 (2%)
Hyperplasia, focal		1 (2%)	
Hyperplasia, alveolar epithelium		1 (2%)	
Histiocytosis		2 (4%)	
#Lung/alveoli	(47)	(50)	(49)
Hemorrhage	1 (2%)		
Inflammation, interstitial		1 (2%)	
Histiocytosis	1 (2%)		

000563

TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Hematopoiesis		1 (2%)	
*Mediastinum	(50)	(50)	(50)
Hematopoiesis			1 (2%)
*Blood	(50)	(50)	(50)
Leukocytosis, neutrophilic		1 (2%)	1 (2%)
#Bone marrow	(48)	(47)	(45)
Congestion, NOS	1 (2%)		
Congestion, acute	4 (8%)	6 (13%)	8 (18%)
Hemorrhage			1 (2%)
Abscess, NOS			1 (2%)
Necrosis, focal	1 (2%)		
Hypoplasia, NOS	2 (4%)		1 (2%)
Hyperplasia, diffuse	7 (15%)	5 (11%)	11 (24%)
Hyperplasia, erythroid		1 (2%)	
Hyperplasia, granulocytic	5 (10%)	17 (36%)	6 (13%)
#Spleen	(47)	(48)	(50)
Depletion, lymphoid	1 (2%)	1 (2%)	1 (2%)
Hypoplasia, lymphoid			1 (2%)
#Splenic follicles	(47)	(48)	(50)
Necrosis, focal			1 (2%)
Atrophy, diffuse	7 (15%)	4 (8%)	3 (6%)
Hyperplasia, diffuse	1 (2%)		1 (2%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	2 (4%)
Hypoplasia, lymphoid	1 (2%)	1 (2%)	2 (4%)
#Splenic red pulp	(47)	(48)	(50)
Congestion, acute		1 (2%)	1 (2%)
Hematopoiesis	19 (40%)	20 (42%)	18 (36%)
#Lymph node	(37)	(37)	(41)
Congestion, acute		2 (5%)	
Hemorrhage	1 (3%)	4 (11%)	4 (10%)
Hyperplasia, reticulum cell		1 (3%)	2 (5%)
Hyperplasia, lymphoid		1 (3%)	
Hematopoiesis	1 (3%)	1 (3%)	1 (2%)
Hypoplasia, lymphoid			1 (2%)
#Mandibular lymph node	(37)	(37)	(41)
Hemorrhage	1 (3%)	1 (3%)	1 (2%)
Depletion, lymphoid			1 (2%)
Hyperplasia, reticulum cell	2 (5%)		
Mastocytosis		1 (3%)	
Hypoplasia, lymphoid		1 (3%)	
#Tracheal lymph node	(37)	(37)	(41)
Hemorrhage	1 (3%)		
#Mediastinal lymph node	(37)	(37)	(41)
Histiocytosis			1 (2%)
#Mesenteric lymph node	(37)	(37)	(41)
Edema, NOS			1 (2%)
Hemorrhage	6 (16%)	3 (8%)	7 (17%)
Necrosis, focal			1 (2%)
Histiocytosis	1 (3%)		
Hyperplasia, reticulum cell	1 (3%)		
Hyperplasia, lymphoid		1 (3%)	2 (5%)
Hematopoiesis	1 (3%)	1 (3%)	
Hypoplasia, lymphoid			1 (2%)
#Renal lymph node	(37)	(37)	(41)
Necrosis, focal			1 (2%)
Hyperplasia, lymphoid			1 (2%)
#Inguinal lymph node	(37)	(37)	(41)
Inflammation, chronic focal			1 (2%)

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TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Lung	(47)	(50)	(49)
Leukocytosis, NOS		2 (4%)	
#Alveolar wall	(47)	(50)	(49)
Leukocytosis, neutrophilic			1 (2%)
#Liver	(50)	(49)	(49)
Leukocytosis, neutrophilic			1 (2%)
Hematopoiesis	4 (8%)	7 (14%)	
#Hepatic sinusoid	(50)	(49)	(49)
Hematopoiesis	1 (2%)		
#Small intestine	(39)	(44)	(38)
Hyperplasia, lymphoid			1 (3%)
#Thymus	(25)	(30)	(17)
Congestion, acute		1 (3%)	
Hemorrhage	1 (4%)		
Inflammation, active chronic	1 (4%)		
Atrophy, diffuse			2 (12%)
#Thymic cortex	(25)	(30)	(17)
Necrosis, NOS		1 (3%)	
#Thymic lymphocytes	(25)	(30)	(17)
Degeneration, NOS	1 (4%)		
Necrosis, NOS		1 (3%)	
Necrosis, diffuse			1 (6%)
CIRCULATORY SYSTEM			
#Heart	(47)	(50)	(50)
Inflammation, acute/chronic		2 (4%)	
#Heart/atrium	(47)	(50)	(50)
Thrombosis, NOS	1 (2%)		
Thrombus, organized			1 (2%)
#Heart/ventricle	(47)	(50)	(50)
Mineralization		1 (2%)	
Histiocytosis		1 (2%)	
#Myocardium	(47)	(50)	(50)
Inflammation, acute/chronic			1 (2%)
Degeneration, NOS		1 (2%)	
Atrophy, focal			1 (2%)
*Renal vein	(50)	(50)	(50)
Inflammation with fibrosis	1 (2%)		
#Prostate	(42)	(44)	(47)
Thrombus, organized	1 (2%)		
DIGESTIVE SYSTEM			
#Salivary gland	(49)	(48)	(49)
Atrophy, focal		5 (10%)	1 (2%)
Atrophy, diffuse	3 (6%)		1 (2%)
#Liver	(50)	(49)	(49)
Congestion, acute		1 (2%)	
Congestion, chronic			1 (2%)
Hemorrhage		1 (2%)	
Inflammation, acute focal			1 (2%)
Inflammation, granulomatous focal			1 (2%)
Inflammation with fibrosis		2 (4%)	
Necrosis, focal	1 (2%)	2 (4%)	
Necrosis, coagulative	3 (6%)	2 (4%)	1 (2%)
Necrosis, ischemic			1 (2%)
Focal cellular change			3 (6%)
Angiectasis	1 (2%)		
#Liver/centrilobular	(50)	(49)	(49)
Degeneration, NOS			1 (2%)
Cytoplasmic vacuolization			2 (4%)

TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Liver/hepatocytes	(50)	(49)	(49)
Degeneration, lipoid			1 (2%)
Necrosis, focal			1 (2%)
Focal cellular change	1 (2%)		1 (2%)
Atrophy, focal			1 (2%)
Atrophy, diffuse		1 (2%)	
Hyperplasia, focal			1 (2%)
*Gallbladder	(50)	(50)	(50)
Cyst, NOS			1 (2%)
#Bile duct	(50)	(49)	(49)
Dilatation, NOS	1 (2%)		
Hyperplasia, cystic	1 (2%)		
#Pancreas	(43)	(43)	(45)
Multiple cysts	1 (2%)		
Hemorrhage, chronic	1 (2%)		
Inflammation, suppurative	1 (2%)		
Inflammation, chronic focal	1 (2%)		
Necrosis, ischemic			1 (2%)
Necrosis, fat			1 (2%)
Histiocytosis			1 (2%)
#Pancreatic acinus	(43)	(43)	(45)
Necrosis, focal	1 (2%)		
Focal cellular change		3 (7%)	
Atrophy, focal	5 (12%)	18 (42%)	29 (64%)
Atrophy, diffuse			2 (4%)
*Esophageal lumen	(50)	(50)	(50)
Hemorrhage	1 (2%)		
#Esophagus	(48)	(50)	(48)
Lacerated wound	2 (4%)	1 (2%)	
#Periesophageal tissue	(48)	(50)	(48)
Inflammation, active chronic	1 (2%)		
#Stomach	(44)	(46)	(39)
Inflammation, suppurative		1 (2%)	
Ulcer, acute		1 (2%)	
Inflammation, acute/chronic		1 (2%)	
Inflammation, pyogranulomatous	1 (2%)		
Hyperkeratosis			1 (3%)
Acanthosis	1 (2%)		1 (3%)
#Gastric mucosa	(44)	(46)	(39)
Ulcer, acute	1 (2%)		
#Gastric fundal gland	(44)	(46)	(39)
Metaplasia, NOS			1 (3%)
Metaplasia, squamous	1 (2%)		
#Cardiac stomach	(44)	(46)	(39)
Hyperkeratosis		1 (2%)	
Acanthosis		1 (2%)	
#Small intestine	(39)	(44)	(38)
Inflammation, acute necrotizing	1 (3%)		
Histiocytosis			1 (3%)
#Small intestine/mucous membrane	(39)	(44)	(38)
Atrophy, focal		1 (2%)	1 (3%)
Atrophy, diffuse		1 (2%)	
#Intestinal villus	(39)	(44)	(38)
Atrophy, NOS		1 (2%)	
Atrophy, diffuse		2 (5%)	1 (3%)

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TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Colon	(43)	(47)	(42)
Parasitism	3 (7%)	4 (9%)	5 (12%)
#Colonic crypt of Lieberkuhn	(43)	(47)	(42)
Atrophy, diffuse			1 (2%)
#Cecum	(43)	(47)	(42)
Inflammation, chronic necrotizing			1 (2%)
Parasitism	1 (2%)		
URINARY SYSTEM			
#Urinary bladder/cavity	(41)	(46)	(41)
Hemorrhage	1 (2%)		
#Kidney	(50)	(47)	(49)
Mineralization	1 (2%)	1 (2%)	
Hydronephrosis		1 (2%)	
Congestion, acute			1 (2%)
Inflammation, interstitial		1 (2%)	
Pyelonephritis, acute	2 (4%)	1 (2%)	4 (8%)
Pyelonephritis, chronic		3 (6%)	2 (4%)
Nephropathy	1 (2%)		
Hyperplasia, tubular cell		1 (2%)	
#Kidney/cortex	(50)	(47)	(49)
Mineralization	2 (4%)		3 (6%)
Cyst, NOS	2 (4%)		1 (2%)
Nephropathy	21 (42%)	17 (36%)	22 (45%)
Infarct, focal	1 (2%)		
Infarct, healed	1 (2%)		
Metaplasia, osseous			1 (2%)
#Kidney/glomerulus	(50)	(47)	(49)
Atrophy, diffuse		1 (2%)	
#Kidney/tubule	(50)	(47)	(49)
Mineralization		1 (2%)	
Dilatation, NOS	1 (2%)	1 (2%)	
Inflammation, acute suppurative		1 (2%)	
Cytoplasmic vacuolization	1 (2%)	1 (2%)	1 (2%)
Atrophy, focal			2 (4%)
Atrophy, diffuse		3 (6%)	
#Kidney/pelvis	(50)	(47)	(49)
Hydronephrosis		2 (4%)	2 (4%)
Inflammation, suppurative		1 (2%)	
Inflammation, chronic focal	1 (2%)		
*Ureter	(50)	(50)	(50)
Inflammation chronic suppurative		1 (2%)	
#Urinary bladder	(41)	(46)	(41)
Lymphocytic inflammatory infiltrate			1 (2%)
Inflammation, acute diffuse	1 (2%)		
Inflammation, chronic focal		1 (2%)	2 (5%)
Inflammation, chronic diffuse		1 (2%)	
Inflammation, chronic necrotizing			2 (5%)
Hyperplasia, epithelial			1 (2%)
#Urinary bladder/mucous membrane	(41)	(46)	(41)
Necrosis, diffuse	1 (2%)		
*Prostatic urethra	(50)	(50)	(50)
Necrosis, NOS	1 (2%)		

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TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM			
#Pituitary	(39)	(40)	(39)
Cyst, NOS	2 (5%)	2 (5%)	1 (3%)
Multiple cysts			1 (3%)
Congestion, acute		1 (3%)	3 (8%)
Hyperplasia, focal			1 (3%)
#Anterior pituitary	(39)	(40)	(39)
Embryonal duct cyst		1 (3%)	
#Adrenal	(44)	(45)	(46)
Hypertrophy, focal		1 (2%)	
Hyperplasia, focal	2 (5%)		
#Adrenal cortex	(44)	(45)	(46)
Lymphocytic inflammatory infiltrate		1 (2%)	
Hypertrophy, focal	2 (5%)	5 (11%)	4 (9%)
#Zona fasciculata	(44)	(45)	(46)
Necrosis, focal			1 (2%)
#Adrenal medulla	(44)	(45)	(46)
Degeneration, NOS			1 (2%)
Hyperplasia, focal		1 (2%)	
#Periadrenal tissue	(44)	(45)	(46)
Hemorrhage		1 (2%)	
#Thyroid	(45)	(48)	(45)
Cyst, NOS			1 (2%)
Hyperplasia, cystic			1 (2%)
Hyperplasia, follicular cell	3 (7%)		
#Thyroid follicle	(45)	(48)	(45)
Atrophy, focal	1 (2%)		
Hyperplasia, cystic		1 (2%)	2 (4%)
#Parathyroid	(13)	(31)	(21)
Thyroglossal duct cyst	1 (8%)		
Hyperplasia, diffuse	1 (8%)		
#Pancreatic islets	(43)	(43)	(45)
Hyperplasia, focal			2 (4%)
REPRODUCTIVE SYSTEM			
*Penis	(50)	(50)	(50)
Inflammation, acute suppurative		1 (2%)	
*Prepuce	(50)	(50)	(50)
Epidermal inclusion cyst	1 (2%)		
Inflammation with fibrosis	1 (2%)		
*Preputial gland	(50)	(50)	(50)
Dilatation, NOS	1 (2%)		
Cyst, NOS	1 (2%)		1 (2%)
Inflammation, acute	1 (2%)		
Inflammation, acute/chronic	1 (2%)		
#Prostate	(42)	(44)	(47)
Mineralization		1 (2%)	
Hemorrhage	1 (2%)		
Inflammation, suppurative	1 (2%)		1 (2%)
Inflammation, acute diffuse		1 (2%)	
Inflammation, acute suppurative	1 (2%)	6 (14%)	6 (13%)
Inflammation, acute/chronic		2 (5%)	2 (4%)
Inflammation, chronic focal		1 (2%)	1 (2%)
Inflammation, chronic suppurative			1 (2%)
Atrophy, diffuse			1 (2%)
*Seminal vesicle	(50)	(50)	(50)
Dilatation, NOS	7 (14%)	2 (4%)	8 (16%)
Hemorrhage	1 (2%)		
Inflammation, chronic focal	1 (2%)		
Inflammation with fibrosis	1 (2%)		
Hyperplasia, focal	2 (4%)		1 (2%)

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TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
REPRODUCTIVE SYSTEM (Continued)			
#Testis	(45)	(44)	(44)
Hemorrhage		1 (2%)	
Metaplasia, osseous			1 (2%)
Dysplasia, NOS		1 (2%)	
#Testis/tubule	(45)	(44)	(44)
Mineralization	1 (2%)	5 (11%)	
Degeneration, NOS	1 (2%)	1 (2%)	1 (2%)
Atrophy, focal			1 (2%)
Atrophy, diffuse		3 (7%)	3 (7%)
*Epididymis	(50)	(50)	(50)
Lymphocytic inflammatory infiltrate		1 (2%)	
Inflammation, acute/chronic	2 (4%)	1 (2%)	2 (4%)
Inflammation, chronic focal	1 (2%)		
Inflammation, chronic suppurative			1 (2%)
Granuloma, spermatic			4 (8%)
Inflammation with fibrosis			1 (2%)
Necrosis, focal			1 (2%)
Atrophy, diffuse			1 (2%)
Hyperplasia, focal	1 (2%)		
*Scrotum	(50)	(50)	(50)
Inflammation, chronic focal			1 (2%)
NERVOUS SYSTEM			
#Brain/meninges	(48)	(48)	(46)
Inflammation, acute/chronic	1 (2%)	1 (2%)	
#Brain/ependyma	(48)	(48)	(46)
Hemorrhage			1 (2%)
#Brain	(48)	(48)	(46)
Mineralization	14 (29%)	15 (31%)	21 (46%)
Hydrocephalus, NOS			1 (2%)
Hemorrhage	1 (2%)		1 (2%)
#Cerebral white matter	(48)	(48)	(46)
Mineralization	1 (2%)		1 (2%)
#Brain/thalamus	(48)	(48)	(46)
Mineralization		1 (2%)	1 (2%)
SPECIAL SENSE ORGANS			
*Eye	(50)	(50)	(50)
Phthisis bulbi			1 (2%)
*Eye/cornea	(50)	(50)	(50)
Inflammation, chronic suppurative		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*Joint of lower extremity	(50)	(50)	(50)
Inflammation, chronic suppurative			1 (2%)
Abscess, chronic	1 (2%)		
*Intercostal muscle	(50)	(50)	(50)
Inflammation, necrotizing granulomatous		1 (2%)	
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Foreign body, NOS	2 (4%)	1 (2%)	
Inflammation, acute suppurative	2 (4%)	1 (2%)	
Inflammation, pyogranulomatous		1 (2%)	

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TABLE C4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
BODY CAVITIES (Continued)			
*Peritoneum	(50)	(50)	(50)
Necrosis, fat			1 (2%)
*Peritoneal cavity	(50)	(50)	(50)
Abscess, chronic	1 (2%)		
*Pleural cavity	(50)	(50)	(50)
Foreign body, NOS	2 (4%)		
Hemorrhage	1 (2%)		
Inflammation, acute suppurative	2 (4%)		
*Pleura	(50)	(50)	(50)
Inflammation, pyogranulomatous		1 (2%)	
*Subpleural tissue	(50)	(50)	(50)
Lymphocytic inflammatory infiltrate		1 (2%)	
*Mediastinal pleura	(50)	(50)	(50)
Hemorrhage	1 (2%)		
*Pericardial cavity	(50)	(50)	(50)
Hemorrhage	1 (2%)		
Inflammation, chronic suppurative	1 (2%)		
*Pericardium	(50)	(50)	(50)
Inflammation, chronic suppurative		1 (2%)	
*Epicardium	(50)	(50)	(50)
Hemorrhage	1 (2%)		
Inflammation, acute suppurative	1 (2%)		
Inflammation, necrotizing granulomatous		1 (2%)	
ALL OTHER SYSTEMS			
*Multiple organs	(50)	(50)	(50)
Congestion, acute	2 (4%)	1 (2%)	1 (2%)
Periorbital region			
Inflammation, chronic suppurative			1 ( )
SPECIAL MORPHOLOGY SUMMARY			
None			

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

000570

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APPENDIX D

SUMMARY OF LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

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TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Trichoepithelioma			1 (2%)
*Subcutaneous tissue	(50)	(50)	(50)
Fibrosarcoma		2 (4%)	1 (2%)
RESPIRATORY SYSTEM			
#Lung	(50)	(50)	(49)
Adenocarcinoma, NOS, metastatic			2 (4%)
Alveolar/bronchiolar adenoma	4 (8%)	4 (8%)	2 (4%)
Alveolar/bronchiolar carcinoma	1 (2%)	3 (6%)	2 (4%)
Fibrosarcoma, metastatic		2 (4%)	
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Malignant lymphoma, NOS	3 (6%)	1 (2%)	2 (4%)
Malignant lymphoma, lymphocytic type	1 (2%)	4 (8%)	3 (6%)
Malignant lymphoma, histiocytic type	5 (10%)	2 (4%)	1 (2%)
Malignant lymphoma, mixed type	3 (6%)	7 (14%)	2 (4%)
#Splenic follicles	(47)	(50)	(49)
Malignant lymphoma, histiocytic type			1 (2%)
Malignant lymphoma, mixed type	1 (2%)		
#Mediastinal lymph node	(45)	(41)	(45)
Fibrosarcoma, metastatic		1 (2%)	
#Pancreatic lymph node	(45)	(41)	(45)
Malignant lymphoma, lymphocytic type			1 (2%)
#Mesenteric lymph node	(45)	(41)	(45)
Sarcoma, NOS, unclear primary or metastatic			1 (2%)
CIRCULATORY SYSTEM			
*Multiple organs	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)		
*Subcutaneous tissue	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)	
#Bone marrow	(45)	(43)	(46)
Hemangiosarcoma		1 (2%)	
#Spleen	(47)	(50)	(49)
Hemangiosarcoma		1 (2%)	
#Splenic red pulp	(47)	(50)	(49)
Hemangiosarcoma		1 (2%)	
#Ovary	(44)	(45)	(46)
Hemangioma	1 (2%)		
DIGESTIVE SYSTEM			
#Liver	(50)	(50)	(50)
Hepatocellular adenoma			2 (4%)
Hepatocellular carcinoma	2 (4%)	3 (6%)	3 (6%)
#Cardiac stomach	(46)	(47)	(48)
Squamous cell papilloma	1 (2%)		
URINARY SYSTEM			
None			

000574

TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM			
#Pituitary	(43)	(40)	(40)
Adenoma, NOS	2 (5%)	4 (10%)	
#Pituitary intermedia	(43)	(40)	(40)
Adenoma, NOS		1 (3%)	1 (3%)
#Adrenal	(48)	(45)	(45)
Pheochromocytoma	1 (2%)		1 (2%)
#Adrenal/capsule	(48)	(45)	(45)
Carcinoma, NOS	1 (2%)		
Adenoma, NOS		1 (2%)	
#Adrenal medulla	(48)	(45)	(45)
Pheochromocytoma			1 (2%)
#Thyroid	(48)	(48)	(44)
Follicular cell adenoma	3 (6%)	3 (6%)	
#Pancreatic islets	(47)	(50)	(48)
Islet cell carcinoma	1 (2%)		
REPRODUCTIVE SYSTEM			
*Mammary gland	(50)	(50)	(50)
Adenocarcinoma, NOS	1 (2%)	2 (4%)	2 (4%)
Fibroadenoma		1 (2%)	
#Uterus	(49)	(48)	(47)
Squamous cell carcinoma, in situ			1 (2%)
Endometrial stromal polyp	1 (2%)		1 (2%)
#Ovary	(44)	(45)	(46)
Papillary cystadenoma, NOS	1 (2%)		
Papillary cystadenocarcinoma NOS		1 (2%)	
Teratoma, NOS		1 (2%)	
NERVOUS SYSTEM			
None			
SPECIAL SENSE ORGANS			
*Harderian gland	(50)	(50)	(50)
Adenocarcinoma, NOS			2 (4%)
MUSCULOSKELETAL SYSTEM			
*Bone/lower extremity	(50)	(50)	(50)
Osteoma	1 (2%)		
BODY CAVITIES			
*Thoracic cavity	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic		1 (2%)	
ALL OTHER SYSTEMS			
None			
ANIMAL DISPOSITION SUMMARY			
Animals initially in study	50	50	50
Natural death	6	4	8
Moribund sacrifice	3	7	5
Terminal sacrifice	41	38	30
Accidentally killed, nda		1	7

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TABLE D1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
TUMOR SUMMARY			
Total animals with primary tumors**	27	31	26
Total primary tumors	35	44	31
Total animals with benign tumors	15	13	9
Total benign tumors	15	14	9
Total animals with malignant tumors	18	23	20
Total malignant tumors	20	29	21
Total animals with secondary tumors##		3	2
Total secondary tumors		4	2
Total animals with tumors uncertain--benign or malignant		1	
Total uncertain tumors		1	
Total animals with tumors uncertain--primary or metastatic			1
Total uncertain tumors			1

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
** Primary tumors: all tumors except secondary tumors
# Number of animals examined microscopically at this site
Secondary tumors: metastatic tumors or tumors invasive into an adjacent organ

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT: VEHICLE CONTROL

ANIMAL NUMBER	047	044	040	028	030	010	037	020	039	001	002	003	004	005	006	007	008	009	011	012	013	014	015	016	017
WEEKS ON STUDY	046	073	077	082	089	093	093	095	103	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X					X				X										
Alveolar/bronchiolar carcinoma																									X
Trachea	+	+	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+
Spleen	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Malignant lymphoma, mixed type																									
Lymph nodes	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Thymus	+	+	−	+	−	−	−	+	−	−	+	+	+	−	+	−	+	+	+	−	+	+	−	+	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma		X																							
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	N	N	+	+	+	N	+	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pancreas	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma		X																							
Small intestine	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	−	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+
Adenoma, NOS													X												X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, NOS																									
Pheochromocytoma																									
Thyroid	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma									X														X		
Parathyroid	−	−	−	−	−	+	−	−	+	+	+	+	+	+	+	−	−	−	+	+	+	+	−	+	−
Pancreatic islets	−	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islet cell carcinoma																									
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	+
Adenocarcinoma, NOS							X																		
Uterus	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endometrial stromal polyp																									
Ovary	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
Papillary cystadenoma, NOS																						X			
Hemangioma																								X	
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MUSCULOSKELETAL SYSTEM																									
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Osteoma																									
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Hemangiosarcoma									X																
Malignant lymphoma, NOS	X				X																		X		
Malignant lymphoma, lymphocytic type																									
Malignant lymphoma, histiocytic type			X					X												X					X
Malignant lymphoma, mixed type			X																						

+: Tissue examined microscopically
−: Required tissue not examined microscopically
X: Tumor incidence
N: Necropsy, no autolysis, no microscopic examination
S: Animal missexed

No tissue information submitted
C Necropsy, no histology due to protocol
A Autolysis
M Animal missing
B No necropsy performed

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TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: VEHICLE CONTROL (Continued)

ANIMAL NUMBER	018	019	021	022	023	024	025	026	027	029	031	032	033	034	035	036	038	041	042	043	045	046	048	049	050	TOTAL TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X																							4
Alveolar/bronchiolar carcinoma																										1
Trachea	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	45
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Malignant lymphoma, mixed type			X																							1
Lymph nodes	+	+	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	45
Thymus	+	+	+	−	+	+	+	+	+	−	−	+	+	+	+	+	+	+	−	+	+	+	+	+	−	34
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																							X			2
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	46
Squamous cell papilloma																										1
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	45
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	45
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	46
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−	+	+	43
Adenoma, NOS																										2
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, NOS																							X			1
Pheochromocytoma																								X		1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell adenoma		X																								3
Parathyroid	+	−	+	−	+	−	−	+	−	+	−	+	−	+	+	+	−	+	+	−	+	+	+	+	+	29
Pancreatic islets	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Islet cell carcinoma												X														1
REPRODUCTIVE SYSTEM																										
Mammary gland	+	N	N	N	N	N	N	N	N	N	+	+	N	+	N	N	+	N	+	N	N	N	N	N	+	*50
Adenocarcinoma, NOS																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endometrial stromal polyp																									X	1
Ovary	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	−	+	44
Papillary cystadenoma, NOS																										1
Hemangioma																										1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	49
MUSCULOSKELETAL SYSTEM																										
Bone	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Osteoma											X															1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Hemangiosarcoma																										1
Malignant lymphoma, NOS																										3
Malignant lymphoma, lymphocytic type					X																					1
Malignant lymphoma, histiocytic type	X																									5
Malignant lymphoma, mixed type										X											X					3

* Animals necropsied

TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT: LOW DOSE

ANIMAL NUMBER	042	048	055	057	018	032	015	013	026	046	049	021	031	092	003	004	005	006	007	009	010	011	012	014	016
WEEKS ON STUDY	052	056	072	072	080	080	095	097	099	099	099	103	105	105	105	105	105	105	105	105	105	105	105	105	105
INTEGUMENTARY SYSTEM																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	N	+	+	+	+	+	+	+	+	+
Fibrosarcoma																									
Hemangiosarcoma																									
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma						X													X						
Alveolar/bronchiolar carcinoma															X					X					
Fibrosarcoma, metastatic																									
Trachea	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	−	+	+
Hemangiosarcoma				X																					
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma				X																X					
Lymph nodes	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	−	+	+	+	+	+	−
Fibrosarcoma, metastatic																									
Thymus	+	−	−	+	−	+	+	−	−	+	−	−	+	+	+	−	+	+	+	+	−	+	−	+	+
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																		X							
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	+	N	N	+	N	N	+	+	+	+	N	+	+	N	+	+	+	+	+	+	N	+	+	+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esophagus	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	−	+	−	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Adenoma, NOS																				X					X
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Adenoma, NOS																									
Thyroid	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell adenoma																					X		X		
Parathyroid	−	+	+	−	+	−	+	−	+	+	−	+	+	+	−	−	−	+	+	+	−	+	+	+	−
REPRODUCTIVE SYSTEM																									
Mammary gland	N	+	+	+	N	N	N	+	N	N	N	+	+	N	N	N	N	N	N	N	N	N	N	N	N
Adenocarcinoma, NOS								X				X													
Fibroadenoma																									
Uterus	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
Ovary	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+
Papillary cystadenocarcinoma, NOS															X										
Teratoma, NOS																									
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BODY CAVITIES																									
Pleura	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Alveolar/bronchiolar carcinoma, metastatic															X										
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, NOS																									
Malignant lymphoma, lymphocytic type								X																	
Malignant lymphoma, histiocytic type		X				X																			
Malignant lymphoma, mixed type							X				X													X	

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TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	017	019	020	022	023	024	027	028	029	030	031	032	033	034	035	036	039	040	041	043	044	045	047	048	050	TOTAL TISSUES TUMORS
WEEKS ON STUDY	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	105	
INTEGUMENTARY SYSTEM																										
Subcutaneous tissue	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibrosarcoma							X														X					2
Hemangiosarcoma																							X			1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma						X										X										4
Alveolar/bronchiolar carcinoma							X																			3
Fibrosarcoma, metastatic							X														X					2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	43
Hemangiosarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										2
Lymph nodes	−	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	41
Fibrosarcoma, metastatic																					X					1
Thymus	+	+	−	+	+	+	+	−	+	+	−	−	+	−	+	+	−	+	−	+	−	+	+	+	+	32
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
Salivary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma									X													X				3
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	N	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	47
ENDOCRINE SYSTEM																										
Pituitary	+	−	+	+	+	+	+	−	+	+	−	+	+	+	+	+	+	+	−	+	−	+	−	+	+	40
Adenoma, NOS						X						X										X				5
Adrenal	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−	+	−	+	+	45
Adenoma, NOS																			X							1
Thyroid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell adenoma																							X			3
Parathyroid	−	+	+	+	+	−	+	−	+	−	+	+	+	−	+	+	+	+	+	−	−	+	+	+	−	32
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	*50
Adenocarcinoma, NOS																										2
Fibroadenoma					X																					1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Ovary	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Papillary cystadenocarcinoma, NOS																										1
Teratoma, NOS											X															1
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
BODY CAVITIES																										
Pleura	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Alveolar/bronchiolar carcinoma, metast																										1
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, NOS												X														1
Malignant lymphoma, lymphocytic type													X	X											X	4
Malignant lymphoma, histiocytic type																										2
Malignant lymphoma, mixed type																	X		X		X			X		7

*Animals necropsied

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TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT: HIGH DOSE

ANIMAL NUMBER	037	093	092	030	028	034	035	026	013	011	010	014	048	047	048	049	050	016	023	039	001	004	005	006	007
WEEKS ON STUDY	052	074	077	077	081	081	083	084	090	092	093	094	094	094	094	094	094	098	099	100	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	N	+
Trichoepithelioma																									
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	N	+
Fibrosarcoma																				X					
RESPIRATORY SYSTEM																									
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Adenocarcinoma, NOS, metastatic				X																					
Alveolar/bronchiolar adenoma													X												
Alveolar/bronchiolar carcinoma																									
Trachea	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	−	−	−	−
HEMATOPOIETIC SYSTEM																									
Bone marrow	+	+	+	+	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Malignant lymphoma, histiocytic type																									
Lymph nodes	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	−	−	+	+	+	−	+	+	+
Sarcoma, NOS, unclear primary or metastatic																									
Malignant lymphoma, lymphocytic type																									
Thymus	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−
CIRCULATORY SYSTEM																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
DIGESTIVE SYSTEM																									
Salivary gland	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																							X		
Hepatocellular carcinoma																		X							
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder & common bile duct	N	N	+	N	+	N	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+
Pancreas	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
Stomach	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small intestine	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Large intestine	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Urinary bladder	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+
ENDOCRINE SYSTEM																									
Pituitary	+	+	+	+	+	+	−	+	+	+	+	+	−	+	+	−	+	−	+	+	+	−	+	+	−
Adenoma, NOS								X																	
Adrenal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	+
Pheochromocytoma																								X	
Thyroid	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	−	−
Parathyroid	−	+	+	−	+	−	+	−	+	−	−	+	+	+	+	−	+	+	−	−	+	−	−	−	−
REPRODUCTIVE SYSTEM																									
Mammary gland	N	N	+	+	N	N	+	+	+	N	+	N	N	N	N	N	N	N	+	N	+	N	N	N	N
Adenocarcinoma, NOS				X															X						
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
Squamous cell carcinoma, in situ																									
Endometrial stromal polyp																				X					
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+
NERVOUS SYSTEM																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Adenocarcinoma, NOS																									X
ALL OTHER SYSTEMS																									
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Malignant lymphoma, NOS										X															
Malignant lymphoma, lymphocytic type		X									X												X		
Malignant lymphoma, histiocytic type									X																
Malignant lymphoma, mixed type								X										X							

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TABLE D2. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	008	009	012	015	017	018	019	020	021	022	024	025	027	029	031	032	033	036	038	040	041	042	043	044	045	TOTAL TISSUES TUMORS
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	
INTEGUMENTARY SYSTEM																										
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Trichoepithelioma																									X	1
Subcutaneous tissue	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	*50
Fibrosarcoma																										1
RESPIRATORY SYSTEM																										
Lungs and bronchi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma, NOS, metastatic																			X							2
Alveolar/bronchiolar adenoma																X										2
Alveolar/bronchiolar carcinoma																						X			X	2
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	42
HEMATOPOIETIC SYSTEM																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	46
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Malignant lymphoma, histiocytic type					X																					1
Lymph nodes	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Sarcoma, NOS, unclear primary or meta										X																1
Malignant lymphoma, lymphocytic type									X																	1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	44
CIRCULATORY SYSTEM																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																										
Salivary gland	−	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma	X																									2
Hepatocellular carcinoma						X														X						3
Bile duct	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder & common bile duct	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	N	+	+	+	+	+	+	+	+	*50
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Small intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Large intestine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	45
ENDOCRINE SYSTEM																										
Pituitary	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+	+	+	+	−	−	+	+	+	40
Adenoma, NOS																										1
Adrenal	+	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	−	+	+	+	+	45
Pheochromocytoma															X											2
Thyroid	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	44
Parathyroid	+	+	−	+	−	+	−	+	+	+	+	−	+	+	+	−	−	+	−	+	+	−	+	+	+	29
REPRODUCTIVE SYSTEM																										
Mammary gland	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenocarcinoma, NOS																										2
Uterus	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	47
Squamous cell carcinoma, in situ																	X									1
Endometrial stromal polyp																										1
Ovary	+	+	+	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	46
NERVOUS SYSTEM																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
Harderian gland	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Adenocarcinoma, NOS																			X							2
ALL OTHER SYSTEMS																										
Multiple organs, NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	*50
Malignant lymphoma, NOS																							X			2
Malignant lymphoma, lymphocytic type																										3
Malignant lymphoma, histiocytic type																										1
Malignant lymphoma, mixed type																										2

* Animals necropsied

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TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	60 mg/kg	120 mg/kg
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	4/50 (8%)	4/50 (8%)	2/49 (4%)
Adjusted Rates (b)	9.4%	9.9%	6.0%
Terminal Rates (c)	3/41 (7%)	3/38 (8%)	1/29 (3%)
Week of First Observation	93	80	94
Life Table Tests (d)	P=0.400N	P=0.607	P=0.470N
Incidental Tumor Tests (d)	P=0.243N	P=0.638	P=0.319N
Cochran-Armitage Trend Test (d)	P=0.282N		
Fisher Exact Test (d)		P=0.643	P=0.349N
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	2/49 (4%)
Adjusted Rates (b)	2.4%	7.9%	6.9%
Terminal Rates (c)	1/41 (2%)	3/38 (8%)	2/29 (7%)
Week of First Observation	104	105	104
Life Table Tests (d)	P=0.270	P=0.278	P=0.380
Incidental Tumor Tests (d)	P=0.270	P=0.278	P=0.380
Cochran-Armitage Trend Test (d)	P=0.391		
Fisher Exact Test (d)		P=0.309	P=0.492
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	5/50 (10%)	7/50 (14%)	4/49 (8%)
Adjusted Rates (b)	11.8%	17.6%	12.7%
Terminal Rates (c)	4/41 (10%)	6/38 (16%)	3/29 (10%)
Week of First Observation	93	80	94
Life Table Tests (d)	P=0.495	P=0.335	P=0.592
Incidental Tumor Tests (d)	P=0.495N	P=0.360	P=0.561N
Cochran-Armitage Trend Test (d)	P=0.449N		
Fisher Exact Test (d)		P=0.380	P=0.513N
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	1/50 (2%)	4/50 (8%)	4/50 (8%)
Adjusted Rates (b)	2.4%	10.0%	10.9%
Terminal Rates (c)	1/41 (2%)	3/38 (8%)	2/30 (7%)
Week of First Observation	104	97	74
Life Table Tests (d)	P=0.088	P=0.165	P=0.127
Incidental Tumor Tests (d)	P=0.167	P=0.187	P=0.210
Cochran-Armitage Trend Test (d)	P=0.146		
Fisher Exact Test (d)		P=0.181	P=0.181
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Overall Rates (a)	5/50 (10%)	2/50 (4%)	2/50 (4%)
Adjusted Rates (b)	11.4%	4.2%	5.6%
Terminal Rates (c)	3/41 (7%)	0/38 (0%)	1/30 (3%)
Week of First Observation	77	65	84
Life Table Tests (d)	P=0.206N	P=0.238N	P=0.321N
Incidental Tumor Tests (d)	P=0.062N	P=0.161N	P=0.156N
Cochran-Armitage Trend Test (d)	P=0.146N		
Fisher Exact Test (d)		P=0.218N	P=0.218N
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	4/50 (8%)	7/50 (14%)	2/50 (4%)
Adjusted Rates (b)	9.3%	17.2%	5.3%
Terminal Rates (c)	3/41 (7%)	5/38 (13%)	0/30 (0%)
Week of First Observation	82	95	83
Life Table Tests (d)	P=0.446N	P=0.233	P=0.444N
Incidental Tumor Tests (d)	P=0.192N	P=0.273	P=0.180N
Cochran-Armitage Trend Test (d)	P=0.297N		
Fisher Exact Test (d)		P=0.262	P=0.339N

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TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	60 mg/kg	120 mg/kg
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	13/50 (26%)	14/50 (28%)	10/50 (20%)
Adjusted Rates (b)	27.8%	31.9%	25.3%
Terminal Rates (c)	8/41 (20%)	9/38 (24%)	4/30 (13%)
Week of First Observation	46	65	74
Life Table Tests (d)	P=0.519N	P=0.442	P=0.534N
Incidental Tumor Tests (d)	P=0.102N	P=0.550	P=0.119N
Cochran-Armitage Trend Test (d)	P=0.281N		
Fisher Exact Test (d)		P=0.500	P=0.318N
Circulatory System: Hemangiosarcoma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	2.4%	7.3%	0.0%
Terminal Rates (c)	0/41 (0%)	2/38 (5%)	0/30 (0%)
Week of First Observation	103	78	
Life Table Tests (d)	P=0.455N	P=0.288	P=0.567N
Incidental Tumor Tests (d)	P=0.332N	P=0.366	P=0.338N
Cochran-Armitage Trend Test (d)	P=0.378N		
Fisher Exact Test (d)		P=0.309	P=0.500N
Circulatory System: Hemangioma or Hemangiosarcoma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted Rates (b)	4.8%	7.3%	0.0%
Terminal Rates (c)	1/41 (2%)	2/38 (5%)	0/30 (0%)
Week of First Observation	103	78	
Life Table Tests (d)	P=0.272N	P=0.473	P=0.313N
Incidental Tumor Tests (d)	P=0.184N	P=0.552	P=0.182N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Test (d)		P=0.500	P=0.247N
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	4.4%	7.9%	9.5%
Terminal Rates (c)	1/41 (2%)	3/38 (8%)	2/30 (7%)
Week of First Observation	73	105	98
Life Table Tests (d)	P=0.298	P=0.469	P=0.395
Incidental Tumor Tests (d)	P=0.373	P=0.509	P=0.522
Cochran-Armitage Trend Test (d)	P=0.412		
Fisher Exact Test (d)		P=0.500	P=0.500
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	5/50 (10%)
Adjusted Rates (b)	4.4%	7.9%	16.0%
Terminal Rates (c)	1/41 (2%)	3/38 (8%)	4/30 (13%)
Week of First Observation	73	105	98
Life Table Tests (d)	P=0.085	P=0.469	P=0.130
Incidental Tumor Tests (d)	P=0.117	P=0.509	P=0.194
Cochran-Armitage Trend Test (d)	P=0.158		
Fisher Exact Test (d)		P=0.500	P=0.218
Pituitary Gland: Adenoma			
Overall Rates (a)	2/43 (5%)	4/40 (10%)	0/40 (0%)
Adjusted Rates (b)	5.6%	12.9%	0.0%
Terminal Rates (c)	2/36 (6%)	4/31 (13%)	0/24 (0%)
Week of First Observation	104	105	
Life Table Tests (d)	P=0.355N	P=0.269	P=0.331N
Incidental Tumor Tests (d)	P=0.355N	P=0.269	P=0.331N
Cochran-Armitage Trend Test (d)	P=0.245N		
Fisher Exact Test (d)		P=0.304	P=0.265N

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TABLE D3. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	60 mg/kg	120 mg/kg
Thyroid Gland: Follicular Cell Adenoma			
Overall Rates (a)	3/48 (6%)	3/48 (6%)	0/44 (0%)
Adjusted Rates (b)	7.3%	7.9%	0.0%
Terminal Rates (c)	2/40 (5%)	3/38 (8%)	0/26 (0%)
Week of First Observation	103	105	
Life Table Tests (d)	P=0.182N	P=0.636	P=0.202N
Incidental Tumor Tests (d)	P=0.139N	P=0.651N	P=0.126N
Cochran-Armitage Trend Test (d)	P=0.115N		
Fisher Exact Test (d)		P=0.661	P=0.138N
All Sites: Benign Tumors			
Overall Rates (a)	15/50 (30%)	13/50 (26%)	9/50 (18%)
Adjusted Rates (b)	33.9%	33.1%	26.4%
Terminal Rates (c)	12/41 (29%)	12/38 (32%)	6/30 (20%)
Week of First Observation	73	80	83
Life Table Tests (d)	P=0.309N	P=0.499N	P=0.347N
Incidental Tumor Tests (d)	P=0.140N	P=0.440N	P=0.141N
Cochran-Armitage Trend Test (d)	P=0.101N		
Fisher Exact Test (d)		P=0.412N	P=0.121N
All Sites: Malignant Tumors			
Overall Rates (a)	18/50 (36%)	23/50 (46%)	20/50 (40%)
Adjusted Rates (b)	36.6%	50.7%	49.9%
Terminal Rates (c)	10/41 (24%)	16/38 (42%)	11/30 (37%)
Week of First Observation	46	65	74
Life Table Tests (d)	P=0.131	P=0.183	P=0.172
Incidental Tumor Tests (d)	P=0.472N	P=0.280	P=0.484N
Cochran-Armitage Trend Test (d)	P=0.380		
Fisher Exact Test (d)		P=0.208	P=0.418
All Sites: All Tumors			
Overall Rates (a)	27/50 (54%)	31/50 (62%)	26/50 (52%)
Adjusted Rates (b)	54.0%	68.6%	64.1%
Terminal Rates (c)	18/41 (44%)	24/38 (63%)	16/30 (53%)
Week of First Observation	46	65	74
Life Table Tests (d)	P=0.175	P=0.212	P=0.230
Incidental Tumor Tests (d)	P=0.361N	P=0.329	P=0.346N
Cochran-Armitage Trend Test (d)	P=0.460N		
Fisher Exact Test (d)		P=0.272	P=0.500N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidences at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the vehicle controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

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TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT

	Vehicle Control	Low Dose	High Dose
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*Skin	(50)	(50)	(50)
Abscess, NOS	1 (2%)		
Inflammation, acute/chronic		1 (2%)	
Ulcer, chronic			1 (2%)
Inflammation, chronic suppurative			1 (2%)
Fibrosis, diffuse			1 (2%)
Pigmentation, NOS	2 (4%)		27 (54%)
Acanthosis		1 (2%)	
*Subcutaneous tissue	(50)	(50)	(50)
Metaplasia, cartilaginous	1 (2%)		
RESPIRATORY SYSTEM			
#Peritracheal tissue	(47)	(47)	(42)
Inflammation, acute focal			1 (2%)
#Lung/bronchiole	(50)	(50)	(49)
Vegetable foreign body			1 (2%)
#Lung	(50)	(50)	(49)
Congestion, acute	1 (2%)	6 (12%)	3 (6%)
Hemorrhage	6 (12%)	5 (10%)	3 (6%)
Inflammation, interstitial		1 (2%)	1 (2%)
Pneumonia, giant cell	1 (2%)		
Pneumonia, aspiration			1 (2%)
Hyperplasia, alveolar epithelium		1 (2%)	
Histiocytosis	2 (4%)	2 (4%)	2 (4%)
#Lung/alveoli	(50)	(50)	(49)
Histiocytosis			1 (2%)
HEMATOPOIETIC SYSTEM			
*Multiple organs	(50)	(50)	(50)
Hematopoiesis			1 (2%)
*Skin	(50)	(50)	(50)
Hyperplasia, lymphoid	1 (2%)		
#Bone marrow	(45)	(43)	(46)
Congestion, acute	2 (4%)	4 (9%)	2 (4%)
Necrosis, focal	1 (2%)		
Hypoplasia, NOS	2 (4%)	3 (7%)	6 (13%)
Atrophy, focal		1 (2%)	
Hyperplasia, focal		1 (2%)	
Hyperplasia, diffuse	9 (20%)	5 (12%)	1 (2%)
Myelofibrosis			1 (2%)
Hyperplasia, granulocytic	3 (7%)	6 (14%)	3 (7%)
#Splenic follicles	(47)	(50)	(49)
Atrophy, diffuse	4 (9%)	4 (8%)	3 (6%)
Hyperplasia, diffuse	2 (4%)	2 (4%)	2 (4%)
Hyperplasia, reticulum cell		1 (2%)	
Hyperplasia, lymphoid	6 (13%)	9 (18%)	13 (27%)
Hypoplasia, lymphoid		2 (4%)	3 (6%)
#Splenic red pulp	(47)	(50)	(49)
Congestion, acute	2 (4%)		1 (2%)
Pigmentation, NOS	2 (4%)		
Hyperplasia, lymphoid	1 (2%)		
Hematopoiesis	18 (38%)	20 (40%)	15 (31%)

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TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
HEMATOPOIETIC SYSTEM (Continued)			
#Lymph node	(45)	(41)	(45)
Congestion, acute			1 (2%)
Hemorrhage			1 (2%)
Inflammation, chronic focal			1 (2%)
Hyperplasia, reticulum cell			1 (2%)
Hyperplasia, lymphoid	1 (2%)	2 (5%)	
#Mandibular lymph node	(45)	(41)	(45)
Hyperplasia, plasma cell	1 (2%)		
Hyperplasia, reticulum cell	1 (2%)		
Hyperplasia, lymphoid		2 (5%)	1 (2%)
#Tracheal lymph node	(45)	(41)	(45)
Hyperplasia, lymphoid			1 (2%)
#Mediastinal lymph node	(45)	(41)	(45)
Hyperplasia, lymphoid		1 (2%)	
#Mesenteric lymph node	(45)	(41)	(45)
Hemorrhage			1 (2%)
Hemorrhage, chronic			1 (2%)
Inflammation, acute suppurative			1 (2%)
Inflammation, chronic suppurative			1 (2%)
Hematopoiesis			1 (2%)
#Renal lymph node	(45)	(41)	(45)
Hemorrhage			1 (2%)
Hyperplasia, lymphoid	1 (2%)		
#Lung	(50)	(50)	(49)
Leukocytosis, NOS			1 (2%)
Hyperplasia, lymphoid		2 (4%)	
#Liver	(50)	(50)	(50)
Hyperplasia, lymphoid	2 (4%)		2 (4%)
Hematopoiesis	2 (4%)	3 (6%)	2 (4%)
#Pancreas	(47)	(50)	(48)
Hyperplasia, lymphoid		1 (2%)	2 (4%)
#Pancreatic interstitial tissue	(47)	(50)	(48)
Hyperplasia, lymphoid	2 (4%)		
#Peyer's patch	(45)	(48)	(49)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	1 (2%)
#Kidney	(50)	(49)	(49)
Hyperplasia, lymphoid	2 (4%)		1 (2%)
#Kidney/cortex	(50)	(49)	(49)
Hyperplasia, lymphoid		1 (2%)	
#Kidney/pelvis	(50)	(49)	(49)
Hyperplasia, lymphoid	1 (2%)	1 (2%)	
#Ovary	(44)	(45)	(46)
Hyperplasia, lymphoid		1 (2%)	
#Adrenal	(48)	(45)	(45)
Hematopoiesis			1 (2%)
#Thymus	(34)	(32)	(44)
Cyst, NOS		1 (3%)	
Hemorrhage	1 (3%)		
Depletion, lymphoid			1 (2%)
Histiocytosis			1 (2%)
#Thymic medulla	(34)	(32)	(44)
Hyperplasia, focal	1 (3%)		
Hyperplasia, lymphoid	1 (3%)		1 (2%)
#Thymic lymphocytes	(34)	(32)	(44)
Necrosis, diffuse			1 (2%)
CIRCULATORY SYSTEM			
#Lung	(50)	(50)	(49)
Arteriosclerosis, NOS			1 (2%)
#Heart	(50)	(50)	(49)
Periarteritis		1 (2%)	1 (2%)

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TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
CIRCULATORY SYSTEM (Continued)			
#Heart/atrium	(50)	(50)	(49)
Thrombus, organized			1 (2%)
#Heart/ventricle	(50)	(50)	(49)
Inflammation, acute/chronic		1 (2%)	
#Liver	(50)	(50)	(50)
Thrombus, organized	1 (2%)	1 (2%)	
#Urinary bladder	(46)	(47)	(45)
Periarteritis		1 (2%)	
#Uterus/endometrium	(49)	(48)	(47)
Thrombus, organized	1 (2%)		
#Thyroid	(48)	(48)	(44)
Periarteritis		1 (2%)	
DIGESTIVE SYSTEM			
#Salivary gland	(47)	(48)	(46)
Atrophy, focal	4 (9%)	3 (6%)	8 (17%)
Atrophy, serous	1 (2%)	3 (6%)	
#Salivary gland interstitial tissue	(47)	(48)	(46)
Inflammation, acute/chronic	1 (2%)		
#Liver	(50)	(50)	(50)
Congestion, acute			4 (8%)
Hemorrhage	2 (4%)		1 (2%)
Inflammation, acute/chronic	11 (22%)	5 (10%)	2 (4%)
Degeneration, lipoid			1 (2%)
Necrosis, focal	1 (2%)		
Infarct, focal		1 (2%)	
Pigmentation, NOS		1 (2%)	
Focal cellular change	1 (2%)		
Hyperplasia, nodular		1 (2%)	
Histiocytosis		1 (2%)	
#Liver/centrilobular	(50)	(50)	(50)
Degeneration, lipoid			1 (2%)
Necrosis, diffuse		1 (2%)	
Atrophy, diffuse			1 (2%)
#Liver/hepatocytes	(50)	(50)	(50)
Hemorrhage	1 (2%)		
Degeneration, lipoid			1 (2%)
Necrosis, focal		1 (2%)	2 (4%)
Necrosis, coagulative	1 (2%)		
Necrosis, ischemic	2 (4%)		
Cytoplasmic vacuolization			1 (2%)
Focal cellular change		2 (4%)	
Atrophy, diffuse	1 (2%)		
Dysplasia, NOS	2 (4%)	1 (2%)	
*Gallbladder	(50)	(50)	(50)
Hyperplasia, cystic	1 (2%)		
#Bile duct	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)		
#Pancreas	(47)	(50)	(48)
Inflammation, acute/chronic	1 (2%)		
Inflammation, chronic suppurative	1 (2%)		
Necrosis, fat			1 (2%)
#Pancreatic duct	(47)	(50)	(48)
Necrosis, NOS			1 (2%)
#Pancreatic acinus	(47)	(50)	(48)
Inflammation with fibrosis			1 (2%)
Atrophy, focal	7 (15%)	25 (50%)	41 (85%)
Atrophy, diffuse	1 (2%)	1 (2%)	
Hyperplasia, focal			1 (2%)

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TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
DIGESTIVE SYSTEM (Continued)			
#Periesophageal tissue	(50)	(49)	(48)
Foreign body, NOS		1 (2%)	
Inflammation, acute suppurative		1 (2%)	
#Stomach	(46)	(47)	(48)
Mineralization	1 (2%)		
Ulcer, NOS		1 (2%)	
Inflammation, acute suppurative		1 (2%)	
Inflammation, acute/chronic			1 (2%)
Inflammation with fibrosis		1 (2%)	
Hyperkeratosis	2 (4%)		
Acanthosis	2 (4%)	1 (2%)	
Metaplasia, NOS		1 (2%)	
Metaplasia, squamous		1 (2%)	
#Gastric mucosa	(46)	(47)	(48)
Inflammation, acute/chronic		1 (2%)	
Acanthosis		1 (2%)	
#Gastric fundal gland	(46)	(47)	(48)
Dilatation, NOS			1 (2%)
Edema, NOS	1 (2%)		
Atrophy, focal	1 (2%)		
Hyperplasia, focal	1 (2%)		
#Forestomach	(46)	(47)	(48)
Hyperplasia, epithelial		1 (2%)	
#Cardiac stomach	(46)	(47)	(48)
Inflammation, acute/chronic			1 (2%)
#Small intestine	(45)	(48)	(49)
Parasitism			1 (2%)
Atrophy, focal		1 (2%)	
Atrophy, pressure		1 (2%)	
#Intestinal villus	(45)	(48)	(49)
Atrophy, focal			1 (2%)
#Colon	(45)	(48)	(48)
Parasitism	2 (4%)	1 (2%)	2 (4%)
URINARY SYSTEM			
#Kidney	(50)	(49)	(49)
Glomerulonephritis, membranous	1 (2%)		
Glomerulonephritis, chronic	2 (4%)		
#Kidney/cortex	(50)	(49)	(49)
Glomerulonephritis, membranous		1 (2%)	
Inflammation, acute/chronic	1 (2%)		
Nephropathy	11 (22%)	9 (18%)	12 (24%)
Infarct, healed	1 (2%)		
Metaplasia, osseous			1 (2%)
#Kidney/tubule	(50)	(49)	(49)
Mineralization	1 (2%)		
Degeneration, NOS	1 (2%)		
Degeneration, granular		1 (2%)	
Atrophy, focal	1 (2%)		
*Ureter	(50)	(50)	(50)
Mineralization	1 (2%)		
#Urinary bladder	(46)	(47)	(45)
Congestion, acute	1 (2%)		
ENDOCRINE SYSTEM			
#Pituitary	(43)	(40)	(40)
Cyst, NOS		3 (8%)	3 (8%)
Hemorrhage		1 (3%)	
Hemorrhagic cyst	3 (7%)		
Hemorrhage, chronic			1 (3%)

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TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
ENDOCRINE SYSTEM			
#Pituitary (Continued)	(43)	(40)	(40)
Hypertrophy, focal		1 (3%)	
Hyperplasia, focal	6 (14%)	2 (5%)	
Hyperplasia, chromophobe cell	3 (7%)		
#Anterior pituitary	(43)	(40)	(40)
Hyperplasia, focal	1 (2%)		
#Adrenal	(48)	(45)	(45)
Hemorrhage		1 (2%)	
Inflammation, acute suppurative		1 (2%)	
#Adrenal/capsule	(48)	(45)	(45)
Fibrosis, focal	1 (2%)		
#Adrenal cortex	(48)	(45)	(45)
Congestion, NOS			1 (2%)
Congestion, acute			1 (2%)
Degeneration, NOS		1 (2%)	
Hypertrophy, focal	2 (4%)	2 (4%)	1 (2%)
#Thyroid	(48)	(48)	(44)
Embryonal duct cyst		1 (2%)	
Cyst, NOS	1 (2%)	1 (2%)	
Hyperplasia, follicular cell	3 (6%)	3 (6%)	
Histiocytosis	1 (2%)		
#Thyroid follicle	(48)	(48)	(44)
Atrophy, focal		1 (2%)	
Atrophy, diffuse			1 (2%)
REPRODUCTIVE SYSTEM			
#Uterus	(49)	(48)	(47)
Dilatation, NOS		12 (25%)	16 (34%)
Hemorrhage		2 (4%)	
Inflammation, suppurative			1 (2%)
Inflammation, acute suppurative	2 (4%)		
Polypoid hyperplasia		1 (2%)	
#Uterus/endometrium	(49)	(48)	(47)
Edema, NOS			1 (2%)
Hemorrhage	1 (2%)		
Inflammation, acute suppurative	2 (4%)		
Hypoplasia, NOS		2 (4%)	2 (4%)
Atrophy, focal			1 (2%)
Hyperplasia, focal			1 (2%)
Hyperplasia, diffuse		2 (4%)	2 (4%)
Hyperplasia, cystic	45 (92%)	42 (88%)	31 (66%)
#Uterus/myometrium	(49)	(48)	(47)
Inflammation, acute/chronic	1 (2%)		
#Ovary/parovarian	(44)	(45)	(46)
Hemorrhagic cyst			1 (2%)
#Ovary	(44)	(45)	(46)
Dilatation, NOS		1 (2%)	
Cyst, NOS	10 (23%)	10 (22%)	8 (17%)
Follicular cyst, NOS			1 (2%)
Multiple cysts	2 (5%)		
Hemorrhage	1 (2%)		
Hemorrhagic cyst	2 (5%)	2 (4%)	2 (4%)
Inflammation, acute suppurative	2 (5%)		
Necrosis, fat		1 (2%)	1 (2%)
Pigmentation, NOS			3 (7%)
Atrophy, senile			1 (2%)
Hyperplasia, cystic		2 (4%)	
Histiocytosis	1 (2%)		
#Ovary/follicle	(44)	(45)	(46)
Hemorrhagic cyst	1 (2%)		

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TABLE D4. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR GAVAGE STUDY OF MALONALDEHYDE, SODIUM SALT (Continued)

	Vehicle Control	Low Dose	High Dose
NERVOUS SYSTEM			
#Brain/meninges	(49)	(48)	(50)
Hemorrhage	1 (2%)		
Inflammation, acute/chronic	3 (6%)		
Fibrosis, multifocal			1 (2%)
#Brain	(49)	(48)	(50)
Mineralization	10 (20%)	18 (38%)	20 (40%)
Inflammation, acute/chronic			1 (2%)
SPECIAL SENSE ORGANS			
*Eye/cornea	(50)	(50)	(50)
Inflammation, acute diffuse			1 (2%)
MUSCULOSKELETAL SYSTEM			
None			
BODY CAVITIES			
*Mediastinum	(50)	(50)	(50)
Foreign body, NOS			2 (4%)
Hemorrhage			1 (2%)
Inflammation, acute suppurative			2 (4%)
Inflammation, chronic focal			1 (2%)
*Peritoneum	(50)	(50)	(50)
Necrosis, fat	2 (4%)		
*Peritoneal cavity	(50)	(50)	(50)
Necrosis, fat	1 (2%)	3 (6%)	1 (2%)
*Pleural cavity	(50)	(50)	(50)
Foreign body, NOS			1 (2%)
Hematoma, organized			1 (2%)
Inflammation, acute suppurative			1 (2%)
*Pleural mesothelium	(50)	(50)	(50)
Inflammation, acute/chronic		1 (2%)	
*Epicardium	(50)	(50)	(50)
Inflammation, acute/chronic			1 (2%)
ALL OTHER SYSTEMS			
None			
SPECIAL MORPHOLOGY SUMMARY			
None			

* Number of animals receiving complete necropsy examinations; all gross lesions including masses examined microscopically.
# Number of animals examined microscopically at this site

000591

APPENDIX E

GENETIC TOXICOLOGY OF MALONALDEHYDE, SODIUM SALT

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TABLE E1. MUTAGENICITY OF MALONALDEHYDE, SODIUM SALT, IN *SALMONELLA TYPHIMURIUM* (a)

Strain	Dose (µg/plate)	Revertants/plate (b)					
		−S9		+S9 (hamster)		+S9 (rat)	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA100	0	141 ± 10.7	100 ± 2.3	160 ± 3.4	151 ± 20.5	161 ± 7.8	150 ± 3.8
	33	--	--	153 ± 4.3	118 ± 13.5	162 ± 13.6	125 ± 4.0
	100	117 ± 8.4	99 ± 9.7	155 ± 3.5	125 ± 7.2	164 ± 10.8	142 ± 5.8
	333	120 ± 6.0	84 ± 3.2	150 ± 5.7	138 ± 13.0	148 ± 11.2	135 ± 9.7
	1,000	113 ± 6.8	98 ± 2.7	150 ± 12.7	169 ± 10.7	157 ± 16.3	158 ± 2.7
	3,333	113 ± 7.1	95 ± 3.2	167 ± 9.1	162 ± 3.0	160 ± 13.7	182 ± 8.5
	10,000	115 ± 0.3	97 ± 2.0	--	--	--	--
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (c)	1,417 ± 76.4	1,034 ± 140.2	2,712 ± 147.0	2,550 ± 299.1	1,114 ± 45.0	1,940 ± 283.6
TA1535	0	11 ± 1.7	16 ± 1.0	13 ± 3.4	21 ± 1.5	10 ± 1.2	13 ± 2.2
	33	--	--	19 ± 3.0	18 ± 3.5	11 ± 1.5	13 ± 0.9
	100	14 ± 1.2	14 ± 0.6	12 ± 0.3	18 ± 1.2	15 ± 1.8	13 ± 0.6
	333	9 ± 2.7	17 ± 2.6	9 ± 2.4	12 ± 1.0	11 ± 0.9	17 ± 0.7
	1,000	12 ± 3.7	12 ± 1.5	12 ± 0.3	15 ± 0.3	12 ± 1.3	16 ± 1.9
	3,333	10 ± 1.5	14 ± 1.3	11 ± 2.5	17 ± 1.5	5 ± 1.0	13 ± 1.5
	10,000	5 ± 0.3	8 ± 1.7	--	--	--	--
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (c)	983 ± 64.1	571 ± 16.0	312 ± 16.8	216 ± 11.6	193 ± 10.5	220 ± 13.0
TA1537	0	13 ± 3.0	7 ± 1.7	13 ± 0.3	12 ± 4.1	17 ± 1.7	10 ± 2.9
	33	--	--	19 ± 2.0	7 ± 0.3	21 ± 2.0	8 ± 0.7
	100	14 ± 1.3	8 ± 0.7	18 ± 3.2	6 ± 1.8	16 ± 3.2	6 ± 2.3
	333	14 ± 1.5	7 ± 0.0	18 ± 3.2	11 ± 1.0	18 ± 2.1	6 ± 1.0
	1,000	16 ± 0.3	8 ± 1.2	17 ± 2.4	11 ± 2.0	21 ± 2.8	5 ± 1.2
	3,333	14 ± 1.0	6 ± 0.9	18 ± 1.9	11 ± 2.5	14 ± 4.7	6 ± 0.9
	10,000	7 ± 1.2	2 ± 0.3	--	--	--	--
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (c)	758 ± 40.5	154 ± 7.0	372 ± 30.3	231 ± 45.4	252 ± 23.2	153 ± 9.8
TA98	0	26 ± 1.8	15 ± 1.7	38 ± 7.2	25 ± 2.5	39 ± 3.1	24 ± 2.9
	33	--	--	32 ± 3.7	23 ± 2.3	29 ± 2.4	25 ± 1.0
	100	31 ± 4.2	16 ± 1.2	43 ± 3.9	22 ± 1.2	33 ± 3.4	22 ± 2.2
	333	34 ± 4.2	18 ± 0.6	42 ± 4.9	29 ± 4.7	31 ± 2.4	19 ± 1.8
	1,000	26 ± 3.2	16 ± 0.7	34 ± 3.7	31 ± 3.2	36 ± 1.5	24 ± 3.5
	3,333	26 ± 4.4	16 ± 3.8	44 ± 3.7	27 ± 1.2	36 ± 5.8	18 ± 2.3
	10,000	15 ± 0.3	17 ± 3.8	--	--	--	--
	Trial summary	Negative	Negative	Negative	Negative	Negative	Negative
	Positive control (c)	162 ± 7.1	292 ± 14.4	942 ± 29.6	2,121 ± 390.8	463 ± 15.3	1,162 ± 175.6

(a) Study performed at Case Western Reserve University. The detailed protocol is presented in Haworth et al. (1983). Cells and study compound or solvent (distilled water) were incubated in the absence of exogenous metabolic activation (−S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague Dawley rat liver. High dose was limited by toxicity or solubility but did not exceed 10 mg/plate; 0 µg/plate dose is the solvent control.
(b) Revertants are presented as mean ± standard error from three plates.
(c) Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was used with TA98, sodium azide was used with TA100 and TA1535, and 9-aminoacridine was used with TA1537.

000593

TABLE E2. MUTAGENICITY OF MALONALDEHYDE, SODIUM SALT, IN MOUSE L5178Y LYMPHOMA CELLS (a,b)

Compound	Concentration (µg/ml)	Cloning Efficiency (percent)	Relative Total Growth (percent)	Mutant Count	Mutant Fraction (c)
Trial 1					
Distilled water		72.3 ± 3.2	100.0 ± 32.0	112.0 ± 9.0	51.3 ± 2.0
Malonaldehyde, sodium salt					
	125	71.3 ± 9.2	128.7 ± 10.5	166.7 ± 11.3	(d) 79.3 ± 6.4
	250	79.3 ± 9.9	88.7 ± 5.8	157.7 ± 10.7	67.0 ± 5.0
	500	63.0 ± 12.0	59.0 ± 4.0	268.0 ± 23.0	(d) 145.5 ± 15.5
	1,000	Lethal	--	--	--
Methyl methanesulfonate					
	5	46.3 ± 10.2	46.7 ± 9.8	787.7 ± 122.0	(d) 596.7 ± 71.5
Trial 2					
Distilled water		89.5 ± 2.5	100.0 ± 2.3	106.8 ± 7.0	39.8 ± 2.3
Malonaldehyde, sodium salt					
	300	83.7 ± 3.3	69.7 ± 3.5	165.7 ± 7.5	(d) 66.0 ± 1.2
	400	74.7 ± 4.4	67.0 ± 3.1	226.7 ± 8.4	(d) 101.7 ± 3.7
	500	84.3 ± 2.4	54.0 ± 1.5	273.0 ± 24.6	(d) 107.7 ± 7.2
	600	73.3 ± 1.9	51.0 ± 7.6	222.7 ± 15.2	(d) 101.0 ± 6.1
	800	58.0 ± 4.0	35.3 ± 2.8	360.3 ± 11.3	(d) 210.3 ± 19.0
	1,000	54.3 ± 6.6	11.3 ± 0.9	442.0 ± 21.4	(d) 279.3 ± 27.4
Methyl methanesulfonate					
	5	52.0 ± 8.5	28.7 ± 3.5	610.0 ± 46.5	(d) 405.3 ± 48.0

(a) Study performed at Litton Bionetics, Inc. The experimental protocol is presented in detail by Myhr et al. (1985) and follows the basic format of Clive et al. (1979). The highest dose of study compound is determined by solubility or toxicity and may not exceed 5 mg/ml. Cells (6×10^5/ml) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency.
(b) Mean ± standard error of replicate trials of approximately 3×10^6 cells each. All data are evaluated statistically for both trend and peak response ($P<0.05$ for at least one of the three highest dose sets). Both responses must be significantly ($P<0.05$) positive for a chemical to be considered mutagenic. If only one of these responses is significant, the call is "equivocal"; the absence of both trend and peak response results in a "negative" call.
(c) Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF per 1×10^6 cells treated); MF = mutant fraction.
(d) Significant positive response; occurs when the relative mutant fraction (average MF of treated culture/average MF of solvent control) is greater than or equal to 1.6.

000594

TABLE E3. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY MALONALDEHYDE, SODIUM SALT (a)

Compound	Dose (µg/ml)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hours in BrdU	Relative SCEs/Cell (percent) (b)
−S9 (c)								
Trial No. 1--Summary: Positive								
Medium		50	1,042	393	0.38	7.9	26.5	--
Malonaldehyde, sodium salt								
	14.9	50	1,049	394	0.38	7.9	26.5	100.0
	49.8	50	1,044	479	0.46	9.6	26.5	121.5
	149	50	1,041	596	0.57	11.9	26.5	150.6
Mitomycin C								
	0.002	50	1,045	660	0.63	13.2	26.5	167.1
	0.010	10	208	331	1.59	33.1	26.5	419.0
Trial No. 2--Summary: Positive								
Medium		50	1,040	428	0.41	8.6	26.0	--
Malonaldehyde, sodium salt								
	15	50	1,048	529	0.50	10.6	26.0	123.3
	50	50	1,036	568	0.55	11.4	26.0	132.6
	150	50	1,045	726	0.69	14.5	26.0	168.6
	300	50	1,038	1,018	0.98	20.4	26.0	237.2
Mitomycin C								
	0.002	50	1,036	496	0.48	9.9	26.0	115.1
	0.010	10	209	93	0.44	9.3	26.0	108.1
+S9 (d)								
Trial No. 1--Summary: Positive								
Medium		50	1,047	453	0.43	9.1	26.0	--
Malonaldehyde, sodium salt								
	149	50	1,043	438	0.42	8.8	26.0	96.7
	498	50	1,047	533	0.51	10.7	26.0	117.6
	1,490	50	1,042	691	0.66	13.8	26.0	151.6
Cyclophosphamide								
	0.500	50	1,034	416	0.40	8.3	26.0	91.2
	2.500	10	206	292	1.42	29.2	26.0	320.9
Trial No. 2--Summary: Positive								
Medium		50	1,047	470	0.45	9.4	26.0	--
Malonaldehyde, sodium salt								
	150	50	1,049	543	0.52	10.9	26.0	116.0
	500	50	1,042	655	0.63	13.1	26.0	139.4
	1,000	50	1,039	613	0.59	12.3	26.0	130.9
	2,000	50	1,032	818	0.79	16.4	(e) 30.0	174.5
Cyclophosphamide								
	0.500	100	2,092	2,049	0.98	20.5	26.0	218.1
	2.500	10	213	460	2.16	46.0	26.0	489.4

000595

TABLE E3. INDUCTION OF SISTER CHROMATID EXCHANGES IN CHINESE HAMSTER OVARY CELLS BY MALONALDEHYDE, SODIUM SALT (Continued)

(a) Study performed at Bioassay Systems Corp. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or medium as described in (c) or (d) below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.
(b) SCEs/cell of culture exposed to study chemical relative to those of culture exposed to medium
(c) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or medium for 2 hours at 37° C. Then BrdU was added, and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and colcemid was added, and incubation was continued for 2-3 hours.
(d) In the presence of S9, cells were incubated with study compound or medium for 2 hours at 37° C. Then cells were washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with colcemid present for the final 2-3 hours. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.
(e) Because some chemicals induce a delay in the cell division cycle, harvest times are occasionally extended to maximize the proportion of second division cells available for analysis.

TABLE E4. INDUCTION OF CHROMOSOMAL ABERRATIONS IN CHINESE HAMSTER OVARY CELLS BY MALONALDEHYDE, SODIUM SALT (a)

Dose (µg/ml)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
−S9 (b)				
Trial 1--Harvest time 10.5 hours				
Medium				
	100	8	0.08	7
Malonaldehyde, sodium salt				
81.8	100	6	0.06	6
245	100	5	0.05	5
409	100	3	0.03	3
Summary: Negative				
Mitomycin C				
5	100	69	0.69	46
+S9 (d)				
Trial 1--Harvest time 12.0 hours				
Medium				
	100	2	0.02	2
Malonaldehyde, sodium salt				
409	100	3	0.03	3
1,640	100	2	0.02	2
3,270	100	5	0.05	4
Summary: Negative				
Cyclophosphamide				
50	100	38	0.38	33
−S9 (b)				
Trial 2--Harvest time 20.0 hours (c)				
Medium				
	100	5	0.05	4
Malonaldehyde, sodium salt				
42.9	100	4	0.04	4
71.5	100	6	0.06	6
143	100	5	0.05	5
215	100	4	0.04	3
Summary: Negative				
Mitomycin C				
1	10	13	1.3	50
5	10	81	8.1	90

(a) Study performed at Bioassay Systems Corporation. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway et al. (1985). Briefly, Chinese hamster ovary cells were incubated with study compound or solvent (medium) as indicated in (b) or (d). Cells were arrested in first metaphase by addition of colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.
(b) In the absence of S9, Chinese hamster ovary cells were incubated with study compound or solvent (medium) for 8-10 hours at 37° C. Cells were then washed, and fresh medium containing colcemid was added for an additional 2-3 hours followed by harvest.
(c) Because of significant chemically induced cell-cycle delay, incubation time before addition of colcemid was lengthened to provide sufficient metaphases at harvest.
(d) In the presence of S9, cells were incubated with study compound or solvent (medium) for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8-10 hours. Colcemid was added for the last 2-3 hours of incubation before harvest. S9 was from the liver of Aroclor 1254-induced male Sprague Dawley rats.

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TABLE E5. INDUCTION OF SEX-LINKED RECESSIVE LETHAL MUTATIONS IN DROSOPHILA BY MALONALDEHYDE, SODIUM SALT (a)

Route of Exposure	Dose (ppm)	Incidence of Deaths (percent)	Incidence of Sterility (percent)	No. of Lethals/No. of X Chromosomes Tested			Overall Total (b)
				Mating 1	Mating 2	Mating 3	
Injection	10,000	22	8	3/2,388	3/2,105	1/1,472	7/5,965 (0.12%)
	0			2/2,476	4/2,388	6/2,195	12/7,059 (0.17%)
Feeding	25,000	24	11	4/2,602	2/1,992	0/1,177	6/5,771 (0.10%)
	0			1/3,784	2/2,863	1/2,128	4/8,775 (0.05%)

(a) Study performed at Bowling Green State University. A detailed protocol of the sex-linked recessive lethal assay is presented in Zimmering et al. (1985). (Exposure by feeding was done by allowing 24-hour-old Canton-S males to feed for 3 days on a solution of the study chemical dissolved in 5% sucrose. In the injection experiments, 24-hour-old Canton-S males were treated with a solution of the chemical dissolved in 0.7% saline and allowed 24 hours to recover.) Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three broods of 3, 2, and 2 days; sample sperm from successive matings were treated as spermatozoa (mating 1), spermatids (mating 2), and spermatocytes (mating 3). F_1 heterozygous females were crossed to their siblings and placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters; no clusters were found. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; these were retested. Results were not significant at the 5% level (Margolin et al., 1983).
(b) Combined total of number of lethal mutations/number of X chromosomes tested for three mating trials

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APPENDIX F

SENTINEL ANIMAL PROGRAM

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TABLE F1	MURINE VIRUS ANTIBODY DETERMINATIONS FOR RATS AND MICE IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT	173

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APPENDIX F. SENTINEL ANIMAL PROGRAM

I. Methods

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via viral serology on sera from extra (sentinel) animals in the study rooms. These animals are untreated, and these animals and the study animals are both subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Fifteen $B6C3F_1$ mice and 15 F344/N rats of each sex are selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group are killed at 6, 12, and 18 months on study. Data from animals surviving 24 months are collected from 5/50 randomly selected vehicle control animals of each sex and species. The blood from each animal is collected and clotted, and the serum is separated. The serum is cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the viral antibody titers. The following tests are performed:

	Hemagglutination Inhibition	Complement Fixation	ELISA
Mice	PVM (pneumonia virus of mice) Reo 3 (reovirus type 3) GDVII (Theiler's encephalomyelitis virus) Poly (polyoma virus) MVM (minute virus of mice) Ectro (infectious ectromelia) Sendai	M. Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus) MHV (6, 12, 18 mo)	MHV (mouse hepatitis virus) (24 mo)
Rats	PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai	RCV (rat coronavirus)	

II. Results

Results are presented in Table F1.

000601

TABLE F1. MURINE VIRUS ANTIBODY DETERMINATIONS FOR RATS AND MICE IN THE TWO-YEAR GAVAGE STUDIES OF MALONALDEHYDE, SODIUM SALT (a)

Interval (months)	No. of Animals	Positive Serologic Reaction for
RATS		
6	6/10	KRV
12	5/10	KRV
18	3/10	KRV
24	4/10	KRV
MICE		
6	0/10	None positive
12	2/10	MVM
18	1/7	GDVII
24	5/10	Reo 3

(a) Blood samples were taken from sentinel animals at 6, 12, and 18 months after the start of dosing and from the vehicle control animals just before they were killed; samples were sent to Microbiological Associates (Bethesda, MD) for determination of antibody titers.

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APPENDIX G

INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Pelleted Diet: December 1979 to January 1982

(Manufactured by Zeigler Bros., Inc., Gardners, PA)

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TABLE G3	NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION	177
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TABLE G1. INGREDIENTS OF NIH 07 RAT AND MOUSE RATION (a)

Ingredients (b)	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Brewer's dried yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

(a) NCI, 1976; NIH, 1978
(b) Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE G2. VITAMINS AND MINERALS IN NIH 07 RAT AND MOUSE RATION (a)

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D-activated animal sterol
K_3	2.8 g	Menadione activity
d-α-Tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 µg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

(a) Per ton (2,000 lb) of finished product

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TABLE G3. NUTRIENT COMPOSITION OF NIH 07 RAT AND MOUSE RATION (a)

Nutrients	Mean ± Standard Deviation	Range	No. of Samples
Crude protein (percent by weight)	24.29 ± 0.81	22.7-26.1	24
Crude fat (percent by weight)	4.81 ± 0.38	4.1-5.5	24
Crude fiber (percent by weight)	3.31 ± 0.50	1.4-4.3	24
Ash (percent by weight)	6.76 ± 0.44	5.83-7.43	24
Amino Acids (percent of total diet)			
Arginine	1.260	1.21-1.31	2
Cystine	0.395	0.39-0.40	2
Glycine	1.175	1.15-1.20	2
Histidine	0.553	0.530-0.576	2
Isoleucine	0.908	0.881-0.934	2
Leucine	1.905	1.85-1.96	2
Lysine	1.250	1.20-1.30	2
Methionine	0.310	0.306-0.314	2
Phenylalanine	0.967	0.960-0.974	2
Threonine	0.834	0.840-0.827	2
Tryptophan	0.175	0.171-0.178	2
Tyrosine	0.587	0.566-0.607	2
Valine	1.085	1.05-1.12	2
Essential Fatty Acids (percent of total diet)			
Linoleic	2.37		1
Linolenic	0.308		1
Arachidonic	0.008		1
Vitamin			
Vitamin A (IU/kg)	10,192 ± 2,534	6,700-17,000	24
Vitamin D (IU/kg)	6,300		1
α-Tocopherol (ppm)	37.6	31.1-44.0	2
Thiamine (ppm) (b)	16.2 ± 4.5	7.4-27.0	23
Riboflavin (ppm)	6.9	6.1-7.4	2
Niacin (ppm)	75	65-85	2
Pantothenic acid (ppm)	30.2	29.8-30.5	2
Pyridoxine (ppm)	7.2	5.6-8.8	2
Folic acid (ppm)	2.1	1.8-2.4	2
Biotin (ppm)	0.24	0.21-0.27	2
Vitamin B_{12} (ppb)	12.8	10.6-15.0	2
Choline (ppm)	3,315	3,200-3,430	2
Minerals			
Calcium (percent)	1.34 ± 0.20	0.81-1.69	24
Phosphorus (percent)	1.01 ± 0.08	0.82-1.10	24
Potassium (percent)	0.809	0.772-0.846	2
Chloride (percent)	0.557	0.479-0.635	2
Sodium (percent)	0.304	0.258-0.349	2
Magnesium (percent)	0.172	0.166-0.177	2
Sulfur (percent)	0.278	0.270-0.285	2
Iron (ppm)	418	409-426	2
Manganese (ppm)	90.8	86.0-95.5	2
Zinc (ppm)	55.1	54.2-56.0	2
Copper (ppm)	12.68	9.65-15.70	2
Iodine (ppm)	2.58	1.52-3.64	2
Chromium (ppm)	1.86	1.79-1.93	2
Cobalt (ppm)	0.57	0.49-0.65	2

(a) One or two batches of feed analyzed for nutrients reported in this table were manufactured in January and/or April 1983.
(b) One batch (7/22/81) was not analyzed for thiamine.

TABLE G4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION

Contaminants	Mean ± Standard Deviation	Range	No. of Samples
Arsenic (ppm)	0.39 ± 0.23	<0.05-1.06	24
Cadmium (ppm) (a)	0.11 ± 0.07	<0.05-0.40	24
Lead (ppm)	0.91 ± 0.51	0.50-2.65	24
Mercury (ppm) (b)	< 0.05		24
Selenium (ppm)	0.29 ± 0.09	0.10-0.52	24
Aflatoxins (ppb) (b,c)	<10	<10-<5	24
Nitrate nitrogen (ppm) (d,e)	7.00 ± 3.70	<0.1-13.0	24
Nitrite nitrogen (ppm) (d,e)	1.45 ± 1.02	<0.1-4.0	24
BHA (ppm) (f,g)	3.83 ± 3.88	<0.2-13.0	24
BHT (ppm) (f)	2.97 ± 1.74	0.8-7.6	24
Aerobic plate count (CFU/g) (h)	48,786 ± 32,701	5,500-120,000	22
Aerobic plate count (CFU/g) (i)	70,970 ± 81,410	5,500-320,000	24
Coliform (MPN/g) (j)	39 ± 57	<3-240	20
Coliform (MPN/g) (k)	270 ± 580	<3-2,400	24
E. coli (MPN/g) (l)	<3		24
Total nitrosamines (ppb) (m,n)	7.63 ± 6.67	2.2-24.5	21
Total nitrosamines (ppb) (m,o)	29.77 ± 64.59	2.2-273	24
N-Nitrosodimethylamine (ppb) (m,n)	5.81 ± 6.30	1.1-20.0	21
N-Nitrosodimethylamine (ppb) (m,o)	27.79 ± 64.31	1.1-272	24
N-Nitrosopyrrolidine (ppb)	1.44 ± 0.89	0.5-3.5	24
Pesticides (ppm)			
α-BHC (b,p)	<0.01		24
β-BHC (b)	<0.02		24
γ-BHC-Lindane (b)	<0.01		24
δ-BHC (b)	<0.01		24
Heptachlor (b)	<0.01		24
Aldrin (b)	<0.01		24
Heptachlor epoxide (b)	<0.01		24
DDE (b)	<0.01		24
DDD (b)	<0.01		24
DDT (b)	<0.01		24
HCB (b)	<0.01		24
Mirex (b)	<0.01		24
Methoxychlor (q)	<0.05	0.09 (8/26/81)	24
Dieldrin (b)	<0.01		24
Endrin (b)	<0.01		24
Telodrin (b)	<0.01		24
Chlordane (b)	<0.05		24
Toxaphene (b)	<0.1		24
Estimated PCBs (b)	<0.2		24
Ronnel (b)	<0.01		24
Ethion (b)	<0.02		24
Trithion (b)	<0.05		24
Diazinon (q)	<0.1	0.2 (4/27/81)	24
Methyl parathion (b)	<0.02		24
Ethyl parathion (b)	<0.02		24
Malathion (r)	0.10 ± 0.07	<0.05-0.27	24
Endosulfan I (b)	<0.01		24
Endosulfan II (b)	<0.01		24
Endosulfan sulfate (b)	<0.03		24

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TABLE G4. CONTAMINANT LEVELS IN NIH 07 RAT AND MOUSE RATION (Continued)

(a) Three batches contained more than 0.1 ppm.
(b) All values were less than the detection limit, given in the table as the mean.
(c) The detection limit was reduced from 10 ppb to 5 ppb after 7/81.
(d) Source of contamination: alfalfa, grains, and fish meal
(e) Two batches contained less than 0.1 ppm.
(f) Source of contamination: soy oil and fish meal
(g) Six batches contained less than 0.5 ppm.
(h) CFU = colony forming units; mean, standard deviation, and range exclude two extreme values (300,000 and 320,000) obtained for batches produced on 12/21/79 and 2/26/80.
(i) Mean, standard deviation, and range include the two extreme values given in footnote (h).
(j) Mean, standard deviation, and range exclude four very high values in the range of 1,100-2,400 obtained for batches produced on 2/4/80, 2/26/80, 5/29/80, and 12/16/80.
(k) Mean, standard deviation, and range include the very high values listed in footnote (j).
(l) MPN = most probable number; all values were less than 3 MPN/g.
(m) All values were corrected for percent recovery.
(n) Mean, standard deviation, and range exclude three very high values in the range of 115-280 ppb obtained for batches produced on 1/26/81, 2/23/81 and 4/27/81.
(o) Mean, standard deviation, and range include the very high values given in footnote (n).
(p) BHC = hexachlorocyclohexane or benzene hexachloride
(q) One observation was above the detection limit. The value and the date it was obtained are listed under the range.
(r) Nine batches contained more than 0.05 ppm.

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APPENDIX H

AUDIT SUMMARY

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APPENDIX H. AUDIT SUMMARY

The experimental data, documents, pathology materials, and draft Technical Report for the 2-year toxicology and carcinogenesis studies of malonaldehyde, sodium salt, in rats and mice were audited for accuracy, consistency, and completeness. Animal exposures for the 2-year studies began in February 1980. The laboratory experiments were conducted for the NTP by Battelle Columbus Laboratories (Columbus, Ohio) under a subcontract with Tracor Jitco, Inc. The retrospective audit was conducted at the NTP Archives in May 1986 by Program Resources, Inc., William L. Oller, Ph.D., Principal Investigator. The other individuals who conducted the audit are listed in the full report that is on file at the NIEHS.

The audit included, as minimum requirements, a review of:

(1) All records concerning animal receipt, quarantine, randomization, and disposition prior to study start.
(2) All chemistry records.
(3) Body weight and clinical observation data for a random 10% sample of the study animals.
(4) Ten percent random sample of the dose preparation records.
(5) All inlife records concerning environmental conditions, palpable masses, mortality, and animal identification.
(6) All postmortem records for individual animals concerning identification, disposition codes, condition codes, and correlation between gross observations and microscopic diagnoses.
(7) Wet tissues from a random 10% sample of the study animals to verify animal identification and to examine for untrimmed potential lesions.
(8) Slides and blocks of tissues from all vehicle control and high dose animals to examine for proper match and inventory.
(9) Tabulated pathology diagnoses for a random 10% of study animals to verify computer data entry.

Review of the inlife data revealed no discrepancies or problems that would influence the validity of the studies. The number of masses (10 in the rat and 9 in the mouse studies) that were observed clinically but did not correlate with necropsy findings was small. The review of analytical chemistry data revealed no discrepancies. Review of the pathology documents resulted in a change of the disposition code for one rat and eight mice from natural death or moribund kill to accidental death because of gavage trauma. Review of the pathology specimens revealed no discrepancies that would influence interpretation of the study results.

The minor discrepancies identified in this audit were adequately resolved or were considered not to affect the interpretation of these studies of malonaldehyde, sodium salt. Thus, the records and specimens examined in the audit support the data and results presented in the NTP Technical Report.

*U.S. Government Printing Office : 1988 - 241-221/80379

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NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PRINTED AS OF JULY 1988

TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-p-dioxin (Dermal)
206	Dibromochloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane (Inhalation)
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butylbenzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-*p* phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
229	Guar Gum
230	Agar
231	Stannous Chloride
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	D-Mannitol
238	Ziram
239	Bis(2 chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos
250	Benzyl Acetate
251	Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
267	Propylene Oxide
269	Telone II®
271	HC Blue No 1
272	Propylene
273	Trichloroethylene (Four strains of rats)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
281	H.C. Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethy)phosphonium Sulfate and Tetrakis(hydroxymethy)phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C I Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2 methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4 Hexylresorcinol
332	Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
336	Penicillin VK
337	Nitrofurazone
339	2-Amino-4-nitrophenol

These NTP Technical Reports are available for sale from the National Technical Information Service, U S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge (and while supplies last) from the NTP Public Information Office, National Toxicology Program, P O. Box 12233, Research Triangle Park, NC 27709.

Carcinogens Listed in the Eleventh Report

Part A. Known to be Human Carcinogens.

Name or synonym	Page No III-
Aflatoxins	8
Alcoholic Beverage Consumption	10
4-Aminobiphenyl	13
Analgesic Mixtures Containing Phenacetin (See Phenacetin and Analgesic Mixtures Containing Phenacetin)	212
Arsenic Compounds, Inorganic	18
Asbestos	21
Azathioprine	25
Benzene	26
Benzidine (See Benzidine and Dyes Metabolized to Benzidine)	28
Beryllium and Beryllium Compounds	32
1,3-Butadiene	37
1,4-Butanediol Dimethanesulfonate (Myleran®)	39
Cadmium and Cadmium Compounds	42
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Pages 000614 - 000620 have been removed in accordance with copyright laws. Please see appended bibliography list of the references that have been removed from this request.

DETERMINATION OF THE ACUTE ORAL TOXICITY OF PROPANE-1,3-DIOL IN RATS

At the request of : Degussa,
Frankfurt, W. Germany

Test material : A 1 l sample of the test substance was received from the principal on the 1st May, 1979. It was a colourless liquid designated propane-1,3-diol.

Test animals : Young adult albino rats (Wistar-derived) from the Institute's colony were used. The body weights of males varied from 206 to 292 g, those of females from 102 to 139 g.
The rats were housed in groups of five in screen-bottomed, stainless steel cages in a well-ventilated room, maintained at 22 - 24 °C.
Before dosing the rats were fasted overnight.

Experimental design : After some preliminary observations the test substance was given undiluted by gavage to groups of five males and five females in single doses of 9.0, 10.8, 13.0, 15.6 or 18.7 ml per kg body weight. After treatment the rats received stock diet and tap water ad libitum. They were observed for signs of intoxication during a 14 day period, after which autopsies were carried out on the survivors. The LD50 was calculated according to the method of Weil (Biometrics 8 (1952) 249-263).

JFile 12253
DNA# 28410

Results : Within a few hours after dosing the rats showed sluggishness, sedation and ataxia. Unconsciousness was frequently observed and preceeded death. Most of the deaths occurred between 8 and 23 hours after treatment.

DEGUSSA AG - US-IT - NR.
79 0077 DKT

SJU - 08c-5000-373

One female died on the 4th and one male on the 7th post-treatment day.
After 7 days the survivors recovered gradually and looked quite healthy again at the end of the observation period. Macroscopic examination of the survivors did not reveal any treatment-related gross alterations.
The doses applied and the mortality figures are shown in the following table.

Dose (ml/kg)	Mortality number males	Mortality number females	Mortality %
9.0	0/5	0/5	0
10.8	0/5	1/5	10
13.0	2/5	0/5	20
15.6	3/5	2/5	50
18.7	5/5	5/5	100

From the mortality figures the LD50 of propane-1,3-diol was calculated to be 14.9 ml per kg body weight with 13.7 and 16.3 as the 95 % confidence limits. Therefore, the material can be classified as practically non-toxic.

CIVO - TNO
Dept. Biological Toxicology
[redacted]
M.Th. Spanjers Dr. H.P. Til

CIVO-TNO
12-06-79/RV

14.9 x 1.05

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Degussa-US-IT-No.: 94-0059-DGM

IUA-No. 5112

CCR PROJECT 476902

CHROMOSOME ABERRATION ASSAY IN CHINESE HAMSTER V79 CELLS IN VITRO

WITH

1,3-Propanediol

JH 12259
DWH 28416

REPORT

Study Completion Date:

December 13, 1994

DEGUSSA AG - US-IT - NR.
94 0059 DGM

RCC
Group

000633

COPY OF GLP CERTIFICATE

HESSISCHES MINISTERIUM
FÜR UMWELT, ENERGIE
UND BUNDESANGELEGENHEITEN

GLP-Bescheinigung

Bescheinigung

Hiermit wird bestätigt, daß die Prüfungseinrichtung(en)
Cytotest Cell Research GmbH & Co KG
In den Leppsteinswiesen 19
in 6101 Roßdorf
(Ort, Anschrift)
der RCC Holding Verwaltung GmbH
(Firma)
am 03.08., 04.08., 05.08. und 06.08.92
(Datum)
von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist (sind).

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.

Toxikologische Eigenschaften

Im Auftrag

(Dr. Hecker)

Wiesbaden, den 28.09.1992

Certificate

It is hereby certified that the test facility(ies)
Cytotest Cell Research GmbH & Co KG
In den Leppsteinswiesen 19
in 6101 Roßdorf
(location, address)
of RCC Holding Verwaltung GmbH
(company name)
on 03.08., 04.08., 05.08. and 06.08.92
(date)
was (were) inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

Toxicological properties

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PREFACE

GENERAL

Sponsor:	Initiative Umweltrelevante Altstoffe e.V. Kennedyallee 93 60596 Frankfurt
Study Monitor:	Dr. Mayr DEGUSSA AG Industrielle Toxikologie Zweigniederlassung Wolfgang 63403 Hanau-Wolfgang
Testing Facility:	C C R CYTOTEST CELL RESEARCH GMBH & CO. KG D-64380 Roßdorf
CCR Project No.:	476902
Test Article:	1,3-Propanediol
Title:	Chromosome Aberration Assay in Chinese Hamster V79 Cells *in vitro* with 1,3-Propanediol

PROJECT STAFF

Management:	Markus Arenz
Study Director:	Dr. Wolfgang Völkner
Head of Quality Assurance Unit	
until August 14, 1994:	Dr. Christiane Helmrich
since August 15, 1994:	Frauke Hermann

SCHEDULE

Date of Protocol:	July 06, 1994
Dates of Amendments to Protocol:	
1st Amendment:	August 12, 1994
2nd Amendment:	December 10, 1994
Start of Pre-Test:	July 14, 1994
Start of Experiment:	August 17, 1994
End of Experiment:	October 04, 1994
Date of Draft:	November 17, 1994
Date of Report:	December 13, 1994

PROJECT STAFF SIGNATURES

Study Director: Dr. Wolfgang Völkner

Date: December 13, 1994

Management: Markus Arenz

Date: December 13, 1994

QUALITY ASSURANCE

The study was performed in compliance with:

"Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Anlage 1 ("Annex 1"), dated August 01, 1994 (BGBl. I 1994 S. 1703)."

"The OECD Principles of Good Laboratory Practice", Paris 1981.

GUIDELINES

This study was conducted according to the procedures indicated by the following internationally accepted guidelines and recommendations:

First Addendum to the OECD Guideline for Testing of Chemicals, Section 4, No. 473, adopted May 26, 1983, *in vitro* Mammalian Cytogenetic Test"

EEC Directive 92/69, L 383 A, Annex V, B 10, dated December 29, 1992.

ARCHIVING

C C R will archive the following data for 30 years:

raw data, protocol and copy of report.

The following specimen and samples will be archived for at least 12 years:

sample of test article, microscopic slides.

No raw data or material relating to the study will be discarded without the sponsor's prior consent.

STATEMENT OF COMPLIANCE

Project Number: 476902

Test Article: 1,3-Propanediol

Study Director: Dr. Wolfgang Völkner

Title: Chromosome Aberration Assay in Chinese Hamster V79 Cells *in vitro* with 1,3-Propanediol

This study performed in the testing facility of CCR was conducted in compliance with the Good Laboratory Practice Regulations:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Anlage 1 ("Annex 1"), dated August 01, 1994 (BGBl. I S. 1703)."

"The OECD Principles of Good Laboratory Practice", Paris 1981.

There were no circumstances that may have affected the quality or integrity of the study.

Study Director

C C R
Dr. Wolfgang Völkner

Date: December 14 1996

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QUALITY ASSURANCE UNIT

C C R, Cytotest Cell Research GmbH & Co KG,
In den Leppsteinswiesen 19, D-64380 Roßdorf

STATEMENT

Project Number: 476902

Test Article: 1,3-Propanediol

Study Director: Dr. Wolfgang Völkner

Title: Chromosome Aberration Assay in Chinese Hamster V79 Cells *in vitro* with 1,3-Propanediol

This report was audited by the Quality Assurance Unit and the conduct of this study was inspected on the following dates.

Phases and Dates of QAU Inspections / Audits		Dates of Reports to the Study Director and to Management	
Protocol Audit:	July 06, 1994	July	06, 1994
Process Inspection:	August 29, 1994	August	29, 1994
Draft Audit:	November 29, 1994	November	29, 1994

Head of
Quality Assurance Unit Frauke Hermann

Date: December 19, 1994

000640

SUMMARY

The test article 1,3-Propanediol was assessed for its potential to induce structural chromosomal aberrations in V79 cells of the Chinese hamster *in vitro* in two independent experiments.

The chromosomes were prepared 18 h and 28 h (experiment II) after start of treatment with the test article, which was dissolved in culture medium. The treatment interval was 4 h with metabolic activation, 18 h and 28 h without metabolic activation. In each experimental group two parallel cultures were set up. Per culture 100 metaphases were scored for structural chromosomal aberrations.

The following concentrations were evaluated:

Experiment I

without S9 mix:	with S9 mix:
18 h: 250; 1000; 2500 µg/ml	18 h: 500; 2500; 5000 µg/ml

Experiment II

without S9 mix:	with S9 mix:
18 h: 375; 1250; 2500 µg/ml	18 h: 2500; 3750; 5000 µg/ml
28 h: 1250 µg/ml	28 h: 5000 µg/ml

The concentration range of the test article had been determined in a pre-test using the XTT-test and a qualitative evaluation of cell densitiy and morphology as indicator for toxicity response. In the absence as well as in the presence of S9 mix no toxic effects could be observed up to the maximum recommended concentration of 5000 µg/ml.
However, in the cytogenetic experiments in the absence of metabolic activation an evaluation was only possible up to concentrations of 1250 µg/ml (28 h) and 2500 µg/ml, respectively (18 h) due to low cell and metaphase numbers combined with poor metaphase quality after administration of higher concentrations. With S9 mix test groups treated with 5000 µg/ml were scorable in the respective preparation intervals.
Within the evaluated concentration ranges (with and without S9 mix) the mitotic indices were not substantially reduced.

In both independent experiments, there were statistically significant and biologically relevant increases in cells with structural aberrations after treatment with 2500 µg/ml of the test article at fixation intervals 18 h without S9 mix.
In the presence of S9 mix no increases of the aberration frequencies after administration of the test article were observed.

In both experiments, no increase in the frequencies of polyploid metaphases was found after treatment with the test article compared to the frequencies of the controls.

Appropriate reference mutagens were used as positive controls. They induced significant increases in cells with structural chromosomal aberrations.

CONCLUSION

In conclusion, it can be stated that in the study described and under the experimental conditions reported, the test article induced structural chromosomal aberrations as determined by the chromosomal aberration test in the V79 Chinese hamster cell line.

Therefore, 1,3-Propanediol is considered to be mutagenic in this chromosome aberration test.

OBJECTIVE

AIMS OF THE STUDY

This *in vitro* assay was performed to assess the potential of the test article to induce structural chromosomal aberrations by means of two independent chromosomal aberration experiments in the Chinese hamster cell line V79.

REASONS FOR THE STUDY

In vitro methods are valuable when it is desirable to accurately control the concentration and exposure time of cells to the test article under study. However, due to the limited capacity for metabolic activation of potential mutagens an exogenous metabolic activation system is necessary.
This *in vitro* test is an assay for the detection of structural chromosomal aberrations. These aberrations are frequently lethal to the damaged cells. However, cytogenetic damage in somatic cells is an indicator of a potential to induce more subtle chromosomal damage that is compatible with cell division. Similar damage induced in germinal cells may lead to heritable cytogenetic abnormalities. Heritable cytogenetic abnormalities are known to have deleterious effects in man, e.g. induction of neoplastic events or birth defects. Also, chromosome abnormalities in somatic cells may become one of the reasons, why a transformed cell population results possibly leading to neoplastic processes.

Chromosomal aberrations are generally evaluated in first post treatment mitoses. With the majority of chemical mutagens, induced aberrations are of the chromatid type, but chromosome type aberrations also occur.

For treatment, cells should be in exponential growth to ensure that there are cells in all stages of the cell cycle (i.e. an asynchronous population). Since the normal cell cycle time is 12 h (see page 14) and the guidelines require fixation times of about 1.5-fold of the normal cell cycle, a fixation time of around 18 hours is appropriate. Because there may be chemicals which induce very extensive mitotic delay at clastogenic concentrations or may be clastogenic only when cells have passed through more than one cell cycle since the beginning of treatment in the second experiment an additional later sampling time (28 h) should be included.
At fixation time 18 h, a minimum of three doses of the test article should be used. The highest dose should cause a significant reduction in mitotic index (by 50 % - 80 %), the lowest dose should be on the borderline of toxicity, and the intermediate dose should give some degree of mitotic inhibition. In the additional delayed fixation time (28 h) in the second experiment the same dose, which induced a suitable degree of mitotic inhibition at the earlier fixation time, should be chosen.
The frequency of polyploid and endoreduplicated cells should also be noted so that the level in control cultures can be monitored and their induction by the test agent can be recorded especially when a late sampling time is used.
To validate the test, reference mutagens are tested in parallel to the test article.

MATERIALS AND METHODS

THE TEST ARTICLE

The test article and the information concerning the test article were provided by the sponsor.

Name:	1,3-Propanediol
Batch No.:	EK 33/92-1
Aggregate State at RT:	liquid
Colour:	colorless
Purity:	99.8 % /GC, area % H_2O 0.1 % / Karl Fischer titration
Analysis:	4783/94 dated July 12, 1994
Molecular Weight:	not indicated by the sponsor
Stability:	Pure: stable for years In solvent: stable in H_2O
Storage:	room temperature
Expiration Date:	July 01, 1996

On the day of the experiment (immediately before treatment), the test article was dissolved in culture medium. The solvent was chosen according to its solubility properties and its non-toxicity.

THE CONTROLS

The Negative Controls

Concurrent solvent controls (culture medium) were performed.

The Positive Control Substances

Without metabolic activation

Name:	EMS; Ethylmethanesulfonate
Supplier:	Merck-Schuchardt, D-85662 Hohenbrunn
Catalogue no.:	820774 (purity > 98 %)
Dissolved in:	nutrient medium
Final Concentration:	600 µg/ml = 4.8 mM

Solution prepared on day of experiment.

The stability of the positive control substance in solution was proven by the mutagenic response in the expected range.

With metabolic activation

Name:	CPA; Cyclophosphamide
Supplier:	SERVA, D-69042 Heidelberg
Catalogue no.:	17681 (purity ≥ 99.5 %)
Dissolved in:	nutrient medium
Final Concentration:	0.47 µg/ml = 1.7 µM

The stability of CPA at room temperature is good. At 25 °C only 3.5 % of its potency is lost after 24 hours (9).

THE TEST SYSTEM

Reasons for the Choice of the Cell Line V79

The V79 cell line has been used successfully for many years in *in vitro* experiments. Especially the high proliferation rate (doubling time of clone V79/T5 in stock cultures: 12 h, determined on March 17, 1994) and a high plating efficiency of untreated cells (as a rule more than 50 %) both necessary for the appropriate performance of the study, recommend the use of this cell line. The cells have a stable karyotype with a modal chromosome number of 22.

Lacking metabolic activities of cells under *in vitro* conditions are a disadvantage of assays with cell cultures as many chemicals only develop mutagenic potential when they are metabolized by the mammalian organism. However, metabolic activation of chemicals can be achieved at least partially by supplementing the cell cultures with liver microsome preparations (S9 mix).

Cell Cultures

Large stocks of the V79 cell line (supplied by LMP, Technical University Darmstadt, D-64287 Darmstadt) were stored in liquid nitrogen in the cell bank of C C R allowing the repeated use of the same cell culture batch in experiments. Before freezing, each batch was screened for mycoplasma contamination and checked for karyotype stability. Consequently, the parameters of the experiments remained similar because of the reproducible characteristics of the cells.

Thawed stock cultures were propagated at 37 °C in 80 cm^2 plastic flasks (GREINER, D-72632 Frickenhausen). About 5 x 10^5 cells per flask were seeded in 15 ml of MEM (minimal essential medium; SEROMED; D-12247 Berlin) supplemented with 10 % fetal calf serum (FCS; Boehringer Mannheim, D-68261 Mannheim). The cells were subcultured twice weekly. The cell cultures were incubated at 37 °C in an atmosphere with 4.5 % carbon dioxide (95.5 % air).

MAMMALIAN MICROSOMAL FRACTION S9 MIX

S9 (Preparation by C C R)

The S9 liver microsomal fraction was obtained from the livers of 8 - 12 weeks old male rats strain Wistar HanIbm* (BRL, CH-4414 Füllinsdorf; weight approx. 220 - 320 g) which received a single i.p. injection of 500 mg/kg b.w. Aroclor 1254 (Antechnika, D-76275 Ettlingen) in olive oil 5 days previously.

After cervical dislocation the livers of the animals were removed, washed in 150 mM KCl and homogenized. The homogenate, diluted 1+3 with KCl was centrifuged twice at 9.000 g for 10 minutes (4 °C). A stock of the supernatant containing the microsomes was frozen in ampoules of 2 or 3 ml and stored at -70 °C. Small numbers of the ampoules were kept at -20 °C for only several weeks before use. The protein content was determined using the analysis kit of Bio-Rad Laboratories, D-80939 München: Bio-Rad protein assay, Catalogue No. 500 000 6.

The protein concentration in the S9 preparation is usually between 20 and 45 mg/ml. In both experiments the protein concentration was 29.0 mg/ml (Lot. No.: 060694).

S9 Mix

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/ml in the cultures. Cofactors were added to the S9 mix to reach the following concentrations:

8 mM $MgCl_2$
33 mM KCl
5 mM glucose-6-phosphate
4 mM NADP
in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames et al. (1).

PRE-TEST FOR TOXICITY

A pre-test was performed in order to determine the toxicity of the test article. The general culturing and experimental conditions in this pre-test were the same as described below for the mutagenicity experiment.

The following two methods were used:

Qualitative assessment:

In a qualitative assessment, high density cultures (approx. 200000 cells/slide) were treated with the test article for simulating the conditions of the main experiment. A qualitative evaluation of cell number and cell morphology was made 4 h and 18 - 20 h after start of treatment.

XTT-Assay:

The XTT-assay is based on the cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye by hydrogenase activity in active mitochondria. 18 - 20 h after treatment with the test article the XTT-assay was initiated by adding a mixture of XTT-labeling reagent with an electron coupling reagent (PMS). After 4 h of incubation the absorption was read at 450 nm (690 nm reference) using an ELISA reader (SLT, Labinstruments Austria, A-5082 Grödig). The viabilities of the cells were calculated as percentages of the solvent controls and reported tabularly.

DOSE SELECTION

According to the results from this pre-test 6 concentrations (18 h interval) were chosen to be applied in the chromosomal aberration assay.

The highest concentration used in the pre-test was (5000 µg/ml). As no toxic effects were observed up to this concentration the cytogenetic experiments were performed with the following concentrations:

Experiment I

with and without S9 mix:
18 h: 100; 250; 500; 1000; 2500; 5000 µg/ml

Experiment II

with and without S9 mix:
18 h: 375; 750; 1250; 2500; 3750; 5000 µg/ml
28 h: 1250; 2500; 3750; 5000 µg/ml

The treatment interval was 4 h with metabolic activation, 18 h and 28 h without metabolic activation. Per concentration duplicate cultures were used.

Toxic effects (low number of remaining/surviving cells, low metaphase number, partially combined with poor metaphase quality) were determined qualitatively for all test groups in both experiments.

In cytogenetic experiment I, in the absence of S9 mix cultures after treatment with 2500 µg/ml as highest concentration were evaluated for cytogenetic damage; in experiment II, 2500 µg/ml (18 h) and 1250 µg/ml as highest concentrations were used.

In the presence of S9 mix, in both experiments cultures treated with 5000 µg/ml (18 and 28 h [exp. II]) as top concentrations could be evaluated.

The cytogenetic evaluation of higher concentrations in the respective intervals without S9 mix was impossible due to toxic effects (reduced cell density and low metaphase number, partially combined with poor metaphase quality).

EXPERIMENTAL PERFORMANCE

Seeding of the Cultures

Exponentially growing stock cultures more than 50 % confluent were trypsinized at 37 °C for approximately 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium and a single cell suspension was prepared. The trypsin concentration was 0.2 % in Ca-Mg-free salt solution (Trypsin: Difco Laboratories, Detroit, USA).

The Ca-Mg-free salt solution was composed as follows (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose	1000 mg
$NaHCO_3$	350 mg

Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/l EDTA (Ethylene diamine tetraacetic acid).

The cells were seeded into Quadriperm dishes (Heraeus, D-63450 Hanau) which contained microscopic slides (at least 2 chambers per dish and test group). In each chamber 1×10^4 - 6×10^4 cells were seeded with regard to preparation time. The medium was MEM + 10 % FCS (complete medium).

Treatment

Exposure time 4 hours (with S9 mix):

In both independent experiments, after 48 h (28 h preparation interval) and 55 h (18 h preparation interval) the culture medium was replaced with serum-free medium containing different concentrations of the test article and 50 µl/ml S9 mix.

After 4 h the cultures were washed twice with "Saline G" and then the cells were cultured in complete medium for the remaining culture time.

The "Saline G" solution is composed as follows (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose	1100 mg
$Na_2HPO_4.7H_2O$	290 mg
KH_2PO_4	150 mg
pH is adjusted to 7.2	

Exposure time 18 and 28 hours (without S9 mix):

In both independent experiments, after 48 h (28 h preparation interval) and 55 h (18 h preparation interval) the culture medium was replaced with complete medium (10 % FCS) containing different concentrations of the test article without S9 mix. This medium was not changed until preparation of the cells.

All cultures were incubated at 37 °C in a humidified atmosphere with 4.5 % CO_2 (95.5 % air).

Preparation of the Cultures

15.5 and 25.5 h after the start of the treatment colcemid was added (0.2 µg/ml culture medium) to the cultures. 2.5 h later, the cells were treated on the slides in the chambers with hypotonic solution (0.4 % KCl) for 20 min at 37 °C. After incubation in the hypotonic solution the cells were fixed with 3 + 1 methanol + glacial acetic acid. Per experiment both slides per group were prepared. After fixation the cells were stained with Giemsa (E. Merck, D-64293 Darmstadt).

Analysis of Metaphase Cells

Evaluation of the cultures was performed (according to standard protocol of the "Arbeitsgruppe der Industrie, Cytogenetik" (4)) using NIKON microscopes with 100x oil immersion objectives. Breaks, fragments, deletions, exchanges and chromosomal disintegrations were recorded as structural chromosomal aberrations. Gaps were recorded as well but not included in the calculation of the aberration rates. At least 100 well spread metaphases per culture were scored for cytogenetic damage on coded slides. Only metaphases with characteristic chromosome numbers of 22 ± 1 were included in the analysis. To describe a cytotoxic effect the mitotic index (% cells in mitosis) was determined. In addition, the number of polyploid cells was scored (% polyploid metaphases; in the case of this aneuploid cell line polyploid means a near tetraploid karyotype).

DATA RECORDING

The data generated were recorded in the raw data file. The results are presented in tabular form, including experimental groups with the test article, negative and positive controls.

ACCEPTABILITY OF THE ASSAY

The chromosomal aberration assay is considered acceptable if it meets the following criteria:

a) The number of aberrations found in the negative and/or solvent controls falls within the range of historical laboratory control data: 0.00 % - 4.00 %.

b) The positive control substances should produce significant increases of the number of cells with structural chromosomal aberrations.

EVALUATION OF RESULTS

A test article is classified as mutagenic if it induces reproducibly either a concentration-related increase in the number of structural chromosomal aberrations or a significant and reproducible positive response for at least one of the test points.

A test article producing reproducibly neither a concentration-related increase in the number of structural chromosomal aberrations nor a significant and reproducibly positive response at anyone of the test points is considered non-mutagenic in this system.

This can be confirmed by means of the chi-square test.

However, both biological and statistical significance should be considered together.

BIOMETRY

Statistical significance at the five per cent level ($p < 0.05$) was evaluated by means of the chi-square test. Evaluation was performed only for cells carrying aberrations exclusive gaps.

Experiment I

Solvent control versus		fixation interval	S9 mix	p-value
Test group	250 µg/ml	18 h	-	n.t.
"	1000 µg/ml	18 h	-	n.t.
"	2500 µg/ml	18 h	-	0.01 > p > 0.001*
"	500 µg/ml	18 h	+	n.t.
"	2500 µg/ml	18 h	+	0.9 > p > 0.1
"	5000 µg/ml	18 h	+	0.9 > p > 0.1
Negative control versus Positive controls				
EMS	600 µg/ml	18 h	-	0.001 > p > 0*
CPA	0.47 µg/ml	18 h	+	0.001 > p > 0*

Experiment II

Solvent control versus		fixation interval	S9 mix	p-value
Test group	375 µg/ml	18 h	-	n.t.
"	1250 µg/ml	18 h	-	n.t.
"	2500 µg/ml	18 h	-	0.001 > p > 0*
"	2500 µg/ml	18 h	+	0.9 > p > 0.1
"	3750 µg/ml	18 h	+	0.9 > p > 0.1
"	5000 µg/ml	18 h	+	n.t.
"	1250 µg/ml	28 h	-	n.t.
"	5000 µg/ml	28 h	+	0.9 > p > 0.1
Negative control versus Positive controls				
EMS	600 µg/ml	18 h	-	0.001 > p > 0*
CPA	0.47 µg/ml	18 h	+	0.001 > p > 0*

n.t. = not tested as the aberration rate is equal or lower than the control rate

* = aberration rate is statistically significantly higher than the control rate

RESULTS

TABLES OF RESULTS

Table &tbl&: Pre-test for toxicity

In the pre-test the toxicity of the test article was examined using the XTT-assay which is based on the cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye by hydrogenase activity in active mitochondria.
Cytotoxicity of 1,3-Propanediol to cultures of Chinese hamster cell line V79.

concentration per ml	S9 mix	XTT-absorption (OD 450/690 nm) (mean±SD)	percentage of viable cells* relative to the solvent control
blank		0.168 ± 0.007	
negative control	-	1.351 ± 0.104	97.1
solvent control			
culture medium	-	1.386 ± 0.081	100.0
10.0 µg	-	1.306 ± 0.120	93.4
30.0 µg	-	1.412 ± 0.089	102.1
100.0 µg	-	1.500 ± 0.095	109.4
300.0 µg	-	1.501 ± 0.104	109.4
600.0 µg	-	1.470 ± 0.079	106.9
1000.0 µg	-	1.581 ± 0.097	116.0
2500.0 µg	-	1.571 ± 0.122	115.2
5000.0 µg	-	1.589 ± 0.096	116.7
blank		0.162 ± 0.012	
negative control	+	1.391 ± 0.087	101.6
solvent control	+		
culture medium	+	1.372 ± 0.083	100.0
10.0 µg	+	1.494 ± 0.081	110.1
30.0 µg	+	1.505 ± 0.102	111.0
100.0 µg	+	1.543 ± 0.145	114.1
300.0 µg	+	1.513 ± 0.130	111.7
600.0 µg	+	1.576 ± 0.147	116.9
1000.0 µg	+	1.621 ± 0.224	120.6
2500.0 µg	+	1.551 ± 0.118	114.8
5000.0 µg	+	1.489 ± 0.173	109.7

* corrected for blank

ABBREVIATIONS

The following abbreviations are used in the tables with structural chromosomal aberrations:
g = gap; ig = iso-gap; gaps are achromatic lesions of chromatid or chromosome type where no dislocation of chromosomal material is visible (independent of the size of the achromatic region).
b = break; ib = iso-break; f = fragment; if = iso-fragment; d = deletion; id = iso-deletion; ma = multiple aberration (= more than 4 events in one cell [excluding gaps]; only exchanges are recorded additionally in these cells); ex = chromatid type exchange; cx = chromosome type exchange; cd = chromosomal disintegration (= pulverization)

Experiment I

Table 2: Number of Polyploid Cells and Mitotic Index
experiment I; fixation interval 18 h; without and with metabolic activation

	conc. per ml	S9 mix	fixation interval	polyploid cells* culture 1	culture 2	total	mean	mitotic index (%)** culture 1	culture 2	abs.	rel.***
Solvent control MEDIUM		-	18 h	2	4	6	**3.0**	14.2	16.4	15.3	**100.0**
Positive control EMS	600.0 µg	-	18 h	2	1	3	**1.5**	14.3	14.7	14.5	**94.8**
Test article	250.0 µg	-	18 h	3	1	4	**2.0**	15.3	11.4	13.4	**87.3**
"	1000.0 µg	-	18 h	3	3	6	**3.0**	13.6	11.2	12.4	**81.0**
"	2500.0 µg	-	18 h	4	3	7	**3.5**	13.9	9.0	11.5	**74.8**
Solvent control MEDIUM		+	18 h	4	2	6	**3.0**	14.1	14.5	14.3	**100.0**
Positive control CPA	0.47 µg	+	18 h	3	1	4	**2.0**	11.3	11.0	11.2	**78.0**
Test article	500.0 µg	+	18 h	2	4	6	**3.0**	16.4	14.4	15.4	**107.7**
"	2500.0 µg	+	18 h	0	3	3	**1.5**	16.4	14.3	15.4	**107.3**
"	5000.0 µg	+	18 h	4	1	5	**2.5**	17.3	17.6	17.5	**122.0**

* The number of polyploid cells was determined in 100 cells per culture of each test group.
** The mitotic index was determined in 1000 cells per culture of each test group.
*** For the positive control groups, the relative values of the mitotic index are related to the negative controls; for the test article treatment groups the values are related to the solvent controls

Table 3: Structural chromosomal aberrations
experiment I; fixation interval 18 h; without metabolic activation

	conc. per ml	S9 mix		cells scored	mean % aberrant cells incl. gaps	excl. gaps	exchanges	types of aberrations found -gaps- g	ig	-chromatid type- b	f	d	ex	-chromosome type- ib	if	id	cx	-other- ma	cd
Solvent control MEDIUM		-	cult. 1	100				1	0	1	0	0	0	0	0	0	0	0	0
			cult. 2	100				1	0	1	0	0	0	0	0	0	0	0	0
			-total-	200	1.5	1.0	0.0	2	0	2	0	0	0	0	0	0	0	0	0
Positive control EMS	600.0 µg	-	cult. 1	100				5	0	4	3	0	18	3	2	0	0	2	0
			cult. 2	100				18	0	1	3	0	9	1	0	0	0	0	0
			-total-	200	25.0	17.0	10.5	23	0	5	6	0	27	4	2	0	0	2	0
Test article	250.0 µg	-	cult. 1	100				0	0	0	0	0	0	0	0	0	0	0	0
			cult. 2	100				1	0	1	0	0	0	0	1	0	0	0	0
			-total-	200	1.5	1.0	0.0	1	0	1	0	0	0	0	1	0	0	0	0
"	1000.0 µg	-	cult. 1	100				1	0	1	1	0	0	0	0	0	0	0	0
			cult. 2	100				0	0	0	0	0	1	0	0	0	0	0	0
			-total-	200	1.5	1.0	0.5	1	0	1	1	0	1	0	0	0	0	0	0
"	2500.0 µg	-	cult. 1	100				6	0	5	2	1	3	0	0	0	0	0	0
			cult. 2	100				3	1	2	7	0	0	1	0	0	0	0	0
			-total-	200	9.5	7.0	1.0	9	1	7	9	1	3	1	0	0	0	0	0

For abbreviations see RESULTS/ABBREVIATIONS

Table 4: Structural chromosomal aberrations
experiment I; fixation interval 18 h; with metabolic activation

	conc. per ml	S9 mix		cells scored	mean % aberrant cells incl. gaps	excl. gaps	exchanges	types of aberrations found -gaps- g	ig	-chromatid type- b	f	d	ex	-chromosome type- ib	if	id	cx	-other- ma	cd
Solvent control MEDIUM		+	cult. 1	100				1	0	0	1	0	0	0	0	0	0	0	0
			cult. 2	100				0	1	0	0	0	0	0	0	0	0	0	0
			-total-	200	1.5	0.5	0.0	1	1	0	1	0	0	0	0	0	0	0	0
Positive control CPA	0.47 µg	+	cult. 1	100				1	0	4	2	0	19	2	0	0	0	0	0
			cult. 2	100				3	0	3	3	0	6	0	0	0	0	0	0
			-total-	200	15.5	14.0	10.0	4	0	7	5	0	25	2	0	0	0	0	0
Test article	500.0 µg	+	cult. 1	100				1	0	0	0	0	0	0	0	0	0	0	0
			cult. 2	100				0	0	0	0	0	0	0	0	0	0	0	0
			-total-	200	0.5	0.0	0.0	1	0	0	0	0	0	0	0	0	0	0	0
"	2500.0 µg	+	cult. 1	100				1	0	1	1	0	0	0	0	0	0	0	0
			cult. 2	100				3	0	0	0	0	0	0	0	0	0	0	0
			-total-	200	3.0	1.0	0.0	4	0	1	1	0	0	0	0	0	0	0	0
"	5000.0 µg	+	cult. 1	100				0	0	0	0	0	0	0	0	0	0	0	0
			cult. 2	100				1	0	3	0	0	0	0	0	0	0	0	0
			-total-	200	1.5	1.0	0.0	1	0	3	0	0	0	0	0	0	0	0	0

For abbreviations see RESULTS/ABBREVIATIONS

Experiment II

Table 5: Number of Polyploid Cells and Mitotic Index
experiment II; fixation intervals 18 h and 28 h; without and with metabolic activation

	conc. per ml	S9 mix	fixation interval	polyploid cells* culture 1	culture 2	total	mean	mitotic index (%)** culture 1	culture 2	abs.	rel.***
Solvent control MEDIUM		-	18 h	3	2	5	**2.5**	10.7	15.2	13.0	**100.0**
Positive control EMS	600.0 µg	-	18 h	2	3	5	**2.5**	8.5	8.4	8.5	**65.3**
Test article	375.0 µg	-	18 h	2	0	2	**1.0**	13.3	14.4	13.9	**106.9**
"	1250.0 µg	-	18 h	3	1	4	**2.0**	10.1	15.5	12.8	**98.8**
"	2500.0 µg	-	18 h	1	3	4	**2.0**	13.1	13.6	13.4	**103.1**
Solvent control MEDIUM		+	18 h	1	3	4	**2.0**	16.6	16.8	16.7	**100.0**
Positive control CPA	0.47 µg	+	18 h	3	2	5	**2.5**	8.3	10.4	9.4	**56.0**
Test article	2500.0 µg	+	18 h	0	2	2	**1.0**	15.1	13.8	14.5	**86.5**
"	3750.0 µg	+	18 h	2	1	3	**1.5**	16.9	18.1	17.5	**104.8**
"	5000.0 µg	+	18 h	2	2	4	**2.0**	14.5	15.6	15.1	**90.1**
Solvent control MEDIUM		-	28 h	0	3	3	**1.5**	11.0	13.1	12.1	**100.0**
Test article	1250.0 µg	-	28 h	4	3	7	**3.5**	11.1	13.8	12.5	**103.3**
Solvent control MEDIUM		+	28 h	3	1	4	**2.0**	10.7	14.8	12.8	**100.0**
Test article	5000.0 µg	+	28 h	2	2	4	**2.0**	12.2	17.8	15.0	**117.6**

* The number of polyploid cells was determined in 100 cells per culture of each test group.
** The mitotic index was determined in 1000 cells per culture of each test group.
*** For the positive control groups, the relative values of the mitotic index are related to the negative controls; for the test article treatment groups the values are related to the solvent controls

Table 6: Structural chromosomal aberrations
experiment II; fixation interval 18 h; without metabolic activation

	conc. per ml	S9 mix	cells scored	mean % aberrant cells incl. gaps	excl. gaps	exchanges	types of aberrations found -gaps- g	ig	-chromatid type- b	f	d	ex	-chromosome type- ib	if	id	cx	-other- ma	cd
Solvent control MEDIUM		-	cult. 1 100				1	0	1	0	1	0	0	0	0	0	0	0
			cult. 2 100				0	0	0	3	0	0	1	0	0	0	0	0
			-total- 200	3.5	3.0	0.0	1	0	1	3	1	0	1	0	0	0	0	0
Positive control EMS	600.0 µg	-	cult. 1 100				3	0	6	3	0	4	2	0	0	0	0	0
			cult. 2 100				10	0	8	7	0	4	4	1	0	0	0	0
			-total- 200	19.5	15.0	3.5	13	0	14	10	0	8	6	1	0	0	0	0
Test article	375.0 µg	-	cult. 1 100				4	0	0	0	0	0	0	0	0	0	0	0
			cult. 2 100				2	0	1	1	0	0	0	0	0	0	0	0
			-total- 200	4.0	1.0	0.0	6	0	1	1	0	0	0	0	0	0	0	0
"	1250.0 µg	-	cult. 1 100				3	0	1	0	0	0	0	0	0	0	0	0
			cult. 2 100				3	0	1	0	0	0	0	0	0	0	0	0
			-total- 200	3.0	1.0	0.0	6	0	2	0	0	0	0	0	0	0	0	0
"	2500.0 µg	-	cult. 1 100				4	0	7	5	1	4	1	0	0	0	3	0
			cult. 2 100				1	0	7	2	0	3	1	1	0	0	0	0
			-total- 200	15.0	13.0	3.0	5	0	14	7	1	7	2	1	0	0	3	0

For abbreviations see RESULTS/ABBREVIATIONS

Table 7: Structural chromosomal aberrations
experiment II; fixation interval 18 h; with metabolic activation

	conc. per ml	S9 mix		cells scored	mean % aberrant cells incl. gaps	excl. gaps	exchanges	types of aberrations found -gaps- g	ig	-chromatid type- b	f	d	ex	-chromosome type- ib	if	id	cx	-other- ma	cd
Solvent control MEDIUM		+	cult. 1	100				1	0	1	1	0	2	0	0	0	0	0	0
			cult. 2	100				1	0	1	0	0	0	0	0	0	0	0	0
			-total-	200	3.0	2.0	0.5	2	0	2	1	0	2	0	0	0	0	0	0
Positive control CPA	0.47 µg	+	cult. 1	100				4	1	11	9	2	4	5	1	0	0	0	0
			cult. 2	100				2	1	4	4	0	12	3	0	0	0	1	0
			-total-	200	22.5	21.0	8.0	6	2	15	13	2	16	8	1	0	0	1	0
Test article	2500.0 µg	+	cult. 1	100				2	0	0	1	1	0	1	0	0	0	0	0
			cult. 2	100				2	0	3	1	0	0	0	0	0	0	0	0
			-total-	200	5.5	3.5	0.0	4	0	3	2	1	0	1	0	0	0	0	0
"	3750.0 µg	+	cult. 1	100				1	0	2	2	0	0	0	0	0	0	0	0
			cult. 2	100				0	0	0	0	0	1	0	0	0	0	0	0
			-total-	200	3.0	2.5	0.5	1	0	2	2	0	1	0	0	0	0	0	0
"	5000.0 µg	+	cult. 1	100				2	0	1	1	0	0	0	0	0	0	0	0
			cult. 2	100				0	1	0	0	0	0	0	0	0	0	0	0
			-total-	200	2.5	1.0	0.0	2	1	1	1	0	0	0	0	0	0	0	0

For abbreviations see RESULTS/ABBREVIATIONS

Table 8: Structural chromosomal aberrations
experiment II; fixation interval 28 h; without and with metabolic activation

	conc. per ml	S9 mix		cells scored	mean % aberrant cells incl. gaps	excl. gaps	exchanges	types of aberrations found -gaps- g	ig	-chromatid type- b	f	d	ex	-chromosome type- ib	if	id	cx	-other- ma	cd
Solvent control MEDIUM		-	cult. 1	100				0	0	1	0	0	0	0	0	0	0	0	0
			cult. 2	100				0	0	2	1	0	0	0	0	0	0	0	0
			-total-	200	2.0	2.0	0.0	0	0	3	1	0	0	0	0	0	0	0	0
Test article	1250.0 µg	-	cult. 1	100				4	0	0	0	0	0	0	1	0	1	0	0
			cult. 2	100				4	0	0	0	0	0	2	0	0	0	0	0
			-total-	200	4.5	1.5	0.5	8	0	0	0	0	0	2	1	0	1	0	0
Solvent control MEDIUM		+	cult. 1	100				0	0	0	0	0	0	0	0	0	0	0	0
			cult. 2	100				0	0	3	0	0	0	0	0	0	0	0	0
			-total-	200	1.5	1.5	0.0	0	0	3	0	0	0	0	0	0	0	0	0
Test article	5000.0 µg	+	cult. 1	100				0	0	1	1	0	0	0	1	0	0	0	0
			cult. 2	100				1	0	1	0	0	0	0	0	0	0	0	0
			-total-	200	2.5	2.0	0.0	1	0	2	1	0	0	0	1	0	0	0	0

For abbreviations see RESULTS/ABBREVIATIONS

DISCUSSION

The test article 1,3-Propanediol was assessed for its potential to induce structural chromosomal aberrations in V79 cells of the Chinese hamster *in vitro* in the absence and presence of metabolic activation by S9 mix. Two independent experiments were performed.

The chromosomes were prepared 18 h and 28 h (experiment II only) after start of treatment with the test article, which was dissolved in culture medium. The treatment interval was 4 h with metabolic activation, 18 h and 28 h without metabolic activation.

In each experimental group two parallel cultures were set up. Per culture 100 metaphases were scored for structural chromosomal aberrations.

The following concentrations were evaluated:

Experiment I

without S9 mix:	with S9 mix:
18 h: 250; 1000; 2500 µg/ml	18 h: 500; 2500; 5000 µg/ml

Experiment II

without S9 mix:	with S9 mix:
18 h: 375; 1250; 2500 µg/ml	18 h: 2500; 3750; 5000 µg/ml
28 h: 1250 µg/ml	28 h: 5000 µg/ml

In the pre-test on toxicity (XTT-test) in the absence and presence of S9 mix after treatment with concentrations up to 5000 µg/ml no cytoxicity was observed.

In the absence as well as in the presence of S9 mix, in both experiments the mitotic indices were not substantially reduced after treatment with the test article.

However, due to low number of cells and metaphases and poor quality of metaphases in the experiments without metabolic activation only concentrations up to 2500 µg/ml (18 h) and 1250 µg/ml (28 h, exp. II) leaded to scorable preparations.

In both experiments, in the presence of S9 mix the test article did not increase the frequency of cells with aberrations. The aberration rates of the cells after treatment with the test article (exp.I: 0.0 % - 1.0 % [18 h]; exp. II: 1.0 % - 3.5 %, [18 h]; 2.0 % [28 h]) were near to the range of the solvent control values (exp. I: 0.5 %; exp. II: 2.0 % [18 h] 1.5 % [28 h]) and in the range of our historical control data: 0.0 % -

4.0 %. In both experiments, in the absence of S9 mix the statistical evaluation revealed a significant difference between the treatment group (2500 µg/ml [7.0 % and 13.0 % aberrant cells]) versus the corresponding controls (1.0 % and 3.0 %aberrant cells).
Taking into account our historical negative control data range the statistical significance is regarded as being biologically relevant. The cells carrying exchanges observed in exp. II (3.0 %) at a concentration of 2500 µg/ml give additional evidence for a clastogenic potential of the test article.

Tables 2 and 5 show the occurrence of polyploid metaphases. In both experiments, no increase in the rate of polyploid metaphases (exp. I: 1.5 % - 3.5 %; exp. II: 1.0 % - 3.5 %) as compared to the rates of the controls (exp. I: 3.0 %; exp. II: 1.5 % - 2.5 %) were found after treatment with the test article.

In both experiments, EMS (600 µg/ml) and CPA (0.47 µg/ml) were used as positive controls. They showed significant increases in cells with structural chromosomal aberrations.

In conclusion, it can be stated that in the study described and under the experimental conditions reported, the test article 1,3-Propanediol induced reproducibly structural chromosomal aberrations in the V79 Chinese hamster cell line.

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SALMONELLA TYPHIMURIUM

REVERSE MUTATION ASSAY

WITH

1,3-Propanediol

J 12258
DN# 2846

REPORT

Study Completion Date:

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GLP-Bescheinigung

Bescheinigung

Hiermit wird bestätigt, daß die Prüfungseinrichtung(en)
Cytotest Cell Research GmbH & Co KG
In den Leppsteinswiesen 19
in 6101 Roßdorf
(Ort, Anschrift)
der RCC Holding Verwaltung GmbH
(Firma)
am 03.08., 04.08., 05.08. und 06.08.92
(Datum)
von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist (sind).

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.

Toxikologische Eigenschaften

Certificate

It is hereby certified that the test facility(ies)
Cytotest Cell Research GmbH & Co KG
In den Leppsteinswiesen 19
in 6101 Roßdorf
(location, address)
of RCC Holding Verwaltung GmbH
(company name)
on 03.08., 04.08., 05.08. and 06.08.92
(date)
was (were) inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

Toxicological properties

Im Auftrag

[redacted]

(Dr. Hecker)

Wiesbaden, den 28.09.1992

HESSISCHES MINISTERIUM FÜR UMWELT, ENERGIE UND BUNDESANGELEGENHEITEN

000666

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PREFACE

GENERAL

Sponsor:	Initiative Umweltrelevante Altstoffe e.V. Kennedyallee 93 D-60596 Frankfurt; Germany
Monitor:	Dr. Mayr DEGUSSA AG Industrielle Toxikologie Zweigniederlassung Wolfgang 63403 Hanau-Wolfgang
Testing Facility:	C C R CYTOTEST CELL RESEARCH GMBH & CO. KG D-64380 Roßdorf, F.R.G.
CCR Project No.:	476901
Test Article:	1,3-Propanediol
Title:	Salmonella typhimurium Reverse Mutation Assay with 1,3-Propanediol

PROJECT STAFF

Management:	Markus Arenz
Study Director:	Eric Wollny
Quality Assurance Unit:	Frauke Hermann

SCHEDULE

Date of Protocol:	July	06, 1994
Date of Amendments to Protocol:	August	12, 1994 (1st)
	December	10, 1994 (2nd)
Start of Pre-Experiment:	August	17, 1994
End of Pre-Experiment:	August	19, 1994
Start of Experiment I:	August	17, 1994
End of Experiment I:	August	29, 1994
Start of Experiment II:	August	30, 1994
End of Experiment II:	September	01, 1994
Date of Draft:	November	28, 1994
Date of Report:	December	13, 1994

PROJECT STAFF SIGNATURES

Study Director: Dr. Eric Wollny

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Date: December 13, 1994

Management: Markus Arenz

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Date: December 13, 1994

QUALITY ASSURANCE

The study was performed in compliance with:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Anlage 1 ("Annex 1"), dated March 14, 1990 (BGBL. I S. 521).

"OECD Principles of Good Laboratory Practice", Paris, 1981

GUIDELINES

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

First Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 471, "Salmonella typhimurium, Reverse Mutation Assay", adopted May 26, 1983 and

EEC Directive 92/69, L 383 A, Annex V, B 14, dated December 29, 1992.

ARCHIVING

C C R, D-64380 Roßdorf/F.R.G. will archive the following data for 30 years: Raw data, protocol, and copy of report.

The following sample will be archived for at least 12 years:

Sample of test article.

No raw data or material relating to the study will be discarded without the sponsor's prior consent.

STATEMENT OF COMPLIANCE

Project Number: 476901

Test Article : 1,3-Propanediol

Study Director: Dr. Eric Wollny

Title : Salmonella typhimurium
Reverse Mutation Assay
with 1,3-Propanediol

This study performed in the testing facilities of CCR was conducted in compliance with Good Laboratory Practice Regulations:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Anlage 1 ("Annex 1"), dated March 14, 1990

OECD Principles of Good Laboratory Practice, Paris, 1981

There were no circumstances that may have affected the quality or integrity of the study.

Study Director

C C R
Dr. Eric Wollny

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Date: December 16, 1994

QUALITY ASSURANCE UNIT

C C R, Cytotest Cell Research GmbH & Co. KG,
In den Leppsteinswiesen 19, D-64380 Roßdorf, F.R.G.

STATEMENT

Project Number: 476901

Test Article : 1,3-Propanediol

Study Director: Dr. Eric Wollny

Title : Salmonella typhimurium Reverse Mutation Assay with 1,3-Propanediol

This report was audited by the Quality Assurance Unit and the conduct of this study was inspected on the following dates:

Phases and Dates of QAU Inspections/Audits		Dates of Reports to the Study Director and to Management	
Protocol Audit:	July 12, 1994	July	12, 1994
Study Inspection:	August 17, 1994	August	17, 1994
Draft Audit:	November 14, 1994	November	14, 1994

Head of Quality Assurance Unit — Frauke Hermann

[redacted].....

Date: December 16, 1994

SUMMARY

This study was performed to investigate the potential of 1,3-Propanediol to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 100, and TA 102.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test article was tested at the following concentrations:

33.3; 100.0; 333.3; 1000.0; 2500.0; and 5000.0 µg/plate

No toxic effects occurred in the test groups with and without metabolic activation.

The plates incubated with the test article showed normal background growth up to 5000.0 µg/plate with and without S9 mix in all strains used.

No substantial increases in revertant colony numbers of any of the five tester strains were observed following treatment with 1,3-Propanediol at any dose level, either in the presence or absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of significance.

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

CONCLUSION

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test article did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

Therefore, 1,3-Propanediol is considered to be non-mutagenic in this Salmonella typhimurium reverse mutation assay.

OBJECTIVE

AIMS OF THE STUDY

The experiments were performed to assess the potential of the test article to induce gene mutations by means of two independent Salmonella typhimurium reverse mutation assays. Experiment I was performed as a plate incorporation assay. Since negative result was obtained in this experiment, experiment II was performed as a pre-incubation assay.

REASONS FOR THE STUDY

The most widely used assays for detecting gene mutations are those using bacteria. They are relatively simple and rapid to perform, and give reliable data on the ability of an agent to interact with DNA and produce mutations.

In spite of great differences between bacterial and eukaryotic cells with respect to structure and function there is a relationship between mutagenicity in bacteria and carcinogenicity in mammals described in literature (7,8).
Reverse mutation assays determine the frequency with which an agent repairs or suppresses the effect of the forward mutation. The genetic target presented to an agent is therefore small, specific and selective. Several bacterial strains, or a single strain with multiple markers are necessary to overcome the effects of mutagen specificity. The reversion of bacteria from growth-dependence on a particular amino acid to growth in the absence of that amino acid (reversion from auxothrophy to prototrophy) is the most widely used marker.
The Salmonella typhimurium histidine (his) reversion system measures $his^- \longrightarrow his^+$ reversions. The S. typhimurium strains are constructed to differentiate between base pair (TA 1535, TA 100, TA 102) and frameshift (TA 1537, TA 98) mutations.

According to the direct plate incorporation and the pre-incubation method the bacteria are exposed to the test article with and without metabolic activation and plated on selective medium. After a suitable period of incubation, revertant colonies are counted.

To establish a dose response effect five dose levels with adequately spaced intervals are to be tested. The maximum dose level was 5000.0 µg/plate, unless limited by toxicity or solubility of the test article.

To validate the test, reference mutagens are tested in parallel to the test article.

MATERIALS AND METHODS

THE TEST ARTICLE

The test article and the information concerning the test article were provided by the sponsor.

Name:	1,3-Propanediol
Batch No.:	EK 33/92-1
Aggregate State at RT:	liquid
Colour:	colourless
Analysis:	4783/94 dated July 12, 1994
Purity:	≥ 99.8 % / GC, Area % H_2O ≤ 0.1 % / Karl Fischer titration
Stability:	Pure: stable for years In solvent: stable in H_2O
Storage:	room temperature
Expiration Date:	July 01, 1996

On the day of the experiment, the test article 1,3-Propanediol was dissolved in aqua bidest..

THE CONTROLS

The Negative Controls

Concurrent untreated and solvent controls were performed.

The Positive Control Substances

Without metabolic activation

Strains:	TA 1535, TA 100
Name:	sodium azide, NaN_3
Supplier:	SERVA, D-69042 Heidelberg, F.R.G.
Catalogue No.:	30175
Purity:	at least 99 %
Dissolved in:	aqua dest.
Concentration:	10 µg/plate

Strains:	TA 1537, TA 98
Name:	4-nitro-o-phenylene-diamine, 4-NOPD
Supplier:	SIGMA, D-82041 Deisenhofen, F.R.G.
Catalogue No.:	N 9504
Purity:	> 99.9 %
Dissolved in:	DMSO
Concentration:	10 µg/plate

Strain:	TA 102
Name:	methyl methane sulfonate, MMS
Supplier:	MERCK-SCHUCHARDT, D-85662 Hohenbrunn, F.R.G.
Catalogue No.:	820775
Purity:	> 99.0 %
Dissolved in:	aqua dest.
Concentration:	5.0 µl/plate*

* = in deviation to protocol, updating

With metabolic activation

Strains:	TA 1535, TA 1537, TA 98, TA 100, TA 102
Name:	2-aminoanthracene, 2-AA
Supplier:	SIGMA, D-82041 Deisenhofen, F.R.G.
Catalogue No.:	A 1381
Purity:	97.5 %
Dissolved in:	DMSO
Concentration:	2.5 µg/plate (10.0 µg/plate in TA 102)

The stability of the positive control substances in solution was unknown but a mutagenic response in the expected range is sufficient evidence of biological stability. The dilutions of the stock solutions were prepared on the day of the experiment and used immediately.

THE TEST SYSTEM

Characterisation of the Salmonella typhimurium Strains

The strains are derived from S. typhimurium strain LT2 and due to a mutation in the histidine locus are histidine dependent. Additionally due to the "deep rough" (rfa-minus) mutation they possess a faulty lipopolysaccharide envelope which enables substances to penetrate the cell wall more easily. A further mutation causes a reduction in the activity of an excision repair system. The latter alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named "uvrB-minus". In the strains TA 98, TA 100 and TA 102 the R-factor plasmid pKM 101 carries the ampicillin resistance marker. The strain TA 102 does not contain the uvrB$^-$-mutation. Additionally TA 102 contains the multicopy plasmid pAQ1, which carries the hisG428 mutation and a tetracycline resistance gene. TA 102 contains the ochre mutation in hisG gene.

In summary, the mutations of the TA strains used in this study can be described as follows:

Salmonella typhimurium

TA 1537:	his C 3076; rfa$^-$; uvrB$^-$;		: frame shift mutations
TA 98:	his D 3052; rfa$^-$; uvrB$^-$;	R-factor:	" "
TA 1535:	his G 46; rfa$^-$; uvrB$^-$;		:base-pair substitutions
TA 100:	his G 46; rfa$^-$; uvrB$^-$;	R-factor:	" "
TA 102:	his G 428; rfa$^-$; uvrB$^+$;	R-factor:	" "

Regular checking of the properties of the strains with regard to membrane permeability, ampicillin- and tetracycline-resistance as well as spontaneous mutation rates is performed in the laboratory of C C R according to Ames et al. (1). In this way it was ensured that the experimental conditions set down by Ames were fulfilled.

The bacterial strains were obtained from Dr. Heinz Träger, Knoll AG, D-67008 Ludwigshafen, F.R.G.

Storage

The strain cultures were stored as stock cultures in ampoules with nutrient broth + 5 % DMSO (MERCK, D-64293 Darmstadt) in liquid nitrogen.

Precultures

From the thawed ampoules of the strains 0.5 ml suspension was transferred to 250 ml Erlenmeyer flasks containing 20 ml nutrient medium. A solution of 20 µl ampicillin was added to the strains TA 98, TA 100 and TA 102 and, additionally, a solution of 20 µl tetracycline was added to strain TA 102. This nutrient medium contains per litre:

8 g Merck Nutrient Broth (MERCK, D-64293 Darmstadt)
5 g NaCl (MERCK, D-64293 Darmstadt)

The bacterial culture was incubated in a shaking water bath for 10 hours at 37° C.

Selective Agar

2.0 % Vogel-Bonner-Glucose-Minimal-Agar was used as selective agar. Each petri dish was filled with 20 ml of this nutrient medium. Sterilisations were performed at 121° C in an autoclave.

Overlay Agar

The overlay agar contains per litre:

6.0 g Merck Agar Agar*
6.0 g NaCl*
10.5 mg L-histidine x HCl x H_2O*
12.2 mg biotin*

* (MERCK, D-64293 Darmstadt)

Sterilisations were performed at 121° C in an autoclave.

MAMMALIAN MICROSOMAL FRACTION S9 MIX

The bacteria used in these assays do not possess the enzyme systems which, in mammals, are known to convert promutagens into active DNA damaging metabolites. In order to overcome this major drawback an exogenous metabolic system is added in form of mammalian microsome enzyme activation mixture.

S9 (Preparation by C C R)

The S9 liver microsomal fraction was obtained from the livers of 8 - 12 weeks old male Wistar rats, strain WU (Charles River Wiga GmbH, D-97633 Sulzfeld, F.R.G.; weight approx. 150 - 200 g) which received a single i.p. injection of 500 mg/kg b.w. Aroclor 1254 (Antechnika, D-76275 Ettlingen, F.R.G.) in olive oil 5 days previously.

After cervical dislocation the livers of the animals were removed, washed in 150 mM KCl and homogenised. The homogenate, was diluted 1+3 in KCl and centrifuged cold at 9,000 g for 10 minutes. A stock of the supernatant containing the microsomes was frozen in ampoules of 2, 3 or 5 ml and stored at -80° C. Small numbers of the ampoules are kept at -20° C for only one week before use. The standardisation of the protein content was made using the analysis kit of Bio-Rad Laboratories, D-80939 München: Bio-Rad protein assay, Catalogue 500 000 6 (6).

The protein concentration in the S9 preparation was 45 mg/ml (lot 240194) in the pre-experiment and in experiment I, and 44.8 (lot 291193) in experiment I and experiment II.

S9 Mix

Before the experiment an appropriate quantity of S9 supernatant was thawed and mixed with S9 co-factor solution. The amount of S9 supernatant was 15% v/v. The composition of the co-factor solution was concentrated to yield the following concentrations in the S9 mix:

8 mM $MgCl_2$
33 mM KCl
5 mM glucose-6-phosphate
5 mM NADP
in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment the S9 mix was stored in an ice bath. The S9 mix preparation was performed according to Ames et al.(2).

PRE-EXPERIMENT FOR TOXICITY

To evaluate the toxicity of the test article a pre-experiment was performed with strains TA 98 and TA 100. 8 concentrations were tested for toxicity and mutation induction with each 3 plates. The experimental conditions in this pre-experiment were the same as described below for the experiment I (plate incorporation test).

Toxicity of the test article can be evidenced by a reduction in the number of spontaneous revertants, a clearing of the bacterial background lawn, or by degree of survival of treated cultures.

DOSE SELECTION

According to the results of the pre-experiment the concentrations applied in the main experiments were chosen.

The maximum concentration was 5000.0 µg/plate. The concentration range included two logarithmic decades. In this study six adequately spaced concentrations were tested. Two independent experiments were performed.

As the results of the pre-experiment were in accordance with the criteria described below (EVALUATION OF RESULTS), these data are reported as a part of the main experiment I.

According to the dose selection criteria the test article was tested at the following concentrations:

33.3; 100.0; 333.3; 1000.0; 2500.0; and 5000.0 µg/plate

EXPERIMENTAL PERFORMANCE

For each strain and dose level, including the controls three plates were used as a minimum.

The following materials were mixed in a test tube and poured onto the selective agar plates:

100 µl	Test solution at each dose level, solvent control, negative control, or reference mutagen solution (positive control),
500 µl	S9 mix (for test with metabolic activation) or S9 mix substitution-buffer (for test without metabolic activation),
100 µl	Bacteria suspension (cf. test system, pre-culture of the strains),
2000 µl	Overlay agar

In the pre-incubation assay 100 µl test solution, 500 µl S9 mix / S9 mix substitution buffer and 100 µl bacterial suspension were mixed in a test tube and incubated at 37°C for 60 minutes. After pre-incubation 2.0 ml overlay agar (45°) was added to each tube. The mixture was poured on minimal agar plates.

After solidification the plates were incubated upside down for at least 48 hours at 37° C in the dark.

DATA RECORDING

The colonies were counted using the AUTOCOUNT (Artek Systems Corporation, BIOSYS GmbH, D-61184 Karben; F.R.G.). The counter was connected to an IBM AT compatible PC with printer which printed out the individual values and the means from the plates for each concentration together with standard deviations and enhancement factors as compared to the spontaneous reversion rates (see tables of results). If precipitation of the test article precluded automatic counting the revertant colonies were counted by hand.

EVALUATION OF RESULTS

The generally accepted conditions for the evaluation of the results are:

- corresponding background growth on both negative controls and test plates
- normal range of spontaneous reversion rates.

Range of spontaneous reversion frequencies*				
1535	1537	98	100	102
10 - 29	5 - 28	15 - 57	77 - 189	121 - 293

* These values refer to the negative control without metabolic activation and represent our historical control range in 1993

Due to international guidelines a statistical evaluation of the results is recommended. However, no evaluated statistical procedure can be recommended for analysis of data from the bacterial assays at this time (5).

A test article is considered positive if either a dose related and reproducible increase in the number of revertants or a significant and reproducible increase for at least one test concentration is induced.

A test article producing neither a dose related and reproducible increase in the number of revertants nor a significant and reproducible positive response at any one of the test points is considered non-mutagenic in this system.

A significant response is described as follows:

A test article is considered mutagenic if in strain TA 100 and TA 102 the number of reversions is at least twice as high and in strains TA 1535, TA 1537, and TA 98 it is at least three times higher as compared to the spontaneous reversion rate (4).

Also, a dose-dependent and reproducible increase in the number of revertants is regarded as an indication of possibly existing mutagenic potential of the test article regardless whether the highest dose induced the above described enhancement factors or not.

BIOMETRY

No appropriate statistical method is available (5).

RESULTS

PRE-EXPERIMENT FOR TOXICITY

To evaluate the toxicity of the test article a pre-study was performed with strains TA 98 and TA 100.

The results are given in the following table:

Substance	Concentration per plate µg	Revertants per plate TA 98 -	TA 98 +	TA 100 -	TA 100 +*
Negative control	-	31	42	99	159
Solvent control	-	26	40	99	158
4-NOPD	10.0	245	/	/	/
Sodium azide	10.0	/	/	619	/
2-aminoanthracene	2.5	/	468	/	965
Test article	3.3	24	41	106	177
" "	10.0	30	48	98	168
" "	33.3	25	45	100	163
" "	100.0	30	45	93	160
" "	333.3	26	50	102	156
" "	1000.0	24	47	93	172
" "	2500.0	26	43	97	156
" "	5000.0	31	43	105	170

* - = without S9 mix; + = with S9 mix
/ not performed

The plates with the test article showed normal background growth up to 5000.0 µg/plate in strain TA 98 and TA 100, respectively.

According to the dose selection criteria, the test article was tested at the following concentrations:

33.3; 100.0; 333.3; 1000.0; 2500.0; and 5000.0 µg/plate

TABLES OF RESULTS

EXPERIMENT I

PLATE INCORPORATION TEST

Test article: 1.3-Propanediol

S9 mix from : Rat liver (Batch R 291193)

Test strain : TA 1535

Concentration µg/plate	Plate 1	Plate 2	Plate 3	Revertants/plate mean	s.d.	factor+
without S9 mix						
Negative control	17	16	9	14	4.4	
Solvent control	17	21	18	19	2.1	1.0
33.3	10	16	14	13	3.1	0.7
100.0	24	11	16	17	6.6	0.9
333.3	24	16	22	21	4.2	1.1
1000.0	18	17	14	16	2.1	0.9
2500.0	9	13	18	13	4.5	0.7
5000.0	8	14	13	12	3.2	0.6
Positive control Sodium azide (10µg/plate)	917	903	835	885	43.9	47.4
with S9 mix						
Negative control	19	17	28	21	5.9	
Solvent control	17	18	18	18	0.6	1.0
33.3	18	17	12	16	3.2	0.9
100.0	23	19	12	18	5.6	1.0
333.3	17	22	27	22	5.0	1.2
1000.0	21	22	29	24	4.4	1.4
2500.0	22	25	13	20	6.2	1.1
5000.0	16	24	17	19	4.4	1.1
Positive control 2-aminoanthracene (2.5µg/plate)	284	300	200	261	53.7	14.8

$$+ \text{ enhancement factor} = \frac{\Sigma \text{ revertants/concentr. test article}}{\Sigma \text{ revertants/solvent control}}$$

Test article: 1.3-Propanediol

S9 mix from : Rat liver (Batch R 291193)

Test strain : TA 1537

Concentration µg/plate	Plate 1	2	3	Revertants/plate mean	s.d.	factor+
without S9 mix						
Negative control	24	24	19	22	2.9	
Solvent control	28	17	13	19	7.8	1.0
33.3	19	27	26	24	4.4	1.2
100.0	26	17	26	23	5.2	1.2
333.3	18	15	12	15	3.0	0.8
1000.0	16	28	25	23	6.2	1.2
2500.0	20	31	15	22	8.2	1.1
5000.0	13	23	19	18	5.0	0.9
Positive control 4-nitro-o phenylene diamine (4-NOPD) 10 µg/plate	65	70	50	62	10.4	3.2
with S9 mix						
Negative control	33	29	22	28	5.6	
Solvent control	17	25	27	23	5.3	1.0
33.3	22	16	24	21	4.2	0.9
100.0	22	26	30	26	4.0	1.1
333.3	29	32	27	29	2.5	1.3
1000.0	20	29	20	23	5.2	1.0
2500.0	17	13	25	18	6.1	0.8
5000.0	16	31	24	24	7.5	1.0
Positive control 2-aminoanthracene (2.5µg/plate)	87	95	89	90	4.2	3.9

$$+ \text{ enhancement factor} = \frac{\Sigma \text{ revertants/concentr. test article}}{\Sigma \text{ revertants/solvent control}}$$

Test article: 1.3-Propanediol

S9 mix from : Rat liver (Batch R 240194)

Test strain : TA 98

Concentration µg/plate	Plate 1	2	3	Revertants/plate mean	s.d.	factor+
without S9 mix						
Negative control	36	29	27	31	4.7	
Solvent control	23	25	31	26	4.2	1.0
33.3	28	22	26	25	3.1	1.0
100.0	27	32	31	30	2.6	1.1
333.3	22	32	23	26	5.5	1.0
1000.0	20	29	22	24	4.7	0.9
2500.0	28	22	27	26	3.2	1.0
5000.0	28	37	29	31	4.9	1.2
Positive control 4-nitro-o-phenylene-diamine (4-NOPD) 10 µg/plate	267	217	250	245	25.4	9.3
with S9 mix						
Negative control	40	41	45	42	2.6	
Solvent control	38	38	45	40	4.0	1.0
33.3	46	50	39	45	5.6	1.1
100.0	43	47	44	45	2.1	1.1
333.3	46	45	59	50	7.8	1.2
1000.0	49	47	44	47	2.5	1.2
2500.0	41	49	40	43	4.9	1.1
5000.0	40	47	43	43	3.5	1.1
Positive control 2-aminoanthracene (2.5 µg\plate)	485	425	494	468	37.5	11.6

$$+ \text{ enhancement factor} = \frac{\Sigma \text{ revertants/concentr. test article}}{\Sigma \text{ revertants/solvent control}}$$

000687

Test article: 1.3-Propanediol

S9 mix from : Rat liver (Batch R 240194)

Test strain : TA 100

Concentration µg/plate	Plate 1	2	3	Revertants/plate mean	s.d.	factor+
without S9 mix						
Negative control	102	106	90	99	8.3	
Solvent control	94	101	103	99	4.7	1.0
33.3	108	100	93	100	7.5	1.0
100.0	87	99	92	93	6.0	0.9
333.3	101	100	105	102	2.6	1.0
1000.0	103	88	87	93	9.0	0.9
2500.0	110	103	79	97	16.3	1.0
5000.0	92	116	106	105	12.1	1.1
Positive control Sodium azide (10µg/plate)	610	596	651	619	28.6	6.2
with S9 mix						
Negative control	148	158	170	159	11.0	
Solvent control	142	156	175	158	16.6	1.0
33.3	172	161	156	163	8.2	1.0
100.0	140	185	156	160	22.8	1.0
333.3	154	153	162	156	4.9	1.0
1000.0	174	174	167	172	4.0	1.1
2500.0	151	139	177	156	19.4	1.0
5000.0	159	180	170	170	10.5	1.1
Positive control 2-aminoanthracene (2.5µg/plate)	968	972	954	965	9.5	6.1

$$+ \text{ enhancement factor} = \frac{\Sigma \text{ revertants/concentr. test article}}{\Sigma \text{ revertants/solvent control}}$$

000688

Test article: 1.3-Propanediol

S9 mix from : Rat liver (Batch R 291193)

Test strain : TA 102

Concentration µg/plate	Plate 1	2	3	Revertants/plate mean	s.d.	factor+
without S9 mix						
Negative control	170	157	179	169	11.1	
Solvent control	181	190	186	186	4.5	1.0
33.3	187	178	186	184	4.9	1.0
100.0	162	164	167	164	2.5	0.9
333.3	159	181	196	179	18.6	1.0
1000.0	171	198	194	188	14.6	1.0
2500.0	203	181	198	194	11.5	1.0
5000.0	169	199	187	185	15.1	1.0
Positive control methyl methane sulfonate, MMS (5µl/plate)	730	744	715	730	14.5	3.9
with S9 mix						
Negative control	225	272	281	259	30.1	
Solvent control	239	235	259	244	12.9	1.0
33.3	264	250	207	240	29.7	1.0
100.0	218	249	240	236	15.9	1.0
333.3	238	235	293	255	32.7	1.0
1000.0	273	303	297	291	15.9	1.2
2500.0	269	278	276	274	4.7	1.1
5000.0	276	245	245	255	17.9	1.0
Positive control 2-aminoanthracene (10µg/plate)	749	757	758	755	4.9	3.1

$$+ \quad \text{enhancement factor} = \frac{\Sigma \text{ revertants/concentr. test article}}{\Sigma \text{ revertants/solvent control}}$$

TABLES OF RESULTS

EXPERIMENT II

PRE-INCUBATION TEST

Test article: 1.3-Propanediol

S9 mix from : Rat liver (Batch R 291193)

Test strain : TA 1535

Concentration µg/plate	Plate 1	Plate 2	Plate 3	Revertants/plate mean	s.d.	factor+
without S9 mix						
Negative control	7	7	6	7	0.6	
Solvent control	7	4	5	5	1.5	1.0
33.3	2	10	12	8	5.3	1.5
100.0	3	5	4	4	1.0	0.8
333.3	5	1	6	4	2.6	0.8
1000.0	7	3	10	7	3.5	1.2
2500.0	11	6	11	9	2.9	1.7
5000.0	6	13	8	9	3.6	1.7
Positive control sodium azide NaN_3 (10µg/plate)	265	253	271	263	9.2	49.3
with S9 mix						
Negative control	4	13	12	10	4.9	
Solvent control	10	5	10	8	2.9	1.0
33.3	7	4	11	7	3.5	0.9
100.0	9	8	8	8	0.6	1.0
333.3	6	8	10	8	2.0	1.0
1000.0	11	20	10	14	5.5	1.6
2500.0	9	8	11	9	1.5	1.1
5000.0	14	18	11	14	3.5	1.7
Positive control 2-aminoanthracene (2.5µg/plate)	43	38	36	39	3.6	4.7

$$+ \text{ enhancement factor} = \frac{\Sigma \text{ revertants/concentr. test article}}{\Sigma \text{ revertants/solvent control}}$$

Test article: 1.3-Propanediol

S9 mix from : Rat liver (Batch R 291193)

Test strain : TA 1537

Concentration µg/plate	Plate 1	2	3	Revertants/plate mean	s.d.	factor+
without S9 mix						
Negative control	21	25	19	22	3.1	
Solvent control	18	26	22	22	4.0	1.0
33.3	18	17	29	21	6.7	1.0
100.0	23	25	18	22	3.6	1.0
333.3	19	24	20	21	2.6	1.0
1000.0	23	21	23	22	1.2	1.0
2500.0	28	21	19	23	4.7	1.0
5000.0	15	21	21	19	3.5	0.9
Positive control 4-Nitro-o-phenylene-diamine (10µg/plate)	127	154	134	138	14.0	6.3
with S9 mix						
Negative control	35	26	29	30	4.6	
Solvent control	32	35	28	32	3.5	1.0
33.3	31	36	28	32	4.0	1.0
100.0	29	27	26	27	1.5	0.9
333.3	31	29	35	32	3.1	1.0
1000.0	34	28	30	31	3.1	1.0
2500.0	31	33	29	31	2.0	1.0
5000.0	28	32	31	30	2.1	1.0
Positive control 2-aminoanthracene (2.5µg/plate)	151	137	141	143	7.2	4.5

$$+ \text{ enhancement factor} = \frac{\Sigma \text{ revertants/concentr. test article}}{\Sigma \text{ revertants/solvent control}}$$

000692

Test article: 1.3-Propanediol

S9 mix from : Rat liver (Batch R 291193)

Test strain : TA 98

Concentration µg/plate	Plate 1	2	3	Revertants/plate mean	s.d.	factor+
without S9 mix						
Negative control	30	25	16	24	7.1	
Solvent control	17	20	22	20	2.5	1.0
33.3	16	24	20	20	4.0	1.0
100.0	20	25	18	21	3.6	1.1
333.3	18	21	21	20	1.7	1.0
1000.0	17	18	22	19	2.6	1.0
2500.0	23	26	27	25	2.1	1.3
5000.0	19	28	28	25	5.2	1.3
Positive control 4-Nitro-o-phenylene-diamine (10µg/plate)	143	150	157	150	7.0	7.6
with S9 mix						
Negative control	19	17	24	20	3.6	
Solvent control	24	20	21	22	2.1	1.0
33.3	20	21	30	24	5.5	1.1
100.0	18	17	17	17	0.6	0.8
333.3	24	18	25	22	3.8	1.0
1000.0	32	19	23	25	6.7	1.1
2500.0	21	29	18	23	5.7	1.0
5000.0	19	16	26	20	5.1	0.9
Positive control 2-aminoanthracene 2.5 µg\plate	179	191	221	197	21.6	9.1

$$+ \text{ enhancement factor} = \frac{\Sigma \text{ revertants/concentr. test article}}{\Sigma \text{ revertants/solvent control}}$$

* no revertant colonies observed

Test article: 1.3-Propanediol

S9 mix from : Rat liver (Batch R 291193)

Test strain : TA 100

Concentration µg/plate	Plate 1	2	3	Revertants/plate mean	s.d.	factor+
without S9 mix						
Negative control	94	80	63	79	15.5	
Solvent control	94	89	93	92	2.6	1.0
33.3	73	95	77	82	11.7	0.9
100.0	84	83	75	81	4.9	0.9
333.3	96	73	85	85	11.5	0.9
1000.0	69	67	72	69	2.5	0.8
2500.0	69	77	58	68	9.5	0.7
5000.0	69	84	97	83	14.0	0.9
Positive control sodium azide (10µg/plate)	221	231	258	237	19.1	2.6
with S9 mix						
Negative control	116	141	153	137	18.9	
Solvent control	145	119	119	128	15.0	1.0
33.3	141	135	118	131	11.9	1.0
100.0	124	154	131	136	15.7	1.1
333.3	126	135	156	139	15.4	1.1
1000.0	128	121	125	125	3.5	1.0
2500.0	137	140	151	143	7.4	1.1
5000.0	126	138	114	126	12.0	1.0
Positive control 2-aminoanthracene (2.5µg/plate)	401	410	406	406	4.5	3.2

$$+ \text{ enhancement factor} = \frac{\Sigma \text{ revertants/concentr. test article}}{\Sigma \text{ revertants/solvent control}}$$

Test article: 1.3-Propanediol

S9 mix from : Rat liver (Batch R 291193)

Test strain : TA 102

Concentration µg/plate	Plate 1	Plate 2	Plate 3	Revertants/plate mean	s.d.	factor+
without S9 mix						
Negative control	176	145	138	153	20.2	
Solvent control	139	152	163	151	12.0	1.0
33.3	151	138	163	151	12.5	1.0
100.0	158	151	167	159	8.0	1.0
333.3	144	176	168	163	16.7	1.1
1000.0	159	144	150	151	7.5	1.0
2500.0	162	176	153	164	11.6	1.1
5000.0	159	147	138	148	10.5	1.0
Positive control methyl methane sulfonate (5.0 µl/plate)	831	879	911	874	40.3	5.8
with S9 mix						
Negative control	231	185	140	185	45.5	
Solvent control	168	149	145	154	12.3	1.0
33.3	165	187	177	176	11.0	1.1
100.0	239	207	188	211	25.8	1.4
333.3	176	198	194	189	11.7	1.2
1000.0	185	173	188	182	7.9	1.2
2500.0	175	190	188	184	8.1	1.2
5000.0	175	207	203	195	17.4	1.3
Positive control 2-aminoanthracene (10µg/plate)	610	587	636	611	24.5	4.0

$$+ \text{ enhancement factor} = \frac{\Sigma \text{ revertants/concentr. test article}}{\Sigma \text{ revertants/solvent control}}$$

SUMMARY OF RESULTS

Test article: 1.3-Propanediol

S9 mix from : rat liver (Batch R 291193 + R 240194)

without S9 mix

Revertants/plate
mean from three plates

Concentration µg/plate	TA 1535 I	TA 1535 II	TA 1537 I	TA 1537 II	TA 98 I	TA 98 II	TA 100 I	TA 100 II	TA 102 I	TA 102 II
Neg. contr.	14	7	22	22	31	24	99	79	169	153
Solv. contr.	19	5	19	22	26	20	99	92	186	151
33.3	13	8	24	21	25	20	100	82	184	151
100.0	17	4	23	22	30	21	93	81	164	159
333.3	21	4	15	21	26	20	102	85	179	163
1000.0	16	7	23	22	24	19	93	69	188	151
2500.0	13	9	22	23	26	25	97	68	194	164
5000.0	12	9	18	19	31	25	105	83	185	148
Positive controls										
Sodium azide (10µg/plate)	885	263					619	237		
4-Nitro-o-phenylene-diamine (10µg/plate)			62	138	245	150				
methyl methane sulfonate 5 µl/plate)									730	874

with S9 mix

Revertants/plate
mean from three plates

Concentration µg/plate	TA 1535 I	TA 1535 II	TA 1537 I	TA 1537 II	TA 98 I	TA 98 II	TA 100 I	TA 100 II	TA 102 I	TA 102 II
Neg. contr.	21	10	28	30	42	20	159	137	259	185
Solv. contr.	18	8	23	32	40	22	158	128	244	154
33.3	16	7	21	32	45	24	163	131	240	176
100.0	18	8	26	27	45	17	160	136	236	211
333.3	22	8	29	32	50	22	156	139	255	189
1000.0	24	14	23	31	47	25	172	125	291	182
2500.0	20	9	18	31	43	23	156	143	274	184
5000.0	19	14	24	30	43	20	170	126	255	195
Positive control										
2-amino-anthracene (2.5µg/plate, 10 µg/plate in TA 102)	261	39	90	143	468	197	965	406	755	611

/ = not performed

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DISCUSSION

The test article 1,3-Propanediol was assessed for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA 1535, TA 1537, TA 98, TA 100, and TA 102.

The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration and the controls, were tested in triplicate. The test article was tested at the following concentrations:

33.3; 100.0; 333.3; 1000.0; 2500.0; and 5000.0 µg/plate

No toxic effects occurred in the test groups with and without metabolic activation.

The plates incubated with the test article showed normal background growth up to 5000.0 µg/plate with and without S9 mix in all strains used.

No substantial increases in revertant colony numbers of any of the five tester strains were observed following treatment with 1,3-Propanediol at any concentration level, either in the presence or absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of significance.

Appropriate reference mutagens were used as positive controls. They showed a distinct increase in induced revertant colonies.

In conclusion, it can be stated that during the described mutagenicity test and under the experimental conditions reported, the test article did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used.

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DISTRIBUTION OF THE REPORT

Sponsor	2x (original, copy)
Study Director	1x (copy)

Degussa-US-IT-No.: 94-0060-DGM

IUA-No. 5113

CCR PROJECT 476903

GENE MUTATION ASSAY IN CHINESE HAMSTER V79 CELLS IN VITRO (V79 / HPRT)

WITH

1,3-Propanediol

REPORT

Study Completion Date:

December 13, 1994

JF#-1260
DW#-28417

DEGUSSA AG - US-IT - NR.
94 0060 DGM

RCC
Group

000700

COPY OF GLP CERTIFICATE

HESSISCHES MINISTERIUM
FÜR UMWELT, ENERGIE
UND BUNDESANGELEGENHEITEN

GLP-Bescheinigung

Bescheinigung

Hiermit wird bestätigt, daß die Prüfungseinrichtung(en)
Cytotest Cell Research GmbH & Co KG
In den Leppsteinswiesen 19
in 6101 Roßdorf
(Ort, Anschrift)
der RCC Holding Verwaltung GmbH
(Firma)
am 03.08., 04.08., 05.08. und 06.08.92
(Datum)
von der für die Überwachung zuständigen Behörde über die Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist (sind).

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.

Toxikologische Eigenschaften

Im Auftrag

(Dr. Hecker)

Wiesbaden, den 28.09.1992

Certificate

It is hereby certified that the test facility(ies)
Cytotest Cell Research GmbH & Co KG
In den Leppsteinswiesen 19
in 6101 Roßdorf
(location, address)
of RCC Holding Verwaltung GmbH
(company name)
on 03.08., 04.08., 05.08. and 06.08.92
(date)
was (were) inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

Toxicological properties

000701

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PREFACE

GENERAL

Sponsor:	Initiative Umweltrelevante Altstoffe e.V. Kennedyallee 93 60596 Frankfurt
Study Monitor:	Dr. Mayr DEGUSSA AG Industrielle Toxikologie Zweigniederlassung Wolfgang 63403 Hanau-Wolfgang
Testing Facility:	C C R CYTOTEST CELL RESEARCH GMBH & CO. KG D-64380 Roßdorf, F.R.G.
CCR Project No.:	476903
Test Article:	1,3-Propandiol
Title:	Gene Mutation Assay in Chinese Hamster V79 Cells *in vitro* (V79/HPRT) with 1,3-Propandiol

PROJECT STAFF

Management:	Markus Arenz
Study Director:	Dr. Albrecht Poth
Quality Assurance Unit	
until August 14, 1994:	Dr. Christiane Helmrich
since August 15, 1994:	Frauke Hermann

SCHEDULE

Date of Protocol:	July 06, 1994
Date of Amendment (1st):	August 12, 1994
Date of Amendment (2nd):	December 10, 1994
Start of Pre-Test:	July 14, 1994
Start of Experiments:	August 16, 1994
End of Experiments:	October 10, 1994
Date of Draft:	October 26, 1994
Date of Report:	

000703

PROJECT STAFF SIGNATURES

Study Director: Dr. Eric Wollny

.....

Date: December 13, 1994

Management: Markus Arenz

.....

Date: December 13, 1994

QUALITY ASSURANCE

The study was performed in compliance with:

"The OECD Principles of Good Laboratory Practice", Paris 1981.

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Anlage 1 ("Annex 1"), dated March 14, 1990 (BGBL. I S. 521) (regulates Good Laboratory Practice)

GUIDELINES

This study was conducted according to the procedures indicated by the following internationally accepted guidelines and recommendations:

Second Addendum to the OECD Guideline for Testing of Chemicals, Section 4, No. 476, adopted April 4, 1984, "*In vitro* Mammalian Cell Gene Mutation Tests"

EEC Directive 87/302, L 133, p. 61 - 63

EPA; 40 CFR; Ch.I; Part 798; Detection of gene mutation in somatic cells in culture; pp. 717-720 (7-1-86 Edition)

ARCHIVING

C C R, D-64380 Roßdorf/F.R.G. will archive the following data for 30 years:
raw data, protocol and copy of report.

The following sample will be archived for at least 12 years:
sample of test article.

No raw data or material relating to the study will be discarded without the sponsor's prior consent.

STATEMENT OF COMPLIANCE

Project Number: 476903

Test Article : 1,3-Propanediol

Study Director: Dr. Eric Wollny

Title : Gene Mutation Assay in Chinese Hamster V79 Cells *in vitro* (V79/HPRT) with 1,3-Propanediol

This study, performed in the testing facility of CCR was conducted in compliance with Good Laboratory Practice Regulations:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Anhang 1 ("Annex 1"), dated March 14, 1990

"OECD Principles of Good Laboratory Practice", Paris, 1981

There were no circumstances that may have affected the quality or integrity of the study.

Study Director

C C R
Dr. Eric Wollny

.....

Date: December 16, 1994

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QUALITY ASSURANCE UNIT

C C R, Cytotest Cell Research GmbH & Co. KG,
In den Leppsteinswiesen 19, D-64380 Roßdorf, F.R.G.

STATEMENT

Project Number: 476903

Test Article : 1,3-Propanediol

Study Director: Dr. Eric Wollny

Title : Gene Mutation Assay in Chinese Hamster V79 Cells *in vitro* (V79/HPRT) with 1,3-Propanediol

This report was audited by the Quality Assurance Unit and the conduct of this study was inspected on the following dates:

Phases and Dates of QAU Inspections/Audits		Dates of Reports to the Study Director and to Management	
Protocol Audit	July 06, 1994	July	06, 1994
Process Inspection	August 29, 1994	August	29, 1994
Process Inspection	Sept. 06, 1994	Sept.	06, 1994
Draft Audit	Nov. 22, 1994	Nov.	22, 1994

Head of
Quality Assurance Unit Frauke Hermann

[redacted] .....

Date: December 14, 1994

SUMMARY

The study was performed to investigate the potential of 1,3-Propanediol to induce gene mutations at the HPRT locus in V79 cells of the Chinese hamster.

The assay was performed in two independent experiments, using identical procedures, both with and without liver microsomal activation.

The test article was tested with the following concentrations:

Experiment I and II:
with and without S9 mix:
250.0; 1000.0; 2500.0 and 5000.0 µg/ml

According to the pre-test on toxicity the concentration ranges were selected to yield concentration-related toxic effects. The highest concentration produced a low level of survival (only without metabolic activation) and the survival at the lowest concentration was approximately in the range of the negative control.

Up to the highest investigated concentration no relevant increase in mutant colony numbers was obtained in two independent experiments.

Appropriate reference mutagens were used as positive controls and showed a distinct increase in induced mutant colonies.

CONCLUSION

In conclusion it can be stated that during the described mutagenicity test and under the experimental conditions reported the test article did not induce gene mutations at the HPRT locus in V79 cells.

Therefore, 1,3-Propanediol is considered to be non-mutagenic in this HPRT assay.

OBJECTIVE

AIMS OF THE STUDY

This *in vitro* assay is performed to assess the potential of the test article to induce gene mutations by means of two independent HGPRT experiments using the Chinese hamster cell line V79.

RELEVANCE OF THE TEST SYSTEM

In vitro methods are valuable when it is desirable to accurately control the concentration and exposure time of cells to the test article under study. However, due to the limited capacity for metabolic activation of potential mutagens an exogenous metabolic activation system is necessary.

This *in vitro* test is an assay for the detection of forward gene mutations in mammalian cells. Gene mutations are considered to be an initial step in the carcinogenic process (2).

The V79 cells are exposed to the test article both with and without exogenous metabolic activation. At a defined time interval after treatment the descendants of the treated original population are monitored for the loss of functional HPRT enzyme.

HPRT (hypoxanthine-guanine phosphoribosyl transferase) catalyzes the conversion of the nontoxic 6TG (6-thioguanine) to its toxic ribophosphorylated derivative. Therefore, cells deficient in HPRT due to a forward mutation are resistant to 6TG. These cells are able to proliferate in the presence of 6TG whereas the non-mutated cells die. However, the mutant phenotype requires a period of time before it is completely expressed. The phenotypic expression is achieved by allowing exponential growth of the cells for 7 - 9 days. The expression period is terminated by adding 6TG to the culture medium (3).

Mutant frequency is determined by seeding known numbers of cells in medium containing the selective agent to detect mutant cells, and in medium without selective agent to determine the surviving cells. After a suitable period the colonies are counted. Mutant frequencies are calculated from the number of mutant colonies corrected for cell survival.

In order to establish a concentration response effect of the test article at least four concentration levels are tested. These concentration levels should yield a concentration related toxic effect. The highest concentration level should induce a reduced level of survival.

To demonstrate the sensitivity of the test system reference mutagens are tested in parallel to the test article.

MATERIALS AND METHODS

THE TEST ARTICLE

The test article and the information concerning the test article were provided by the sponsor.

Name:	1,3-Propanediol
CAS No.:	504-63-2
Batch No.:	EK 33/92-1
Aggregate State at RT:	liquid
Colour:	colourless
Molecular Weight:	not indicated by the sponsor
Purity:	≥ 99.8 % / GC, area % H_2O ≤ 0.1 % /Karl Fischer titration
Analysis:	4783/94 dated: July 12, 94
Stability:	Pure: stable for years In solvent: stable in H_2O
Storage:	room temperature
Expiration Date:	July 01, 1996

On the day of the experiment (immediately before treatment), the test article was dissolved in culture medium (MEM without FCS). The solvent was chosen according to its solubility properties and its non-toxicity for the cells.

THE CONTROLS

The Negative Controls

Concurrent negative and solvent controls were performed.

The Positive Control Substances

Without metabolic activation

Name:	EMS; Ethylmethanesulfonate
Supplier:	Merck-Schuchardt, D-85662 Hohenbrunn, F.R.G.
Catalogue no.:	820774; (Purity: > 98%)
Dissolved in:	nutrient medium (MEM without FCS)
Final Concentration:	0.6 mg/ml = 4.8 mM

Solution prepared on day of experiment.

With metabolic activation

Name:	DMBA; 7,12-dimethylbenz(a)anthracene
Supplier:	SIGMA CHEMIE GMBH, D-82041 Deisenhofen
Catalogue no.:	D 3254; (Purity: appr. 95%)
Dissolved in:	DMSO, Dimethylsulfoxide; final concentration in nutrient medium 1 %
Final Concentration:	3.85 µg/ml = 15.0 µM

The stability of both positive control substances in solution is unknown, but a mutagenic response in the expected mutation range is sufficient evidence of biological stability. The dilutions of the stock solutions were prepared on the day of the experiment and used immediatelly.

THE TEST SYSTEM

Reasons for the Choice of the Cell Line V79

The V79 cell line has been used successfully in *in vitro* experiments for many years. Especially the high proliferation rate (doubling time 12 - 16 h in stock cultures) and a good cloning efficiency of negative control cells (as a rule more than 50 %) both necessary for the appropriate performance of the study, recommend the use of this cell line. The cells have a stable karyotype with a modal chromosome number of 22 (3).

Cell Cultures

Large stocks of the V79 cell line (supplied by Laboratory for Mutagenicity Testing; Technical University; D-64287 Darmstadt; F.R.G.) are stored in liquid nitrogen in the cell bank of C C R allowing the repeated use of the same cell culture batch in experiments. Before freezing, the level of spontaneous mutants was depressed by treatment with HAT-medium as described in [3]. Each batch is screened for mycoplasma contamination and checked for karyotype stability and spontaneous mutant frequency. Consequently, the parameters of the experiments remain similar because of the reproducible characteristics of the cells.

Thawed stock cultures are propagated at 37 °C in 80 cm^2 plastic flasks (GREINER, D-72632 Frickenhausen, F.R.G.). About 5 x 10^5 cells are seeded into each flask with 15 ml of MEM (minimal essential medium; SEROMED, D-12247 Berlin, F.R.G.) supplemented with 10 % fetal calf serum (FCS; Boehringer Mannheim, 68261-Mannheim, F.R.G.). The cells are subcultured twice weekly. The cell cultures are incubated at 37 °C in a 4.5 % carbon dioxide atmosphere (95.5% air).

For the selection of mutants the medium is supplemented with 11 µg/ml thioguanine (6TG, SIGMA GmbH, D-82041 Deisenhofen, F.R.G.).

MAMMALIAN MICROSOMAL FRACTION S9 MIX

Lacking metabolic activities of cells under *in vitro* conditions are a disadvantage of assays with cell cultures as many chemicals only develop a mutagenic potential when they are metabolized by the mammalian organism. However, metabolic activation of chemicals can be achieved at least partially by supplementing the cell cultures with mammalian liver microsome preparations (S9 mix).

S9 (Preparation by C C R)

The S9 liver microsomal fraction was obtained from the liver of 8 - 12 weeks old male rats, strain Wistar HanIbm in deviation to the protocol (BRL, CH-4414 Füllingsdorf; body weight approx. 220 - 320 g) which received a single i.p. injection of 500 mg/kg b.w. Aroclor 1254 (Antechnika, D-76275 Ettlingen, F.R.G.) in olive oil 5 days previously.

After cervical dislocation, the livers of the animals were removed, washed in 150 mM KCl and homogenized. The homogenate was diluted 1:4 in KCl and centrifuged twice at 9,000 g at 4 °C for 10 minutes. A stock of the supernatant containing the microsomes was frozen in ampoules of 2 or 5 ml and stored at -70 °C. Small numbers of the ampoules were kept at -20 °C for only one week before use. The protein content was determined using the analysis kit of Bio-Rad Laboratories, D-80939 München: Bio-Rad protein assay, Catalogue 500 000 6.

The protein concentration in the S9 preparation was 29.0 mg/ml (lot. no.: 060694)in the main experiments and 26.4 mg/ml (lot. no.: 190494) in the pre-test.

S9 Mix

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution to result in a final protein concentration of 0.75 mg/ml in the cultures. Cofactors were added to the S9 mix to reach following concentrations:

8 mM $MgCl_2$
33 mM KCl
5 mM glucose-6-phosphate
4 mM NADP
in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.

During the experiment, the S9 mix was stored in an ice bath.
The S9 mix preparation was performed according to Ames et al.(1).

PRE-TEST ON TOXICITY

A pre-test was performed in order to determine the concentration range for the mutagenicity experiments. The general culturing and experimental conditions in this pre-test were the same as described below for the mutagenicity experiment.
The following method was used in the pre-test:

XTT-Assay:

The XTT-assay is based on the cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye by hydrogenase activity in active mitochondria. 18 - 20 h after treatment with the test article the XTT-assay was initiated by adding a mixture of XTT-labeling reagent with an electron coupling reagent (PMS). After 4 h of incubation the absorption was read at 450 nm (690 nm reference) using an ELISA reader (SLT, Labinstruments Austria, A-5082 Grödig). The viabilities of the cells were calculated as percentages of the solvent controls and reported tabularly.

DOSE SELECTION

According to the recommendations of the guidelines (see page 5), several concentrations (usually at least four) of the test article should be used. These should yield a concentration-related toxic effect. The highest concentration should produce a low level of survival and the survival in the lowest concentration should approximate the negative control. Relatively insoluble substances should be tested up to their limit of solubility under culture conditions. For freely-soluble nontoxic substances the maximum concentration should be as recommended in the in vitro cytogenetic assay, namely 5 mg/ml or 10 mM. If the maximum concentration is based on cytotoxicity the cloning efficiency should be reduced to less than 50 % and/or culture growth at subcultivation should be at least 20% of the corresponding solvent control.

In the pre-test (XTT-Assay) no toxic effects could be observed up to the highest tested concentraton of 5.0 mg/ml neither with nor without metabolic activation (see table PRE-TEST ON TOXICITY; page 20). Therefore, the main experiments were performed with four concentrations ranging from 250.0 to 5000.0 µg/ml without and with metabolic activation.

The test article was tested with the following concentrations:

Experiment I and II:
with and without S9 mix:
250.0; 1000.0; 2500.0 and 5000.0 µg/ml

EXPERIMENTAL PERFORMANCE

Seeding

Three days old exponentially growing stock cultures (more than 50 % confluent) were trypsinized at 37 °C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium and a single cell suspension was prepared. The trypsin concentration for all subculturing steps was 0.2 % in Ca-Mg-free salt solution (Trypsin: Difco Laboratories, Detroit, USA).

The Ca-Mg-free salt solution was composed as follows (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose	1000 mg
$NaHCO_3$	350 mg

Prior to the trypsin treatment the cells were rinsed with Ca-Mg-free salt solution containing 200 mg/l EDTA (ethylene diamine tetraacetic acid).

The cell suspension was seeded into plastic culture flasks (Greiner, D-72632 Frickenhausen). Approximately 1.5 x 10^6 (single culture) and 5 x 10^2 cells (in duplicate) were seeded in MEM with 10 % FCS (complete medium) for the determination of mutation rate and toxicity, respectively (see experimental scheme).

Treatment

After 24 h the medium was replaced with serum-free medium containing the test article, either without S9 mix or with 50 µl/ml S9 mix. After 4 h this medium was replaced with complete medium following two washing steps with "saline G".

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The "saline G" solution was composed as follows (per litre):

NaCl	8000 mg
KCl	400 mg
Glucose	1100 mg
$Na_2HPO_4 \cdot 7H_2O$	290 mg
KH_2PO_4	150 mg

pH is adjusted to 7.2

Experimental Scheme:

Segment a): Procedure for determination of toxicity
Segment b): Procedure for determination of mutation rates.

Day 1: Subculturing of a log-phase culture which showed an initial spontaneous mutation rate at the beginning of the experiment of 4.0 (experiment I) and 5.1 (experiment II) mutants per 10^6 cells.

a) About 500 cells in 5 ml medium/25 cm^2-plastic-flask for cloning efficiency; in duplicate per experimental point
b) 1.5 x 10^6 cells in 30 ml medium/175 cm^2-plastic-flask for the mutagenicity test, 1 flask per experimental point

Day 2: Treatment of a) and b)

Day 5: Subculturing of b) in 175 cm^2-plastic-flasks
1.5 x 10^6 cells in 30 ml medium/175 cm^2-plastic-flasks

Day 9: Fixation and staining of colonies in a)-flasks
determination of concentration-related cloning efficiency

Day 9: Subculturing of b) in five 80 cm^2-plastic-flasks containing selective medium: mutant selection (about 3-5 x 10^5 cells/flask);
subculturing of b) in two 25 cm^2-flasks for cloning efficiency (about 500 cells/flask)

Day16: Fixation and staining of colonies in b) - derived flasks seeded on day 9 (cloning efficiency).

Day18: Fixation and staining of colonies in b) - derived flasks seeded on day 9 (mutant selection).

The cultures were incubated at 37° C in a humidified atmosphere with 4.5 % CO_2. The colonies were stained with 10 % methylene blue in 0.01 % KOH solution (E. Merck, D-64293 Darmstadt, F.R.G.).

The stained colonies with more than 50 cells were counted. In doubt the colony size was checked with a preparation microscope (Nikon, D-40407 Düsseldorf, F.R.G.).

DATA RECORDING

The data generated were recorded in the raw data. The results are presented in tabular form, including experimental groups with the test article, negative and positive controls.

ACCEPTABILITY OF THE ASSAY

The gene mutation assay is considered acceptable if it meets the following criteria:

a) the numbers of mutant colonies per 10^6 cells found in the negative and/or solvent controls fall within the laboratory historical control data range: 0 - 45 mutants/10^6 cells.

b) the positive control substances must produce a significant increase in mutant colony frequencies.

c) the cloning efficiency (absolute value) of the negative and/or solvent controls must exceed 50 %.

The data of this study comply with the above mentioned criteria [a) and b) see mutation rate, tables III and VI, c) see tables II and V, factor calculated referring to the C.E. of the untreated cultures].

EVALUATION OF RESULTS

A test article is classified as positive if it induces either a concentration-related increase of the mutant frequency or a reproducible and positive response for one of the test points.

A test article producing neither a concentration- related increase of the mutant frequency nor a and reproducible positive response at any of the test points is considered non-mutagenic in this system.

A significant response is described as follows:

The test article is classified as mutagenic if it induces reproducibly with one of the concentrations a mutation frequency that is three times higher than the spontaneous mutation frequency in the experiment.

The test article is classified as mutagenic if there is a reproducible concentration-related increase of the mutation frequency. Such evaluation may be considered also in the case that a threefold increase of the mutant frequency is not observed.

However, in a case by case evaluation this decision depends on the level of the corresponding negative control data. If there is by chance a low spontaneous mutation rate in the range normally found (0 - 45 mutants per 10^6 cells) a concentration-related increase of the mutations within this range has to be discussed.

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BIOMETRY

Since the distribution of mutant cells does not follow known statistical models, an adequate statistical method is not available.

RESULTS

PRE-TEST ON TOXICITY

concentration µg/ml	extinction (450/690 nm) mean	standard deviation	% of the corresponding control *
without S9 mix			
blancs	0.161	0.032	
negative control	1.196	0.112	95.04
medium control	1.250	0.087	100.00
10.0	1.401	0.117	113.87
30.0	1.379	0.092	111.85
100.0	1.407	0.091	114.42
300.0	1.468	0.093	120.02
600.0	1.444	0.110	117.81
1000.0	1.433	0.117	116.80
2500.0	1.451	0.186	118.46
5000.0	1.416	0.065	115.24
with S9 mix			
blancs	0.162	0.012	
negative control	1.391	0.087	101.57
medium control	1.372	0.083	100.0
10.0	1.494	0.081	110.08
30.0	1.505	0.102	110.99
100.0	1.543	0.145	114.13
300.0	1.513	0.130	111.65
600.0	1.576	0.147	116.86
1000.0	1.621	0.224	120.58
2500.0	1.551	0.118	114.79
5000.0	1.489	0.173	109.67

* corrected with the blanc

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TABLES OF RESULTS

WITH

1,3-Propanediol

EXPERIMENT I

Table I: Toxicity data; experiment I

	conc. per ml	S9 mix	number of cells per flask* seeded I/II	found I	found II	mean	CEZ** absolute	CEZ*** relative	cells/ml at 1st sub-cultivation	cell density; % of control
Column	1	2	3	4	5	6	7	8	9	10
Negative control (untreated cells)			527	455	430	442.5	84.0			
Solvent control with medium	0.00 µg	-	527	326	254	290.0	55.0	100.0	3246667	100.0
Positive control with EMS	0.60 mg	-	527	201	247	224.0	42.5	77.2	3080000	94.9
Test article	250.00 µg	-	527	219	284	251.5	47.7	86.7	3793333	116.8
"	1000.00 µg	-	527	29	22	25.5	4.8	8.8	3173333	97.7
"	2500.00 µg	-	527	0	0	0.0	0.0	0.0	2506667	77.2
"	5000.00 µg	-	527	0	0	0.0	0.0	0.0	1226667	37.8
Solvent control with medium	0.00 µg	+	527	296	306	301.0	57.1	100.0	2268667	100.0
Solvent control with DMSO	0.00 µg	+	527	280	279	279.5	53.0	100.0	3200000	100.0
Positive control with DMBA	3.85 µg	+	527	160	157	158.5	30.1	56.7	2080000	65.0
Test article	250.00 µg	+	527	288	270	279.0	52.9	92.7	3420000	150.7
"	1000.00 µg	+	527	286	288	287.0	54.5	95.3	3100000	136.6
"	2500.00 µg	+	527	286	282	284.0	53.9	94.4	2960000	130.5
"	5000.00 µg	+	527	276	276	276.0	52.4	91.7	3093333	136.4

* only colonies with more than 50 cells 7 days after seeding were scored
** CE absolute (value column 6 / value column 3 x 100)
***.CE relative (value column 6 / value column 6 of corresponding control x 100)

Table II: Mutagenicity data; experiment I (part 1: cell survival)

	conc. per ml	S9 mix	number of cells per flask* seeded I/II	found I	found II	mean	factor** calculated	cells seeded	cells*** survived
Column	1	2	3	4	5	6	7	8	9
Solvent control with medium	0.00 µg	-	510	365	424	394.5	0.77	404000	311080
Positive control with EMS	0.60 mg	-	509	280	300	290.0	0.57	404000	230280
Test article	250.00 µg	-	506	376	320	348.0	0.69	384000	264960
"	1000.00 µg	-	509	379	389	384.0	0.75	399000	299250
"	2500.00 µg	-	505	402	357	379.5	0.75	399000	299250
"	5000.00 µg	-	504	342	429	385.5	0.76	402000	305520
Solvent control with medium	0.00 µg	+	506	317	321	319.0	0.63	483000	304290
Solvent control with DMSO	0.00 µg	+	502	350	368	359.0	0.72	478000	344160
Positive control with DMBA	3.85 µg	+	502	238	292	265.0	0.53	426000	225780
Test article	250.00 µg	+	510	379	344	361.5	0.71	486000	345060
"	1000.00 µg	+	500	305	336	320.5	0.64	426000	272640
"	2500.00 µg	+	500	255	260	257.5	0.52	469000	243880
"	5000.00 µg	+	507	317	303	310.0	0.61	435000	265350

* only colonies with more than 50 cells 7 days after seeding in normal medium were scored
** factor calculated (value column 6 / value column 3)
*** cells survived after plating in TG containing medium (value column 8 x value column 7)

Table III: Mutagenicity data; experiment I (part 2: mutation rates)

	conc. per ml	S9 mix	number of mutant colonies per flask* found after plating in TG medium I	II	III	IV	V	mean	standard deviation	mutant** colonies per 10^6 cells
Column	1	2	3	4	5	6	7	8	9	10
Solvent control with medium	0.00 µg	-	7	2	1	2	4	3.2	2.4	10.3
Positive control with EMS	0.60 mg	-	75	86	73	71	92	79.4	9.1	344.8
Test article	250.00 µg	-	1	0	1	1	2	1.0	0.7	3.8
"	1000.00 µg	-	3	2	6	1	1	2.6	2.1	8.7
"	2500.00 µg	-	0	1	0	0	0	0.2	0.4	0.7
"	5000.00 µg	-	0	1	1	1	1	0.8	0.4	2.6
Solvent control with medium	0.00 µg	+	0	0	1	0	0	0.2	0.4	0.7
Solvent control with DMSO	0.00 µg	+	0	1	0	2	0	0.6	0.9	1.7
Positive control with DMBA	3.85 µg	+	116	140	101	134	103	118.8	17.7	526.2
Test article	250.00 µg	+	1	0	1	0	0	0.4	0.5	1.2
"	1000.00 µg	+	2	0	0	0	1	0.6	0.9	2.2
"	2500.00 µg	+	0	4	3	2	3	2.4	1.5	9.8
"	5000.00 µg	+	3	0	1	0	0	0.8	1.3	3.0

* only colonies with more than 50 cells 8 days after seeding in TG medium were scored
** value column 8 (this page) x 10^6 / value column 9 of table II

TABLES OF RESULTS

WITH

1,3-Propanediol

EXPERIMENT II

Table IV: Toxicity data; experiment II

	conc. per ml	S9 mix	number of cells per flask* seeded I/II	found I	found II	mean	CE%** absolute	CE%*** relative	cells/ml at 1st sub-cultivation	cell density; % of control
Column	1	2	3	4	5	6	7	8	9	10
Negative control (untreated cells)			560	472	456	464	82.9			
Solvent control with medium	0.00 µg	-	560	383	303	343.0	61.3	100.0	2457333	100.0
Positive control with EMS	0.60 mg	-	560	216	262	239.0	42.7	69.7	2276667	92.6
Test article	250.00 µg	-	560	305	353	329.0	58.8	95.9	2520000	102.6
"	1000.00 µg	-	560	1	1	1.0	0.2	0.3	2223333	90.5
"	2500.00 µg	-	560	0	0	0.0	0.0	0.0	1846667	75.1
"	5000.00 µg	-	560	0	0	0.0	0.0	0.0	354667	14.4
Solvent control with medium	0.00 µg	+	560	342	318	330.0	58.9	100.0	3453333	100.0
Solvent control with DMSO	0.00 µg	+	560	293	352	322.5	57.6	100.0	3126667	100.0
Positive control with DMBA	3.85 µg	+	560	195	197	196.0	35.0	60.8	2673333	85.5
Test article	250.00 µg	+	560	321	328	324.5	57.9	98.3	2826667	81.9
"	1000.00 µg	+	560	302	310	306.0	54.6	92.7	2720000	78.8
"	2500.00 µg	+	560	268	309	288.5	51.5	87.4	2820000	81.7
"	5000.00 µg	+	560	355	294	324.5	57.9	98.3	2506667	72.6

* only colonies with more than 50 cells 7 days after seeding were scored
** CE absolute (value column 6 / value column 3 x 100)
*** CE relative (value column 6 / value column 6 of corresponding control x 100)

Table V: Mutagenicity data; experiment II (part 1: cell survival)

	conc. per ml	S9 mix	number of cells per flask* seeded I/II	found I	found II	mean	factor** calculated	cells seeded	cells*** survived
Column	1	2	3	4	5	6	7	8	9
Solvent control with medium	0.00 µg	-	511	322	343	332.5	0.65	395950	257368
Positive control with EMS	0.60 mg	-	503	213	198	205.5	0.41	400500	164205
Test article	250.00 µg	-	509	324	332	328.0	0.64	418350	267744
"	1000.00 µg	-	505	310	329	319.5	0.63	410500	258615
"	2500.00 µg	-	502	343	332	337.5	0.67	442500	296475
"	5000.00 µg	-	503	327	342	334.5	0.67	427500	286425
Solvent control with medium	0.00 µg	+	501	393	381	387.0	0.77	470000	361900
Solvent control with DMSO	0.00 µg	+	502	440	427	433.5	0.86	420000	361200
Positive control with DMBA	3.85 µg	+	563	390	395	392.5	0.70	453000	317100
Test article	250.00 µg	+	601	466	446	456.0	0.76	480000	364800
"	1000.00 µg	+	504	412	396	404.0	0.80	391500	313200
"	2500.00 µg	+	572	390	398	394.0	0.69	489000	337410
"	5000.00 µg	+	506	500	487	493.5	0.98	390000	382200

* only colonies with more than 50 cells 7 days after seeding in normal medium were scored
** factor calculated (value column 6 / value column 3)
*** cells survived after plating in TG containing medium (value column 8 x value column 7)

Table VI: Mutagenicity data; experiment II (part 2: mutation rates)

	conc. per ml	S9 mix	number of mutant colonies per flask* found after plating in TG medium I	II	III	IV	V	mean	standard deviation	mutant** colonies per 10^6 cells
Column	1	2	3	4	5	6	7	8	9	10
Solvent control with medium	0.00 µg	-	9	5	4	7	3	5.6	2.4	21.8
Positive control with EMS	0.60 mg	-	125	122	145	131	136	131.8	9.1	802.7
Test article	250.00 µg	-	0	0	2	2	0	0.8	1.1	3.0
"	1000.00 µg	-	1	2	0	0	4	1.4	1.7	5.4
"	2500.00 µg	-	2	3	4	4	3	3.2	0.8	10.8
"	5000.00 µg	-	5	2	5	4	4	4.0	1.2	14.0
Solvent control with medium	0.00 µg	+	0	2	1	0	2	1.0	1.0	2.8
Solvent control with DMSO	0.00 µg	+	0	1	1	0	0	0.4	0.5	1.1
Positive control with DMBA	3.85 µg	+	121	122	119	127	112	120.2	5.4	379.1
Test article	250.00 µg	+	3	4	1	4	2	2.8	1.3	7.7
"	1000.00 µg	+	1	0	1	2	1	1.0	0.7	3.2
"	2500.00 µg	+	3	2	1	0	1	1.4	1.1	4.1
"	5000.00 µg	+	1	3	0	1	1	1.2	1.1	3.1

* only colonies with more than 50 cells 8 days after seeding in TG medium were scored
** value column 8 (this page) x 10^6 / value column 9 of table V

RESULTS AND DISCUSSION

The test article 1,3-Propanediol was assessed for its potential to induce gene mutations at the HPRT locus using V79 cells of the Chinese hamster.

The study was performed in two independent experiments, using identical procedures, both with and without liver microsomal activation.

In both experiments with liver microsomal activation, up to the highest concentration of 5000.0 µg/ml, neither a reduction of the cloning efficiency of the cells nor a reduced cell density at the first subcultivation was observed (see Table I and IV, Column 8 and 10; page 22 and 26).

Without metabolic activation the cloning efficiency of the cells in both experiments showed heavily toxic effects at 1000.0, 2500.0 and 5000.0 µg/ml in both experiments (see Table I and IV, Column 8 and 10; page 22 and 26).
However, the cell density at the first subcultivation was only slightly reduced at 2500.0 µg/ml (77.2 % in experiment I and 75.1 % in experiment II, respectively. At the highest investigated concentration a distinct reduction of the cell density at first subcultivation was observed (37.8 % in experiment I and 14.4 % in experiment II, respectively).

Taking into account the mutation rates found in the groups treated with the test article compared to the negative and solvent controls it can be concluded that no relevant increase of gene mutations was observed. The test article did not induce a reproducible concentration-related increase in mutant colony numbers. The mutant values of the groups treated with the test article were in the range of the negative controls.

The more than three fold increases (compared to the corresponding solvent control) in mutant colony numbers at 2500.0 (experiment I and II) and at 250.0 µg/ml (experiment II) with metabolic activation are considered not to be relevant, since these effects are due to the relatively low level of the corresponding solvent control and the obtained values are within our historical control data range (0-45 mutants/10^6 cells).

In this study in both experiments (with and without S9 mix) the range of the negative controls was from 0.7 up to 21.8 mutants per 10^6 cells; the range of the groups treated with the test article was from 0.7 up to 14.0 mutants per 10^6 cells.

EMS (0.6 mg/ml) and DMBA (3.85 µg/ml) were used as positive controls and showed a distinct increase in induced mutant colonies.

In conclusion, it can be stated that in this mutagenicity assay and under the experimental conditions reported the test article did not induce gene mutations at the HPRT locus in V79 cells.

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Study Director	1x (copy)

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Food and Drug Administration
Memorandum of Telephone conversation

Date: August 26, 2009.
Time: 3:00 pm
Location: 4300 River Road, College Park, MD 20740- RM 2073.
Subject: 1,3-propanediol submission.

Participants:

David Wood	DuPont Tate & Lyle Bio Products (865-458-5681)
Erika Rath	CANTOX

FDA/CFSAN/OFAS

Susan Carlson	HFS 255
Bianca Farias	HFS 255 (ORISE Fellow)
Moraima Ramos Valle	HFS 255

On August 26, 2009, FDA representatives called Mr. Wood, to discuss some issues regarding his GRAS submission for 1,3-propanediol. We explained Mr. Wood the following: the statement of the "availability of the data" needs to follow the language available in the proposed rule; and that since the intended use of the ingredient will be in accordance with 21 CFR 184.1666, a regulation that allows for use in meat and poultry products, we will need either a 4th copy of the submission or a statement saying that the intended use excludes the use in meat and poultry. In addition, we mentioned that the submission does not provide any contact information, so we asked them to please provide contact information as well to clearly state who should be contacted in case we have any questions.

Mr. Wood clarify that the intended use does not include the use in meat or poultry and that they will send a letter providing contact information, a revised availability of information statement, as well as a statement excluding the use of the ingredient in meat and poultry.

DuPont Tate & Lyle BioProducts

198 Blair Bend Drive
Loudon, TN 37774-6562

August 28, 2009

RECEIVED
SEP 08 2009
BY:

Office of Food Additive Safety (HFS-225)
Center for Food Safety and Applied Nutrition
Food and Drug Administration
5100 Paint Branch Pkwy
College Park, MD 20740

RE: **GRAS EXEMPTION CLAIM: 1,3-PROPANEDIOL**

Dear Sir or Madam,

DuPont Tate & Lyle Bio Products previously submitted a GRAS Exemption claim for 1,3-propanediol for use as a food ingredient as an alternate to 1,2-propanediol. Based on discussions with the agency, DuPont Tate & Lyle Bio Products respectfully submits the following additional information to amend Section I **(GRAS EXEMPTION CLAIM)** of the original documents.

B. Name and Address of Notifier

David Wood
DuPont Tate & Lyle Bio Products
198 Blair Bend Road
Loudon, Tennessee
37774
Telephone: (865) 408-1969
Email: David.W.Wood@usa.dupont.com

D. Conditions of Intended Use in Food

1,3-Propanediol is intended for use as a food ingredient as an alternate to 1,2-propanediol. The maximum individual intended food use levels of 1,3-propanediol in food, discussed in greater detail in Section IV.B herein, are the same as the current GRAS use of 1,2-propanediol, as specified in 21 CFR 184.1666, except that DuPont Tate & Lyle Bio Products wishes to exclude from this notification the following categories: meats, fish and poultry, animal food (feed), infant formula, and ingredients used in infant formula. This exclusion is for business considerations only.

F. Availability of Information

The data and information that serve as the basis for the GRAS determination, as well as the information that has become available since the GRAS determination, will be sent to the FDA upon request, or are available for the FDA's review and copying at reasonable times as arranged by DuPont Tate & Lyle Bio Products,198 Blair Bend Road, Loudon, Tennessee 37774 (contact: David Wood, telephone: (865) 408-1969; email: David.W.Wood@usa.dupont.com).

Respectfully,

David Wood
DuPont Tate & Lyle Bio Products
198 Blair Bend Road
Loudon, Tennessee 37774

SUBMISSION END

Reference List for Industry Submission, GRN 000302

Pages	*Author*	*Title*	*Publish Date*	*Publisher*	*BIB_Info*
000069 - 000074	Gingell, Ralph; Kirkpatrick, Jeannie B.; Steup, David R.	Subchronic Toxicity Study of 1, 3-Propanediol Administered Orally to Rats	2000	International Journal of Toxicology	Volume 19, pgs 27-32
000614 - 000620	Pollitt, R.J.; Fowler, B.; Sardharwalla, I.B.; Edwards, M.A.; Gray, R.G.F.	Increased excretion of propan-1,3-diol and 3-hydroxypropionic acid apparently caused by abnormal bacterial metabolism in the gut	1987	Clinica Chimica Acta	Volume 169, pgs 151-158
000623 - 000632	Summerfleld, Frank W.; Tappel, Al L.	Cross-Linking of DNA In Liver And Testes Of Rats Fed 1, 3-Propanediol	1984	Chem-Biol Interactions	Volume 50, pgs 87-96

NA- Not applicable