NTP TECHNICAL REPORT

ON THE

PRENATAL DEVELOPMENTAL TOXICITY STUDIES

OF 4-METHYLCYCLOHEXANEMETHANOL

(CAS NO. 34885-03-5)

IN SPRAGUE DAWLEY (Hsd:Sprague Dawley SD) RATS

(GAVAGE STUDIES)

Scheduled Peer Review Date: 2019

NOTICE

NTP DART-02

National Toxicology Program

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

Although the NTP has conducted numerous Developmental and Reproductive Toxicology (DART) Studies since the inception of the Program, it was only in 2009 that the Program formulated levels of evidence criteria for drawing conclusions as to the developmental and/or reproductive toxicity of a compound based on the conditions employed in the study. The studies described in this DART Report series are designed and conducted to characterize and evaluate the developmental and/or reproductive toxicity of selected substances in laboratory animals. Substances selected for NTP DART studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP DART Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's developmental or reproductive toxicity potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

NTP Technical Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*). Additional information regarding this study may be requested through Central Data Management (CDM) at cdm@niehs.nih.gov. Toxicity data are available through NTP's Chemical Effects in Biological Systems (CEBS) database: https://www.niehs.nih.gov/research/resources/databases/cebs/index.cfm

NTP TECHNICAL REPORT

ON THE

PRENATAL DEVELOPMENTAL TOXICITY STUDIES

OF 4-METHYLCYCLOHEXANEMETHANOL

(CAS NO. 34885-03-5)

IN SPRAGUE DAWLEY (Hsd:Sprague Dawley SD) RATS

(GAVAGE STUDIES)

Scheduled Peer Review Date: 2019

NOTICE

This DRAFT Technical Report is distributed solely for the purpose of predissemination peer review under the applicable information quality guidelines. It has not been formally disseminated by the NTP. It does not represent and should not be construed to represent NTP determination or policy.

NTP DART-02

National Toxicology Program

National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

COLLABORATORS

National Toxicology Program
Evaluated and interpreted results and reported findings

A.T.D. Watson, Ph.D., Study Scientist
S.S. Auerbach, Ph.D.
C.R. Blystone, Ph.D.
B.J. Collins, M.S.P.H.
M.C. Cora, D.V.M.
H.C. Cunny, Ph.D.
J.M. Fostel, Ph.D.
P.M. Foster, Ph.D. (Retired)
M.J. Hooth, Ph.D.
A.P. King-Herbert, D.V.M.
G.E. Kissling, Ph.D. (Retired)
S.A. Masten, Ph.D.
B.S. McIntyre, Ph.D.
K.R. Ryan, Ph.D
K.A. Shipkowski, Ph.D.
K.R. Shockley, Ph.D.
V.L. Sutherland, Ph.D.
S. Waidyanatha, Ph.D.
N.J. Walker, Ph.D.

Southern Research
Conducted studies and evaluated fetal findings

C.D. Hébert, Ph.D., Principal Investigator
K.K. Daniels, Ph.D.
A. Penman, Ph.D.
A.H. Stokes, Ph.D.

Social & Scientific Systems, Inc.
Provided statistical analyses

M.V. Smith, Ph.D., Principal Investigator
L.J. Betz, M.S.
S.F. Harris, M.S.
J.D. Krause, Ph.D.
C.G. Leach, M.S.

Dynamac Corporation
Prepared quality assessment audits

S. Brecher, Ph.D., Principal Investigator
S. Iyer, B.S.
V.S. Tharakan, D.V.M.

CONTRIBUTORS

National Toxicology Program
Provided oversight of report audit and preparation

M.D. Stout, Ph.D.
M.K. Vallant, B.S., M.T.

Vistronix
Prepared data for report

P. Brown, B.S.
H. Gong, M.S.
C. Martini, B.S.
C. Myers, M.S.
N. Sayers, B.S.
M. Shaw, B.S.
R. Whittlesey, M.S.

MRI Global
Conducted pre-test chemistry activities and prepared dose formulations

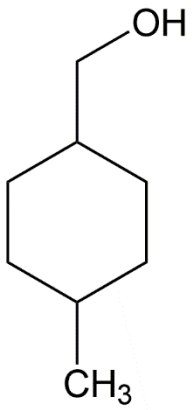
J. Algaier, Ph.D., Principal Investigator
L. Siemann, B.S.
K. Aillon, Ph.D.

Biotechnical Services, Inc.
Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
K.K. Coker, Ph.D.
B.F. Hall, M.S.
L.M. Harper, B.S.
D.C. Serbus, Ph.D.

CONTENTS

ABSTRACT



4-METHYLCYCLOHEXANEMETHANOL

CAS No. 34885-03-5

Chemical Formula: $C_8H_{16}O$ Molecular Weight: 128.21

Synonyms: Cyclohexanemethanol, 4-methyl-; MCHM

4-Methylcyclohexanemethanol (MCHM) is sold as a mixture and is used to reduce impurities in mined coal. On January 9, 2014, an estimated 10,000 gallons of a mixture containing 75% MCHM leaked into the Elk River upstream of the intake for West Virginia American Water Company's Elk River plant. Upon review of the available toxicity literature for MCHM, the Centers for Disease Control and Prevention and the Agency for Toxic Substances and Disease Registry set a drinking water advisory level of 1 parts per million (ppm) for MCHM, and nominated MCHM and other chemicals present in the Elk River spill to the National Toxicology Program (NTP) for toxicity evaluation. Due to the potential for exposure of pregnant women to MCHM and the absence of adequate developmental toxicity data, the NTP conducted studies to characterize the toxicity of MCHM in a regulatory accepted *in vivo* rat model system that assesses the potential harm to the developing conceptus and pregnant rat. Time-mated pregnant Harlan Sprague Dawley rats (Hsd:Sprague Dawley SD) received MCHM (99.8% pure) in corn oil via gavage from implantation on gestation day (GD) 6 to GD 20, the day before expected parturition. The

potential for MCHM to induce overt maternal and fetal toxicity was examined in a dose range-finding study followed by a prenatal developmental toxicity study.

DOSE RANGE-FINDING PRENATAL DEVELOPMENTAL TOXICITY STUDY

Time-mated female rats (n=10/dose level) were administered 0, 150, 300, 600, or 900 mg MCHM/kg body weight per day in corn oil by gavage (2 mL/kg) from GD 6 to GD 20. Control females (0 mg/kg) received corn oil vehicle.

All dams in the 900 mg/kg group were euthanized on GD 8 due to clinical observations indicating overt toxicity (ataxia, cold to touch, clear ocular discharge, excessive salivation, lethargy/hypoactivity, and/or piloerection); three dams from the 600 mg/kg group displayed similar clinical observations and were removed from study. Body weight gain from GD 6 to 21 in the 600 mg/kg group was 44% lower than that of the vehicle control and was associated with a 13% reduction in feed consumption during the same interval. No signs of maternal toxicity were observed in the 150 or 300 mg/kg dose groups.

Dams administered 600 mg/kg displayed higher post-implantation loss (53%) and lower gravid uterine weight. MCHM exposure did not affect the number of live fetuses per litter or fetal sex ratio. However, fetal weights were 12% and 39% lower in the 300 and 600 mg/kg exposure groups, respectively. There were no external malformations or variations attributed to MCHM exposure.

PRENATAL DEVELOPMENTAL TOXICITY STUDY

Due to the maternal toxicity observed at 600 and 900 mg/kg in the dose range-finding study, time-mated female rats (n=25/dose level) were administered 0, 50, 100, 200, or 400 mg MCHM/kg body weight per day in corn oil (2 mL/kg) by gavage from GD 6 to GD 20. Vehicle control animals (0 mg/kg) received corn oil vehicle.

No clinical observations of toxicity were observed in dams in any dose group. Dams administered 400 mg/kg MCHM had significantly lower (11%) mean body weight gains compared to vehicle control dams. Dams administered MCHM had slightly higher feed consumption. Alterations in dam clinical chemistry included

reductions in total protein and globulin concentrations that occurred in a dose-related manner in dams administered ≥100 mg/kg.

Dams administered 400 mg/kg exhibited lower gravid uterine weight. There were no exposure-related effects on the number of live fetuses per litter or fetal sex ratio. Fetal body weight was lower (15%) in the 400 mg/kg group. Visceral and skeletal examination identified several anomalies that were attributed to MCHM exposure. Misshapen adrenal glands (malformation) and discolored adrenal glands and kidneys (variations) were observed in fetuses in the 400 mg/kg group. Malformations and variations of the ribs, sternebrae, and vertebrae were also present in the same exposure group. Findings of misaligned costal cartilage (variation); seventh, right costal cartilage not fused to the sternum (malformation); and an increase in short, cervical supernumerary ribs (SNR) and full, thoracolumbar SNR (malformations) were significantly higher in the 400 mg/kg group. Together, the total incidence of all malformations of the ribs, sternebrae, SNR, and vertebrae were present in 1.0, 1.1, 2.2, 2.8 and 15.7% of fetuses from the 0, 50, 100, 200, and 400 mg/kg groups; these findings were present in 13, 14, 14, 26, and 57% of litters, respectively.

The maternal no-observed-effect level (NOEL) was 50 mg/kg based on MCHM-related changes in clinical chemistry at doses ≥100 mg/kg, reduced maternal body weight gain at 400 mg/kg, and overt toxicity observed at doses ≥600 mg/kg in the dose range-finding study. The minimal MCHM-related changes in maternal clinical chemistry would not be expected to impact fetal development. MCHM-related effects (lower fetal weight and specific and total axial skeletal malformations) were observed in fetuses exposed to 400 mg/kg, indicating a fetal NOEL of 200 mg/kg. These findings suggest a significant margin of exposure (>10^3 fold) exists between both the maternal and fetal NOELs in the rat and the estimated exposure of 0.04 mg/kg/day in pregnant women at the 1 ppm MCHM advisory level.

CONCLUSIONS

Under the conditions of this prenatal study, there was *clear evidence* of developmental toxicity of MCHM in Hsd:Sprague Dawley rats based on reduced fetal weight, adrenal malformations, and increased malformations of the axial skeleton (short cervical SNR, full thoracolumbar SNR, and costal cartilage not fused to the sternum).

These findings occurred in fetuses of dams administered 400 mg/kg and in the absence of overt maternal toxicity.

Summary of Exposure-Related Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Maternal Parameters					
Animals on study	25	25	25	25	25
Number pregnant	23	21	22	19	21
Number died or euthanized moribund	0	0	0	0	0
Clinical Observations	None	None	None	None	None
Body Weight and Feed Consumption[a]					
Necropsy body weight	370.9 ± 5.7**	381.5 ± 4.3	370.1 ± 5.8	368.8 ± 5.6	356.9 ± 4.9
Body weight change GD 6 to 21	131.4 ± 3.9**	141.5 ± 4.3	130.1 ± 4.8	129.6 ± 4.9	116.5 ± 4.1*
Feed consumption GD 6 to 21	20.7 ± 0.26**	21.2 ± 0.35	21.2 ± 0.26	21.9 ± 0.23**	22.1 ± 0.29**
Necropsy Observations	None	None	None	None	None
Clinical Pathology					
Hematology	None	None	None	None	None
Clinical chemistry	None	None	↓ Total protein (6%) ↓ Globulin (10%)	↓ Total protein (5%) ↓ Globulin (9%)	↓ Total protein (8%) ↓ Globulin (10%)
Developmental/Fetal Parameters					
Number of litters examined	23	21	22	19	21
Number of live fetuses evaluated	296	283	279	247	254
Number of live fetuses per litter[b]	12.87 ± 0.64	13.48 ± 0.58	12.68 ± 0.71	13.00 ± 0.81	12.10 ± 0.79
Number of early resorptions	21	16	12	14	20
Number of late resorptions	1	2	0	0	0
Number of dead fetuses	0	0	0	0	0
Number of whole litter resorptions	0	0	1	0	0
Percent post-implantation loss	8.02 ± 2.47	6.54 ± 3.13	8.09 ± 4.50	5.18 ± 1.96	7.76 ± 2.51
Fetal body weight per litter[a]	5.14 ± 0.07**	5.16 ± 0.08	5.14 ± 0.07	4.98 ± 0.09	4.39 ± 0.09**
Male fetal body weight per litter	5.28 ± 0.06**	5.30 ± 0.08	5.28 ± 0.07	5.12 ± 0.09	4.46 ± 0.09**
Female fetal body weight per litter	4.99 ± 0.07**	5.00 ± 0.08	4.98 ± 0.07	4.82 ± 0.09	4.34 ± 0.12**
Gravid uterine weight[a]	91.76 ± 4.05**	96.88 ± 3.61	90.41 ± 4.83	88.57 ± 4.88	75.58 ± 4.20**
External Findings	None	None	None	None	None
Visceral Findings[c]					
Abdominal Viscera					
Adrenal, total, discolored — [V]					
Fetuses	0 (0.0)*	1 (0.4)	0 (0.0)	0 (0.0)	3 (1.2)
Litters	0 (0.00)*	1 (4.76)	0 (0.00)	0 (0.00)	3 (14.29)
Adrenal, total, misshapen — [M]					
Fetuses	0 (0.0)*	1 (0.4)	0 (0.0)	0 (0.0)	3 (1.2)
Litters	0 (0.00)*	1 (4.76)	0 (0.00)	0 (0.00)	3 (14.29)

Summary of Exposure-Related Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Visceral Findings (continued)					
Urinary Tract					
Kidney, total, discolored — [V]					
Fetuses	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	3 (1.2)
Litters	0 (0.00)*	1 (4.76)	0 (0.00)	0 (0.00)	3 (14.29)
Skeletal Findings					
Ribs					
Costal cartilage, total, misaligned — [V]					
Fetuses	0 (0.0)**	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.2)
Litters	0 (0.00)*	0 (0.00)	0 (0.00)	0 (0.00)	2 (9.52)
Costal cartilage, 7th right, not fused to sternum — [M]					
Fetuses	0 (0.0)**	0 (0.0)	0 (0.0)	2 (0.8)	4 (1.6)*
Litters	0 (0.00)**	0 (0.00)	0 (0.00)	1 (5.26)	4 (19.05)*
Sternebrae					
Sternebra, total, unossified or misaligned — [V]					
Fetuses	0 (0.0)**	1 (0.4)	0 (0.0)	0 (0.0)	7 (2.8)**
Litters	0 (0.00)**	1 (4.76)	0 (0.00)	0 (0.00)	6 (28.57)**
Vertebrae					
Thoracic centrum, total, incomplete ossification or unossified — [V]					
Fetuses	0 (0.0)**	1 (0.4)	0 (0.0)	0 (0.0)	4 (1.6)*
Litters	0 (0.00)*	1 (4.76)	0 (0.00)	0 (0.00)	3 (14.29)
Supernumerary rib					
Cervical, total, short — [M]					
Fetuses	0 (0.0)**	0 (0.0)	0 (0.0)	0 (0.0)	6 (2.4)**
Litters	0 (0.00)**	0 (0.00)	0 (0.00)	0 (0.00)	3 (14.29)
Thoracolumbar, total, full — [M]					
Fetuses	2 (0.7)**##	1 (0.4)	6 (2.2)	5 (2.0)	26 (10.2)**##
Litters	2 (8.70)**	1 (4.76)	3 (14.29)	4 (21.05)	7 (33.33)

Level of evidence of developmental toxicity: Clear evidence

* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column);
** P≤0.01.
Statistically significant (P≤0.01) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column) in litter-based analysis of fetuses
[a] Results given in grams. Data are displayed as mean ± standard error.
[b] Data are displayed as mean ± standard error.
[c] Upper row denotes the number of affected fetuses and (%) and lower row the number of affected litters and (%)
GD = Gestation Day
[M] = Malformation
[V] = Variation

EXPLANATION OF LEVELS OF EVIDENCE FOR DEVELOPMENTAL TOXICITY

The NTP describes the results of individual studies of chemical agents and other test articles, and notes the strength of the evidence for conclusions regarding each study. Generally, each study is confined to a single laboratory animal species, although in some instances, multiple species may be investigated under the purview of a single study report. Negative results, in which the study animals do not exhibit evidence of developmental toxicity, do not necessarily imply that a test article is not a developmental toxicant, but only that the test article is not a developmental toxicant under the specific conditions of the study. Positive results demonstrating that a test article causes developmental toxicity in laboratory animals under the conditions of the study are assumed to be relevant to humans, unless data are available that demonstrate otherwise. In addition, such positive effects should be assumed to be primary effects, unless there is clear evidence that they are secondary consequences of excessive maternal toxicity. Given that developmental events are intertwined in the reproductive process, effects on developmental toxicity may be detected in reproductive studies. Evaluation of such developmental effects should be based on the NTP Criteria for Levels of Evidence for Developmental Toxicity.

It is critical to recognize that the "levels of evidence" statements described herein describe only developmental **hazard**. The actual determination of **risk** to humans requires exposure data that are not considered in these summary statements.

Five categories of evidence of developmental toxicity are used 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 cannot be evaluated because of major design or performance flaws **(inadequate study)**. Application of these criteria requires professional judgment by individuals with ample experience and an understanding of the animal models and study designs employed. For each study, conclusion statements are made using one of the following five categories to describe the findings. These categories refer to the strength of the evidence of the experimental results and not to potency or mechanism.

Levels of Evidence for Evaluating Developmental System Toxicity

- **Clear evidence** of developmental toxicity is demonstrated by data that indicate a dose-related[a] effect on one or more of its four elements (embryo-fetal death, structural malformations, growth retardation, or functional deficits) that is not secondary to overt maternal toxicity.
- **Some evidence** of developmental toxicity is demonstrated by dose-related effects on one or more of its four elements (embryo-fetal death, structural malformations, growth retardation, or functional deficits), but where there are greater uncertainties or weaker relationships with regard to dose, severity, magnitude, incidence, persistence, and/or decreased concordance among affected endpoints.
- **Equivocal evidence** of developmental toxicity is demonstrated by marginal or discordant effects on developmental parameters that may or may not be related to the test article.
- **No evidence** of developmental toxicity is demonstrated by data from a study with appropriate experimental design and conduct that are interpreted as showing no biologically relevant effects on developmental parameters that are related to the test article.
- **Inadequate study** of developmental toxicity is demonstrated by a study that, because of major design or performance flaws, cannot be used to determine the occurrence of developmental toxicity.

When a conclusion statement for a particular study is selected, consideration must be given to key factors that would support the selection of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of developmental toxicity studies in laboratory animals, particularly with respect to interrelationships between endpoints, impact of the change on development, relative sensitivity of endpoints, normal background incidence, and specificity of the effect. For those evaluations that may be on the borderline between two adjacent levels, some factors to consider in selecting the level of evidence of developmental toxicity are given below:

- Increases in severity and/or prevalence (more individuals and/or more affected litters) as a function of dose generally strengthen the level of evidence, keeping in mind that the specific manifestation may be different with increasing dose. For example, malformations may be observed at a lower dose level, but higher doses may produce embryo-fetal death.
- Effects seen in many litters may provide stronger evidence than effects confined to one or a few litters, even if the incidence within those litters is high.
- Because of the complex relationship between maternal physiology and development, evidence for developmental toxicity may be greater for a selective effect on the embryo-fetus or pup.
- Concordant effects (syndromic) may strengthen the evidence of developmental toxicity. Single endpoint changes by themselves may be weaker indicators of effect than concordant effects on multiple endpoints related by a common process or mechanism.
- In order to be assigned a level of "clear evidence" the endpoint(s) evaluated should normally show a statistical increase in the deficit, or syndrome, on a litter basis.
- In general, the more animals affected, the stronger the evidence; however, effects in a small number of animals across multiple, related endpoints should not be discounted, even in the absence of statistical significance for the individual endpoint(s). In addition, rare malformations with low incidence, when interpreted in the context of historical controls, may be biologically important.
- Consistency of effects across generations in a multigenerational study may strengthen the level of evidence. However, if effects are observed in the F_1 generation but not in the F_2 generation (or the effects occur at a lesser frequency in the F_2 generation), this may be due to survivor selection for resistance to the effect (i.e., if the effect is incompatible with successful reproduction, then the affected individuals will not produce offspring).
- Transient changes (e.g., pup weight decrements, reduced ossification in fetuses) by themselves may be weaker indicators of an effect than persistent changes.
- Uncertainty about the occurrence of developmental toxicity in one study may be lessened by effects (even if not identical) that are observed in a second species.

- Insights from supportive studies (e.g., toxicokinetics, ADME, computational models, structure-activity relationships) and developmental findings from other *in vivo* animal studies (NTP or otherwise) should be drawn upon when interpreting the biological plausibility of an effect.
- New assays and techniques need to be appropriately characterized to build confidence in their utility: their usefulness as indicators of effect is increased if they can be associated with changes in traditional endpoints.

http://ntp.niehs.nih.gov/go/10003

[a] The term “dose-related” describes any dose relationship, recognizing that the test article-related responses for some endpoints may be non-monotonic due to saturation of exposure or effect, overlapping dose-response behaviors, change in manifestation of the effect at different dose levels, or other phenomena.

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft NTP Prenatal Developmental Toxicity Study Report on 4-methylcyclohexanemethanol in 2019 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 in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of developmental toxicity and other observed toxic responses.

SUMMARY OF PEER REVIEW PANEL COMMENTS

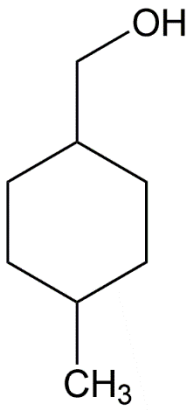
NOTE: A summary of the Peer Review Panel's remarks will appear in a future draft of this report.

OVERVIEW

The Centers for Disease Control and Prevention and the Agency for Toxic Substances and Disease Registry nominated chemicals associated with the Elk River spill in West Virginia to the National Toxicology Program (NTP) for toxicology studies. In response, the NTP performed research to evaluate the toxicity of 4-methylcyclohexanemethanol and the additional chemical components of crude 4-methylcyclohexanemethanol through various short-term studies. The goals of this research program were as follows: 1) to evaluate the teratogenic, immunotoxic, and genotoxic potential of 4-methylcyclohexanemethanol; 2) to identify sensitive biological effects of the spill chemicals and provide additional information about the levels at which there are no adverse effects; and 3) to use efficient, medium-, and high-throughput methods to predict qualitative and quantitative toxicological properties of all chemicals spilled into the Elk River.

These goals were addressed utilizing prenatal developmental toxicity studies in rats, dermal irritation and hypersensitivity studies in mice, short-term toxicogenomic studies in rats, medium-throughput screening assessments in lower animal models, and cell-based high-throughput screening assays. The information and results presented in this Prenatal Developmental Toxicity Study Report are specific to the prenatal developmental toxicity studies on 4-methylcyclohexanemethanol; however, further information about the NTP research program related to the Elk River spill in West Virginia is available at https://ntp.niehs.nih.gov/go/wvspill.

INTRODUCTION



4-METHYLCYCLOHEXANEMETHANOL

CAS No. 34885-03-5

Chemical Formula: $C_8H_{16}O$ Molecular Weight: 128.21

Synonyms: Cyclohexanemethanol, 4-methyl-; MCHM

CHEMICAL AND PHYSICAL PROPERTIES

4-Methylcyclohexanemethanol (MCHM) is an organic compound with a molecular weight of 128.21 g/mol and a density of 0.9074 g/cm^3. MCHM has estimated boiling and melting points of 203.7° C and -12.0° C, respectively, and a vapor pressure of 0.0588 mm Hg at 25° C. It has a low estimated water solubility of 2.024×10^3 mg/L (at 25° C) and an estimated log K_{ow} of 2.55. MCHM is a clear, colorless oil and has been reported to have an alcohol or licorice-like odor (Lide and Milne, 1994; USEPA, 2014; Eastman Chemical, 2015).

PRODUCTION, USE, AND HUMAN EXPOSURE

The production volume of MCHM is not available, but it is typically sold as a crude mixture for use as a frothing agent to remove impurities during the processing of coal. This crude mixture may contain 68% to 89% MCHM with

other components including 4-(methoxymethyl)cyclohexanemethanol (4% to 22%), water (4% to 10%), methyl 4-methylcyclohexanecarboxylate (5%), dimethyl 1,4-cyclohexanedicarboxylate (1%), methanol (1%), and 1,4-cyclohexanedimethanol (1% to 2%) (Eastman Chemical, 2015). Exposure to MCHM may occur via dermal or inhalation routes during the handling or use of the chemical. Of the *cis*- and *trans*- isomers, the *trans*-MCHM is thought to be the dominant source of the licorice odor with an air odor threshold concentration of 0.060 ppb/v (Foreman *et al.*, 2015; Gallagher *et al.*, 2015).

On January 9, 2014, approximately 10,000 gallons of a mixture of chemicals containing predominantly MCHM leaked into the Elk River upstream of the intake for West Virginia American Water Company's Elk River plant, a municipal water source serving approximately 300,000 people in Charleston, WV (West Virginia, 2015). A number of chemicals were identified in the spill, including crude MCHM (estimated at 88.5%), propylene glycol ether, and dipropylene glycol phenyl ether (WVPC, 2014). Of the crude MCHM in the mixture, MCHM alone was estimated to be 75% of the entire 10,000-gallon spill (CDC, 2014). This spill temporarily contaminated 15% of the state's tap water, and prior to flushing, concentrations of MCHM in tap water ranged from less than 10 to 420 ppb; levels of the other components of crude MCHM were not measured (Whelton *et al.*, 2015). Concentrations of MCHM were also measured at the intake (up to 3.35 ppm) and posttreatment (up to 2.4 ppm) (West Virginia, 2015). Exposure to 2.4 ppm is approximately equivalent to 0.07 mg/kg body weight per day for an adult (70 kg) consuming 2 L of water a day, 0.10 mg/kg per day for a pregnant woman (58 kg) consuming 2.5 L of water per day, and 0.24 mg/kg per day for a child (10 kg) consuming 1 L of water a day. Levels of the other components of crude MCHM were not measured.

REGULATORY STATUS

Workplace exposure limits for MCHM are currently unavailable. Immediately following the Elk River spill, the Centers for Disease Control and Prevention (CDC) and the Agency for Toxic Substances and Disease Registry (ATSDR) established a short-term drinking water limit of 1 ppm based on the approximate weight (10 kg) and drinking water intake (1 L) of a child (CDC, 2014). Evaluation of the same data using differing adjustment factors resulted in a calculated health advisory level of 120 ppb (TERA, 2014).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

There are no studies on the absorption, distribution, metabolism, or excretion of MCHM in experimental animals or humans in the literature.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

There are no guideline reproductive or developmental toxicity studies of MCHM in the literature. In a 28-day repeat oral exposure study, no effects were observed on the histology of the testis or ovaries (Eastman Kodak, 1990a). The NTP conducted developmental toxicity studies in alternative models including the nematode (*Caenorhabditis elegans*) and zebrafish (*Danio rerio*). MCHM did not display signs of toxicity in growth, development, feeding, or reproduction in the nematode assay (NTP, 2015a). In zebrafish, no effects on embryonic and early larval development toxicity were observed following exposure to MCHM at concentrations up to 100 μM (12.8 ppm) for 120 hours (NTP, 2015b). Some indications of developmental neurotoxicity were identified in the zebrafish model with reduced embryo reaction to light pulses at concentrations of 35 to 100 μM MCHM (NTP, 2015c).

Humans

There are no studies on the reproductive or developmental toxicity of MCHM in humans in the literature.

GENERAL TOXICITY

Experimental Animals

The acute dermal toxicity of MCHM was evaluated in male and female rats [Crl:CD(SD)BR] administered a single topical dose (0, 2, 6, or 20 mL/kg) (Eastman Kodak, 1990b). A dermal LD_{50} value of 3.6 mL/kg was determined for both males and females.

Oral LD_{50} values for MCHM were determined from an acute oral toxicity study in rats [Crl:CD(SD)BR] administered a single dose (0, 625, 1,250, or 2,500 mg/kg) by oral gavage (Eastman Kodak, 1990b). LD_{50} values of 1,768 and 884 mg/kg were calculated for male and female rats, respectively.

Administration of 0.5 mL MCHM in a guinea pig dermal irritation study (Crl:(HA)BR Hartley; n=5) resulted in strong irritation 24 hours after an occluded single dose (Eastman Kodak, 1990b). In the same guinea pig species, a repeated (nine applications over 11 days) skin test of 0.5 mL MCHM found an increased irritant response with moderate edema, moderate necrosis, and moderate to strong eschars (Eastman Kodak, 1990b). MCHM was not found to be a skin sensitizer in the guinea pig (n=10) after application of 0.05 mL to the footpad and was determined to be a moderate eye irritant in New Zealand white rabbits after a single application of 0.1 mL (Eastman Kodak, 1990b).

In skin irritation and sensitization studies by the NTP, mild skin irritation was induced in BALB/c mice after 3 days of application of 0%, 2%, 20%, or 50% MCHM solution in 4:1 acetone:olive oil (vehicle); hypersensitivity was not induced by the same concentrations (NTP, 2015d, 2019).

In a preliminary study to determine doses for a 28-day toxicity study, two male and two female rats were orally administered 0, 200, 400, or 800 mg MCHM/kg body weight per day via oral gavage for 5 days. Clinical signs of ataxia and decreased activity were observed in both sexes at 800 mg/kg, and one female rat was euthanized (Eastman Kodak, 1990a).

Male and female rats exposed to MCHM (0, 25, 100, or 400 mg/kg per day) by oral gavage, 5 days per week for 4 weeks exhibited increased liver weights, kidney tubular degeneration, and inflammation at 400 mg/kg (Eastman Kodak, 1990a). A no-observed-effect level (NOEL) was set at 100 mg/kg. The results of this study were used by ASTDR to establish the short-term drinking water limit of 1 ppm for MCHM.

MCHM was found to activate a low number of genes associated with gene expression pathways within the rat liver after 5 days of exposure (NTP, 2016). Benchmark dose values of these pathways ranged from 107 to 495 mg/kg per

day. In the same study, no significant changes were observed in kidney gene expression. Other effects observed in the 5-day toxicogenomic study included decreases in thymus weight at 300 and 500 mg/kg per day and an increase in triglycerides and a slight increase in liver weight at 500 mg/kg per day.

Humans

No direct studies of MCHM exposure on human health have been conducted. An indirect measurement of potential human health effects following the Elk River spill was conducted via a survey of 498 households and found that 159 households reported an illness related to the spill (Schade *et al.*, 2015). These health effects consisted of rash or skin irritation, nausea or vomiting, diarrhea or abdominal cramps, headache or dizziness, or eye irritation.

GENETIC TOXICITY

MCHM was evaluated for genetic toxicity by the NTP. It was negative in a micronucleus assay after 5 days of exposure in Hsd:Sprague Dawley SD rats (NTP, 2015e). In addition, MCHM was negative up to 1,000 μg/plate in two *Salmonella typhimurium* strains and one *Escherichia coli* strain (NTP, 2015f).

STUDY RATIONALE

The CDC and the ATSDR nominated MCHM and other chemicals associated with the Elk River spill to the NTP for toxicity evaluation. In response, the NTP conducted a number of studies of relatively short duration to provide information relevant to the potential exposures of the Charleston, WV, residents. Due to the potential exposure to pregnant women and the developing fetus during gestation and concern for potential future exposures, the NTP conducted prenatal developmental toxicity studies in rats to assess potential MCHM toxicity.

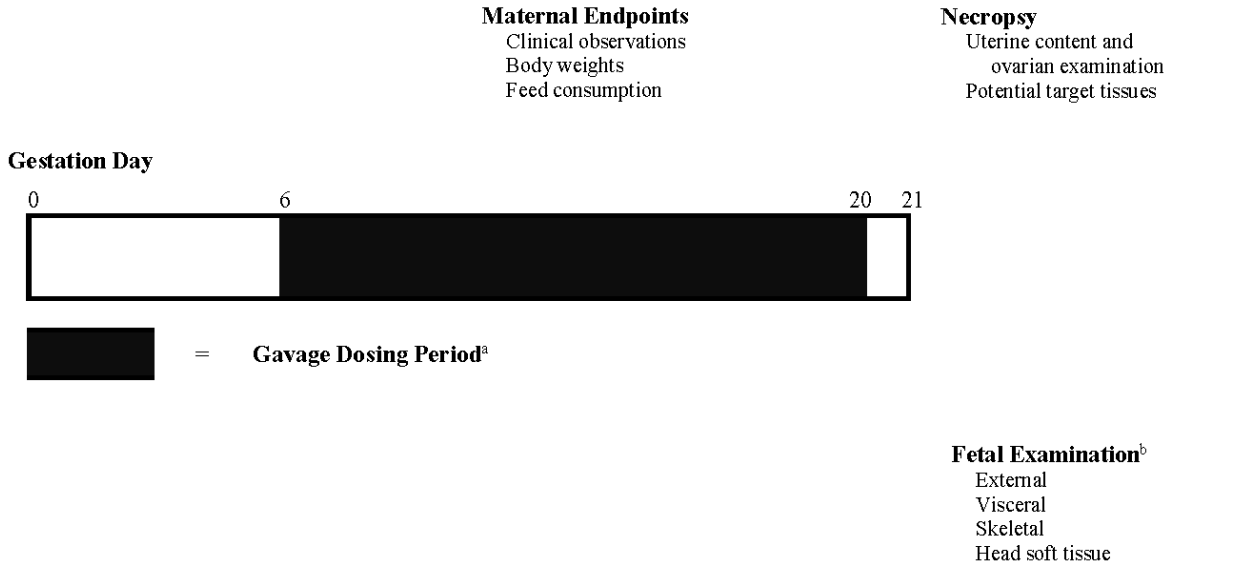
MATERIALS AND METHODS

OVERVIEW OF PRENATAL DEVELOPMENTAL TOXICITY STUDY DESIGNS

Prenatal developmental toxicity studies are conducted to ascertain if *in utero* exposure to a test agent results in embryo-fetal death, structural malformations/variations, growth retardation, or functional deficits that are not secondary to overt maternal toxicity. Overt maternal toxicity has been shown to impact normal embryo-fetal growth and development (e.g., excessively lower maternal body weight gains and lower fetal weights, increased maternal stress in mice, and cleft palate) (Chernoff *et al.*, 1990; USEPA, 1991; Tyl, 2012). However, the presence of maternal toxicity should not *a priori* negate an apparent fetal response. Rather, given the maternal/embryo-fetal interrelationship, fetal findings should be interpreted considering the maternal responses. Conversely, pregnant animals should be administered dose levels of test agent to the extent feasible (or limit dose) to obtain maximal dam and fetal exposure, thereby sufficiently challenging the test system to identify potential developmental hazards (OECD, 2001).

The conduct of a dose range-finding study aids in the determination of dose selection when the potential for test agent-induced maternal toxicity is unknown and can provide preliminary information on embryo-fetal outcomes (e.g., postimplantation loss, changes in fetal weight, external defects) and informs the prenatal developmental toxicity study design. In the prenatal developmental toxicity study, fetal examination is expanded to include examination of the fetal viscera, head (soft tissue and skeletal components), and the skeleton for osseous and cartilaginous defects. Abnormalities are separated into malformations that are permanent structural changes that may adversely affect survival, development, or function or variations that are a divergence beyond the usual range of structural constitution that may not adversely affect survival or health (USEPA, 1991), consistent with that described by Makris *et al.* (2009). The general study design for the dose range-finding and prenatal developmental toxicity studies is presented in Figure 1.

FIGURE 1
Design of Dose Range-Finding and Prenatal Developmental Toxicity Studies in the Rat



[a] Animals were exposed once daily from gestation day (GD) 6 to 20 and necropsied on GD 21.
[b] All fetuses were given an external examination (including inspection of the oral cavity). Fetuses in the prenatal developmental toxicity study were also subjected to visceral and skeletal examinations with approximately 50% of the heads examined for soft tissue alterations.

PROCUREMENT AND CHARACTERIZATION

4-Methylcyclohexanemethanol

4-Methylcyclohexanemethanol (MCHM) was obtained from TCI America (Portland, OR) in one lot (KDY3F). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at MRIGlobal (Kansas City, MO) for the study laboratory at Southern Research (Birmingham, AL) (Appendix C).

The chemical, a clear colorless liquid, was identified as MCHM using Fourier transform infrared (FTIR) and proton and carbon-13 nuclear magnetic resonance spectroscopy, gas chromatography (GC) with mass spectrometry detection. In addition, boiling point, density, and octanol:water partition coefficient were determined. Purity of the test article was determined by elemental analyses and GC with flame ionization detection (FID) and two columns with differing polarities.

Karl Fischer titration indicated 0.209% water. Elemental analyses for carbon, hydrogen, and nitrogen were consistent with the theoretical values for MCHM. GC/FID analysis by one system detected two major peaks with a combined area of 99.97% of the total peak area and no impurities with areas ≥0.05% of the total peak area. The relative areas of the two major peaks indicated that MCHM consisted of 67.99% *cis* and 31.98% *trans* isomers. GC/FID by a second system detected two major peaks with a combined relative area of 99.83% [with relative areas of 67.80% (*cis*) and 32.03% (*trans*) isomers)], and two minor impurities totaling 0.13% of the total peak area. The overall purity of lot KDY3F was determined to be greater than or equal to 99.8%.

Stability studies of the bulk chemical were conducted using GC/FID. These studies indicated that MCHM was stable as a bulk chemical for 2 weeks when stored in amber glass vials under an inert headspace, sealed with aluminum caps with Teflon®-lined septa at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature under an inert headspace in amber glass bottles. Reanalyses of the bulk chemical were performed using FTIR and GC/FID, and no degradation of the bulk chemical was detected.

Corn Oil

Corn oil was obtained from Spectrum Laboratory Products, Inc. (Gardena, CA), in two lots (1CK0678 and 2DG0376) that were used as the vehicle in the dose range-finding and prenatal developmental toxicity studies, respectively. A solubility study of MCHM was performed by the analytical chemistry laboratory; after 17 days under refrigerated conditions, the test article remained soluble in corn oil at up to 600 mg/mL with no remixing required. Both lots contained peroxide levels less than the rejection level of 3 mEq/kg corn oil.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once for the dose range-finding study and once for the prenatal developmental toxicity study by mixing the appropriate amount of MCHM with corn oil. Stability studies of 0.20 and 2.0 mg/mL formulations were performed by the analytical chemistry laboratory using GC/FID. For the 0.20 mg/mL formulation, stability was confirmed for at least 42 days when the formulation was stored under ambient or refrigerated conditions, protected from light, and for 3 hours under simulated animal room conditions. In addition,

for the 2.0 mg/mL formulation, stability was confirmed for at least 44 days when stored under ambient or refrigerated conditions, protected from light.

Analyses of the dose formulations of MCHM were conducted by the analytical chemistry laboratory using GC/FID. During the dose range-finding study, the dose formulations were analyzed once; all four dose formulations were within 10% of the target concentrations (Table C3). Animal room samples of these dose formulations were also analyzed; all four animal room samples were within 10% of the target concentrations. During the prenatal developmental toxicity study, the dose formulations were analyzed once; animal room samples of these dose formulations were analyzed twice (Table C4). All four dose formulations analyzed were within 10% of the target concentrations and all eight animal room samples were within 10% of the target concentrations.

ANIMAL SOURCE

Female Sprague Dawley (Hsd:Sprague Dawley SD) rats for use in the dose range-finding and prenatal developmental toxicity studies were obtained from Envigo (formerly Harlan Laboratories, Inc., Indianapolis, IN). Sexually mature (12 to 13 weeks old) females were time-mated overnight at the vendor and were received on gestation day (GD) 1 or 2 for both the dose range-finding and prenatal developmental toxicity studies. GD 0 was defined as the day positive evidence of mating was observed.

ANIMAL HEALTH SURVEILLANCE

Disease screening was not conducted at the laboratory in the rats; however, rats were obtained from a commercial colony free of the following rat pathogens: Sendai virus, pneumonia virus of mice, sialodacryoadenitis virus, Kilham rat virus, Toolan's H1 virus, rat minute virus, reovirus, rat theilovirus, lymphocytic choriomeningitis virus, hantavirus, mouse adenovirus, rat parvovirus, *Mycoplasma pulmonis*, and *Pneumocystis carinii*.

ANIMAL WELFARE

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by AAALAC International. Studies were

approved by the Southern Research Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

EXPERIMENTAL DESIGN

In the dose range-finding and prenatal developmental toxicity studies, time-mated rats were housed individually, provided NIH-07 feed and water *ad libitum*, and observed at least twice daily for viability (morning and afternoon, with at least 6 hours between observations). Clinical observations were recorded at least once from GD 3 through GD 5 and then daily during dosing (GD 6 through GD 20) until removal (1 to 3 hours after dosing). Dams were weighed daily from GD 3 through GD 21 (dose range-finding study) or on arrival, on GD 3, and daily from GD 6 through GD 21 (prenatal developmental toxicity study). Feed consumption was recorded for GD 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18, and 18 to 21. Details of the study design including animal source and identification, diet, water, husbandry, environmental conditions, euthanasia, necropsy, and fetal evaluations are summarized in Table 1. Information on feed composition and contaminants is provided in Appendix D.

On GD 21, dams were weighed, euthanized with CO_2, and examined for gross lesions of the thoracic and abdominal cavities. The gravid uterus as well as the ovary, liver, and adrenal glands were excised and weighed (organs for prenatal developmental toxicity study only), and any placental findings were recorded. The numbers of implantation sites and corpora lutea visible on the surface of each ovary were recorded. Uterine contents were examined for pregnancy status, and the numbers and locations of all live and dead fetuses and resorptions were recorded. Resorptions were classified as early or late. Early resorptions included a conceptus characterized by a grossly necrotic mass that had no recognizable fetal form and presence of nidation sites ("pregnant by stain"). Late resorptions were characterized by grossly necrotic but recognizable fetal form with placental remnants visible (Suckow *et al.*, 2006; Hayes and Kruger, 2014). Post-implantation loss was calculated as the number of dead and resorbed conceptuses divided by the total number of implantations (multiplied by 100). For each uterus with no macroscopic evidence of implantation, the uterus was stained with 10% (v/v) ammonium sulfide to visualize any possible implantation sites (Salewski, 1964). In the dose range-finding study, the left and right kidney and liver were also weighed.

Adult females that were euthanized moribund, delivered early, or found dead received a gross necropsy that included an examination of the thoracic and abdominal viscera for evidence of dosing trauma or toxicity. The uterus of each female was examined and stained, if necessary, to determine pregnancy status. Dams were not retained for further examination.

Dose Range-Finding Study

Time-mated rats were individually identified by tail marking and randomized by GD 3 body weight stratification into five treatment groups using the Instem™ Provantis® (version 8) electronic data collection system.

Groups of 10 time-mated female rats were administered 0 (vehicle control), 150, 300, 600, or 900 mg MCHM/kg body weight per day, based on the most recent body weight, in corn oil by gavage from GD 6 to GD 20. The top dose of 900 mg MCHM/kg per day was selected based on the limited data available for MCHM, which includes a 5-day oral gavage study of doses up to 800 mg MCHM/kg body weight per day (n=2 animals/sex/dose) that was used to select doses for a subsequent 28-day subchronic study (Eastman Kodak, 1990a). Vehicle control animals received the corn oil vehicle alone; the dosing volume was 2 mL/kg body weight.

On GD 21, fetuses were removed from the uterus, individually weighed (live fetuses only), and examined externally for morphologic alterations, including inspection of the oral cavity for cleft palate. Live fetuses were euthanized by intraperitoneal injection of a commercially available solution of sodium pentobarbital followed by bilateral pneumothorax and/or decapitation. External findings were recorded as developmental variations or malformations. Fetuses were not retained following completion of the external examination.

Prenatal Developmental Toxicity Study

On receipt (GD 1 or 2), time-mated rats were individually identified by tail marking and were randomized by GD 3 body weight stratification into five treatment groups using the Instem™ Provantis® (version 8) electronic data collection system.

Groups of 25 time-mated female rats were administered 0 (vehicle control), 50, 100, 200, or 400 mg MCHM/kg per day, based on the most recent body weight, in corn oil by gavage from GD 6 to GD 20. Vehicle control animals received corn oil vehicle alone; the dosing volume was 2 mL/kg.

On GD 21, fetuses were removed from the uterus, and live fetuses individually weighed. The uteri of animals that did not appear pregnant were examined for nidations (implantation sites) by staining with 0.5% ammonium sulfide (Salewski, 1964; Tyl and Marr, 2006). All fetuses were examined externally for alterations, including inspection of the oral cavity for cleft palate. Live fetuses were subsequently euthanized by oral administration of sodium pentobarbital. Fetal sex was confirmed by inspection of gonads *in situ*. All fetuses were examined for soft tissue alterations under a stereomicroscope (Staples, 1974; Stuckhardt and Poppe, 1984). The heads were removed from approximately half of the fetuses in each litter and fixed in Bouin's solution and subsequently examined by free-hand sectioning (Thompson, 1967). Fetuses were eviscerated, fixed in ethanol, macerated in potassium hydroxide, stained with alcian blue and alizarin red, and examined for subsequent cartilage and osseous alterations (Marr *et al.*, 1992; Tyl and Marr, 2006). External, visceral, and skeletal fetal alterations were recorded as developmental variations or malformations.

Additionally, blood was collected by cardiac puncture from the dams at the time of euthanasia for clinical pathology. Blood was collected into tubes containing potassium EDTA as the anticoagulant for hematology or no anticoagulant for clinical chemistry samples. The contents of the tubes containing anticoagulant were mixed by gentle inversion and maintained at room temperature. Samples obtained for hematology and clinical chemistry were analyzed on the day the samples were collected. Hematology analyses were performed on an Advia 120 analyzer (Siemens Healthcare Diagnostics, Tarrytown, NJ), except manual hematocrit determinations were performed using a microcentrifuge. Platelet, leukocyte, and erythrocyte morphology and nucleated erythrocytes were assessed using smears stained with a Romanowsky-type aqueous stain in a HemaTek Slide Stainer (Siemens Healthcare Diagnostics, Tarrytown, NJ). Serum samples for clinical chemistry analyses were centrifuged, the serum harvested, and analyses performed on a Cobas c501 analyzer (Roche Diagnostic Corp., Indianapolis, IN). The parameters measured are listed in Table 1.

TABLE 1
Experimental Design and Materials and Methods in the Prenatal Developmental Toxicity Gavage Studies of 4-Methylcyclohexanemethanol

Dose Range-Finding Study	Prenatal Developmental Toxicity Study
Study Laboratory Southern Research (Birmingham, AL)	Southern Research (Birmingham, AL)
Strain and Species Sprague Dawley (Hsd:Sprague Dawley SD) rats	Sprague Dawley (Hsd:Sprague Dawley SD) rats
Animal Source Envigo (formerly Harlan Laboratories, Inc., Indianapolis, IN)	Envigo (formerly Harlan Laboratories, Inc., Indianapolis, IN)
Day of Arrival Gestation day (GD) 1 or 2 (August 20, 2014)	GD 1 or 2 (November 12, 14, or 19, 2014)
Average Age on Arrival 13 weeks	12 to 13 weeks
Weight Range at Randomization 206.7 to 266.2 g on GD 3	218.6 to 271.9 g on GD 3
Calendar Day of First Dose (GD 6) and Last Dose (GD 20) GD 6 (August 24 or 25, 2014) and GD 20 (September 7 or 8, 2014); staggered start	GD 6 (November 16, 17, 18, 19, or 23, 2014) and GD 20 (November 30 or December 1, 2, 3, or 7, 2014); staggered start
Duration of Dosing GD 6 to 20, once daily	GD 6 to 20, once daily
Size of Study Groups 10 time-mated females	25 time-mated females
Method of Randomization and Identification Time-mated animals were uniquely identified on day of receipt by ink tail marking and assigned to exposure group by body weight stratified randomization of GD 3 body weights using Instem Provantis® (version 8) electronic data collection system. Each animal was assigned a unique animal number in Provantis®. This number was linked to the respective marking and all data collected during the study was associated with the Provantis® animal number.	Same as dose range-finding study
Animals per Cage 1	1
Diet Irradiated NIH-07 pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed at least weekly	Same as dose range-finding study
Water Tap water (Birmingham, AL, municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as dose range-finding study
Cages Solid bottom polycarbonate (Lab Products, Seaford, DE), changed weekly	Same as dose range-finding study

TABLE 1
Experimental Design and Materials and Methods in the Prenatal Developmental Toxicity Gavage Studies of 4-Methylcyclohexanemethanol

Dose Range-Finding Study	Prenatal Developmental Toxicity Study
Bedding Certified irradiated Sani-Chips® hardwood cage bedding (P.J. Murphy Forest Products Corporation, Montville, NJ), changed weekly	Same as dose range-finding study
Cage Filters Reemay® spunbonded polyester (Andico, Birmingham, AL), changed every 2 weeks	Same as dose range-finding study
Racks Stainless steel (Lab Products, Inc.), changed every 2 weeks	Same as dose range-finding study
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 17/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 19/hour
Doses 0, 150, 300, 600, or 900 mg/kg in corn oil (dosing volume 2 mL/kg)	0, 50, 100, 200, or 400 mg/kg in corn oil (dosing volume 2 mL/kg)
Type and Frequency of Observation of Dams Observed for viability twice daily from GD 3 through GD 20. Clinical observations (out of cage) were recorded at least once from GD 3 through GD 5 and then daily beginning GD 6 at 1 to 3 hours post dose, and at the end of the study. Animals were weighed daily beginning on GD 3. Feed consumption was recorded at 3-day intervals from GD 3 through GD 21.	Observed for viability twice daily from GD 3 through GD 20. Clinical observations (out of cage) were recorded at least once from GD 3 through GD 5 and then daily beginning GD 6 at 1 to 3 hours post dose, and at the end of the study. Animals were weighed on the day of arrival, on GD 3, and daily beginning on GD 6. Feed consumption was recorded at 3-day intervals from GD 3 through GD 21.
Primary Method of Euthanasia 100% CO_2 (dams) or intraperitoneal injection of solution containing sodium pentobarbital followed by bilateral pneumothorax and/or decapitation (fetuses GD ≥ 15)	Same as dose range-finding study

TABLE 1
Experimental Design and Materials and Methods in the Prenatal Developmental Toxicity Gavage Studies of 4-Methylcyclohexanemethanol

Dose Range-Finding Study	Prenatal Developmental Toxicity Study
Necropsy and Postmortem Evaluation of Females	
On GD 21, terminal body weights, kidney and liver weights, and gravid uterine weights were recorded and the uterine contents examined. The number of corpora lutea on each ovary was recorded. The number and location of all fetuses (live or dead) and resorptions (early or late) and the total number of implantation sites were recorded; if no macroscopic evidence of pregnancy, the uterus was stained with a 10% aqueous solution of ammonium sulfide to visualize potential evidence of implantation sites.	On GD 21, terminal body weights and gravid uterine weights were recorded and the uterine contents examined. The number of corpora lutea on each ovary was recorded. The number and location of all fetuses (live or dead) and resorptions (early or late) and the total number of implantation sites were recorded; if no macroscopic evidence of pregnancy, the uterus was stained with a 10% aqueous solution of ammonium sulfide to visualize potential evidence of implantation sites.
For early removals, gross necropsy including an examination of the thoracic and abdominal viscera was performed. The uterus of each dam was examined to determine pregnancy status, or, if no evidence of pregnancy, stained with a 10% aqueous solution of ammonium sulfide to visualize possible early implantation sites.	There were no early removals.
Clinical Pathology	
None	Blood was collected from surviving dams by cardiac puncture at the time of euthanasia for hematology and clinical chemistry analyses. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, platelet, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical Chemistry:*** urea nitrogen, creatinine, glucose, calcium, phosphorus, total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, direct bilirubin, cholesterol, triglycerides, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, and creatine kinase
Fetal Evaluation	
Live fetuses were counted, sexed, weighed, and examined for external morphologic abnormalities that included inspection of the oral cavity for cleft palate.	Live fetuses were counted, sexed, weighed, and examined for external morphologic abnormalities that included inspection of the oral cavity for cleft palate. Placental morphology was also evaluated. Live fetuses were euthanized and then examined for visceral morphologic abnormalities by fresh dissection. The sex of each fetus was confirmed by internal examination. The heads from approximately one half of the fetuses in each litter were fixed, sectioned, and examined. All fetuses were eviscerated, fixed, stained, and examined for skeletal developmental variations, malformations, or other morphologic findings.

STATISTICAL METHODS

In both the dose range-finding study and the main study, statistical analyses were performed on data from pregnant females that survived until the end of the study and were examined on GD 21 and from live fetuses. Statistical analyses were performed using SAS 9.3 (SAS Institute, Cary NC).

Descriptive Statistics

Maternal Parameters: Disposition of pregnant females is presented as the number of animals that were moribund or survived to the end of the study (Tables 4 and 8). Summaries of maternal clinical observations are presented as the total number of animals with the observation and the first day of onset (Tables A1 and B1).

Maternal body weights were measured daily starting at GD 3 and reported as means in Figures 2 and 3 (also see Tables A2 and B2). Terminal maternal body weights at GD 21, were adjusted for gravid uterine weight by subtracting the gravid uterine weight from the dam's body weight. Body weight gains were calculated over each 3-day-interval and from GD 6 to GD 21. Daily feed consumption was averaged over each 3-day-interval and from GD 6 to GD 21. These continuous variables, in addition to gravid uterine weights, other organ weights, hematology, and clinical chemistry were summarized with means and standard errors.

Placental and Fetal Parameters: Data on uterine contents are reported as means and standard errors of counts per dam/litter (corpora lutea, implants, resorptions, dead fetuses) and as total numbers of occurrences (resorptions, dead fetuses) and are presented in Tables 5 and 10. Data from females that were not pregnant or that did not survive to the end of the study were not included. Post-implantation loss is calculated as a percentage of the number of implants per dam. Fetal findings are reported as means and standard errors of counts per litter (numbers of live fetuses, male fetuses, female fetuses), means and standard errors of litter means (fetal weight, male fetal weight, female fetal weight) and total numbers of occurrences (total number of live fetuses). In addition, several calculated variables are reported, including the percentage of live male and female fetuses per litter.

Incidences of morphologic findings from the gross, external, visceral, skeletal and head examinations of pathology of placentae and fetuses are presented as number and percentage of affected fetuses and as number and percentage of affected litters. Fetal findings listing dam and fetus identification number are provided in Table B7.

Analysis of Maternal Parameters and Uterine Contents

Maternal organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Non-normally distributed variables, such as food consumption, hematology, clinical chemistry, and uterine content endpoints, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). For normally distributed and non-normally distributed variables, Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-related trends at $P < 0.01$ to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis.

Fetal body weights were analyzed using mixed effects linear models, with litter as a random effect to account for potential within-litter correlations. To test for a linear trend, dose was entered into the model as its numeric value and its significance was evaluated. For pairwise comparisons with the control group, a second mixed effects model with dose entered into the model as a categorical variable was estimated, followed by the Dunnett (1955)-Hsu (1992) multiple comparisons test.

Analysis of Incidences of Gross Pathology and Morphology Findings

Incidences of gross findings, malformations, and variations in the fetuses were summarized and analyzed as number of litters affected and as number of fetuses affected. Incidences of gross findings, malformations and numbers of litters affected were analyzed using the Cochran-Armitage trend test (Armitage, 1955) and Fisher's exact test (Gart *et al.*, 1979). Incidences of numbers of fetuses affected were analyzed using mixed effects logistic regression in which the litter was a random effect in order to account for potential litter effects (Zorrilla, 1997; Pendergast *et al.*, 2005; Li *et al.*, 2011). For each fetal finding, an initial mixed effects logistic regression model incorporated dose as

its numeric value to assess the significance of an exposure-related trend; a subsequent logistic regression model incorporated dose as a categorical variable to assess the significance of contrasts of each dose group with the control group. To conduct the mixed effects logistic regression analyses, at least one finding was required per dose group and the correlation matrix describing the relationship between litters was required to be "positive definite." If the mixed effects logistic regression failed to converge or did not meet the specified criteria, two separate analyses were used to bracket the true P value. The Cochran-Armitage trend test and Fisher's exact test were used with litter as the experimental unit to calculate the upper limit for the true P value and with fetus as the experimental unit to calculate the lower limit for the true P value.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP developmental and reproductive toxicity studies. However, historical control data are often helpful in interpreting potential exposure-related effects, particularly for uncommon fetal findings that occur at a very low incidence. For meaningful comparisons, the conditions for studies in the historical control database must be generally similar. Significant factors that may affect the background incidences of fetal findings at a variety of sites are diet, sex, strain/stock, route of exposure, study type, and/or laboratory that conducted the study. The NTP historical control database for teratology studies contains all fetal evaluations (e.g., teratology studies or modified one generation studies) for each laboratory. In general, the historical control database for a given study includes studies using the same route of administration and study design. However, historical control data for rats in this Prenatal Developmental Toxicity Study Report contain data from gavage studies conducted at Southern Research. The concurrent controls are included in the historical control data set. NTP historical controls are available online at https://ntp.niehs.nih.gov/go/historical_controls.

QUALITY ASSURANCE METHODS

The dose range-finding and prenatal developmental toxicity studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). Records from these studies were submitted to the NTP Archives. The prenatal developmental toxicity study was audited retrospectively by an independent quality assessment contractor. Separate audits covered completeness and accuracy of the final study

data tables for the dose range-finding and prenatal developmental toxicity studies and a draft of this NTP Prenatal Developmental Toxicity Study Report. Audit procedures and findings are presented in the reports and are on file at the NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Prenatal Developmental Toxicity Study Report.

RESULTS

DOSE RANGE-FINDING STUDY IN RATS

Maternal Findings

Viability and Clinical Observations

Dose-related mortality and clinical observations of toxicity occurred in dams in the 600 and 900 mg/kg groups (Tables 2 and A1). The 900 mg/kg group was euthanized by gestation day (GD) 8 due to overt toxicity. Clinical observations in these animals included ataxia, cold to touch, clear ocular discharge, excessive salivation, lethargy/hypoactivity, and/or piloerection. Additionally, three animals in the 600 mg/kg group were euthanized on GD 9 to 10 with clinical observations similar to those that were observed in the 900 mg/kg group. No clinical observations of toxicity occurred in dams administered 300 mg/kg or less.

TABLE 2
Maternal Disposition of Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	900 mg/kg
Time-mated females	10	10	10	10	10
Pregnant (on GD 21)	9	9	8	7	0
Euthanized moribund — pregnant	0	0	0	3[a]	2[b]
Group removal	0	0	0	0	8[c]
Delivered early	0	1	1	0	0
Non-pregnant (on GD 21)	1	0	1	0	0

[a] Dams were euthanized on GD 9 and GD 10.
[b] Dams were euthanized on GD 8.
[c] Remaining dams from the 900 mg/kg group were removed on GD 8.

Body Weights and Feed Consumption

Average body weights of the 600 and 900 mg/kg groups were 9% and 14% lower than those of the vehicle controls on GD 8, respectively (Table A2). Maternal body weight gain (GD 6 to 21) was 44% lower than that in the vehicle controls in dams administered 600 mg/kg that were necropsied on GD 21 (Figure 2, Table 3). This lower weight gain was associated with the body weight loss that occurred on GD 6 to 9 and towards the end of gestation (Tables 3 and A2). Body weights and body weight gains of the 150 and 300 mg/kg groups were similar to those of the vehicle controls throughout gestation. When adjusted for gravid uterine weight (at necropsy), maternal body weight was 2% and 4% higher in 150 and 300 mg/kg groups, respectively, and 5% lower in the 600 mg/kg group than that of the vehicle controls (Table 5).

Mean feed consumption from GD 6 to 21 was 13% less than that of the vehicle controls in dams administered 600 mg/kg. This overall reduction was due to a 52% lower feed consumption during GD 6 to 9, which was associated with lower body weights and negative weight gains during that interval (Tables 3 and 4). Feed consumption in the 150 and 300 mg/kg groups was similar to or greater than that of the vehicle controls throughout gestation.

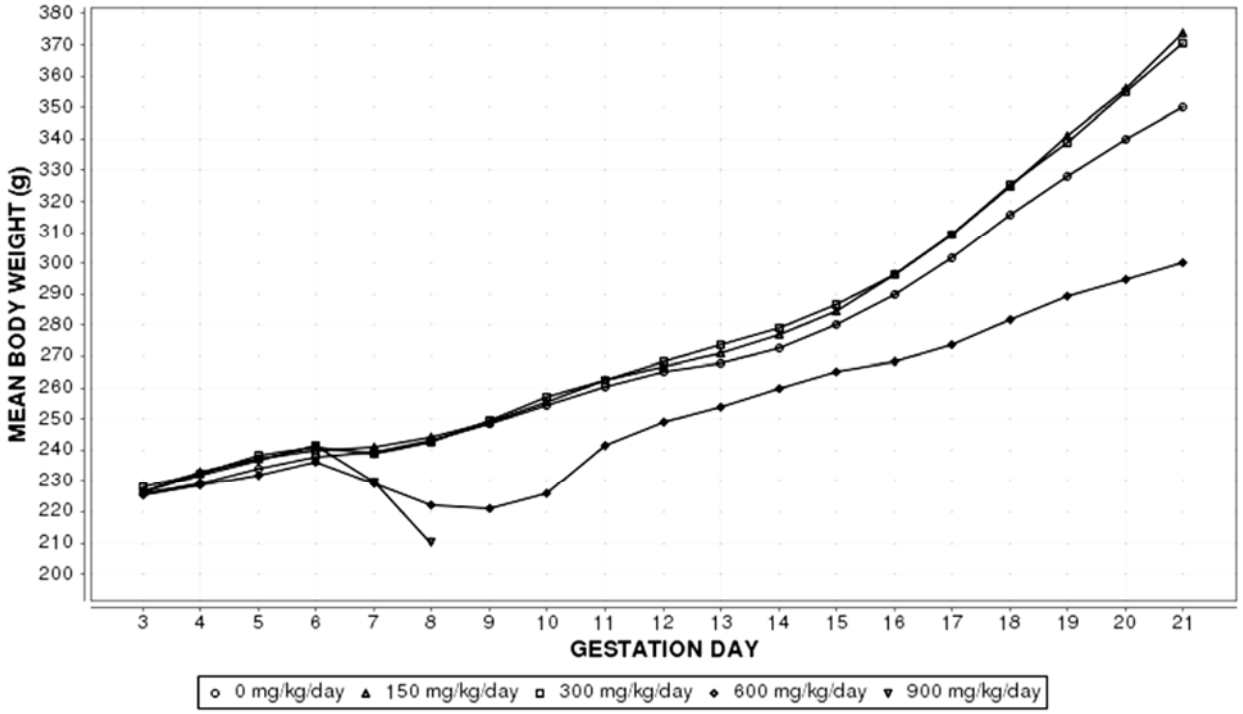


FIGURE 2
Maternal Growth Curves for Pregnant Rats Administered 4-Methylcyclohexanemethanol by Gavage in the Dose Range-Finding Study
Information for statistical significance in maternal weights is provided in Tables 3 and A2.

TABLE 3
Summary of Maternal Body Weight Gains of Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	900 mg/kg
Gestation Day Interval					
6 to 21	112.1 ± 9.4 (9)	132.2 ± 7.8 (9)	129.6 ± 5.0 (9)	63.1 ± 16.8** (7)	
3 to 6	11.8 ± 0.7 (9)	13.7 ± 1.8 (10)	12.9 ± 1.5 (9)	10.7 ± 1.1 (10)	14.9 ± 1.4 (9)
6 to 9	10.5 ± 0.8** (9)	9.2 ± 1.2 (10)	8.6 ± 1.4 (9)	-14.9 ± 5.2** (10)	
9 to 12	16.9 ± 0.9 (9)	18.0 ± 1.0 (10)	19.1 ± 1.1 (9)	18.0 ± 2.4 (7)	
12 to 15	15.2 ± 2.1 (9)	17.4 ± 1.8 (10)	18.3 ± 0.7 (9)	16.2 ± 3.1 (7)	
15 to 18	35.0 ± 3.7 (9)	40.5 ± 2.4 (10)	38.6 ± 2.3 (9)	16.4 ± 5.1** (7)	
18 to 21	34.5 ± 3.8 (9)	49.1 ± 2.9 (9)	45.1 ± 2.4 (9)	18.4 ± 8.0* (7)	

* Statistically significant (P≤0.05) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P≤0.01
[a] Body weight gains for pregnant animals are given in grams. Data are displayed as mean ± standard error. Number of dams weighed is given in parentheses.

TABLE 4
Summary of Maternal Feed Consumption of Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	900 mg/kg
Gestation Day Interval					
6 to 21	19.5 ± 0.33 (9)	20.8 ± 0.56 (9)	21.8 ± 0.51* (9)	17.0 ± 1.07 (7)	
3 to 6	17.8 ± 0.27 (9)	18.6 ± 0.58 (10)	18.6 ± 0.48 (9)	17.0 ± 0.38 (10)	17.8 ± 0.40 (9)
6 to 9	17.7 ± 0.37** (9)	17.7 ± 0.47 (10)	17.4 ± 0.57 (9)	8.4 ± 1.67** (10)	
9 to 12	18.8 ± 0.32 (9)	19.7 ± 0.44 (10)	21.3 ± 0.66* (9)	16.2 ± 0.97 (7)	
12 to 15	18.6 ± 0.45* (9)	19.5 ± 0.44 (9)	22.0 ± 0.63** (9)	18.7 ± 1.37 (7)	
15 to 18	21.5 ± 0.68 (9)	23.3 ± 0.76 (10)	24.3 ± 0.65* (9)	20.2 ± 1.28 (7)	
18 to 21	20.9 ± 0.63 (9)	23.4 ± 0.70 (9)	24.1 ± 0.84* (9)	19.1 ± 1.33 (7)	

* Statistically significant (P≤0.05) trend (by Jonckheere's test) or pairwise comparison (by Shirley's or Dunn's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P≤0.01
[a] Feed consumption for pregnant animals is given in grams/day. Data are displayed as mean ± standard error. Number of dams with feed consumption measured is given in parentheses.

Maternal and Litter Observations

Gross observations in the 600 mg/kg group at necropsy consisted of stomachs distended with food and were considered dose related (this occurred in three moribund euthanasia animals and in two animals at scheduled euthanasia on GD 21) (Table A3). One moribund 600 mg/kg animal was observed to have a dilated bladder; it is not clear if this was related to chemical administration. Increased relative kidney and liver weights in the 600 mg/kg group were considered to be secondary to the decreased body weights of dams in this group (Table A4).

The number of pregnant females and the mean numbers of corpora lutea and implantation sites were similar across the dose groups (Table 5). One 150 mg/kg dam delivered on GD 20, and one 300 mg/kg dam delivered in the morning of GD 21 shortly before scheduled necropsy; these early deliveries were considered incidental and not related to chemical exposure (Tables 2 and 5).

There was an exposure-related effect on percent post-implantation loss as a result of an increase in the number of early and late resorptions and dead fetuses noted in the 600 mg/kg group (Table 5). The numbers of live and dead fetuses and early and late resorptions in the 150 and 300 mg/kg groups were similar to those in the vehicle controls. The fetal sex ratios were similar across the dose groups.

There was an exposure-related effect on fetal weights at doses of 300 and 600 mg/kg (Table 5). Compared to the vehicle controls, mean fetal weights per litter were decreased 12% and 39% in the 300 and 600 mg/kg groups, respectively, and the magnitudes of these effects were similar in male and female fetuses. In the 150 mg/kg group, mean fetal weight per litter was marginally less (4%) than that in the vehicle controls.

TABLE 5
Summary of Uterine Content Data for Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	900 mg/kg
Pregnancy Summary					
Mated females	10	10	10	10	10
Pregnant females	9	10	9	10	9
Pregnant females examined on GD 21[a]	9*	9	8	7	0
Corpora lutea per female[b]	16.67 ± 1.24 (9)	16.20 ± 0.47 (10)	16.22 ± 0.94 (9)[c]	15.71 ± 0.75 (7)	
Implantations per female[b]	11.67 ± 1.67 (9)	13.56 ± 0.94 (9)	14.50 ± 0.82 (8)	15.00 ± 0.79 (7)	
Percent post-implantation loss[b]	9.81 ± 2.71 (9)	5.68 ± 3.36 (9)	2.08 ± 1.40 (8)	53.34 ± 17.46 (7)	
Total resorptions per litter[b]	1.22 ± 0.40 (9)	0.56 ± 0.29 (9)	0.25 ± 0.16 (8)	8.00 ± 2.86 (7)	
Early resorptions per litter[b]	1.11 ± 0.42 (9)	0.56 ± 0.29 (9)	0.13 ± 0.13 (8)	7.57 ± 2.92 (7)	
Late resorptions per litter[b]	0.11 ± 0.11 (9)	0.00 ± 0.00 (9)	0.13 ± 0.13 (8)	0.43 ± 0.30 (7)	
Dead fetuses per litter[b]	0.00 ± 0.00 (9)	0.00 ± 0.00 (9)	0.00 ± 0.00 (8)	0.29 ± 0.29 (7)	
Number of early resorptions	10	5	1	53	
Number of late resorptions	1	0	1	3	
Number of dead fetuses	0	0	0	2	
Number of whole litter resorptions[a]	0*	0	0	2	
Live Fetuses[b]					
Number of live fetuses	94	117	114	47	
Live fetuses per litter	10.44 ± 1.54 (9)	13.00 ± 1.17 (9)	14.25 ± 0.92 (8)	6.71 ± 2.52 (7)	
Live male fetuses per litter	4.56 ± 1.25 (9)	5.44 ± 0.84 (9)	6.63 ± 0.56 (8)	2.71 ± 1.19 (7)	
Live female fetuses per litter	5.89 ± 1.09 (9)	7.56 ± 1.02 (9)	7.63 ± 0.84 (8)	4.00 ± 1.51 (7)	
Percent live male fetuses per litter	37.25 ± 8.84 (9)	42.87 ± 5.60 (9)	47.25 ± 3.98 (8)	34.72 ± 10.21 (5)	
Fetal Weight[d]					
Fetal weight per litter (g)	5.34 ± 0.16 (9)**	5.11 ± 0.11 (9)	4.72 ± 0.07 (8)*	3.28 ± 0.37 (5)**	
Male weight per litter (g)	5.38 ± 0.17 (8)**	5.26 ± 0.11 (9)	4.79 ± 0.08 (8)**	3.71 ± 0.30 (4)**	
Female weight per litter (g)	5.25 ± 0.17 (9)**	4.98 ± 0.09 (9)	4.64 ± 0.08 (8)*	3.23 ± 0.37 (5)**	
Gravid Uterine Weight[e]					
Gravid uterine weight	75.88 ± 10.09 (9)	88.80 ± 7.15 (9)	92.78 ± 4.70 (8)	40.59 ± 14.31 (7)*	
Terminal body weight (g)	349.9 ± 11.9 (9)	373.9 ± 11.5 (9)	370.5 ± 8.1 (9)	300.0 ± 16.1 (7)*	
Adjusted body weight (g)	274.06 ± 2.81 (9)	285.06 ± 5.93 (9)	280.16 ± 6.02 (8)	259.40 ± 5.77 (7)	

Values are reported per litter as mean ± standard error (n) and do not include non-pregnant animals or those that did not survive to end of study.
* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column)
** P≤0.01
[a] Statistical analysis performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests
[b] Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests
[c] One dam in the 300 mg/kg group delivered early on GD 21, shortly before scheduled necropsy, and was examined for corpora lutea.
[d] Statistical analysis performed using a mixed effects linear model with litter as a random effect (trend and pairwise)
[e] Statistical analysis performed by Jonckheere's (trend) and Williams' or Dunnett's (pairwise) tests; adjusted body weight = terminal body weight minus gravid uterine weight

Fetal Findings

External

There were no external morphologic abnormalities attributed to MCHM administration at 150, 300, or 600 mg/kg (Tables A6 and A7).

Dose Selection Rationale for the Prenatal Developmental Toxicity Study in Rats

Due to overt maternal toxicity in dams administered 600 or 900 mg/kg and lack of significant maternal toxicity in those dosed with 300 mg/kg in the dose range-finding study, a high dose of 400 mg/kg was selected for the subsequent prenatal developmental toxicity study. The dose of 400 mg/kg was estimated to induce minimal maternal toxicity. A low dose of 50 mg/kg was included to extend the dose-response assessment. Thus, dose concentrations of 0, 50, 100, 200, and 400 mg/kg were selected for the prenatal developmental toxicity gavage study.

PRENATAL DEVELOPMENTAL TOXICITY STUDY IN RATS

Maternal Findings

Viability and Clinical Observations

No animals were removed from the study prior to schedule necropsy (Table 6). No clinical observations of toxicity were observed in dams at any dose (Table B1).

TABLE 6
Maternal Disposition of Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Time-mated females	25	25	25	25	25
Pregnant (on GD 21)	23	21	22	19	21
Non-pregnant (on GD 21)	2	4	3	6	4

Body Weights and Feed Consumption

There was no effect on absolute body weight from GD 6 to 21. Overall maternal weight gain from GD 6 to 21 was 11% lower than that of vehicle controls in dams in administered 400 mg/kg (Figure 3, Table 7). The overall decrease in 400 mg/kg dams reflects lower (41%) weight gains over the GD 6 to 9 interval, a rebound (25% increase) from GD 9 to 12, and lower (18%) weight gain over the GD 18 to 21 interval. The lower weight gain in late gestation in dams exposed to 400 mg/kg was associated with lower gravid uterine weight. Mean adjusted body weight at necropsy (minus gravid uterine weight) was unaffected with MCHM administration (Table 10).

Overall feed consumption (GD 6 to 21) was marginally increased 6% to 7% in the 200 and 400 mg/kg groups, respectively, compared to the vehicle control group (Table 8). Dams in the 400 mg/kg group displayed an increase in feed consumption (11%) from GD 9 to 12, which corresponds to the increased weight gain observed over the same interval.

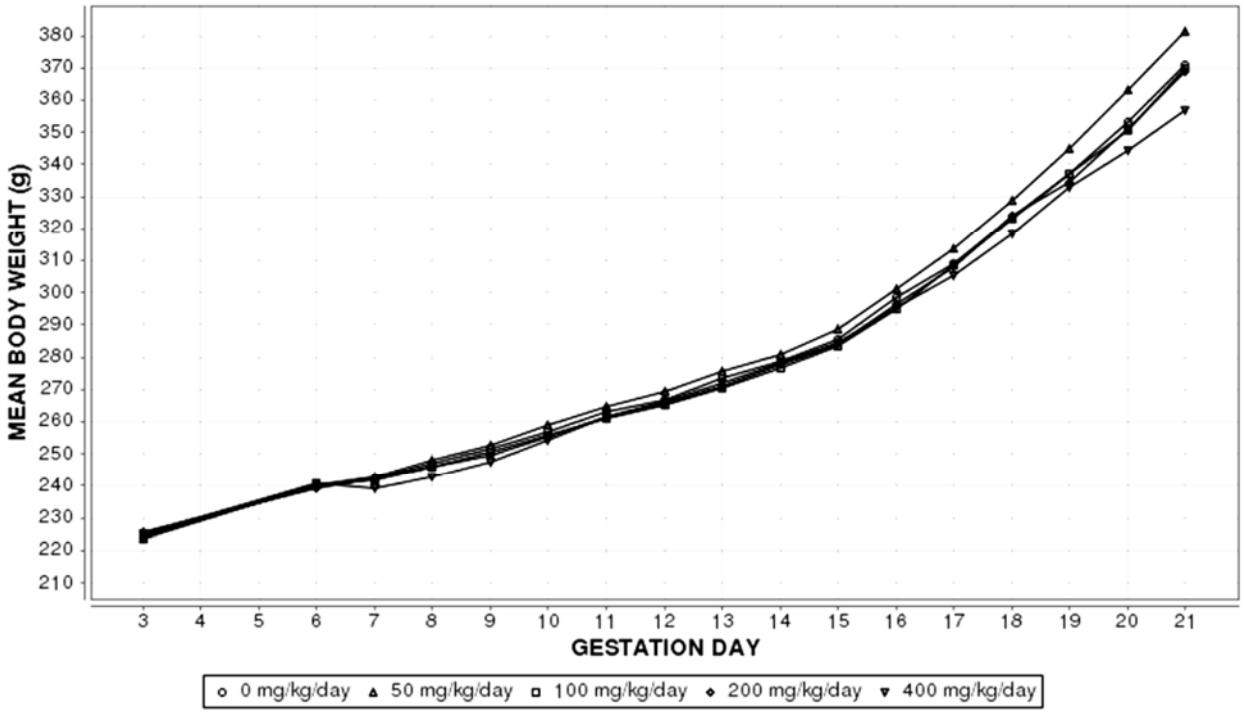


FIGURE 3
Maternal Growth Curves for Pregnant Rats Administered 4-Methylcyclohexanemethanol by Gavage in the Prenatal Developmental Toxicity Study
Information for statistical significance in maternal weights is provided in Tables 7 and B2.

TABLE 7
Summary of Maternal Body Weight Gains of Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Gestation Day Interval					
6 to 21	131.4 ± 3.9** (23)	141.5 ± 4.3 (21)	130.1 ± 4.8 (22)	129.6 ± 4.9 (19)	116.5 ± 4.1* (21)
3 to 6	15.1 ± 2.2 (23)	16.3 ± 2.1 (21)	16.0 ± 2.3 (22)	13.8 ± 1.2 (19)	15.2 ± 1.9 (21)
6 to 9	12.3 ± 0.6** (23)	12.9 ± 1.0 (21)	10.8 ± 0.5 (22)	10.2 ± 0.9 (19)	7.2 ± 0.9** (21)
9 to 12	14.9 ± 0.6** (23)	16.7 ± 0.7 (21)	14.5 ± 0.6 (22)	16.1 ± 1.0 (19)	18.6 ± 0.7** (21)
12 to 15	18.8 ± 0.8 (23)	19.3 ± 0.5 (21)	18.4 ± 1.6 (22)	18.5 ± 1.8 (19)	18.2 ± 0.8 (21)
15 to 18	38.3 ± 1.4 (23)	39.9 ± 3.0 (21)	39.5 ± 1.9 (22)	39.8 ± 2.1 (19)	33.9 ± 1.6 (21)
18 to 21	47.1 ± 1.9** (23)	52.8 ± 2.7 (21)	47.0 ± 2.4 (22)	44.9 ± 2.7 (19)	38.6 ± 2.5* (21)

* Statistically significant (P≤0.05) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P≤0.01
[a] Body weight gains for pregnant animals are given in grams. Data are displayed as mean ± standard error. Number of dams is given in parentheses.

TABLE 8
Summary of Maternal Feed Consumption of Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Gestation Day Interval					
6 to 21	20.7 ± 0.26** (23)	21.2 ± 0.35 (21)	21.2 ± 0.26 (22)	21.9 ± 0.23** (19)	22.1 ± 0.29** (21)
3 to 6	19.0 ± 0.34 (23)	18.6 ± 0.37 (21)	18.9 ± 0.24 (22)	19.1 ± 0.33 (19)	19.5 ± 0.40 (21)
6 to 9	19.0 ± 0.31 (23)	18.9 ± 0.40 (21)	19.0 ± 0.31 (22)	19.8 ± 0.52 (19)	17.9 ± 0.45 (21)
9 to 12	19.5 ± 0.35** (23)	20.3 ± 0.37 (21)	19.8 ± 0.31 (22)	20.3 ± 0.30 (19)	21.7 ± 0.37** (21)
12 to 15	20.2 ± 0.29** (23)	20.3 ± 0.47 (21)	20.4 ± 0.33 (22)	21.1 ± 0.27 (19)	22.2 ± 0.36** (21)
15 to 18	22.6 ± 0.40** (23)	22.6 ± 0.59 (21)	23.5 ± 0.47 (22)	24.5 ± 0.39** (19)	24.9 ± 0.52** (21)
18 to 21	22.4 ± 0.38* (23)	23.7 ± 0.49 (21)	23.3 ± 0.54 (22)	23.7 ± 0.54 (19)	23.8 ± 0.61 (21)

* Statistically significant (P≤0.05) trend (by Jonckheere's test) or pairwise comparison (by Shirley's or Dunn's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P≤0.01
[a] Feed consumption for pregnant animals is given in grams/day. Data are displayed as mean ± standard error. Number of dams with feed consumption measured is given in parentheses.

Maternal and Litter Observations

There were no maternal gross observations at any dose level (Table B3).

Due to the influence of pregnancy status on hematology and clinical chemistry, these data are presented only for pregnant animals (Table B4). Total protein concentrations were decreased (≤ 10%) in the 100, 200, and 400 mg/kg groups; this change was driven by a 10% decrease in the globulin concentrations in the same dose groups (Table 9). A mild decrease (6%) in calcium concentration was observed in the 400 mg/kg group. Calcium is mainly bound to albumin in circulation; therefore, decreases in albumin result in decreases in serum total calcium concentrations (vs. serum ionized calcium). Although there was not a significant decrease in albumin concentration in the 400 mg/kg group, there is a significant downward trend with albumin being 7% lower in the 400 mg/kg group compared to the vehicle control group.

TABLE 9
Selected Clinical Chemistry Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Urea nitrogen (mg/dL)	17.9 ± 0.6** (23)	17.7 ± 0.5 (21)	17.5 ± 0.5 (22)	18.7 ± 1.0 (19)	20.8 ± 0.6** (21)
Glucose (mg/dL)	181.2 ± 10.3** (23)	153.9 ± 7.4 (21)	176.0 ± 11.1 (22)	150.6 ± 10.6* (19)	141.3 ± 9.3** (21)
Calcium (mg/dL)	12.2 ± 0.2* (22)	11.7 ± 0.2 (21)	11.8 ± 0.2 (22)	11.7 ± 0.2 (19)	11.4 ± 0.3* (20)
Total protein (g/dL)	5.17 ± 0.11** (23)	4.93 ± 0.10 (21)	4.84 ± 0.10* (22)	4.91 ± 0.15* (19)	4.74 ± 0.14** (21)
Albumin (g/dL)	3.07 ± 0.09* (23)	2.93 ± 0.07 (21)	2.95 ± 0.10 (22)	3.01 ± 0.12 (19)	2.85 ± 0.13 (21)
Globulin (g/dL)	2.10 ± 0.05** (23)	2.01 ± 0.06 (21)	1.89 ± 0.04** (22)	1.90 ± 0.04** (19)	1.89 ± 0.06** (21)
Triglycerides (mg/dL)	145.5 ± 21.0** (23)	114.8 ± 18.0 (21)	94.6 ± 12.6 (22)	108.4 ± 14.0 (19)	194.6 ± 31.8* (21)
Alkaline phosphatase (IU/L)	138.4 ± 6.9 (23)	142.0 ± 4.7 (21)	131.7 ± 4.2 (22)	134.9 ± 5.4 (19)	157.0 ± 6.2* (21)

* Statistically significant (P<0.05) trend (by Jonckheere's test) or pairwise comparison (by Shirley's or Dunn's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P<0.01
[a] Data are presented as mean ± standard error (number of animals); statistical tests were performed on unrounded data.

Glucose concentrations were decreased in the 200 mg/kg (17%) and 400 mg/kg (22%) groups, and triglyceride concentrations were increased (34%) in the 400 mg/kg group (Table 9). Although the mechanism is not known, these changes may indicate alterations in carbohydrate and lipid metabolism or may be due to differences in feed consumption relative to control animals. Blood urea nitrogen (BUN) was mildly increased (16%) in the 400 mg/kg group, while creatinine concentration was unchanged. A common cause of mildly increased BUN levels is decreased water intake (dehydration); while there are no other hematologic or biochemical changes to support this and water consumption was not assessed, a decrease in water intake was considered the most likely reason for this finding.

Alkaline phosphatase (ALP) activity was elevated (13%) in the 400 mg/kg group (Table 9). This hepatic enzyme may increase due to cholestasis, however, bilirubin was unchanged and the increase in ALP was minimal. The intestinal isoenzyme of ALP (along with the osseous isoenzyme) is the dominant form of serum ALP in rats and food intake is known to have a pronounced effect on serum ALP activity in rats (Pickering and Pickering 1978; Waner and Nyska, 1994). Thus, the minimal increases in ALP activity in the 400 mg/kg group may be due to increased feed consumption that was observed in this dose group during gestation, or alternatively, may represent normal biological variability.

The number of corpora lutea, implantation sites, and fetuses per litter were similar across the dose groups (Table 10). There was no effect on embryo-fetal viability with exposure to MCHM. The fetal sex ratios were similar across the dose groups.

The mean numbers of live fetuses and early/late resorptions were similar across the dose groups. There was a significant decrease (15%) in fetal weights (males and females combined) in the 400 mg/kg group. There was no effect of MCHM exposure on fetal weights in the 50, 100, and 200 mg/kg groups.

TABLE 10
Summary of Uterine Content Data for Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Pregnancy Summary					
Mated females	25	25	25	25	25
Pregnant females	23	21	22	19	21
Pregnant females examined on GD 21[a]	23	21	22	19	21
Corpora lutea per female[b]	17.17 ± 0.43 (23)	16.67 ± 0.85 (21)	17.50 ± 0.70 (22)	18.47 ± 1.11 (19)	16.81 ± 0.63 (21)
Implantations per female[b]	13.83 ± 0.55 (23)	14.33 ± 0.37 (21)	13.23 ± 0.69 (22)	13.74 ± 0.80 (19)	13.05 ± 0.74 (21)
Percent post-implantation loss[b]	8.02 ± 2.47 (23)	6.54 ± 3.13 (21)	8.09 ± 4.50 (22)	5.18 ± 1.96 (19)	7.76 ± 2.51 (21)
Total resorptions per litter[b]	0.96 ± 0.20 (23)	0.86 ± 0.36 (21)	0.55 ± 0.14 (22)	0.74 ± 0.27 (19)	0.95 ± 0.30 (21)
Early resorptions per litter[b]	0.91 ± 0.21 (23)	0.76 ± 0.28 (21)	0.55 ± 0.14 (22)	0.74 ± 0.27 (19)	0.95 ± 0.30 (21)
Late resorptions per litter[b]	0.04 ± 0.04 (23)	0.10 ± 0.10 (21)	0.00 ± 0.00 (22)	0.00 ± 0.00 (19)	0.00 ± 0.00 (21)
Dead fetuses per litter[b]	0.00 ± 0.00 (23)	0.00 ± 0.00 (21)	0.00 ± 0.00 (22)	0.00 ± 0.00 (19)	0.00 ± 0.00 (21)
Number of early resorptions	21	16	12	14	20
Number of late resorptions	1	2	0	0	0
Number of dead fetuses	0	0	0	0	0
Number of whole litter resorptions[a]	0	0	1	0	0
Live Fetuses[b]					
Number of live fetuses	296	283	279	247	254
Live fetuses per litter	12.87 ± 0.64 (23)	13.48 ± 0.58 (21)	12.68 ± 0.71 (22)	13.00 ± 0.81 (19)	12.10 ± 0.79 (21)
Live male fetuses per litter	6.26 ± 0.41 (23)	7.14 ± 0.59 (21)	7.05 ± 0.67 (22)	6.26 ± 0.64 (19)	6.05 ± 0.49 (21)
Live female fetuses per litter	6.61 ± 0.42 (23)	6.33 ± 0.51 (21)	5.64 ± 0.51 (22)	6.74 ± 0.58 (19)	6.05 ± 0.56 (21)
Percent live male fetuses per litter	49.89	51.95	54.80	49.29	51.02
Fetal Weight[c]					
Fetal weight per litter (g)	5.14 ± 0.07 (23)**	5.16 ± 0.08 (21)	5.14 ± 0.07 (21)	4.98 ± 0.09 (19)	4.39 ± 0.09 (21)**
Male weight per litter (g)	5.28 ± 0.06 (23)**	5.30 ± 0.08 (21)	5.28 ± 0.07 (21)	5.12 ± 0.09 (19)	4.46 ± 0.09 (21)**
Female weight per litter (g)	4.99 ± 0.07 (22)**	5.00 ± 0.08 (21)	4.98 ± 0.07 (21)	4.82 ± 0.09 (18)	4.34 ± 0.12 (21)**
Gravid Uterine Weight[d]					
Gravid uterine weight	91.76 ± 4.05 (23)**	96.88 ± 3.61 (21)	90.41 ± 4.83 (22)	88.57 ± 4.88 (19)	75.58 ± 4.20 (21)**
Terminal body weight (g)	370.9 ± 5.7 (23)**	381.5 ± 4.3 (21)	370.1 ± 5.8 (22)	368.8 ± 5.6 (19)	356.9 ± 4.9 (21)
Adjusted body weight (g)	279.10 ± 2.70 (23)	284.61 ± 2.55 (21)	279.65 ± 2.61 (22)	280.23 ± 2.47 (19)	281.34 ± 3.23 (21)

Values are reported per litter as mean ± standard error (n) and do not include non-pregnant animals or those that did not survive to end of study.
(g) = grams
** Statistically significant (P≤0.01) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column)
[a] Statistical analysis performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests
[b] Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests
[c] Statistical analysis performed using a mixed effects linear model with litter as a random effect (trend and pairwise)
[d] Statistical analysis performed by Jonckheere's (trend) and Williams' or Dunnett's (pairwise) tests; adjusted body weight = terminal body weight minus gravid uterine weight

Fetal Findings

External

Low incidences of malformations (one to two fetuses) of absent anus and thread-like tail (occurred in the same fetus), bent tail, and omphalocele were noted. These malformations were observed in fetuses in the 400 mg/kg group but were not considered to be due to MCHM administration (Tables B6 and B7).

Visceral

Visceral malformations and variations were limited to findings in the adrenal glands and kidneys at 400 mg/kg. These findings included misshaped and/or discolored adrenal glands and discolored kidneys (Tables 11 and B6). These three findings occurred together in one 50 mg/kg fetus and in each of three 400 mg/kg fetuses from three different litters (Tables 11, B6, and B8). No other visceral findings were considered to be MCHM-related.

TABLE 11
Summary of Selected Visceral Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Total number of fetuses	296	283	279	247	254
Visceral					
Number of fetuses examined	296	283	279	247	254
Number of litters examined	23	21	21	19	21
Abdominal Viscera					
Adrenal, total, discolored — [V][a]					
Fetuses[b]	0 (0.0)*	1 (0.4)	0 (0.0)	0 (0.0)	3 (1.2)
Litters	0 (0.00)*	1 (4.76)	0 (0.00)	0 (0.00)	3 (14.29)
Adrenal, total, misshapen — [M][a]					
Fetuses	0 (0.0)*	1 (0.4)	0 (0.0)	0 (0.0)	3 (1.2)
Litters	0 (0.00)*	1 (4.76)	0 (0.00)	0 (0.00)	3 (14.29)
Urinary Tract					
Kidney, total, discolored — [V][a]					
Fetuses	0 (0.0)*	1 (0.4)	0 (0.0)	0 (0.0)	3 (1.2)
Litters	0 (0.00)*	1 (4.76)	0 (0.00)	0 (0.00)	3 (14.29)

Upper row denotes number of affected fetuses and (%) and lower row the number of affected litters and (%)

* Statistically significant ($P \leq 0.05$) trend according to the Cochran-Armitage (trend) or Fisher exact (pairwise) tests. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column

[a] Historical incidence for prenatal developmental toxicity gavage studies: Fetuses: 0/1,326 (0%); Litters 0/104 (0%)

[b] Statistical analysis of fetuses performed by mixed effects logistic regression models with litter-based adjustments found no statistically significant trend or pairwise comparison.

[M] = Malformation

[V] = Variation

Head

There were no chemical-related increases in the incidences of variations or malformations of the skull in exposed fetuses (Tables B6 and B7).

Skeletal

Several skeletal anomalies were observed in fetuses in the 400 mg/kg group. These variations and malformations occurred along the axial skeleton and included anomalies in the costal cartilage, sternebrae, and the presence of cervical and thoracolumbar supernumerary ribs (SNR).

The incidence of the seventh, right costal cartilage not fusing to the sternum was increased in the 400 mg/kg group (Tables 12, B6, and B8). Additionally, misaligned costal cartilage was observed in the 4th and 5th right ribs in one fetus and in multiple sites in two fetuses from different litters. When combined, there was a significant increase in the total litter incidences of misaligned costal cartilage in three fetuses from two litters in the 400 mg/kg group.

Increases in the number of unossified 2nd, 5th, and 6th sternebrae were observed in the 400 mg/kg group; in one fetus, this occurred in multiple sites (Tables 12, B6, and B8). In addition, there was an increase in the total incidences of unossified (1st thoracic) or incompletely ossified (12th and 13th thoracic) vertebrae of the thoracic centrum due to the occurrence in four fetuses from three litters in the 400 mg/kg group. The sternal and vertebral ossification delays were consistent with the small size of the affected fetuses and with the reduced fetal weight at this exposure level.

There was an increase in the number of full thoracolumbar and short cervical supernumerary ribs (SNR). These malformations were significantly increased in the 400 mg/kg group (Tables 12 and B6).

Overall, the total incidences of skeletal malformations of the axial skeleton (ribs, sternebrae, SNR, and vertebrae) were increased in an exposure-dependent manner. These malformations exceeded the historical control incidences (both fetal and litter-based) observed in NTP prenatal developmental toxicity studies (Tables 12 and B6).

TABLE 12
Summary of Selected Skeletal Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Total number of fetuses	296	283	279	247	254
Skeletal: Body					
Number of fetuses examined	296	283	279	247	254
Number of litters examined	23	21	21	19	21
Ribs					
Costal cartilage, 4th right, misaligned — [V]					
Fetuses	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Costal cartilage, 5th right, misaligned — [V]					
Fetuses	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Costal cartilage, multiple sites, misaligned — [V]					
Fetuses	0 (0.0)*	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.8)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Costal cartilage, total, misaligned — [V]					
Fetuses	0 (0.0)**	0 (0.0)	0 (0.0)	0 (0.0)	3 (1.2)
Litters	0 (0.00)*	0 (0.00)	0 (0.00)	0 (0.00)	2 (9.52)
Costal cartilage, 7th right, not fused to sternum — [M][a]					
Fetuses	0 (0.0)**	0 (0.0)	0 (0.0)	2 (0.8)	4 (1.6)*
Litters	0 (0.00)**	0 (0.00)	0 (0.00)	1 (5.26)	4 (19.05)*
Sternebrae					
Sternebra, 2nd, unossified — [V]					
Fetuses	0 (0.0)**	1 (0.4)	0 (0.0)	0 (0.0)	4 (1.6)*
Litters	0 (0.00)**	1 (4.76)	0 (0.00)	0 (0.00)	4 (19.05)*
Sternebra, 5th, unossified — [V]					
Fetuses	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	2 (0.8)
Litters	0 (0.00)	1 (4.76)	0 (0.00)	0 (0.00)	2 (9.52)
Sternebra, 6th, unossified — [V]					
Fetuses	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)	2 (0.8)
Litters	0 (0.00)	1 (4.76)	0 (0.00)	0 (0.00)	2 (9.52)
Sternebra, multiple sites, unossified — [V]					
Fetuses	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Sternebra, total, unossified — [V]					
Fetuses	0 (0.0)**	1 (0.4)	0 (0.0)	0 (0.0)	6 (2.4)**
Litters	0 (0.00)**	1 (4.76)	0 (0.00)	0 (0.00)	5 (23.81)*
Sternebra, 4th, misaligned — [V]					
Fetuses	0 (0.0)*	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.8)
Litters	0 (0.00)*	0 (0.00)	0 (0.00)	0 (0.00)	2 (9.52)
Sternebra, total, unossified or misaligned — [V]					
Fetuses	0 (0.0)**	1 (0.4)	0 (0.0)	0 (0.0)	7 (2.8)**
Litters	0 (0.00)**	1 (4.76)	0 (0.00)	0 (0.00)	6 (28.57)**

TABLE 12
Summary of Selected Skeletal Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Total number of fetuses	296	283	279	247	254
Skeletal: Body (continued)					
Number of fetuses examined	296	283	279	247	254
Number of litters examined	23	21	21	19	21
Vertebrae					
Thoracic centrum, total, incomplete ossification — [V]					
Fetuses	0 (0.0)*	1 (0.4)	0 (0.0)	0 (0.0)	3 (1.2)
Litters	0 (0.00)	1 (4.76)	0 (0.00)	0 (0.00)	2 (9.52)
Thoracic centrum, total, incomplete ossification or unossified — [V]					
Fetuses	0 (0.0)**	1 (0.4)	0 (0.0)	0 (0.0)	4 (1.6)*
Litters	0 (0.00)*	1 (4.76)	0 (0.00)	0 (0.00)	3 (14.29)
Supernumerary rib					
Cervical, total, short — [M][b]					
Fetuses	0 (0.0)**	0 (0.0)	0 (0.0)	0 (0.0)	6 (2.4)**
Litters	0 (0.00)**	0 (0.00)	0 (0.00)	0 (0.00)	3 (14.29)
Thoracolumbar, total, short — [V]					
Fetuses	76 (25.8)**	56 (19.8)	79 (28.3)	87 (35.2)*	77 (30.3)
Litters	21 (91.30)	19 (90.48)	17 (80.95)	17 (89.47)	19 (90.48)
Thoracolumbar, total, full — [M][c]					
Fetuses	2 (0.7)**##	1 (0.4)	6 (2.2)	5 (2.0)	26 (10.3)**##
Litters	2 (8.70)**	1 (4.76)	3 (14.29)	4 (21.05)	7 (33.33)*
Ribs, sternebrae, supernumerary rib, or vertebrae, total					
Incomplete ossification or unossified, total — [V]					
Fetuses	0 (0.0)**	2 (0.7)	0 (0.0)	0 (0.0)	9 (3.6)**
Litters	0 (0.00)**	2 (9.52)	0 (0.00)	0 (0.00)	7 (33.33)**
Malformation, total — [M][d]					
Fetuses	3 (1.0)**##	3 (1.1)	6 (2.2)	7 (2.8)	40 (15.8)**##
Litters	3 (13.04)**	3 (14.29)	3 (14.29)	5 (26.32)	12 (57.14)**

Upper row denotes number of affected fetuses and (%) and lower row the number of affected litters and (%)
Statistically significant (P≤0.01) according to mixed effects logistic regression with litter-based adjustments. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
* Statistically significant (P≤0.05) trend according to the Cochran-Armitage (trend) or Fisher exact (pairwise) tests. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P≤0.01
[M] = Malformation
[V] = Variation
Historical incidence for gavage studies:
[a] Fetuses: 2/1,324 (0.15%), range 0% – 0.41%; Litters 2/104 (1.92%), range 0% – 5.56%
[b] Fetuses: 1/1,324 (0.08%), range 0% – 0.35 %; Litters 1/104 (0.96%), range 0% – 4.35%
[c] Fetuses: 14/1,324 (1.06%), range 0.34% – 3.35%; Litters 13/104 (12.5%), range 4.76% – 31.6%
[d] Fetuses: 18/1,324 (1.36%), range 0.68% – 3.35%; Litters 17/104 (16.3%), range 9.5% – 31.6%

DISCUSSION AND CONCLUSIONS

4-Methylcyclohexanemethanol (MCHM) is sold as a crude mixture (containing 68% to 89% MCHM) and is used to remove impurities during the processing of coal (Eastman Chemical, 2016). On January 9, 2014, an estimated 10,000 gallons of a mixture containing 75% MCHM leaked into the Elk River upstream of the intake for the West Virginia American Water Company's Elk River plant (West Virginia, 2015). In response, the Centers for Disease Control and Prevention (CDC) and the Agency for Toxic Substances and Disease Registry recommended a 1 ppm drinking water health advisory level based on the available toxicity data, and nominated MCHM and other chemicals present in the Elk River spill for toxicologic evaluation by the NTP. Due to the potential for exposure of pregnant women to MCHM and the absence of developmental toxicity data, the NTP conducted toxicity studies in Hsd:Sprague Dawley SD rats to evaluate the potential effects of MCHM on pregnant rats and fetal development. The guideline prenatal developmental toxicity studies in this report provide important animal data to address the adequacy of the 1 ppm advisory level in protecting sensitive human populations.

MCHM dose levels selected for the range-finding study were 0, 150, 300, 600, and 900 mg/kg body weight per day. All dams in the 900 mg/kg group and three dams in the 600 mg/kg group displayed clinical signs of overt toxicity and were euthanized moribund and/or removed from the study by gestational day (GD) 10. The remaining 600 mg/kg dams experienced reduced body weight gain during gestation and reduced gravid uterine weight compared to vehicle control dams. Increased post-implantation loss was also observed at this dose, and while not statistically significant, this finding indicates test article-related toxicity to dams and/or pups. No signs of maternal toxicity were present at 300 mg/kg; however, exposure to MCHM resulted in lower fetal body weights in the 300 and 600 mg/kg groups. Thus, based on maternal toxicity at or above 600 mg/kg in the dose range-finding study, 400 mg/kg was selected as the high dose for the prenatal developmental toxicity study.

In the prenatal developmental toxicity study, no signs of overt toxicity were evident in dams following daily gavage of 0, 50, 100, 200, or 400 mg/kg. Total protein concentrations were decreased in dams administered 100 mg/kg or

greater, which was driven by a decrease in globulin concentrations in the same dams. Several serum proteins comprise the globulin fraction and include proteins produced by both B-lymphocytes (immunoglobulins) and hepatocytes (e.g., haptoglobulin, complement, C-reactive protein); however, the mechanism of the decreased globulins in this study is not known and the toxicologic significance of these changes is unclear.

Exposure to MCHM significantly impacted embryo-fetal development. Fetal body weight was decreased in an exposure-dependent manner in both the dose range-finding and prenatal developmental toxicity studies with lower fetal body weight observed at levels greater than or equal to 300 mg/kg. Reduced fetal body weight is a common toxicologic response following *in utero* exposure and is responsible for lowest-observable-adverse-effect levels (LOAEL) in 75% of NTP prenatal developmental toxicity studies conducted in rats (Chernoff *et al.*, 2008). Fetal body weight reductions may also be associated with lower maternal body weight and body weight gain. Such a finding may be secondary to maternal stress and/or toxicity as maternal feed restriction studies in the rat and rabbit demonstrate a relationship between reduced maternal weight gain and lower fetal body weights (Niztsche, 2017). The present study, however, demonstrated that the MCHM-related reduction in fetal body weight occured at exposure levels where the adjusted maternal body weight remained unaffected in the 300 and 400 mg/kg dams.

Examination of visceral organs revealed exposure-related increases in kidney variations as well as adrenal variations and malformations. A total of three fetuses from three litters in the 400 mg/kg group had misshapen adrenals and discolored adrenals and kidneys. MCHM-related effects on the kidney have been previously observed following exposure for 28 days in adult Sprague Dawley rats. Both males (2/5) and females (2/5) displayed proximal tubular degeneration at 400 mg/kg per day and males displayed slight increases in relative kidney weights at 25, 100, and 400 mg/kg per day (Eastman Kodak, 1990a). While no external or skeletal findings were seen in these three fetuses from the current study, one fetus had additional variations (absence of innominate artery, discolored ovary) and a malformation of the left ovary (misshapen). Despite the low incidences of adrenal and kidney findings, these occurred at rates greater than those observed in the historical controls for the Harlan Sprague-Dawley rat used by the NTP in prenatal developmental toxicity studies.

MCHM-related teratogenicity was also evidenced by increased incidences of skeletal malformations. These included exposure-related increases in the incidences of cartilage not fused to the sternum and full thoracolumbar supernumerary ribs (SNR); the latter demonstrating a statistically significant increase in fetuses exposed to 400 mg/kg. Full thoracolumbar SNR were observed in 10% and 33% of fetuses and litters, respectively, in the 400 mg/kg group. Both measures were significantly higher than those in concurrent vehicle controls and exceeded the historical control incidence in fetuses (0.3% to 3.5%) and litters (5% to 32%) observed in Harlan Sprague Dawley rats used in prenatal developmental toxicity studies conducted by the NTP.

Despite varying levels of background incidence, an increase in thoracolumbar SNR is a common finding following *in utero* exposure to a range of dissimilar test agents in the rat and mouse model. Notable examples include methanol (Rogers *et al.*, 1991), tri-*n*-butyltin acetate (Noda *et al.*, 1991), nitrous oxide (Fujinaga *et al.*, 1989), sodium salicylate (Foulon *et al.*, 1999), retinoic acid, valproic acid, and bromoxynil (Rogers *et al.*, 1991; Kawanishi *et al.*, 2003). An increased incidence of SNR has been attributed to alterations of the underlying mechanisms responsible for anteriorization/posteriorization patterning of the axial skeleton (Branch *et al.*, 1996; Connelly and Rogers, 1997; Kawanishi *et al.*, 2003; Wéry *et al.*, 2003). Alternatively, an increase in SNR has been attributed to maternal stress; however, this effect may be species-dependent as maternal restraint induced an elevated incidence of SNR in mice but not in rats (Beyer and Chernoff, 1986). Given the absence of overt maternal stress or toxicity in dams administered 400 mg/kg MCHM, the observed increase in SNR at this dose is likely due to MCHM treatment; however, the underlying mechanism(s) responsible for the increase in SNR cannot be ascertained under the conditions of this study. The exposure-related increase in full thoracolumbar SNR in this study is biologically significant. Full thoracolumbar SNR have been shown to persist through postnatal development following sodium salicylate exposure to Sprague Dawley dams on GD 9 and longitudinal assessment of pups from postnatal day (PND) 1 to PND 54 (Foulon *et al.*, 2000). A similar finding was observed in bromoxynil- (Chernoff *et al.*, 1991) and acetazolamide-induced SNR in mice (Beck, 1983). Therefore, the exposure-related increase of full thoracolumbar SNR in the present study may represent a permanent structural change that is unlikely to be remodeled and resolved with continued growth.

MCHM also resulted in exposure-related increases in other skeletal anomalies including misaligned costal cartilage and unossified or incomplete ossification of the sternebrae and thoracic centra. While these variations may not represent adverse functional deficits *per se*, it is important to note their incidence in conjunction with other endpoints associated with developmental delay. The overall delays in ossification and reduction in fetal body weight suggests that MCHM exposure results in an overall growth retardation. Several studies demonstrate a similar relationship between fetal body weight and ossification delays (Khera, 1981; Daston and Seed, 2007); however, delays in ossification may resolve during subsequent postnatal development as shown in rats exposed to ethylene glycol *in utero* (Marr *et al*., 1992).

Taken together, the data from the range-finding and prenatal developmental toxicity studies demonstrate the potential for MCHM to adversely affect fetal development in the Harlan Sprague Dawley rat. A maternal NOEL of 50 mg/kg was identified based on changes in clinical chemistry observed at doses ≥100 mg/kg, as well as reduced weight gain at 400 mg/kg and overt toxicity observed at 600 and 900 mg/kg in the dose-range finding study. The minimal alterations in clinical chemistry would not be expected to impact fetal development. A fetal NOEL of 200 mg/kg was based on findings of reduced fetal body weight and increased incidence of skeletal malformations at 400 mg/kg. These data provide important context to address the recommended 1 ppm health advisory level for drinking water set by the CDC. Consumption of drinking water containing 1 ppm MCHM represents an equivalent exposure of 0.03 mg/kg in adults (70 kg adult consuming 2 L per day), 0.04 mg/kg/day in pregnant women (58 kg female consuming 2.5 L per day), and 0.1 mg/kg/day in children (10 kg child consuming 1 L per day). Thus, there exists a significant margin of exposure ($>10^3$) between the fetal NOEL of 200 mg/kg identified in the present study and the estimated human exposure at the 1 ppm screening level for MCHM.

CONCLUSIONS

Under the conditions of this prenatal study, there was clear evidence of developmental toxicity of MCHM in Hsd:Sprague Dawley rats based on reduced fetal weight, adrenal malformations, and increased malformations of the axial skeleton (short cervical SNR, full thoracolumbar SNR, and costal cartilage not fused to the sternum). These findings occurred in fetuses of dams administered 400 mg/kg and in the absence of overt maternal toxicity.

REFERENCES

Advanced Chemistry Development (ACD) (1994-2014). SciFinder® Spectrum ID 34885035-HNMR. Predicted NMR data calculated using Advanced Chemistry Development, Inc., Software V11.01. ACD Labs, Toronto, Ontario, Canada.

Allen, C.F.H., Ball, W.L., and Young, D.M. (1933). Dihydro-*p*-tolualdehyde. *Can. J. Res.* **9**, 169-174.

American Chemical Society (ACS) (2014a). SciFinder® Spectrum ID BR08924.

American Chemical Society (ACS) (2014b). SciFinder® Spectrum ID NC_05067.

Armitage, P. (1955). Tests for linear trends in proportions and frequencies. *Biometrics* **11**, 375-386.

Beck, S.L. (1983). Assessment of adult skeletons to detect prenatal exposure to trypan blue in mice. *Teratology* **28**, 271-285.

Beyer, P.E., and Chernoff, N. (1986). The induction of supernumerary ribs in rodents: Role of the maternal stress. *Teratog. Carcinog. Mutagen.* **6**, 419-429.

Branch, S., Rogers, J.M., Brownie, C.F., and Chernoff, N. (1996). Supernumerary lumbar rib: Manifestation of basic alteration in embryonic development of ribs. *J. Appl. Toxicol.* **16**, 115-119.

Centers for Disease Control and Prevention (CDC) (2014). Summary Report of Short-term Screening Level Calculation and Analysis of Available Animal Studies for MCHM. Center for Disease Control and Prevention, Atlanta, GA. <http://emergency.cdc.gov/chemical/MCHM/westvirginia2014/mchm.asp>

Chernoff, N., Setzer, W., Miller, D.B., Rosen, M.B., and Rogers, J.M. (1990). Effects of chemically induced maternal toxicity on prenatal development in the rat. *Teratology* **42**, 651-658.

Chernoff, N., Rogers, J.M., Turner, C.I., and Francis, B.M. (1991). Significance of supernumerary ribs in rodent developmental toxicity studies: Postnatal persistence in rats and mice. *Fundam. Appl. Toxicol.* **17**, 448-453.

Chernoff, N., Rogers, E.H., Gage, M.I., and Francis, B.M. (2008). The relationship of maternal and fetal toxicity in developmental toxicology bioassays with notes on the biological significance of the "no observed adverse effect level." *Reprod. Toxicol.* **25**, 192-202.

Code of Federal Regulations (CFR) **21**, Part 58.

Connelly, L.E., and Rogers, J.M. (1997). Methanol causes posteriorization of cervical vertebrae in mice. *Teratology* **55**, 138-144.

Daston, G., and Seed, J. (2007). Skeletal malformations and variations in developmental toxicity studies: Interpretation issues for human risk assessment. *Birth Defects Res. B. Dev. Reprod. Toxicol.* **80**, 421-424.

Dixon, W.J., and Massey, F.J., Jr. (1957). *Introduction to Statistical Analysis*, 2nd ed., pp. 276-278, 412. McGraw-Hill Book Company, Inc., New York.

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

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

Eastman Chemical Company (2015). Safety Data Sheet: Crude MCHM. Version 3.0, Eastman Chemical Company, Kingsport, TN.

Eastman Chemical Company (2016). Safety Data Sheet: Crude MCHM. Version 3.1, Eastman Chemical Company, Kingsport, Tennessee.

Eastman Kodak Company (1990a). Four-week Oral Toxicity Study of 4-Methylcyclohexane Methanol in the Rat. TX-89-296; HAEL No. 89-0081; Accession. No. 907670. Toxicological Sciences Laboratory, Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY. <http://www.eastman.com/Literature_Center/Misc/Pure_Distilled_MCHM-28-Day_Oral_Feeding_Study.pdf>

Eastman Kodak Company (1990b). Acute Toxicity of 4-Methylcyclohexane Methanol. TX-90-5; HAEL No. 89-0081; Accession No. 907670. Toxicological Sciences Laboratory, Health and Environment Laboratories, Eastman Kodak Company, Rochester, NY. <http://www.eastman.com/Literature_Center/Misc/Pure_Distilled_MCHM-Acute_Toxicity_Battery_Containing_5_Study_Reports.pdf>

Foreman, W.T., Rose. D.L., Chambers, D.B., Crain, A.S., Murtagh, L.K., Thakellapalli, H., and Wang, K.K. (2015). Determination of (4-methylcyclohexyl)methanol isomers by heated purge-and-trap GC/MS in water samples from the 2014 Elk River, West Virginia, chemical spill. *Chemosphere* **131**, 217-224.

Foulon, O., Girard, H., Pallen, C., Urtizberea, M., Repetto-Larsay, M., and Blacker, A.M. (1999). Induction of supernumerary ribs with sodium salicylate. *Reprod. Toxicol.* **13**, 369-374.

Foulon, O., Jaussely, C., Repetto, M., Urtizberea, M., and Blacker, A.M. (2000). Postnatal evolution of supernumerary ribs in rats after a single administration of sodium salicylate. *J. Appl. Toxicol.* **20**, 205-209.

Fujinaga, M., Baden, J.M., and Mazze, R.I. (1989). Susceptible period of nitrous oxide teratogenicity in Sprague-Dawley rats. *Teratology* **40**, 439-444.

Gallagher, D.L., Phetxumphou, K., Smiley, E., and Dietrich, A.M. (2015). Tale of two isomers: Complexities of human odor perception for *cis*- and *trans*-4-methylcyclohexane methanol from the chemical spill in West Virginia. *Environ. Sci. Technol.* **49**, 1319-1327.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *JNCI* **62**, 957-974.

Hayes, A.W., and Kruger, C.L. (2014). *Hayes' Principles and Methods of Toxicology*, 6th ed., pp. 1670-1672. CRC Press, Boca Raton, FL.

Hsu, J.C. (1992). The factor analytic approach to simultaneous inference in the general linear model.. *J. Computat. Graphic. Stat.* **1**, 151-168.

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

Kawanishi, C.Y., Hartig, P., Bobseine, K.L., Schmid, J., Cardon, M., Massenburg, G., and Chernoff, N. (2003). Axial skeletal and Hox expression domain alterations induced by retinoic acid, valproic acid, and bromoxynil during murine development. *J. Biochem. Mol. Toxicol.* **17**, 346–356.

Khera, K.S. (1981). Common fetal aberrations and their teratologic significance: A review. *Toxicol. Sci.* **1**, 13-18.

Li, B., Lingsma, H.F., Steyerberg, E.W., and Lesaffre, E. (2011). Logistic random effects regression models: A comparison of statistical packages for binary and ordinal outcomes. *BMC Med. Res. Methodol.* **11**, 11-17.

Lide, D.R., and Milne, G.W.A., Eds. (1994). Handbook of Data on Organic Compounds, 3rd ed., Vol. I, No. 2228. CRC Press, Inc., Boca Raton, FL.

Makris, S.L., Solomon, H.M., Clark, R., Shiota, K., Barbellion, S., Buschmann, J., Ema, M., Fujiwara, M., Grote, K., Hazelden, K.P., Hew, K.W., Horimoto, M., Ooshima, Y., Parkinson, M., and Wise, D.L. (2009). Terminology of developmental abnormalities in common laboratory mammals (version 2). *Reprod. Toxicol*. **28**, 371-434.

Marr, M.C., Price, C.J., Myers, C.B., and Morrissey, R.E. (1992). Developmental stages of the CD (Sprague-Dawley) rat skeleton after maternal exposure to ethylene glycol. *Teratology* **46**, 169-181.

National Institute of Standards and Technology (NIST) (2008). Mass Spectral Library, Version d.OF; *trans*, ID 18215, *cis*, ID 18228. NIST, Gaithersburg, MD.

National Toxicology Program (NTP) (2015a). West Virginia Chemical Spill: Nematode (*Caenorhabditis elegans*) Toxicity Study. March 2015 Update. National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC. <https://ntp.niehs.nih.gov/ntp/research/areas/wvspill/celegans_wvupdate_march2015_508.pdf>

National Toxicology Program (NTP) (2015b). West Virginia Chemical Spill: Zebrafish Developmental Toxicity Study. June 2015 Update. National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC. <https://ntp.niehs.nih.gov/ntp/research/areas/wvspill/zebrafish_update_june2015_508.pdf>

National Toxicology Program (NTP) (2015c). West Virginia Chemical Spill: Zebrafish Photomotor Response Study. August 2015 Update. National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC. <https://ntp.niehs.nih.gov/ntp/research/areas/wvspill/zebrafish_update_aug2015_508.pdf>

National Toxicology Program (NTP) (2015d). West Virginia Chemical Spill: Mouse Dermal Irritation and Hypersensitivity Study. June 2015 Update. National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC. <https://ntp.niehs.nih.gov/ntp/research/areas/wvspill/mouse_dermal_wvupdate_508.pdf>

National Toxicology Program (NTP) **(**2015e**)**. West Virginia Chemical Spill: 5-Day Toxicogenomic Studies: *In Vivo* Micronucleus Assay Component. February 2015 Update. National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC. <https://ntp.niehs.nih.gov/ntp/research/areas/wvspill/micronucleus_wvfeb2015_508.pdf>

National Toxicology Program (NTP) (2015f). West Virginia Chemical Spill: Bacterial Mutagenicity Study. July 2015 Update. National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC. <https://ntp.niehs.nih.gov/ntp/research/areas/wvspill/bacterial_mutagenesis_july2015_508.pdf>

National Toxicology Program (NTP) (2016). West Virginia Chemical Spill: 5-Day Rat Toxicogenomic Studies. July 2016 Update. National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC. <https://ntp.niehs.nih.gov/ntp/research/areas/wvspill/tgmx_update_july2016_508.pdf>

National Toxicology Program (NTP) (2019). NTP Technical Report on the Dermal Hypersensitivity and Irritancy Studies of 4-Methylcyclohexanemethanol (CAS No. 34885-03-5) and Crude 4-Methylcyclohexanemethanol Administered Topically to Female BALB/c Mice. Immunotoxicity Report Series No. 01. National Institutes of Health, Public Health Service, U.S. Department of Health and Human Services, Research Triangle Park, NC (in preparation).

Nitztsche, D. (2017). Effect of maternal feed restriction on prenatal development in rats and rabbits: A review of published data. *Regulatory Toxicol. Pharmacol.* **90**, 95-103.

Noda, T., Morita, S., Yamano, T., Shijmizu, M., Nakamuru, T., Saitoh, M., and Yamada, A. (1991). Teratogenicity study of tri-*n*-butyltin acetate in rats by oral administration. *Toxicol. Lett.* **55**, 109-115.

Organisation for Economic Co-operation and Development (OECD) (2001). OECD Guideline for the Testing of Chemicals, Test No. 44, Prenatal Developmental Toxicity Study. OECD, Paris.

Pendergast, J.F., Gagne, S.J., and Lindstrom, M.J. (2005). Correlated Binary Data. In *Encyclopedia of Biostatistics*. John Wiley and Sons, London.

Pickering, R.G., and Pickering, C.E. (1978). Studies of rat alkaline phosphatase. II. Some applications of the methods for detecting the isoenzymes of plasma alkaline phosphatase in rats. *Arch. Toxicol*. **39**, 267-287.

Rogers, J.M., Francis, B.M., Barbee, B.D., and Chernoff, N. (1991). Developmental toxicity of bromoxynil in mice and rats. *Fundam. Appl. Toxicol.* **17**, 442-447.

Salewski, E. (1964). Farbemethode zum makroskopischen Nachweis von Implantationsstellen am Uterus der Ratte. Naunyn-Schmiedebergs. *Arch. Exp. Pathol. Pharmakol.* **247**, 367.

Schade, C.P., Wright, N., Gupta, R., Latif, D.A., Jha, A., and Robinson, J. (2015). Self-reported household impacts of large-scale chemical contamination of the public water supply, Charleston, West Virginia, USA. *PLoS One* **10**, e0126744 (doi: 10.1371/journal.pone.0126744).

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

Staples, R.E. (1974). Detection of visceral alterations in mammalian fetuses. *Teratology* **9**, A37-A38.

Stuckhardt, J.L., and Poppe, S.M. (1984). Fresh visceral examination of rat and rabbit fetuses used in teratogenicity testing. *Teratog. Carcinog. Mutagen.* **4**, 181-188.

Suckow, M.A., Weisbroth, S.H., and Franklin, C.L. (2006). *The Laboratory Rat*, 2nd ed., Elsevier, Amsterdam.

Thompson, R.F. (1967). Basic Neuroanatomy. In *Foundations of Physiological Psychology* (R.F. Thompson, Ed.), Chapter 4, pp. 79-82. Harper and Row Publishers, New York.

Toxicology Excellence for Risk Assessment (TERA) (2014). Report of Expert Panel Review of Screening Levels for Exposure to Chemicals from the January 2014 Elk River Spill. Toxicology Excellence for Risk Assessment, West Virginia Testing Assessment Project (WV TAP), West Virginia Division of Homeland Security and Emergency Management, Charleston, WV.

Tyl, R.W. (2012). Commentary on the role of maternal toxicity on developmental toxicity. *Birth Defects Res. B Dev. Reprod. Toxicol.* **95**, 262-266.

Tyl, R.W., and Marr, M.C. (2006). Developmental toxicity texting – methodology. In *Developmental and Reproductive Toxicology* (R.D. Hood, Ed.), 2nd ed., pp. 201-261. Taylor and Francis Group, New York.

United States Environmental Protection Agency (USEPA) (1991). Guidelines for Developmental Toxicity Risk Assessment. EPA Document No. EPA/600/FR-91/001. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC.

United States Environmental Protection Agency (USEPA) (2014). Estimation Programs Interface Suite™ for Microsoft Windows©, Ver. 4.1. United States Environmental Protection Agency, Washington, DC. <https://www.epa.gov/tsca-screening-tools/estimation-program-interface-epi-suite-tm-program-modifications-new-features>

Waner, T., and Nyska, A. (1994). The influence of fasting on blood glucose, triglycerides, cholesterol, and alkaline phosphatase in rats. *Vet. Clin. Pathol.* **23**, 78-80.

Wéry, N., Narotsky, M.G., Pacico, N., Kavlock, R.J., Picard, J.J., and Gofflot, F. (2003). Defects in cervical vertebrae in boric acid-exposed rat embryos are associated with anterior shifts of *hox* gene expression domains. *Birth Defects Res. Part A - Clin. Mol. Teratol.* **67**, 59-67.

West Virginia (2015). After Action Review: Emergency Response to January 9, 2014, Freedom Industries Chemical Leak. State of West Virginia: Office of the Governor; Homeland Security and Emergency Management; and West Virginia National Guard, Charleston, WV. <http://www.governor.wv.gov/Documents/After%20Action%20Review.PDF> Accessed September 21, 2016.

West Virginia Poison Center (WVPC) (2014). Factsheet: Freedom Industries, Elk River Chemical Spill, February 10, 2014. West Virginia Poison Center (Charleston, WV) and Mid-Atlantic Center for Children's Health and the Environment, Georgetown University, Washington, DC. <http://www.legis.state.wv.us/legisdocs/2014/committee/interim/water/Documents/Bureau%20for%20Public%20Health%20Packet.pdf> Accessed September 21, 2016.

Whelton, A.J., McMillan, L., Connell, M., Kelley, K.M., Gill, J.P., White, K.D., Gupta, R., Dey, R., and Novy, C. (2015). Residential tap water contamination following the freedom industries chemical spill: Perceptions, water quality, and health impacts. *Environ. Sci. Technol.* **49**, 813-823.

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

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

Williams, D.A. (1986). A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. *Biometrics* **42**, 183-186.

Zorrilla, E.P. (1997). Multiparous species present problems (and possibilities) to developmentalists. *Dev. Psychobiol.* **30**, 141-150.

APPENDIX A
SUMMARY OF FINDINGS IN RATS IN THE DOSE RANGE-FINDING GAVAGE STUDY OF 4-METHYLCYCLOHEXANEMETHANOL

TABLE A1
Summary of Clinical Observations for Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	900 mg/kg
Pregnant Rats					
n	9	10	9	10	9
Aggressive	0	0	0	1 (GD 10)	0
Alopecia	0	0	1 (GD 18)	0	0
Ataxia, no grade recorded	0	0	0	7 (GD 7)	6 (GD 7)
Cold to touch	0	0	0	1 (GD 8)	5 (GD 7)
Discharge, eye, bilateral, clear	0	0	0	0	6 (GD 7)
Discharge, nose/snout, brown	2 (GD 19)	5 (GD 18)	3 (GD 18)	2 (GD 18)	0
Discharge, nose/snout, clear	0	0	0	3 (GD 15)	1 (GD 7)
Discharge, nose/snout, red	1 (GD 17)	0	0	3 (GD 14)	0
Discharge, vagina, brown	0	0	0	1 (GD 20)	0
Discharge, vagina, red	1 (GD 20)	0	0	2 (GD 14)	0
Hunched	0	0	0	2 (GD 10)	0
Hypoactivity	0	0	0	3 (GD 7)	4 (GD 7)
Lethargic	0	0	0	0	3 (GD 7)
Piloerection	0	0	0	3 (GD 9)	5 (GD 7)
Salivation	0	0	0	1 (GD 7)	2 (GD 7)
Non-pregnant Rats					
n	1	0	1	0	1
Ataxia, no grade recorded	0	0	0	0	1 (SD 2)
Piloerection	0	0	0	0	1 (SD 2)

[a] Cumulative number of animals with the observation and the first day of observation onset (displayed in parentheses). n = number of animals; GD = gestation phase; SD = study phase for females that were not pregnant.

TABLE A2
Summary of Mean Maternal Body Weights of Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg		150 mg/kg			300 mg/kg		
	Weight (g)	N[b]	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N
GD3	226.0 ± 4.2	9	226.2 ± 4.1	100.09	10	227.9 ± 4.8	100.84	9
GD4	229.1 ± 4.8	9	232.7 ± 4.4	101.57	10	232.4 ± 4.4	101.44	9
GD5	234.0 ± 4.5	9	236.9 ± 4.5	101.24	10	238.4 ± 4.3	101.88	9
GD6	237.8 ± 4.3	9	239.8 ± 4.4	100.84	10	240.8 ± 4.8	101.26	9
GD7	239.5 ± 4.7*	9	241.1 ± 4.7	100.67	10	238.9 ± 4.8	99.75	9
GD8	243.3 ± 4.3**	9	244.4 ± 5.0	100.45	10	242.8 ± 4.0	99.79	9
GD9	248.4 ± 4.5**	9	249.1 ± 4.7	100.28	10	249.4 ± 4.1	100.40	9
GD10	254.5 ± 5.2*	9	255.7 ± 5.2	100.47	10	257.0 ± 4.9	100.98	9
GD11	260.1 ± 4.8	9	262.3 ± 5.5	100.85	10	262.5 ± 4.7	100.92	9
GD12	265.2 ± 5.2	9	267.0 ± 5.5	100.68	10	268.5 ± 4.9	101.24	9
GD13	267.9 ± 5.6	9	271.3 ± 5.3	101.27	10	273.9 ± 5.0	102.24	9
GD14	272.4 ± 5.8	9	277.1 ± 5.6	101.73	10	278.9 ± 5.1	102.39	9
GD15	280.4 ± 6.5	9	284.4 ± 6.3	101.43	10	286.8 ± 5.2	102.28	9
GD16	289.7 ± 7.3	9	296.1 ± 6.4	102.21	10	296.4 ± 5.6	102.31	9
GD17	301.6 ± 8.6	9	309.1 ± 7.3	102.49	10	309.2 ± 6.3	102.52	9
GD18	315.5 ± 9.4	9	324.9 ± 8.2	102.98	10	325.3 ± 7.0	103.11	9
GD19	328.3 ± 10.6	9	340.9 ± 9.5	103.84	10	339.0 ± 7.3	103.26	9
GD20	339.8 ± 12.2	9	355.9 ± 10.0	104.74	10	354.9 ± 8.4	104.44	9
GD21	349.9 ± 11.9	9	373.9 ± 11.4	106.86	9	370.5 ± 8.1	105.89	9

	600 mg/kg			900 mg/kg		
	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N
GD 3	225.1 ± 3.5	99.60	10	226.3 ± 3.5	100.13	9
GD4	228.8 ± 3.5	99.87	10	231.8 ± 3.2	101.18	9
GD5	231.8 ± 4.0	99.06	10	236.5 ± 2.8	101.07	9
GD6	235.8 ± 3.8	99.16	10	241.2 ± 3.2	101.43	9
GD7	228.9 ± 3.8	95.57	10	229.6 ± 4.3	95.87	9
GD8	222.3 ± 5.2**	91.37	10	210.0 ± 3.9**	86.31	4
GD9	220.9 ± 6.0**	88.93	10			
GD10	225.7 ± 6.7**	88.68	9			
GD11	241.3 ± 5.2*	92.77	7			
GD12	248.9 ± 5.5	93.85	7			
GD13	254.0 ± 4.0	94.81	7			
GD14	259.7 ± 6.2	95.34	7			
GD15	265.2 ± 6.8	94.58	7			
GD16	268.3 ± 7.1	92.61	7			
GD17	273.7 ± 7.5*	90.75	7			
GD18	281.6 ± 10.0*	89.26	7			
GD19	289.4 ± 11.5*	88.15	7			
GD20	294.7 ± 12.8*	86.73	7			
GD21	300.0 ± 16.1*	85.74	7			

* Statistically significant ($P \leq 0.05$) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** $P < 0.01$
[a] Data are displayed as mean ± standard error by gestation day (GD).
[b] Number of surviving dams

TABLE A3
Summary of Gross Pathology Findings in Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	900 mg/kg
Disposition Summary					
Animals initially in study	10	10	10	10	10
Early deaths					
Euthanized, moribund				3	10
Survivors					
Terminal euthanasia	10	10	10	7	
Number of animals examined	10	10	10	10	10
Alimentary System					
Esophagus	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Large intestines	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Liver	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Pancreas	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Small intestines	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Stomach	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	5 (50)	10 (100)
Enlarged	0	0	0	5 (50)*	0
Cardiovascular System					
Heart	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Endocrine System					
Adrenal gland	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
General Body System					
None					
Genital System					
Ovary	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Uterus	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Vagina	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Hematopoietic System					
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Spleen	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Thymus	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)

TABLE A3
Summary of Gross Pathology Findings in Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg	900 mg/kg
Integumentary System					
Skin	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Musculoskeletal System					
None					
Nervous System					
None					
Respiratory System					
Lungs with bronchi	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Trachea	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Special Senses System					
None					
Urinary System					
Kidney, left	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Kidney, right	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Ureter	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	10 (100)	10 (100)
Urinary bladder	(10)	(10)	(10)	(10)	(10)
No visible lesion	10 (100)	10 (100)	10 (100)	9 (90)	10 (100)
Dilation				1 (10%)	

[a] Number of animals examined (displayed in parentheses) and number of animals with observation.
* Statistically significant ($P \leq 0.05$) trend according to the Cochran-Armitage (trend) or Fisher exact (pairwise) tests comparison. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.

TABLE A4
Summary of Maternal Organ Weights and Organ Weight Ratios for Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
n	9	9	9	7
Necropsy body wt.	349.9 ± 11.9	373.9 ± 11.5	370.5 ± 8.1	300.0 ± 16.1*
R. Kidney				
Absolute	0.83 ± 0.02	0.89 ± 0.03	0.91 ± 0.02*	0.87 ± 0.02
Relative	2.39 ± 0.13**	2.38 ± 0.06	2.47 ± 0.07	2.96 ± 0.20**
L. Kidney				
Absolute	0.83 ± 0.02	0.83 ± 0.04	0.86 ± 0.02	0.84 ± 0.03
Relative	2.38 ± 0.10	2.22 ± 0.07	2.33 ± 0.07	2.84 ± 0.18*
Liver				
Absolute	13.82 ± 0.67	15.15 ± 0.61	15.13 ± 0.41	14.96 ± 0.75
Relative	39.55 ± 1.38**	40.53 ± 1.12	40.86 ± 0.86	50.33 ± 2.64**

* Statistically significant (P≤0.05) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight. Data are displayed as mean ± standard error. No data are available for the 900 mg/kg group due to 100% mortality.

TABLE A5
Summary of Placental Findings in Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	150 mg/kg	300 mg/kg	600 mg/kg
Number of placentas examined	94	117	114	47
Placental Findings				
Number of placentas examined	94	117	114	47
Number of dams examined	9	9	8	5
Visible lesions present	0	0	0	0

TABLE A6
Summary of Fetal External Findings in Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg		150 mg/kg		300 mg/kg		600 mg/kg	
Number of fetuses examined	94		117		114		47	
External								
Number of fetuses examined	94		117		114		47	
Number of litters examined	9		9		8		5	
Extremities								
Limb, hind, left or hind, right, hyperflexion — [V]								
Fetuses	0	(0.00)	1	(0.85)	1	(0.88)	0	(0.00)
Litters	0	(0.00)	1	(11.11)	1	(12.50)	0	(0.00)
Limb, hind, left, hyperflexion — [V]								
Fetuses	0	(0.00)	0	(0.00)	1	(0.88)	0	(0.00)
Litters	0	(0.00)	0	(0.00)	1	(12.50)	0	(0.00)
Limb, hind, right, hyperflexion — [V]								
Fetuses	0	(0.00)	1	(0.85)	0	(0.00)	0	(0.00)
Litters	0	(0.00)	1	(11.11)	0	(0.00)	0	(0.00)
Tail, bent — [M]								
Fetuses	1	(1.06)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(11.11)	0	(0.00)	0	(0.00)	0	(0.00)
Tail, bent or curled — [M]								
Fetuses	1	(1.06)	0	(0.00)	0	(0.00)	1	(2.13)
Litters	1	(11.11)	0	(0.00)	0	(0.00)	1	(20.00)
Tail, curled — [M]								
Fetuses	0	(0.00)	0	(0.00)	0	(0.00)	1	(2.13)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(20.00)
Trunk								
General, omphalocele — [M]								
Fetuses	1	(1.06)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(11.11)	0	(0.00)	0	(0.00)	0	(0.00)

[a] Number of fetuses and (%) (upper row) or litters and (%) (lower row) with the observation
Statistical analysis of litters performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests found no statistically significant trend or pairwise comparison.
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression models, where the dam identification was the random effect, found no statistically significant trend or pairwise comparison.
[V] = Variation
[M] = Malformation

TABLE A7
Summary of Total Fetal Findings in Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg		150 mg/kg		300 mg/kg		600 mg/kg	
All Exams								
Number of fetuses	94		117		114		47	
Number of litters	9		9		8		5	
Malformation								
Affected fetuses	2	(2.13)	0		0		1	(2.13)
Affected litters	2	(22.22)	0		0		1	(20.00)
Variation								
Affected fetuses	0		1	(0.85)	1	(0.88)	0	
Affected litters	0		1	(11.11)	1	(12.50)	0	
External								
Number of fetuses	94		117		114		47	
Number of litters	9		9		8		5	
Malformation								
Affected fetuses	2	(2.13)	0		0		1	(2.13)
Affected litters	2	(22.22)	0		0		1	(20.00)
Variation								
Affected fetuses	0		1	(0.85)	1	(0.88)	0	
Affected litters	0		1	(11.11)	1	(12.50)	0	

[a] Number of fetuses (%) (upper row) or litters (%) (lower row) affected by each classification of developmental observation.
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression models, where the dam identification was the random effect, found no statistically significant trend or pairwise comparison.
Statistical analysis of litters performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests found no statistically significant trend or pairwise comparison.

APPENDIX B
SUMMARY OF FINDINGS IN RATS IN THE PRENATAL DEVELOPMENTAL TOXICITY GAVAGE STUDY OF 4-METHYLCYCLOHEXANEMETHANOL

TABLE B1
Summary of Clinical Observations for Rats
in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Pregnant Rats					
n	23	21	22	19	21
Discharge, vagina, brown	4 (GD 14)	3 (GD 14)	5 (GD 14)	6 (GD 14)	4 (GD 16)
Discharge, vagina, clear	2 (GD 18)	8 (GD 18)	5 (GD 19)	9 (GD 19)	7 (GD 18)
Discharge, vagina, red	6 (GD 13)	4 (GD 13)	5 (GD 14)	6 (GD 14)	1 (GD 14)
Rales	0	0	0	0	1 (GD 17)
Scab, tip of tail	1 (GD 19)	0	0	0	1 (GD 15)
Sore, tip of tail	0	0	0	0	2 (GD 9)
Non-pregnant Rats					
n	2	4	3	6	4
Discharge, vagina, clear	0	1 (SD 18)	0	1 (SD 15)	2 (SD 15)
Discharge, vagina, red	0	0	0	0	1 (SD 17)

[a] Cumulative number of animals with the observation and the first day of observation onset (displayed in parentheses). n = number of animals; GD = gestation phase; SD = study phase for females that were not pregnant.

TABLE B2
Summary of Mean Maternal Body Weights of Rats
in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg		50 mg/kg			100 mg/kg		
	Weight (g)	N[b]	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N
GD3	224.3 ± 3.0	23	223.7 ± 3.1	99.73	21	224.0 ± 2.8	99.87	22
GD6	239.5 ± 2.5	23	240.0 ± 2.2	100.21	21	240.0 ± 2.0	100.21	22
GD7	242.2 ± 2.4	23	242.9 ± 2.3	100.29	21	241.6 ± 2.2	99.75	22
GD8	247.1 ± 2.4	23	247.8 ± 2.4	100.28	21	245.8 ± 2.3	99.47	22
GD9	251.7 ± 2.5	23	252.9 ± 2.4	100.48	21	250.8 ± 2.2	99.64	22
GD10	257.0 ± 2.7	23	259.0 ± 2.4	100.78	21	255.9 ± 2.2	99.57	22
GD11	262.9 ± 2.6	23	264.4 ± 2.4	100.57	21	260.9 ± 2.2	99.24	22
GD12	266.6 ± 2.7	23	269.5 ± 2.5	101.09	21	265.3 ± 2.3	99.51	22
GD13	273.5 ± 2.9	23	275.8 ± 2.4	100.84	21	270.6 ± 2.5	98.94	22
GD14	278.5 ± 2.9	23	280.8 ± 2.5	100.83	21	276.6 ± 2.5	99.32	22
GD15	285.4 ± 3.0	23	288.8 ± 2.5	101.19	21	283.6 ± 2.9	99.37	22
GD16	298.5 ± 3.6	23	301.0 ± 2.7	100.84	21	294.9 ± 2.9	98.79	22
GD17	309.1 ± 3.9	23	313.5 ± 2.9	101.42	21	308.3 ± 3.5	99.74	22
GD18	323.7 ± 4.2	23	328.7 ± 3.7	101.54	21	323.1 ± 4.0	99.81	22
GD19	337.2 ± 4.5	23	345.0 ± 3.4	102.31	21	336.8 ± 4.5	99.88	22
GD20	353.4 ± 5.1*	23	363.1 ± 3.6	102.74	21	350.7 ± 5.0	99.24	22
GD21	370.9 ± 5.7**	23	381.5 ± 4.3	102.86	21	370.1 ± 5.8	99.78	22

	200 mg/kg			400 mg/kg		
	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N
GD3	225.4 ± 2.4	100.49	19	225.2 ± 3.2	100.40	21
GD6	239.2 ± 1.9	99.87	19	240.5 ± 2.6	100.42	21
GD7	242.5 ± 2.1	100.12	19	239.0 ± 2.6	98.68	21
GD8	245.7 ± 2.0	99.43	19	243.0 ± 2.8	98.34	21
GD9	249.5 ± 2.0	99.13	19	247.6 ± 2.9	98.37	21
GD10	255.2 ± 2.1	99.30	19	254.0 ± 2.9	98.83	21
GD11	261.5 ± 1.9	99.47	19	261.3 ± 3.0	99.39	21
GD12	265.6 ± 2.2	99.62	19	266.2 ± 2.9	99.85	21
GD13	270.9 ± 2.2	99.05	19	271.8 ± 2.7	99.38	21
GD14	277.9 ± 2.1	99.78	19	278.0 ± 2.6	99.82	21
GD15	284.1 ± 1.9	99.54	19	284.4 ± 3.0	99.65	21
GD16	296.3 ± 2.6	99.26	19	295.3 ± 3.1	98.93	21
GD17	307.8 ± 3.0	99.58	19	305.2 ± 3.8	98.74	21
GD18	323.9 ± 3.5	100.06	19	318.3 ± 3.6	98.33	21
GD19	334.7 ± 4.7	99.26	19	333.1 ± 3.7	98.78	21
GD20	351.3 ± 5.0	99.41	19	344.5 ± 4.3	97.48	21
GD21	368.8 ± 5.6	99.43	19	356.9 ± 4.9	96.23	21

* Statistically significant ($P \leq 0.05$) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend is indicated in the vehicle control column. No statistically significant pairwise comparisons with the vehicle control group were found for body weights.

** $P < 0.01$

[a] Data are displayed as mean ± standard error by gestation day (GD).

[b] Number of surviving dams

TABLE B3
Summary of Gross Pathology Findings in Rats
in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Disposition Summary					
Animals initially in study	25	25	25	25	25
Early deaths					
Survivors					
Scheduled sacrifice, terminal	25	25	25	25	25
Number of animals examined	25	25	25	25	25
Alimentary System					
Esophagus	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Intestine, large, cecum	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Intestine, large, colon	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Intestine, large, rectum	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Intestine, small, duodenum	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Intestine, small, ileum	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Intestine, small, jejunum	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Liver	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Pancreas	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Stomach, forestomach	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Stomach, glandular	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Cardiovascular System					
Heart	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Endocrine System					
Adrenal gland	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
General Body System					
None					
Genital System					
Ovary	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Uterus	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	21 (84)	24 (96)	23 (92)
Dilation; fluid-filled			3 (12)	1 (4)	2 (8)

TABLE B3
Summary of Gross Pathology Findings in Rats
in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Genital System (continued)					
Uterus (continued)	(25)	(25)	(25)	(25)	(25)
Fluid			1 (4)		
Fluid; all			4 (16)		2 (8)
Fluid; brown			1 (4)		1 (4)
Fluid; clear			1 (4)		
Fluid; red			1 (4)		
Fluid; yellow					1 (4)
Vagina	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Hematopoietic System					
Lymph node, mesenteric	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Spleen	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Thymus	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Integumentary System					
Skin	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Musculoskeletal System					
None					
Nervous System					
None					
Respiratory System					
Lung	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Trachea	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Special Senses System					
None					
Urinary System					
Kidney	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Ureter	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)
Urinary bladder	(25)	(25)	(25)	(25)	(25)
No visible lesion	25 (100)	25 (100)	25 (100)	25 (100)	25 (100)

[a] Number of animals examined (displayed in parentheses) and number of animals with observation. No statistically significant trend (by Cochran-Armitage test) or pairwise comparison (Fisher exact test)

TABLE B4
Summary of Clinical Pathology Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Hematology					
n	22	20	21	19	19
Hematocrit (auto) (%)	38.9 ± 0.6	37.2 ± 0.5	39.3 ± 0.6	39.4 ± 0.6	38 ± 0.6
Percent of control		96	101	101	98
Hematocrit (spun) (%)	36.6 ± 0.5	35.1 ± 0.5	38.1 ± 0.8	37.2 ± 0.5	36.4 ± 0.5
Percent of control		96	104	102	99
Hemoglobin (g/dL)	11.6 ± 0.2	11.2 ± 0.2	11.8 ± 0.2	11.9 ± 0.2	11.5 ± 0.2
Percent of control		96	102	103	99
Erythrocytes (10^6/µL)	6.16 ± 0.08	5.95 ± 0.06	6.26 ± 0.08	6.21 ± 0.09	5.92 ± 0.08
Percent of control		97	102	101	96
Reticulocytes (10^5/µL)	2.82 ± 0.063	2.965 ± 0.153	2.794 ± 0.087	2.704 ± 0.088	3.081 ± 0.084
Percent of control		105	99	96	109
Nucleated erythrocytes/100 leukocytes	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.4 ± 0.3
Percent of control		165	105	0	405
Mean cell volume (fL)	63.1 ± 0.3	62.6 ± 0.5	62.7 ± 0.4	63.4 ± 0.5	64.1 ± 0.4
Percent of control		99	99	100	102
Mean cell hemoglobin (pg)	18.8 ± 0.1**	18.8 ± 0.2	18.9 ± 0.1	19.1 ± 0.1	19.4 ± 0.1**
Percent of control		100	100	102	103
Mean cell hemoglobin concentration (g/dL)	29.9 ± 0.1*	30 ± 0.1	30.2 ± 0.1	30.2 ± 0.1	30.3 ± 0.1
Percent of control		101	101	101	102
Platelets (10^3/µL)	1,269 ± 46	1,311 ± 40	1,221 ± 55	1,243 ± 64	1,170 ± 55
Percent of control		103	96	98	92
Leukocytes (10^3/µL)	6.232 ± 0.572	6.186 ± 0.597	6.289 ± 0.551	6.018 ± 0.56	5.524 ± 0.466
Percent of control		99	101	97	89
Segmented neutrophils (10^3/µL)	1.55 ± 0.13	1.59 ± 0.17	1.68 ± 0.14	1.53 ± 0.14	1.43 ± 0.12
Percent of control		103	109	99	92
Lymphocytes (10^3/µL)	4.13 ± 0.42	4.04 ± 0.38	4.1 ± 0.41	4.06 ± 0.42	3.71 ± 0.33
Percent of control		98	99	98	90
Monocytes (10^3/µL)	0.31 ± 0.04	0.36 ± 0.07	0.3 ± 0.03	0.25 ± 0.03	0.25 ± 0.03
Percent of control		116	97	82	81
Basophils (10^3/µL)	0.03 ± 0	0.05 ± 0.02	0.04 ± 0	0.02 ± 0	0.03 ± 0
Percent of control		177	129	89	97
Eosinophils (10^3/µL)	0.15 ± 0.03**	0.09 ± 0.02	0.1 ± 0.02	0.09 ± 0.03*	0.06 ± 0.01**
Percent of control		61	68	60	39
Large unstained cells (10^3/µL)	0.068 ± 0.009	0.073 ± 0.013	0.069 ± 0.011	0.055 ± 0.007	0.052 ± 0.006
Percent of control		107	101	81	76

TABLE B4
Summary of Clinical Pathology Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Clinical Chemistry					
n	23	21	22	19	21
Urea nitrogen (mg/dL)	17.9 ± 0.6**	17.7 ± 0.5	17.5 ± 0.5	18.7 ± 1.0	20.8 ± 0.6**
Percent of control		99	98	105	116
Creatinine (mg/dL)	0.4 ± 0.01	0.4 ± 0.01	0.4 ± 0.01	0.4 ± 0.01	0.39 ± 0.01
Percent of control		99	98	99	95
Glucose (mg/dL)	181.2 ± 10.3**	153.9 ± 7.4	176.0 ± 11.1	150.6 ± 10.6*	141.3 ± 9.3**
Percent of control		85	97	83	78
Calcium (mg/dL)	12.2 ± 0.2*[b]	11.7 ± 0.2	11.8 ± 0.2	11.7 ± 0.2	11.4 ± 0.3*[c]
Percent of control		96	97	96	94
Phosphorus (mg/dL)	10.27 ± 0.37[b]	9.58 ± 0.29	9.90 ± 0.32	9.55 ± 0.32	9.60 ± 0.49
Percent of control		93	96	93	93
Total protein (g/dL)	5.17 ± 0.11**	4.93 ± 0.10	4.84 ± 0.10*	4.91 ± 0.15*	4.74 ± 0.14**
Percent of control		96	94	95	92
Albumin (g/dL)	3.07 ± 0.09*	2.93 ± 0.07	2.95 ± 0.1	3.01 ± 0.12	2.85 ± 0.13
Percent of control		95	96	98	93
Globulin (g/dL)	2.096 ± 0.052**	2.005 ± 0.059	1.886 ± 0.041**	1.900 ± 0.039**	1.886 ± 0.064**
Percent of control		96	90	91	90
Albumin/globulin ratio	1.482 ± 0.052	1.483 ± 0.054	1.579 ± 0.065	1.583 ± 0.045	1.55 ± 0.089
Percent of control		100	107	107	105
Total bilirubin (mg/dL)	<0.200	<0.200	<0.200	<0.200	<0.200
Percent of control		100	100	100	100
Direct bilirubin (mg/dL)	<0.200	<0.200	<0.200	<0.200	<0.200
Percent of control		100	100	100	100
Cholesterol (mg/dL)	89.5 ± 2.1	95.7 ± 2.7	89.4 ± 2.4	96.6 ± 4.5	87.4 ± 3.1
Percent of control		107	100	108	98
Triglycerides (mg/dL)	145.5 ± 21.0**	114.8 ± 18.0	94.6 ± 12.6	108.4 ± 14.0	194.6 ± 31.8*
Percent of control		79	65	74	134
Alanine aminotransferase (IU/L)	66.39 ± 2.50*	67.33 ± 3.12	68.45 ± 1.40	66.37 ± 1.98	77.19 ± 3.67
Percent of control		101	103	100	116
Alkaline phosphatase (IU/L)	138.4 ± 6.9	142 ± 4.7	131.7 ± 4.2	134.9 ± 5.4	157 ± 6.2*
Percent of control		103	95	98	113
Aspartate aminotransferase (IU/L)	90.35 ± 5.38	100.57 ± 14.24	93.14 ± 7.91	77.63 ± 5.13	121.14 ± 13.72
Percent of control		111	103	86	134
Creatine kinase (IU/L)	233.3 ± 18.6	414.4 ± 186.5	239.1 ± 20.9	195 ± 15.4	268.9 ± 31.3
Percent of control		178	102	84	115

* Statistically significant (P<0.05) trend (by Jonckheere's test) or pairwise comparison (by Shirley's or Dunn's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.

** P<0.01

[a] Data are presented as mean ± standard error, with percent of control calculated as (dosed group mean / vehicle control group mean × 100). Percent of control was calculated and statistical tests were performed on unrounded data. For values less than the limit of detection, the limit of detection is given as the mean.

[b] n=22

[c] n=20

TABLE B5
Summary of Placental Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Number of placentas examined	296	283	279	247	254
Placental Findings					
Number of placentas examined	296	283	279	247	253[a]
Number of dams examined	23	21	21	19	21
Visible lesions present	0	0	0	0	0

[a] An examination was not conducted for one placenta from one dam in the 400 mg/kg group.

TABLE B6
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Number of fetuses examined	296	283	279	247	254
External					
Number of fetuses examined	296	283	279	247	254
Number of litters examined	23	21	21	19	21
Anogenital					
Anus, absent — [M]					
Fetuses	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.39)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Body - General					
Body, subcutaneous hemorrhage — [GF]					
Fetuses	1 (0.34)	4 (1.41)	3 (1.08)	1 (0.40)	3 (1.18)
Litters	1 (4.35)	3 (14.29)	1 (4.76)	1 (5.26)	3 (14.29)
Extremities					
Tail, bent — [M]					
Fetuses	0 (0.0)	2 (0.71)	0 (0.0)	0 (0.0)	0 (0.0)
Litters	0 (0.00)	2 (9.52)	0 (0.00)	0 (0.00)	0 (0.00)
Tail, thread-like — [M]					
Fetuses	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.39)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Trunk					
General, omphalocele — [M]					
Fetuses	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.39)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Visceral					
Number of fetuses examined	296	283	279	247	254
Number of litters examined	23	21	21	19	21
Abdominal Viscera					
Adrenal, left, discolored — [V]					
Fetuses	0 (0.0)**	0 (0.00)	0 (0.00)	0 (0.00)	3 (1.18)
Litters	0 (0.00)**	0 (0.00)	0 (0.00)	0 (0.00)	3 (14.29)
Adrenal, left, misshapen — [M]					
Fetuses	0 (0.0)**	0 (0.00)	0 (0.00)	0 (0.00)	3 (1.18)
Litters	0 (0.00)**	0 (0.00)	0 (0.00)	0 (0.00)	3 (14.29)
Adrenal, right, discolored — [V]					
Fetuses	0 (0.00)	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)
Litters	0 (0.00)	0 (4.76)	0 (0.00)	0 (0.00)	0 (0.00)
Adrenal, right, misshapen — [M]					
Fetuses	0 (0.00)	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)
Litters	0 (0.00)	0 (4.76)	0 (0.00)	0 (0.00)	0 (0.00)
Adrenal, total, discolored — [V]					
Fetuses	0 (0.0)*	1 (0.35)	0 (0.0)	0 (0.0)	3 (1.18)
Litters	0 (0.00)*	1 (4.76)	0 (0.00)	0 (0.00)	3 (14.29)
Adrenal, total, misshapen — [M]					
Fetuses	0 (0.0)*	1 (0.35)	0 (0.0)	0 (0.0)	3 (1.18)
Litters	0 (0.00)*	1 (4.76)	0 (0.00)	0 (0.00)	3 (14.29)
Heart					
Aortic valve, misshapen — [M]					
Fetuses	12 (4.05)*	8 (2.83)	9 (3.23)	7 (2.83)	17 (6.69)
Litters	8 (34.78)	6 (28.57)	5 (23.81)	4 (21.05)	9 (42.86)
Pulmonary valve, misshapen — [M]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.39)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)

TABLE B6
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Visceral (continued)					
Number of fetuses examined	296	283	279	247	254
Number of litters examined	23	21	21	19	21
Major Vessels					
Aorta, dilated — [M]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.39)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Aortic arch, dilated — [M]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.39)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Carotid artery, bilateral, malpositioned — [V]					
Fetuses	1 (0.34)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Litters	1 (4.35)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Innominate artery, absent — [V]					
Fetuses	7 (2.36)	6 (2.12)	6 (2.15)	5 (2.02)	11 (4.33)
Litters	4 (17.39)	3 (14.29)	3 (14.29)	4 (21.05)	7 (33.33)
Innominate artery, absent or short — [V]					
Fetuses	12 (4.05)	7 (2.47)	10 (3.58)	6 (2.43)	12 (4.72)
Litters	7 (30.43)	4 (19.05)	4 (19.05)	4 (21.05)	7 (33.33)
Innominate artery, short — [V]					
Fetuses	5 (1.69)	1 (0.35)	4 (1.43)	1 (0.40)	1 (0.39)
Litters	4 (17.39)	1 (4.76)	2 (9.52)	1 (5.26)	1 (4.76)
Reproductive Tract					
Ovary, left, discolored — [V]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.39)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Ovary, left, misshapen — [M]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.39)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Testis, bilateral, malpositioned — [M]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.40)	1 (0.39)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.26)	1 (4.76)
Thoracic Viscera					
Thymus, supernumerary — [V]					
Fetuses	4 (1.35)	0 (0.0)	2 (0.72)	1 (0.40)	0 (0.00)
Litters	3 (13.04)	0 (0.00)	2 (9.52)	1 (5.26)	0 (0.00)
Urinary Tract					
Kidney, left, discolored — [V]					
Fetuses	0 (0.00)**	0 (0.00)	0 (0.00)	0 (0.00)	3 (1.18)
Litters	0 (0.00)**	0 (0.00)	0 (0.00)	0 (0.00)	3 (14.29)
Kidney, right, discolored — [V]					
Fetuses	0 (0.00)	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)
Litters	0 (0.00)	1 (4.76)	0 (0.00)	0 (0.00)	0 (0.00)
Kidney, total, discolored — [V]					
Fetuses	0 (0.00)*	1 (0.35)	0 (0.00)	0 (0.00)	3 (1.18
Litters	0 (0.00)*	1 (4.76)	0 (0.00)	0 (0.00)	3 (14.29)
Renal pelvis, left, dilated — [V]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.40)	0 (0.0)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.26)	0 (0.00)
Ureter, left, dilated — [V]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.40)	0 (0.00)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.26)	0 (0.00)

TABLE B6
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Head					
Number of fetuses examined	155	146	145	128	131
Number of litters examined	23	21	21	19	21
No visible lesions present					
Skeletal: Body					
Number of fetuses examined	295	283	279	247	253
Number of litters examined	23	21	21	19	21
Hindlimb					
Metatarsal, unossified — [V]					
Fetuses	0 (0.00)	0 (0.00)	1 (0.36)	0 (0.00)	0 (0.00)
Litters	0 (0.00)	0 (0.00)	1 (4.76)	0 (0.00)	0 (0.00)
Pelvic Girdle					
Pubis, bilateral, incomplete ossification — [V]					
Fetuses	0 (0.00)	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)
Litters	0 (0.00)	1 (4.76)	0 (0.00)	0 (0.00)	0 (0.00)
Ribs					
Costal cartilage, 4th right, misaligned — [V]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.40)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Costal cartilage, 5th right, misaligned — [V]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.40)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Costal cartilage, 7th right, not fused to sternum — [M]					
Fetuses	0 (0.00)**	0 (0.00)	0 (0.00)	2 (0.81)	4 (1.58)*
Litters	0 (0.00)**	0 (0.00)	0 (0.00)	1 (5.26)	4 (19.05)*
Costal cartilage, multiple sites, misaligned — [V]					
Fetuses	0 (0.00)*	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.79)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Costal cartilage, total, misaligned — [V]					
Fetuses	0 (0.00)**	0 (0.00)	0 (0.00)	0 (0.00)	3 (1.19)
Litters	0 (0.00)*	0 (0.00)	0 (0.00)	0 (0.00)	2 (9.52)
Rib, 7th left, fused — [M]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.40)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Rib, 7th right, absent — [M]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.40)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Rib, multiple sites, wavy — [V]					
Fetuses	0 (0.00)	1 (0.35)	0 (0.00)	0 (0.00)	0 (0.00)
Litters	0 (0.00)	1 (4.76)	0 (0.00)	0 (0.00)	0 (0.00)
Ribs, sternebrae, supernumerary rib, or vertebrae					
Malformation, total — [M]					
Fetuses	3 (1.02)**##	3 (1.06)	6 (2.15)	7 (2.83)	40 (15.81)**##
Litters	3 (13.04)**	3 (14.29)	3 (14.29)	5 (26.32)	12 (57.14)**
Total, incomplete ossification or unossified — [V]					
Fetuses	0 (0.00)**	2 (0.71)	0 (0.00)	0 (0.00)	9 (3.56)**
Litters	0 (0.00)**	2 (9.52)	0 (0.00)	0 (0.00)	7 (33.33)**
Sternebrae					
Sternebra, 2nd, unossified — [V]					
Fetuses	0 (0.00)**	1 (0.35)	0 (0.00)	0 (0.00)	4 (1.58)*
Litters	0 (0.00)**	1 (4.76)	0 (0.00)	0 (0.00)	4 (19.05)*
Sternebra, 4th, misaligned — [V]					
Fetuses	0 (0.00)*	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.79)
Litters	0 (0.00)*	0 (0.00)	0 (0.00)	0 (0.00)	2 (9.52)

TABLE B6
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Skeletal: Body (continued)					
Number of fetuses examined	295	283	279	247	253
Number of litters examined	23	21	21	19	21
Sternebrae (continued)					
Sternebra, 5th, supernumerary site — [V]					
Fetuses	1 (0.34)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Litters	1 (4.35)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Sternebra, 5th, unossified — [V]					
Fetuses	0 (0.00)	1 (0.35)	0 (0.00)	0 (0.00)	2 (0.79)
Litters	0 (0.00)	1 (4.76)	0 (0.00)	0 (0.00)	2 (9.52)
Sternebra, 6th, split — [M]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.40)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Sternebra, 6th, supernumerary site — [V]					
Fetuses	0 (0.00)	0 (0.00)	1 (0.36)	0 (0.00)	0 (0.00)
Litters	0 (0.00)	0 (0.00)	1 (4.76)	0 (0.00)	0 (0.00)
Sternebra, 6th, unossified — [V]					
Fetuses	0 (0.00)	1 (0.35)	0 (0.00)	0 (0.00)	2 (0.79)
Litters	0 (0.00)	1 (4.76)	0 (0.00)	0 (0.00)	2 (9.52)
Sternebra, multiple sites, unossified — [V]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.40)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Sternebra, total, supernumerary site — [V]					
Fetuses	1 (0.34)	0 (0.00)	1 (0.36)	0 (0.00)	0 (0.00)
Litters	1 (4.35)	0 (0.00)	1 (4.76)	0 (0.00)	0 (0.00)
Sternebra, total, unossified — [V]					
Fetuses	0 (0.00)**	1 (0.35)	0 (0.00)	0 (0.00)	6 (2.37)**
Litters	0 (0.00)**	1 (4.76)	0 (0.00)	0 (0.00)	5 (23.81)*
Sternebra, total, unossified or misaligned — [V]					
Fetuses	0 (0.0)**	1 (0.35)	0 (0.00)	0 (0.00)	7 (2.77)**
Litters	0 (0.00)**	1 (4.76)	0 (0.00)	0 (0.00)	6 (28.57)**
Xiphoid cartilage, split — [M]					
Fetuses	1 (0.34)	2 (0.71)	0 (0.00)	0 (0.00)	3 (1.19)
Litters	1 (4.35)	2 (9.52)	0 (0.00)	0 (0.00)	3 (14.29)
Supernumerary rib					
Cervical, bilateral, short — [M]					
Fetuses	0 (0.00)**	0 (0.00)	0 (0.00)	0 (0.00)	3 (1.19)
Litters	0 (0.00)**	0 (0.00)	0 (0.00)	0 (0.00)	3 (14.29)
Cervical, left, short — [M]					
Fetuses	0 (0.00)*	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.79)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Cervical, right, short — [M]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.40)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Cervical, total, short — [M]					
Fetuses	0 (0.00)**	0 (0.00)	0 (0.00)	0 (0.00)	6 (2.37)**
Litters	0 (0.00)**	0 (0.00)	0 (0.00)	0 (0.00)	3 (14.29)
Thoracolumbar, bilateral, full — [M]					
Fetuses	1 (0.34)**	0 (0.00)	2 (0.72)	1 (0.40)	10 (3.95)**
Litters	1 (4.35)	0 (0.00)	2 (9.52)	1 (5.26)	3 (14.29)
Thoracolumbar, bilateral, short — [V]					
Fetuses	34 (11.53)	18 (6.36)*#	25 (8.96)	23 (9.31)	21 (8.30)
Litters	15 (65.22)	9 (42.86)	12 (57.14)	12 (63.16)	11 (52.38)
Thoracolumbar, left, full — [M]					
Fetuses	0 (0.00)**	1 (0.35)	4 (1.43)	3 (1.21)	13 (5.14)**
Litters	0 (0.00)**	1 (4.76)	2 (9.52)	3 (15.79)	6 (28.57)**

TABLE B6
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg		50 mg/kg		100 mg/kg		200 mg/kg		400 mg/kg	
Skeletal: Body (continued)										
Number of fetuses examined	295		283		279		247		253	
Number of litters examined	23		21		21		19		21	
Supernumerary Rib (continued)										
Thoracolumbar, left, short — [V]										
Fetuses	27	(9.15)	30	(10.60)	34	(12.19)	47	(19.03)**##	27	(10.67)
Litters	17	(73.91)	14	(66.67)	14	(66.67)	16	(84.21)	14	(66.67)
Thoracolumbar, right, full — [M]										
Fetuses	1	(0.34)*	0	(0.00)	0	(0.00)	1	(0.40)	3	(1.19)
Litters	1	(4.35)*	0	(0.00)	0	(0.00)	1	(5.26)	3	(14.29)
Thoracolumbar, right, short — [V]										
Fetuses	15	(5.08)**##	8	(2.83)	20	(7.17)	17	(6.88)	29	(11.46)**#
Litters	10	(43.48)	7	(33.33)	9	(42.86)	8	(42.11)	12	(57.14)
Thoracolumbar, total, full — [M]										
Fetuses	2	(0.68)**##	1	(0.35)	6	(2.15)	5	(2.02)	26	(10.28)**##
Litters	2	(8.70)**	1	(4.76)	3	(14.29)	4	(21.05)	7	(33.33)*
Thoracolumbar, total, short — [V]										
Fetuses	76	(25.76)**	56	(19.79)	79	(28.32)	87	(35.22)*	77	(30.43)
Litters	21	(91.30)	19	(90.48)	17	(80.95)	17	(89.47)	19	(90.48)
Vertebrae										
Presacral vertebrae, greater than 26 — [V]										
Fetuses	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(0.40)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.76)
Thoracic arch, 7th left, supernumerary — [M]										
Fetuses	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(0.40)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.76)
Thoracic arch, 7th right, fused — [M]										
Fetuses	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(0.40)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.76)
Thoracic arch, multiple sites, misshapen — [M]										
Fetuses	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(0.40)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.76)
Thoracic centrum, 12th, incomplete ossification — [V]										
Fetuses	0	(0.00)	1	(0.35)	0	(0.00)	0	(0.00)	2	(0.79)
Litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)	2	(9.52)
Thoracic centrum, 13th, incomplete ossification — [V]										
Fetuses	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(0.40)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.76)
Thoracic centrum, 1st, unossified — [V]										
Fetuses	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(0.40)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.76)
Thoracic centrum, total, incomplete ossification — [V]										
Fetuses	0	(0.00)*	1	(0.35)	0	(0.0)	0	(0.0)	3	(1.19)
Litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)	2	(9.52)
Thoracic centrum, total, incomplete ossification or unossified — [V]										
Fetuses	0	(0.00)**	1	(0.35)	0	(0.0)	0	(0.0)	4	(1.58)*
Litters	0	(0.00)*	1	(4.76)	0	(0.00)	0	(0.00)	3	(14.29)
Thoracic vertebra, multiple sites, misaligned — [V]										
Fetuses	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(0.40)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.76)

TABLE B6
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Skeletal: Skull					
Number of fetuses examined	141	137	134	119	123
Number of litters examined	23	21	21	19	21
Skull					
Frontal, general, interparietal or parietal, total, incomplete or isolated ossification — [V]					
Fetuses	4 (2.84)	0 (0.00)	1 (0.75)	4 (3.36)	2 (1.63)
Litters	4 (17.39)	0 (0.00)	1 (4.76)	4 (21.05)	2 (9.52)
Frontal, bilateral, incomplete ossification — [V]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.81)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
General, isolated ossification site — [V]					
Fetuses	4 (2.84)	0 (0.0)	1 (0.75)	4 (3.36)	1 (0.81)
Litters	4 (17.39)	0 (0.00)	1 (4.76)	4 (21.05)	1 (4.76)
General, supernumerary site — [V]					
Fetuses	2 (1.42)	2 (1.46)	2 (1.49)	1 (0.84)	0 (0.00)
Litters	1 (4.35)	2 (9.52)	2 (9.52)	1 (5.26)	0 (0.00)
Interparietal, incomplete ossification — [V]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.81)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Parietal, bilateral, incomplete ossification — [V]					
Fetuses	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.81)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.76)
Zygomatic, bilateral, misshapen — [M]					
Fetuses	0 (0.00)	1 (0.73)	0 (0.00)	0 (0.00)	0 (0.00)
Litters	0 (0.00)	1 (4.76)	0 (0.00)	0 (0.00)	0 (0.00)

Upper row denotes number of affected fetuses and (%) and lower row the number of affected litters and (%)
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
Statistically significant at P≤0.05
P≤0.01
Statistical analysis of litters performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
* Statistically significant at P≤0.05
** P≤0.01
[M] = Malformation
[GF] = Gross finding
[V] = Variation

TABLE B7
Summary of Total Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol[a]

	0 mg/kg		50 mg/kg		100 mg/kg		200 mg/kg		400 mg/kg	
All Exams										
Number of fetuses	296		283		279		247		254	
Number of litters	23		21		21		19		21	
Malformation										
Affected fetuses	15	(5.07)**##	14	(4.95)	15	(5.38)	15	(6.07)	59	(23.23)**##
Affected litters	10	(43.48)*	11	(52.38)	7	(33.33)	10	(52.63)	15	(71.43)
Variation										
Affected fetuses	92	(31.08)**#	65	(22.97)*	86	(30.82)	98	(38.68)*	99	(38.98)*
Affected litters	21	(91.30)	20	(95.24)	18	(85.71)	17	(89.47)	20	(95.24)
Gross Finding										
Affected fetuses	1	(0.34)	4	(1.41)	3	(1.08)	1	(0.40)	3	(1.18)
Affected litters	1	(4.35)	3	(14.29)	1	(4.76)	1	(5.26)	3	(14.29)
External										
Number of fetuses	296		283		279		247		254	
Number of litters	23		21		21		19		21	
Malformation										
Affected fetuses	0		2	(0.71)	0		0		2	(0.79)
Affected litters	0		2	(9.52)	0		0		2	(9.52)
Gross Finding										
Affected fetuses	1	(0.34)	4	(1.41)	3	(1.08)	1	(0.40)	3	(1.18)
Affected litters	1	(4.35)	3	(14.29)	1	(4.76)	1	(5.26)	3	(14.29)
Visceral										
Number of fetuses	296		283		279		247		254	
Number of litters	23		21		21		19		21	
Malformation										
Affected fetuses	12	(4.05)**##	9	(3.18)	9	(3.23)	8	(3.24)	24	(9.45)**#
Affected litters	8	(34.78)	7	(33.33)	5	(23.81)	5	(26.32)	12	(57.14)
Variation										
Affected fetuses	16	(5.41)	8	(2.83)	12	(4.30)	8	(3.24)	14	(5.51)
Affected litters	10	(43.48)	5	(23.81)	6	(28.57)	6	(31.58)	9	(42.86)
Head										
Number of fetuses	155		146		145		128		131	
Number of litters	23		21		21		19		21	
No visible lesions present										
Skeletal - Body										
Number of fetuses	296		283		279		247		253	
Number of litters	23		21		21		19		21	
Malformation										
Affected fetuses	3	(1.02)**##	3	(1.06)	6	(2.15)	7	(2.83)	40	(15.81)**##
Affected litters	3	(13.04)**	3	(14.29)	3	(14.29)	5	(26.32)	12	(57.14)**
Variation										
Affected fetuses	76	(25.76)**#	59	(20.85)	79	(28.32)	87	(35.22)*	88	(34.78)*
Affected litters	21	(91.30)	19	(90.48)	17	(80.95)	17	(89.47)	20	(95.24)

TABLE B7
Summary of Total Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Skeletal - Skull					
Number of fetuses	141	137	134	119	123
Number of litters	23	21	21	19	21
Malformation					
Affected fetuses	0	1 (0.73)	0	0	0
Affected litters	0	1 (4.76)	0	0	0
Variation					
Affected fetuses	6 (4.26)	2 (1.46)	3 (2.24)	5 (4.20)	2 (1.63)
Affected litters	5 (21.74)	2 (9.52)	2 (9.52)	5 (26.32)	2 (9.52)

Upper row denotes number of affected fetuses and (%) and lower row the number of affected litters and (%)
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
Statistically significant at P≤0.05
P≤0.01
Statistical analysis of litters performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
* Statistically significant at P≤0.05
** P≤0.01

TABLE B8
Fetal Findings Cross Reference of Dams and Fetuses
in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Number of fetuses examined	296	283	279	247	254
Number of dams examined	23	21	21	19	21
Visceral					
Number of fetuses examined	296	283	279	247	254
Number of dams examined	23	21	21	19	21
Abdominal Viscera					
Adrenal, left, discolored — [V]					
					101 (5)
					112 (4)
					114 (11)
Adrenal, left, misshapen — [M]					
					101 (5)
					112 (4)
					114 (11)
Adrenal, right, discolored — [V]					
		31 (6)			
Adrenal, right, misshapen — [M]					
		31 (6)			
Heart					
Aortic valve, misshapen — [M]					
	3 (12)	33 (10)	57 (5)	87 (13,15)	103 (1,6,14)
	14 (2,11)	34 (8)	61 (2,4,8,9)	92 (3,7)	104 (6)
	15 (1,8)	35 (8)	65 (4)	96 (4)	105 (1)
	16 (4)	39 (7,11)	67 (10)	98 (2,9)	107 (10,12,13,14)
	18 (17)	40 (8)	75 (6,7)		111 (5)
	19 (12)	43 (3,15)			114 (12)
	20 (13)				119 (5,13)
	22 (5,6,9)				123 (1)
					125 (2,7,11)
Pulmonary valve, misshapen — [M]					
					123 (6)
Major Vessels					
Aorta, dilated — [M]					
					117 (11)
Aortic arch, dilated — [M]					
					105 (4)
Carotid artery, bilateral, malpositioned — [V]					
	25 (4)				
Innominate artery, absent — [V]					
	6 (1,3)	41 (8,11)	66 (2)	79 (2)	101 (5)
	7 (12)	43 (1,2,9)	72 (6)	82 (1)	103 (13)
	23 (9,13)	50 (1)	73 (3,6,10,13)	89 (6,7)	104 (6)
	25 (4,5)			100 (1)	106 (8)
					113 (5,14)
					117 (3,4)
					123 (8,12,13)
Innominate artery, short — [V]					
	1 (5)	34 (5)	59 (1,2,7)	79 (8)	104 (3)
	16 (3,6)		66(3)		
	20 (15)				
	25 (7)				

TABLE B8
Fetal Findings Cross Reference of Dams and Fetuses
in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Visceral (continued)					
Number of fetuses examined	296	283	279	247	254
Number of dams examined	23	21	21	19	21
Reproductive Tract					
Ovary, left, discolored — [V]					101 (5)
Ovary, left, misshapen — [M]					101 (5)
Testis, bilateral, malpositioned — [M]				76 (1)	101 (12)
Thoracic Viscera					
Thymus, supernumerary — [V]	14 (15) 15 (1) 18 (11,13)		64 (14) 70 (6)	90 (1)	
Urinary Tract					
Kidney, left, discolored — [V]					101 (5) 112 (4) 114 (11)
Kidney, right, discolored — [V]		31 (6)			
Renal pelvis, left, dilated — [V]				93 (1)	
Ureter, left, dilated — [V]				93 (1)	
Skeletal - Skull					
Number of fetuses examined	141	137	134	119	123
Number of dams examined	23	21	21	19	21
Skull					
Frontal, bilateral, incomplete ossification — [V]					102 (10)
General, isolated ossification site — [V]	4 (14) 10 (6) 14 (2) 19 (4)		69 (8)	79 (6) 82 (12) 87 (10) 98 (12)	112 (4)
General, supernumerary site — [V]	15 (2,12)	31 (6) 40 (6)	69 (10) 75 (14)	76 (4)	
Interparietal, incomplete ossification — [V]					102 (10)
Parietal, bilateral, incomplete ossification — [V]					102 (10)
Zygomatic, bilateral, misshapen — [M]		47 (12)			

TABLE B8
Fetal Findings Cross Reference of Dams and Fetuses
in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Skeletal – Body					
Number of fetuses examined	296	283	279	247	254
Number of dams examined	23	21	21	19	21
Appendicular Skeleton					
General, not examined	4 (9)		53 (6)		104 (11) 114 (5)
Hindlimb					
Metatarsal, unossified — [V]			53 (1)		
Pelvic Girdle					
Pubis, bilateral, incomplete ossification — [V]		46 (1)			
Ribs					
Costal cartilage, 4th right, misaligned — [V]					109 (8)
Costal cartilage, 5th right, misaligned — [V]					109 (8)
Costal cartilage, 7th right, not fused to sternum — [M]				100 (1,2)	105 (1) 107 (4) 115 (1) 117 (10)
Costal cartilage, multiple sites, misaligned — [V]					106 (4,13)
Rib, 7th left, fused — [M]					123 (9)
Rib, 7th right, absent — [M]					123 (9)
Rib, multiple sites, wavy — [V]		33 (3)			
Sternebrae					
Sternebra, 2nd, unossified — [V]		46 (1)			102 (6) 106 (4) 116 (4) 117 (10)
Sternebra, 4th, misaligned — [V]					106 (4) 109 (8)
Sternebra, 5th, supernumerary site — [V]	23 (1)				
Sternebra, 5th, unossified — [V]		46 (1)			106 (4) 115 (13)
Sternebra, 6th, split — [M]					107 (14)
Sternebra, 6th, supernumerary site — [V]			55 (3)		

TABLE B8
Fetal Findings Cross Reference of Dams and Fetuses
in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Skeletal – Body (continued)					
Number of fetuses examined	296	283	279	247	254
Number of dams examined	23	21	21	19	21
Sternebrae (continued)					
Sternebra, 6th, unossified — [V]					
		46 (1)			102 (6)
					115 (13)
Sternebra, multiple sites, unossified — [V]					
					115 (6)
Xiphoid cartilage, split — [M]					
	22 (10)	30 (5)			101 (8)
		46 (1)			107 (14)
					113 (5)
Supernumerary Rib					
Cervical, bilateral, short — [M]					
					105 (11)
					112 (2)
					117 (5)
Cervical, left, short — [M]					
					117 (4,7)
Cervical, right, short — [M]					
					105 (2)
Thoracolumbar, bilateral, full — [M]					
	25 (11)		57 (13)	86 (12)	105 (4,5,9,12,13)
			73 (4)		119 (1,5,10,12)
					123 (8)
Thoracolumbar, bilateral, short — [V]					
	1 (3,5,7,11,14)	28 (13)	52 (8)	76 (7,9)	101 (4,9,12)
	5 (11)	31 (1,6,7,11)	53 (1,3)	79 (5)	104 (11,13,14)
	6 (5,10)	34 (5,10)	54 (5)	81 (13)	105 (1)
	7 (4,10,11)	35 (8,14)	55 (6)	82 (3,6,10)	107 (14)
	8 (3,8)	36 (2)	57 (1,6,12)	86 (1,7,10,14)	109 (8)
	10 (3,7,11)	39 (7)	61 (2)	87 (5,6,12)	111 (4)
	11 (10,14)	43 (4,6,8,15,16)	64 (1)	89 (8)	113 (4)
	12 (1,2)	46 (2)	65 (10)	90 (5)	114 (8)
	14 (9,10)	47 (6)	69 (12)	93 (16)	116 (7,10)
	16 (3,8,11)		72 (4,7,9)	97 (3,7,8,11)	119 (2,3,13)
	17 (5,8)		73 (1,2,3,5,6,11, 12,13)	99 (8)	123 (3,6,10,13)
	19 (15)			100 (9)	
	23 (11)		74 (4,9)		
	24 (6)				
	25 (2,4,5,10)				
Thoracolumbar, left, full — [M]					
		41 (11)	54 (3,14,15)	82 (13)	104 (10)
			73 (10)	86 (3)	105 (6,14)
				97 (12)	110 (7)
					119 (4,7,8)
					123 (4,5)
					125 (4,7,11,12)

TABLE B8
Fetal Findings Cross Reference of Dams and Fetuses
in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Skeletal – Body					
Number of fetuses examined	296	283	279	247	254
Number of dams examined	23	21	21	19	21
Supernumerary Rib (continued)					
Thoracolumbar, left, short — [V]					
	1 (8,15)	26 (3)	52 (7)	76 (3,4,13,14,17)	101 (2)
	4 (13,15)	28 (5)	53 (2,5,6,7,10)	79 (12)	102 (1,5)
	5 (2,5)	29 (13)	54 (7,9)	81 (4,9,10)	103 (11,14)
	6 (9)	30 (1,9,10,11)	55 (3,8,10,12)	84 (4,8)	104 (3,7)
	7 (3)	32 (9)	57 (8,11)	85 (4,10)	105 (2,3,7)
	8 (11)	33 (2)	59 (7,8,11,14)	86 (13)	107 (2,13,15)
	10 (4)	35 (2,6)	60 (8)	87 (3,7,9,14,15)	110 (6)
	12 (3)	36 (3,4,10,13)	61 (15)	89 (2,12)	111 (8)
	14 (1)	39 (5)	67 (11)	90 (6,7,10)	112 (10,11)
	15 (11)	40 (16)	69 (7,8)	92 (5,7,8,9)	113 (9,11)
	16 (4,9)	41 (3,5,9,10,14, 16)	70 (5)	93 (5)	116 (3,9)
	17 (1,3,9)		72 (1,2,3,10)	96 (7,13,15)	117 (7)
	18 (3)	43 (2,3,14)	74 (3,5,8,10)	97 (6,9,10)	119 (6,9,14)
	19 (3,4,6,12)	47 (8,11)	75 (3,8)	98 (5,9)	123 (7,12)
	20 (4,15)	49 (1,4)		99 (2,3,4)	
	21 (1)			100 (1,2,7,8,10, 11,12)	
	22 (4)				
Thoracolumbar, right, full — [M]					
	17 (9)			81 (4)	104 (9)
					105 (3)
					107 (2)
Thoracolumbar, right, short — [V]					
	6 (15)	27 (1)	53 (11)	79 (10,14)	101 (8)
	8 (5)	34 (12)	54 (6,11,14,15)	82 (13)	104 (6,8,10,12)
	10 (2,5,9)	36 (7,11)	61 (12,16)	84 (6)	105 (6,8,14)
	11 (12)	38 (14)	64 (5,6)	85 (7,9,12)	106 (5)
	14 (11)	39 (14)	67 (8)	86 (3,9,11)	109 (10)
	15 (3)	43 (13)	70 (6)	89 (1,4,5)	110 (3,7)
	18 (5)	47 (12)	72 (6,13,14)	90 (9,12,15)	116 (11)
	19 (9)		73 (10)	97 (12)	117 (6)
	23 (1,3,6)		75 (4,5,12,13,15)		119 (4,7,8,11)
	25 (1,9)				122 (4)
					123 (2,4,5,11)
					125 (3,4,7,10,11, 12)
Vertebrae					
Presacral vertebrae, greater than 26 — [V]					
					105 (13)
Thoracic arch, 7th left, supernumerary — [M]					
					123 (9)
Thoracic arch, 7th right, fused — [M]					
					123 (9)
Thoracic arch, multiple sites, misshapen — [M]					
					123 (9)

TABLE B8
Fetal Findings Cross Reference of Dams and Fetuses
in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

	0 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Skeletal – Body					
Number of fetuses examined	296	283	279	247	254
Number of dams examined	23	21	21	19	21
Vertebrae (continued)					
Thoracic centrum, 12th, incomplete ossification — [V]		34 (2)			105 (2) 112 (6)
Thoracic centrum, 13th, incomplete ossification — [V]					105 (12)
Thoracic centrum, 1st, unossified — [V]					115 (6)
Thoracic vertebra, multiple sites, misaligned — [V]					123 (9)
External					
Number of fetuses examined	296	283	279	247	254
Number of dams examined	23	21	21	19	21
Anogenital					
Anus, absent — [M]					107 (14)
Body - General					
Body, subcutaneous hemorrhage — [GF]	4 (15)	28 (9,11) 30 (9) 46 (1)	54 (13,14,15)	90 (7)	115 (5) 117 (6) 125 (10)
Extremities					
Tail, Bent — [M]		33 (7) 34 (8)			
Tail, thread-like — [M]					107 (14)
Trunk					
General, omphalocele — [M]					123 (3)

Findings are reported by dam ID number and fetus ID number (displayed in parentheses).
[V] = Variation
[M] = Malformation
[GF] = Gross finding

APPENDIX C
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

4-Methylcyclohexanemethanol

4-Methylcyclohexanemethanol (MCHM) was obtained from TCI America (Portland, OR) in one lot (KDY3F) that was used in the dose range-finding study and the prenatal developmental toxicity study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at MRIGlobal (Kansas City, MO) for the study laboratory at Southern Research (Birmingham, AL). Reports on analyses performed in support of the MCHM studies are on file at the National Institute of Environmental Health Sciences.

Lot KDY3F of the test chemical, a clear colorless liquid, was identified as MCHM using Fourier transform infrared (FTIR), proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy, and gas chromatography (GC) with mass spectrometry (MS) detection. In addition, boiling point, density, and octanol:water partition coefficient were measured. All spectra were consistent with isomers of the proposed structure and literature spectra of MCHM (ACD, 1994-2014; NIST, 2008; ACS, 2014a,b). Two components tentatively identified as *cis* and *trans* isomers of MCHM were observed for the test article using GC/MS. Representative FTIR and FT proton NMR spectra are presented in Figures C1 and C2, respectively. The boiling point of the test chemical was 199.4° C [consistent with a literature reference value of 202° C (Allen *et al*., 1933)], the relative density was 0.9203 g/mL, and the octanol:water partition coefficient was 353 (resulting in a log P of 2.55).

The moisture content of lot KDY3F was determined using Karl Fischer titration. Elemental analyses for carbon, hydrogen, and nitrogen were conducted by Galbraith Laboratories, Inc. (Knoxville, TN). The purity profile was determined using GC with flame ionization detection (FID) and two columns with differing polarities.

For lot KDY3F, Karl Fischer titration indicated 0.209% water. Elemental analyses for carbon, hydrogen, and nitrogen were consistent with the theoretical values for MCHM. GC/FID analysis by system A (Table C1) detected two major peaks with a combined area of 99.97% of the total peak area and no impurities with areas ≥0.05% of the total peak area. The relative areas of the two major peaks indicated that MCHM consisted of 67.99% *cis* and 31.98% *trans* isomers. GC/FID by system B detected two major peaks with a combined relative area of 99.83% [with relative areas of 67.80% (*cis*) and 32.03% (*trans*) isomers)], and two minor impurities totaling 0.13% of the total peak area. The overall purity of lot KDY3F was determined to be greater than or equal to 99.8%.

Stability studies of the bulk chemical were conducted using GC/FID by a system similar to system A. These studies indicated that MCHM was stable as a bulk chemical for 2 weeks when stored in amber glass vials under an inert headspace, sealed with aluminum caps with Teflon®-lined septa at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature under an inert headspace in amber glass bottles. Reanalyses of the bulk chemical were performed during the animal studies by the analytical chemistry laboratory using FTIR and GC/FID by system C and no degradation of the bulk chemical was detected.

Corn Oil

Corn oil was obtained from Spectrum Laboratory Products, Inc. (Gardena, CA), in two lots (ICK0678 and 2DG0376) that were used as the vehicle in the dose range-finding and prenatal developmental toxicity studies, respectively. A solubility study of MCHM was performed by the analytical chemistry laboratory; after 17 days under refrigerated conditions, the test article remained soluble in corn oil at up to 600 mg/mL with no remixing required. Both lots contained peroxide levels less than the rejection level of 3 meQ/kg corn oil.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once for the dose range-finding study and once for the prenatal developmental toxicity study by mixing the appropriate amount of MCHM with corn oil to give the required concentrations (Table C2). The dose formulations were stored at room temperature in amber glass bottles with Teflon® lined caps, protected from light, for up to 27 (dose range-finding study) or 39 (prenatal developmental toxicity study) days.

Stability studies of 0.20 and 2.0 mg/mL formulations were performed by the analytical chemistry laboratory with GC/FID by system C (Table C1). For the 0.2 mg/mL formulation, stability was confirmed for at least 42 days when the formulation was stored under ambient or refrigerated conditions, protected from light, and for 3 hours under simulated animal room conditions. In addition, for the 2.0 mg/mL formulation, stability was confirmed for at least 44 days when stored under ambient or refrigerated conditions, protected from light.

Analyses of the dose formulations of MCHM were conducted by the analytical chemistry laboratory using GC/FID by system C. During the dose range-finding study, the dose formulations were analyzed once; all four dose formulations were within 10% of the target concentrations (Table C3). Animal room samples of these dose formulations were also analyzed; all four animal room samples were within 10% of the target concentrations. During the prenatal developmental toxicity study, the dose formulations were analyzed once; animal room samples of these dose formulations were analyzed twice (Table C4). All four dose formulations analyzed were within 10% of the target concentrations and all eight animal room samples were within 10% of the target concentrations.

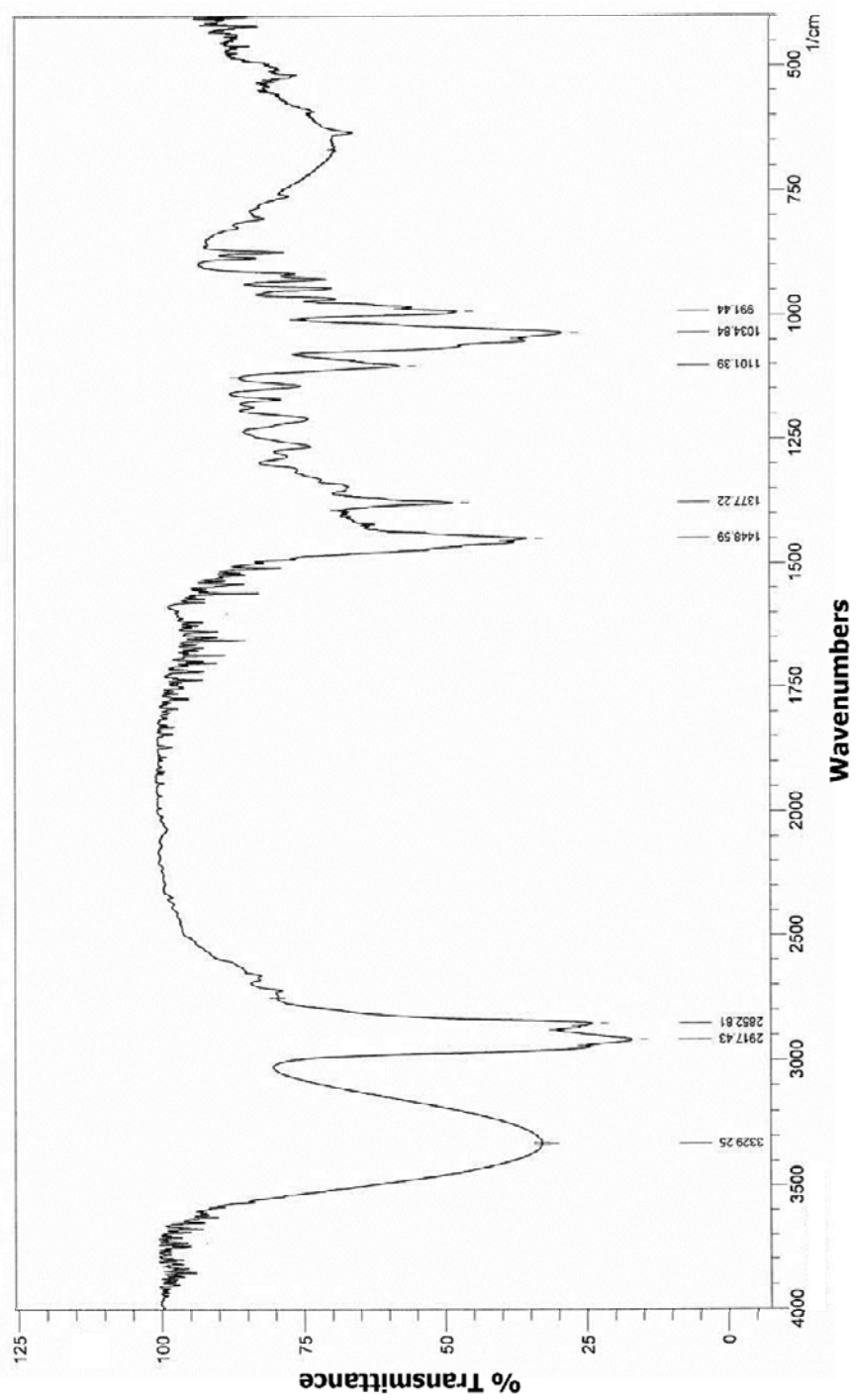


FIGURE C1
Fourier Transform Infrared Absorption Spectrum of 4-Methylcyclohexanemethanol

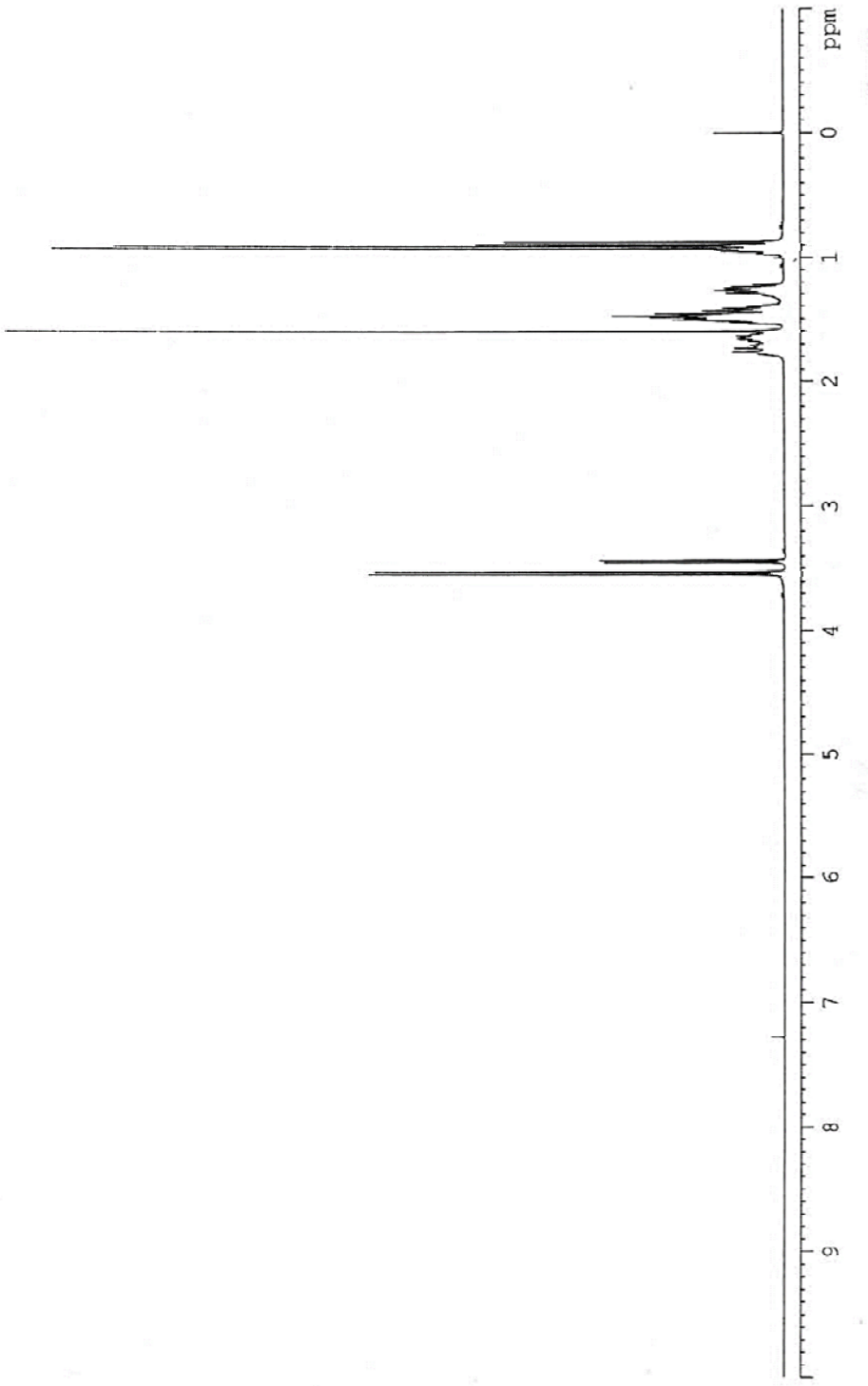


FIGURE C2
Fourier Transform Proton Nuclear Magnetic Resonance Spectrum of 4-Methylcyclohexanemethanol

TABLE C1
Gas Chromatography Systems Used in the Gavage Studies of 4-Methylcyclohexanemethanol[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	Zebron™ ZB-624, 30 m × 0.53 mm, 3.0 µm film (Phenomenex, Torrance, CA)	Helium at 5 mL/minute	50° C for 2 minutes, then 10° C/minute to 240° C, held for 4 minutes
System B			
Flame ionization	Agilent DB-1, 30 m × 0.53 mm, 1.5 µm film (Agilent Technologies, Inc., Santa Clara, CA)	Helium at 5 mL/minute	50° C for 2 minutes, then 10° C/minute to 240° C, held for 4 minutes
System C			
Flame ionization	Rtx®-VMS, 30 m × 0.53 mm, 3.0 µm film (Restek, Bellefonte, PA)	Helium at 5 mL/minute	50 C° for 2 minutes, then 10° C/minute to 240° C, held for 4 minutes

[a] The gas chromatographs were manufactured by Agilent Technologies, Inc. (Santa Clara, CA).

TABLE C2
Preparation and Storage of Dose Formulations in the Gavage Studies of 4-Methylcyclohexanemethanol

Dose Range-Finding Study	Prenatal Developmental Toxicity Study
Preparation	
The appropriate amounts of 4-methylcyclohexanemethanol were weighed into 500 mL volumetric flasks, diluted to near volume with corn oil, swirled to dissolve, diluted to volume with corn oil, mixed by inversion, and then stirred with a magnetic stir bar for 30 minutes. The dose formulations were prepared once.	Same as the dose range-finding study except that the formulations were prepared in 1 L volumetric flasks.
Chemical Lot Number	
KDY3F	KDY3F
Maximum Storage Time	
27 days	39 days
Storage Conditions	
Stored in amber glass bottles with Teflon®-lined caps, protected from light, at room temperature	Stored in amber glass bottles with Teflon®-lined caps, protected from light, at room temperature
Study Laboratory	
Southern Research (Birmingham, AL)	Southern Research (Birmingham, AL)

TABLE C3
Results of Analyses of Dose Formulations Administered to Female Rats in the Dose Range-Finding Gavage Study of 4-Methylcyclohexanemethanol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
August 13, 2014	August 13, 2014	75	75.82	+1
		150	152.0	+1
		300	303.5	+1
		450	456.0	+1
	September 11, 2014[b]	75	75.36	0
		150	152.1	+1
		300	302.6	+1
		450	448.5	0

[a] Results of triplicate analyses. Dosing volume=2 mL/kg; 75 mg/mL=150 mg/kg, 150 mg/mL=300 mg/kg, 300 mg/mL=600 mg/kg; 450 mg/mL=900 mg/kg.
[b] Animal room samples

TABLE C4
Results of Analyses of Dose Formulations Administered to Female Rats in the Prenatal Developmental Toxicity Gavage Study of 4-Methylcyclohexanemethanol

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
October 30, 2014	October 30, 2014	25	24.78	–1
		50	49.67	–1
		100	99.84	0
		200	199.3	0
	December 3, 2014[b]	25	24.88	0
		50	50.57	+1
		100	100.5	+1
		200	199.6	0
	December 10, 2014[b]	25	25.00	0
		50	50.09	0
		100	99.83	0
		200	200.1	0

[a] Results of triplicate analyses. Dosing volume=2 mL/kg; 25 mg/mL=50 mg/kg, 50 mg/mL=100 mg/kg, 100 mg/mL=200 mg/kg, 200 mg/mL=400 mg/kg.
[b] Animal room samples

APPENDIX D
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE D1
Ingredients of NIH-07 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground #2 yellow shelled corn	24.25
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	3.00
Soy oil (without preservatives)	2.50
Dried brewer's yeast	2.00
Dried molasses	1.50
Calcium phosphate, dibasic (USP)	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.40
Choline chloride (70% choline)	0.10

TABLE D2
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	4,600,000 IU	D-activated animal sterol
K	2.8 mg	Dimethylpyrimidinol bisulfite
E	20 IU	α-Tocopheryl acetate
Niacin	30 mg	
Folic acid	2.2 mg	
d-Pantothenic acid	18.0 mg	*d*-Calcium pantothenate
Riboflavin	3.4 mg	
Thiamin	10 mg	Thiamine mononitrate
B_{12}	45.4 µg	
Pyridoxine	5.9 mg	Pyridozine hydrochloride
Biotin	140 mg	*d*-Biotin
Minerals		
Iron	120 mg	Iron sulfate
Zinc	16 mg	Zinc oxide
Manganese	60 mg	Manganese oxide
Copper	4.0 mg	Copper sulfate
Iodine	1.4 mg	Calcium iodate
Cobalt	0.4 mg	Cobalt carbonate

[a] Per kg of finished product

TABLE D3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	25.1		1
Crude fat (% by weight)	0.5		1
Crude fiber (% by weight)	3.15		1
Ash (% by weight)	6.33		1
Amino Acids (% of total diet)			
Arginine	1.375 ± 0.065	1.3 – 1.49	8
Cystine	0.321 ± 0.035	0.274 – 0.372	8
Glycine	1.145 ± 0.077	1.06 – 1.31	8
Histidine	0.516 ± 0.023	0.497 – 0.553	8
Isoleucine	0.982 ± 0.025	0.952 – 1.03	8
Leucine	1.996 ± 0.054	1.93 – 2.08	8
Lysine	1.261 ± 0.032	1.22 – 1.32	8
Methionine	0.487 ± 0.015	0.468 – 0.515	8
Phenylalanine	1.091 ± 0.020	1.07 – 1.12	8
Threonine	0.919 ± 0.032	0.883 – 0.961	8
Tryptophan	0.280 ± 0.022	0.266 – 0.326	8
Tyrosine	0.855 ± 0.039	0.785 – 0.894	8
Valine	1.134 ± 0.0245	0.11 – 1.17	8
Essential Fatty Acids (% of total diet)			
Linoleic	2.33 ± 0.211	2.04 – 2.59	8
Linolenic	0.25 ± 0.028	0.217 – 0.296	8
Vitamins			
Vitamin A (IU/kg)	6,020		1
α–Tocopherol (ppm)	48.07 ± 4.38	40.3 – 52.73	8
Thiamine[b] (ppm)	16.1		1
Riboflavin (ppm)	14.3 ± 3.58	10 – 19.8	8
Niacin (ppm)	99.4 ± 9.10	87 – 112	8
Pantothenic acid (ppm)	45.6 ± 3.13	40.4 – 51.1	8
Pyridoxine[b] (ppm)	12.33 ± 2.25	9.63 – 15.6	8
Folic acid (ppm)	2.47 ± 0.550	1.68 – 3.09	8
Biotin (ppm)	0.342 ± 0.125	0.25 – 0.64	8
Vitamin B_{12} (ppb)	50.21 ± 7.47	41.8 – 61.6	8
Choline (as chloride) (ppm)	1,776 ± 197	1,570 – 2,200	8
Minerals			
Calcium (%)	1.170		1
Phosphorus (%)	0.885		1
Potassium (%)	0.829 ± 0.036	0.77 – 0.88	8
Chloride (%)	0.625 ± 0.102	0.441 – 0.8	8
Sodium (%)	0.368 ± 0.047	0.318 – 0.469	8
Magnesium (%)	0.183 ± 0.009	0.170 – 0.194	8
Iron (ppm)	376.3 ± 52.5	276 – 455	8
Manganese (ppm)	91.03 ± 7.93	80.7 – 104	8
Zinc (ppm)	64.07 ± 11.32	52.4 – 89.2	8
Copper (ppm)	14.11 ± 2.91	11.9 – 21.1	8
Iodine (ppm)	1.71 ± 0.886	0.54 – 3.45	8
Chromium (ppm)	3.96 ± 0.033	3.91 – 4.00	8
Cobalt (ppm)	0.53 ± 0.293	0.01 – 0.963	8

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine)

TABLE D4
Contaminant Levels in NIH-07 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.472		1
Cadmium (ppm)	0.069		1
Lead (ppm)	0.169		1
Mercury (ppm)	<0.02		1
Selenium (ppm)	0.523		1
Aflatoxins (ppb)	<5.00		1
Nitrate nitrogen[c] (ppm)	10		1
Nitrite nitrogen[c] (ppm)	<0.61		1
BHA[d] (ppm)	2.37		1
BHT[d] (ppm)	<1.0		1
Aerobic plate count (CFU/g)	10		1
Coliform (MPN/gm)	3.0		1
Escherichia coli (MPN/g)	<3		1
Salmonella (MPN/g)	Negative		1
Total nitrosamines (ppb)	4.6		1
N–Nitrosodimethylamine (ppb)	0		1
N–Nitrosopyrrolidine (ppb)	4.6		1
Pesticides (ppm)			
α-BHC	<0.01		1
β-BHC	<0.02		1
γ-BHC	<0.01		1
δ-BHC	<0.01		1
Heptachlor	<0.01		1
Aldrin	<0.01		1
Heptachlor epoxide	<0.01		1
DDE	<0.01		1
DDD	<0.01		1
DDT	<0.01		1
HCB	<0.01		1
Mirex	<0.01		1
Methoxychlor	<0.05		1
Dieldrin	<0.01		1
Endrin	<0.01		1
Telodrin	<0.01		1
Chlordane	<0.05		1
Toxaphene	<0.10		1
Estimated PCBs	<0.20		1
Ronnel	<0.01		1
Ethion	<0.02		1
Trithion	<0.05		1
Diazinon	<0.10		1
Methyl chlorpyrifos	0.041		1
Methyl parathion	<0.02		1
Ethyl parathion	<0.02		1
Malathion	0.164		1
Endosulfan I	<0.01		1
Endosulfan II	<0.01		1
Endosulfane sulfate	<0.03		1

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal