NTP TECHNICAL REPORT

ON THE

PRENATAL DEVELOPMENTAL TOXICITY STUDIES

OF DIMETHYLAMINOETHANOL BITARTRATE

(CAS No. 5988-51-2)

IN SPRAGUE DAWLEY (Hsd:Sprague Dawley SD) RATS

(GAVAGE STUDIES)

Scheduled Peer Review Date: 2019

NTP DART-04

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 DIMETHYLAMINOETHANOL BITARTRATE

(CAS No. 5988-51-2)

IN SPRAGUE DAWLEY (Hsd:Sprague Dawley SD) RATS

(GAVAGE STUDIES)

Scheduled Peer Review Date: 2019

NTP DART-04

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

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

Southern Research
Conducted studies and evaluated findings

C.D. Hébert, Ph.D., Principal Investigator
E. Mylchreest, Ph.D., Study Director
A.H. Stokes, Ph.D., Study Director

Social & Scientific Systems, Inc.
Provided statistical analyses

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

RTI Laboratories
Conducted pre-test chemistry activities and dose formulations

R.A. Fernando, Ph.D.
S.D Cooper, B.S.
J. Blake, B.S.

CONTRIBUTORS

National Toxicology Program
Oversaw Quality Assessment Contract

G.K. Roberts, Ph.D.

Vistronix
Prepared data for report

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

Dynamac Corporation
Prepared quality assessment audits

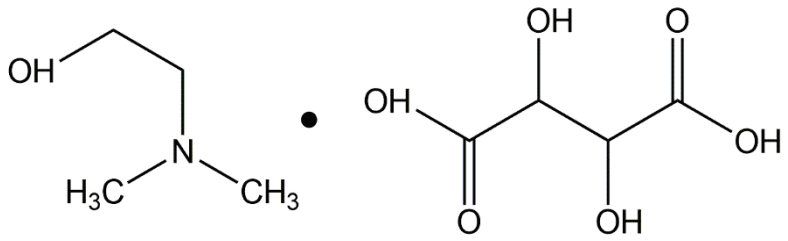
S. Brecher, Ph.D., Principal Investigator
S. Iyer, B.S.
V.S. Tharakan, D.V.M.

Biotechnical Services, Inc.
Prepared Prenatal Developmental Toxicity Report

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

CONTENTS

ABSTRACT



DIMETHYLAMINOETHANOL BITARTRATE

CAS No. 5988-51-2

Chemical Formula: $C_4H_{11}NO \bullet C_4H_6O_6$ Molecular Weight: 239.23

Synonyms: 2-Dimethylaminoethanol bitartrate; 2-dimethylaminoethanol tartrate; dimethylethanolamine bitartrate; *N,N*-dimethylethanolamine-tartaric acid salt; ethanol, 2-(dimethylamino)-, [R(R*,R*)]-2,3-dihydroxybutanedioate; ethanol, 2-(dimethylamino)-, tartrate

Dimethylaminoethanol is a close structural analog of choline, an essential nutrient. Dietary supplements containing dimethylaminoethanol bitartrate, a salt of dimethylaminoethanol, are marketed to improve memory and general cognitive function due to the ability of dimethylaminoethanol to increase levels of acetylcholine in the brain. Human exposure to dimethylaminoethanol may also occur through occupational and industrial routes (e.g., spray painting, beverage can lacquering, etc.). Dimethylaminoethanol was nominated by the National Institute of Environmental Health Sciences (NIEHS) for toxicologic characterization due to concerns for widespread human exposure through its use in industrial and consumer products. Due to limited literature indicating that dimethylaminoethanol may be a teratogen and reproductive toxicant and the possibility for widespread exposure to the salt form of dimethylaminoethanol (dimethylaminoethanol bitartrate) as a dietary supplement in women of childbearing age, the National Toxicology Program (NTP) conducted prenatal developmental toxicology studies in Sprague Dawley (Hsd:Sprague Dawley SD) rats. In these studies, time-mated female rats received dimethylaminoethanol bitartrate in sterile water by gavage from implantation on gestation day (GD) 6 to the day before expected parturition (GD 20). In order to identify dose levels that would appropriately challenge the model

system, dimethylaminoethanol bitartrate-related maternal and fetal toxicity was examined in the dose range-finding study followed by the prenatal developmental toxicity study.

DOSE RANGE-FINDING PRENATAL DEVELOPMENTAL TOXICITY STUDY

Groups of 10 time-mated female rats were administered 0, 250, 500, or 1,000 mg dimethylaminoethanol bitartrate/kg body weight per day in sterile water by gavage from GD 6 to GD 20. Vehicle control (0 mg/kg) animals received sterile water.

There were no indications of maternal or fetal toxicity in the dose range-finding study. All animals survived to study termination. There were no dose-related effects on maternal body weights, body weight gains, body weights corrected for live litter size, or feed consumption. The number of pregnant animals, mean number of corpora lutea, dead fetuses, early and late resorptions, and fetal sex ratio were similar across all treatment groups. There was a significant positive trend in the mean number of live female fetuses per litter relative to dose. There were no exposure-related fetal findings.

PRENATAL DEVELOPMENTAL TOXICITY STUDY

As no maternal toxicity was observed in the dose range-finding study, groups of 25 time-mated female rats were administered 0, 250, 500, or 1,000 mg dimethylaminoethanol bitartrate/kg body weight per day in sterile water by gavage from GD 6 to GD 20. Vehicle control (0 mg/kg) animals received sterile water. In this study, dimethylaminoethanol bitartrate was well-tolerated and there were no significant effects on mortality, maternal body weights, body weight gains, body weights corrected for litter size, or feed consumption during gestation. One dam each in the 1,000 mg/kg group was euthanized moribund (GD 21) or found dead (GD 10), but these deaths were not considered dose-related. Clinical observations were limited to single or sporadic incidences with the exception of brown or red vaginal discharge, which was observed between GD 14 and GD 21 in 10/20, 3/20, 4/20, and 10/24 dams in the 0, 250, 500, and 1,000 mg/kg groups, respectively. There were no notable placental or other maternal gross observations at necropsy with the exception of a significant, but not biologically relevant, positive trend in mean absolute liver weight.

The number of pregnant animals, mean number of corpora lutea, implantations, litter size, live fetuses per litter, and fetal sex ratio were similar across all treatment groups.

External and visceral malformations were limited to common background findings and singular or sporadic incidences. There were no observed incidences of fetal head, specifically brain, abnormalities. Skeletal malformations and variations occurred predominantly in the ribs. A significant increase in the incidence of total, short thoracolumbar ribs (a variation) was observed in the 1,000 mg/kg group, along with a significant positive trend. Additionally, there was a significant increase in the number of supernumerary sites, or ossification sites, in the skull in 1,000 mg/kg fetuses as well as a significant positive trend across all groups. These effects may be reversible (supernumerary ribs) or of uncertain biological significance (supernumerary sites in the skull); however, in the absence of maternal toxicity or effects on fetal body weight, the increased incidences of extra ossification sites in two separate locations, each occurring through two different skeletal developmental pathways, suggest that these effects may be related to dimethylaminoethanol bitartrate exposure.

CONCLUSIONS

Under the conditions of this prenatal study, there was *equivocal evidence* of developmental toxicity of dimethylaminoethanol bitartrate in Hsd:Sprague Dawley SD rats based on increased incidences of short thoracolumbar ribs and supernumerary sites in the skull in the absence of overt maternal toxicity.

Summary of Exposure-Related Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Maternal Parameters:				
Animals on study	25	25	25	25
Number pregnant	20	20	20	24
Number found dead	0	0	0	1
Number euthanized moribund	0	0	0	1
Number euthanized - early delivery	1	0	0	1
Clinical Observations	None	None	None	None
Body Weight and Feed Consumption[a]				
Terminal body weight	359.6 ± 8.8	375.2 ± 5.3	380.3 ± 5.1	367.1 ± 6.5
Body weight change GD 6 to 21	120.5 ± 7.5	135.2 ± 4.7	140.0 ± 4.2*	127.7 ± 5.8
Feed consumption GD 6 to 21	21.2 ± 0.4	21.6 ± 0.4	22.0 ± 0.3	21.5 ± 0.4
Necropsy Observations	None	None	None	None
Developmental/Fetal Parameters:				
Number of litters examined	19	20	20	22
Number of live fetuses evaluated	209	265	260	249
Number of live fetuses per litter[b]	11.00 ± 1.12	13.25 ± 0.60	13.00 ± 0.56	11.32 ± 1.07
Number of early resorptions	9	10	10	14
Number of late resorptions	0	0	0	0
Number of dead fetuses	0	0	0	11
Number with whole litter resorptions	0	0	0	0
Percent post-implantation loss[b]	5.05 ± 1.57	3.80 ± 1.53	3.45 ± 1.10	11.17 ± 5.56
Fetal body weight per litter[a]	5.38 ± 0.15	5.26 ± 0.05	5.33 ± 0.06	5.22 ± 0.09
Male fetal weight per litter	5.44 ± 0.16 (18)	5.37 ± 0.05 (19)	5.50 ± 0.06 (20)	5.51 ± 0.09 (20)
Female fetal weight per litter	5.15 ± 0.12 (18)	5.14 ± 0.06 (20)	5.18 ± 0.07 (20)	5.21 ± 0.08 (20)
Gravid uterine weight[a]	80.18 ± 7.36	95.85 ± 3.93	96.17 ± 3.82	85.25 ± 6.38
External Findings	None	None	None	None
Visceral Findings	None	None	None	None
Skeletal Findings[c]				
Supernumerary rib				
Thoracolumbar short, total – [V]				
Fetuses	56 (26.79)**##	56 (21.21)	59 (22.69)	100 (38.46)**#
Litters	17 (89.47)	18 (90.00)	18 (90.00)	19 (86.36)
Skull				
General, supernumerary site – [V]				
Fetuses	1 (1.0)**##	3 (2.34)	2 (1.59)	13 (10.16)**#
Litters	1 (5.56)**	3 (15.00)	2 (10.00)	10 (50.00)**
Level of evidence of developmental toxicity: Equivocal 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≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column) in litter-based analysis of fetuses
P≤0.01
[a] Results given in grams. Data are displayed as mean ± standard error.
[b] Data are displayed as mean ± standard error.
[c] Upper row denotes number of affected fetuses (%) and lower row the number of affected litters (%)
GD = Gestational Day
[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

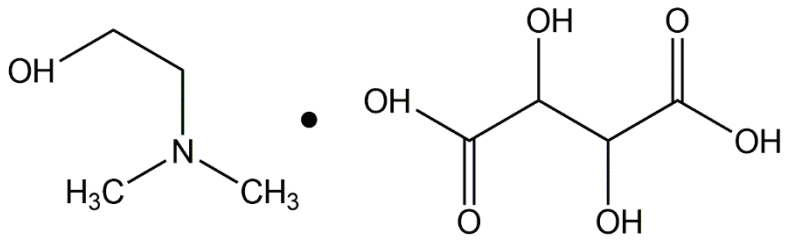
The members of the Peer Review Panel who evaluated the draft NTP Technical Report on dimethylaminoethanol bitartrate in 2019, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have 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 carcinogenic activity and other observed toxic responses.

SUMMARY OF PEER REVIEW PANEL COMMENTS

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

INTRODUCTION



DIMETHYLAMINOETHANOL BITARTRATE

CAS No. 5988-51-2

Chemical Formula: $C_4H_{11}NO \bullet C_4H_6O_6$ Molecular Weight: 239.23

Synonyms: 2-Dimethylaminoethanol bitartrate; 2-dimethylaminoethanol tartrate; dimethylethanolamine bitartrate; *N,N*-dimethylethanolamine-tartaric acid salt; ethanol, 2-(dimethylamino)-, [R(R*,R*)]-2,3-dihydroxybutanedioate; ethanol, 2-(dimethylamino)-, tartrate

CHEMICAL AND PHYSICAL PROPERTIES

Dimethylaminoethanol bitartrate, a salt of dimethylaminoethanol, is produced from dimethylaminoethanol and tartaric acid. Dimethylaminoethanol bitartrate is a white powder with a molecular weight of 239.23 g/mol and a melting point range of 109° to 113° C; it is also soluble in water (*Merck*, 2006; Sigma-Aldrich, 2014).

There is limited information available on the bitartrate form, but dimethlyaminoethanol, the free base, is a colorless liquid with a molecular weight of 89.136 g/mol, a melting point of –65° C, and a boiling point of 134.1° C (HSDB, 2015). Dimethylaminoethanol has a density of 0.8866 g/cm^3 (at 20° C), a log K_{ow} of –0.55 (at 23° C), and a vapor pressure of 21 mm Hg torr (at 20° C). It has also been reported to have an amine or fishy odor. Dimethylaminoethanol is moderately flammable (HSDB, 2015).

PRODUCTION, USE, AND HUMAN EXPOSURE

A production volume of 100 to 250 million pounds was reported for dimethylaminoethanol in 2015 based on information submitted under the 2016 Toxic Substances Control Act Inventory Update Rule (USEPA, 2016). Dimethylaminoethanol was also included in the 2004 Organisation for Economic Cooperation and Development (OECD, 2004) List of High Production Volume Chemicals, which included chemicals produced at levels greater than 1,000 tons per year. The USEPA Chemical Data Reporting (CDR) database (2011) listed eight United States-based industries producing dimethylaminoethanol; two of those companies reported annual production volumes of 38,000 and 79,000 pounds dimethylaminoethanol (USEPA, 2011).

Dimethylaminoethanol is used in a variety of industrial and consumer applications, including as a catalyst in the curing of epoxy resins and polyurethanes, as an inhibitor of corrosion, and as an amino resin stabilizer. Dimethylaminoethanol is also used as a chemical intermediate in the synthesis of antihistamines, local anesthetics, dyes, textiles, and emulsifiers/solubilizers in water-based paints (HSDB, 2015). Based on the OECD (1996) Screening Information Data Set, it is estimated that 50% of dimethylaminoethanol produced is utilized in the manufacturing of flocculants for wastewater treatment, 20% is used in the manufacturing of flexible and rigid polyurethane foams and lacquers, 20% is used in the manufacturing of water-based paints and surface coatings, and 10% is utilized to produce ion exchange resins, pharmaceuticals, and corrosion inhibitors.

Occupational exposure to dimethylaminoethanol is believed to primarily involve workers in the spray painting and beverage can lacquering industries. There is some concern for the release of dimethylaminoethanol into the environment from these industrial sources (Pitts *et al.*, 1981).

Non-occupational exposure to dimethylaminoethanol can occur through the consumption of squid and fish (NTP, 2002). Dimethylaminoethanol may also be released from sealants, architectural coatings, furniture and cabinet coatings, polyurethane foam cushions, and carpets in homes, buildings, and vehicles (De Silva, 1977; Rothe and Cordelair, 2001); however, the primary concern for non-occupational exposure is through pharmaceuticals and dietary supplements.

Dimethylaminoethanol is a close structural analog of choline (*N,N,N*-trimethylaminoethanol), an essential nutrient. Dimethylaminoethanol supplies the brain with choline, where it is acetylated by choline acetylase to form acetylcholine (De Silva, 1977). Dimethylaminoethanol salts (i.e., *p*-acetamidobenzoate) have been used to treat central nervous system disorders in humans, specifically those considered to be associated with decreased cholinergic neuron function (Stenbäck *et al.*, 1988). Dimethylaminoethanol salts have also been used to manage learning and behavioral problems, Huntington's chorea, chronic fatigue, and neurasthenia (De Silva, 1977; Hendler and Rorvik, 2001; HSDB, 2015). The prescription drug Deaner® (deanol *p*-acetamidobenzoate) was used in the United States for over 20 years to treat learning and behavioral problems in children, but was withdrawn from the market in 1983 due to better alternatives becoming available. The dimethylaminoethanol chemical structure is also part of many different pharmaceutical formulations, including a variety of antihistamines, antiemetics, local anesthetics, and tamoxifen (NTP, 2002).

A number of dietary supplements on the market contain dimethylaminoethanol, most commonly in the form of dimethylaminoethanol bitartrate, and these supplements are purported to improve memory and general cognitive function. The recommended dosage for these supplements varies greatly between products, with recommended adult doses ranging from 100 to 500 mg dimethylaminoethanol/day. One dimethylaminoethanol bitartrate supplement currently on the market contains 351 mg dimethylaminoethanol bitartrate, which corresponds to approximately 130 mg dimethylaminoethanol (Source Naturals®, 2017a). Dietary supplements containing dimethylaminoethanol are also marketed to treat symptoms of attention deficit hyperactivity disorder in children and on average contain 100 mg dimethylaminoethanol (as dimethylaminoethanol bitartrate) (Nature's Plus, 2017; Source Naturals®, 2017b).

REGULATORY STATUS

No regulatory limits for dimethylaminoethanol exposure have been established by NIOSH or the EPA. Individual industrial companies have established workplace exposure limits for dimethylaminoethanol; for example, DuPont has an 8-hour time-weighted average (TWA) of 2 ppm for dimethylaminoethanol (NTP, 2002). Federal regulations currently applicable to dimethylaminoethanol include the following: 40 CFR Part 60 [Subpart YYY – Standards of Performance for Volatile Organic Compounds (VOC) Emissions from Synthetic Organic Chemical Manufacturing

Industry (SOCMI) Wastewater], which regulates the emission of volatile organic compounds (VOCs) from wastewater; 40 CFR § 63.100ff (Subpart F – National Emission Standards for Organic Hazardous Air Pollutants from the Synthetic Organic Chemical Manufacturing Industry), which regulates air pollution emitted by chemical manufacturers that produce dimethylaminoethanol (or mixtures containing dimethylaminoethanol); 21 CFR § 173.20 [Secondary Direct Food Additives Permitted in Food for Human Consumption (Subpart A – Polymer Substances and Polymer Adjuvants for Food Treatment)], which regulates membranes used for food packaging that were produced by reactions with dimethylaminoethanol; and 21 CFR § 175.300 (Indirect Food Additives: Adhesives and Components of Coatings), which states that dimethylaminoethanol, when used in coating emulsions, may be used as an adjuvant at no more than 2% (by weight).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

Data on absorption, distribution, metabolism, and excretion of dimethylaminoethanol bitartrate could not be located in the literature, and there are limited data for dimethylaminoethanol or other dimethylaminoethanol salts or esters in the literature. Briefly, in male Wistar rats, following an intravenous dose of 11 mg/kg (120 mmol/kg) [^{14}C]dimethylaminoethanol or 30 mg/kg (120 mmol/kg) cyprodenate, 0.16% and 0.2%, respectively, of the administered dose was measured in the plasma 6 minutes after dosing (Dormard *et al.*, 1975). Only 33% of the administered dose was eliminated in urine by 24 hours. The *N*-oxide of dimethylaminoethanol was determined to be the primary urinary metabolite in rodents; the N-oxide constituted 13.5% of the dose eliminated in urine within 24 hours. In another study in rats, following an intravenous dose of 30 mg/kg (0.13 mmol/kg) [^{14}C]cyprodenate maleate, the cyclohexylpropionic acid ester of dimethylaminoethanol, approximately 2.41, 1.30, and 0.20% of the dose was found in the liver, brain, and plasma, respectively, 5 minutes after dosing. In male mice, 10 minutes after an intraperitoneal dose of 300 mg/kg (3.30 mmol/kg) dimethylaminoethanol, approximately 280 nmol dimethylaminoethanol/g plasma was detected (Zahniser *et al.*, 1977). Daily oral exposures (duration not provided) of chinchilla rabbits to dimethylaminoethanol as deanol acetamidobenzoate (0.48 µg, 1.8 nmol) or dimethylaminoethanol (10.40 µg, 4.5 nmol), resulted in plasma concentrations of 6 to 7 µM (1.6 to 1.9 µg/mL) deanol acetamidobenzoate or 12 to 18 µM (1.1 to 1.6 µg/mL) dimethylaminoethanol. The chemical could not be

detected in the plasma 36 hours after the end of the treatment period. Concentrations in the cerebrospinal fluid were similar to measurements in the plasma (Ceder and Schuberth, 1977).

NTP investigated the absorption, distribution, metabolism, and excretion of dimethylaminoethanol in male and female Wistar Han rats and B6C3F1/N mice (Shipkowski *et al.,* 2019). Within 24 hours following gavage administration of 10, 50, or 500 mg/kg in rats, 57% to 62% of the administered dose was excreted in urine, 4% to 5% was expired as CO_2, and <1% was recovered in feces and as volatile organic compounds demonstrating that dimethylaminoethanol was well absorbed and rapidly excreted. A significant amount of the administered dose was retained in tissues with 34%, 30%, and 24% of the 10, 50, and 500 mg/kg doses, respectively, recovered 24 hours after administration. Following a single oral dose of 500 mg/kg in rats, [^{14}C]dimethylaminoethanol-derived radioactivity was highest at 2 hours and declined over time with 84%, 24%, 15%, and 13% recovered at 2, 24, 72, and 168 hours, respectively, following administration. Of the tissues examined, liver, kidney, lung, and thyroid gland contained the highest concentrations; tissue to blood ratio was higher than 1 in all examined tissues. Excretion in mice was somewhat different than in rats, with increasing dose (10, 50, and 500 mg/kg), the administered dose recovered at 24 hours in urine increased (16%, 18%, and 43%), expired as CO_2 decreased (22%, 16%, and 13%), and that remaining concentrations in tissues decreased. The tissues with the highest levels of radioactivity were the liver, kidney, thyroid gland, lung, spleen, adipose, and uterus.

Urinary products identified following gavage administration of dimethylaminoethanol were the *N*-oxide metabolite and unmetabolized parent compound; the amounts of each depended on the dose, sex, and species. *N,N*-dimethylglycine was identified as a minor metabolite; dimethylamine and *N,N*-dimethylnitrosamine were not detected. As the dose increased, the concentration of parent compound generally increased compared to that of the *N*-oxide, except in male rats, suggesting saturation of metabolism. In male rats, the *N*-oxide metabolite was not detected at the low dose; however, at the higher doses, male rats produced more *N*-oxide than male mice and female rats and mice (Shipkowski *et al.,* 2019).

Following administration of 500 mg/kg dimethylaminoethanol in male Wistar Han rats, serum choline levels were moderately elevated within 12 hours of administration (Shipkowski *et al.,* 2019). In order to understand the effects

of choline disposition by dimethylaminoethanol, male and female rats were given three consecutive daily gavage doses of 100 or 500 mg/kg dimethylaminoethanol prior to administration of a single dose of 100 mg/kg [^{14}C]choline. The disposition data revealed only modest effects on choline disposition. In an investigation by Schlenk (1990) following intracerebral injection of [^{14}C]dimethylaminoethanol in rats, brain levels of phosphatidylethanolamine were found to increase over the 7-hour observation period and were 10- to 40-fold higher than levels of phosphodimethylethanolamine. Analysis of brain tissue from mice administered [^{14}C]dimethylaminoethanol or *p*-chlorophenoxyacetate, a dimethylaminoethanol derivative, indicated the presence of phosphoryl-dimethylaminoethanol and phosphatidyl-dimethylaminoethanol; phosphatidyl-dimethylaminoethanol is believed to be the end-metabolite of dimethylaminoethanol (Miyazaki *et al.*, 1976). Mice administered dimethylaminoethanol had increased concentrations and rate of turnover of free choline in the blood and kidneys (NTP, 2002). Jope and Jenden (1979) reported increased choline concentrations in the plasma and brain of rats treated with dimethylaminoethanol. The extent to which dimethylaminoethanol is methylated and substituted into acetylcholine is not well understood; however, it has been suggested that, once it crosses the blood-brain barrier, dimethylaminoethanol is methylated to form choline and subsequently incorporated into acetylcholine (NTP, 2002). Dimethylaminoethanol is reported to undergo metabolism via the phospholipid cycle, resulting in the production of phosphoryldimethylethanolamine and glycerophosphatidylcholine (Dormard *et al.*, 1975).

Humans

In a study utilizing human volunteers, 33% of an injected dose of 1 g (10 mmol) dimethylaminoethanol was excreted unchanged (Beard and Noe, 1981). It was proposed that the remaining compound may have been demethylated to ethanolamine and utilized in normal metabolic pathways.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY

Experimental Animals

Choline is an essential nutrient; however, mammals are limited in their ability to synthesize choline and therefore much of it is acquired through dietary sources. Choline is required for many biological processes, in particular nervous system development, and choline deficiency has been associated with the development of neural tube

defects (NTDs) (Fisher *et al.*, 2001, 2002). As dimethylaminoethanol is a structural analog of choline, there is potential for dimethylaminoethanol to disrupt choline uptake and metabolism and interfere with biological processes such as development.

Pregnant Sprague Dawley rats fed a choline-deficient diet supplemented with either 0.8% choline, 1% *N*-methylaminoethanol, or 1% dimethylaminoethanol (10,000 mg/kg feed) from gestational day (GD) 6 to postnatal day (PND) 14 had significantly reduced offspring survival (Zahniser *et al.*, 1978). Despite no effects on pregnancy progression, litter size, or pup body weight, only 18/253 pups from exposed dams survived to at least PND 3, while pups from control dams survived to at least PND 15. Pups from dams fed the dimethylaminoethanol-supplemented diet had increased levels of glycogen and fatty infiltration in their livers; levels of dimethylaminoethanol (72.2 ± 12.7 nmol/g) were measured in the brains of these pups as well. Choline and acetylcholine levels were increased (53% and 36%, respectively) in the brains of pups from dams fed the dimethylaminoethanol-supplemented diet relative to dams fed the choline-deficient diet.

Exposure to dimethylaminoethanol via inhalation for 90 days did not induce any histopathologic changes in the gonads of rats (NTP, 2002).

Inhalation exposure to dimethylaminoethanol (10, 30, or 100 ppm) induced maternal toxicity in pregnant Fischer 344 rats, which was indicated by decreases in body weight (30 and 100 ppm) and ocular effects (30 and 100 ppm) (Leung *et al.*, 1996). Reported gestational effects included significant decreases in the number of viable implants per litter, the percentage of live fetuses per litter, and litter size (all at 10 ppm), as well as a significant decrease in the percentage of male fetuses in rats exposed to 30 ppm dimethylaminoethanol.

Skeletal alterations were observed in the fetuses of pregnant Fischer 344 rats exposed to 10 to 100 ppm dimethylaminoethanol via inhalation from GD 6 to GD 15 (Tyl *et al.*, 1987). Recorded skeletal variations were sporadic and included increased incidences of split cervical centra 1, 2, 3, and/or 4, and bilobed thoracic centrum 1. Decreased incidences of poorly ossified cervical centrum 6, bilobed thoracic centrum 9, bilobed sternebrae 5, and unossified proximal phalanges of the forelimb were also reported. A no-observed-adverse-effect level (NOAEL) of

100 ppm or greater was estimated for embryofetal toxicity and teratogenicity, while a NOAEL of 10 ppm was estimated for maternal toxicity.

Exposure to dimethylaminoethanol (0, 250, 375, 500, or 750 µM) *in vitro* for 26 hours altered choline uptake and metabolism in neurulating mouse embryos (collected at GD 9), resulting in significant dose-dependent increases in the incidence and severity of malformations (Fisher *et al.*, 2001, 2002). These malformations included neural tube defects, craniofacial hypoplasia, caudal dysgenesis, and abnormal circulation. The average amount of embryonic protein was also decreased in embryos exposed to 375 µM dimethylaminoethanol or greater.

Humans

There are no studies in the literature on the reproductive or developmental toxicity of dimethylaminoethanol in humans. Decreases in choline are associated with neural tube defects in humans; however, the data is somewhat inconsistent (Shaw *et al.*, 2004, 2009; Mills *et al.*, 2014). Dimethylaminoethanol is not recommended for use during pregnancy or lactation (Hendler and Rorvik, 2001; NTP, 2002).

GENERAL TOXICITY

Experimental Animals

Reported oral LD_{50} values for rats range from 2,000 to 6,000 mg/kg (22.44 to 67.31 mmol/kg) dimethylaminoethanol (Hartung and Cornish, 1968; Beard and Noe, 1981).

Rats orally exposed to 890, 1,250, or 1,800 mg/kg dimethylaminoethanol for 14 days exhibited signs of toxicity early in the dosing period at the mid and high doses (Union Carbide, 1986). Signs of toxicity included sluggishness, discharge around the eyes and nose, kyphosis, and prostration; however, these abated by days 2 to 5 in surviving animals. Observations at necropsy included red, mottled lungs, dark fluid in the stomach and intestine, and reddened stomach.

Exposure to dimethylaminoethanol via inhalation has been demonstrated to induce toxicity and mortality in rats. Exposure concentration-related mortality was observed in rats exposed to 1,668, 2,408, or 3,311 ppm dimethylaminoethanol for 4 hours and observed for 14 days (Klonne *et al.*, 1987). Rats from all exposure groups were observed with signs of toxicity including lacrimation, nasal discharge, respiratory difficulties, decreased motor activity, and weight loss.

Exposure of F344 rats to 98, 288, or 586 ppm dimethylaminoethanol via inhalation, 6 hours/day over 11 days (nine exposures total), caused 100% mortality at 586 ppm and some mortality at 288 ppm (Klonne *et al.*, 1987). Histologic lesions in the 288 and 98 ppm groups were observed primarily in the upper respiratory tract and in the eyes (288 ppm only). In a related study, F344 rats exposed to 8, 24, or 76 ppm dimethylaminoethanol via inhalation for 6 hours/day, 5 days/week for 13 weeks were observed with corneal opacity and alterations in nasal tissue at 24 and 76 ppm (Klonne *et al.*, 1987).

Dimethylaminoethanol did not induce genotoxicity when evaluated in the *Salmonella typhimurium* assay, *Drosophila melanogaster* sex-linked recessive lethal assay, sister chromatid exchange assays, or hypoxanthine-guanine phosphoribosyl transferase forward gene mutation tests (HGPT) (Murray and Cummins, 1979; Union Carbide, 1987, 1988; Zeiger *et al.*, 1987; Leung and Ballantyne, 1997; NTP, 2002). There were no significant increases in micronucleated erythrocytes in Swiss-Webster mice exposed to 270 to 860 mg/kg dimethylaminoethanol (Leung and Ballantyne, 1997).

Humans

No serious side effects were reported in humans treated orally with up to 1,200 mg dimethylaminoethanol/day (13.46 mmol/day) (Gosselin *et al.*, 1984).

Oral administration of 10 to 20 mg (0.042 to 0.084 mmol) dimethylaminoethanol tartrate in humans caused mild mental stimulation (Beard and Noe, 1981). Doses of 20 mg/day (0.084 mmol) gradually increased muscle tone and the frequency of convulsions in more susceptible individuals. Larger doses (unspecified) induced insomnia, muscle tenseness, and sporadic muscle twitches.

Humans treated with dimethylaminoethanol to relieve tardive dyskinesia exhibited severe cholinergic side effects, including nasal and oral secretion, dyspnea, and respiratory failure (Mehta *et al.*, 1976; Nesse and Carroll, 1976). A meta-analysis of randomized controlled trials of dimethylaminoethanol indicated that not only was it no more effective at treating tardive dyskinesia than placebo, but treatment with dimethylaminoethanol was also associated with a significantly increased risk of adverse outcomes (Soares and McGrath, 1999; Tammenmaa *et al.*, 2012).

STUDY RATIONALE

Dimethylaminoethanol was nominated by the National Institute of Environmental Health Sciences (NIEHS) for toxicologic characterization due to the potential for widespread human exposure through its use in industrial and consumer products. Dimethylaminoethanol is structurally similar to choline, an essential nutrient, and may impact choline uptake and synthesis in the body. Due to limited literature indicating that dimethylaminoethanol may be a teratogen and reproductive toxicant, and the possibility for widespread exposure to the salt form of dimethylaminoethanol (dimethylaminoethanol bitartrate) as a dietary supplement in women of childbearing age, the NTP conducted prenatal developmental toxicology studies to assess the effects of oral dimethylaminoethanol administration in pregnant rats and on fetal development.

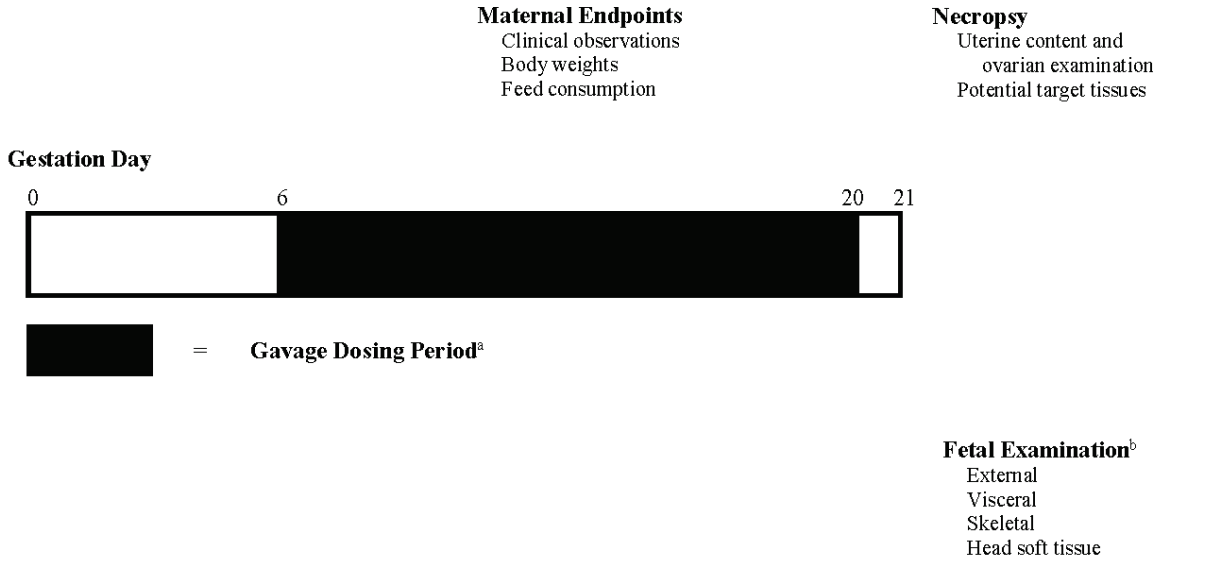
MATERIALS AND METHODS

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, can provide preliminary information on embryo-fetal outcomes (e.g., post-implantation loss, changes in fetal weight, external defects) and inform 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 in the rat is presented in Figure 1.

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



[a] Animals were gavaged 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

Dimethylaminoethanol Bitartrate (DMAE)

Dimethylaminoethanol bitartrate was obtained from Bayville Chemical Supply Company, Inc. (Deer Park, NY) in one lot (159AK) that was used in the dose range-finding and prenatal developmental toxicity studies (Appendix C). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at RTI International (Research Triangle Park, NC) for the study laboratory at Southern Research (Birmingham, AL). Lot 159AK of the chemical, a white crystalline powder, was identified as dimethylaminoethanol bitartrate using Fourier transform infrared (FTIR) and proton and carbon-13 nuclear magnetic resonance spectroscopy. The exact mass for dimethylaminoethanol and tartaric acid was determined using ultra high-performance liquid chromatography (UHPLC) coupled with time-of-flight mass spectrometry (TOF MS) detection. In addition, the melting point of the bulk chemical was determined.

The moisture content of lot 159AK was measured by a desorption type Karl Fischer analysis and by thermogravimetric analysis (TGA); these procedures were conducted by Robertson Microlit Laboratory

(Ledgewood, NJ). The purity of lot 159AK was determined by Galbraith Laboratories, Inc. (Knoxville, TN) using elemental analyses and by the analytical chemistry laboratory initially using UHPLC/TOF-MS with a hydrophilic interaction liquid chromatography (HILIC) gradient in the positive mode for dimethylaminoethanol and the negative mode for tartaric acid and subsequently using gas chromatography (GC) with MS detection. Assays for volatile organic impurities in the bulk chemical were conducted by the analytical chemistry laboratory using two GC systems with flame ionization detection (FID).

The Karl Fischer analysis indicated approximately 12% water and TGA results indicated an estimated maximum water value not greater than 3.5%, based on the mass lost in the temperature range matching the boiling point of water. Elemental analyses for carbon, hydrogen, nitrogen, and oxygen were consistent with the structural composition of dimethylaminoethanol bitartrate and theoretical values. UHPLC/TOF-MS indicated one major peak in chromatograms accounting for greater than 99.9% of the total peak area relative to either the dimethylaminoethanol or tartaric acid peaks. GC/MS indicated one major peak accounting for 100% of the total peak area; the spectrum was consistent with a library reference spectrum (NIST, 2005). A residual solvent screening assay using GC/FID by a second system tentatively identified the presence of ethanol at 0.325% (w/w). The overall purity of lot 159AK was determined to be 96% or greater.

Stability studies of lot 159AK were conducted using the same HILIC gradient UHPLC/TOF-MS system used for the bulk chemical purity assessment. Results indicated that both dimethylaminoethanol and the counterion tartaric acid were stable in the bulk chemical for at least 14 days when stored in amber glass vials sealed with Teflon®-lined caps at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature in sealed glass bottles. Reanalyses of the bulk chemical were performed prior to the dose range-finding study and after the prenatal developmental toxicity study using FTIR spectroscopy (dose range-finding study only) and GC/FID by a fourth system, and no degradation of the bulk chemical was detected.

Sterile Water for Irrigation, USP

The sterile water vehicle was obtained from Baxter Healthcare Corporation (Cleveland, MS) in two lots (G105999 and G110783); lot G10599 was used in the dose range-finding study and lot G110783 was used in the prenatal developmental toxicity study.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once for each study by mixing dimethylaminoethanol bitartrate with sterile water to give the required concentrations (Table C2). The dose formulations were stored at room temperature in sealed amber glass bottles for up to 42 days.

The analytical chemistry laboratory performed syringeability studies of a 300 mg/mL formulation using a 22-gauge gavage needle and syringe and stability studies of a 10 mg/mL formulation using GC/FID. Syringeability was confirmed and stability was confirmed for at least 42 days for dimethylaminoethanol formulations stored in sealed clear glass bottles both refrigerated and up to room temperature and for 3 hours under simulated animal room conditions.

Additional stability studies of formulations of dimethylaminoethanol bitartrate in sterile water were performed to monitor the relative stability of the tartaric acid counterion and the pH of the formulations. Two formulations (25.0 and 22.5 mg/mL) of dimethylaminoethanol bitartrate in sterile water were prepared for direct comparison to standards of tartaric acid at concentrations equivalent to those present in the formulations. After diluting into the range of a validated analytical method, a 300 mg/mL formulation was also analyzed using HPLC. Formulations were determined to have a stable tartaric acid concentration for at least 42 days. The pH of the formulations across the concentration range 2.5 to 300 mg/mL was between 3.4 and 3.6; based on the 10 mg/mL formulation, the pH of the formulation did not change over the period of 42 days when stored either refrigerated or at room temperatures.

Periodic analyses of the dose formulations of dimethylaminoethanol bitartrate were conducted by the analytical chemistry laboratory using GC/FID. During the dose range-finding study, the dose formulations were analyzed once; all three of the dose formulations were within 10% of the target concentrations. Animal room samples of

these dose formulations were also analyzed and all three were within 10% of the target concentrations. During the prenatal developmental toxicity study, the dose formulations were analyzed once; all three dose formulations analyzed were used and all three animal room samples were within 10% of the target concentrations.

ANIMAL SOURCE

Female Sprague Dawley (Hsd:Sprague Dawley SD) rats were obtained from Envigo (formerly Harlan Laboratories, Inc., Indianapolis, IN) for use in the dose range-finding and prenatal developmental toxicity studies (Table 1). 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; 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, hanta virus, mouse adenovirus, rat parvovirus, *Mycoplasma pulmonis*, and *Pneumocystis carinii*.

ANIMAL WELFARE

Animal care and use were 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 both dimethylaminoethanol bitartrate 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). Clinical observations were recorded on GD 3 (prenatal developmental toxicity study only), and on GD 6 through GD 21 until removal, typically twice daily (at the time of dose administration and cage side post-dose). Dams in the dose range-finding study were weighed daily from GD 3 through 21, and those in the prenatal developmental toxicity study were weighed on the day of arrival, on GD 3, and on GD 6 through GD 21. Feed consumption was recorded for GD 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 are provided in Appendix D.

On GD 21, rats were weighed, euthanized with CO_2 inhalation, and examined for gross lesions of the thoracic and abdominal cavities. The liver and gravid uterus were excised and weighed (liver for the 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 number and location 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 or presence of nidation sites ("pregnant by stain"). Late resorptions were characterized by grossly necrotic but recognizable fetal forms with placental remains visible (Suckow *et al.*, 2006; Hayes and Kruger, 2014). Post-implantation loss was calculated as the number of dead plus 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 early implantation sites (Salewski, 1964).

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 four groups (vehicle control, low, mid, or high) using Southern Research's Instem™ Provantis® (version 8) electronic data collection system.

Groups of 10 time-mated female rats were administered 0 (vehicle control), 250, 500, or 1,000 mg dimethylaminoethanol bitartrate/kg/day, based on the most recent body weight, in sterile water by gavage from GD 6 to GD 20. Vehicle control animals received sterile water alone; the dosing volume was 5 mL/kg body weight.

A high dose of 1,000 mg/kg dimethylaminoethanol bitartrate was selected for the dose range-finding study as 1,000 mg/kg is the limit dose recommended by the Organisation for Economic Co-operation and Development (OECD) for prenatal developmental toxicity studies (OECD, 2001).

On GD 21, fetuses were removed from the uterus, individually weighed (live fetuses only), and examined externally for alterations, including inspection of the oral cavity for cleft palate. Live fetuses were euthanized by decapitation or with intraperitoneal injection of a commercially available solution containing sodium pentobarbital followed by bilateral pneumothorax and/or decapitation. 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 randomized, based on GD 3 body weight stratification, into four groups (vehicle control, low, mid, or high) using Southern Research's Instem™ Provantis® (version 8) electronic data collection system. Dams were delivered in three groups, at least 2 days apart, to allow for a staggered study start.

Groups of 25 time-mated female rats were administered 0 (vehicle control), 250, 500, or 1,000 mg dimethylaminoethanol bitartrate/kg per day, based on the most recent body weight, in sterile water by gavage from GD 6 to GD 20. Vehicle control animals received sterile water alone; the dosing volume was 5 mL/kg body weight.

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.

TABLE 1
Experimental Design and Materials and Methods in the Prenatal Developmental Toxicity Gavage Studies of Dimethylaminoethanol Bitartrate in Rats

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 February 5, 2014 [Gestation Day (GD) 1 or 2]	April 9, 2014 (GD 1 or 2) April 11, 2014 (GD 1 or 2) April 16, 2014 (GD 1 or 2)
Average Age on Arrival 12 weeks	12 to 13 weeks
Weight Range at Randomization 224.8 – 262.3 g	210.3 – 241.4 g (April 7, 2014) 212.7 – 255.4 g (April 8, 2014) 217.5 – 241.8 g (April 9, 2014) 220.2 – 246.4 g (April 10, 2014) 207.8 – 238.8 g (April 14, 2014)

TABLE 1
Experimental Design and Materials and Methods in the Prenatal Developmental Toxicity Gavage Studies of Dimethylaminoethanol Bitartrate in Rats

Dose Range-Finding Study	Prenatal Developmental Toxicity Study
Calendar Day of First Dose and Last Dose GD 6 (February 9, 2014) and GD 20 (February 24, 2014)	GD 6 (April 13, 2014) and GD 21 (April 27, 2014) GD 6 (April 14, 2014) and GD 21 (April 28, 2014) GD 6 (April 15, 2014) and GD 21 (April 29, 2014) GD 6 (April 16, 2014) and GD 21 (April 30, 2014) GD 6 (April 20, 2014) and GD 21 (May 4, 2014)
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 indelible 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 tattoo 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 Certified Rodent Diet wafer diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as dose range-finding study
Water Tap water (Birmingham Water Works Co., Birmingham, AL, municipal supply) via automatic watering system, available *ad libitum*	Same as dose range-finding study
Cages Solid bottom polycarbonate cages (Lab Products, Inc., Seaford, DE), changed weekly	Same as dose range-finding 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® spun-bonded polyester (Andico, Birmingham, AL), changed every 2 weeks	Same as dose range-finding study
Racks Stainless steel (Lab Products, Inc., Seaford, DE), 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 10/hour	Same as dose range-finding study

TABLE 1
Experimental Design and Materials and Methods in the Prenatal Developmental Toxicity Gavage Studies of Dimethylaminoethanol Bitartrate in Rats

Dose Range-Finding Study	Prenatal Developmental Toxicity Study
Doses	
0, 250, 500, or 1,000 mg/kg in sterile water (dosing volume 5 mL/kg)	0, 250, 500, or 1,000 mg/kg in sterile water (dosing volume 5 mL/kg)
Type and Frequency of Observation of Dams	
Observed for viability twice daily from GD 3 through GD 20. Clinical observations were recorded daily from GD 6 until necropsy [prior to dosing (out of cage) and at least 1 to 3 hours post-dose (cageside)]. Animals were weighed daily beginning on GD 3. Feed consumption was recorded at 3-day intervals from GD 6 through GD 21.	Observed for viability twice daily from GD 3 through GD 20. Clinical observations were recorded once daily on GD 3 (out of cage) and at least 1 to 3 hours post-dose (cageside) from GD 6 through GD 20. Animals were weighed on the day of arrival, on GD 3, and on GD 6 through GD 21. Feed consumption was recorded at 3-day intervals from GD 6 through GD 21.
Primary Method of Euthanasia	
100% CO_2 (adults) or intraperitoneal injection of a solution containing sodium pentobarbital followed by bilateral pneumothorax and/or decapitation (fetuses)	100% CO_2 (adults) or decapitation or intraperitoneal injection of a solution containing sodium pentobarbital followed by bilateral pneumothorax and/or decapitation (fetuses)
Necropsy and Postmortem Evaluation of Females	
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.	On GD 21, terminal body, liver, and gravid uterine weights were recorded. Uterine contents were 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.
There were no early removals.	For animals removed early, gross necropsy including an examination of the thoracic and abdominal vicera was performed. The uterus of each female 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.
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 visceral and 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, found dead, 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 on GD 3 and reported as means with standard error bars in Figures 2 and 3 (also see Tables A2 and B2). Terminal maternal body weights on 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 and other organ weights, 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, and female fetuses), means and standard errors of litter means (fetal weight, male fetal weight, and 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 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 B6.

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 feed consumption 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 a dose-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 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. Historical control data for rats in this Prenatal Developmental Toxicity Study Report represent 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 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

All dams survived to end of the study (Table 2). There were no treatment-related clinical observations (Table A1)

TABLE 2
Maternal Disposition of Rats in the Dose Range-Finding Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Time-mated females	10	10	10	10
Pregnant females examined (on GD 21)	9	7	10	8
Non-pregnant (on GD 21)	1	3	0	2

Body Weights and Feed Consumption

There were no dose-related effects on maternal body weight gain during gestation (Figure 2 and Tables 3 and A2). One 1,000 mg/kg dam lost body weight between gestation day (GD) 15 and GD 18 (loss of 15.5 g) and between GD 18 and GD 21 (loss of 23.7 g), which coincided with decreased feed consumption in the same animal. This body weight decrease was not considered treatment related.

Mean feed consumption for either the intervals or the overall dosing period was not affected by administration of dimethylaminoethanol bitartrate (Table 4).

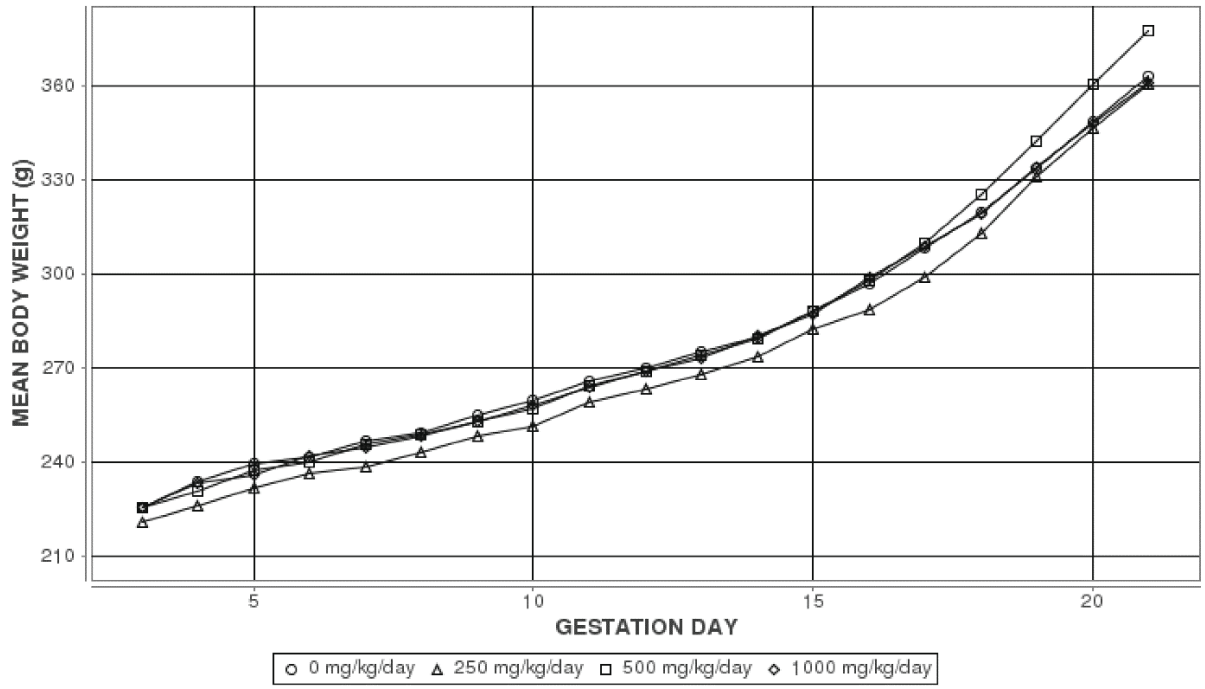


FIGURE 2
Maternal Growth Curves for Pregnant Rats Administered Dimethylaminoethanol Bitartrate by Gavage in the Dose Range-Finding Study
Information for statistical significance in maternal weights is provided in Tables 3 and A2.

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

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Gestation Day Interval				
6 to 21	121.5 ± 10.5 (9)	124.4 ± 12.9 (7)	137.5 ± 5.5 (10)	119.0 ± 15.9 (8)
3 to 6	15.7 ± 2.7 (9)	15.1 ± 1.1 (7)	14.8 ± 1.0 (10)	16.5 ± 1.8 (8)
6 to 9	13.9 ± 1.1 (9)	11.9 ± 1.4 (7)	12.9 ± 1.5 (10)	10.9 ± 1.0 (8)
9 to 12	14.9 ± 1.2 (9)	15.0 ± 0.9 (7)	16.1 ± 1.1 (10)	15.9 ± 0.6 (8)
12 to 15	18.0 ± 2.0 (9)	19.3 ± 2.3 (7)	19.0 ± 1.7 (10)	18.1 ± 1.5 (8)
15 to 18	31.5 ± 2.8 (9)	30.4 ± 4.1 (7)	37.3 ± 2.6 (10)	31.9 ± 7.0 (8)
18 to 21	43.3 ± 5.1 (9)	47.8 ± 5.6 (7)	52.3 ± 1.6 (10)	42.2 ± 9.7 (8)

Statistical analysis performed by Jonckheere's test (trend) and Williams' or Dunnett's test (pairwise comparison) found no statistically significant trend or pairwise comparison.

[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 Dimethylaminoethanol Bitartrate[a]

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Gestation Day Interval				
6 to 21	21.8 ± 0.4 (9)	22.0 ± 0.7 (7)	22.3 ± 0.6 (10)	21.7 ± 0.8 (8)
6 to 9[b]	19.3 ± 0.2 (4)	19.3 ± 0.9 (3)	18.9 ± 1.1 (5)	19.1 ± 0.3 (4)
9 to 12	21.6 ± 0.5 (9)	21.2 ± 0.8 (7)	22.0 ± 0.5 (10)	21.9 ± 0.7 (8)
12 to 15	21.6 ± 0.4 (9)	21.6 ± 0.9 (7)	21.2 ± 0.6 (10)	21.3 ± 0.3 (8)
15 to 18	23.4 ± 0.5 (9)	23.2 ± 0.8 (7)	24.3 ± 0.8 (10)	24.0 ± 1.0 (8)
18 to 21	23.6 ± 0.7 (9)	24.6 ± 1.0 (7)	24.6 ± 0.5 (10)	22.2 ± 3.0 (8)

Statistical analysis performed by Jonckheere's test (trend) and Shirley's or Dunn's test (pairwise comparison) found no statistically significant trend or pairwise comparison.

[a] Feed consumption for pregnant animals in grams/day. Data are displayed as mean ± standard error. Number of dams with feed consumption measured is given in parentheses.

[b] Due to a technical error, feed consumption was not recorded for a number of dams on GD 9.

Maternal and Litter Observations

Gross observations at necropsy were limited to skin discoloration in one 1,000 mg/kg rat and fluid in the uterus in one 250 mg/kg rat and one 1,000 mg/kg rat (Table A3). These observations were not considered related to chemical administration.

The number of pregnant animals, the mean number of corpora lutea, dead fetuses, early and late resorptions, and sex ratio were similar across all treatment groups (Table 5). There was a significant positive trend in the mean number of live female fetuses per litter relative to dose (Table 5). There was no effect on mean fetal body weight per litter.

TABLE 5
Summary of Uterine Content Data of Rats in the Dose Range-Finding Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Pregnancy Summary				
Mated females	10	10	10	10
Pregnant females	9	7	10	8
Pregnant females examined on GD 21[a]	9	7	10	8
Corpora lutea per female[b]	14.89 ± 0.54 (9)	16.00 ± 1.23 (7)	15.40 ± 0.65 (10)	14.75 ± 0.65 (8)
Implantations per female[b]	11.33 ± 1.38 (9)	11.86 ± 1.90 (7)	13.10 ± 0.71 (10)	14.00 ± 0.65 (8)
Percent post-implantation loss[b]	10.22 ± 7.35 (9)	0.95 ± 0.95 (7)	2.30 ± 1.19 (10)	3.42 ± 1.30 (8)
Total resorptions per litter[b]	0.56 ± 0.29 (9)	0.14 ± 0.14 (7)	0.30 ± 0.15 (10)	0.50 ± 0.19 (8)
Early resorptions per litter[b]	0.56 ± 0.29 (9)	0.14 ± 0.14 (7)	0.30 ± 0.15 (10)	0.50 ± 0.19 (8)
Late resorptions per litter[b]	0.00 ± 0.00 (9)	0.00 ± 0.00 (7)	0.00 ± 0.00 (10)	0.00 ± 0.00 (8)
Dead fetuses per litter[b]	0.00 ± 0.00 (9)	0.00 ± 0.00 (7)	0.00 ± 0.00 (10)	0.00 ± 0.00 (8)
Number of early resorptions	5	1	3	4
Number of late resorptions	0	0	0	0
Number of whole litter resorptions[a]	0	0	0	0
Number of dead fetuses	0	0	0	0
Live Fetuses[b]				
Number of live fetuses	97	82	128	108
Live fetuses per litter	10.78 ± 1.55 (9)	11.71 ± 1.86 (7)	12.80 ± 0.71 (10)	13.50 ± 0.60 (8)
Live male fetuses per litter	5.56 ± 1.09 (9)	7.00 ± 1.31 (7)	7.10 ± 0.80 (10)	6.38 ± 0.65 (8)
Live female fetuses per litter	5.22 ± 0.88 (9)*	4.71 ± 1.02 (7)	5.70 ± 0.83 (10)	7.13 ± 0.30 (8)
Percent live male fetuses per litter	44.84 ± 8.15 (9)	64.57 ± 8.10 (7)	55.99 ± 5.40 (10)	46.54 ± 2.99 (8)
Fetal Weight[c]				
Fetal body weight per litter (g)	5.39 ± 0.05 (9)	5.39 ± 0.17 (7)	5.40 ± 0.05 (10)	5.08 ± 0.40 (8)
Male fetal weight per litter (g)	5.53 ± 0.07 (8)	5.45 ± 0.16 (7)	5.52 ± 0.06 (10)	5.21 ± 0.41 (8)
Female fetal weight per litter (g)	5.26 ± 0.09 (9)	5.18 ± 0.15 (6)	5.25 ± 0.05 (10)	4.97 ± 0.39 (8)
Gravid Uterine Weight[d]				
Gravid uterine weight (g)	80.57 ± 10.81 (9)	84.13 ± 14.08 (7)	94.56 ± 4.66 (10)	93.54 ± 5.54 (8)
Terminal body weight (g)	363.1 ± 12.0 (9)	360.8 ± 12.9 (7)	377.8 ± 7.3 (10)	361.3 ± 16.0 (8)
Adjusted body weight (g)	282.56 ± 4.18 (9)	276.63 ± 5.67 (7)	283.24 ± 4.73 (10)	267.80 ± 11.29 (8)

Values are reported per litter as mean ± standard error (n) and do not include non-pregnant animals or those that did not survive to the end of the study.
(g) = grams.
* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column)
[a] Statistical analysis performed by Cochran-Armitage (trend) and Fisher's 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

No external malformations or variations were observed in either the vehicle control or exposed groups (Table A4).

Dose Selection Rationale for the Prenatal Developmental Toxicity Study in Rats

No maternal toxicity was observed in the dose range-finding study up to the limit dose of 1,000 mg/kg. Thus, dose concentrations of 250, 500, and 1,000 mg/kg dimethylaminoethanol bitartrate were chosen for the subsequent prenatal developmental toxicity gavage study.

PRENATAL DEVELOPMENTAL TOXICITY STUDY IN RATS

Maternal Findings

Viability and Clinical Observations

One 1,000 mg/kg dam was euthanized moribund on GD 21 following observations of dehydration, coldness to touch, brown discoloration in both eyes, and hypoactivity, but it is unclear if this condition was related to treatment (Table 6). Offspring from this dam were examined and included in fetal assessments. A second 1,000 mg/kg dam was found dead on GD 10; however, this death was determined to be the result of a gavage accident due to observed breathing difficulties following dosing. One vehicle control dam and one 1,000 mg/kg dam delivered prior to scheduled C-section on GD 21, and were therefore euthanized on GD 19 and 20, respectively. All other dams survived to the end of the study.

Clinical observations were generally limited to single or sporadic incidences with the exception of vaginal discharge (Table B1). Brown or red vaginal discharge was observed between GD 14 and GD 21 in 10, 3, 4, and 10 dams in the 0, 250, 500, and 1,000 mg/kg groups, respectively; however, due to a lack of a dose response, the observations of vaginal discharge were not considered dose related.

TABLE 6
Maternal Disposition of Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Time-mated females	25	25	25	25
Pregnant females examined (on GD 21)	19	20	20	21
Delivered early	1[a]	0	0	1[b]
Euthanized moribund	0	0	0	1[c]
Found dead	0	0	0	1[d]
Non-pregnant (on GD 21)	5	5	5	1

[a] Dam removed on GD 19
[b] Dam removed on GD 20
[c] Dam removed on GD 21; dam and offspring were included in maternal and fetal assessments.
[d] Dam removed on GD 10

Body Weights and Feed Consumption

There were no dose-related effects on maternal body weight gain during gestation in any dose group (Figure 3 and Tables 7 and B2); however, there was a significant increase (approximately 16%) in body weight gain in the 500 mg/kg dams relative to vehicle controls between GD 6 and GD 21.

Mean feed consumption values were similar between vehicle control and dosed dams (Table 8). The 1,000 mg/kg pregnant dam euthanized moribund on GD 21 exhibited decreased feed consumption between GDs 15 and 21.

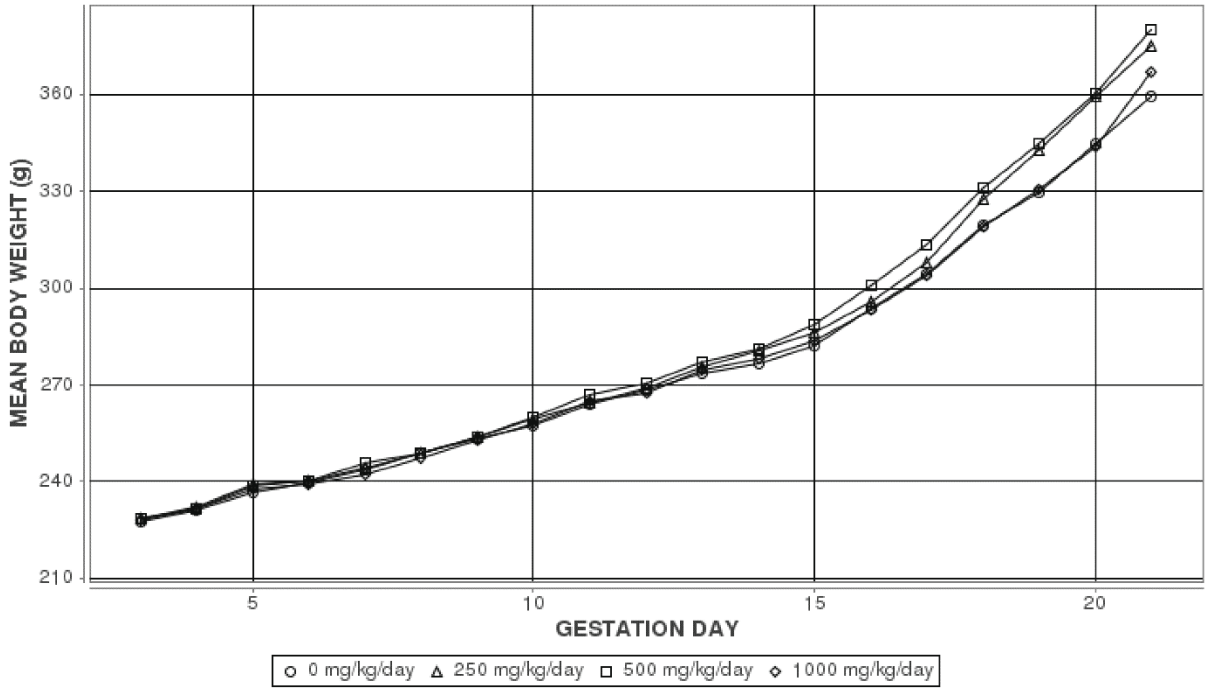


FIGURE 3
Maternal Growth Curves for Pregnant Rats Administered Dimethylaminoethanol Bitartrate by Gavage in the Prenatal Developmental Toxicity Study
Information for statistical significance in maternal weights is provided in Tables 7 and B2.

TABLE 7
Summary of Maternal Body Weight Gains of Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate[a]

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Gestation Day Interval				
6 to 21	120.5 ± 7.5 (19)	135.2 ± 4.7 (20)	140.0 ± 4.2* (20)	127.7 ± 5.8 (21)
3 to 6	11.8 ± 0.9 (20)	11.3 ± 0.9 (20)	11.6 ± 1.2 (20)	11.1 ± 0.8 (24)
6 to 9	13.6 ± 0.6 (20)	14.1 ± 1.0 (20)	13.7 ± 0.9 (20)	13.3 ± 1.1 (24)
9 to 12	15.2 ± 0.8 (20)	15.0 ± 0.9 (20)	16.4 ± 1.0 (20)	14.8 ± 0.9 (23)
12 to 15	13.6 ± 1.9 (20)	17.2 ± 2.3 (20)	18.1 ± 1.3 (20)	15.6 ± 2.1 (23)
15 to 18	37.7 ± 3.5 (20)	41.2 ± 1.9 (20)	42.8 ± 1.5 (20)	35.4 ± 4.7 (23)
18 to 21	41.6 ± 3.1 (19)	47.8 ± 2.3 (20)	49.0 ± 2.1 (20)	43.2 ± 2.1 (21)

Statistical analysis performed by Jonckheere's test found no statistically significant trend.
* Statistically significant (P≤0.05) pairwise comparison by Williams' or Dunnett's test.
[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 8
Summary of Maternal Feed Consumption of Rats in the Prenatal Developmental Toxicity Study of Dimethylaminoethanol Bitartrate[a]

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Gestation Day Interval				
6 to 21	21.2 ± 0.4 (19)	21.6 ± 0.4 (20)	22.0 ± 0.3 (20)	21.5 ± 0.4 (22)
6 to 9	19.9 ± 0.3 (20)	20.0 ± 0.3 (20)	19.6 ± 0.4 (20)	19.1 ± 0.5 (24)
9 to 12	20.5 ± 0.3 (20)	20.9 ± 0.3 (20)	20.6 ± 0.3 (20)	20.7 ± 0.3 (23)
12 to 15	20.3 ± 0.6 (19)	21.2 ± 0.5 (20)	21.5 ± 0.4 (20)	21.0 ± 0.4 (23)
15 to 18	23.1 ± 0.4* (20)	22.7 ± 0.7 (20)	24.5 ± 0.4 (20)	23.7 ± 0.8 (23)
18 to 21	22.1 ± 0.5 (19)	23.4 ± 0.6 (20)	23.8 ± 0.5 (20)	22.6 ± 1.1 (22)

Statistical analysis performed by Shirley's or Dunn's test found no statistically pairwise comparison.
* Statistically significant (P≤0.05) trend (by Jonckheere's test).
[a] Feed consumption for pregnant animals 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 dose-related gross observations noted at necropsy (Table B3). There was a significant positive trend in mean absolute liver weight (2.5%, 3.3%, and 7.2% in the 250, 500, and 1,000 mg/kg groups respectively); however, these weights were only marginally greater than the vehicle control weight, and the relative liver weights were similar across all groups (Table 9). Therefore, this trend was not deemed treatment related. The 1,000 mg/kg pregnant dam euthanized moribund on GD 21 had the smallest recorded liver weight (8.81 g) of any animal in the study; mean absolute liver weights for all dose groups ranged from approximately 13.96 to 14.96 g.

There were no effects on pregnancy status or litter size following administration of dimethylaminoethanol bitartrate (Table 10). The mean number of corpora lutea and implantations for all dosed groups were also similar to the vehicle control group. The 1,000 mg/kg pregnant dam euthanized moribund on GD 21 had 11 dead fetuses and one early resorption, which contributed to an increase in percent post-implantation loss that was not treatment related or representative of the entire dose group. There were no treatment-related effects on the number of live fetuses per litter, live male fetuses per litter, or live female fetuses per litter. Fetal body weights (male and female, or separate) were similar across all treatment groups.

TABLE 9
Summary of Liver Weights of Rats in the Prenatal Developmental Toxicity Study of Dimethylaminoethanol Bitartrate[a]

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
n	19	20	20	21
Terminal body wt.	359.6 ± 8.8	375.2 ± 5.3	380.3 ± 5.1	367.1 ± 6.5
Absolute	13.96 ± 0.37*	14.31 ± 0.34	14.42 ± 0.36	14.96 ± 0.25
Relative	38.91 ± 0.66	38.10 ± 0.64	37.89 ± 0.75	41.00 ± 1.01

Statistical analysis performed by Williams' or Dunnett's tests found no statistically significant pairwise comparison.
* Significant trend (P≤0.05) by Jonckheere's test
[a] Liver weights (absolute weights) and body weights are given in grams; liver-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight. Data are displayed as mean ± standard error.

TABLE 10
Summary of Uterine Content Data of Rats in the Prenatal Developmental Toxicity Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Pregnancy Summary				
Mated females	25	25	25	25
Pregnant females	20	20	20	24
Pregnant females examined on GD 21[a]	19	20	20	22[b]
Corpora lutea per female[c]	15.74 ± 0.57 (19)	15.85 ± 0.45 (20)	16.70 ± 0.73 (20)	15.86 ± 0.64 (22)
Implantations per female[c]	11.47 ± 1.11 (19)	13.75 ± 0.54 (20)	13.50 ± 0.60 (20)	12.45 ± 0.87 (22)
Percent post-implantation loss[c]	5.05 ± 1.57 (19)	3.80 ± 1.53 (20)	3.45 ± 1.10 (20)	11.17 ± 5.56 (22)
Total resorptions per litter[c]	0.47 ± 0.12 (19)	0.50 ± 0.20 (20)	0.50 ± 0.17 (20)	0.64 ± 0.26 (22)
Early resorptions per litter[c]	0.47 ± 0.12 (19)	0.50 ± 0.20 (20)	0.50 ± 0.17 (20)	0.64 ± 0.26 (22)
Late resorptions per litter[c]	0.00 ± 0.00 (19)	0.00 ± 0.00 (20)	0.00 ± 0.00 (20)	0.00 ± 0.00 (22)
Dead fetuses per litter[c]	0.00 ± 0.00 (19)	0.00 ± 0.00 (20)	0.00 ± 0.00 (20)	0.50 ± 0.50 (22)
Number of early resorptions	9	10	10	14
Number of late resorptions	0	0	0	0
Number of whole litter resorptions[a]	0	0	0	0
Number of dead fetuses	0	0	0	11
Live Fetuses[c]				
Number of live fetuses	209	265	260	249
Live fetuses per litter	11.00 ± 1.12 (19)	13.25 ± 0.60 (20)	13.00 ± 0.56 (20)	11.32 ± 1.07 (22)
Live male fetuses per litter	5.21 ± 0.68 (19)	6.10 ± 0.55 (20)	6.10 ± 0.55 (20)	5.77 ± 0.67 (22)
Live female fetuses per litter	5.79 ± 0.69 (19)	7.15 ± 0.50 (20)	6.90 ± 0.48 (20)	5.55 ± 0.60 (22)
Percent live male fetuses per litter	47.53 ± 5.21 (19)	44.76 ± 3.98 (20)	46.61 ± 3.23 (20)	50.42 ± 4.39 (21)
Fetal Weight[d]				
Fetal body weight per litter (g)	5.38 ± 0.15 (19)	5.26 ± 0.05 (20)	5.33 ± 0.06 (20)	5.40 ± 0.09 (21)
Male fetal weight per litter (g)	5.44 ± 0.16 (18)	5.37 ± 0.05 (19)	5.50 ± 0.06 (20)	5.51 ± 0.09 (20)
Female fetal weight per litter (g)	5.15 ± 0.12 (18)	5.14 ± 0.06 (20)	5.18 ± 0.07 (20)	5.21 ± 0.08 (20)
Gravid Uterine Weight[e]				
Gravid uterine weight (g)	80.18 ± 7.36 (19)	95.85 ± 3.93 (20)	96.17 ± 3.82 (20)	85.25 ± 6.38 (22)
Terminal body weight (g)	359.6 ± 8.8 (19)	375.2 ± 5.3 (20)	380.3 ± 5.1 (20)	361.4 ± 8.5 (22)
Adjusted body weight (g)	279.42 ± 3.68 (19)	279.34 ± 3.77 (20)	284.12 ± 2.90 (20)	276.17 ± 4.59 (22)

Values are reported per litter as mean ± standard error (n) and do not include non-pregnant animals or those that did not survive to the end of the study. (g) = grams.

[a] Statistical analysis performed by Cochran-Armitage (trend) and Fisher's exact (pairwise) tests

[b] The dam euthanized moribund on GD 21 was included in the uterine content assessment.

[c] Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests

[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

Subcutaneous hemorrhage was observed in fetuses from all exposure groups with a higher incidence in the 1,000 mg/kg group; however, three of the five affected fetuses in the 1,000 mg/kg group were from the same dam (Tables 11 and B4) and the incidences were low and not significantly increased. Malformations observed in the vehicle control and exposed groups were limited to single incidences each of meningocele, omphalocele, and bent right hind limb and sporadic incidences of bent tail.

TABLE 11
Summary of Selected External Fetal Findings in the Prenatal Developmental Toxicity Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Total number of fetuses	209	265	260	260
External				
Number of fetuses examined	209	265	260	260
Number of litters examined	19	20	20	22
Body: General				
Body, subcutaneous hemorrhage — [GF]				
Fetuses	1 (0.48)	2 (0.75)	1 (0.38)	5 (1.92)
Litters	1 (5.26)	2 (10.00)	1 (5.00)	3 (13.64)
Extremities				
Limb, hind, right, bent — [M]				
Fetuses	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.38)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.55)
Tail, bent — [M]				
Fetuses	0 (0.0)	1 (0.38)	1 (0.38)	2 (0.77)
Litters	0 (0.00)	1 (5.00)	1 (5.00)	1 (4.55)
Head				
Head, meningocele — [M]				
Fetuses	1 (0.48)	0 (0.0)	0 (0.0)	0 (0.0)
Litters	1 (5.26)	0 (0.00)	0 (0.00)	0 (0.00)
Trunk				
General, omphalocele — [M]				
Fetuses	0 (0.0)	1 (0.38)	0 (0.0)	0 (0.0)
Litters	0 (0.00)	1 (5.00)	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 for litter data and for fetal data (without litter effect) performed by Cochran-Armitage (trend) and Fisher's 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 found no statistically significant trend or pairwise comparison
[M] = Malformation
[GF] = Gross Finding

Visceral

There were single incidences of visceral malformations in the heart of vehicle control and exposed groups that included large right atrium, thick bilateral ventricular wall, and ventricular septal defect (Tables 12 and B4). Misshapen aortic valve, a malformation, was observed in all exposure groups at singular or low incidences. Visceral malformations in the major vessels of control and exposed groups were limited to single incidences of absent aortic arch, atresia of the innominate artery, and dilated pulmonary artery/trunk. Visceral variations occurred predominantly in the vessels and included absent or short innominate arteries, which are a common background variation; these were noted in both vehicle control and exposed groups. The incidences of absent innominate artery in the 1,000 mg/kg group were significantly increased; however, innominate artery variations are a common finding noted in approximately 5% of control rat fetuses (Scott *et al.*, 1997).

TABLE 12
Summary of Selected Visceral Fetal Findings in the Prenatal Developmental Toxicity Study of Dimethylaminoethanol Bitartrate

	0 mg/kg		250 mg/kg		500 mg/kg		1,000 mg/kg	
Total number of fetuses	209		265		260		260	
Visceral								
Number of fetuses examined	209		265		260		260	
Number of litters examined	19		20		20		22	
Heart								
Aortic valve, misshapen — [M]								
Fetuses	2	(0.96)	1	(0.38)	3	(1.15)	1	(0.38)
Litters	2	(10.53)	1	(5.00)	3	(15.00)	1	(4.55)
Atrium, right, large — [M]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.38)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.55)
Ventricle, bilateral, thick wall — [M]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	0	(0.00)
Ventricle, bilateral, ventricular septum defect — [M]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	0	(0.00)

TABLE 12
Summary of Selected Visceral Fetal Findings in the Prenatal Developmental Toxicity Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Major vessels				
Aortic arch, absent — [M]				
Fetuses	0 (0.0)	0 (0.0)	1 (0.38)	0 (0.0)
Litters	0 (0.00)	0 (0.00)	1 (5.00)	0 (0.00)
Innominate artery, atresia — [M]				
Fetuses	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.38)
Litters	0 (0.00)	0 (0.00)	0 (0.00)	1 (4.55)
Innominate artery, absent — [V]				
Fetuses	3 (1.44)**##	4 (1.51)	6 (2.31)	12 (4.62)*#
Litters	3 (15.79)*	4 (20.00)	4 (20.00)	10 (45.45)*
Innominate artery, short — [V]				
Fetuses	6 (2.87)	7 (2.64)	8 (3.08)	5 (1.92)
Litters	4 (21.05)	7 (35.00)	6 (30.00)	4 (18.18)
Pulmonary artery/trunk, dilated — [M]				
Fetuses	1 (0.48)	0 (0.0)	0 (0.0)	0 (0.0)
Litters	1 (5.26)	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 for litter data and for fetal data (without litter effects) performed by Cochran-Armitage (trend) and Fisher's exact (pairwise) tests
* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column);
** P≤0.01
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression
\# Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column) in litter-based analysis of fetuses;
P≤0.01
[M] = Malformation
[V] = Variation

Head

There were no exposure-related effects of dimethylaminoethanol bitartrate administration on the incidence of fetal head, specifically brain, abnormalities (Table B4).

Skeletal

Skeletal malformations and variations occurred predominantly in the ribs of fetuses from all exposure groups (Tables 13 and B4). Single or low incidences of nodulated costal cartilage (6th, 8th, and 9th), a variation, were observed in vehicle control, 500 mg/kg, and 1,000 mg/kg groups. There was a negative trend in the incidences of total (sum of left, right, and bilateral), full thoracolumbar ribs (malformation), while a significant increase in the incidence of left, bilateral, and total short thoracolumbar ribs (variation) was observed in the 1,000 mg/kg group, along with a significant positive trend.

There were increased incidences of isolated ossification sites in the skulls of fetuses in the 1,000 mg/kg group (Tables 13 and B4). There was a significant increase in the number of supernumerary sites, or extra bones, in the skull (around the frontonasal suture) in 1,000 mg/kg fetuses as well as a significant positive trend across all groups.

TABLE 13
Summary of Selected Skeletal Fetal Findings in the Prenatal Developmental Toxicity Study of Dimethylaminoethanol Bitartrate

	0 mg/kg		250 mg/kg		500 mg/kg		1,000 mg/kg	
Total number of fetuses	209		265		260		260	
Skeletal: Body								
Number of fetuses examined	209		264		260		260	
Number of litters examined	19		20		20		22	
Ribs								
Costal cartilage, 6th right, nodulated — [V]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	2	(0.77)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	2	(9.09)
Costal cartilage, 8th right, nodulated — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(5.00)	0	(0.00)
Costal cartilage, 9th right, nodulated — [V]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	0	(0.00)
Supernumerary rib								
Thoracolumbar, left, full — [M]								
Fetuses	3	(1.44)*	1	(0.38)	0	(0.0)	0	(0.0)
Litters	3	(15.79)*	1	(5.00)	0	(0.00)	0	(0.00)
Thoracolumbar, right, full — [M]								
Fetuses	3	(1.44)	2	(0.76)	1	(0.38)	1	(0.38)
Litters	3	(15.79)	2	(10.00)	1	(5.00)	1	(4.55)
Thoracolumbar, bilateral, full — [M]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	0	(0.00)
Thoracolumbar, full, total — [M]								
Fetuses	7	(3.35)**##	3	(1.14)	1	(0.38)*#	1	(0.38)*#
Litters	6	(31.58)*	3	(15.00)	1	(5.00)*	1	(4.55)*
Thoracolumbar, left, short — [V]								
Fetuses	23	(11.0)*#	32	(12.12)	26	(10.0)	45	(17.31)*#
Litters	14	(73.68)	17	(85.00)	16	(80.00)	17	(77.27)
Thoracolumbar, right, short — [V]								
Fetuses	16	(7.66)	13	(4.92)	9	(3.46)*#	16	(6.15)
Litters	10	(52.63)	10	(50.00)	8	(40.00)	10	(45.45)
Thoracolumbar, bilateral, short — [V]								
Fetuses	17	(8.13)**##	11	(4.17)	24	(9.23)	39	(15.0)*
Litters	8	(42.11)*	7	(35.00)	11	(55.00)	16	(72.73)*
Thoracolumbar, short, total — [V][a]								
Fetuses	56	(26.79)**##	56	(21.21)	59	(22.69)	100	(38.46)**#
Litters	17	(89.47)	18	(90.00)	18	(90.00)	19	(86.36)

TABLE 13
Summary of Selected Skeletal Fetal Findings in the Prenatal Developmental Toxicity Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Skeletal: Skull				
Number of fetuses examined	100	128	126	128
Number of litters examined	18	20	20	20
Skull				
General, isolated ossification site — [V]				
Fetuses	1 (1.0)	5 (3.91)	3 (2.38)	3 (2.34)
Litters	1 (5.56)	5 (25.00)	2 (10.00)	2 (10.00)
General, supernumerary site — [V][b]				
Fetuses	1 (1.0)**##	3 (2.34)	2 (1.59)	13 (10.16)**#
Litters	1 (5.56)**	3 (15.00)	2 (10.00)	10 (50.00)**

Upper row denotes number of affected fetuses (%) and lower row the number of affected litters (%)
Statistical analysis for litter data and for fetal data (without litter effects) performed by Cochran-Armitage (trend) and Fisher's exact (pairwise) tests
* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column);
** P≤0.01
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression
\# Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column) in litter-based analysis of fetuses;
P≤0.01
[M] = Malformation
[V] = Variation
[a] Historical incidence for prenatal developmental toxicity gavage studies: fetuses: 247/1,324 (18.7%), range 9.9%-26.8%; litters: 83/104 (79.8%), range 66.7%-91.3%
[b] Historical incidence for prenatal developmental toxicity gavage studies: fetuses: 11/637 (1.73%), range 0.70%-2.94%; litters: 7/102 (6.86%), range 4.35%-11.11%

DISCUSSION AND CONCLUSIONS

Dimethylaminoethanol is a close structural analog of choline (*N,N,N*-trimethylaminoethanol), an essential nutrient. Dimethylaminoethanol supplies the brain with choline, where it is then acetylated to form acetylcholine (De Silva, 1977). Choline is required for many biological processes, including nervous system development, and choline deficiency has been associated with neural tube defects (NTDs) in both rodents and humans (Fisher *et al.*, 2001, 2002; Shaw *et al.*, 2004, 2009; Mills *et al.*, 2014). Human exposure to dimethylaminoethanol may occur through occupational routes (e.g. spray painting, beverage can lacquering, etc.) (Pitts *et al.*, 1981), however the primary route of exposure is through consumption of dimethylaminoethanol as a dietary supplement. Dietary supplements containing dimethylaminoethanol bitartrate, a salt of dimethylaminoethanol, are marketed to improve memory and general cognitive function due to the ability of dimethylaminoethanol to increase levels of acetylcholine in the brain. Dimethylaminoethanol bitartrate supplements are also purported to treat symptoms of ADHD in children.

There are indications in the literature that exposure to dimethylaminoethanol may induce developmental and reproductive toxicity, including decreases in viable implants, litter size, and pup survival and increases in fetal skeletal variations (Zahniser *et al.*, 1978; Tyl *et al.*, 1987; Leung, *et al.*, 1996). Due to the potential for widespread human exposure and concerns for use by pregnant women and children, the NTP conducted prenatal developmental toxicity studies to assess the effects of oral dimethylaminoethanol bitartrate administration in pregnant rats and on fetal development.

There were no indications of maternal or fetal toxicity in the dose range-finding study following exposure to 0, 250, 500, or 1,000 mg/kg dimethylaminoethanol bitartrate. As no maternal toxicity was observed in the dose range-finding study, identical dose levels of 250, 500, and 1,000 mg/kg dimethylaminoethanol bitartrate were chosen for the prenatal developmental toxicity study. Dimethylaminoethanol bitartrate was well tolerated in the prenatal developmental toxicity study, allowing for a complete evaluation of embryo-fetal development following exposure. There were no significant effects on maternal survival, body weights, or food consumption. One dam each in the

1,000 mg/kg group was euthanized moribund (GD 21) or found dead (GD 10), but these deaths were not considered dose-related. Marginal increases in liver weights were observed and similar results have been reported in the literature with dimethylaminoethanol (45 to 890 mg/kg) in the diet for 90 days causing increased liver weights with no associated histopathologic changes (Huntsman Corporation, 2007). The marginal increases observed in this study were not considered to be toxicologically significant.

Exposure to dimethylaminoethanol bitartrate did not affect any pregnancy or litter parameters. The dam administered 1,000 mg/kg dimethylaminoethanol bitartrate and euthanized moribund on GD 21 had 11 dead fetuses and one early resorption, which contributed to an increase in post-implantation loss that, given it was a single incidence, was not exposure related or representative of the entire dose group. Fetal body weight, a sensitive indicator of embryo-fetal toxicity, was unaffected at any dose level in the dose range-finding and prenatal developmental toxicity studies.

There were minimal significant findings of increased incidences in variations of short thoracolumbar ribs (short supernumerary ribs) and supernumerary sites in the skull. The incidence of short supernumerary ribs occurred in 86% to 90% of litters across all exposure groups and was significantly increased in the 1,000 mg/kg fetuses, with an 11% increase in exposed fetuses relative to the vehicle controls. This increase was considered exposure related as the percentage of affected fetuses was outside the NTP historical control range (9.90%-26.79% for fetuses); however, this did not correspond to an increase in full thoracolumbar ribs (a malformation), and this is also a common background variation in the Sprague Dawley rat strain used in this study. The manifestation of short supernumerary ribs is short-lived, as this particular variation is considered reversible during postnatal development (Wickramaratne, 1988; Foulon *et al.*, 2000; Chernoff and Rogers, 2004). The incidence of supernumerary sites in the skull was significantly increased in 1,000 mg/kg fetuses (approximately 10%) relative to the vehicle controls (1%) and outside of the NTP historical control range (0.70%-2.94% for fetuses). This increase was considered to be treatment-related; however, the toxicologic significance of this finding is unclear. There is minimal available animal data evaluating whether these supernumerary sites cause biological consequences later in life or resolve; however, in clinical research, these findings are commonly used as diagnostic markers for certain syndromes and genetic disorders such as craniosynostosis and osteogenesis imperfecta (Bellary *et al.*, 2013).

When examined individually, the noted effects are likely reversible (supernumerary ribs) or of uncertain biological significance (supernumerary sites in the skull). However, the increased incidence of extra ossification sites in two separate locations could be considered an indication of altered development in exposed fetuses, as the incidences are outside of the NTP historical control range and occurred in the absence of decreased fetal weight and maternal toxicity. Skull and rib development occur via two different skeletal developmental pathways, endochondral ossification (ribs) and intramembranous ossification (skull) (Lefebvre and Bhattaram, 2010). During endochondral ossification, a cartilage template is gradually replaced with bone via osteoblasts that secrete extracellular bone matrix which undergoes mineralization to form bone. Subsequent growth requires remodeling by bone-resorbing osteoclasts followed by deposition of new bone matrix by osteoblasts. Intramembranous ossification, on the other hand, occurs in the absence of cartilage template and involves condensation of mesenchymal stem cells (osteoblast precursors) at sites of future skull bones. These precursor cells differentiate to become osteoblasts which secrete bone extracellular matrix that undergoes mineralization to form ossified bone. Both pathways require functioning osteoblasts and osteoclasts, and while differential gene expression patterns are required for each of these developmental pathways, there are genes common to both pathways (i.e., *Runx2*) (Lefebvre and Bhattaram, 2010). These data suggest that dimethylaminoethanol bitartrate exposure may be affecting multiple skeletal development pathways, and therefore while the observed rib and skull findings are of unclear biological significance, they may have been related to exposure

CONCLUSIONS

Under the conditions of this prenatal study, there was *equivocal evidence* of developmental toxicity of dimethylaminoethanol bitartrate in Hsd:Sprague Dawley SD rats based on increased incidences of short thoracolumbar ribs and supernumerary sites in the skull in the absence of overt maternal toxicity.

REFERENCES

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

Beard, R.R., and Noe, J.T. (1981). Aliphatic and alicyclic amines. In *Patty's Industrial Hygiene and Toxicology,* 3rd ed. (G.D. Clayton and F.E. Clayton, Eds.), Vol. 2B, pp. 3135-3173. John Wiley & Sons, Inc., New York.

Bellary, S.S., Steinberg, A., Mirzayan, N., Shirak, M., Tubbs, R.S., Cohen-Gadol, A.A., and Loukas, M. (2013). Wormian bones: A review. *Clin. Anat.* **26**, 922-927.

Ceder, G., and Schuberth, J. (1977). 2-Dimethylaminoethanol (Deaner) in body fluids. *J. Pharm. Pharmacol.* **29**, 373-374.

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., and Rogers, J.M. (2004). Supernumerary ribs in developmental toxicity bioassays and in human populations: Incidence and biological significance. *J. Toxicol. Environ. Health B Crit. Rev.* **7**, 437-449.

Clares, B., Ruiz, M.A., Morales, M.E., Tamayo, J.A., and Lara, V.G. (2010). *J. Cosmet. Sci*. **61**, 269-278.

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

Code of Federal Regulations (CFR) **21**, § 173.20.

Code of Federal Regulations (CFR) **21**, § 175.300.

Code of Federal Regulations (CFR) **40**, Part 60.YYY.

Code of Federal Regulations (CFR) **40**, § 63.100ff.

De Silva, L. (1977). Biochemical mechanisms and management of choreiform movement disorders. *Drugs* **14**, 300-310.

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.

Dormard, Y., Levron, J.C., and Benakis, A. (1975). Pharmacokinetic study of maleate acid of 2-(N,N-dimethylaminoethanol-^{14}C)-cyclohexylpropionate (cyprodenate) and of N,N-dimethylaminoethanol-^{14}C in animals. *Arzneimittelforschung* **25**, 194-201.

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.

Fisher, M.C., Zeisel, S.H., Mar, M.-H., and Sadler, T.W. (2001). Inhibitors of choline uptake and metabolism cause developmental abnormalities in neurulating mouse embryos. *Teratology* **64**, 114-122.

Fisher, M.C., Zeisel, S.H., Mar, M.-H., and Sadler, T.W. (2002). Perturbations in choline metabolism cause neural tube defects in mouse embryos *in vitro*. *FASEB J.* **16**, 619-621.

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.

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.

Gosselin, R.E., Smith, R.P., and Hodge, H.C., Eds. (1984). Ingredients Index: Deanol. In *Clinical Toxicology of Commercial Products,* 5th ed., Vol. 2, p. 240. Wilkins and Wilkens Company, Baltimore, MD.

Hartung, R., and Cornish, H.H. (1968). Cholinesterase inhibition in the acute toxicity of alkyl-substituted 2-aminoethanols. *Toxicol. Appl. Pharmacol.* **12**, 486-494.

Hayes, A.W., and Kruger, C.L. (2014). *Hayes' Principles and Methods of Toxicology.* 6th ed., pp. 1670-1674. CRC Press, New York.

Hazardous Substances Data Bank (HSDB) (2015). 2-Dimethylaminoethanol. CASRN: 108-01-0. HSDB No. 1329. National Library of Medicine, Bethesda, MD. Profile updated 10/19/2015. <https://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+1329>

Hendler, S.S., and Rorvik, D., Eds. (2001). Deanol. In *PDR for Nutritional Supplements*, pp. 123-124. Thomson Healthcare, Montvale, NJ.

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

Huntsman Corporation (2007). Technical Bulletin: N,N-Dimethylethanolamine (DMEA).

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

Jope, R.S., and Jenden, D.J. (1979). Dimethylaminoethanol (deanol) metabolism in rat brain and its effect on acetylcholine synthesis. *J. Pharmacol. Exp. Ther.* **211**, 472-479.

Klonne, D.R., Dodd, D.E., Pritts, I.M., Nachreiner, D.J., Fowler, E.H., Troup, C.M., Homan, E.R., and Ballantyne, B. (1987). Dimethylethanolamine: Acute 2-week and 13-week inhalation toxicity studies in rats. *Fundam. Appl. Toxicol.* **9**, 512-521.

Lefebvre, V., and Bhattaram, P. (2010). Vertebrate skeletogenesis. *Curr. Top. Dev. Biol.* **90**, 291-317.

Leung, H.-W., Tyl, R.W., Ballantyne, B., and Klonne, D.R. (1996). Developmental toxicity study in Fischer 344 rats by whole-body exposure to *N,N*-dimethylethanolamine vapor. *J. Appl. Toxicol.* **16**, 533-538.

Leung, H.-W., and Ballantyne, B. (1997). Evaluation of genotoxic potential of alkylalkanolamines. *Mutat. Res.* **393**, 7-15.

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**, 77.

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.

Mehta, D., Mehta, S., and Mathew, P. (1976). Failure of deanol in treating tardive dyskinesia. *Am. J. Psychiatry* **133**, 1467.

The Merck Index (2006). 14th ed. (M.J. O'Neil, Ed.), p. 480. Merck and Company, Inc., Whitehouse Station, NJ.

Mills, J.L., Fan, R., Brody, L.C., Liu, A., Ueland, P.M., Wang, Y., Kirke, P.N., Shane, B., and Molloy, A.M. (2014). Maternal choline concentrations during pregnancy and choline-related genetic variants as risk factors for neural tube defects. *Am. J. Clin. Nutr.* **100**, 1069-1074.

Miyazaki, H., Nambu, K., Minaki, Y., Hashimoto, M., and Nakamura, K. (1976). Comparative studies on the metabolism of beta-dimethylaminoethanol in the mouse brain and liver following administration of beta-dimethylaminoethanol and its p-chlorophenoxyacetate meclofenoxate. *Chem. Pharm. Bull. (Tokyo)* **24**, 763-769.

Murray, M.P., and Cummins, J.E. (1979). Mutagenic activity of epoxy embedding reagents employed in electron microscopy. *Environ. Mutagen.* **1**, 307-313.

National Institute of Standards and Technology (NIST) Mass Spectral Library (2005). (Wiley Registry, 8th ed.) Revision D06.00, version G1035B, entry 78256. Wiley and Sons, Inc., New York.

National Toxicology Program (NTP) (2002). Dimethylethanolamine (DMAE) [108-01-0] and selected salts and esters. Review of Toxicological Literature (Update). <https://ntp.niehs.nih.gov/ntp/htdocs/chem_background/exsumpdf/dmae_update_110002_508.pdf> Accessed June 22, 2017.

Nature's Plus (2017). Pedi-Active (Phosphatidylserion, DMAE Complex). <http://naturesplus.com/products/productdetail.php?productNumber=3000> Accessed June 22, 2017.

Nesse, R., and Carroll, B.J. (1976). Cholinergic side-effects associated with deanol. *Lancet* **2**, 50-51.

Organisation for Economic Cooperation and Development (OECD) (1996). Screening Information Data Set. *N,N*-Dimethylamino-2-ethanol. Processed by International Register of Potentially Toxic Chemicals. A contribution to the International Programme on Chemical Safety, United Nations, New York and Geneva.

Organisation for Economic Cooperation and Development (OECD) (2001). OECD Guideline for the Testing of Chemicals: Prenatal Developmental Toxicity Study (OECD 414).

Organisation for Economic Cooperation and Development (OECD) (2004). The 2004 OECD List of High Production Volume Chemicals. OECD Environment Directorate, Environment, Health and Safety Division, Paris.

Pendergast, J.F., Gange, S.J., and Lindstrom, M.J. (2005). Correlated Binary Data. In *Encyclopedia of Biostatistics.* John Wiley & Sons, Ltd., Hoboken, NJ. <https://doi.org/10.1002/0470011815.b2a10018>

Pitts, J.N., Jr., Winer, A.M., and Carter, W.P.L. (1981). Chemical Consequences of Air Quality Standards and of Control Implementation Programs, Report No. ARB-R-80/131, NTIS Order No. PB81-137697, p.408. Statewide Air Pollution Research Center, University of California, Riverside, CA.

Rothe, J., and Cordelair, H. (2001). New catalysts for low VOC in flexible slabstock foam. *J. Cell. Plast.* **37**, 207-220.

Salewski, E. (1964). Färbemethode zum makroskopischen nachweis von implantationsstellen am uterus der ratte. *Arch. Pathol. Exp. Pharmakol*. **247**, p. 367.

Schlenk, D.K. (1990). Dimethylaminoethanol. In *Ethel Browning's Toxicity and Metabolism of Industrial Solvents.* 2nd ed. (D.R. Buhler and D.J. Reed, Eds.), Vol. 2, pp. 417-422. Elsevier Science Publishers, New York.

Scott, W.J., Jr., Resnick, E., Hummler, H., Clozel, J.P., and Bürgin, H. (1997). Cardiovascular alterations in rat fetuses exposed to calcium channel blockers. *Reprod. Toxicol.* **11**, 207-214.

SDBSWeb (2019). SDBS No. 3992. <http://sdbs.riodb.aist.go.jp/sdbs/cgi-bin/direct>frame top.cgi?lang=eng (Accessed January 18, 2019)

Shaw, G.M., Carmichael, S.L., Yang, W., Selvin, S., and Schaffer, D.M. (2004). Periconceptional dietary intake of choline and betaine and neural tube defects in offspring. *Am. J. Epidemiol.* **160**, 102-109.

Shaw, G.M., Finnell, R.H., Blom, H.J., Carmichael, S.L., Vollset, S.E., Yang, W., and Ueland, P.M. (2009). Choline and risk of neural tube defects in a folate-fortified population. *Epidemiology* **20**, 714-719.

Shipkowski, K.A., McDonald, J.D., Sanders, J.M., and Waidyanatha, S. (2019). Comparative disposition of dimethylaminoethanol and choline in rats and mice following oral and intravenous administration. *Toxicol. Appl. Pharmacol.* (in preparation).

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

Sigma-Aldrich (2014). Safety Data Sheet: 2-Dimethylaminoethanol (+)-bitartrate salt. <http://www.sigmaaldrich.com> Accessed December 1, 2016.

Soares, K.V.S., and McGrath, J.J. (1999). The treatment of tardive dyskinesia – A systematic review and meta-analysis. *Schizophr. Res.* **39**, 1-16.

Source Naturals® (2017a). Dimethylaminoethanol Bitartrate (DMAE). <http://www.sourcenaturals.com/products/GP1101> Accessed June 22, 2017.

Source Naturals® (2017b). Attentive Child™. <http://www.sourcenaturals.com/products/GP1028> Accessed June 22, 2017.

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

Stenbäck, F., Weisburger, J.H., and Williams, G.M. (1988). Effect of lifetime administration of dimethylaminoethanol on longevity, aging changes, and cryptogenic neoplasms in C3H mice. *Mech. Ageing Dev.* **42**, 129-138.

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.

Tammenmaa, I., McGrath, J., Sailas, E.E.S., and Soares-Weiser, K. (2012). Cholinergic medication for neuroleptic-induced tardive dyskinesia. *Cochrane Database Syst. Rev.* **3**, 1-33.

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.

Tyl, R.W., Homan, E.R., Klonne, D.R., Pritts, I.M., Fowler, E.H., Fisher, L.C., France, K.A., Rebick, T.A., Beskitt, J.L., and Ballantyne, B. (1987). Developmental toxicity evaluation of inhaled N,N-dimethylethanolamine (DMEA) in Fischer 344 rats. *Toxicologist* **7**, 173.

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.

Union Carbide (1986). Initial Submission: N,N-dimethylethanolamine: Acute Toxicity and Primary Irritancy Studies (Project Report) with Cover Sheet and Letter Dated 05/05/92. Environmental Protection Agency (EPA) Toxic Substances Control Act (TSCA) Section 8ECP Test Submission. Doc. No. 88-920002183. Fiche No. OTS0536318 (1).

Union Carbide. (1987). Dimethylethanolamine (DMEA). *Salmonella*/microsomes (Ames) bacterial mutagenicity assay. Project Report 50-85. Bushy Run Research Centre.

Union Carbide. (1988). Dimethylethanolamine (DMEA). *In vitro* genotoxicity studies: CHO/HGPRT gene mutation test and sister chromatid exchange assay. Project Report 50-130. Bushy Run Research Centre.

U.S. 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.

U.S. Environmental Protection Agency (USEPA) (2011). Chemical Data Reporting (CDR) Database. <https://chemview.epa.gov/chemview> Accessed January 16, 2018.

U.S. Environmental Protection Agency (USEPA) (2016). Toxic Substances Control Act (TSCA) Inventory Update Rule (IUR). <https://www.epa.gov/chemical-data-reporting/2016-chemical-data-reporting-results> Accessed June 22, 2017.

U.S. Environmental Protection Agency (USEPA) (2018). Dimethylaminoethanol. USEPA Chem Dashboard. <https://comptox.epa.gov/dashboard/dsstoxdb/results?search=dimethylaminoethanol#properties> Accessed October 1, 2018.

Wickramaratne, G.A. de S. (1988). The post-natal fate of supernumerary ribs in rat teratogenicity studies. *J. Appl. Toxicol.* **8**, 91-94.

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.

Zahniser, N.R., Chou, D., and Hanin, I. (1977). Is 2-dimethylaminoethanol (deanol) indeed a precursor of brain acetylcholine? A gas chromatographic evaluation. *J. Pharmacol. Exp. Ther.* **200**, 545-559.

Zahniser, N.R., Katyal, S.L., Shih, T.-M., Hanin, I., Moossy, J., Martinez, A.J., and Lombardi, B. (1978). Effects of *N*-methylaminoethanol, and *N,N*-dimethylaminoethanol in the diet of pregnant rats on neonatal rat brain cholinergic and phospholipid profile. *J. Neurochem.* **30**, 1245-1252.

Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., Mortelmans, K., and Speck, W. (1987). *Salmonella* mutagenicity tests: III. Results from the testing of 255 chemicals. *Environ. Mutagen.* **9**, 1-110.

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 DIMETHYLAMINOETHANOL BITARTRATE

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

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Pregnant Rats				
n	9	7	10	8
Vaginal discharge, clear	0	0	0	1 (GD 20)
Discolored head, brown	0	0	0	1 (GD 21)
Sore tip of tail	0	0	0	1 (GD 20)

[a] Cumulative number of animals with the observation and the first day of observation onset (displayed in parentheses).
n = number of dams; GD = gestation day

TABLE A2
Summary of Maternal Body Weights of Rats in the Dose Range-Finding Gavage Study of Dimethylaminoethanol Bitartrate[a]

	0 mg/kg		250 mg/kg			500 mg/kg			1,000 mg/kg		
	Weight (g)	N[b]	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N
Mean Body Weights											
GD 3	225.9 ± 4.3	9	221.2 ± 3.7	97.9	7	225.6 ± 3.0	99.9	10	225.9 ± 4.3	100.0	8
GD 4	234.0 ± 2.6	9	226.2 ± 4.5	96.7	7	230.8 ± 3.5	98.6	10	233.3 ± 4.1	99.7	8
GD 5	239.5 ± 3.0	9	231.8 ± 4.1	96.8	7	237.5 ± 3.1	99.2	10	235.8 ± 4.1	98.5	8
GD 6	241.6 ± 2.7	9	236.3 ± 3.6	97.8	7	240.4 ± 3.2	99.5	10	242.3 ± 4.3	100.3	8
GD 7	246.9 ± 3.2	9	238.4 ± 4.1	96.6	7	245.8 ± 3.3	99.6	10	244.7 ± 4.5	99.1	8
GD 8	249.7 ± 3.4	9	243.5 ± 4.3	97.5	7	248.9 ± 3.9	99.7	10	248.6 ± 4.0	99.5	8
GD 9	255.4 ± 3.4	9	248.2 ± 3.8	97.2	7	253.2 ± 3.7	99.1	10	253.2 ± 4.2	99.1	8
GD 10	259.6 ± 3.4	9	251.6 ± 4.0	96.9	7	257.1 ± 3.7	99.1	10	258.5 ± 4.7	99.6	8
GD 11	265.8 ± 3.9	9	259.2 ± 3.8	97.5	7	264.5 ± 4.1	99.5	10	264.0 ± 4.9	99.3	8
GD 12	270.3 ± 3.5	9	263.3 ± 3.5	97.4	7	269.3 ± 3.5	99.6	10	269.2 ± 4.0	99.6	8
GD 13	275.2 ± 4.0	9	267.9 ± 3.8	97.3	7	274.5 ± 4.0	99.7	10	273.1 ± 4.4	99.2	8
GD 14	279.8 ± 4.2	9	273.8 ± 4.5	97.8	7	279.4 ± 3.8	99.9	10	280.3 ± 4.4	100.2	8
GD 15	288.3 ± 4.7	9	282.5 ± 4.6	98.0	7	288.2 ± 4.4	100.0	10	287.3 ± 4.5	99.6	8
GD 16	296.9 ± 5.4	9	288.9 ± 4.6	97.3	7	297.8 ± 4.6	100.3	10	299.1 ± 4.6	100.7	8
GD 17	308.3 ± 6.2	9	299.2 ± 5.9	97.0	7	310.1 ± 5.1	100.6	10	308.7 ± 5.2	100.1	8
GD 18	319.8 ± 7.1	9	312.9 ± 7.6	97.8	7	325.5 ± 6.3	101.8	10	319.1 ± 7.4	99.8	8
GD 19	333.7 ± 9.0	9	330.9 ± 9.2	99.1	7	342.5 ± 6.2	102.6	10	334.0 ± 10.2	100.1	8
GD 20	348.5 ± 10.0	9	346.7 ± 10.9	99.5	7	360.8 ± 7.1	103.5	10	348.3 ± 13.6	99.9	8
GD 21	363.1 ± 12.0	9	360.8 ± 12.9	99.3	7	377.8 ± 7.3	104.0	10	361.3 ± 16.0	99.5	8

[a] Data are displayed as mean ± standard error by gestation day (GD). No statistically significant trends (by Jonckheere's test) or pairwise comparisons (by Williams' or Dunnett's test) were found for body weights.
[b] Number of surviving dams

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

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Disposition Summary				
Females initially in study	10	10	10	10
Survivors				
Terminal euthanasia	10	10	10	10
Number of animals examined	10	10	10	10
Alimentary System				
Esophagus	(10)	(10)	(10)	(10)
Intestine, large, cecum	(10)	(10)	(10)	(10)
Intestine, large, colon	(10)	(10)	(10)	(10)
Intestine, large, rectum	(10)	(10)	(10)	(10)
Intestine, small, duodenum	(10)	(10)	(10)	(10)
Intestine, small, ileum	(10)	(10)	(10)	(10)
Intestine, small, jejunum	(10)	(10)	(10)	(10)
Liver	(10)	(10)	(10)	(10)
Pancreas	(10)	(10)	(10)	(10)
Stomach, forestomach	(10)	(10)	(10)	(10)
Stomach, glandular	(10)	(10)	(10)	(10)
Cardiovascular System				
Heart	(10)	(10)	(10)	(10)
Endocrine System				
Adrenal gland	(10)	(10)	(10)	(10)
General Body System				
None				
Genital System				
Ovary	(10)	(10)	(10)	(10)
Uterus	(10)	(10)	(10)	(10)
Fluid, clear		1		1
Vagina	(10)	(10)	(10)	(10)
Hematopoietic System				
Lymph node, mesenteric	(10)	(10)	(10)	(10)
Spleen	(10)	(10)	(10)	(10)
Thymus	(10)	(10)	(10)	(10)
Integumentary System				
Skin	(10)	(10)	(10)	(10)
Discoloration				1
Musculoskeletal System				
None				

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

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Nervous System				
None				
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Trachea	(10)	(10)	(10)	(10)
Special Senses System				
None				
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Ureter	(10)	(10)	(10)	(10)
Urinary bladder	(10)	(10)	(10)	(10)

[a] Number of animals examined at the site (displayed in parentheses) and number of animals with observation

TABLE A4
Summary of Fetal External Findings in Rats in the Dose Range-Finding Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Total number of fetuses	97	82	128	108
External				
Number of fetuses examined	97	82	128	108
Number of litters examined	9	7	10	8
No visible lesions present				

APPENDIX B
SUMMARY OF FINDINGS IN RATS IN THE PRENATAL DEVELOPMENTAL TOXICITY GAVAGE STUDY OF DIMETHYLAMINOETHANOL BITARTRATE

TABLE B1
Summary of Clinical Observations for Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate[a]

	0 mg/kg		250 mg/kg		500 mg/kg		1,000 mg/kg	
Pregnant Rats								
n	20		20		20		24	
Breathing, abnormal lung noise	0		0		0		2	(GD 6)
Cold to touch	0		0		0		1	(GD 21)
Dehydrated	0		0		0		1	(GD 20)
Discharge nose/snout, red	0		0		0		1	(GD 14)
Discharge vagina, brown	8	(GD 14)	3	(GD 15)	4	(GD 15)	9	(GD 14)
Discharge vagina, clear	2	(GD 21)	5	(GD 15)	4	(GD) 15	2	(GD 15)
Discharge vagina, red	3	(GD 14)	0		0		1	(GD 19)
Hypoactivity	0		0		0		1	(GD 20)
Scab, tip of tail	1	(GD 19)	0		0		0	
Sore, tail	0		0		0		1	(GD 12)
Stained eye, brown	0		0		0		1	(GD 21)
Stained face, red	0		0		0		1	(GD 14)
Stained nose/snout, brown	0		0		1	(GD 16)	1	(GD 14)
Stained nose/snout, red	0		0		2	(GD 14)	3	(GD 14)
Non-Pregnant Rats								
n	5		5		5		1	
Discharge, vagina, clear	1	(SD 11)	0		0		0	
Scab, tip of tail	0		0		1	(SD 18)	0	
Sore, tip of tail	0		0		1	(SD 17)	0	
Stained nose/snout, brown	0		0		1	(SD 10)	0	

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

TABLE B2
Summary of Maternal Body Weights of Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate[a]

	0 mg/kg		250 mg/kg			500 mg/kg			1,000 mg/kg		
	Weight (g)	N[b]	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N	Weight (g)	Weight (% of controls)	N
Mean Body Weights											
GD 3	227.8 ± 2.3	20	228.7 ± 1.9	100.4	20	228.6 ± 2.3	100.4	20	228.3 ± 2.1	100.2	24
GD 4	231.2 ± 2.3	20	232.0 ± 1.9	100.3	20	231.4 ± 2.4	100.1	20	231.4 ± 2.1	100.1	24
GD 5	236.8 ± 2.6	20	239.0 ± 1.8	100.9	20	238.7 ± 2.5	100.8	20	237.7 ± 2.0	100.4	24
GD 6	239.6 ± 2.4	20	240.0 ± 1.7	100.1	20	240.3 ± 2.5	100.3	20	239.4 ± 2.2	99.9	24
GD 7	243.7 ± 2.4	20	244.2 ± 2.0	100.2	20	245.8 ± 2.4	100.9	20	242.4 ± 2.2	99.5	24
GD 8	248.6 ± 2.5	20	248.8 ± 2.1	100.1	20	248.9 ± 2.2	100.1	20	247.5 ± 2.1	99.6	24
GD 9	253.2 ± 2.5	20	254.0 ± 2.1	100.3	20	254.0 ± 2.5	100.3	20	252.7 ± 2.2	99.8	24
GD 10	257.6 ± 2.6	20	259.6 ± 2.2	100.8	20	260.0 ± 2.6	100.9	20	258.0 ± 2.3	100.2	24
GD 11	263.9 ± 2.7	20	264.7 ± 2.2	100.3	20	266.9 ± 2.5	101.1	20	265.1 ± 2.4	100.5	23
GD 12	268.4 ± 2.7	20	269.0 ± 2.2	100.2	20	270.4 ± 2.4	100.8	20	267.7 ± 2.5	99.8	23
GD 13	273.6 ± 3.0	20	275.6 ± 2.3	100.7	20	277.0 ± 2.7	101.3	20	274.6 ± 2.6	100.4	23
GD 14	276.8 ± 3.2	20	280.8 ± 2.8	101.4	20	281.3 ± 2.9	101.7	20	278.2 ± 2.9	100.5	23
GD 15	281.9 ± 3.5	20	286.2 ± 3.1	101.5	20	288.5 ± 2.8	102.3	20	283.4 ± 3.4	100.5	23
GD 16	293.7 ± 4.2	20	295.7 ± 3.8	100.7	20	300.6 ± 2.9	102.3	20	293.4 ± 3.7	99.9	23
GD 17	304.6 ± 4.7	20	307.7 ± 4.9	101.0	20	313.4 ± 3.2	102.9	20	304.1 ± 4.5	99.8	23
GD 18	319.6 ± 5.9	20	327.4 ± 3.7	102.4	20	331.3 ± 3.4	103.7	20	318.8 ± 5.8	99.7	23
GD 19	329.5 ± 6.6	20	342.7 ± 3.9	104.0	20	344.8 ± 4.0	104.7	20	330.5 ± 7.0	100.3	23
GD 20	344.9 ± 8.1	19	359.2 ± 4.7	104.1	20	360.2 ± 4.5	104.4	20	343.5 ± 7.7	99.6	23
GD 21	359.6 ± 8.8	19	375.2 ± 5.3	104.3	20	380.3 ± 5.1	105.8	20	367.1 ± 6.5	102.1	21

[a] Data are displayed as mean ± standard error by gestation day (GD). No statistically significant trends (by Jonckheere's test) or pairwise comparisons (by Williams' or Dunnett's test) were found for body weights.
[b] Number of surviving dams

TABLE B3
Summary of Gross Pathology Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate[a]

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Disposition Summary				
Females initially in study	25	25	25	25
Early deaths				
Euthanized early - pregnant	1			1
Moribund				1
Natural death				1
Survivors				
Terminal euthanasia	24	25	25	22
Number of animals examined	25	25	25	25
Alimentary System				
Esophagus	(25)	(25)	(25)	(25)
Intestine, large, cecum	(25)	(25)	(25)	(25)
Intestine, large, colon	(25)	(25)	(25)	(25)
Intestine, large, rectum	(25)	(25)	(25)	(25)
Intestine, small, duodenum	(25)	(25)	(25)	(25)
Intestine, small, ileum	(25)	(25)	(25)	(25)
Intestine, small, jejunum	(25)	(25)	(25)	(25)
Liver	(25)	(25)	(25)	(25)
Pancreas	(25)	(25)	(25)	(25)
Stomach	(25)	(25)	(25)	(25)
Cardiovascular System				
Heart	(25)	(25)	(25)	(25)
Endocrine System				
Adrenal gland	(25)	(25)	(25)	(25)
General Body System				
Cavity, thoracic	(0)	(1)	(0)	(0)
Fluid		1		
Genital System				
Ovary	(25)	(25)	(25)	(25)
Uterus	(25)	(25)	(25)	(25)
Dilation	1			
Fluid	1			
Vagina	(25)	(25)	(25)	(25)
Hematopoietic System				
Lymph node, mesenteric	(25)	(25)	(25)	(25)
Spleen	(25)	(25)	(25)	(25)
Thymus	(25)	(25)	(25)	(25)
Integumentary System				
Skin	(25)	(25)	(25)	(25)

TABLE B3
Summary of Gross Pathology Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Lung	(25)	(25)	(25)	(25)
Trachea	(25)	(25)	(25)	(25)
Special Senses System				
None				
Urinary System				
Kidney	(25)	(25)	(25)	(25)
Ureter	(25)	(25)	(25)	(25)
Urinary bladder	(25)	(25)	(25)	(25)

[a] Number of animals examined at the site (displayed in parentheses) and number of animals with observation

TABLE B4
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg		250 mg/kg		500 mg/kg		1,000 mg/kg	
Total number of fetuses examined	209		265		260		260	
External								
Number of fetuses examined	209		265		260		260	
Number of litters examined	19		20		20		22	
Body: General								
Body, subcutaneous hemorrhage — [GF]								
Fetuses	1	(0.48)	2	(0.75)	1	(0.38)	5	(1.92)
Litters	1	(5.26)	2	(10.00)	1	(5.00)	3	(13.64)
Extremities								
Limb, hind, right, bent — [M]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.38)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.55)
Tail, bent — [M]								
Fetuses	0	(0.0)	1	(0.38)	1	(0.38)	2	(0.77)
Litters	0	(0.00)	1	(5.00)	1	(5.00)	1	(4.55)
Head								
Head, meningocele — [M]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	0	(0.00)
Trunk								
General, omphalocele — [M]								
Fetuses	0	(0.0)	1	(0.38)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.00)	0	(0.00)	0	(0.00)
Visceral								
Number of fetuses examined	209		265		260		260	
Number of litters examined	19		20		20		22	
General								
General, fluid-filled thorax — [GF]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.38)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.55)
Heart								
Aortic valve, misshapen — [M]								
Fetuses	2	(0.96)	1	(0.38)	3	(1.15)	1	(0.38)
Litters	2	(10.53)	1	(5.00)	3	(15.00)	1	(4.55)
Atrium, right, large — [M]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.38)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.55)
Ventricle, bilateral, thick wall — [M]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	0	(0.00)
Ventricle, bilateral, ventricular septum defect — [M]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	0	(0.00)
Major vessels								
Aortic arch, absent — [M]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(5.00)	0	(0.00)
Carotid artery, left, malpositioned — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(5.00)	0	(0.00)

TABLE B4
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg		250 mg/kg		500 mg/kg		1,000 mg/kg	
Visceral (continued)								
Number of fetuses examined	209		265		260		260	
Number of litters examined	19		20		20		22	
Major vessels (continued)								
Innominate artery, absent — [V]								
Fetuses	3	(1.44)**##	4	(1.51)	6	(2.31)	12	(4.62)*#
Litters	3	(15.79)*	4	(20.00)	4	(20.00)	10	(45.45)*
Innominate artery, atresia — [M]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.38)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.55)
Innominate artery, short — [V]								
Fetuses	6	(2.87)	7	(2.64)	8	(3.08)	5	(1.92)
Litters	4	(21.05)	7	(35.00)	6	(30.00)	4	(18.18)
Pulmonary artery/trunk, dilated — [M]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	0	(0.00)
Subclavian artery, right, dilated — [V]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.38)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.55)
Thoracic viscera								
Thymus, supernumerary — [V]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.38)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.55)
Urinary tract								
Renal pelvis, dilated — [V]								
Fetuses	0	(0.0)	1	(0.38)	1	(0.38)	1	(0.38)
Litters	0	(0.00)	1	(5.00)	1	(5.00)	1	(4.55)
Renal pelvis, bilateral, dilated — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(5.00)	0	(0.00)
Renal pelvis, left, dilated — [V]								
Fetuses	0	(0.0)	1	(0.38)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.00)	0	(0.00)	0	(0.00)
Renal pelvis, right, dilated — [V]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	1	(0.38)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.55)
Ureter, dilated — [V]								
Fetuses	3	(1.44)	3	(1.13)	2	(0.77)	2	(0.77)
Litters	2	(10.53)	3	(15.00)	2	(10.00)	2	(9.09)
Ureter, bilateral, dilated — [V]								
Fetuses	1	(0.48)	1	(0.38)	1	(0.38)	0	(0.0)
Litters	1	(5.26)	1	(5.00)	1	(5.00)	0	(0.00)
Ureter, left, dilated — [V]								
Fetuses	1	(0.48)	2	(0.75)	1	(0.38)	2	(0.77)
Litters	1	(5.26)	2	(10.00)	1	(5.00)	2	(9.09)
Ureter, right, dilated — [V]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	0	(0.00)
Head								
Number of fetuses examined	109		137		134		132	
Number of litters examined	18		20		20		22	
No visible lesions present								

TABLE B4
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg		250 mg/kg		500 mg/kg		1,000 mg/kg	
Skeletal: Body								
Number of fetuses examined	209		264		260		260	
Number of litters examined	19		20		20		22	
Pelvic girdle								
Pubis, left, incomplete ossification — [V]								
Fetuses	0	(0.0)	0	(0.0)	0	(0.0)	2	(0.77)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.55)
Ribs								
Costal cartilage, 6th right, nodulated — [V]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	2	(0.77)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	2	(9.09)
Costal cartilage, 8th right, nodulated — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(5.00)	0	(0.00)
Costal cartilage, 9th right, nodulated — [V]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	0	(0.00)
Supernumerary rib								
Thoracolumbar, full — [M]								
Fetuses	7	(3.35)**##	3	(1.14)	1	(0.38)*#	1	(0.38)*#
Litters	6	(31.58)*	3	(15.00)	1	(5.00)*	1	(4.55)*
Thoracolumbar, short — [V]								
Fetuses	56	(26.79)**##	56	(21.21)	59	(22.69)	100	(38.46)**#
Litters	17	(89.47)	18	(90.00)	18	(90.00)	19	(86.36)
Thoracolumbar, bilateral, full — [M]								
Fetuses	1	(0.48)	0	(0.0)	0	(0.0)	0	(0.0)
Litters	1	(5.26)	0	(0.00)	0	(0.00)	0	(0.00)
Thoracolumbar, bilateral, short — [V]								
Fetuses	17	(8.13)**##	11	(4.17)	24	(9.23)	39	(15.0)*
Litters	8	(42.11)*	7	(35.00)	11	(55.00)	16	(72.73)*
Thoracolumbar, left, full — [M]								
Fetuses	3	(1.44)*	1	(0.38)	0	(0.0)	0	(0.0)
Litters	3	(15.79)*	1	(5.00)	0	(0.00)	0	(0.00)
Thoracolumbar, left, short — [V]								
Fetuses	23	(11.0)*#	32	(12.12)	26	(10.0)	45	(17.31)*#
Litters	14	(73.68)	17	(85.00)	16	(80.00)	17	(77.27)
Thoracolumbar, right, full — [M]								
Fetuses	3	(1.44)	2	(0.76)	1	(0.38)	1	(0.38)
Litters	3	(15.79)	2	(10.00)	1	(5.00)	1	(4.55)
Thoracolumbar, right, short — [V]								
Fetuses	16	(7.66)	13	(4.92)	9	(3.46)*#	16	(6.15)
Litters	10	(52.63)	10	(50.00)	8	(40.00)	10	(45.45)
Vertebrae								
Thoracic centrum, 10th, incomplete ossification — [V]								
Fetuses	0	(0.0)	0	(0.0)	1	(0.38)	0	(0.0)
Litters	0	(0.00)	0	(0.00)	1	(5.00)	0	(0.00)
Thoracic centrum, 11th, incomplete ossification — [V]								
Fetuses	0	(0.0)	1	(0.38)	0	(0.0)	0	(0.0)
Litters	0	(0.00)	1	(5.00)	0	(0.00)	0	(0.00)

TABLE B4
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Skeletal: Skull				
Number of fetuses examined	100	128	126	128
Number of litters examined	18	20	20	20
Skull				
General, isolated ossification site — [V]				
Fetuses	1 (1.0)	5 (3.91)	3 (2.38)	3 (2.34)
Litters	1 (5.56)	5 (25.00)	2 (10.00)	2 (10.00)
General, supernumerary site — [V]				
Fetuses	1 (1.0)**##	3 (2.34)	2 (1.59)	13 (10.16)**#
Litters	1 (5.56)**	3 (15.00)	2 (10.00)	10 (50.00)**

Upper row denotes number of affected fetuses and (%) and lower row the number of affected litters and (%)
Statistical analysis for litter data and for fetal data (without litter effects) performed by Cochran-Armitage (trend) and Fisher's exact (pairwise) tests
* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column);
** P≤0.01
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression
\# Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column) in litter-based analysis of fetuses;
P≤0.01
[M] = Malformation
[V] = Variation
[GF] = Gross Finding

TABLE B5
Summary of Total Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg		250 mg/kg		500 mg/kg		1,000 mg/kg	
All Exams								
Number of fetuses	209		265		260		260	
Number of litters	19		20		20		22	
Malformation								
Affected fetuses	9	(4.31)	6	(2.26)	6	(2.31)	7	(2.69)
Affected litters	8	(42.11)	5	(25.00)	5	(25.00)	4	(18.18)
Variation								
Affected fetuses	69	(33.01)**##	76	(28.68)	78	(30.00)	127	(48.85)**##
Affected litters	17	(89.47)	20	(100.00)	20	(100.00)	21	(95.45)
Gross Finding								
Affected fetuses	3	(1.44)	2	(0.75)	1	(0.38)	6	(2.31)
Affected litters	2	(10.53)	2	(10.00)	1	(5.00)	4	(18.18)
External								
Number of fetuses	209		265		260		260	
Number of litters	19		20		20		22	
Malformation								
Affected fetuses	1	(0.48)	2	(0.75)	1	(0.38)	3	(1.15)
Affected litters	1	(5.26)	2	(10.00)	1	(5.00)	2	(9.09)
Gross Finding								
Affected fetuses	1	(0.48)	2	(0.75)	1	(0.38)	5	(1.92)
Affected litters	1	(5.26)	2	(10.00)	1	(5.00)	3	(13.64)
Visceral								
Number of fetuses	209		265		260		260	
Number of litters	19		20		20		22	
Malformation								
Affected fetuses	4	(1.91)	1	(0.38)	4	(1.54)	3	(1.15)
Affected litters	4	(21.05)	1	(5.00)	4	(20.00)	3	(13.64)
Variation								
Affected fetuses	11	(5.26)	14	(5.28)	16	(6.15)	19	(7.31)
Affected litters	7	(36.84)	11	(55.00)	9	(45.00)	14	(63.64)
Gross Finding								
Affected fetuses	0	(0.00)	0	(0.00)	0	(0.00)	1	(0.38)
Affected litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(4.55)
Skeletal: Body								
Number of fetuses	209		264		260		260	
Number of litters	19		20		20		22	
Malformation								
Affected fetuses	7	(3.35)**##	3	(1.14)	1	(0.38)*#	1	(0.38)*#
Affected litters	6	(31.58)*	3	(15.00)	1	(5.00)*	1	(4.55)*
Variation								
Affected fetuses	58	(27.75)**##	57	(21.59)	60	(23.08)	102	(39.23)**#
Affected litters	17	(89.47)	18	(90.00)	18	(90.00)	19	(86.36)

TABLE B5
Summary of Total Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg		250 mg/kg		500 mg/kg		1,000 mg/kg	
Skeletal: Skull								
Number of fetuses	100		128		126		128	
Number of litters	18		20		20		20	
Variation								
Affected fetuses	2	(2.00)**##	8	(6.25)	5	(3.97)	16	(12.50)**#
Affected litters	2	(11.11)*	7	(35.00)	4	(20.00)	10	(50.00)*

Upper row denotes number of affected fetuses and (%) and lower row the number of affected litters and (%)
Statistical analysis for litter data and for fetal data (without litter effects) performed by Cochran-Armitage (trend) and Fisher's exact (pairwise) tests
* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column);
** P≤0.01
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression
\# Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column) in litter-based analysis of fetuses;
P≤0.01

TABLE B6
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Total number of fetuses examined	209	265	260	260
Total number of dams examined	19	20	20	22
Placental				
Number of fetuses examined	209	265	260	260
Number of dams examined	19	20	20	22
Placentae				
Placenta, bipartite — [GF]	3 (8)			
Placenta, fused — [GF]	15 (7)			
Visceral				
Number of fetuses examined	209	265	260	260
Number of dams examined	19	20	20	22
General				
General, fluid-filled thorax — [GF]				76 (11)
Heart				
Aortic valve, misshapen — [M]	15 (3) 18 (3)	33 (10)	58 (12) 62 (13) 71 (3)	91 (8)
Atrium, right, large — [M]				76 (11)
Ventricle, bilateral, thick wall — [M]	16 (3)			
Ventricle, bilateral, ventricular septum defect — [M]	16 (3)			
Major vessels				
Aortic arch, absent — [M]			60 (3)	
Carotid artery, left, malpositioned — [V]			72 (2)	
Innominate artery, absent — [V]	3 (13) 16 (1) 19 (10)	30 (11) 31 (9) 32 (3) 41 (9)	55 (8,9) 60 (3) 61 (13) 72 (2,7)	78 (5) 79 (8) 81 (1) 82 (6) 83 (3) 85 (8) 87 (7) 89 (16) 91 (4) 96 (1,9,10)

TABLE B6
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Visceral (continued)				
Number of fetuses examined	209	265	260	260
Number of dams examined	19	20	20	22
Major vessels				
Innominate artery, atresia — [M]				95 (6)
Innominate artery, short — [V]	9 (3,6) 10 (7,8) 16 (3) 25 (7)	31 (5) 33 (9) 36 (5) 37 (3) 41 (10) 43 (15) 50 (7)	52 (9,11) 57 (2) 62 (12) 64 (4) 71 (4,11) 72 (1)	76 (11) 83 (1,10) 94 (11) 95 (4)
Pulmonary artery/trunk, dilated — [M]	24 (1)			
Subclavian artery, right, dilated — [V]				81 (1)
Thoracic viscera				
Thymus, supernumerary — [V]				76 (11)
Urinary tract				
Renal pelvis, bilateral, dilated — [V]			61 (4)	
Renal pelvis, left, dilated — [V]		29 (6)		
Renal pelvis, right, dilated — [V]				92 (3)
Ureter, bilateral, dilated — [V]	16 (1)	41 (11)	61 (4)	
Ureter, left, dilated — [V]	18 (8)	29 (6) 34 (5)	64 (5)	87 (8) 92 (3)
Ureter, right, dilated — [V]	16 (2)			
Skeletal: Body				
Number of fetuses examined	209	264	260	260
Number of dams examined	19	20	20	22
Appendicular skeleton				
General, not examined		43 (15)		
Pelvic girdle				
Pubis, left, incomplete ossification — [V]				85 (4,6)
Ribs				
Costal cartilage, 6th right, nodulated — [V]	10 (12)			97 (9) 100 (11)
Costal cartilage, 8th right, nodulated — [V]			68 (10)	
Costal cartilage, 9th right, nodulated — [V]	6 (1)			

TABLE B6
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Skeletal: Body (continued)				
Number of fetuses examined	209	264	260	260
Number of dams examined	19	20	20	22
Supernumerary rib				
Thoracolumbar, bilateral, full — [M]	6 (3)			
Thoracolumbar, bilateral, short — [V]	1 (6,12) 3 (4) 6 (11) 10 (5) 13 (1,5) 18 (9,13) 23 (3,4,6,8,11) 25 (3,9,11)	28 (2,3,7,11) 33 (9,11) 34 (1) 36 (10) 42 (6) 44 (12) 46 (4)	51 (2,6,14) 59 (5,12) 60 (5) 61 (14) 63 (5,6,8) 65 (2,13) 67 (1,9,10,11) 68 (1,4) 70 (15) 72 (6,9,10) 73 (3,10)	76 (1,5) 77 (5,6) 78 (6) 82 (13) 83 (14) 84 (5,8) 85 (6) 87 (5,8) 89 (12,14,16) 90 (4,5,6,13) 92 (7,8,10) 94 (8,11,12,13) 95 (2,3) 96 (12,15) 99 (4) 100 (4,5,8,10, 12,13,14,16)
Thoracolumbar, left, full — [M]	10 (3) 23 (13) 25 (1)	49 (15)		
Thoracolumbar, left, short — [V]	1 (2,11,14) 3 (3) 4 (13) 6 (4) 8 (12,17) 10 (1) 11 (7) 13 (4) 15 (2) 16 (3) 17 (1,10,13) 18 (3,4,6) 23 (7,10) 25 (6,13)	26 (2,5) 28 (8,9) 29 (9) 31 (11,14) 32 (15) 33 (7,8) 34 (2) 36 (2,8) 37 (1,10) 38 (5,9) 39 (3,7,9) 40 (2,13) 42 (5,14) 43 (11) 44 (1,8,11) 49 (4,13) 50 (11,14)	51 (5,7,13) 52 (11) 53 (3) 55 (1,6) 58 (5,10) 59 (3) 60 (6,8) 61 (9) 63 (2,13) 65 (11) 68 (5,7,10) 69 (2,3) 70 (8) 71 (10) 72 (7,11) 73 (14)	77 (4,7) 82 (3,5,9,11,14,16) 83 (4,12) 84 (2) 85 (4,11) 87 (3,4) 89 (4,7) 90 (7,10,12) 91 (1,4,9) 92 (6,12) 94 (4,7) 95 (1,6,9,10,12) 96 (2,7,11) 97 (1,8,11) 98 (1,2,5,6) 99 (9) 100 (1,3)
Thoracolumbar, right, full — [M]	16 (3) 18 (3) 25 (6)	31 (14) 33 (6)	63 (13)	94 (4)

TABLE B6
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
Skeletal: Body (continued)				
Number of fetuses examined	209	264	260	260
Number of dams examined	19	20	20	22
Thoracolumbar, right, short — [V]				
	1 (5,13)	28 (4)	51 (10,11)	78 (2)
	5 (4)	32 (9)	53 (6)	83 (5)
	6 (6)	33 (5)	58 (3)	84 (3)
	8 (8,13)	34 (7,9)	60 (7)	87 (9)
	9 (2,12)	39 (6)	64 (4)	89 (5,10)
	10 (3)	42 (8)	67 (6)	90 (2,9)
	17 (11,14)	43 (12)	68 (11)	94 (1,6)
	18 (10)	44 (3)	71 (7)	96 (1,3,13)
	19 (4)	49 (15,16)		97 (2,12)
	25 (1,7,12)	50 (4,13)		100 (7)
Vertebrae				
Thoracic centrum, 10th, incomplete ossification — [V]				
			72 (12)	
Thoracic centrum, 11th, incomplete ossification — [V]				
		34 (3)		
Skeletal: Skull				
Number of fetuses examined	100	128	126	128
Number of dams examined	18	20	20	20
Skull				
General, isolated ossification site — [V]				
	18 (12)	26 (2)	65 (10,12)	83 (8)
		28 (6)	72 (8)	98 (4,6)
		40 (10)		
		43 (14)		
		44 (8)		
General, supernumerary site — [V]				
	15 (10)	31 (2)	64 (6)	76 (2,4,9)
		32 (8)	68 (8)	79 (5)
		43 (4)		83 (2)
				84 (8)
				87 (2,4)
				89 (12)
				91 (12)
				92 (4)
				96 (10)
				98 (8)
Head				
Number of fetuses examined	109	137	134	132
Number of dams examined	18	20	20	22
External				
Number of fetuses examined	209	265	260	260
Number of dams examined	19	20	20	22
Body, general				
Body, subcutaneous hemorrhage — [GF]				
	3 (9)	29 (13)	71 (8)	77 (1,7,10)
		43 (14)		79 (9)
				85 (2)

TABLE B6
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Dimethylaminoethanol Bitartrate

	0 mg/kg	250 mg/kg	500 mg/kg	1,000 mg/kg
External (continued)				
Number of fetuses examined	209	265	260	260
Number of dams examined	19	20	20	22
Extremities				
Limb, hind, right, bent — [M]				76 (7)
Tail, bent — [M]		41 (14)	63 (6)	91 (7,16)
Head				
Head, meningocele — [M]	16 (3)			
Trunk				
General, omphalocele — [M]		26 (4)		

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

APPENDIX C
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Dimethylaminoethanol Bitartrate

Dimethylaminoethanol bitartrate was obtained from Bayville Chemical Supply Company, Inc. (Deer Park, NY) in one lot (159AK) that was used in the dose range-finding and prenatal developmental toxicity studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at RTI International (Research Triangle Park, NC) for the study laboratory at Southern Research (Birmingham, AL). Reports on analyses performed in support of the dimethylaminoethanol bitartrate studies are on file at the National Institute of Environmental Health Sciences.

Lot 159AK of the chemical, a white crystalline powder, was identified as dimethylaminoethanol bitartrate using Fourier transform infrared (FTIR) and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy. The exact mass for dimethylaminoethanol and tartaric acid was determined using ultra high-performance liquid chromatography (UHPLC) with time-of-flight mass spectrometry (TOF MS) detection. In addition, the melting point of the bulk chemical was determined. All spectra were consistent with the structure and literature spectra (Clares, *et al.*, 2010; SDBSWeb, 2019) of dimethylaminoethanol bitartrate. UHPLC/TOF-MS results measured values within 3.9 ppm or less of the theoretical mass values for dimethylaminoethanol and tartaric acid. The melting point (USEPA, 2018) was in good agreement with literature values. Representative FTIR and proton and carbon-13 NMR spectra are presented in Figures C1, C2, and C3, respectively.

The moisture content of lot 159AK was measured by a desorption type Karl Fischer analysis and by thermogravimetric analysis (TGA); these procedures were conducted by Robertson Microlit Laboratory (Ledgewood, NJ). The purity of lot 159AK was determined by Galbraith Laboratories, Inc. (Knoxville, TN) using elemental analyses and by the analytical chemistry laboratory initially using UHPLC/TOF MS with a hydrophilic interaction liquid chromatography (HILIC) gradient in the positive mode for dimethylaminoethanol and the negative mode for tartaric acid and subsequently using gas chromatography (GC) with MS detection. The UHPLC/TOF MS system included a 1290 Infinity chromatograph with TOF MS detection (Agilent, Santa Clara, CA) and a Waters Acquity® UPLC BEH Amide (2.1 mm × 50 mm, 1.7 µm particle size) column (Waters Corporation, Milford, MA). The mobile phases consisted of A) 10 mM ammonium acetate (pH 6.8) and B) acetonitrile, programmed with a HILIC gradient of 5% A for 2 minutes, then to 95% A in 5 minutes, held for 0.5 minutes, reversed to 5% A in 2 minutes, and then held at 5% A for 2.5 minutes; the flow rate was 0.5 mL/minute. Assays for volatile organic impurities in the bulk chemical were conducted by the analytical chemistry laboratory using two GC systems with flame ionization detection (FID).

The Karl Fischer desorption analysis indicated approximately 12% water and TGA results indicated an estimated maximum water value not greater than 3.5%, based on the mass lost in the temperature range matching the boiling point of water. Elemental analyses for carbon, hydrogen, nitrogen, and oxygen were consistent with the structural composition of dimethylaminoethanol bitartrate and theoretical values. UHPLC/TOF MS indicated one major peak accounting for greater than 99.9% of the total peak area relative to either the dimethylaminoethanol or tartaric acid peak. GC/MS using system A (Table C1) indicated one major peak accounting for 100% of the total peak area; the spectrum was consistent with a library reference spectrum (NIST, 2005). A residual solvent screening assay using GC/FID by system B tentatively identified the presence of ethanol, chloroform, pyridine, and *N,N*-dimethylformamide. A second quantitative analysis using GC/FID by system C confirmed only the presence of one residual solvent, ethanol at 0.325%; the other analytes were not quantified above their corresponding limits of quantitation (~0.05 to 0.1%). The overall purity of lot 159AK was determined to be 96% or greater.

Stability studies of lot 159AK were conducted using the HILIC gradient UHPLC/TOF-MS system described above for the bulk chemical purity assessment. Results indicated that both dimethylaminoethanol and the counterion tartaric acid were stable in the bulk chemical for at least 14 days when stored in amber glass vials sealed with Teflon®-lined caps at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature in sealed glass bottles. Reanalyses of the bulk chemical were performed prior to the dose range-finding

study and after the prenatal developmental toxicity study using FTIR spectroscopy (dose range-finding study only) and GC/FID by system D, and no degradation of the bulk chemical was detected.

Sterile Water for Irrigation, USP

The sterile water vehicle was obtained from Baxter Healthcare Corporation (Cleveland, MS) in two lots (G105999 and G110783); lot G10599 was used in the dose range-finding study and lot G110783 was used in the prenatal developmental toxicity study.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once for each study by mixing dimethylaminoethanol bitartrate with sterile water to give the required concentrations (Table C2). The dose formulations were stored at room temperature in sealed amber glass bottles for up to 42 days.

The analytical chemistry laboratory performed syringeability studies of a 300 mg/mL formulation using a 22-gauge gavage needle and syringe and stability studies of a 10 mg/mL formulation using GC/FID by system D (Table C1). Syringeability was confirmed and stability was confirmed for at least 42 days for dimethylaminoethanol for dose formulations stored in sealed clear glass bottles both at refrigerated and room temperatures and for 3 hours under simulated animal room conditions.

Additional stability studies of formulations of dimethylaminoethanol bitartrate in sterile water were performed to monitor the relative stability of the tartaric acid counterion and the pH of the formulations. Two formulations (25.0 and 22.5 mg/mL) of dimethylaminoethanol bitartrate in sterile water were prepared for direct comparison to standards of tartaric acid at concentrations equivalent to those present in the formulations. After diluting into the range of a validated analytical method, a 300 mg/mL formulation was also analyzed using HPLC. The HPLC system included a Waters Alliance 2695 instrument (Waters Corporation) with a SIELC Primesep™ D 150 mm × 4.6 mm, 5 µm particle size column (SIELC Technologies, Wheeling, IL), mobile phases A) deionized water (adjusted to pH 2.2 with H_3PO_4), B) acetonitrile, and C) 100 mM NaH_2PO_4 in deionized water (adjusted to pH 2.7 with H_3PO_4), with an isocratic gradient of 30% A: 10% B: and 60% C, ultraviolet detection at 210 nm, and a flow rate of 1.0 mL/minute. Formulations were determined to have a stable tartaric acid concentration for at least 42 days. The pH of the formulations across the concentration range 2.5 to 300 mg/mL was between 3.4 to 3.6; based on the 10 mg/mL formulation, the pH of the formulation did not change over the period of 42 days when stored at either refrigerated or room temperatures.

Periodic analyses of the dose formulations of dimethylaminoethanol bitartrate were conducted by the analytical chemistry laboratory using GC/FID by system D. During the dose range-finding study, the dose formulations were analyzed once; all three of the dose formulations were within 10% of the target concentrations (Table C3). Animal room samples of these dose formulations were also analyzed and all three were within 10% of the target concentrations. During the prenatal developmental toxicity study, the dose formulations were analyzed once; all three dose formulations analyzed were used and all three animal room samples were within 10% of the target concentrations (Table C4).

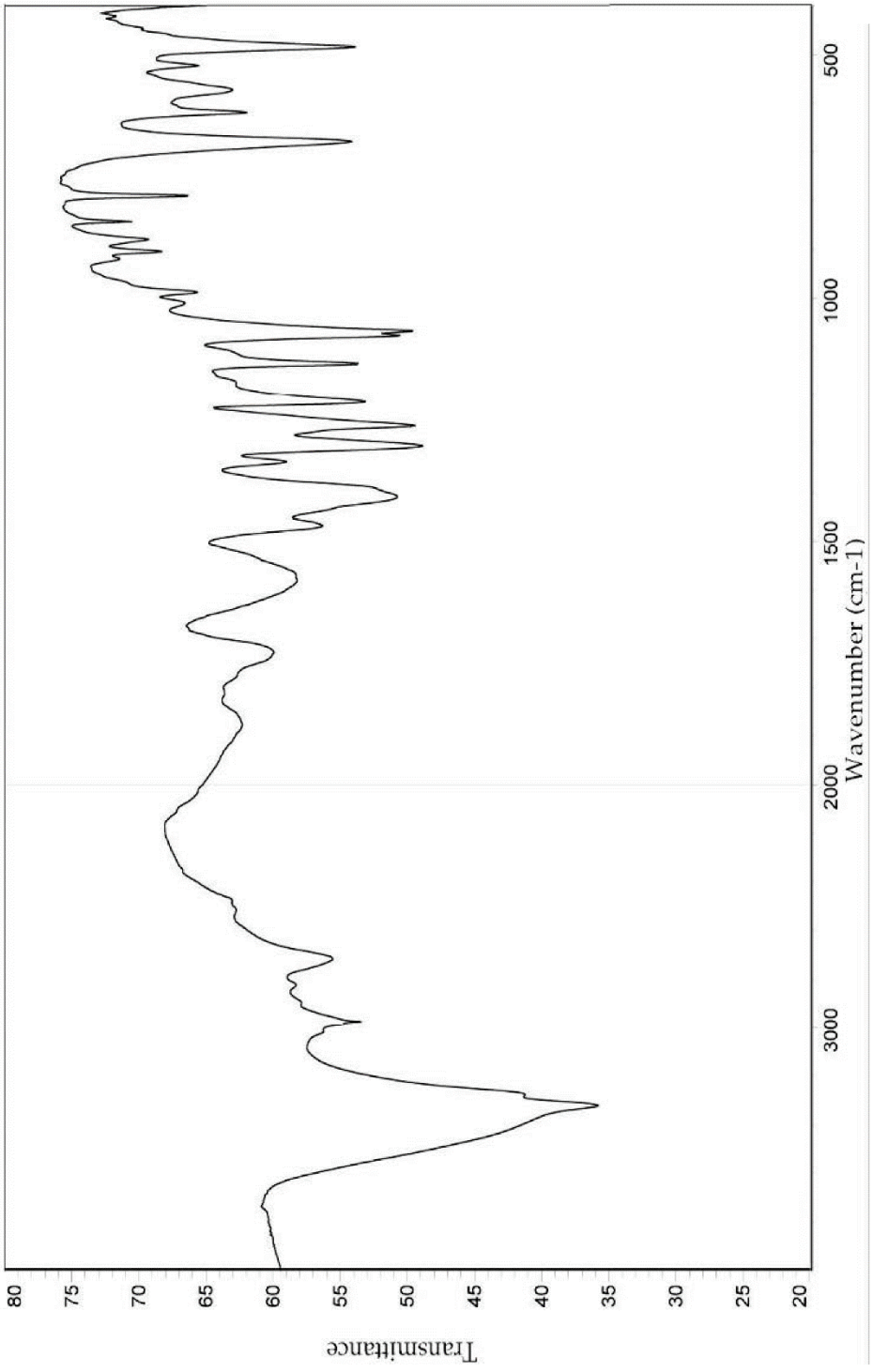


FIGURE C1
Fourier Transform Infrared Absorption Spectrum of Dimethylaminoethanol Bitartrate

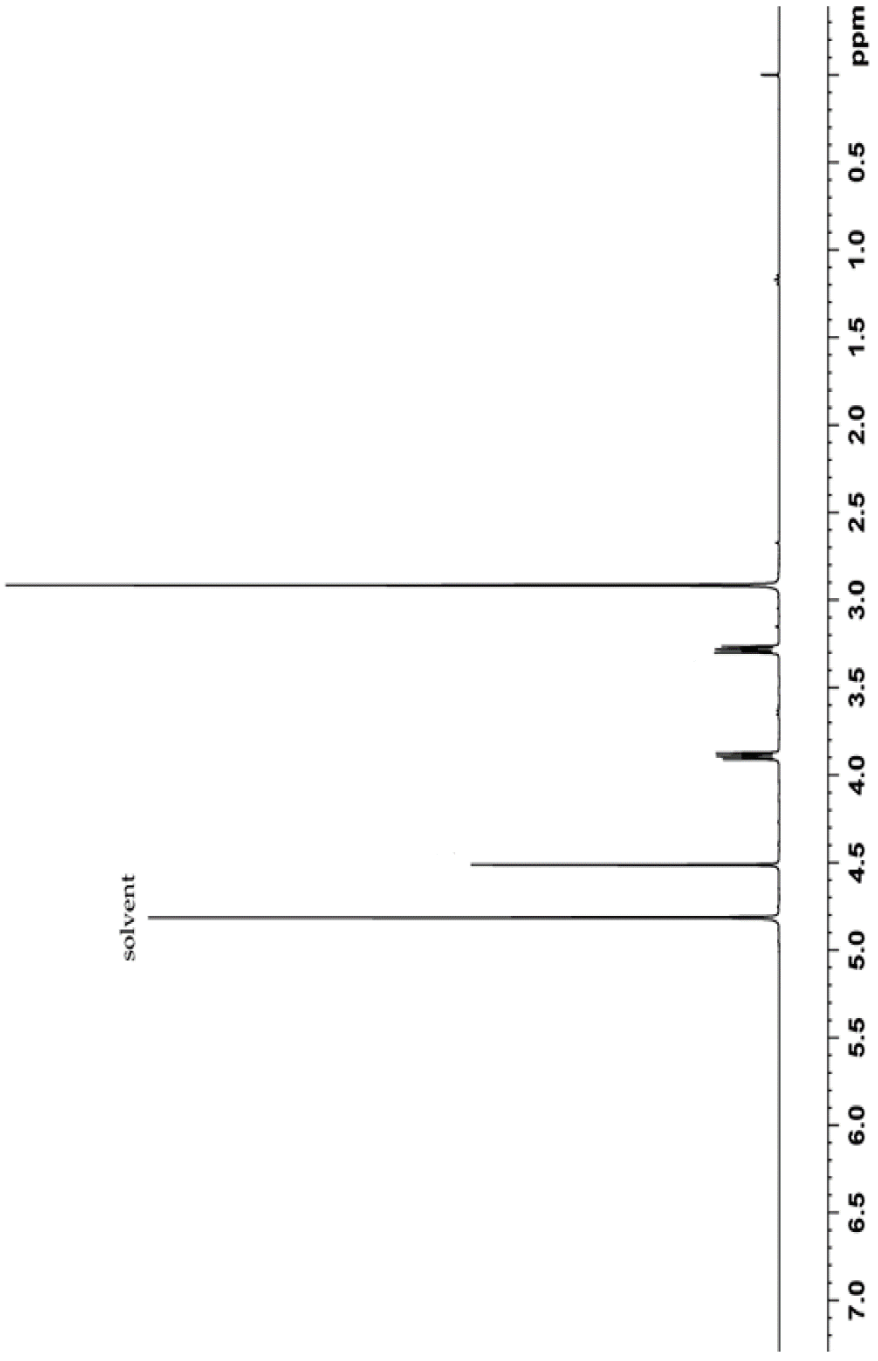


FIGURE C2
Proton Nuclear Magnetic Resonance Spectrum of Dimethylaminoethanol Bitartrate

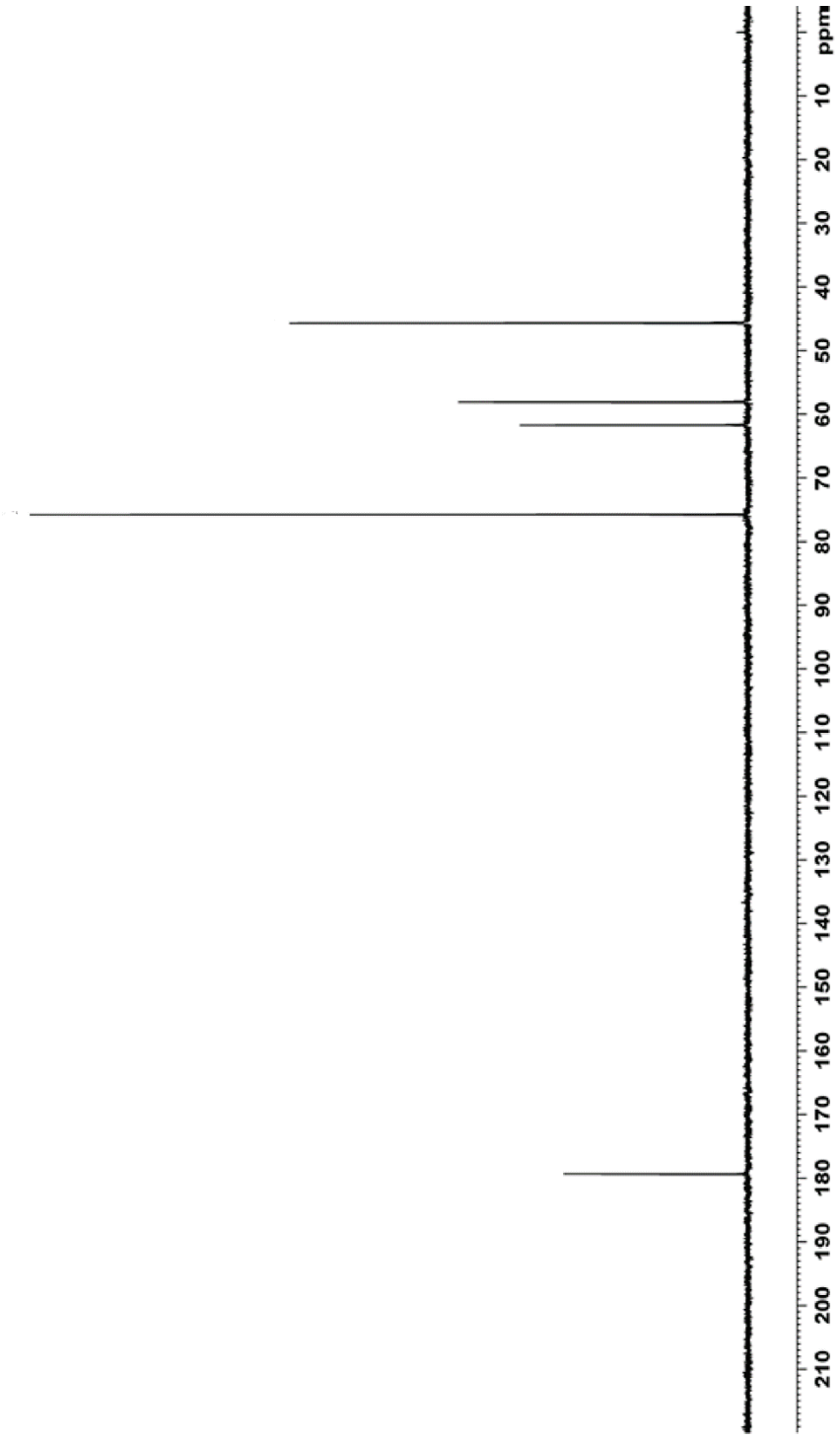


FIGURE C3
Carbon-13 Nuclear Magnetic Resonance Spectrum of Dimethylaminoethanol Bitartrate

TABLE C1
Gas Chromatography Systems Used in the Gavage Studies of Dimethylaminoethanol Bitartrate[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A Mass spectrometry with electron impact ionization (35 to 100 amu)	DB™-WAX, 30 m × 0.32 mm, 0.5 µm film (J&W Scientific, Folsom, CA)	Helium at ~ 1.5 mL/minute	60° C for 4 minutes, then 20° C/minute to 240 ° C, held for 2 minutes
System B Flame ionization	DB™-624, 30 m × 0.53 mm, 3.0 µm film (J&W Scientific)	Helium at ~ 4.5 mL/minute	40° C for 20 minutes, then 20° C/minute to 240° C, held for 20 minutes
System C Flame ionization	Rtx®-200, 30 m × 0.53 mm, 3.0 µm film thickness) (Restek, Bellefonte, PA)	Helium at ~ 4.5 mL/minute	40° C for 20 minutes, then 20° C/minute to 240° C, held for 20 minutes
System D Flame ionization	DB™-WAX, 30 m × 0.53 mm, 1.0 µm film (J&W Scientific)	Helium at ~ 5 mL/minute	80° C for 1 minute, then 10° C/minute to 150° C, held for 7 or 12 minutes

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

TABLE C2
Preparation and Storage of Dose Formulations in the Gavage Studies of Dimethylaminoethanol Bitartrate

Preparation
The dose formulations were prepared by weighing the appropriate amount of dimethylaminoethanol bitartrate into a small glass beaker, transferring the test article into a calibrated glass bottle with sterile water rinses, diluting to final volume with sterile water, and stirring for approximately 15 minutes. The dose formulations were prepared once for each study.

Chemical Lot Number
159AK

Maximum Storage Time
42 days

Storage Conditions
Stored in sealed amber glass bottles at room temperature

Study Laboratory
Southern Research (Birmingham, AL)

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

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
January 27, 2014	January 28-29, 2014	50	50.9	+2
		100	103	+3
		200	203	+2
	February 29, 2014[b]	50	50.0	0
		100	99.1	–1
		200	207	+4

[a] Results of triplicate analyses. Dosing volume=5mL/kg; 50 mg/mL=250 mg/kg, 100 mg/mL=500 mg/kg, 200 mg/mL=1,000 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 Dimethylaminoethanol Bitartrate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
March 31, 2014	April 2-4, 2014	50	53.6	+7
		100	106	+6
April 4, 2014		200	220	+10
	May 9, 2014[b]	50	52.3	+5
		100	104	+4
		200	219	+10

[a] Results of triplicate analyses. Dosing volume=5mL/kg; 50 mg/mL=250 mg/kg, 100 mg/mL=500 mg/kg, 200 mg/mL=1,000 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
Wheat middlings	10.00
Fish meal (60% protein)	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Vitamin premix[a]	0.25
Mineral premix[b]	0.15
Choline chloride (70% choline)	0.10

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

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_3	4,600,000 IU	D-activated animal sterol
K	2.8 g	Dimethylpyrimidinol bisulfite
d-α-Tocopheryl acetate	20.0 g	
Niacin	30.0 g	
Folic acid	2.2 g	
d-Pantothenic acid	18.0 g	*d*-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	45,400.0 µg	
Pyridoxine	5.9 g	Pyridoxine hydrochloride
Biotin	140.0 mg	*d*-Biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product

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

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	23.25 ± 0.636	22.8 – 23.7	2
Crude fat (% by weight)	5.75 ± 0.071	5.7 – 5.8	2
Crude fiber (% by weight)	3.325 ± 0.092	3.26 – 3.39	2
Ash (% by weight)	6.36 ± 0.240	6.19 – 6.53	2
Amino Acids (% of total diet)			
Arginine	1.375 ± 0.065	1.30 – 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	1.11 – 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)	5,950 ± 66.5	5,480 – 6,420	2
α-Tocopherol (ppm)	48.07 ± 4.38	40.3 – 52.73	8
Thiamine (ppm)[b]	11.3 ± 1.13	10.5 – 12.1	2
Riboflavin (ppm)	14.3 ± 3.58	10.0 – 19.8	8
Niacin (ppm)	99.4 ± 9.10	87.0 – 112	8
Pantothenic acid (ppm)	45.6 ± 3.13	40.4 – 51.1	8
Pyridoxine (ppm)[b]	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 (ppm)[b]	1,776 ± 197	1,570 – 2,200	8
Minerals			
Calcium (%)	1.190 ± 0.028	1.17 – 1.21	2
Phosphorus (%)	0.978 ± 0.046	0.945 – 1.01	2
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) or chloride (choline)

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

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.368 ± 0.038	0.341 – 0.395	2
Cadmium (ppm)	0.097 ± 0.010	0.09 – 0.104	2
Lead (ppm)	0.074 ± 0.003	0.072 – 0.076	2
Mercury (ppm)	<0.02		2
Selenium (ppm)	0.519 ± 0.092	0.454 – 0.584	2
Aflatoxins (ppb)	<5.00		2
Nitrate nitrogen (ppm)[c]	25.05 ± 3.041	22.9 – 27.2	2
Nitrite nitrogen (ppm)[c]	<0.61		2
BHA (ppm)[d]	<1.0		2
BHT (ppm)[d]	<1.0		2
Aerobic plate count (CFU/g)	10		2
Coliform (MPN/g)	3.0		2
Escherichia coli (MPN/g)	<3		2
Salmonella (MPN/g)	Negative		2
Total nitrosoamines (ppb)	5.1 ± 7.1	0 – 10.1	2
N-Nitrosodimethylamine (ppb)	1.2 ± 1.6	0 – 2.3	2
N-Nitrosopyrrolidine (ppb)	3.9 ± 5.5	0 – 7.8	2
Pesticides (ppm)			
α-BHC	<0.01		2
β-BHC	<0.02		2
γ-BHC	<0.01		2
δ-BHC	<0.01		2
Heptachlor	<0.01		2
Aldrin	<0.01		2
Heptachlor epoxide	<0.01		2
DDE	<0.01		2
DDD	<0.01		2
DDT	<0.01		2
HCB	<0.01		2
Mirex	<0.01		2
Methoxychlor	<0.05		2
Dieldrin	<0.01		2
Endrin	<0.01		2
Telodrin	<0.01		2
Chlordane	<0.05		2
Toxaphene	<0.10		2
Estimated PCBs	<0.20		2
Ronnel	<0.01		2
Ethion	<0.02		2
Trithion	<0.05		2
Diazinon	<0.10		2
Methyl chlorpyrifos	0.051 ± 0.02	0.037 – 0.065	2
Methyl parathion	<0.02		2
Ethyl parathion	<0.02		2
Malathion	0.022 ± 0.003	0.02 – 0.024	2
Endosulfan I	<0.01		2
Endosulfan II	<0.01		2
Endosulfan sulfate	<0.03		2

[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