NTP TECHNICAL REPORT

ON THE

PRENATAL DEVELOPMENTAL TOXICITY STUDIES

OF TRIS(CHLOROPROPYL) PHOSPHATE

(CAS No. 13674-84-5)

IN SPRAGUE DAWLEY (Hsd:Sprague Dawley SD) RATS

(GAVAGE STUDIES)

Scheduled Peer Review Date: 2019

NOTICE

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

NTP DART-01

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.

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ABSTRACT

TRIS(CHLOROPROPYL) PHOSPHATE*

Chemical Formula: $C_9H_{18}Cl_3O_4P$ Molecular Weight: 327.57

* Test article name represents the mixture.
Trade Names: Amgard TMCP, Antiblaze 80, Antiblaze TMCP, Fyrol PCF

Tris(chloropropyl) phosphate (TCPP) is used as a flame retardant within textiles, furniture (flexible polyurethane foam), and other related products. In addition, it is manufactured for use in construction materials (rigid polyurethane foam), electronic products, paints, coatings, and adhesives. Several flame retardants have been removed from products in commerce due to toxicity concerns and TCPP has been considered as a replacement flame retardant for use in these products. Due to concerns for increased use, and thus increased human exposure, the Consumer Product Safety Commission nominated TCPP for toxicological testing by the National Toxicology Program (NTP). Additional information on the NTP's evaluation of the potential toxicity of TCPP is available at the Program's website (NTP, 2016a); however, the purpose of this report is to summarize and discuss TCPP effects on prenatal development. In these studies, time-mated female Sprague Dawley (Hsd:Sprague Dawley SD) rats received TCPP (95.7% to 97% pure) in 0.5% methylcellulose by gavage from implantation on gestation day (GD) 6 to the day before expected parturition (GD 20). Evidence of TCPP-related maternal and fetal toxicity was examined in the dose range-finding study followed by the standard prenatal developmental toxicity study.

DOSE RANGE-FINDING PRENATAL DEVELOPMENTAL TOXICITY STUDY

Groups of 11 time-mated female rats were administered 0, 300, 650, or 1,000 mg TCPP/kg body weight per day in 0.5% aqueous methylcellulose by gavage from GD 6 to GD 20. Vehicle control (0 mg/kg) animals received aqueous methylcellulose.

Maternal toxicity was observed in the 1,000 mg/kg group as evidenced by seven of eleven dams being either found dead or euthanized moribund. Associated clinical observations in the 1,000 mg/kg group included convulsion,

tremors, prone, gasping, hypoactivity, hunched posture, nasal discharge, stained fur, piloerection, salivation, and rooting (pre- and post-dosing) which occurred throughout gestation. One female in the 650 mg/kg group was euthanized moribund on GD 16 with associated clinical observations including cold to touch, hypoactivity, paleness, ataxia, and labored breathing which may have been related to TCPP exposure. All vehicle control and 300 mg/kg animals survived to study termination. There were no TCPP-related effects on maternal body weights, body weight gain, or feed consumption from GD 6 to GD 20. Additionally, there were no significant exposure-related effects on post-implantation loss, fetal body weights, or fetal sex ratio, although there were limited litters available for assessment in the 1,000 mg/kg TCPP group due to maternal toxicity. Finally, there were no significant exposure-related external fetal findings (including examination of the palate).

PRENATAL DEVELOPMENTAL TOXICITY STUDY

Due to the maternal toxicity observed at 1,000 mg/kg in the dose ranging-finding study, groups of 25 time-mated female rats were administered 0 (n=50), 162.5, 325, or 650 mg TCPP/kg body weight per day in 0.5% aqueous methylcellulose by gavage from GD 6 to GD 20. Vehicle control (0 m/kg) animals received aqueous methylcellulose. Additional animals were added to the vehicle control group to obtain historical control data for both maternal and fetal findings in this strain of rat. In this study, TCPP was well tolerated and there were no exposure-related effects on mortality, maternal body weights, body weight gains, or feed consumption during gestation. Low incidences of clinical observations including nasal discharge, salivation, twitches, ataxia, piloerection, audible respiratory sounds, and hyperactivity were observed in the 650 mg/kg group. Adverse clinical observations were not observed in other groups exposed to TCPP. There were no notable placental or other maternal gross observations at necropsy except for dose-related increases in absolute (9%, 16%, and 26% at 162.5, 325, and 650 mg/kg, respectively) and relative liver weights.

There were no significant effects of TCPP on post-implantation loss, mean fetal body weights, or fetal sex ratio. Likewise, no biologically relevant exposure-related malformations were found in external, visceral, and skeletal fetal exams of groups exposed to TCPP.

CONCLUSIONS

Under the conditions of this prenatal study, there was *no evidence of developmental toxicity* of TCPP in Hsd:Sprague Dawley SD rats administered 162.5, 325, or 650 mg/kg in the absence of overt maternal toxicity.

Summary of Exposure-Related Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg[a]	162.5 mg/kg	325 mg/kg	650 mg/kg
Maternal Parameters				
Animals on study	50	25	25	25
Number pregnant	44	21	21	20
Number died or euthanized moribund	0	0	0	0
Clinical Observations	None	None	None	Ataxia, audible respiratory sounds, hyperactivity, nasal discharge, piloerection, salivation, and twitches
Body Weight and Feed Consumption[b]				
Necropsy body weight	382.3 ± 3.1	386.3 ± 4.2	384.3 ± 5.1	379.4 ± 8.2
Body weight change GD 6 to 21	139.6 ± 2.5	143.6 ± 3.0	139.9 ± 3.8	137.6 ± 6.3
Feed consumption GD 6 to 21	22.8 ± 0.23	22.5 ± 0.33	22.9 ± 0.33	22.2 ± 0.42
Necropsy Observations				
Organ weights	None	9% ↑ in absolute liver weight	16% ↑ in absolute liver weight	26% ↑ in absolute liver weight
Developmental/Fetal Parameters				
Number of litters examined	44	21	21	20
Number of live fetuses evaluated	599	300	270	259
Number of live fetuses per litter[c]	13.61 ± 0.30	14.29 ± 0.37	12.86 ± 0.62	12.95 ± 0.91
Number of early resorptions	22	11	11	15
Number of late resorptions	2	0	0	0
Number of dead fetuses	1	0	2	0
Number of whole litter resorptions	0	0	0	0
Percent post-implantation loss[c]	3.81 ± 1.13	3.42 ± 0.99	4.33 ± 1.19	7.17 ± 4.50
Fetal body weight per litter[b]	5.29 ± 0.04	5.22 ± 0.06	5.42 ± 0.08	5.22 ± 0.07
Male fetal weight per litter	5.42 ± 0.05	5.35 ± 0.06	5.47 ± 0.06	5.34 ± 0.06
Female fetal weight per litter	5.17 ± 0.04	5.08 ± 0.06	5.30 ± 0.09	5.09 ± 0.07
Gravid uterine weight[b]	98.89 ± 1.93	101.98 ± 2.23	95.76 ± 4.06	91.78 ± 5.91
External Findings	None	None	None	None
Visceral Findings	None	None	None	None
Skeletal Findings	None	None	None	None

Level of evidence of developmental toxicity: No evidence

[a] This study had two vehicle control groups. Data from both vehicle control groups were combined and are presented here.
[b] Results given in grams. Data are displayed as mean ± standard error.
[c] Data are displayed as mean ± standard error.
GD = Gestation day

EXPLANATION OF LEVELS OF EVIDENCE FOR DEVELOPMENTAL TOXICITY

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

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

Five categories of evidence of developmental toxicity are used to summarize the strength of the evidence observed in each experiment: two categories for positive results **(clear evidence** and **some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major design or performance flaws **(inadequate study)**. Application of these criteria requires professional judgment by individuals with ample experience and an understanding of the animal models and study designs employed. For each study, conclusion statements are made using one of the following five categories to describe the findings. These categories refer to the strength of the evidence of the experimental results and not to potency or mechanism.

Levels of Evidence for Evaluating Developmental System Toxicity

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM TECHNICAL REPORTS
PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft NTP Prenatal Developmental Toxicity Study Report on tris(chloropropyl) phosphate 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

TRIS(CHLOROPROPYL) PHOSPHATE*

Chemical Formula: $C_9H_{18}Cl_3O_4P$ Molecular Weight: 327.57

* Test article name represents the mixture.
Trade Names: Amgard TMCP, Antiblaze 80, Antiblaze TMCP, Fyrol PCF

Tris(chloropropyl) phosphate (TCPP) is a flame retardant commonly used in consumer products and was nominated to the National Toxicology Program (NTP) by the Consumer Product Safety Commission for toxicological testing. The NTP is evaluating TCPP toxicity on various cellular or molecular targets *in vitro* (e.g., high throughput screening) and *in vivo* following subchronic and chronic exposure to rats and mice. Genotoxicity and immunotoxicity assessments are also under evaluation following TCPP exposure. Further information on the NTP's evaluation of the potential toxicity of TCPP is available at the Program's website (NTP, 2016a); however, the purpose of this report is to summarize and discuss TCPP effects on prenatal development in rats.

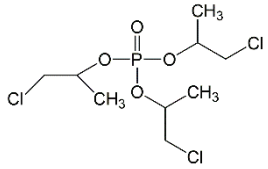
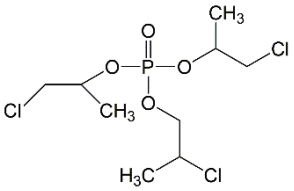
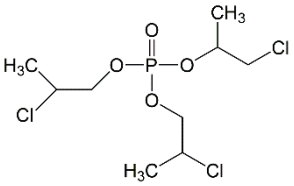
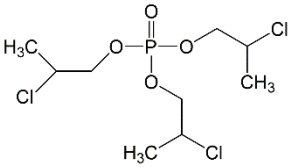
CHEMICAL AND PHYSICAL PROPERTIES

TCPP is a clear colorless liquid mixture (NRC, 2000; EU, 2008; ATSDR, 2012; USEPA, 2015a,b). TCPP has a molar mass of 327.59 g/mol and a relative density of 1.29 g/cm^3 at 25° C. It has an estimated boiling point of greater than 200° C, vapor pressure of less than 2 mm Hg at 25° C, water solubility of 1.6 g/L at 20° C, and log K_{OW} of 2.59.

PRODUCTION, USE, AND HUMAN EXPOSURE

TCPP is produced as an isomeric mixture in a closed system by the reaction of phosphorus oxychloride and propylene oxide to generate a mixture of four isomers (NRC, 2000). The most abundant isomer in commercial products is tris(1-chloro-2-propyl) phosphate (50% to 85%) (Table 1). Additional isomers include bis(2-chloro-1-methylethyl)-2-chloropropyl phosphate (15% to 40%), bis(2-chloropropyl) 2-chloroisopropyl phosphate (<15%),

TABLE 1
Tris(chloropropyl) Phosphate Isomers in Commercial Products

Isomer	CAS Number	Chemical Structure	Percent (w/w) in Commercial Products
Tris(1-chloro-2-propyl) phosphate 2-Propanol, 1-chloro-, 2,2′,2″-phosphate Tris(2-chloro-1-methylethyl) phosphate Tris(2-chloro isopropyl)phosphate	13674-84-5		50% to 85%
Bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate Bis(1-chloro-2-propyl) 2-chloro-1-propyl phosphate Bis(2-chloro isopropyl) 2-chloropropyl phosphate	76025-08-6		15% to 40%
Bis(2-chloropropyl) 2-chloroisopropyl phosphate 2-Chloro-1-methylethyl bis(2-chloropropyl) phosphate Bis(2-chloropropyl) 2-chloro-1-methylethyl phosphate Bis(2-chloro-1-propyl) 1-chloro-2-propyl phosphate	76649-15-5		<15%
Tris(2-chloropropyl) phosphate 1-Propanol, 2-chloro-, phosphate (3:1) Tris(2-chloro-1-propyl) phosphate	6145-73-9		<1%

TCPP isomers [in bold, noted by the United Sates Environmental Protection Agency (USEPA) Registry Name] and common synonyms listed in the USEPA Substance Registry Services database (USEPA, 2016a)

and tris(2-chloropropyl) phosphate (<1%). Variations in manufacturing methods result in commercial formulations that contain different ratios of the four isomers. The TCPP mixture and commercial products are commonly referenced by the major isomer [tris(1-chloro-2-propyl) phosphate] and CAS No. 13674-84-5 (USEPA, 2015a).

The United States production volume of TCPP was approximately 54 million pounds in 2012 (USEPA, 2012). TCPP is used as a flame retardant within textiles, furniture (flexible polyurethane foam), and other related products. In addition, it is manufactured for use in construction materials (rigid polyurethane foam), electronic products,

paints, coatings, and adhesives (USEPA, 2015b). TCPP has been proposed as a substitute for brominated flame retardants as well as a replacement for other chlorinated flame retardants such as tris(2-chloroethyl) phosphate (Wilczynski *et al.*, 1983; WHO, 1998).

Fate and transport of TCPP were recently summarized by the United States Environmental Protection Agency's Design for the Environment Branch (USEPA, 2015a). Available data suggest that TCPP is routinely found in drinking, ground, and surface waters. TCPP is expected to have high mobility in soil based on carbon-water partition values. It is also anticipated to be persistent in the environment based on 28-day biodegradation studies, which suggest the half-life is greater than 60 days (OECD, 2000). TCPP has been detected in sediment, surface water, household dust, indoor air, and ambient air (USEPA, 2015a).

Human exposure to TCPP may occur through inhalation, oral, or dermal contact. The USEPA Office of Pollution Prevention and Toxics suggests that potential occupational exposure to TCPP is likely to occur through inhalation of vapors and dermal exposures during the manufacturing of consumer products containing TCPP (USEPA, 2015b) or when working with consumer products containing TCPP. Since TCPP is considered ubiquitous in the environment, consumers could be exposed by inhalation of vapor, direct skin contact, and incidental ingestion. Exposures may occur in offices, homes, and other indoor environments as a result of use of consumer products such as upholstered furniture containing TCPP. Children may be more susceptible to ingestion due to increased object-to-mouth behaviors (WHO, 1998; USEPA, 2016b).

REGULATORY STATUS

TCPP is listed on the Toxic Substances Control Act (TSCA) Inventory. TCPP is one of several flame retardants included on the TSCA Work Plan, and the USEPA released a problem formulation and initial assessment document for the chlorinated phosphate ester cluster that includes TCPP in August 2015 (USEPA, 2015b). The conclusion of this problem formulation is that the USEPA will assess risks to consumers, the general population, and aquatic organisms following exposure to TCPP and similar chemicals. There are currently no regulations restricting production or use of TCPP in the United States.

The European Union Risk Assessment Report for TCPP indicates no unacceptable risks for workers, consumers, or the general population, with the exception of dermal exposure to workers manufacturing TCPP in relation to effects on fertility and developmental toxicity (EU, 2008). TCPP is currently not registered under the Registration, Evaluation, Authorization, and Restriction of Chemicals (REACH) Regulation; therefore, the European Chemicals Agency (ECHA) has no usable data available about this substance from registered dossiers (ECHA, 2016).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

TCPP is readily absorbed and excreted in rats following gavage administration of 50 µmol [^{14}C]TCPP/kg body weight (Minegishi *et al.*, 1988). In this study, approximately 98% of the administered dose was recovered during 168 hours after dosing. Of the administered dose, 67%, 22%, and 7.7% were recovered in urine, feces, and expired air, respectively, within 48 hours. TCPP was rapidly distributed to tissues with tissue to blood ratios highest in liver and kidney followed by lung, spleen, and adipose during the first 12 hours after administration. Elimination half-life in blood was estimated to be approximately 59 hours. Biliary excretion studies showed that approximately 45% of the administered dose was excreted in bile within 48 hours and that TCPP excreted in feces is likely from biliary excretion.

Additional absorption, disposition, metabolism, excretion, and toxicokinetic data are summarized in the European Union Risk Assessment Report (EU, 2008) and the USEPA Design for the Environment Report on Flame Retardants Used in Flexible Polyurethane Foam: An Alternatives Assessment Update (USEPA, 2015a). These reports indicate that TCPP is readily absorbed and excreted based on animal studies.

Humans

There are no studies on the absorption, distribution, metabolism, or excretion of TCPP in humans in the literature. TCPP metabolism was investigated *in vitro* with human liver microsomes by Van den Eede *et al.* (2013). Incubation of microsomes with TCPP resulted in several Phase I metabolites including bis(1-chloro-2-propyl) phosphate (BCPP), a major metabolite; bis(1-chloro-2-propyl) 1-hydroxy-2-propyl phosphate;

bis(1-chloro-2-propyl) 1-carboxy-2-propyl phosphate; and 1-chloro-2-propyl,1-hydroxy-2-propyl phosphate. No phase II metabolites were detected.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY

Experimental Animals

A prenatal developmental toxicity study conducted by Kawasaki and colleagues (1982) is reported in the literature. Wistar rats were fed a diet containing 0%, 0.01%, 0.1%, or 1% TCPP during gestational days (GD) 0 through 20 (n=11 to 14). Daily TCPP intake for the exposed groups was estimated to be 6, 70, or 625 mg/kg body weight per day, respectively. Following exposure, there were no significant effects on dam survival, feed consumption, or body weight gain during gestation. There were also no effects on the number of implants, resorptions, or live or dead fetuses. An exam of fetal morphology demonstrated no significant external or visceral test article-related effects. While there were no statistically significant increases in the incidences of skeletal abnormalities, dose-related increases in the incidences of cervical ribs and absent 13th ribs were reported, which are suggestive of developmental toxicity.

Summaries of the results of a two-generation reproduction study in Wistar rats exposed to TCPP are presented in various hazard and risk assessment reports (EU, 2008; USEPA, 2015a). It was reported that rats (28 per sex per group) received 0, 100, 333, or 1,000 mg TCPP/kg body weight per day in the diet over two generations. Animals were fed TCPP 10 weeks prior to mating, during mating, and throughout gestation and lactation until study end. No treatment-related clinical observations or mortality in either parental generation were reported. TCPP exposure did not affect pre-coital time, mating index, fecundity index, fertility index, duration of gestation, or post-implantation loss. The mean number of pups delivered was lower in the F_0 and F_1 generations in the mid and high dose groups compared to the control.

Humans

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

GENERAL TOXICITY

Experimental Animals

The toxicity database, which includes published and unpublished data for TCPP, has been summarized in the National Industrial Chemicals Notification and Assessment Scheme (NICNAS, 2016), USEPA's Design for the Environment Branch (USEPA, 2015a), Environmental Health Criteria for Flame Retardants by the World Health Organization (WHO, 1998), SIDS Initial Assessment Profile (OECD, 2000), EU Risk Assessment Report (EU, 2008), and the Agency for Toxic Substances and Disease Registry Toxicological Profile for Phosphate Ester Flame Retardants (ATSDR, 2012).

Reported acute oral LD_{50} values for TCPP range from 1,000 to 4,000 mg/kg body weight in male rats and 2,000 mg/kg in female rats (NRC, 2000; OECD, 2000; EU, 2008). Common clinical observations observed in acute studies included ataxia, hunched posture, lethargy, labored respiration, increased salivation, body tremors, and piloerection. Macroscopic signs of toxicity included hemorrhagic lungs and dark liver and kidneys. The acute dermal LD_{50} in rats and rabbits is reported to be greater than 5,000 mg/kg and the inhalation LC_{50} in rats is greater than 4.6 mg/L (NRC, 2000; OECD, 2000; EU, 2008). The USEPA Design for the Environment Branch assigned a low hazard to TCPP for acute toxicity (USEPA, 2015a).

In a 13-week toxicity study, Fyrol PCF® (i.e., TCPP) administered in feed to Sprague-Dawley rats (20 per sex per concentration) at exposure concentrations from 800 to 20,000 ppm had no effect on mortality, clinical observations, changes in hematology, clinical chemistry, or urinalysis parameters in the study animals (Freudenthal and Henrich, 1999). Body weights were decreased (<12% compared to controls) at the highest exposure concentration in males and females. Significantly increased liver weights were noted in all treated males and in the two highest exposure groups of females (7,500 and 20,000 ppm). Kidney weights were increased in males in the 7,500 and 20,000 ppm groups. Histopathologic evaluation revealed minor changes in the liver, kidney and thyroid gland, which were most prevalent in the two highest exposure concentration groups. Based on these data, the USEPA Design for the Environment Branch assigned a moderate hazard to TCPP for repeat dose toxicity (USEPA, 2015a).

Subsequently, the NTP conducted 13-week studies in rats and mice; the data are available in the NTP CEBS database (NTP, 2016b). TCPP was administered in feed at dietary concentrations of 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm in rats and 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm in mice (10 per sex per group). Rat exposures included a perinatal exposure from GD 6 to postnatal day 21 prior to the 13-week exposure. Briefly, all rat dams exposed to 40,000 ppm (gestation) and all male pups (first week post-weaning) in the 20,000 ppm group were removed due to overt toxicity. Body weights in all exposed rats at weaning were 13% to 30% lower than controls. In mice, no treatment-related mortality or clinical observations of toxicity were observed. Terminal body weights were 12% (rats) and 29% (mice) lower than controls in the 20,000 ppm groups. There were treatment-related increases in relative liver (rats, mice) and thymus (rats) weights. In rats, there were treatment-related increases in the incidences of biliary hyperplasia and increased cellularity of the thymic cortex. In mice, the incidences of hepatocellular hypertrophy and male renal tubule epithelium cytoplasmic alterations were observed to be treatment related. In summary, subchronic exposure to TCPP in feed resulted in reduced dam and pup survival (rats); and the liver, thymus, and kidney were considered primary targets of toxicity.

Humans

No direct studies of tris(chloropropyl) phosphate exposure on human health have been conducted.

STUDY RATIONALE

TCPP was nominated by the Consumer Product Safety Commission in 2005 due to the expected increased use of TCPP as a flame retardant for flexible polyurethane foam used in home furnishings and construction materials (NTP, 2016c). TCPP exposure to consumers via oral, dermal, and inhalation routes was also expected to increase and the publicly available toxicity data at the time was considered limited. One report in the literature suggested that TCPP exposure may impact development (Kawasaki *et al.*, 1982); therefore, the NTP determined that further studies were warranted to characterize the effects of oral TCPP administration in pregnant rats and on fetal development.

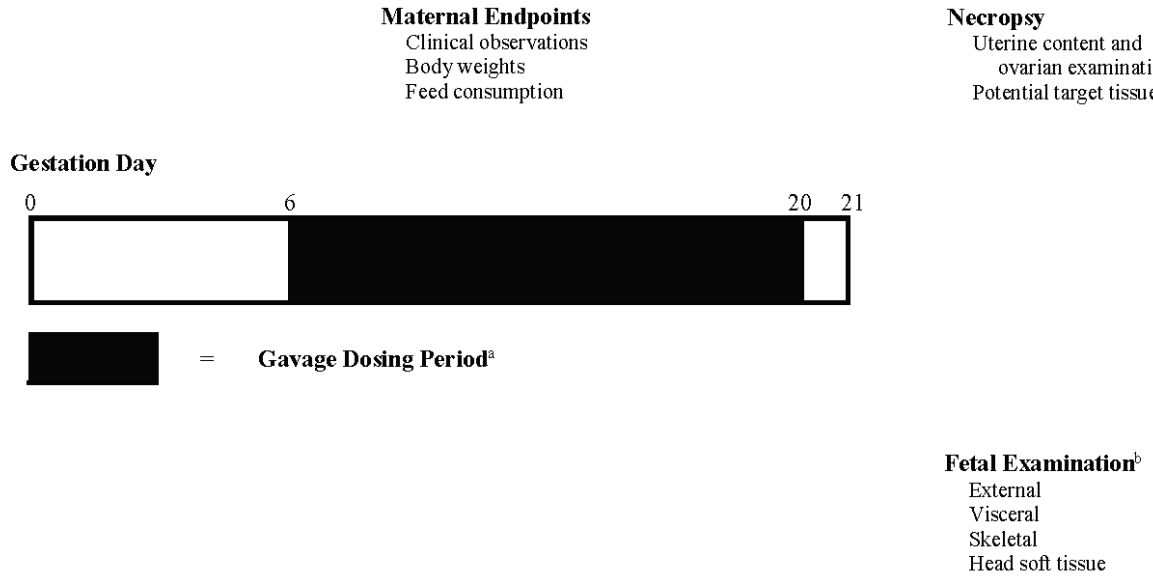
MATERIALS AND METHODS

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, and 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, function, or variations that are a divergence beyond the usual range of structural constitution that may not adversely affect survival or health (USEPA, 1991), consistent with that described by Makris *et al.* (2009). The general study design for the dose range-finding and prenatal developmental toxicity studies is presented in Figure 1.

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



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

PROCUREMENT AND CHARACTERIZATION

Tris(chloropropyl) Phosphate (TCPP)

TCPP was obtained from Albemarle Corporation (Orangeburg, SC) in two lots (101 and 134). Lot 101 was used in the dose range-finding study, and lots 101 and 134 were blended to form lot M072911NP that was stored in two drums and used during the prenatal developmental toxicity study. Identity and purity of lots 101 and 134 were confirmed prior to blending (Table 2). Homogeneity of the blended lot M072911NP was confirmed both within the individual drums and between the two drums. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at MRI Global (Kansas City, MO) (Appendix C).

TABLE 2
Composition of Lots Used in the Prenatal Developmental Toxicity Studies of Tris(chloropropyl) Phosphate in Rats

Analysis	Lot 101[a]	Lot 134	Lot M072911NP[b] Drum 1	Lot M072911NP[b] Drum 2
Elemental (%)				
Carbon	33.02	ND	33.06	32.93
Hydrogen	5.64	ND	5.62	5.55
Nitrogen	0.14	ND	0.09	0.10
Chlorine	31.92	ND	32.00	32.19
Water: Karl Fischer (%)	0.093	ND	0.038	0.039
Acid Number (mg KOH/g)	0.011	ND	0.067	ND
Ester Value (mg KOH/g)	104.7	ND	105.85	ND
Relative Density (g/mL)	1.294	ND	1.296	1.296
Log P				
TCPP Peak 1	2.69	ND	2.59	ND
TCPP Peak 2	2.74	ND	2.65	ND
Purity: GC/FID (%)[c]				
System 1: DB-5				
Sum of TCPP Isomers	96.04	98.79	97.04	97.43
Isomer 1	65.22	71.33	67.57	68.54
Isomer 2	26.80	24.29	25.65	25.21
Isomer 3	3.86	3.07	3.61	3.48
Isomer 4	0.16	0.14	0.21	0.20
Reportable Impurities % (number)[d]	3.76 (7)	1.14 (3)	2.74 (8)	2.35 (8)
System 2: DB-Wax				
Sum of TCPP Isomers	ND	ND	97.50	97.91
Isomer 1	ND	ND	67.84	68.85
Isomer 2	ND	ND	25.87	25.42
Isomer 3	ND	ND	3.61	3.57
Isomer 4	ND	ND	0.18	0.17
Reportable Impurities % (number)[d]	ND	ND	2.49 (6)	2.09 (6)

ND = Not Determined
[a] Used in the dose range-finding study
[b] Lots 101 and 134 were blended to generate lot M072911NP that was used in the prenatal developmental toxicity study
[c] Isomers 1 through 4 were identified as tris(1-chloro-2-propyl) phosphate, bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate, bis(2 chloropropyl) 2-chloroisopropyl phosphate, and tris(2-chloropropyl) phosphate, respectively (Appendix C).
[d] Impurities of ≥ 0.05% are listed

Lots 101, 134, and M072911NP of the test chemical, clear oily liquids, were identified using proton and carbon-13 Fourier transform nuclear magnetic resonance (FT-NMR) spectroscopy. Due to the isomeric complexity of the test article, two-dimensional FT-NMR was performed on lot M072911NP including homonuclear correlation spectroscopy (COSY) and heteronuclear correlation (HETCOR) spectroscopy to confirm the data from the proton and carbon-13 NMR spectra. In addition, lots 101 and M072911NP were analyzed using Fourier transform infrared (FT-IR) and ultraviolet/visible (UV-Vis) spectroscopy and gas chromatography (GC) with mass spectrometry (MS) detection. GC/MS using electron ionization of lots 101 and M072911NP identified one major peak and three other

peaks with similar molecular weights indicating the presence of isomeric compounds (Table 2). The major peak (Isomer 1) identified as tris(1-chloro-2-propyl) phosphate, CAS No. 13674-84-5 matched a literature spectrum (NIST, 2008); the three other isomers were identified as bis(2-chloro-1-methylethyl) 2-chloropropyl phosphate (Isomer 2; CAS No. 76025-08-6), bis(2-chloropropyl) 2-chloroisopropyl phosphate (Isomer 3; CAS No. 76649-15-5), and tris(2-chloropropyl) phosphate (Isomer 4; CAS No. 6145-73-9).

The percents of individual and combined isomers estimated for each lot using GC with flame ionization detection (FID) using two systems are shown in Table 2 and align with the range of percentages reported in commercial products (Table 1). For lots 101 and M072911NP, the following additional analyses were conducted: moisture content by Karl Fischer titration; elemental analyses for carbon, hydrogen, nitrogen, and chlorine (ICON Development Solutions (Whitesboro, NY); octanol:water partition coefficients (log P) of the major peak; density; and acid number and ester value using titration with standardized ~0.001 N sodium hydroxide and ~0.5 N hydrochloric acid are also shown for each isomer, respectively (Table 2). Elemental analyses for carbon, hydrogen, nitrogen, and chlorine for all lots were consistent with the theoretical values for TCPP. The purity of the lots estimated based on the combined percents of four isomers were greater than or equal to 96% (Table 2).

To ensure stability, the test chemical was stored under inert gas at ~25° C, in sealed drums. Periodic analyses of lots 101 and M072911NP of the test chemical were performed prior to and during the animal studies by the analytical chemistry laboratory using FT-NMR and GC/FID; no degradation of the test chemical was detected.

Methylcellulose

Methylcellulose was obtained from Spectrum Quality Products (Gardena, CA) in two lots (YX0540 and 2AJ0439). Lot YX0540 was used as the vehicle in the dose range-finding study and lot 2AJ0439 was used in the prenatal developmental toxicity study. The identity of both lots was confirmed by the analytical chemistry laboratory using FT-IR spectroscopy. The methoxy group content was determined by Galbraith Laboratories (Knoxville, TN) using titration with standardized sodium thiosulfate solution. Methoxy group content was 30.4% and 31.0% for lots YX0540 and 2AJ0439, respectively, both within the accepted range of 27.5% to 31.5%.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

Prior to conducting the dose range-finding study, formulation homogeneity studies at 1.56 mg/mL and 200 mg/mL and stability studies at 1.56 mg/mL were performed by the analytical chemistry laboratory using GC/FID. Homogeneity was confirmed, with the stipulation that high-dose formulations be stirred constantly during use to maintain homogeneity. Stability was confirmed for at least 7 days for dose formulations stored in sealed glass containers at ~5° C and for 3 hours under simulated animal room conditions at 5° C, the 1.56 mg/mL formulation was 93.8%, 88%, and 86.6% of the day 0 value at 7, 14, and 42 days, respectively, suggesting some loss over time. Additional stability studies were conducted at 32.5 and 130 mg/mL prior to the prenatal developmental toxicity study; stability of the 130 mg/mL formulation was confirmed for up to 42 days, while that of the 32.5 mg/mL formulation was confirmed for up to 35 days for formulations stored in sealed glass containers at ~5° C.

The dose formulations were prepared by the analytical chemistry laboratory once (dose range-finding study) or three times (prenatal developmental toxicity study) by mixing TCPP with a 0.5% methylcellulose solution to give the required concentrations with a dosing volume of 5 mL/kg. The dose formulations were stored at ~5° C in sealed glass jars for up to 30 (dose range-finding study) or 15 (prenatal developmental toxicity study) days.

Dose formulations of TCPP were analyzed by the analytical chemistry laboratory using GC/FID. During the dose range-finding study, the formulations were analyzed twice; all 10 dose formulations were within 10% of the target concentrations (Table C3). Animal room samples received on day 36 were also analyzed; two of six were within 10% of the target concentrations and the other four were within 11% to 31%. During the prenatal developmental toxicity study, the dose formulations were prepared three times and analyzed once; all nine dose formulation samples were within 10% of the target concentrations (Table C4). During the preparation of formulations, homogeneity was confirmed using the 32.5 and 130 mg/mL concentrations (Table C4). Animal room samples of each dose formulation were also analyzed; six were within 10% of the target concentrations and three were within 12% to 15%.

ANIMAL SOURCE

Female Sprague Dawley (Hsd:Sprague Dawley SD) rats for use in the dose range-finding and prenatal developmental toxicity studies were obtained from Envigo (formerly Harlan Laboratories, Inc., Dublin, VA, or Indianapolis, IN) (Table 3). This stock is routinely used in NTP studies for toxicity evaluation. Sexually mature (12 to 13 weeks old) females were time-mated overnight at the vendor and were received on gestation day (GD) 2 for the dose range-finding study and on GD 1 or 2 for the prenatal developmental toxicity study. GD 0 was defined as the day that positive evidence of mating was observed. In addition, 10 non-mated females were received for use as sentinels during the prenatal developmental toxicity study.

ANIMAL HEALTH SURVEILLANCE

In accordance with the NTP sentinel animal program (Appendix E), female sentinels were evaluated in the prenatal developmental toxicity study on January 31 and February 15, 2012. All test results were negative.

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 RTI International Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

EXPERIMENTAL DESIGN

In the dose range-finding and prenatal developmental toxicity studies, time-mated rats were housed individually, provided NIH-07 feed and water *ad libitum*, and observed at least twice daily for viability (morning and afternoon). Clinical observations were performed from GD 3 through GD 21 until removal, typically twice daily (at the time of dose administration and cage-side post dose). Females were weighed daily from GD 3 through GD 21. Feed consumption was recorded for GD 3 to 6, 6 to 9, 9 to 12, 12 to 15, 15 to 18, and 18 to 21. Details of the study

design including animal source and identification, diet, water, husbandry, environmental conditions, euthanasia, necropsy, and fetal evaluations are summarized in Table 3. Information on feed composition and contaminants is 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 gravid uterus as well as the ovary, liver, and adrenal glands were excised and weighed (organs for prenatal developmental toxicology 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 (a live fetus is defined as one that responds to stimuli; a dead fetus is defined as a term fetus that does not respond to stimuli and is not markedly autolyzed), 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 form with placental remnants 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. Females were not retained for further examination.

Dose Range-Finding Study

Time-mated rats were individually identified by ear tag and randomized by GD 3 body weight stratification into four groups using RTI International's Instem™ Provantis® (version 8.2.0) electronic data collection system.

Groups of 11 time-mated female rats were administered 0 (vehicle control), 300, 650, or 1,000 mg TCPP/kg body weight (based on the most recent weight) per day in 0.5% aqueous methylcellulose by gavage from GD 6 to GD 20. Vehicle control animals received aqueous methylcellulose alone; the dosing volume was 5 mL/kg. One thousand mg/kg was considered the limit dose for a prenatal developmental study in rats and was chosen as the high dose in the dose range-finding study. Dose selection was supported by developmental and two-generation reproduction studies reported in the literature (Kawasaki *et al.*, 1982; EU, 2008; USEPA, 2015a).

On GD 21, live fetuses of surviving females were counted, sexed, weighed, and examined for external morphological abnormalities, including inspection of the oral cavity for cleft palate. Fetuses were euthanized by intraperitoneal injection of a commercially available solution containing sodium pentobarbital. 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 tattoo and randomized, based on GD 3 body weight stratification, into five groups using RTI's Instem™ Provantis® (version 8.2.0) electronic data collection system. Dams were delivered 2 days apart to allow for a staggered study start.

Groups of 25 time-mated female rats were administered 0 (2 concurrent vehicle control groups), 162.5, 325, or 650 mg TCPP/kg body weight (based on the most recent weight) per day in 0.5% aqueous methylcellulose by gavage from GD 6 to GD 20 (15 days). The additional vehicle control group was included to generate additional control data for both maternal and fetal findings in this strain of rat. At the end of the study, the vehicle control groups were evaluated for reproducibility and combined for assessment of treatment-related effects because they were run concurrently. Vehicle control animals received aqueous methylcellulose alone; the dosing volume was 5 mL/kg.

On GD 21, fetuses were removed from the uterus, and live fetuses individually weighed. The uteri of animals that did not appear pregnant were examined for nidations (implantation sites) by staining with 0.5% ammonium sulfide (Salewski, 1964; Tyl and Marr, 2006). All fetuses were examined externally for alterations, including inspection of

the oral cavity for cleft palate. Live fetuses were subsequently euthanized by oral administration of sodium pentobarbital. Fetal sex was confirmed by inspection of gonads *in situ*. All fetuses were examined for soft tissue alterations under a stereomicroscope (Staples, 1974; Stuckhardt and Poppe, 1984). The heads were removed from approximately half of the fetuses in each litter and fixed in Bouin's solution and subsequently examined by free-hand sectioning (Thompson, 1967). Fetuses were eviscerated, fixed in ethanol, macerated in potassium hydroxide, stained with alcian blue and alizarin red, and examined for subsequent cartilage and osseous alterations (Marr *et al.*, 1992; Tyl and Marr, 2006). External, visceral, and skeletal fetal alterations were recorded as developmental variations or malformations.

TABLE 3
Experimental Design and Materials and Methods in the Prenatal Developmental Toxicity Gavage Studies of Tris(chloropropyl) Phosphate

Dose Range-Finding Study	Prenatal Developmental Toxicity Study
Study Laboratory RTI International (Research Triangle Park, NC)	RTI International (Research Triangle Park, NC)
Strain and Species Sprague Dawley (Hsd:Sprague Dawley SD) rats	Sprague Dawley (Hsd:Sprague Dawley SD) rats
Animal Source Envigo (formerly Harlan Laboratories, Inc., Dublin, VA)	Envigo (formerly Harlan Laboratories, Inc., Dublin, VA)
Day of Arrival Gestation Day (GD) 2	GD 1 or 2
Average Age on Arrival 12 weeks	12 to 13 weeks
Weight Range at Randomization 195.8 to 246.6 g on GD 3	208.3 to 251.6 g on GD 3
Calendar Day of First Dose (GD 6) and Last Dose (GD 20) GD 6 (August 2, 2010) and GD 20 (August 16, 2010)	GD 6 (January 29, 2012) and GD 20 (February 15, 2012); staggered start
Duration of Dosing GD 6 to 20, once daily	GD 6 to 20, once daily
Size of Study Groups 11 time-mated females	50 time-mated females (2 vehicle control groups with 25 each), 25 time-mated females (treated groups)
Method of Randomization and Identification Time-mated animals were uniquely identified on day of receipt by ear tag and assigned to dose group by body weight stratified randomization of GD 3 body weights using Instem Provantis® (version 8) electronic data collection system. Each animal was assigned a unique animal number in Provantis®. This number was linked to the respective marking and all data collected during the study was associated with the Provantis® animal number.	Time-mated animals were uniquely identified on day of receipt by tail tattoo and assigned to dose group by body weight stratified randomization of GD 3 body weights using Instem Provantis® (version 8) electronic data collection system. Each animal was assigned a unique animal number in Provantis®. This number was linked to the respective marking and all data collected during the study was associated with the Provantis® animal number.
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 (Durham, NC, municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as dose range-finding study
Cages Solid bottom polycarbonate cages (Ancare, Bellmore, NY), changed and rotated weekly	Solid bottom polycarbonate cages (Lab Products, Inc., Seaford, DE), changed and rotated weekly

TABLE 3
Experimental Design and Materials and Methods in the Prenatal Developmental Toxicity Gavage Studies of Tris(chloropropyl) Phosphate

Dose Range-Finding Study	Prenatal Developmental Toxicity Study
Bedding Certified irradiated Sani-Chips® hardwood cage bedding (P.J. Murphy Forest Products Corporation, Montville, NJ), changed weekly	Same as dose range-finding study
Cage Filters Filter paper (Ancare, Bellmore, NY), changed weekly	Filter paper (Granville Milling Co., Creedmoor, NC), changed weekly
Racks Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks, rotated once during the study	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
Doses 0, 300, 650, or 1,000 mg/kg in 0.5% methylcellulose (dosing volume 5 mL/kg)	0, 162.5, 325, or 650 mg/kg in 0.5% methylcellulose (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 twice daily from GD 3 until necropsy; [prior to dosing (out of cage) and at 1 to 3 hours post dose (cage side)] beginning on GD 6. Animals were weighed daily beginning on GD 3. Feed consumption was recorded at 3-day intervals from GD 3 through GD 21.	Observed for viability twice daily from GD 3 through GD 20. Clinical observations were recorded once daily from GD 3 until necropsy; [prior to dosing (out of cage), and at 1 to 3 hours post dose (cage side)] from GD 6 through GD 20. Feed consumption was recorded at 3-day intervals from GD 3 through GD 21.
Primary Method of Euthanasia 100% CO_2 (adults) or intraperitoneal injection of a solution containing sodium pentobarbital (fetuses)	100% CO_2 (adults) or oral administration of a solution containing sodium pentobarbital (fetuses)
Necropsy and Postmortem Evaluation of Females On GD 21, terminal body 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 to visualize potential evidence of implantation sites.	On GD 21, terminal body, adrenal glands, liver, ovary, 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 to visualize potential evidence of implantation sites.
For animals removed early, gross necropsy including an examination of the thoracic and abdominal viscera was performed. The uterus of each female was examined to determine pregnancy status or, if no evidence of pregnancy, stained to visualize possible early implantation sites.	There were no early removals.

TABLE 3
Experimental Design and Materials and Methods in the Prenatal Developmental Toxicity Gavage Studies of Tris(chloropropyl) Phosphate

Dose Range-Finding Study	Prenatal Developmental Toxicity Study
Fetal Evaluation Live fetuses were counted, sexed, weighed, and examined for external morphologic abnormalities that included inspection of the oral cavity for cleft palate.	Live fetuses were counted, sexed, weighed, and examined for external morphologic abnormalities that included inspection of the oral cavity for cleft palate. Placental morphology was also evaluated. Live fetuses were euthanized and then examined for visceral morphologic abnormalities by fresh dissection. The sex of each fetus was confirmed by internal examination. The heads from approximately one half of the fetuses in each litter were fixed, sectioned, and examined. All fetuses were eviscerated, fixed, stained, and examined for skeletal developmental variations, malformations, or other morphologic findings.

STATISTICAL METHODS

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

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 at GD 3 and reported as means with standard error bars in Figures 2 and 4 (also see Tables A2 and B2). Terminal maternal body weights at GD 21, were adjusted for gravid uterine weight by subtracting the gravid uterine weight from the dam's body weight. Body weight gains were calculated over each 3-day-interval and from GD 6 to GD 21. Daily feed consumption was averaged over each 3-day-interval and from GD 6 to GD 21. These continuous variables, in addition to gravid uterine weights 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 7 and 12. Data from females that were not pregnant or that did not survive to the end of the study were not included. Post-implantation loss is calculated as a percentage of the number of implants per dam. Fetal findings are reported as means and standard errors of counts per litter (numbers of live fetuses, male fetuses, female fetuses), means and standard errors of litter means (fetal weight, male fetal weight, female fetal weight) and total numbers of occurrences (total number of live fetuses). In addition, several calculated variables are reported, including the percentage of live male fetuses per litter.

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

Analysis of Maternal Parameters and Uterine Contents

Maternal organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Non-normally distributed variables, such as food consumption 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 (Zorilla, 1996; 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 a very low incidence. For meaningful comparisons, the conditions for studies in the historical control database must be generally similar. Significant factors that may affect the background incidences of fetal findings at a variety of sites are diet, sex, strain/stock, route of exposure, study type, and/or laboratory that conducted the study. The NTP historical control database for teratology studies contains all fetal evaluations (e.g., teratology studies or modified one generation studies) for each laboratory. In general, the historical control database for a given study includes studies using the same route of administration and study design. However, historical control data for rats in this Prenatal Developmental Toxicity Study Report contain all studies conducted by the laboratory due to the limited number of studies available. 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 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

Seven of 11 dams in the 1,000 mg/kg group were either found dead or euthanized moribund (Table 4). Associated adverse neurological clinical observations in the 1,000 mg/kg group included convulsions, tremors, and hypoactivity. Additional observations included gasping, hunched posture, nasal discharge, stained fur, piloerection, prone, salivation, and rooting (pre- and post-dosing) which occurred throughout the gestational period (Table A1). One female was euthanized moribund in the 650 mg/kg group on gestation day (GD) 16 with clinical observations including cold to touch, hypoactivity, paleness, ataxia, and labored breathing. All vehicle control (0 mg/kg) and 300 mg/kg animals survived to study termination and showed no treatment-related clinical observations.

TABLE 4
Maternal Disposition of Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	300 mg/kg	650 mg/kg	1,000 mg/kg
Time-mated females	11	11	11	11
Pregnant (on GD 21)	10	11	7	4
Euthanasia moribund – pregnant	0	0	1[a]	5[b]
Found dead - pregnant	0	0	0	1[c]
Non-pregnant (on GD 21)	1	0	3	0
Found dead – non-pregnant	0	0	0	1[d]

[a] Dam removed on GD 16
[b] Dams removed on GD 6, 13, and 20 (3)
[c] Dam found dead on GD 13
[d] Dam found dead on GD 6

Body Weights and Feed Consumption

Maternal body weight gain from GD 9 to 12 was 26% lower in the 1,000 mg/kg group relative to the vehicle control group (Table 5). However, maternal body weight gain from GD 6 to 21 in the 1,000 mg/kg group was similar to that in the vehicle control group. Overall, there were no dose-related effects on maternal body weight gain during gestation (Figure 2; Tables 5 and A2).

In association with transient body weight changes, maternal feed consumption was 19% (GD 6 to 9) and 12% (GD 9 to 12) lower in the 1,000 mg/kg group relative to vehicle controls for those time periods (Table 6). However, feed consumption from GD 6 to 21 by the 1,000 mg/kg group was similar to that in the vehicle control group. Feed consumption in the 300 and 650 mg/kg groups was similar to that in the vehicle control group (Table 6).

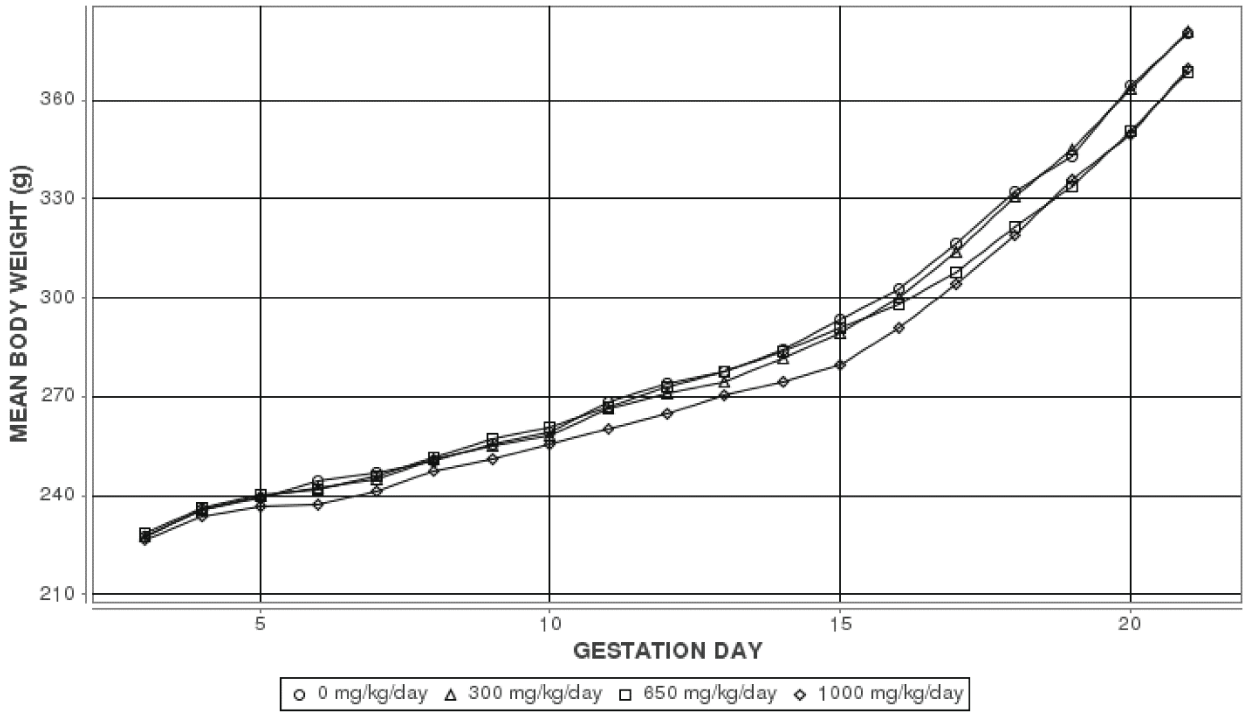


FIGURE 2
Maternal Growth Curves for Pregnant Rats Administered Tris(chloropropyl) Phosphate by Gavage in the Dose Range-Finding Study
Information for statistical significance in maternal weights is provided in Tables 5 and A2.

TABLE 5
Summary of Maternal Body Weight Gains of Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	300 mg/kg	650 mg/kg	1,000 mg/kg
Gestation Day Interval				
6 to 21	136.1 ± 2.6[a] (10)	138.4 ± 4.3 (11)	127.5 ± 17.1 (7)	130.2 ± 12.7 (4)
3 to 6	16.5 ± 2.5 (10)	14.8 ± 2.3 (11)	13.0 ± 1.1 (8)	10.8 ± 1.2 (10)
6 to 9	11.4 ± 0.4 (10)	12.8 ± 1.1 (11)	15.1 ± 1.3 (8)	12.1 ± 1.8 (9)
9 to 12	18.5 ± 1.1* (10)	15.8 ± 0.8 (11)	16.1 ± 0.9 (8)	13.8 ± 1.5* (9)
12 to 15	19.3 ± 0.9 (10)	18.1 ± 0.9 (11)	17.7 ± 1.9 (8)	17.4 ± 2.5 (7)
15 to 18	38.9 ± 1.7 (10)	41.6 ± 1.9 (11)	30.5 ± 9.8 (7)	39.3 ± 3.4 (7)
18 to 21	48.1 ± 0.8 (10)	50.1 ± 2.0 (11)	46.8 ± 4.4 (7)	47.0 ± 7.4 (4)

* Statistically significant (P≤0.05) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dose group column.

[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 6
Summary of Maternal Feed Consumption of Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	300 mg/kg	650 mg/kg	1,000 mg/kg
Gestation Day Interval				
6 to 21	21.8 ± 0.46[a] (10)	21.4 ± 0.44 (11)	21.8 ± 1.21 (7)	21.4 ± 0.76 (4)
3 to 6	19.8 ± 0.54 (10)	19.5 ± 0.42 (11)	19.6 ± 0.55 (8)	19.0 ± 0.55 (10)
6 to 9	19.5 ± 0.53** (10)	17.8 ± 0.51 (11)	17.9 ± 0.86 (8)	15.9 ± 0.71** (9)
9 to 12	20.6 ± 0.43** (10)	19.6 ± 0.48 (11)	19.6 ± 0.47 (8)	18.2 ± 0.69** (9)
12 to 15	20.9 ± 0.52 (10)	20.9 ± 0.34 (11)	21.9 ± 0.57 (8)	21.0 ± 0.79 (7)
15 to 18	24.1 ± 0.63 (10)	23.6 ± 0.62 (11)	24.7 ± 1.71 (7)	24.9 ± 0.62 (7)
18 to 21	23.9 ± 0.54* (10)	25.1 ± 0.62 (11)	24.5 ± 2.79 (7)	26.5 ± 1.07 (4)

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

** P≤0.01

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

Maternal and Litter Observations

The maternal toxicity observed in the 1,000 mg/kg TCPP dose group resulted in only four litters available for assessments, as compared to seven to 11 litters in the other groups (Table 7). In this dose group, there were less implants per female; however, this is a variable endpoint in this strain and because TCPP dosing began on GD 6, this finding is not considered related to treatment. Also, the number of live male fetuses per litter in the 1,000 mg/kg group was significantly lower (by 53%) compared to vehicle controls. Additional observations included nonsignificant decreases in the number of live fetuses per litter and mean gravid uterine weight in the 650 and 1,000 mg/kg groups. These findings were considered to have an uncertain relationship to TCPP exposure due to the small sample size and number of litters available for evaluation. Overall, there were no significant effects of TCPP exposure on embryo-fetal survival (i.e., post-implantation loss) or growth retardation (i.e., fetal weight).

TABLE 7
Summary of Uterine Content Data for Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	300 mg/kg	650 mg/kg	1,000 mg/kg
Pregnancy Summary				
Mated females	11	11	11	11
Pregnant females	10	11	8	10
Pregnant females examined on GD 21[a]	10**	11	7	4*
Corpora lutea per female[b]	16.90 ± 0.66 (10)	15.55 ± 0.77 (11)	16.14 ± 1.37 (7)	16.25 ± 0.48 (4)
Implantations per female[b]	13.90 ± 0.62 (10)	13.45 ± 0.53 (11)	12.43 ± 1.21 (7)	10.75 ± 2.59 (4)
Percent post-implantation loss[b]	2.14 ± 2.14 (10)	0.83 ± 0.83 (11)	6.46 ± 3.99 (7)	1.92 ± 1.92 (4)
Total resorptions per litter[b]	0.30 ± 0.30 (10)	0.09 ± 0.09 (11)	0.29 ± 0.18 (7)	0.25 ± 0.25 (4)
Early resorptions per litter[b]	0.30 ± 0.30 (10)	0.09 ± 0.09 (11)	0.29 ± 0.18 (7)	0.25 ± 0.25 (4)
Late resorptions per litter[b]	0.00 ± 0.00 (10)	0.00 ± 0.00 (11)	0.00 ± 0.00 (7)	0.00 ± 0.00 (4)
Dead fetuses per litter[b]	0.00 ± 0.00 (10)	0.00 ± 0.00 (11)	0.00 ± 0.00 (7)	0.00 ± 0.00 (4)
Number of early resorptions	3	1	2	1
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	136	147	83	42
Live fetuses per litter	13.60 ± 0.69 (10)	13.36 ± 0.58 (11)	11.86 ± 1.44 (7)	10.50 ± 2.53 (4)
Live male fetuses per litter	7.44 ± 0.56* (9)[d]	6.64 ± 0.59 (11)	5.57 ± 1.02 (7)	3.50 ± 0.87** (4)
Live female fetuses per litter	6.11 ± 0.48 (9)[d]	6.73 ± 0.66 (11)	6.29 ± 0.87 (7)	7.00 ± 2.16 (4)
Percent live male fetuses per litter	54.95 ± 2.48 (9)[d]	49.91 ± 4.20 (11)	46.53 ± 6.43 (7)	39.81 ± 10.48 (4)
Fetal Weight[c]				
Fetal weight per litter (g)	5.13 ± 0.06 (9)[d]	5.24 ± 0.09 (11)	5.20 ± 0.10 (7)	5.23 ± 0.22 (4)
Male weight per litter (g)	5.23 ± 0.07 (9)[d]	5.42 ± 0.07 (11)	5.33 ± 0.15 (7)	5.44 ± 0.21 (4)
Female weight per litter (g)	5.01 ± 0.07 (9)[d]	5.07 ± 0.09 (11)	5.15 ± 0.09 (7)	5.06 ± 0.14 (4)
Gravid Uterine Weight[e]				
Gravid uterine weight (g)	98.11 ± 4.01 (10)	96.62 ± 3.35 (11)	86.37 ± 9.78 (7)	74.65 ± 15.90 (4)
Terminal body weight (g)	378.7 ± 5.7 (10)	376.4 ± 6.3 (11)	363.9 ± 20.4 (7)	365.0 ± 10.1 (4)
Adjusted body weight (g)	280.62 ± 4.86 (10)	279.76 ± 4.06 (11)	277.54 ± 11.58 (7)	290.35 ± 11.08 (4)

Values are reported per litter as mean ± standard error (n) and do not include non-pregnant animals or those that did not survive to end of study.
(g) = grams
* Statistically significant (P≤0.05) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column)
** P≤0.01
[a] Statistical analysis performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests
[b] Statistical analysis performed by Jonckheere's (trend) and Shirley's or Dunn's (pairwise) tests
[c] Statistical analysis performed using a mixed effects linear model with litter as a random effect (trend and pairwise)
[d] n=9 litters: Individual sex and fetal body weight data for 14 fetuses were inadvertently not collected for one litter.
[e] Statistical analysis performed by Jonckheere's (trend) and Williams' or Dunnett's (pairwise) tests; adjusted body weight = terminal body weight minus gravid uterine weight

Fetal Findings

External

There were no exposure-related external malformations or variations attributed to TCPP administration at 300, 650, or 1,000 mg/kg (Tables A3, A4, and A5).

Dose Selection Rationale for the Prenatal Developmental Toxicity Study in Rats

Excessive maternal toxicity (animals were euthanized moribund or found dead) in the dose range-finding study was observed at 1,000 mg/kg. At 650 mg/kg, there was an uncertain relationship between TCPP administration and toxicity due to the presence of a single moribund dam with adverse clinical observations. No toxicity was observed at lower doses. Thus, dose concentrations of 0, 162.5, 325, or 650 mg/kg were chosen for the subsequent prenatal developmental toxicity gavage study.

PRENATAL DEVELOPMENTAL TOXICITY STUDY IN RATS

Maternal Findings

Viability and Clinical Observations

No animals were removed from the study prior to scheduled necropsy (Table 8). Dose-related clinical observations were observed in six females in the 650 mg/kg group and included nasal discharge, salivation, twitches, ataxia, piloerection, audible respiratory sounds, and hyperactivity (Table B1). The duration for the majority of the clinical observations was limited to a single day of gestation aside from hyperactivity in one female which was observed over a 7-day period starting on GD 7. There were no dose-related clinical observations in the other TCPP groups or in vehicle control (0 mg/kg) animals.

TABLE 8
Maternal Disposition of Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg[a]	162.5 mg/kg	325 mg/kg	650 mg/kg
Time-mated females	50	25	25	25
Pregnant (on GD 21)	44	21	21	20
Non-pregnant (on GD 21)	6	4	4	5

[a] This study had two vehicle control groups. Data from both vehicle control groups were combined and are presented here.

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 Table 9). Daily mean body weights for dams in each dose group are available in Table B2. Compared to the vehicle control group, maternal feed consumption was 8% to 16% lower over GD 6 to 9 and 9 to 12 for dams in the 650 mg/kg group (Table 10). The feed consumption differences were transient and overall, there was no effect of TCPP administration on maternal feed consumption during gestation (Table 10).

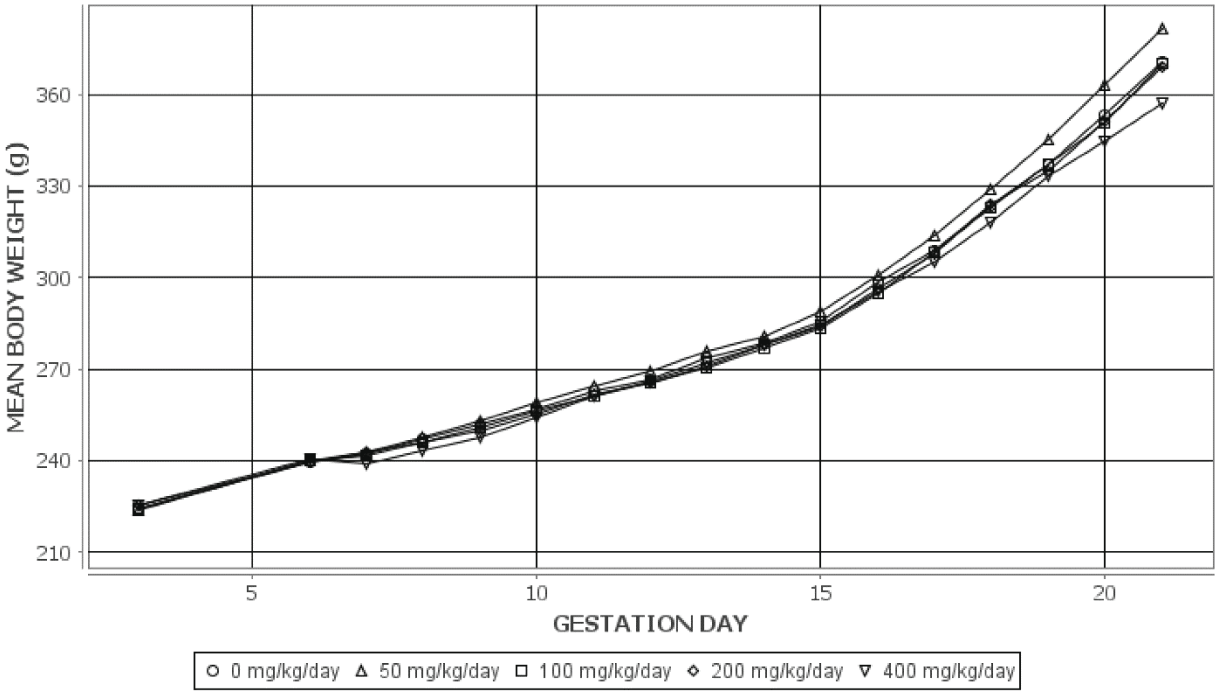


FIGURE 3
Maternal Growth Curves for Pregnant Rats Administered Tris(chloropropyl) Phosphate by Gavage in the Prenatal Developmental Toxicity Study
Information for statistical significance in maternal weights is provided in Tables 9 and B2.

TABLE 9
Summary of Maternal Body Weight Gains of Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	162.5 mg/kg	325 mg/kg	650 mg/kg
Gestation Day Interval				
6 to 21	139.6 ± 2.5[a] (44)	143.6 ± 3.0 (21)	139.9 ± 3.8 (21)	137.6 ± 6.3 (20)
3 to 6	14.4 ± 0.8 (44)	14.1 ± 0.7 (21)	15.7 ± 1.0 (21)	14.1 ± 1.0 (20)
6 to 9	12.9 ± 0.6 (44)	13.0 ± 0.6 (21)	12.5 ± 0.8 (21)	10.6 ± 0.8 (20)
9 to 12	16.8 ± 0.6 (44)	20.5 ± 0.9 (21)	16.8 ± 0.8 (21)	15.4 ± 1.2 (20)
12 to 15	18.9 ± 0.9 (44)	18.1 ± 0.9 (21)	19.6 ± 1.4 (21)	21.2 ± 1.3 (20)
15 to 18	42.4 ± 1.0 (44)	43.7 ± 1.0 (21)	41.9 ± 1.6 (21)	39.0 ± 2.2 (20)
18 to 21	48.8 ± 1.4 (44)	50.8 ± 1.5 (21)	49.0 ± 1.8 (21)	51.4 ± 3.1 (20)

Statistical analysis performed by Jonckheere's test (trend) or 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 10
Summary of Maternal Feed Consumption of Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	162.5 mg/kg	325 mg/kg	650 mg/kg
Gestation Day Interval				
6 to 21	22.8 ± 0.23[a] (44)	22.5 ± 0.33 (21)	22.9 ± 0.33 (21)	22.2 ± 0.42 (20)
3 to 6	20.2 ± 0.42 (44)	18.7 ± 0.24 (21)	19.7 ± 0.50 (21)	19.4 ± 0.55 (20)
6 to 9	20.4 ± 0.32** (44)	19.4 ± 0.37 (21)	18.8 ± 0.41** (21)	17.0 ± 0.46** (20)
9 to 12	21.6 ± 0.34** (44)	20.7 ± 0.41 (21)	21.0 ± 0.38 (21)	19.8 ± 0.54** (20)
12 to 15	21.6 ± 0.22 (44)	21.1 ± 0.37 (21)	22.4 ± 0.55 (21)	21.4 ± 0.29 (20)
15 to 18	24.8 ± 0.34 (44)	25.0 ± 0.42 (21)	25.5 ± 0.53 (21)	25.2 ± 0.74 (20)
18 to 21	25.5 ± 0.36** (44)	26.3 ± 0.51 (21)	27.0 ± 0.38* (21)	27.8 ± 0.66** (20)

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

** $P \leq 0.01$

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

Maternal and Litter Observations

There were no maternal gross observations at necropsy (Table B3). However, there were dose-related increases in absolute (9%, 16%, and 26% at 162.5, 325, and 650 mg/kg, respectively) and relative liver weights (Table 11).

TABLE 11
Summary of Maternal Liver Weights and Liver Weight Ratios for Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate[a]

	0 mg/kg	162.5 mg/kg	325 mg/kg	650 mg/kg
n	44	21	21	20
Necropsy body wt.	378.5 ± 3.0	382.7 ± 4.0	383.1 ± 5.2	375.2 ± 8.3
Liver				
Absolute	14.35 ± 0.19**	15.62 ± 0.32**	16.58 ± 0.27**	18.02 ± 0.56**
Relative	37.93 ± 0.41**	40.78 ± 0.67**	43.39 ± 0.78**	48.09 ± 1.09**

** Statistically significant (P≤0.01) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.

[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.

There were no effects on pregnancy status or litter size following TCPP administration (Table 12). Although there was a 2-fold increase in mean percent post-implantation loss in the 650 mg/kg group as compared to the vehicle controls, this increase is the result of one dam that had nine early resorptions. Given the singular litter incidence, this finding in the 650 mg/kg group was not considered related to TCPP administration. The number of live fetuses per litter was 5% lower in the 325 and 650 mg/kg groups and was accompanied by lower gravid uterine weights (< 7%) at these doses; however, these differences were not statistically significant. There were no exposure-related effects on absolute fetal body weights (male or female).

TABLE 12
Summary of Uterine Content Data for Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	162.5 mg/kg	325 mg/kg	650 mg/kg
Pregnancy Summary				
Mated females	50	25	25	25
Pregnant females	44	21	21	20
Pregnant females examined on GD 21[a]	44	21	21	20
Corpora lutea per female	16.64 ± 0.37 (44)	18.05 ± 0.55 (21)	16.62 ± 0.72 (21)	17.55 ± 0.85 (20)
Implantations per female	14.18 ± 0.29 (44)	14.81 ± 0.38 (21)	13.48 ± 0.65 (21)	13.70 ± 0.67 (20)
Percent post-implantation loss[b]	3.81 ± 1.13 (44)	3.42 ± 0.99 (21)	4.33 ± 1.19 (21)	7.17 ± 4.50 (20)
Total resorptions per litter[b]	0.55 ± 0.16 (44)	0.52 ± 0.15 (21)	0.52 ± 0.15 (21)	0.75 ± 0.45 (20)
Early resorptions per litter[b]	0.50 ± 0.16 (44)	0.52 ± 0.15 (21)	0.52 ± 0.15 (21)	0.75 ± 0.45 (20)
Late resorptions per litter[b]	0.05 ± 0.03 (44)	0.00 ± 0.00 (21)	0.00 ± 0.00 (21)	0.00 ± 0.00 (20)
Dead fetuses per litter[b]	0.02 ± 0.02 (44)	0.00 ± 0.00 (21)	0.10 ± 0.07 (21)	0.00 ± 0.00 (20)
Number of early resorptions	22	11	11	15
Number of late resorptions	2	0	0	0
Number of whole litter resorptions[a]	0	0	0	0
Number of dead fetuses	1	0	2	0
Live Fetuses[b]				
Number of live fetuses	599	300	270	259
Live fetuses per litter	13.61 ± 0.30 (44)	14.29 ± 0.37 (21)	12.86 ± 0.62 (21)	12.95 ± 0.91 (20)
Live male fetuses per litter	6.48 ± 0.28 (44)	7.57 ± 0.41 (21)	6.71 ± 0.54 (21)	6.60 ± 0.54 (20)
Live female fetuses per litter	7.14 ± 0.30 (44)	6.71 ± 0.52 (21)	6.10 ± 0.46 (21)	6.35 ± 0.66 (20)
Percent live male fetuses per litter	47.71 ± 1.88 (44)	53.48 ± 3.05 (21)	50.51 ± 3.79 (21)	50.04 ± 3.96 (20)
Fetal Weight[c]				
Fetal weight per litter (g)	5.29 ± 0.04 (44)	5.22 ± 0.06 (21)	5.42 ± 0.08 (21)	5.22 ± 0.07 (20)
Male fetal weight per litter (g)	5.42 ± 0.05 (44)	5.35 ± 0.06 (21)	5.47 ± 0.06 (20)	5.34 ± 0.06 (19)
Female fetal weight per litter (g)	5.17 ± 0.04 (44)	5.08 ± 0.06 (21)	5.30 ± 0.09 (21)	5.09 ± 0.07 (20)
Gravid Uterine Weight[d]				
Gravid uterine weight (g)	98.89 ± 1.93 (44)	101.98 ± 2.23 (21)	95.76 ± 4.06 (21)	91.78 ± 5.91 (20)
Terminal body weight (g)	378.5 ± 3.0 (44)	382.7 ± 4.0 (21)	383.1 ± 5.2 (21)	375.2 ± 8.3 (20)
Adjusted body weight (g)	279.56 ± 1.81 (44)	280.75 ± 2.23 (21)	287.30 ± 2.38 (21)	283.45 ± 3.85 (20)

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

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

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

[c] Statistical analysis performed using a mixed effects linear model with litter as a random effect (trend and pairwise)

[d] Statistical analysis performed by Jonckheere's (trend) and Williams' or Dunnett's (pairwise) tests; adjusted body weight = terminal body weight minus gravid uterine weight

Fetal Findings

External

In the external exam, various low or single-incidence findings were observed in the head and placenta which were considered unrelated to exposure. The only malformation, a meningoencephalocele in the 162.5 mg/kg group, was also considered unrelated to TCPP exposure due to the single incidence and lack of a dose response (Tables B5 and B6).

Visceral

Malformations and associated variations were observed in the ureter, which included hydroureter malformations (bilateral and unilateral) in the vehicle control and 325 mg/kg groups and distention of the ureter (bilateral and unilateral) across all groups. Although associated, these findings were not considered exposure related because the incidences of findings in groups exposed to TCPP were either similar to or lower than the incidences in vehicle control animals. Various other single-incidence malformations and variations were observed in the abdomen, heart, and thorax following the visceral exam; these findings were either not considered exposure-related or only observed in vehicle control animals. Overall, there were no effects of TCPP exposure on the incidences of fetal visceral abnormalities (Tables 13, B5, and B6).

TABLE 13
Summary of Selected Visceral Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg		162.5 mg/kg		325 mg/kg		650 mg/kg	
Total number of fetuses	599		300		270		259	
Visceral								
Number of fetuses examined	599		299		270		259	
Number of litters examined	44		21		21		20	
Pelvis								
Ureter								
Total, hydroureter — [M]								
Fetuses	1	(0.17)	0	(0.00)	1	(0.37)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	1	(4.76)	0	(0.00)
Bilateral, hydroureter — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Left, hydroureter — [M]								
Fetuses	0	(0.00)	0	(0.00)	1	(0.37)	0	(0.00)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Total, distended — [V]								
Fetuses	92	(15.36)**#	35	(11.71)	14	(5.19)**##	24	(9.27)**
Litters	30	(68.18)	15	(71.43)	5	(23.81)**	12	(60.00)
Bilateral, distended — [V]								
Fetuses	47	(7.85)**#	14	(4.68)*	7	(2.59)**#	10	(3.86)*
Litters	19	(43.18)	10	(47.62)	3	(14.29)*	6	(30.00)
Left, distended — [V]								
Fetuses	22	(3.67)	9	(3.01)	4	(1.48)	9	(3.47)
Litters	16	(36.36)	8	(38.10)	3	(14.29)	7	(35.00)
Right, distended — [V]								
Fetuses	23	(3.84)*	12	(4.01)	3	(1.11)*#	5	(1.93)
Litters	15	(34.09)	4	(19.05)	1	(4.76)**	4	(20.00)

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 the litter effects) were performed by Cochran-Armitage (trend) and Fisher 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 single incidences of malformations in the head soft tissue including enlarged nasal sinus, anophthalmia, and folded retina which were noted in vehicle control animals and groups exposed to TCPP (Tables B5 and B6). The only variation, enlarged lateral ventricle of the brain, was observed in the same fetus with anophthalmia and meningoencephalocele in the 162.5 mg/kg group. Overall, there were no effects of TCPP exposure on the incidences of fetal head abnormalities (Tables B5 and B6).

Skeletal

Skeletal malformations including discontinuous rib cartilage and full lumbar ribs were observed in TCPP exposed animals. However, the incidences of these findings were low and considered not exposure related (Tables 14, B5, and B6). Associated skeletal variations observed in TCPP-treated groups included incomplete ossification of the sternebrae (II and V), floating extra rib, rudimentary ribs (lumbar I), and bipartite or dumbbell ossification of the thoracic centrum. There was a statistically significant increase (trend and pairwise comparison) for the percent of fetuses with rudimentary ribs in all TCPP groups (22%, 23%, 22%) compared to the concurrent controls (14%). While this finding appears to be exposure related, it is not dose dependent. This lack of dose response, a variation of limited biological significance, and lack of any other related effect suggests that it is not toxicologically relevant. Overall, examination of the fetal skeleton for osseous and cartilaginous defects of the skull (~50% of fetuses) and body-only (100% of the fetuses) was not suggestive of a TCPP-related effect (Tables B5 and B6).

TABLE 14
Summary of Selected Skeletal Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg		162.5 mg/kg		325 mg/kg		650 mg/kg	
Total number of fetuses	599		300		270		259	
Skeletal: Body								
Number of fetuses examined	599		300		270		259	
Number of litters examined	44		21		21		20	
Thoracic vertebrae – thoracic centrum								
Fused — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Bipartite ossification, bipartite cartilage — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Unilateral ossification, bipartite cartilage — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Unossified, bipartite cartilage — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Bipartite ossification, normal cartilage — [V]								
Fetuses	2	(0.33)	3	(1.00)	1	(0.37)	1	(0.39)
Litters	2	(4.55)	2	(9.52)	1	(4.76)	1	(5.00)
Bipartite ossification, dumbbell cartilage — [V]								
Fetuses	1	(0.17)	0	(0.00)	2	(0.74)	1	(0.39)
Litters	1	(2.27)	0	(0.00)	2	(9.52)	1	(5.00)
Bipartite ossification, normal or dumbbell cartilage — [V]								
Fetuses	3	(0.50)	3	(1.00)	3	(1.11)	2	(0.77)
Litters	3	(6.82)	2	(9.52)	3	(14.29)	1	(5.00)
Dumbbell ossification, normal cartilage — [V]								
Fetuses	7	(1.17)	4	(1.33)	4	(1.48)	7	(2.70)
Litters	6	(13.64)	3	(14.29)	4	(19.05)	3	(15.00)
Dumbbell ossification, dumbbell cartilage — [V]								
Fetuses	3	(0.50)	1	(0.33)	2	(0.74)	2	(0.77)
Litters	2	(4.55)	1	(4.76)	2	(9.52)	2	(10.00)
Dumbbell ossification, normal or dumbbell cartilage — [V]								
Fetuses	10	(1.67)	5	(1.67)	6	(2.22)	9	(3.47)
Litters	8	(18.18)	4	(19.05)	5	(23.81)	4	(20.00)
Cartilage, normal ossification, dumbbell cartilage — [V]								
Fetuses	0	(0.00)	1	(0.33)	0	(0.00)	0	(0.00)
Litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)
Ribs								
Cartilage, discontinuous — [M]								
Fetuses	0	(0.00)	1	(0.33)	0	(0.00)	0	(0.00)
Litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)
Cartilage, VIII attached to sternum — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Discontinuous — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Floating extra — [V]								
Fetuses	0	(0.00)	0	(0.00)	1	(0.37)	0	(0.00)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)

TABLE 14
Summary of Selected Skeletal Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg		162.5 mg/kg		325 mg/kg		650 mg/kg	
Total number of fetuses	599		300		270		259	
Skeletal: Body (continued)								
Number of fetuses examined	599		300		270		259	
Number of litters examined	44		21		21		20	
Ribs (continued)								
Left, intercostal rib — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Lumbar I full — [M]								
Fetuses	4	(0.67)	4	(1.33)	2	(0.74)	3	(1.16)
Litters	4	(9.09)	3	(14.29)	1	(4.76)	2	(10.00)
Lumbar I rudimentary[a] — [V]								
Fetuses	82	(13.69)**#	65	(21.67)**	61	(22.59)**#	56	(21.62)**
Litters	29	(65.91)	17	(80.95)	17	(80.95)	15	(75.00)
Sternebrae								
Sternebra II, incomplete ossification — [V]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Sternebra V, incomplete ossification —[V]								
Fetuses	2	(0.33)	1	(0.33)	4	(1.48)	0	(0.00)
Litters	2	(4.55)	1	(4.76)	3	(14.29)	0	(0.00)
Sternebra(e), extra ossification site between sternebrae — [V]								
Fetuses	3	(0.50)	1	(0.33)	0	(0.00)	0	(0.00)
Litters	2	(4.55)	1	(4.76)	0	(0.00)	0	(0.00)
Sternebra(e), total, incomplete ossification — [V]								
Fetuses	3	(0.50)	1	(0.33)	4	(1.48)	0	(0.00)
Litters	3	(6.82)	1	(4.76)	3	(14.29)	0	(0.00)
Sternebra(e), misaligned (>2, not V) — [V]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	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 the litter effects) performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests
** Statistically significant (P≤0.01) trend (denoted in vehicle control column) or pairwise comparison (denoted in dosed group column) in litter-based analysis of fetuses.
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.
[M] = Malformation
[V] = Variation
[a] Historical incidence for all routes: Fetuses 114/1,385 (8.23%), range 3.35%-13.69%; Litters 53/97 (54.64%), range 26.32%-65.91%.

DISCUSSION AND CONCLUSIONS

Tris(chloropropyl) phosphate (TCPP) is a high production flame retardant mixture for use in textiles, furniture (flexible polyurethane foam), construction materials (rigid polyurethane foam), electronic products, paints, coatings, and adhesives (Marklund *et al.*, 2003; USEPA, 2015b). TCPP has been proposed as a substitute for brominated flame retardants as well as a replacement for other chlorinated flame retardants such as tris(2-chloroethyl) phosphate which have been identified to be toxic (Wilczynski *et al.*, 1983; WHO, 1998). Based on the potential for increased use and exposure, the Consumer Product Safety Commission nominated TCPP for toxicological testing by the NTP. The NTP is in the process of evaluating TCPP toxicity on various cellular or molecular targets *in vitro* and is testing for subchronic toxicity, chronic toxicity, carcinogenicity, genotoxicity, and immunotoxicity in rodent models. Further information on the NTP's evaluation of the potential toxicity of TCPP is available at the Program's website (NTP, 2016a); however, the purpose of this report is to summarize and discuss TCPP effects on prenatal development in rats due to concerns for exposure to women of childbearing potential and the lack of robust evaluations for developmental toxicity. The current Technical Report presents the findings of the dose range-finding and prenatal developmental toxicity studies of TCPP in Hsd:Harlan Sprague Dawley SD rats.

TCPP doses selected for the range-finding study were 0, 300, 650, 1,000 mg/kg. Maternal toxicity was observed, as evidenced by mortality and morbidity in seven of 11 dams in the 1,000 mg/kg group. In previously conducted studies, TCPP did not affect survival of Wistar Han rats exposed to as much as 1,000 mg TCPP/kg body weight per day in the diet over two generations (EU, 2008; USEPA, 2015a). TCPP also did not affect survival in a developmental toxicity study of Wistar rats given TCPP in their diet (up to 893 mg/kg per day) (Kawasaki *et al.*, 1982; USEPA, 2015a). These data suggest that the route of administration of TCPP could have contributed to maternal toxicity observed in the current range-finding study. Other differences in the exposure paradigms such as exposure duration and strain differences may also have contributed to toxicity.

In the current dose range-finding study, treatment-related clinical observations are reported in dams exposed to 1,000 mg/kg TCPP. These included convulsions, tremors, prone, gasping, hypoactivity, hunched posture, nasal discharge, stained fur, piloerection, salivation, and rooting (pre- and post-dosing) which occurred throughout gestation. One dam in the 650 mg/kg dose range-finding study was euthanized moribund following adverse clinical observations such as ataxia, hypoactivity, piloerection, labored respiration, pale and cold to touch. Due to the singular incidence of overt toxicity and adverse clinical observations in the 650 mg/kg group, the relationship between TCPP exposure and maternal toxicity at this dose was considered uncertain. These findings are consistent with reported clinical observations from acute oral (gavage) toxicity studies which are summarized in the European Union Risk Assessment Report of TCPP (EU, 2008).

Several of the clinical observations (i.e., convulsions, tremors, etc.) observed in the dose range-finding study indicate that high doses of TCPP may be neurotoxic in rats. TCPP belongs to the flame retardant class of organophosphate flame retardants (OPFRs) which are structurally similar to organophosphate pesticides, many of which have previously been shown to affect the nervous system (Slotkin *et al.*, 2009; Slotkin and Seidler, 2011; Munoz-Quezada *et al.*, 2013; Carr *et al.*, 2014). *In vitro* studies in neuronal cells suggest that flame retardants with an organophosphate backbone (i.e., OPFRs) may be toxic to adult and developing nervous systems (Dishaw *et al.*, 2011; Behl *et al.*, 2015), developing zebrafish (Dishaw *et al.*, 2014; Oliveri *et al.*, 2015), and *Caenorhabditis elegans* (Behl *et al.*, 2016; Xu *et al.*, 2017). However, the clinical observations of neurotoxicity observed in the range-finding study were not observed in a rodent developmental toxicity study in the published literature (Kawasaki *et al.*, 1982), possibly due to TCPP administration through feed. Likewise, clinical signs of neurotoxicity were not observed in several repeat dose or reproduction feed studies summarized by risk assessment documents or reported in the literature (EU, 2008; OECD, 2000; USEPA, 2015a; NTP, 2016a). These data suggest that neurological clinical observations are limited to instances of high bolus doses of TCPP.

Based on the dose range-finding study results demonstrating overt maternal toxicity (i.e., mortality) at 1,000 mg/kg, the TCPP doses selected for the current prenatal developmental toxicity study were 0, 162.5, 325, or 650 mg/kg. As predicted, TCPP was well tolerated at all administered doses during the study which allowed for definitive evaluation of TCPP effects on embryo-fetal development. There was no significant effect on maternal survival,

body weights, or feed consumption in the prenatal developmental toxicity study of TCPP. Also as anticipated, adverse clinical observations were observed in 650 mg/kg dams, but not in dams exposed to lower doses. These clinical observations were of low incidence (6 of 25 dams) and short duration (~1 to 3 days) but were considered treatment related. The clinical observations were also similar to those observed in the range-finding study which supports a dose-related effect of TCPP exposure. At necropsy, several organ weights were evaluated and there were dose-related increases in absolute and relative liver weights. Multiple studies have shown that TCPP exposure can increase liver weights and alter liver histopathology in rats and mice following repeat exposures (NRC, 2000; EU, 2008; USEPA, 2015a; NTP, 2016b). Therefore, these results are concordant with previous research and confirm that the liver is a target following TCPP exposure *in vivo*.

Due to excess maternal toxicity in the 1,000 mg/kg group of the dose range-finding study, assessment of developmental toxicity was not possible at this dose. There were some indications of TCPP-related fetal effects in the 650 mg/kg group but these findings were either not statistically significant (increased mean percent post-implantation loss, decreased number of live fetuses per litter, and decreased gravid uterine weight) or of no biological significance due to the magnitude of the effect (i.e., decreased mean fetal body weight per litter). Increasing the number of animals examined from 11 to 25 for the prenatal developmental toxicity study confirmed that TCPP exposure during gestation does not have a significant effect on embryo-fetal toxicity. Overall, there was no evidence of growth retardation following TCPP exposure.

Examination of fetuses for malformations or variations in the prenatal developmental toxicity study demonstrated that TCPP exposure does not cause toxicologically significant external, visceral, or skeletal defects. The only statistically significant exposure-related finding was an increase in the percent of fetuses with lumbar rudimentary ribs. In the NTP's experience, this skeletal variation is variable among this strain of rat. It has been reported that rudimentary ribs are a common finding in laboratory rodents, are considered reversible, and are of limited toxicologic relevance (Wickramaratne, 1988; Foulon *et al.*, 2000).

In the previously published developmental toxicity study in rats (Kawasaki *et al.*, 1982), dose-related increases in the incidences of cervical ribs and absent 13th rib malformations were reported. TCPP exposure in the study by

Kawasaki and colleagues (1982) occurred in feed from gestation days 0 to 20 and was conducted in Wistar rats. These experimental variables (exposure window, route of administration, and rodent strain) may account for the differences in skeletal malformations observed between the Kawasaki *et al.* (1982) report and the prenatal developmental toxicity study described here.

CONCLUSIONS

Under the conditions of this prenatal study, there was *no evidence of developmental toxicity* of TCPP in Hsd:Sprague Dawley SD rats administered 162.5, 325, or 650 mg/kg in the absence of overt maternal toxicity.

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APPENDIX A
SUMMARY OF FINDINGS IN RATS IN THE DOSE RANGE-FINDING GAVAGE STUDY OF TRIS(CHLOROPROPYL) PHOSPHATE

TABLE A1 Summary of Clinical Observations for Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate A-2

TABLE A2 Summary of Maternal Body Weights of Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate A-3

TABLE A3 Summary of Gross Pathology Findings in Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate A-4

TABLE A4 Summary of Fetal External Findings in Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate A-5

TABLE A5 Summary of Total Fetal Findings in Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate A-5

TABLE A6 Fetal Findings Cross Reference of Dams and Fetuses in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate A-6

TABLE A1
Summary of Clinical Observations for Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate[a]

	0 mg/kg	300 mg/kg	650 mg/kg	1,000 mg/kg
Pregnant Rats				
n	10	11	8	10
Ataxia, moderate	0	0	1 (GD 16)	0
Cold to touch	0	0	1 (GD 16)	0
Convulsion	0	0	0	2 (GD 6)
Right eye, discharge, black	0	1 (GD 19)	0	0
Fur, head, stained, red	0	0	1 (GD 8)	1 (GD 8)
Gasping	0	0	0	2 (GD 20)
Hunched	0	0	0	1 (GD 7)
Hypoactive	0	0	1 (GD 16)	2 (GD 6)
Nasal discharge, clear	0	0	0	1 (GD 7)
Nasal discharge, red	0	1 (GD 7)	0	4 (GD 6)
Pale	0	0	1 (GD 16)	0
Piloerection	0	0	1 (GD 16)	4 (GD 6)
Prone	0	0	0	2 (GD 6)
Respiration, labored	0	0	1 (GD 16)	0
Rooting, post dosing	0	0	1 (GD 19)	3 (GD 19)
Rooting, prior to dosing	0	0	0	3 (GD 8)
Salivation, post dosing	0	0	0	6 (GD 6)
Sore(s) on body, head	0	1 (GD 10)	0	0
Tremors, head	0	0	0	4 (GD 6)
Tremors, left forepaw	0	0	0	3 (GD 20)
Tremors, right forepaw	0	0	0	3 (GD 20)
Non-pregnant Rats				
n	1	0	3	1
Found dead	0	0	0	1 (SD 6)
Rooting, post dosing	0	0	1 (SD 19)	0
Salivation, post dosing	0	0	0	1 (SD 6)
Tremors, head	0	0	0	1 (SD 6)

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

TABLE A2
Summary of Maternal Body Weights of Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate[a]

	0 mg/kg		300 mg/kg			650 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	227.7 ± 4.6	10	227.6 ± 3.6	99.96	11	228.7 ± 3.8	100.44	8	226.5 ± 3.2	99.47	10
GD 4	235.8 ± 3.6	10	235.9 ± 3.1	100.04	11	236.4 ± 3.1	100.25	8	233.5 ± 3.3	99.02	10
GD 5	239.4 ± 3.7	10	239.9 ± 3.5	100.21	11	240.5 ± 3.0	100.46	8	236.5 ± 3.4	98.79	10
GD 6	244.2 ± 4.1	10	242.4 ± 3.4	99.26	11	241.8 ± 3.4	99.02	8	237.3 ± 3.2	97.17	10
GD 7	247.0 ± 4.1	10	244.7 ± 3.7	99.07	11	245.7 ± 3.7	99.47	8	241.1 ± 2.9	97.61	9
GD 8	250.5 ± 3.9	10	251.1 ± 4.0	100.24	11	251.4 ± 4.3	100.36	8	247.2 ± 3.3	98.68	9
GD 9	255.6 ± 4.3	10	255.2 ± 4.1	99.84	11	256.9 ± 4.4	100.51	8	251.0 ± 3.9	98.20	9
GD 10	259.0 ± 4.1	10	258.1 ± 3.8	99.65	11	260.8 ± 4.4	100.69	8	255.7 ± 4.0	98.73	9
GD 11	268.4 ± 4.1	10	266.1 ± 3.8	99.14	11	266.9 ± 4.4	99.44	8	260.3 ± 3.9	96.98	9
GD 12	274.1 ± 4.2	10	271.0 ± 4.0	98.87	11	272.9 ± 4.6	99.56	8	264.8 ± 4.7	96.61	9
GD 13	277.6 ± 4.2	10	274.4 ± 4.5	98.85	11	277.6 ± 4.6	100.00	8	270.5 ± 4.8	97.44	9
GD 14	284.3 ± 4.3	10	281.4 ± 4.0	98.98	11	283.6 ± 5.2	99.75	8	274.7 ± 4.7	96.62	7
GD 15	293.4 ± 4.6	10	289.2 ± 4.2	98.57	11	290.6 ± 6.1	99.05	8	279.7 ± 4.0	95.33	7
GD 16	302.4 ± 4.9	10	300.1 ± 4.9	99.24	11	297.8 ± 6.8	98.48	8	290.7 ± 4.9	96.13	7
GD 17	316.2 ± 4.8	10	313.8 ± 5.0	99.24	11	307.9 ± 11.3	97.38	7	304.1 ± 4.7	96.17	7
GD 18	332.2 ± 5.8	10	330.8 ± 5.6	99.58	11	321.7 ± 15.9	96.84	7	319.0 ± 4.5	96.03	7
GD 19	343.1 ± 6.7	10	345.1 ± 5.9	100.58	11	333.6 ± 19.3	97.23	7	335.6 ± 6.1	97.81	7
GD 20	364.5 ± 5.9	10	363.4 ± 6.2	99.70	11	350.4 ± 19.8	96.13	7	349.7 ± 7.3	95.94	7
GD 21	380.3 ± 6.0	10	380.9 ± 6.7	100.16	11	368.5 ± 20.1	96.90	7	369.7 ± 12.4	97.21	4

[a] Data are displayed as mean ± standard error by gestation day (GD). No statistically significant trends (by Jonckheere's test) nor 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 Tris(chloropropyl) Phosphate[a]

	Vehicle Control	300 mg/kg	650 mg/kg	1,000 mg/kg
Disposition Summary				
Animals initially in study	11	11	11	11
Early deaths				
Moribund			1	5
Natural deaths				2
Survivors				
Terminal euthanasia	11	11	10	4
Number of animals examined			1	3
Alimentary System				
Stomach	(0)	(0)	(0)	(1)
Gas				1
Cardiovascular System				
Heart	(0)	(0)	(1)	(0)
Enlarged; atrium; moderate, right			1	
Endocrine System				
Adrenal gland (paired)	(0)	(0)	(0)	(2)
Discoloration, mild, pale				1
Discoloration, moderate, mottled				1
Adrenal glands	(0)	(0)	(1)	(0)
Enlarged, mild			1	
General Body System				
None				
Genital System				
Uterus	(0)	(0)	(1)	(0)
Fluid; red			1	
Hematopoietic System				
None				
Integumentary System				
None				
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
None				
Special Senses System				
None				
Urinary System				
None				

[a] Number of tissues 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 Tris(chloropropyl) Phosphate

	0 mg/kg	300 mg/kg	650 mg/kg	1,000 mg/kg
Number of fetuses examined	136	147	83	42
External				
Number of fetuses examined	136	147	83	42
Number of litters examined	10	11	7	4
General				
Torso, subcutaneous hemorrhage — [V]				
Fetuses	0 (0.00)*	0 (0.00)	2 (2.41)	1 (2.38)
Litters	0 (0.00)*	0 (0.00)	2 (28.57)	1 (25.00)

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 the litter effects) were performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests.
* Statistically significant trend at P≤0.05
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression models, where the dam identification was the random effect, found no statistically significant trend or pairwise comparison.
[V] = Variation

TABLE A5
Summary of Total Fetal Findings in Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	300 mg/kg	650 mg/kg	1,000 mg/kg
All Exams				
Number of fetuses	136	147	83	42
Number of litters	10	11	7	4
Variation				
Affected fetuses	0 (0.00)*	0 (0.00)	2 (2.41)	1 (2.38)
Affected litters	0 (0.00)*	0 (0.00)	2 (28.57)	1 (25.00)
External				
Number of fetuses	136	147	83	42
Number of litters	10	11	7	4
Variation				
Affected fetuses	0 (0.00)*	0 (0.00)	2 (2.41)	1 (2.38)
Affected litters	0 (0.00)*	0 (0.00)	2 (28.57)	1 (25.00)

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 the litter effects) were performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests.
* Statistically significant trend at P≤0.05
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression models, where the dam identification was the random effect, found no statistically significant trend or pairwise comparison.

TABLE A6
Fetal Findings Cross Reference of Dams and Fetuses in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	300 mg/kg	650 mg/kg	1,000 mg/kg
Number of fetuses examined	136	147	83	42
Number of dams examined	10	11	7	4
External				
Number of fetuses examined	136	147	83	42
Number of dams examined	10	11	7	4
General				
Torso, subcutaneous hemorrhage — [V]			28 (8) 32 (10)	43 (8)

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

APPENDIX B
SUMMARY OF FINDINGS IN RATS IN THE PRENATAL DEVELOPMENTAL TOXICITY GAVAGE STUDY OF TRIS(CHLOROPROPYL) PHOSPHATE

TABLE B1 Summary of Clinical Observations for Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate B-2
TABLE B2 Summary of Maternal Body Weights of Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate B-3
TABLE B3 Summary of Gross Pathology Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate B-4
TABLE B4 Summary of Maternal Organ Weights and Organ Weight Ratios for Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate B-5
TABLE B5 Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate B-6
TABLE B6 Summary of Total Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate B-10
TABLE B7 Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate B-12

TABLE B1
Summary of Clinical Observations for Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate[a]

	0 mg/kg		162.5 mg/kg	325 mg/kg	650 mg/kg	
Pregnant Rats						
n	44		21	21	20	
Ataxia, mild	0		0	0	1	(GD 6)
Right eye, discharge, black	1	(GD 19)	0	0	0	
Hyperactive	0		0	0	1	(GD 7)
Nasal discharge, brown	0		0	0	3	(GD 6)
Piloerection	0		0	0	1	(GD 6)
Respiration, audible	0		0	0	2	(GD 16)
Salivation, post dosing	0		0	0	1	(GD 17)
Twitches	0		0	0	1	(GD 17)
Non-pregnant Rats						
n	6		4	4	5	
Eye discharge, bilateral, black	0		0	0	1	(SD 11)
Hunched	0		0	0	1	(SD 9)
Hyperactive	0		0	0	1	(SD 8)
Piloerection	0		0	0	1	(SD 7)
Prone	0		0	0	1	(SD 11)
Respiration, audible	0		0	0	1	(SD 17)
Salivation, moderate	0		0	0	1	(SD 7)
Tremors, head	0		0	0	1	(SD 7)
Tremors, left forelimb	0		0	0	1	(SD 7)
Tremors, right forelimb	0		0	0	1	(SD 7)
Twitches	0		0	0	1	(SD 7)

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

TABLE B2
Summary of Maternal Body Weights of Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate[a]

	0 mg/kg		162.5 mg/kg			325 mg/kg			650 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	228.2 ± 1.5	44	228.7 ± 2.2	100.22	21	228.7 ± 2.2	100.22	21	227.7 ± 2.7	99.78	20
GD 4	232.2 ± 1.6	44	232.2 ± 2.1	100.00	21	233.5 ± 2.3	100.56	21	231.5 ± 2.7	99.70	20
GD 5	237.9 ± 1.5	44	237.7 ± 2.0	99.92	21	238.9 ± 2.4	100.42	21	237.0 ± 2.8	99.62	20
GD 6	242.7 ± 1.6	44	242.8 ± 2.3	100.04	21	244.5 ± 2.7	100.74	21	241.8 ± 3.0	99.63	20
GD 7	246.0 ± 1.6	44	245.1 ± 1.9	99.63	21	245.7 ± 2.4	99.88	21	240.0 ± 2.8	97.56	20
GD 8	249.8 ± 1.5	44	249.3 ± 2.2	99.80	21	251.8 ± 2.5	100.80	21	248.0 ± 2.9	99.28	20
GD 9	255.6 ± 1.6	44	255.8 ± 2.2	100.08	21	257.0 ± 2.6	100.55	21	252.4 ± 3.2	98.75	20
GD 10	260.8 ± 1.6	44	260.9 ± 2.1	100.04	21	262.6 ± 2.7	100.69	21	258.0 ± 2.9	98.93	20
GD 11	266.9 ± 1.7	44	266.1 ± 2.6	99.70	21	268.1 ± 2.8	100.45	21	264.0 ± 3.2	98.91	20
GD 12	272.3 ± 1.7	44	271.3 ± 2.7	99.63	21	273.8 ± 2.8	100.55	21	267.8 ± 3.5	98.35	20
GD 13	278.0 ± 1.8	44	278.1 ± 2.6	100.04	21	279.1 ± 2.8	100.40	21	273.0 ± 3.6	98.20	20
GD 14	282.3 ± 1.8	44	282.6 ± 2.5	100.11	21	285.2 ± 2.9	101.03	21	280.4 ± 3.4	99.33	20
GD 15	291.2 ± 1.9	44	291.8 ± 2.6	100.21	21	293.4 ± 3.4	100.76	21	289.0 ± 4.0	99.24	20
GD 16	302.6 ± 2.1	44	303.5 ± 2.9	100.30	21	304.1 ± 3.3	100.50	21	300.1 ± 4.6	99.17	20
GD 17	316.4 ± 2.2	44	317.8 ± 2.9	100.44	21	318.8 ± 3.5	100.76	21	314.6 ± 6.1	99.43	20
GD 18	333.5 ± 2.4	44	335.5 ± 3.4	100.60	21	335.3 ± 4.1	100.54	21	328.0 ± 5.8	98.35	20
GD 19	348.6 ± 2.5	44	351.7 ± 3.7	100.89	21	349.6 ± 4.3	100.29	21	343.4 ± 6.5	98.51	20
GD 20	366.0 ± 3.0	44	369.0 ± 4.0	100.82	21	366.4 ± 4.7	100.11	21	361.1 ± 7.2	98.66	20
GD 21	382.3 ± 3.1	44	386.3 ± 4.2	101.05	21	384.3 ± 5.1	100.52	21	379.4 ± 8.2	99.24	20

[a] Data are displayed as mean ± standard error by gestation day (GD). No statistically significant trends (by Jonckheere's test) nor 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 Tris(chloropropyl) Phosphate[a]

	0 mg/kg	162.5 mg/kg	325 mg/kg	650 mg/kg
Disposition Summary				
Animals initially in study	50	25	25	25
Survivors				
Terminal euthanasia	50	25	25	25
Number of animals examined	50	25	25	25
Alimentary System				
None				
Cardiovascular System				
None				
Endocrine System				
None				
General Body System				
Complete gross exam	(50)	(25)	(25)	(25)
Exam complete, findings listed	2	1	1	
Genital System				
Cervix	(1)	(0)	(0)	(0)
Cyst, cloudy	1			
Hematopoietic System				
None				
Integumentary System				
None				
Musculoskeletal System				
Skeletal muscle, quadriceps femoris	(1)	(0)	(0)	(0)
Mass, right	1			
Nervous System				
None				
Respiratory System				
Lungs	(0)	(1)	(1)	(0)
Discoloration, mild, mottled		1	1	
Special Senses System				
None				
Urinary System				
None				

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

TABLE B4
Summary of Maternal Organ Weights and Organ Weight Ratios for Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate[a]

	0 mg/kg	162.5 mg/kg	325 mg/kg	650 mg/kg
n	44	21	21	20
Necropsy body wt.	378.5 ± 3.0	382.7 ± 4.0	383.1 ± 5.2	375.2 ± 8.3
Adrenal Glands				
Absolute	0.0727 ± 0.0014	0.0756 ± 0.0023	0.0751 ± 0.0025	0.0780 ± 0.0029
Relative	0.19 ± 0.00	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.01
Liver				
Absolute	14.35 ± 0.19**	15.62 ± 0.32**	16.58 ± 0.27**	18.02 ± 0.56**
Relative	37.93 ± 0.41**	40.78 ± 0.67**	43.39 ± 0.78**	48.09 ± 1.09**
R. Ovary				
Absolute	0.0953 ± 0.0029	0.0937 ± 0.0037	0.0965 ± 0.0042	0.0890 ± 0.0036
Relative	0.25 ± 0.01	0.25 ± 0.01	0.25 ± 0.01	0.24 ± 0.01
L. Ovary				
Absolute	0.0941 ± 0.0025	0.0924 ± 0.0026	0.0902 ± 0.0036	0.0976 ± 0.0042
Relative	0.25 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.26 ± 0.01

** Statistically significant ($P \leq 0.01$) trend (by Jonckheere's test) or pairwise comparison (by Williams' or Dunnett's test). A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.

[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight. Data are displayed as mean ± standard error.

TABLE B5
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg		162.5 mg/kg		325 mg/kg		650 mg/kg	
Number of fetuses examined	599		300		270		259	
External								
Number of fetuses examined	588		297		270		259	
Number of litters examined	43		21		21		20	
Head								
Cranium, meningoencephalocele — [M]								
Fetuses	0	(0.00)	1	(0.34)	0	(0.00)	0	(0.00)
Litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)
Placenta								
Placenta, discolored — [GF]								
Fetuses	0	(0.00)	0	(0.00)	0	(0.00)	1	(0.39)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(5.00)
Placenta, enlarged — [GF]								
Fetuses	0	(0.00)	0	(0.00)	0	(0.00)	1	(0.39)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(5.00)
Placenta, fused — [GF]								
Fetuses	2	(0.34)	2	(0.67)	0	(0.00)	2	(0.77)
Litters	1	(2.33)	1	(4.76)	0	(0.00)	1	(5.00)
Visceral								
Number of fetuses examined	599		299		270		259	
Number of litters examined	44		21		21		20	
Abdomen								
Kidney, left, accessory kidney without ureter — [M]								
Fetuses	0	(0.00)	1	(0.33)	0	(0.00)	0	(0.00)
Litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)
Kidney, right, agenesis — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Renal pelvis, right, dilated — [V]								
Fetuses	2	(0.33)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	2	(4.55)	0	(0.00)	0	(0.00)	0	(0.00)
General								
Abdomen, right side, mass, encapsulated, discolored — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Heart								
Ventricular septum, septum defect — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Pelvis								
Ureter, total, distended — [V]								
Fetuses	92	(15.36)**#	35	(11.71)	14	(5.19)**##	24	(9.27)**
Litters	30	(68.18)	15	(71.43)	5	(23.81)**	12	(60.00)
Ureter, total, hydroureter — [M]								
Fetuses	1	(0.17)	0	(0.00)	1	(0.37)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	1	(4.76)	0	(0.00)
Ureter, bilateral, distended — [V]								
Fetuses	47	(7.85)**#	14	(4.68)*	7	(2.59)**#	10	(3.86)*
Litters	19	(43.18)	10	(47.62)	3	(14.29)*	6	(30.00)
Ureter, bilateral, hydroureter — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)

TABLE B5
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg		162.5 mg/kg		325 mg/kg		650 mg/kg	
Visceral (continued)								
Number of fetuses examined	599		299		270		259	
Number of litters examined	44		21		21		20	
Pelvis (continued)								
Ureter, left, distended — [V]								
Fetuses	22	(3.67)	9	(3.01)	4	(1.48)	9	(3.47)
Litters	16	(36.36)	8	(38.10)	3	(14.29)	7	(35.00)
Ureter, left, hydroureter — [M]								
Fetuses	0	(0.00)	0	(0.00)	1	(0.37)	0	(0.00)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Ureter, right, distended — [V]								
Fetuses	23	(3.84)*	12	(4.01)	3	(1.11)*#	5	(1.93)
Litters	15	(34.09)	4	(19.05)	1	(4.76)**	4	(20.00)
Thorax (excluding heart)								
Aorta, malpositioned — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Aortic arch, agenesis — [M]								
Fetuses	1	(0.17)	1	(0.33)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	1	(4.76)	0	(0.00)	0	(0.00)
Ductus arteriosus, agenesis — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Innominate artery, agenesis — [V]								
Fetuses	9	(1.50)	2	(0.67)	1	(0.37)	4	(1.54)
Litters	7	(15.91)	2	(9.52)	1	(4.76)	4	(20.00)
Lung, left lobe, small — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Head								
Number of fetuses examined	299		148		138		121	
Number of litters examined	44		21		21		18	
Eyes								
Eye, bilateral, anophthalmia — [M]								
Fetuses	0	(0.00)	1	(0.68)	0	(0.00)	0	(0.00)
Litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)
Retina, bilateral, folded — [M]								
Fetuses	0	(0.00)	0	(0.00)	0	(0.00)	1	(0.83)
Litters	0	(0.00)	0	(0.00)	0	(0.00)	1	(5.56)
Head								
Lateral ventricle, left, enlarged half — [V]								
Fetuses	0	(0.00)	1	(0.68)	0	(0.00)	0	(0.00)
Litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)
Nasal sinus, total, enlarged — [M]								
Fetuses	1	(0.33)	1	(0.68)	1	(0.72)	0	(0.00)
Litters	1	(2.27)	1	(4.76)	1	(4.76)	0	(0.00)
Nasal sinus, bilateral, enlarged — [M]								
Fetuses	0	(0.00)	1	(0.68)	0	(0.00)	0	(0.00)
Litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)
Nasal sinus, left, enlarged — [M]								
Fetuses	1	(0.33)	0	(0.00)	1	(0.72)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	1	(4.76)	0	(0.00)

TABLE B5
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg		162.5 mg/kg		325 mg/kg		650 mg/kg	
Skeletal: Body								
Number of fetuses examined	599		300		270		259	
Number of litters examined	44		21		21		20	
Ribs								
Rib cartilage, discontinuous — [M]								
Fetuses	0	(0.00)	1	(0.33)	0	(0.00)	0	(0.00)
Litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)
Rib cartilage, VIII attached to sternum — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Rib, discontinuous — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Rib, floating extra — [V]								
Fetuses	0	(0.00)	0	(0.00)	1	(0.37)	0	(0.00)
Litters	0	(0.00)	0	(0.00)	1	(4.76)	0	(0.00)
Rib, lumbar I full — [M]								
Fetuses	4	(0.67)	4	(1.33)	2	(0.74)	3	(1.16)
Litters	4	(9.09)	3	(14.29)	1	(4.76)	2	(10.00)
Rib, lumbar I rudimentary — [V]								
Fetuses	82	(13.69)**#	65	(21.67)**	61	(22.59)**#	56	(21.62)**
Litters	29	(65.91)	17	(80.95)	17	(80.95)	15	(75.00)
Rib, left, intercostal rib — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Sternebrae								
Sternebra II, incomplete ossification — [V]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Sternebra V, incomplete ossification — [V]								
Fetuses	2	(0.33)	1	(0.33)	4	(1.48)	0	(0.00)
Litters	2	(4.55)	1	(4.76)	3	(14.29)	0	(0.00)
Sternebra(e), extra ossification site between sternebrae — [V]								
Fetuses	3	(0.50)	1	(0.33)	0	(0.00)	0	(0.00)
Litters	2	(4.55)	1	(4.76)	0	(0.00)	0	(0.00)
Sternebra(e), total, incomplete ossification — [V]								
Fetuses	3	(0.50)	1	(0.33)	4	(1.48)	0	(0.00)
Litters	3	(6.82)	1	(4.76)	3	(14.29)	0	(0.00)
Sternebra(e), misaligned (>2, not V) — [V]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Thoracic vertebrae								
Thoracic centrum cartilage, normal ossification, dumbbell cartilage — [V]								
Fetuses	0	(0.00)	1	(0.33)	0	(0.00)	0	(0.00)
Litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)
Thoracic centrum, bipartite ossification, bipartite cartilage — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Thoracic centrum, bipartite ossification, dumbbell cartilage — [V]								
Fetuses	1	(0.17)	0	(0.00)	2	(0.74)	1	(0.39)
Litters	1	(2.27)	0	(0.00)	2	(9.52)	1	(5.00)

TABLE B5
Summary of Fetal External, Visceral, Head, and Skeletal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg		162.5 mg/kg		325 mg/kg		650 mg/kg	
Skeletal: Body (continued)								
Number of fetuses examined	599		300		270		259	
Number of litters examined	44		21		21		20	
Thoracic vertebrae (continued)								
Thoracic centrum, bipartite ossification, normal cartilage — [V]								
Fetuses	2	(0.33)	3	(1.00)	1	(0.37)	1	(0.39)
Litters	2	(4.55)	2	(9.52)	1	(4.76)	1	(5.00)
Thoracic centrum, bipartite ossification, normal or dumbbell cartilage — [V]								
Fetuses	3	(0.50)	3	(1.00)	3	(1.11)	2	(0.77)
Litters	3	(6.82)	2	(9.52)	3	(14.29)	1	(5.00)
Thoracic centrum, dumbbell ossification, dumbbell cartilage — [V]								
Fetuses	3	(0.50)	1	(0.33)	2	(0.74)	2	(0.77)
Litters	2	(4.55)	1	(4.76)	2	(9.52)	2	(10.00)
Thoracic centrum, dumbbell ossification, normal cartilage — [V]								
Fetuses	7	(1.17)	4	(1.33)	4	(1.48)	7	(2.70)
Litters	6	(13.64)	3	(14.29)	4	(19.05)	3	(15.00)
Thoracic centrum, dumbbell ossification, normal or dumbbell cartilage — [V]								
Fetuses	10	(1.67)	5	(1.67)	6	(2.22)	9	(3.47)
Litters	8	(18.18)	4	(19.05)	5	(23.81)	4	(20.00)
Thoracic centrum, fused — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Thoracic centrum, unilateral ossification, bipartite cartilage — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Thoracic centrum, unossified, bipartite cartilage — [M]								
Fetuses	1	(0.17)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	0	(0.00)	0	(0.00)	0	(0.00)
Skeletal: Skull								
Number of fetuses examined	300		152		132		132	
Number of litters examined	44		21		21		20	
Skull								
Interparietal, incomplete ossification — [V]								
Fetuses	1	(0.33)	0	(0.00)	0	(0.00)	0	(0.00)
Litters	1	(2.27)	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 the litter effects) were performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
* Statistically significant at P≤0.05
** P≤0.01
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
Statistically significant at P≤0.05
P≤0.01
[M] = Malformation
[V] = Variation
[GF] = Gross Finding

TABLE B6
Summary of Total Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg		162.5 mg/kg		325 mg/kg		650 mg/kg	
All Exams								
Number of fetuses	599		300		270		259	
Number of litters	44		21		21		20	
Malformation								
Affected fetuses	11	(1.84)	8	(2.67)	4	(1.48)	4	(1.54)
Affected litters	10	(22.73)	7	(33.33)	3	(14.29)	3	(15.00)
Variation								
Affected fetuses	188	(31.39)	102	(34.00)	79	(29.26)	80	(30.89)
Affected litters	41	(93.18)	21	(100.00)	20	(95.24)	17	(85.00)
Gross finding								
Affected fetuses	2	(0.33)	2	(0.67)	0	(0.00)	3	(1.16)
Affected litters	1	(2.27)	1	(4.76)	0	(0.00)	2	(10.00)
External								
Number of fetuses	588		297		270		259	
Number of litters	43		21		21		20	
Malformation								
Affected fetuses	0	(0.00)	1	(0.34)	0	(0.00)	0	(0.00)
Affected litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)
Gross Finding								
Affected fetuses	2	(0.34)	2	(0.67)	0	(0.00)	3	(1.16)
Affected litters	1	(2.33)	1	(4.76)	0	(0.00)	2	(10.00)
Visceral								
Number of fetuses	599		299		270		259	
Number of litters	44		21		21		20	
Malformation								
Affected fetuses	4	(0.67)	2	(0.67)	1	(0.37)	0	(0.00)
Affected litters	4	(9.09)	2	(9.52)	1	(4.76)	0	(0.00)
Variation								
Affected fetuses	103	(17.20)**#	37	(12.37)*	15	(5.56)**##	28	(10.81)**
Affected litters	34	(77.27)*	16	(76.19)	6	(28.57)**	12	(60.00)
Head								
Number of fetuses	299		148		138		121	
Number of litters	44		21		21		18	
Malformation								
Affected fetuses	1	(0.33)	2	(1.35)	1	(0.72)	1	(0.83)
Affected litters	1	(2.27)	2	(9.52)	1	(4.76)	1	(5.56)
Variation								
Affected fetuses	0	(0.00)	1	(0.68)	0	(0.00)	0	(0.00)
Affected litters	0	(0.00)	1	(4.76)	0	(0.00)	0	(0.00)

TABLE B6
Summary of Total Fetal Findings in Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg		162.5 mg/kg		325 mg/kg		650 mg/kg	
Skeletal: Body								
Number of Fetuses	599		300		270		259	
Number of Litters	44		21		21		20	
Malformation								
Affected fetuses	6	(1.00)	5	(1.67)	2	(0.74)	3	(1.16)
Affected litters	6	(13.64)	4	(19.05)	1	(4.76)	2	(10.00)
Variation								
Affected fetuses	99	(16.53)**	75	(25.00)**#	70	(25.93)**#	64	(24.71)**
Affected litters	33	(75.00)	19	(90.48)	19	(90.48)	15	(75.00)
Skeletal: Skull								
Number of Fetuses	300		152		132		132	
Number of Litters	44		21		21		20	
Variation								
Affected fetuses	1	(0.33)	0	(0.00)	0	(0.00)	0	(0.00)
Affected litters	1	(2.27)	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 the litter effects) were performed by Cochran-Armitage (trend) and Fisher exact (pairwise) tests. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
* Statistically significant at P≤0.05
** P≤0.01
Statistical analysis of fetuses with litter-based adjustments performed by mixed effects logistic regression. A significant trend test is indicated in the vehicle control column. A significant pairwise comparison with the vehicle control group is indicated in the dosed group column.
\# Statistically significant at P≤0.05
P≤0.01

TABLE B7
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	162.5 mg/kg	325 mg/kg	650 mg/kg
Number of fetuses examined	599	300	270	259
Number of dams examined	44	21	21	20
External				
Number of fetuses examined	588	297	270	259
Number of dams examined	43	21	21	20
Head				
Cranium, meningoencephalocele — [M]		94 (7)		
Placenta				
Placenta, discolored — [GF]				184 (8)
Placenta, enlarged — [GF]				184 (8)
Placenta, fused — [GF]	246 (13,14)	52 (6,7)		156 (17,18)
Visceral				
Number of fetuses examined	599	299	270	259
Number of dams examined	44	21	21	20
Abdomen				
Kidney, left, accessory kidney without ureter — [M][b]		72 (13)		
Kidney, right, agenesis — [M]	210 (3)			
Renal pelvis, right, dilated — [V]	46 (8)			
	220 (5)			
General				
Abdomen, right side, mass, encapsulated, discolored — [M]	202 (5)			
Heart				
Ventricular septum, septum defect — [M]	42 (9)			

TABLE B7
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	162.5 mg/kg	325 mg/kg	650 mg/kg
Visceral (continued)				
Number of fetuses examined	599	299	270	259
Number of dams examined	44	21	21	20
Pelvis				
Ureter, bilateral, distended — [V]				
	2 (3,6)	52 (13)	118 (4,5,9,14)	156 (14)
	6 (15)	56 (3)	122 (10,12)	166 (9,10,14)
	10 (7)	58 (4,9,10)	148 (1)	178 (2,4,8)
	20 (13)	60 (4)		180 (1)
	24 (7,9,10,13)	68 (10)		182 (4)
	32 (14)	78 (10)		196 (14)
	34 (1,5,6,10,11,12,16)	80 (15)		
	38 (10)	82 (1)		
	40 (12)	88 (8,14)		
	206 (3,8,13,14)	100 (3,4)		
	212 (1)			
	228 (6,7,8)			
	232 (9,11)			
	234 (4,10,14)			
	238 (2)			
	240 (2,3,6,10)			
	242 (2,5,14)			
	246 (2,4,8)			
	248 (4,10,11,12)			
Ureter, bilateral, hydroureter — [M]				
	8 (9)			
Ureter, left, distended — [V]				
	8 (5,11,15)	52 (2)	102 (2,3)	152 (3)
	20 (11)	54 (3,10)	118 (8)	156 (9)
	26 (8)	58 (7)	138 (8)	158 (9)
	36 (1)	66 (11)		160 (2,3,4)
	40 (6)	78 (2)		166 (6)
	202 (1,6)	82 (11)		172 (13)
	204 (8,14)	84 (1)		188 (6)
	206 (5)	90 (16)		
	210 (13)			
	212 (14)			
	214 (7,12)			
	216 (10)			
	224 (11)			
	234 (8)			
	238 (4,6)			
	240 (15)			
Ureter, left, hydroureter — [M]				
			104 (11)	

TABLE B7
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	162.5 mg/kg	325 mg/kg	650 mg/kg
Visceral (continued)				
Number of fetuses examined	599	299	270	259
Number of dams examined	44	21	21	20
Pelvis (continued)				
Ureter, right, distended — [V]				
	10 (1,8)	60 (3,7)	148 (2,5,13)	178 (9,15)
	20 (16)	88 (1,10,13)		180 (4)
	24 (3)	92 (1,8,11)		192 (1)
	34 (3,7,15)	100 (1,7,10,14)		196 (7)
	38 (2,8)			
	206 (6)			
	214 (13)			
	224 (10)			
	230 (1,8)			
	232 (6)			
	234 (11,13)			
	242 (10)			
	246 (5,7)			
	248 (5,14)			
	250 (14)			
Thorax (excluding heart)				
Aorta, malpositioned — [M]				
	42 (9)			
Aortic arch, agenesis — [M]				
	42 (9)	94 (7)		
Ductus arteriosus, agenesis — [M]				
	42 (9)			
Innominate artery, agenesis — [V]				
	6 (8,13)	56 (13)	112 (11)	156 (15)
	22 (4,7)	72 (16)		158 (13)
	40 (1)			160 (1)
	42 (15)			188 (12)
	220 (8)			
	238 (12)			
	246 (6)			
Lung, left lobe, small — [M]				
	42 (9)			
Head				
Number of fetuses examined	299	148	138	121
Number of dams examined	44	21	21	18
Eyes				
Eye, bilateral, Anophthalmia — [M]				
		94 (7)		
Retina, bilateral, folded — [M]				
				168 (10)
Head				
Lateral ventricle, left, enlarged half — [V]				
		94 (7)		
Nasal sinus, bilateral, enlarged — [M]				
		62 (4)		
Nasal sinus, left, enlarged — [M]				
	202 (4)		112 (7)	

TABLE B7
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	162.5 mg/kg	325 mg/kg	650 mg/kg
Skeletal: Body				
Number of fetuses examined	599	300	270	259
Number of dams examined	44	21	21	20
Ribs				
Rib, left, intercostal rib — [M]	46 (2)			
Rib, discontinuous — [M]	46 (2)			
Rib, floating extra — [V]			118 (4)	
Rib, lumbar I full — [M]	2 (11) 22 (14) 228 (12) 242 (10)	58 (1,13) 64 (10) 100 (15)	150 (11,13)	152 (8) 178 (4,10)
Rib, lumbar I rudimentary, Normal cartilage — [V]			116 (4,14)	
Rib, lumbar I rudimentary, Unilateral cartilage — [V]		82 (5)		
Rib, lumbar I rudimentary — [V]	2 (9,10,11,12) 6 (2,8,9,13,15,16) 10 (6) 12 (8) 18 (11) 24 (1,2,3,5,8,12) 32 (4,10) 34 (3) 36 (4,9,13) 38 (6,8,11,12,14) 40 (4) 42 (13) 44 (2,5,8,10,14,16) 46 (1,7,10,12,15) 48 (1,4,7,8,12) 202 (6) 208 (1,2,4,7) 210 (4,10,14) 212 (13) 216 (1) 228 (4,9,12) 232 (14) 236 (1,6,8,10,12) 238 (5,13) 240 (5,13) 242 (2) 246 (2) 248 (3) 250 (1,2,3,4,5,7,13,14)	52 (6,11) 54 (2,4,9) 56 (4,6,8,10,12,13,19) 58 (1,2,3,5,6,7,8,9,12,13,14) 60 (9) 62 (11) 64 (10,15,16) 66 (10,18) 68 (2,3,6) 72 (2,13) 84 (6) 88 (3,4,5,6,7,8,9,10,11,13,14) 90 (2,4,5) 96 (4,5,14,15) 98 (5,9,11,12,13) 100 (5,11,13,15,16)	104 (8) 108 (2,5,7,8,9,13) 110 (2,4,5,8,10,12,13,17) 112 (11,13) 114 (11) 116 (1,9,11) 118 (2) 122 (11) 124 (2,3,5) 132 (2,6) 134 (2,4,6,8,11) 136 (3,7,10) 138 (8,14) 142 (4,5,6,8,9,10,13) 144 (2,9,12) 146 (5,10) 150 (3,4,5,8,9,10,12,13,14)	152 (1,2,3,4,5,6,7,8,9,10,12,13) 156 (7,13,14) 158 (10,12) 160 (2) 166 (6,10,11) 168 (5,6,11) 170 (3,5,11,14,15,16) 172 (2,3,4,8,11,14) 178 (4,5,8) 188 (6,7,8,9,11,17) 190 (5,11,12) 192 (4) 196 (5,7,14) 198 (1,5,7) 200 (6)
Rib cartilage, discontinuous — [M]		66 (7)		
Rib cartilage, VIII attached to sternum — [M]	44 (15)			

TABLE B7
Fetal Findings Cross Reference of Dams and Fetuses in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

	0 mg/kg	162.5 mg/kg	325 mg/kg	650 mg/kg
Skeletal: Body (continued)				
Number of fetuses examined	599	300	270	259
Number of dams examined	44	21	21	20
Sternebrae				
Sternebra II, incomplete ossification — [V]	46 (2)			
Sternebra V, incomplete ossification — [V]	22 (8) 246 (14)	68 (8)	114 (13) 124 (4) 148 (1,13)	
Sternebra (e), extra ossification site between sternebrae — [V]	202 (5,12) 206 (14)	66 (5)		
Sternebra (e), misaligned (>2, not V) — [V]	46 (2)			
Thoracic Vertebrae				
Thoracic centrum, bipartite ossification, bipartite cartilage — [M]	46 (2)			
Thoracic centrum, bipartite ossification, dumbbell cartilage — [V]	44 (11)		104 (1) 134 (8)	178 (7)
Thoracic centrum, bipartite ossification, normal cartilage — [V]	2 (7) 240 (15)	80 (12,15) 84 (1)	148 (13)	178 (5)
Thoracic centrum, dumbbell ossification, dumbbell cartilage — [V]	218 (1,2) 220 (6)	96 (5)	104 (6) 118 (4)	170 (1) 178 (12)
Thoracic centrum, dumbbell ossification, normal cartilage — [V]	34 (16) 44 (10) 212 (17) 238 (3) 240 (6,15) 250 (11)	54 (3) 60 (8,10) 100 (4)	104 (8) 110 (8) 130 (11) 148 (2)	160 (4) 178 (7,8,9,10,13) 188 (2)
Thoracic centrum, fused — [M]	46 (2)			
Thoracic centrum, unilateral ossification, bipartite cartilage — [M]	46 (2)			
Thoracic centrum, unossified, bipartite cartilage — [M]	46 (2)			
Thoracic centrum cartilage, normal ossification, dumbbell cartilage — [V]		94 (11)		
Skeletal: Skull				
Number of fetuses examined	300	152	132	132
Number of dams examined	44	21	21	20
Skull				
Interparietal, Incomplete ossification — [V]	232 (11)			

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

PROCUREMENT AND CHARACTERIZATION C-2
PREPARATION AND ANALYSIS OF DOSE FORMULATIONS C-3
FIGURE C1 Fourier Transform Infrared Absorption Spectrum of Tris(chloropropyl) Phosphate C-4
FIGURE C2 Proton Nuclear Magnetic Resonance Spectrum of Tris(chloropropyl) Phosphate C-5
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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Tris(chloropropyl) Phosphate (TCPP)

TCPP was obtained from Albemarle Corporation (Orangeburg, SC) in two lots (101 and 134). Lot 101 was used in the dose range-finding study, and lots 101 and 134 were blended to form lot M072911NP that was divided into two drums and used during the prenatal developmental toxicity study. Homogeneity of the blended lot M072911NP was confirmed both within the individual drums and between the two drums. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at MRI Global (Kansas City, MO) for the study laboratory at RTI International (Research Triangle Park, NC). Reports on analyses performed in support of the TCPP studies are on file at the National Institute of Environmental Health Sciences.

Lots 101, 134, and M072911NP of the test chemical, clear oily liquids, were identified as TCPP using proton and carbon-13 Fourier transform nuclear magnetic resonance (FT-NMR) spectroscopy. In addition, lots 101 and M072911NP were identified as TCPP using Fourier transform infrared (FT-IR) and ultraviolet/visible (UV-Vis) spectroscopy, gas chromatography (GC) with mass spectrometry (MS) detection, and measurement of density. FT-NMR, FT-IR, and UV-Vis spectra were consistent with the structure of TCPP. Due to the isomeric complexity of the test article, two-dimensional FT-NMR was performed on lot M072911NP including homonuclear correlation spectroscopy (COSY) and heteronuclear correlation (HETCOR) spectroscopy to confirm the data from the proton and carbon-13 NMR spectra. Representative FT-IR and proton NMR spectra are presented in Figures C1 and C2, respectively. GC/MS electron ionization analyses of lots 101 and M072911NP identified one major peak and three isomers with molecular weights of 327.6. The primary isomer (Isomer 1) [tris(1-chloro-2-propyl) phosphate, CAS No. 13674-84-5] matched a literature spectrum (NIST, 2008); the three other isomers were identified as bis (1-chloro-2-propyl) 2-chloro-1-propyl phosphate (Isomer 2; CAS No. 76025-08-6), bis (2-chloro-1-propyl) 1-chloro-2-propyl phosphate (Isomer 3; CAS No. 76649-15-5), and tris(2-chloro-1-propyl) phosphate (Isomer 4; CAS No. 6145-73-9) (Table 2). The densities (at 21° C) of lots 101 and M072911NP were determined to be 1.2936 and 1.2958 to 1.2959, respectively.

The moisture content of lots 101 and M072911NP was determined by Karl Fischer titration. Elemental analyses for carbon, hydrogen, nitrogen, and chlorine were conducted by ICON Development Solutions (Whitesboro, NY) on lots 101 and M072911NP. The purity profiles of lots 101, 134, and M072911NP were determined using GC with flame ionization detection (FID), and octanol:water partition coefficients were determined for the two largest peaks of the profiles for lots 101 and M072911NP. Acid number and ester value were determined for lots 101 and M072911NP using titration with standardized ~0.001 N sodium hydroxide and ~0.5 N hydrochloric acid, respectively.

For lot 101, Karl Fischer titration indicated a water content of 0.093% to 0.100%. Elemental analyses for carbon, hydrogen, nitrogen, and chlorine were consistent with the theoretical values for TCPP. GC/FID analysis by system A (Table C1) detected four TCPP peaks with a combined area of 96.04% of the total peak area and seven reportable impurities with individual areas ≥0.05% of the total peak area. The largest peak in this analysis comprised 65.22% of the total peak area and log P values for the two largest peaks in this profile were determined to be 2.69 and 2.74, respectively. The average acid number for lot 101 was determined to be 0.011 mg potassium hydroxide (KOH)/g and the average ester value was calculated to be 104.7 mg KOH/g. The overall purity of lot 101 was determined to be approximately 96%.

For lot 134, the GC/FID purity profile by system A detected four TCPP peaks with a combined relative area of 98.79% and three reportable impurities ≥0.05% of the total peak area; the largest peak comprised 71.33% of the total peak area. Coupled with the proton and carbon-13 FT-NMR identity confirmation for lot 134, these results indicated that lot 134 was suitable for blending with lot 101 to constitute lot M072911NP.

Lot M072911NP was determined to contain 0.038% to 0.039% water by Karl Fischer titration. Elemental analyses for carbon, hydrogen, nitrogen, and chlorine were consistent with the theoretical values for TCPP. GC/FID by

system A detected four TCPP peaks accounting for 97.04% to 97.43% of the total peak area and eight minor peaks with individual areas ≥0.05% of the total peak area. The major peak in this analysis comprised 67.57% to 68.54% of the total peak area and log P values for the two largest peaks in this profile were determined to be 2.59 and 2.65, respectively. Using a more polar column, GC/FID by system B detected four TCPP peaks with a combined area of 97.50% to 97.91% of the total peak area and six reportable impurities with individual areas ≥0.05% of the total peak area. The major peak in this analysis comprised 67.84% to 68.85% of the total peak area. The average acid number for lot M072911NP was determined to be 0.067 mg KOH/g and the average ester value was calculated to be 105.85 mg KOH/g. The overall purity of lot M072911NP was determined to be 97% or greater. A summary of these analyses is given in Table 2.

Accelerated stability studies of lot 101 of the test chemical were conducted by the analytical chemistry laboratory using GC/FID by system A. Stability of the bulk chemical was confirmed for at least 2 weeks when stored in glass vials sealed with Teflon®-lined crimp caps at temperatures up to 60° C. To ensure stability, the test chemical was stored under inert gas at ~25° C, in sealed drums. Periodic analyses of lots 101 and M072911NP of the test chemical were performed prior to and during the animal studies by the analytical chemistry laboratory using FT-NMR and GC/FID; no degradation of the test chemical was detected.

Methylcellulose

Methylcellulose was obtained from Spectrum Quality Products (Gardena, CA) in two lots (YX0540 and 2AJ0439). Lot YX0540 was used as the vehicle in the dose range-finding study and lot 2AJ0439 was used in the prenatal developmental toxicity study. The identity of both lots was confirmed by the analytical chemistry laboratory using FT-IR spectroscopy; all spectra were consistent with the structure of methylcellulose and the literature (*Aldrich*, 1981). The methoxy group content was determined by Galbraith Laboratories (Knoxville, TN) using titration with standardized sodium thiosulfate solution. Methoxy group content was 30.4% and 31.0% for lots YX0540 and 2AJ0439, respectively, both within the accepted range of 27.5% to 31.5%.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once (dose range-finding study) or three times (prenatal developmental toxicity study) by mixing TCPP with a 0.5% methylcellulose solution to give the required concentrations (Table C2). The dose formulations were stored at ~5° C in sealed glass jars for up to 30 (dose range-finding study) or 15 (prenatal developmental toxicity study) days.

Prior to conducting the dose range-finding study, homogeneity studies of 1.56 mg/mL and 200 mg/mL formulations and stability studies of the 1.56 mg/mL formulation were performed by the analytical chemistry laboratory using GC/FID by system A (Table C1). Homogeneity was confirmed, with the stipulation that high-dose formulations be stirred constantly during use to maintain homogeneity. Stability was confirmed for at least 7 days for dose formulations stored in sealed glass containers at ~5° C and for 3 hours under simulated animal room conditions; at ~5° C, the 1.56 mg/mL formulation was 93.8%, 88%, and 86.6% of the day 0 value at 7, 14, and 42 days, respectively, suggesting some loss over time. Additional stability studies were conducted at 32.5 and 130 mg/mL prior to the prenatal developmental toxicity study; stability of the 130 mg/mL formulation was confirmed for up to 42 days, while that of the 32.5 mg/mL formulation was confirmed for up to 35 days for formulations stored in sealed glass containers at ~5° C.

Periodic analyses of the dose formulations of TCPP were conducted by the analytical chemistry laboratory using GC/FID by system A. During the dose range-finding study, the dose formulations were analyzed one day after preparation and after storage at ~5° C for 7 days; all 10 dose formulation samples were within 10% of the target concentrations (Table C3). Animal room samples received on day 36 were also analyzed; two of six were within 10% of the target concentrations. During the prenatal developmental toxicity study, the dose formulations were prepared three times, and all nine dose formulation samples were within 10% of the target concentrations (Table C4). Animal room samples of these dose formulations were also analyzed; six of nine were within 10% of the target concentrations.

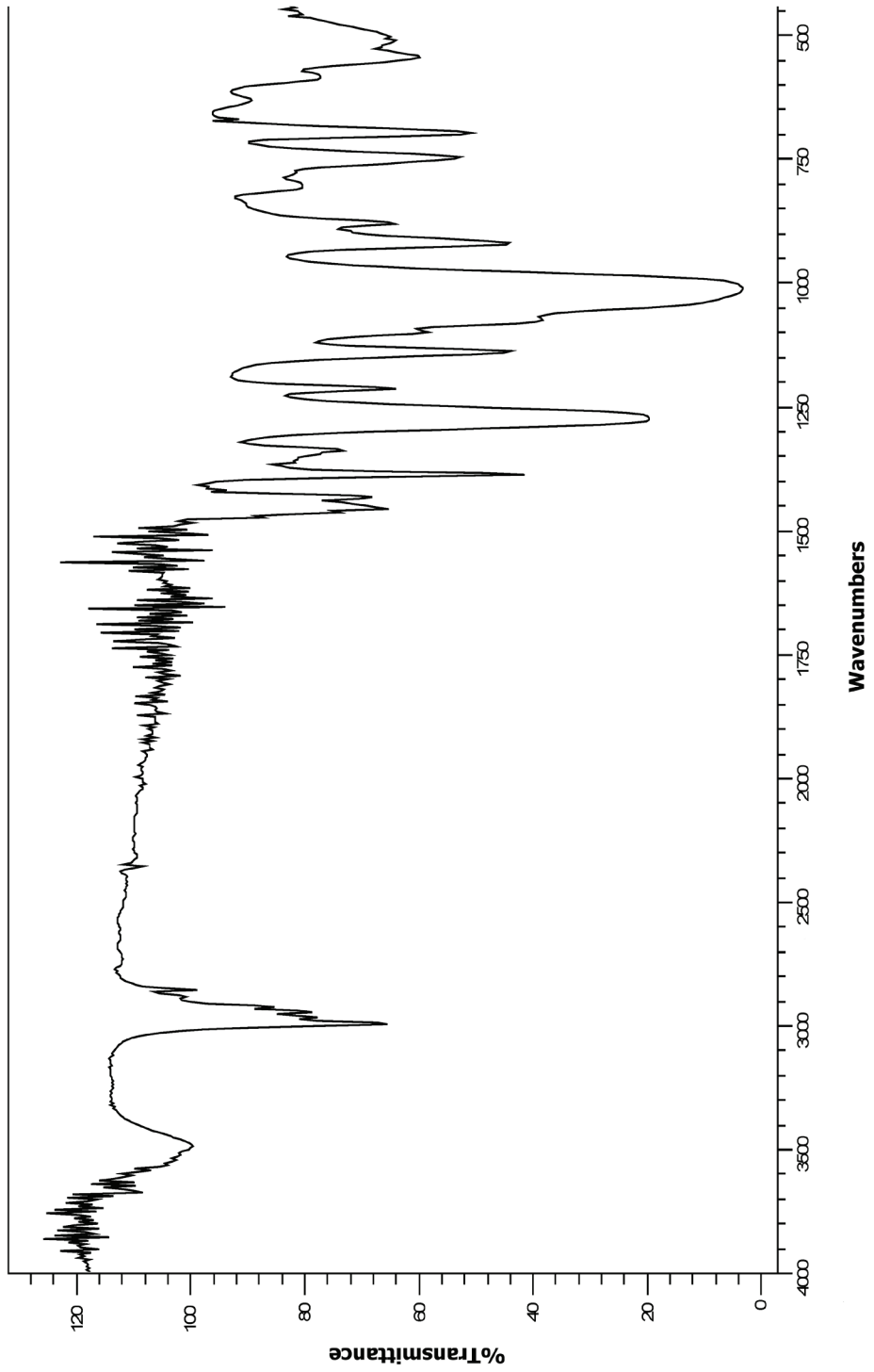

FIGURE C1
Fourier Transform Infrared Absorption Spectrum of Tris(chloropropyl) Phosphate

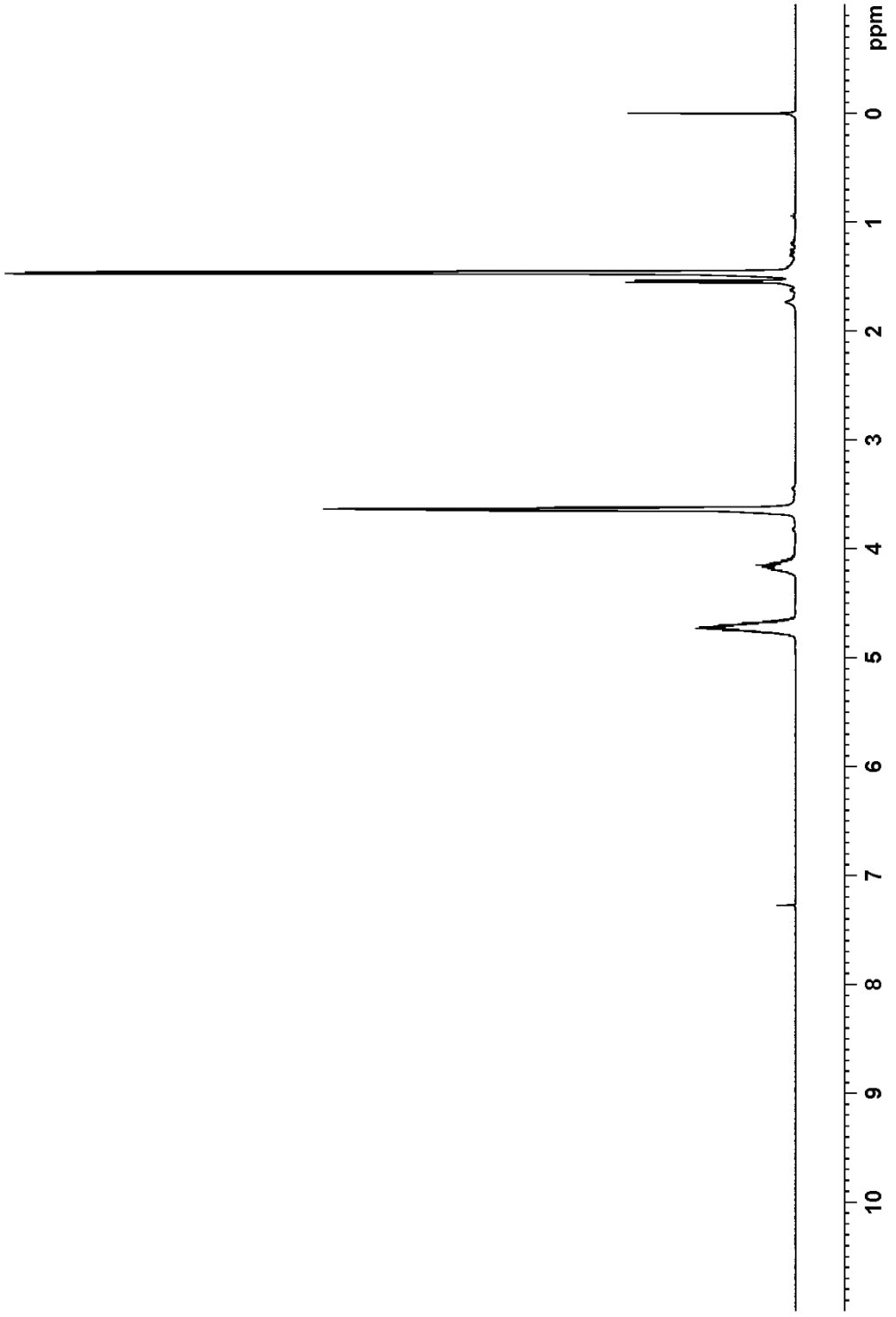

FIGURE C2
Proton Nuclear Magnetic Resonance Spectrum of Tris(chloropropyl) Phosphate

TABLE C1
Gas Chromatography Systems Used in the Gavage Studies of Tris(chloropropyl) Phosphate[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	DB-5, 30 m × 0.53 mm, 1.5 µm film (J&W Scientific, Folsom, CA)	Helium at ~10 mL/minute	160° C for 5 minutes, then 1° C/minute to 180° C, held for 5, 10, 15, or 25 minutes
System B			
Flame ionization	DB™-WAX, 30 m × 0.53 mm, 1.0 µm film (J&W Scientific)	Helium at ~10 mL/minute	200° C for 5 minutes, then 1° C/minute to 230° C, held for 15 minutes

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

TABLE C2
Preparation and Storage of Dose Formulations in the Gavage Studies of Tris(chloropropyl) Phosphate

Dose Range-Finding Study	Prenatal Developmental Toxicity Study
Preparation	
The dosing vehicle was prepared by mixing methylcellulose with heated, deionized water while stirring and then diluting with water to form a 0.5% solution, which was allowed to cool. For the dose formulations, the appropriate amount of TCPP was weighed and transferred into a 2 L beaker, diluted with the vehicle, mixed using a POLYTRON® PT-10-35 homogenizer with a PTA 10TS low-foaming generator for approximately 2 minutes, and then stirred using a stir bar for approximately 5 minutes. The doses were prepared once.	Same as the 3-week dose range-finding study except that the doses were prepared three times.
Chemical Lot Number	
101	M072911NP
Maximum Storage Time	
30 days	15 days
Storage Conditions	
Stored in glass jars with lids at ~5° C	Stored in wide mouth clear glass jars with lids at ~5° C
Study Laboratory	
RTI International (Research Triangle Park, North Carolina)	RTI International (Research Triangle Park, North Carolina)

TABLE C3
Results of Analyses of Dose Formulations Administered to Female Rats in the Dose Range-Finding Gavage Study of Tris(chloropropyl) Phosphate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
July 19, 2010	July 20, 2010[b]	60	60.2	0
		60	60.2	0
		60	60.4	+1
		130	130.3	0
		200	207.3	+4
		200	206.5	+3
		200	204.5	+2
	July 27, 2010	60	59.8	0
		130	129.7	0
		200	205.3	+3
	August 24, 2010[c]	60	75.0[d]	+25
		60	78.5[d]	+31
		130	156.3[d]	+20
		130	143.9[d]	+11
		200	209.4	+5
		200	202.6	+1

[a] Results of triplicate analyses. Dosing volume=5 mL/kg; 60 mg/mL=300 mg/kg, 130 mg/mL=650 mg/kg, 200 mg/mL=1,000 mg/kg
[b] For the 60 and 200 mg/mL dose formulations, the triplicate results are for homogeneity analyses from samples collected from the top, middle, and bottom of the each vessel.
[c] Animal room samples
[d] Formulation was outside the acceptable range of ± 10% of target concentration but determined to not impact the quality or integrity of the study.

TABLE C4
Results of Analyses of Dose Formulations Administered to Female Rats in the Prenatal Developmental Toxicity Gavage Study of Tris(chloropropyl) Phosphate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
January 20, 2012	January 20, 2012	32.5	32.8	+1
		65	64.7	0
		130	130.9	+1
	February 9, 2012[b]	32.5	30.5	–6
		65	55.2	–15
		130	123.2	–5
February 2, 2012	February 2, 2012	32.5	32.2.	–1
		65	64.2	–1
		130	130.3	0
	February 16, 2012[b]	32.5	28.7	–12
		65	57.4	–12
		130	126.7	–3
February 8, 2012	February 8, 2012	32.5	32.9	+1
		65	62.5	–4
		130	125.8	–3
	February 21, 2012[b]	32.5	31.0	–5
		65	61.1	–6
		130	126.9	–2

[a] Results of triplicate analyses. Dosing volume=5 mL/kg; 32.5 mg/mL=162.5 mg/kg, 65 mg/mL=325 mg/kg, 130 mg/mL=650 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 D-2
TABLE D2 Vitamins and Minerals in NIH-07 Rat and Mouse Ration D-2
TABLE D3 Nutrient Composition of NIH-07 Rat and Mouse Ration D-3
TABLE D4 Contaminant Levels in NIH-07 Rat and Mouse Ration D-4

TABLE D1
Ingredients of NIH-07 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.0
Fish meal (60% protein)	10.0
Wheat middlings	10.0
Alfalfa meal (dehydrated, 17% protein)	4.0
Soy oil (without preservatives)	2.5
Dried brewer's yeast	2.0
Calcium phosphate, dibasic (USP)	1.3
Calcium carbonate (USP)	0.5
Sodium chloride	0.5
Premixes (vitamin and mineral)	0.25
Choline chloride (70% choline)	0.09

TABLE D2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	6,062 IU	Stabilized vitamin A palmitate or acetate
D	5,070 IU	D-activated animal sterol
K	3.09 mg	Menadione sodium bisulfite complex
E	22 IU	α-Tocopheryl acetate
Niacin	33 mg	
Folic acid	2.4 mg	
d-Pantothenic acid	19.8 mg	*d*-Calcium pantothenate
Riboflavin	3.8 mg	
Thiamin	11 mg	Thiamine mononitrate
B_{12}	50 µg	
Pyridoxine	6.5 mg	Pyridozine hydrochloride
Biotin	0.15 mg	*d*-Biotin
Minerals		
Iron	132 mg	Iron sulfate
Zinc	18 mg	Zinc oxide
Manganese	66 mg	Manganese oxide
Copper	4.4 mg	Copper sulfate
Iodine	1.5 mg	Calcium iodate
Cobalt	0.44 mg	Cobalt carbonate

[a] Per kg of finished product

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

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	24.7	24.7	1
Crude fat (% by weight)	5.6	5.6	1
Crude fiber (% by weight)	3.42	3.42	1
Ash (% by weight)	7.17	7.17	1
Amino Acids (% of total diet)			
Arginine	1.374 ± 0.049	1.31 – 1.43	5
Cystine	0.325 ± 0.029	0.29 – 0.372	5
Glycine	1.120 ± 0.041	1.06 – 1.16	5
Histidine	0.504 ± 0.008	0.49 – 0.51	5
Isoleucine	0.973 ± 0.018	0.95 – 0.99	5
Leucine	1.982 ± 0.036	1.93 – 2.02	5
Lysine	1.250 ± 0.039	1.22 – 1.32	5
Methionine	0.485 ± 0.011	0.46 – 0.49	5
Phenylalanine	1.086 ± 0.017	1.07 – 1.11	5
Threonine	0.907 ± 0.024	0.88 – 0.94	5
Tryptophan	0.274 ± 0.014	0.26 – 0.29	5
Tyrosine	0.871 ± 0.017	0.85 – 0.89	5
Valine	1.120 ± 0.022	1.11 – 1.16	5
Essential Fatty Acids (% of total diet)			
Linoleic	2.23 ± 0.223	2.04 – 2.59	5
Linolenic	0.24 ± 0.0249	0.22 – 0.28	5
Vitamins			
Vitamin A (IU/kg)	5,350	5,350	1
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	73.72 ± 4.40	69.5 – 78.7	5
Thiamine (ppm)[b]	16	16	1
Riboflavin (ppm)	14.42 ± 3.64	10 – 19.8	5
Niacin (ppm)	95.96 ± 7.28	87 – 106	5
Pantothenic acid (ppm)	45.26 ± 1.487	43.2 – 47.4	5
Pyridoxine (ppm)[b]	11.96 ± 1.948	9.63 – 14.9	5
Folic acid (ppm)	2.39 ± 0.439	1.69 – 2.74	5
Biotin (ppm)	0.3 ± 0.0463	0.25 – 0.37	5
Vitamin B_{12} (ppb)	49.28 ± 8.364	41.8 – 61.6	5
Minerals			
Calcium (%)	1.260	1.260	1
Phosphorus (%)	0.967	0.967	1
Potassium (%)	0.814 ± 0.035	0.769 – 0.865	5
Chloride (%)	0.572 ± 0.076	0.441 – 0.628	5
Sodium (%)	0.344 ± 0.018	0.318 – 0.365	5
Magnesium (%)	0.182 ± 0.006	0.174 – 0.192	5
Iron (ppm)	394.8 ± 38.848	348 – 455	5
Manganese (ppm)	86.2 ± 4.77	80.7 – 93.2	5
Zinc (ppm)	64.66 ± 14.521	52.4 – 89.2	5
Copper (ppm)	13.12 ± 0.949	11.9 – 14.1	5
Iodine (ppm)	1.876 ± 0.986	0.8 – 3.45	5
Chromium (ppm)	1.304 ± 0.225	0.97 – 1.59	5
Cobalt (ppm)	0.532 ± 0.179	0.25 – 0.73	5

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

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

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.587	0.587	1
Cadmium (ppm)	0.096	0.096	1
Lead (ppm)	0.106	0.106	1
Mercury (ppm)	0.016	0.016	1
Selenium (ppm)	0.488	0.488	1
Aflatoxins (ppb)	<5.00		1
Nitrate Nitrogen (ppm)[c]	<10.0		1
Nitrite Nitrogen (ppm)[c]	<0.61		1
BHA (ppm)[d]	<1.0		1
BHT (ppm)[d]	<1.0		1
Aerobic Plate Count (CFU/gm)	<10		1
Coliform (MPN/gm	<3		1
Escherichia coli (MPN/gm)	<10		1
Salmonella (MPN/gm)	Negative		1
Total Nitrosamines (ppb)[e]	8.2	8.2	1
N-Nitrosodimethylamine (ppb)[e]	1.1	1.1	1
N-Nitrosopyrrolidine (ppb)[e]	7.1	7.1	1
Pesticides (ppm)			
α-BHC	<0.01		1
β-BHC	<0.02		1
γ-BHC	<0.01		1
δ-BHC	<0.01		1
Heptachlor	<0.01		1
Aldrin	<0.01		1
Heptachlor epoxide	<0.01		1
DDE	<0.01		1
DDD	<0.01		1
DDT	<0.01		1
HCB	<0.01		1
Mirex	<0.01		1
Methoxychlor	<0.05		1
Dieldrin	<0.01		1
Endrin	<0.01		1
Telodrin	<0.01		1
Chlordane	<0.05		1
Toxaphene	<0.10		1
Estimated PCBs	<0.20		1
Ronnel	<0.01		1
Ethion	<0.02		1
Trithion	<0.05		1
Diazinon	<0.10		1
Methyl chlorpyrifos	0.0706	0.0706	1
Methyl parathion	<0.02		1
Ethyl parathion	<0.02		1
Malathion	0.0295	0.0295	1
Endosulfan I	<0.01		1
Endosulfan II	<0.01		1
Endosulfane sulfate	<0.03		1

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride

[b] For values less than the limit of detection, the detection limit is given as the mean.

[c] Sources of contamination: alfalfa, grains, and fish meal

[d] Sources of contamination: soy oil and fish meal

[e] All values were corrected for percent recovery.

APPENDIX E
SENTINEL ANIMAL PROGRAM

METHODS E-2
RESULTS E-2

SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of test compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) or dosed animals in the study rooms. The sentinel animals and the study animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

Blood samples were collected and allowed to clot, and the serum was separated. All samples were processed appropriately and tested at the Research Animal Diagnostic Laboratory (RADIL), University of Missouri (Columbia, MO) for determination of the presence of pathogens. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the study are also listed.

Blood was collected from five female rats per time of collection for the prenatal developmental toxicity study.

Method and Test	Time of Collection
Multiplex Fluorescent Immunoassay	
H-1 (Toolan's H-1 virus)	Arrival, study termination
KRV (Kilham rat virus)	Arrival, study termination
Mycoplasma pulmonis	Arrival, study termination
PVM (pneumonia virus of mice)	Arrival, study termination
RCV/SDA	
(rat coronavirus/sialodacryoadenitis virus)	Arrival, study termination
RMV (rat minute virus)	Arrival, study termination
RPV (rat parvovirus)	Arrival, study termination
RTV (rat Theilovirus)	Arrival, study termination
Sendai	Arrival, study termination
TMEV (Theiler's murine encephalomyelitis virus)	Arrival, study termination

RESULTS

All test results were negative.