



8EHQ-0502-15134

GE Specialty Chemicals

RECEIVED
OPPT CBIC

General Electric Company
1000 Morgantown Industrial Park
Morgantown, WV 26501

2002 MAY -7 AM 11:31

MA 58609

CERTIFIED MAIL R.R.R.

Contains No CBI

April 26, 2002

TSCA Document Processing Center
EPA Mail Code 7407
Room E-G99
Office of Pollution Prevention and Toxics
US Environmental Protection Agency
401 M Street, SW
Washington, DC 20460



8EHQ-02-15134

Attn: TSCA 8(e) Coordinator

Re: **Phenol, Nonyl-, Phosphite (3:1) (TNPP)**
CAS Number: 26523-78-4

This submission is made by:

General Electric Specialty Materials
GE Specialty Chemicals (GESC)
1000 Morgantown Industrial Park
Morgantown, WV 26501 USA



88020000120

GESC is currently sponsoring a study entitled *Reproductive/Developmental Toxicity Screening Test of Trisnonylphenyl Phosphite (TNPP) Administered via Oral Gavage to CD® (Sprague-Dawley) Rats (Modified OECD 421)* at RTI International (formerly Research Triangle Institute). The laboratory has reported results in the Final Report that GESC is submitting pursuant to current guidance issued by the Environmental Protection Agency (EPA) giving EPA's interpretation of Section 8(e) of the Toxic Substances Control Act. GESC has not determined that these results indicate any potential risk of injury to human health or the environment.

Tris(nonylphenyl)phosphite (TNPP) has been used for decades as a stabilizer in plastics, including plastics intended for food contact applications. Typical applications for TNPP include linear low density polyethylene (LLDPE), high density polyethylene (HDPE), poly-vinyl chloride (PVC), ethylene-vinyl acetate copolymers (EVA) and high impact polystyrene. TNPP is typically used in polymers such as LLDPE at 15 to 2000 ppm (1500 ppm avg.) The manufacture of TNPP is fully automated and normally controlled by 1-2 operators. Workplace exposure is very limited. Personal protective equipment is required when exposure may occur, such as with sampling. Environmental releases are also limited. TNPP

2002 MAY 15 AM 11:20

RECEIVED
OPPT CBIC

has been determined to be hydrolytically unstable per OECD 111 and would be expected to break down in the environment in the event of an accidental release.

Male and female CD® (Sprague-Dawley) rats (the F0 generation) were administered Trisnonylphenyl Phosphite (TNPP; CAS No. 26523-78-4) orally by gavage at 0, 50, 200, or 1000 mg/kg/day in Mazola® corn oil, ten animals/sex/dose, for two weeks of prebreed exposure (males and females) and two weeks of mating (males and females) for F0 parents. F0 females continued to be dosed for three weeks each of gestation and lactation, as were F1 offspring from weaning through scheduled sacrifice, at approximately 85 days of age. Five additional F0 males per group from the control and 1000 mg/kg/day groups were designated as recovery animals and held without dosing for two weeks, after the F0 male dosing period was completed, to evaluate recovery from any possible treatment-related effects identified in the high dose.

Body weights, feed consumption, and clinical signs were recorded for all animals. On the day of birth (postnatal day [pnd] 0), anogenital distance (AGD) was measured and body weights recorded for all live F1 pups in all litters. F1 litters were culled on pnd 4 to yield, as nearly as possible, five males and five females per litter. At weaning, at least one female and one male (whenever possible) from each F1 litter were randomly selected to continue treatment for approximately seven more weeks, with dosing for F1 selected pups begun on pnd 22 until all pups were at least 85 days of age.

F1 postweaning observations and procedures for each retained female included examination for patency of vaginal opening (from pnd 22 until acquisition of vaginal opening). Estrous cyclicity and normality were evaluated by vaginal smears from F1 females taken daily the last three weeks of the postwean exposure period prior to scheduled sacrifice. For each retained F1 male offspring, observations for the cleavage of the balanopreputial gland (preputial separation) began at 35 days of age and continued until acquisition of preputial separation. Andrologic assessments were also performed on the F1 retained males at necropsy. All F0, nonselected F1 weanlings, and retained F1 adults were necropsied, with complete histologic evaluation of five selected from these F0 and F1 males and females in the 0 and 1000 mg/kg/day groups. Histopathology was performed on F1 males and females (five/sex/group) at 0 and 1000 mg/kg/day.

Exposure once daily to TNPP at 0, 50, 200, or 1000 mg/kg/day resulted in mild systemic toxicity in F0 parental males at 1000 mg/kg/day, expressed as reduced weight gains, increased kidney weights, and renal histopathologic findings (presence of renal corticomedullary junction mineralization). Three of ten pregnant F0 parental females from the 1000 mg/kg/day group were found dead on Day 22 of gestation. These deaths may have been related to dystocia, since the dams appeared to be unable to deliver their normal appearing pups. In addition, absolute ovarian weights and ovarian weights relative to terminal body or brain weights were reduced in F0 females at 1000 mg/kg/day. No other consistent, treatment-related effects were observed. There was no evidence of F0 reproductive toxicity in males. The only evidence of F1 offspring toxicity pre- or postnatally was reduced litter size on pnd 4 (but not on pnd 0) and renal histopathology in F1 adult males at 1000 mg/kg/day.

In conclusion, TNPP administered by gavage once daily at 0, 50, 200, and 1000 mg/kg/day to parental F0 CD® (SD) rats, ten/sex/group, through prebreed, mating, gestation, and lactation and direct dosing to F1 offspring from weaning to scheduled sacrifice resulted in adult F0 parental toxicity at 1000 mg/kg/day in males, represented by slightly decreased weight gain, increased kidney weights, and the presence of renal corticomedullary junction mineralization. There was F1 offspring toxicity observed postnatally at 1000 mg/kg/day, expressed as reduced litter size on pnd 4 (although not at pnd 0) and corticomedullary mineralization in the adult F1 male kidneys at 1000 mg/kg/day. Three of ten pregnant F0 females at 1000 mg/kg/day died in late pregnancy, possibly due to dystocia. In addition, ovary weights were decreased at 1000 mg/kg/day in F0 but not F1 adult females. No effects were seen on the developmental landmarks, including the time to vaginal opening or preputial separation, or estrous cycle normality or length. Although there were slight effects seen at the highest dose and the possible occurrence of dystocia at this dose, there were no effects seen at the two lower doses for any parameter measured and all females gave birth normally.

Therefore, under the conditions of this study in rats, the no observable adverse effect levels (NOAELs) for systemic parental toxicity and for reproductive and offspring toxicity were 200 mg/kg/day.

A copy of the Final Report is included with this submission.

Please do not hesitate to contact me if you have general questions at (304) 284-2214 or Dr. Ronald L. Joiner at (413) 448-6323 if you have technical questions.

Sincerely,
GE Specialty Chemicals



Sheri L. Blystone, Ph. D.
Product Steward
(304) 284-2231 (fax)
Sheri.Blystone@gesm.ge.com

cc: Stephen F. Austin
Ronald L. Joiner, Ph. D.

Attachment

FINAL REPORT

TITLE: Reproductive/Developmental Toxicity Screening Test of Trisnonylphenyl Phosphite (TNPP) Administered via Oral Gavage to CD® (Sprague-Dawley) Rats (Modified OECD 421)

AUTHORS: Rochelle W. Tyl, Ph.D., DABT
Bonnie T. Hamby, B.S., LATG
Christina B. Myers, M.S.
Melissa C. Marr, B.A., RLATG

PERFORMING LABORATORY: Center for Life Sciences and Toxicology
Chemistry and Life Sciences
RTI
P. O. Box 12194
Research Triangle Park, NC 27709-2194

SPONSOR: General Electric Company
One Plastic Avenue
Pittsfield, MA 01201

SPONSOR'S REPRESENTATIVE: Ronald L. Joiner, Ph.D.
Manager, Global Toxicology
General Electric Company

STUDY INITIATION DATE: July 19, 2001

IN-LIFE PERFORMANCE DATES: August 29, 2001 to January 8, 2002

EXPERIMENTAL DATES: September 5, 2001 – February 7, 2002

FINAL REPORT DATE: April 10, 2002

RTI IDENTIFICATION NUMBER: 65C-07895.300

Author:

Approved:

 4/10/02
Rochelle W. Tyl, Ph.D., DABT Date
Study Director
Center for Life Sciences and Toxicology
RTI

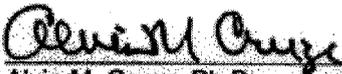
 4/10/02
Alvin M. Cruze, Ph.D. Date
Interim Vice President
Chemistry and Life Sciences
RTI

TABLE OF CONTENTS

	<u>Page</u>
ABSTRACT	6
OBJECTIVES	8
MATERIALS AND METHODS	8
Test Material and Dose Formulations	8
Animals and Husbandry	11
Text Table A. Study Schedule	11
Study Design	14
Figure 1. Study Design	15
Parental F0 Animals, Including Recovery Animals (F0).....	15
Progeny (F1)	17
Necropsy and Histology	18
Full Necropsy at Scheduled Sacrifice (F0 Parents, F1 Weanlings, and Adults)	18
Necropsy of Unscheduled Dead Animals	20
Necropsy of Pnd 4 Culled Pups	20
F1 Male Andrology	20
Reproductive and Offspring Indices	21
Calculation of Standard Reproductive Toxicity Parameters.....	21
Text Table B. Reproductive and Offspring Indices.....	21
Text Table C. Formulas for Calculating Standard Reproductive Toxicity Study Parameters.....	23
Statistical Analyses	24
Personnel	26
Individual Scientist Reports and Protocol/Amendment	27
Storage of Records	27
Compliance.....	27
RESULTS	28
Dose Formulations	28
Fate of F0 Animals	28
F0 Prebreed.....	28
F0 Males	28
Figure 2. F0 Male Body Weights	29
F0 Recovery Males.....	30
Figure 3. Recovery Male Body Weights	30
F0 Females.....	31
Figure 4. F0 Female Body Weights	31
F0 Gestation	32

TABLE OF CONTENTS (continued)

	<u>Page</u>
F0 Lactation.....	32
F0 Reproductive Indices.....	32
F1 Lactation.....	33
Figure 5. F1 Offspring Body Weights by Sex by Litter	34
Figure 5A. Males	34
Figure 5B. Females	34
Necropsy of F0 Parental Animals.....	35
Males	35
Recovery Males.....	36
Females	36
Fate of F1 Animals	38
Males	38
Figure 6. F1 Male Body Weights	39
Females	39
Figure 7. F1 Female Body Weights.....	40
Necropsy of Adult F1 Animals.....	41
Males	41
Females	42
DISCUSSION.....	43
F0 and F1 Adult Systemic Toxicity.....	43
Text Table D. Summary of F0 Adult Systemic Toxicity.....	45
Text Table E. Summary of F1 Adult Systemic Toxicity.....	49
F0 Parental Reproductive Toxicity	53
Text Table F. Summary of F0 Parental Male and Female Reproductive Toxicity	53
F1 Offspring Toxicity	54
Text Table G. Summary of F1 Offspring Toxicity.....	54
CONCLUSIONS.....	56
REFERENCES.....	57
PROTOCOL DEVIATIONS.....	59
Quality Assurance Statement	61

Appendices

Analytical Chemistry Report.....	Appendix I
Individual Animal Data Tables.....	Appendix II
Pathology Report	Appendix III
Protocol and Two Amendments.....	Appendix IV

LIST OF TABLES

Table	Title	Page
1	Analyses of Dosing Formulations.....	63
2	Summary of the Fate of the F0 Males, Recovery Males and F0 Females.....	64
3	Summary and Statistical Analysis of F0 Male Body Weights and Weight Change During the Prebreed and Mating Periods.....	66
4	Summary and Statistical Analysis of F0 Male Feed Consumption During the Prebreed Period.....	67
5	Summary of the F0 Male Clinical Observations During the Prebreed and Mating Periods.....	68
6	Summary and Statistical Analysis of F0 Recovery Male Body Weights and Weight Changes.....	71
7	Summary of the Recovery Male Clinical Observations.....	73
8	Summary and Statistical Analysis of F0 Female Body Weights and Weight Change During the Prebreed, Mating and Post Mating Periods.....	76
9	Summary and Statistical Analysis of F0 Female Feed Consumption During the Prebreed Period.....	78
10	Summary of the F0 Female Clinical Observations During the Prebreed, Mating and Post Mating Holding Periods.....	79
11	Summary and Statistical Analysis of F0 Female Body Weights and Weight Change During the Gestation Period.....	81
12	Summary and Statistical Analysis of F0 Female Feed Consumption During the Gestation Period.....	82
13	Summary of the F0 Female Clinical Observations During the Gestation Period.....	84
14	Summary and Statistical Analysis of F0 Female Body Weights and Weight Change During the Lactation Period.....	87
15	Summary and Statistical Analysis of F0 Female Feed Consumption During the Lactation Period.....	89
16	Summary of the F0 Female Clinical Observations During the Lactation Period.....	91
17	Summary and Statistical Analysis of F0 Reproductive and Lactational Indexes for the F1 Litters.....	95
18	Summary and Statistical Analysis of the F1 Litter Size, Pup Body Weights, Pup Anogenital Distance, Percent Male Pups and Pup Nipple/Areolae Retention During Lactation.....	98
19	Summary of the F1 Pup Clinical Observations on Postnatal Days 0 Through 21.....	103
20	Summary of the F1 Pup Necropsy Findings on Postnatal Days 0 Through 21.....	104

LIST OF TABLES (continued)

Table	Title	Page
21	Summary of the F1 Pup Necropsy Findings for Pups Culled on Postnatal Day 4	105
22	Summary and Statistical Analysis of F1 Pup Organ Weights and Relative Organ Weights on Postnatal Day 21	106
23	Summary of the F1 Pup Necropsy Findings on Postnatal Day 21	110
24	Summary and Statistical Analysis of the F0 Male Organ Weights and Relative Organ Weights.....	111
25	Summary of the F0 Male Gross Necropsy and Microscopic Findings	115
26	Summary and Statistical Analysis of the F0 Recovery Male Organ Weights and Relative Organ Weights.....	118
27	Summary of F0 Recovery Male Gross Necropsy Findings	122
28	Summary and Statistical Analysis of the F0 Female Organ Weights and Relative Organ Weights.....	123
29	Summary of F0 Female Gross Necropsy Findings and Microscopic Findings	127
30	Summary of the Fate of the F1 Males and F1 Females.....	130
31	Summary and Statistical Analysis of the F1 Female Vaginal Opening and the F1 Male Preputial Separation Data	131
32	Summary and Statistical Analysis of the F1 Male Body Weights and Weight Change During the Post Wean Holding Period	132
33	Summary and Statistical Analysis of the F1 Male Feed Consumption During the Post Wean Holding Period	135
34	Summary of the F1 Male Clinical Observations During the Post Wean Holding Period..	138
35	Summary and Statistical Analysis of the F1 Female Body Weights and Weight Change During the Post Wean Holding Period	143
36	Summary and Statistical Analysis of the F1 Female Feed Consumption During the Post Wean Holding Period	146
37	Summary of the F1 Female Clinical Observations During the Post Wean Holding Period	149
38	Summary and Statistical Analysis of the F1 Female Vaginal Cytology During the Post Wean Holding Period	153
39	Summary and Statistical Analysis of the F1 Male Organ Weights, Relative Organ Weights, and Sperm Analysis	154
40	Summary of F1 Male Gross Necropsy Findings and Microscopic Findings	159
41	Summary and Statistical Analysis of the F1 Female Organ Weights and Relative Organ Weights.....	162
42	Summary of F1 Female Gross Necropsy Findings and Microscopic Findings	166

RTI Project No.: 65C-07895.300
RTI Protocol No.: RTI-810

FINAL REPORT

Reproductive/Developmental Toxicity Screening Test of Trisnonylphenyl Phosphite (TNPP)
Administered via Oral Gavage to CD® (Sprague-Dawley) Rats (Modified OECD 421)

ABSTRACT

The purpose of this study is to evaluate the potential for trisnonylphenyl phosphite (TNPP), administered by oral gavage once daily, seven days per week in CD® rats, to cause toxic characteristics from repeated dosing, encompassing two-week prebreed, mating (for both sexes), gestation, and lactation (for F0 females) for F0 parents and direct dosing to F1 offspring from weaning through scheduled sacrifice, at least seven weeks postweaning. This study was performed, to the extent possible, in compliance with the Organization for Economic Cooperation and Development (OECD) Guideline for the Testing of Chemicals (OECD 421; 1995), and under TSCA (U.S. EPA, 1989) and OECD (OECD, 1998) Good Laboratory Practice principles and regulations. This study exceeds the OECD 421 study design as follows: (1) enhanced evaluation of toxicity in the F0 generation, including the evaluation of a recovery group of males; (2) evaluation of developmental landmarks in the F1 generation; and (3) following the F1 offspring to adulthood, with continued exposure and assessments of reproductive structures and functions, including potential effects on sperm.

Male and female CD® (Sprague-Dawley) rats (the F0 generation) were administered TNPP (CAS No. 26523-78-4) orally by gavage at 0, 50, 200, or 1000 mg/kg/day at a dose volume of 5 ml/kg/day in Mazola® corn oil, ten/animals/sex/dose, for two weeks of prebreed exposure (males and females) and two weeks of mating (males and females) for F0 parents. F0 females continued to be dosed for three weeks each of gestation and lactation, as were F1 offspring from weaning through scheduled sacrifice, at approximately 85 days of age. Five additional F0 males per group from the control and 1000 mg/kg/day groups were designated as recovery animals and held without dosing for two weeks, after the F0 male dosing period was completed to evaluate recovery from any possible treatment-related effects identified in the high dose. Body weights and feed consumption for the F0 males and females were recorded at least weekly during the prebreed period for both sexes, for F0 females during gestation and lactation, and F1 offspring from birth through scheduled sacrifice. Clinical signs were recorded at least

once daily for all animals. After the two-week prebreed exposure period, animals were randomly mated within treatment groups for a two-week mating period to produce the F1 generation, with continuing exposure. On the day of birth (postnatal day [pnd] 0), anogenital distance (AGD) was measured and body weights recorded for all live F1 pups in all litters. F1 litters were culled on pnd 4 to yield, as nearly as possible, five males and five females per litter. The culled F1 pups were weighed, euthanized, and necropsied with complete external and visceral examinations. For the remaining F1 pups, survival indices were calculated at least weekly through weaning (pnd 21). At weaning, at least one female and one male (whenever possible) from each F1 litter were randomly selected to continue treatment for approximately seven more weeks, with dosing for F1 selected pups begun on pnd 22 until all pups were at least 85 days of age. F1 postweaning observations and procedures for each retained female included examination for patency of vaginal opening (from pnd 22 until acquisition of vaginal opening). Estrous cyclicity and normality were evaluated by vaginal smears from F1 females taken daily the last three weeks of the postwean exposure period prior to scheduled sacrifice. For each retained F1 male offspring, observations for the cleavage of the balanopreputial gland (preputial separation) began at 35 days of age and continued until acquisition of preputial separation. Andrologic assessments were also performed on the F1 retained males at necropsy. All F0 parental animals, nonselected F1 weanlings, and retained F1 adults were necropsied, with complete histologic evaluation of five selected F0 and F1 males and females in the 0 and 1000 mg/kg/day groups. Histopathology was performed on F1 males and females (five/sex/group) at 0 and 1000 mg/kg/day.

Exposure once daily to TNPP at 0, 50, 200, or 1000 mg/kg/day resulted in mild systemic toxicity in F0 parental males at 1000 mg/kg/day, expressed as reduced weight gains, increased kidney weights, and renal histopathologic findings. Three of ten pregnant F0 parental females from the 1000 mg/kg/day group were found dead on Day 22 of gestation. These deaths may have been related to dystocia, since the dams appeared to be unable to deliver their normal appearing pups. In addition, absolute ovarian weights and ovarian weights relative to terminal body or brain weights were reduced in F0 females at 1000 mg/kg/day. No other consistent, treatment-related effects were observed. There was no evidence of F0 reproductive toxicity in males. The only evidence of F1 offspring toxicity pre- or postnatally was reduced litter size on pnd 4 (but not on pnd 0) and renal histopathology in F1 adult males at 1000 mg/kg/day.

In conclusion, TNPP, administered by gavage once daily at 0, 50, 200, and 1000 mg/kg/day to parental F0 CD® (SD) rats, ten/sex/group, through prebreed, mating, gestation, and lactation and direct dosing to F1 offspring from weaning to scheduled sacrifice, resulted in adult F0 parental toxicity at 1000 mg/kg/day, in males represented by slightly decreased weight gain, increased kidney weights, and the presence of renal corticomedullary junction mineralization. There was F1 offspring toxicity observed postnatally at 1000 mg/kg/day, expressed as reduced litter size on pnd 4 and corticomedullary mineralization in the adult F1 male kidneys at 1000 mg/kg/day. No effects were seen on the developmental landmarks, including the time to vaginal opening or preputial separation, or estrous cycle normality or length. Therefore, under the conditions of this study in rats, the no observable adverse effect levels (NOAELs) for systemic parental toxicity and for reproductive and offspring toxicity were 200 mg/kg/day.

OBJECTIVES

This study was performed to evaluate the potential of TNPP, administered by oral gavage to CD® rats, to produce toxic characteristics from repeated dosing during prebreed, mating (for both sexes), gestation, and lactation (for F0 females) for F0 parents and direct dosing to F1 offspring from weaning to scheduled sacrifice, at least seven weeks postweaning. Assessment of male and female reproductive performance, such as gonadal function, mating behavior, conception, development of the conceptus, and parturition was made. Offspring survival, growth and development through lactation, and weaning through reproductive development until adulthood were also evaluated.

MATERIALS AND METHODS

Test Material and Dose Formulations

The test material, TNPP (CAS No. 26523-78-4), was received from Dover Chemical Corporation, 3676 Davis Road NW, Dover, OH. The test material was a light-colored liquid with phenolic odor and was identified by the supplier as Batch No. 173T060401 with a purity of 99.98%, as specified in the Certificate of Analysis (attachment to protocol in Appendix IV).

The purity of TNPP, used as the test material, was assumed to be 100% for dose formulation purposes (i.e., all measurements of the test material were made by weight and not corrected for the purity of TNPP). The vehicle was Mazola® corn oil (CAS No. 8001-30-7).

For dosing formulations, TNPP was weighed into tared, color-coded glass beakers for each concentration, and the appropriate amount of vehicle was added. The formulations were stirred and then transferred to two labeled, wide-mouth amber bottles.

For analysis of the dosed formulations, a compound stock solution A, at a concentration of 250 mg/g, was made by weighing 2.5 g of TNPP into a tared vial. Mazola® corn oil was added to achieve a final weight of 10 g and then stirred for 30 minutes. A compound stock solution B, at a concentration of 200 mg/g, was made by weighing 2 g of TNPP into a tared vial. Mazola® corn oil was added to a final weight of 10 g total and then stirred for 30 minutes. Matrix standards were prepared by transferring the necessary volume amounts of stock A or B to tared 50-ml centrifuge tubes. The transfer weights were recorded, and Mazola® corn oil was added to each sample to obtain a 1-g solution weight, combined with a 1-ml aliquot of internal standard (n-butyrophenone, 6.1 mg/ml) and 24 ml of acetone. The tubes were vortexed and centrifuged at high speed for ten minutes. Aliquots of the top layers were filtered and transferred to autosampler vials for analysis. Matrix blanks were prepared by adding 25 ml of acetone to a weighed 1-ml aliquot of corn oil. Each sample was vortexed and then centrifuged at high speed. An aliquot was filtered and transferred to a vial for analysis. Each sample was analyzed by single injection using high performance liquid chromatography with a Model 680 Automated Gradient Controller (Waters Corporation), with a Model 510 and M6000 pump and a Model 710B WISP autosampler (Waters Corporation). The column was a ABZ Plus (Supelco), 4.6 m x 50 mm; 5 µm, 120Å (Agilent), the guard column was a Polaris C18-A Metaguard (Metachem), and the detector was a Model 757 Absorbance Detector (Applied Biosystems). The regression equation for the vehicle standard data was calculated, and the data were plotted for a visual evaluation of linearity. The concentration of TNPP was calculated in the dose formulation samples (mg/ml) from the peak area ratio for each sample and the linear regression equation.

Standards for acceptable accuracy of mixing were: the mean of the analyzed samples was within $\pm 10\%$ of nominal, and the % RSD (Relative Standard Deviation) for triplicate samples did not exceed 10%. If one or both of these standards was not met, the dosing solutions

were not administered to the animals until the problem was resolved by analysis of the archived sample of the formulation.

Homogeneity and stability of the test material in Mazola® corn oil, for the ranges used in this study, were determined by RTI as follows. Prior to and during the performance of this study, aliquots of doses, encompassing the range of concentrations that were to be used in the study (5.0 and 200 mg/ml; actual concentrations used for the study were 10, 40, and 200 mg/ml), were used to assess stability of the dose suspensions. The low-dose formulation was prepared by weighing approximately 1 g TNPP in a 400-ml beaker precalibrated to 200 ml. An approximate 200-ml aliquot of the vehicle (Mazola® corn oil) was added gradually to the calibration line, and the solution was stirred for at least 30 minutes before sampling and storage. Triplicate 1-ml aliquots were collected from the top, middle, and bottom of the beaker. The aliquots were transferred to tared 50-ml centrifuge tubes and the weights recorded. A 1-ml aliquot of internal standard solution (n-butyrophenone, 6.6 mg/ml) was added to each sample, followed by 24 ml of acetone. The samples were vortexed for one minute and centrifuged at high speed for ten minutes. An aliquot was carefully removed from the top layer, filtered through a 0.45 µm filter, and analyzed by HPLC as described above. For the high dose, a total of 40 g TNPP was placed in a 400-ml beaker precalibrated to 200 ml. Mazola® corn oil was added to the 200-ml mark, and the solution was stirred for at least 30 minutes before sampling. Triplicate 1-ml aliquots were analyzed as for the low dose (see above).

For stability studies, the doses mixed for the low and high homogeneity study were stored under refrigerated or ambient conditions prior to sampling. The refrigerated samples were removed from the refrigerator on the day of sampling (day 3, 9, 21, and 34) and brought to ambient conditions. The formulations were stirred for 30 minutes prior to sampling. Triplicate 1-g aliquots were removed and analyzed as described above. Dose formulations at 5.0 and 200 mg/ml were stable for at least 34 days when stored in the refrigerator. Dosing simulation studies indicated that the formulations were stable for at least four hours at ambient temperature when exposed to air and light. All dose formulations used in the study had analytical values of 94.3 to 110% of target concentrations. Since all formulations were within 10% of nominal, nominal values were used for reporting and discussing. Vehicle control formulations contained no TNPP, with an estimated detection limit of 0.2 mg/ml. Details of the methods and results of the dose formulations and analyses are presented in the Analytical Chemistry Report (Appendix I).

Target doses of 0, 50, 200, and 1000 mg/kg/day were selected, based on the results of a two-week, range-finding study. All RTI technical staff involved in the in-life portion of this study, except for the Study Director, Laboratory Supervisor, and personnel involved in formulation and analyses of the dosing solutions, were not aware of the doses (groups were identified by color and Rx codes).

Animals and Husbandry

Fifty virgin female and 60 virgin male outbred albino CD® (Sprague-Dawley) rats (CrI:CD®[SD] IGS BR) were received from Charles River Breeding Laboratories, Raleigh, NC (males and females from area R10 and R12, respectively) on August 29, 2001; the animals were approximately 63 days old. (The actual dates of all major phases of the study are presented in Text Table A.)

Text Table A. Study Schedule

Event	Dates ^a
Animals arrived at RTI	08/29/01
Quality control – blood for viral antibody titers	08/29/01
Weighing and randomization	09/04/01
F0 prebreed exposure (2 weeks)	09/5/01 to 09/19/01
F0 mating (2 weeks)	09/19/01 to 10/03/01
F0 gestation (approximately 22 days)	09/20/01 to 10/16/01
Recovery male holding period	10/03/01 to 10/17/01
F0 male necropsy	10/04/01
Recovery male necropsy	10/17/01
F0 lactation (pnd 0 to pnd 21)	10/11/01 to 11/07/01
F0 female necropsy/F1 pnd 21 pups	11/01/01 to 11/07/01
F1 dosing	11/02/01 to 01/07/02
Necropsy of F1 adults	01/07/02 to 01/08/02

^a A range of dates is provided, when appropriate, since study females became pregnant on different days during the two-week mating period.

The animals were quarantined for approximately one week, during which time they were weighed and examined by a veterinarian. Representative animals were subjected to fecal examination and serum viral antibody analysis. For serum viral antibody analysis, within one day after receipt, five rats per sex were arbitrarily chosen from the shipment of animals, sacrificed, and blood collected for assessment of viral antibody status. Heat-inactivated serum was sent to BioReliance, Rockville, MD, for their Level 1 Rat Antibody Screen. The viral screen consisted of evaluation for the presence of antibodies against the following: Toolan H-1 virus (H-1), Sendai virus, pneumonia virus of mice (PVM), rat coronavirus/sialodacryoadenitis (RCV/SDA), Kilham rat virus (KRV), CAR *Bacillus* (CARB), and *Mycoplasma pulmonis* (*M. Pul.*). Results of the physical examination, serology, and parasitology were negative for signs of infectious disease; the animals were considered to be in good health and suitable for use in this study.

During the quarantine period, after selection of quality control animals and study animals, two rats per sex were randomly selected and designated as sentinels. They were singly housed in the study room(s) in polycarbonate solid-bottom cages with bedding, and provided feed and water *ad libitum* (as described below for study animals). They were examined once daily by cage-side observation for morbidity or mortality at the same time as clinical observations or morbidity/mortality checks for the study animals. No sentinels exhibited any clinical signs, morbidity, or mortality. At the time of necropsy of retained F1 offspring, the sentinels were terminated, blood samples collected, and serum samples prepared. All sentinel serum samples were submitted to BioReliance, Rockville, MD, for serological evaluation (see above). Analysis of serum (as described above) from sentinels sacrificed during the necropsy of the F0 parental animals was negative.

There were 90 animals (50 males and 40 females) assigned to the study at the initiation of the F0 prebreed exposure period (ten/sex/group were designated as parental animals, and five additional males in the control and high dose were designated as recovery males). F0 animals were uniquely identified prior to initiation of the study by ear tag. Selected F1 weanlings were identified by eartag and F1 pups prior to weaning were not uniquely identified. The weight variation of the study animals at initiation did not exceed $\pm 20\%$ of the mean weight for each sex. The method and numbers for identification were documented in the study records.

All adult animals were euthanized by CO₂ asphyxiation. F1 pups culled on pnd 4 were sacrificed by decapitation. Animals received with the initial shipment, but not used in the study, were removed from the study room prior to the start of the treatment period and used for methods development and training of the RTI staff. Records were kept documenting the fate of all animals received for the study.

The experiment was carried out under standard laboratory conditions. The animals were individually housed during the quarantine period and upon the initiation of the treatment period in solid-bottom polycarbonate cages with stainless-steel wire lids (Laboratory Products, Rochelle Park, NJ) with Sani-Chip® cage litter (P.J. Murphy Forest Products Corp., Montville, NJ). Study animals were housed two per cage (one male:one female from the same dose level) during the mating period. Females were caged separately and individually once they were successfully mated (or at the end of the mating period). F0 females were housed with their F1 litters during lactation. Postwean, retained F1 males and females were housed singly until necropsy. All animals were housed in the RTI Animal Research Facility for the duration of the study. All animal rooms were on a 12-hour light cycle per day and were air-conditioned; temperature and relative humidity (RH) were continuously monitored, controlled, and recorded using an automatic system (Siebe/Barber-Colman Network 8000 System, Version 4.4.1, Loves Park, IL). The protocol-mandated temperature range was 66-77°F (22+3°C), and the RH range was 30-70% (OECD, 1995; NRC, 1996). The F0 animals (and F1 pups during lactation) were housed in Room 305 of the Animal Research Facility, and the F1 animals postweaning were housed in Room 402 (one F0 female, No. 68, was moved to Room 402 on pnd 20 of her F1 litter, one day prior to weaning). Temperature and RH readings for Room 305 from September 5 to November 6, 2001, were 70.8 to 76.6°F and 44.5 to 63.9% RH. Temperature and RH readings for Room 402 from November 1, 2001, to January 8, 2002, were 70.7 to 73.8°F and 47.6 to 67.5% RH. One deviation to the protocol occurred in Room 305 on September 24, 2001, when the RH was above that specified in the protocol for one hour with a reading of 92.1% RH (see Protocol Deviations before the QA Statement).

Purina Certified Rodent Chow (No. 5002, PMI Feeds, Inc., St. Louis, MO; batch numbers documented in the study records) was available *ad libitum*. The analyses of each feed batch for nutrient levels and possible contaminants were performed by the supplier, examined by the Study Director, and maintained in the study records. There was a slight increase in the levels of

malathion in the feed analyses for three batches of Purina Certified Rodent Chow (Lot No. L0124351-2, L0125799-2, and L0122048-1), with levels of 0.17, 0.03, and 0.04 ppm, respectively (specification for malathione is <0.02 ppm), but this had no effect on the study.

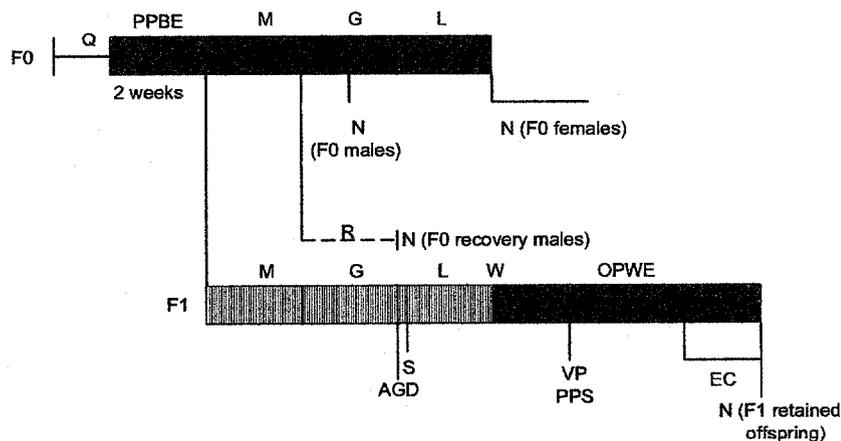
Water (tap water; source: City of Durham, Department of Water Resources, Durham, NC) was available *ad libitum* by plastic water bottles with butyl rubber stoppers and stainless-steel sipper tubes. Contaminant levels of the Durham City water were measured at regular intervals by the supplier per EPA specifications. The tap water was analyzed by Balazs Analytical Laboratories, Inc. (Sunnyvale, CA) and U.S. Biosystems (Boca Raton, FL). There were no known contaminants that may have affected the outcome of this study.

Study Design

A graphic representation of the study design is presented in Figure 1. The study began with ten males/group and ten females/group. In addition, five males were assigned to each of the 0 and 1000 mg/kg/day groups and were designated as recovery males.

Exposure began for all F0 animals on September 5, 2001, when they were approximately ten weeks old. The doses were chosen based on a range-finding study, employing doses of 0, 100, 300, or 1000 mg/kg/day, administered by oral gavage at 5 ml/kg for ten days. There were no effects in either sex, on any parameters examined, at any dose employed in the range-finding study. Because 1000 mg/kg/day is designated as a "limit" dose per OECD testing guidelines, the doses chosen for this study were 0, 50, 200, and 1000 mg/kg/day. Animals were assigned to the different groups by means of randomization stratified by body weight, such that the body weights of all groups by sex were homogeneous at study initiation. The range of F0 male body weights at the start of the prebreed exposure period was 328.2 to 361.8 g. The range for F0 females was 214.6 to 241.7 g.

Figure 1. Study Design



■ Direct exposure via oral gavage

▨ Possible indirect exposure from transplacental and/or translactational exposure

--- No dosing

KEY:

Q = Quarantine

PPBE = Parental prebreed exposure (two wks)

M = Mating (two wks)

G = Gestation (three wks)

L = Lactation (three wks)

N = Necropsy

AGD = F1 pup anogenital distance on pnd 0

R = Recovery period, 5 males in high and control groups for two wks

W = Weaning

OPWE = Offspring postwean exposure (7 wks)

S = Standardize litters to 10 pups on pnd 4

VP = Acquisition of vaginal patency (females)

PPS = Acquisition of preputial separation (males)

EC = Estrous cyclicity

Parental F0 Animals, Including Recovery Animals (F0)

Animals of the F0 generation were dosed with vehicle control or 50, 200, or 1000 mg/kg of TNPP in Mazola® corn oil at 5 ml/kg, based on the most recent body weight. Dosing was done once daily by oral gavage with an appropriate-sized syringe fitted with a 16 g two-inch stainless-steel curved dosing needle (Perfektum®, Popper and Sons, New Hyde Park, NY). Parental animals were dosed daily, beginning with the prebreed period until the day prior to necropsy. Recovery males were dosed daily during the prebreed and mating periods of the study animals, then held an additional two weeks without dosing.

Observations for mortality were made twice daily (a.m. and p.m.), and the general condition of all animals was checked daily. Clinical examinations were conducted and recorded daily throughout the course of the study. This record included the time of onset and degree and duration of symptoms. These cage-side observations included, but were not limited to, changes

in: skin and fur, eyes, mucous membranes, respiratory and circulatory system, autonomic and central nervous system, somatomotor activity, and behavioral pattern.

The body weights of the F0 male rats were determined and recorded initially and then weekly throughout mating. The body weights of F0 female rats were recorded in the same manner until confirmation of mating. During gestation, F0 females were weighed on gd 0, 7, 14, and 20. Dams producing litters were weighed on lactational days (pnd) 0, 4, 7, 14, and 21, and body weight gains were computed.

Feed consumption measurements were recorded weekly for all F0 parental study animals during the prebreed treatment period. During pregnancy of F0 females, feed consumption was recorded for gd 0-7, 7-14, and 14-20. During lactation of the F1 litters, maternal feed consumption was measured for pnd 0-7, 7-14, and 14-21, although maternal feed consumption after pnd 14 was confounded by the contribution from the pups since the pups were self-feeding by this time. Feed consumption was not measured during the cohabitation period since two adult animals (breeding pair) were in the same cage. Feed consumption collection periods corresponded to the collection of the animals' body weight data.

After two weeks of dosing, the animals were mated on the basis of one male to one female, selected randomly within each dose group, for a period of 14 days with no change in mating partners. The observation of vaginal sperm or copulation plug was considered evidence of successful mating. Females were examined daily during the cohabitation period for the presence of sperm or copulation plug in the vaginal tract. The observation of vaginal sperm or copulation plug was considered evidence of successful mating. The day vaginal sperm (or plug) were observed was designated gd 0 (Hafez, 1970). Once vaginal sperm were observed, the male and female from that mating pair were individually housed. Any female that did not show evidence of successful mating after 14 days of cohabitation was weighed weekly, and treatment continued for 26 days or until delivery occurred. If a female without a confirmed gd 0 date was, in fact, pregnant and delivered a litter, her lactational information was collected as described below. Beginning on gd 20, each female was observed twice daily (a.m. and p.m.) for evidence of littering. On the day of birth (pnd 0), AGD was measured and body weight recorded for all live F1 pups in all litters. Body weight was recorded for all live pups on pnd 4 prior to culling and euthanasia. The dams were allowed to rear their young to pnd 21. On pnd 21, each litter was weaned. When each F1 litter had reached pnd 21, at least one male and one female pup, if

possible, for a total of ten/sex/dose, was randomly selected. Each litter was represented at least once per sex, if possible, until a total of ten per sex per treatment group was attained. These selected F1 animals were administered TNPP by gavage at the same dose as their parents, seven days per week, for at least seven more weeks. All F0 parental animals in all groups were subjected to a complete gross necropsy, where selected organs were weighed and/or retained in fixative for possible subsequent histopathologic evaluation. Two weeks after treatment ended, the recovery males were subjected to a complete gross necropsy, with reproductive and selected organs weighed and/or retained in fixative for possible subsequent histopathological evaluation.

Progeny (F1)

All F1 pups were counted, sexed, weighed, and examined as soon as possible after birth (date of birth designated pnd 0) to determine the number of viable and stillborn pups from each litter. Thereafter, litters were evaluated for survival on pnd 4, 7, 14, and at weaning (pnd 21). Individual AGD and body weight were recorded on pnd 0 for all F1 offspring.

On pnd 4, the size of each litter was adjusted to ten by eliminating extra pups by random selection to yield, as nearly as possible, five males and five females per litter. The OECD 421 study design (OECD, 1995) specifies termination of the study on pnd 4, with external and internal examination of the F1 pups at this time. The modified study design used in this study provides for continuation of the F1 offspring, with continuing exposure until sexual maturity. To provide data on the pnd 4 pups, the pups culled to standardize litters on pnd 4 were euthanized and subjected to a complete gross (external and visceral) necropsy, gross lesions retained in fixative, and nonretained tissues and carcasses from the necropsied pups discarded.

All live pups were counted, sexed, weighed individually, and examined grossly at birth (pnd 0), pnd 4, 7, 14 and at weaning (pnd 21). The body weights and sexes were recorded on an individual basis, but the pups were not uniquely identified. AGD was recorded with the individual pup weight on pnd 0 for all F1 pups, and the presence or absence of retained nipples and areolae on the ventrum was recorded for F1 male offspring at approximately pnd 11-13. All pups were examined for physical abnormalities at birth and throughout the preweaning and postwean period. All pups dying during lactation were necropsied, when possible, to investigate the cause of death.

At weaning (pnd 21), at least one female and one male (whenever possible) from each F1 litter, for a total of ten/sex/group, were selected on a random basis to continue treatment for approximately seven more weeks (dosing for F1 selected pups began on pnd 22 and continued until all pups were at least 70 days of age).

F1 postweaning observations and procedures for each retained female included examination for patency of vaginal opening (from pnd 22 until acquisition of vaginal opening) and determination of estrus cyclicity and normality evaluated by vaginal smears taken daily the last three weeks of the postwean exposure period prior to scheduled sacrifice. For each retained male offspring, observations for the cleavage of the balanopreputial gland (preputial separation) began at 35 days of age and continued until acquisition of preputial separation. All retained F1 postweanling were weighed weekly (with clinical observations daily) until scheduled necropsy.

All retained F1 adults, and nonselected F1 weanlings in all groups were subjected to a complete gross necropsy, where selected organs were weighed and/or retained in fixative for possible subsequent histopathologic evaluation.

Necropsy and Histology

Full Necropsy at Scheduled Sacrifice (F0 Parents, F1 Weanlings, and Adults)

All F0 parental animals (including control and high dose F0 recovery males, five/group) and retained F1 adults were subjected to a complete gross necropsy, with selected organs (see below) weighed. Uterine nidation scars were recorded for F0 females. The following organs were weighed and retained in 10% neutral buffered formalin, except one testis and one epididymis per F1 male, which were retained in Bouin's fixative for 24 hours and then stored in 70% ethanol. The other testis was frozen at approximately -20°C , and the other epididymis had its cauda removed prior to fixation; both testes and epididymides from F0 parental males were fixed as described above for F1 adult males. Selected organs weighed were:

Ovaries (pair)	Prostate	Epididymides (pair)
Uterus with cervix and vagina	Testes (pair)	
Seminal vesicles with coagulating glands and their fluids (pair)		

In addition, the following organs were weighed and saved in 10% neutral buffered formalin from five arbitrarily selected F0 and F1 adult males and females per group (and the five males from the recovery control and high dose groups):

Liver	Heart	Kidneys (pair)
Adrenals (pair)	Brain (including cerebrum, cerebellum, and pons)	
Spleen	Thymus	

In addition to the organs listed above, the organs listed below were also saved in 10% neutral buffered formalin from the same selected five F0 and F1 adult males and females per group (and the five males from the recovery control and high dose groups):

Spinal cord	Thyroid	Stomach
Urinary bladder	Peripheral nerve (sciatic nerve)	
Bone marrow (femur)		
Small and large intestines (including Peyer's patches)		
Trachea and lungs (preserved by inflation with fixative and then immersion fixed)		
Lymph nodes (one cervical, near route of administration; and one mesenteric, distant from the route of administration)		
All gross lesions		

Full histopathology of the organs listed above was performed for the selected five high dose and control F0 and F1 males and females (with special emphasis on stages of spermatogenesis in the male gonads and histopathology of interstitial testicular cell structure). The pathology report, with individual and summarized data, is presented in Appendix III.

The following organs were weighed from the nonselected F1 weanlings, subjected to necropsy:

Brain	Ovaries (pair)	Epididymides (pair)
Spleen	Uterus with cervix and vagina	
Thymus	Testes (pair)	

Sacrifice of the F0 parental males occurred after the two-week mating period, sacrifice of the recovery males occurred two weeks after the four-week dosing period, and sacrifice of the F0

maternal animals occurred after F1 litters were weaned. The fixed (buffered neutral 10% formalin) uteri from any females of the F0 generation failing to produce a litter were stained with potassium ferricyanide for confirmation of pregnancy. This staining procedure did not interfere with subsequent histopathologic evaluation. Nonselected F1 offspring were sacrificed at weaning. Sacrifice of retained F1 offspring occurred when they were approximately 85 days of age.

All organ weights were reported as absolute and as relative to terminal body weight and brain weight.

Necropsy of Unscheduled Dead Animals

No unscheduled deaths occurred in the F0 and F1 males. One unscheduled death occurred in the F0 recovery males at 1000 mg/kg/day. Unscheduled deaths occurred in the F0 females at 50 and 1000 mg/kg/day (one and four, respectively).

Necropsy of Pnd 4 Culled Pups

All F1 pups culled on pnd 4, when the litters were standardized, were subjected to a complete external and visceral examination, including examination of all thoracic and abdominal organs, bisection of kidneys, and heart dissection.

F1 Male Andrology

At the time of sacrifice, one testis from each F1 male was frozen at approximately -20°C for subsequent enumeration of testicular homogenization-resistant spermatid heads for high dose and control males. If treatment-related changes in the number of testicular homogenization-resistant spermatid heads were observed in the high dose group, then these evaluations were extended to the mid and low dose group animals (from retained frozen testes). In addition, one cauda epididymis from each F1 male was immediately removed, weighed, and seminal fluid from the cauda was assessed for sperm number, motility, and morphology. Sperm motility (motile and progressively motile) was assessed immediately after necropsy for all males; number and morphology were evaluated using appropriately retained sperm samples initially from the high dose and control males. If treatment-related andrological changes had been observed in the high dose, then these evaluations would have been extended to the mid and low dose animals (from retained sperm samples). Sperm motility and number were assessed using an HTM-IVOS

(Version 10.8 S) Automated Sperm Analysis System (Hamilton-Thorne Research, Beverly, MA). Sperm morphology was assessed manually on 200 sperm per male (fixed and stained with Eosin Y).

Reproductive and Offspring Indices

The indices for reproductive performance and gestational and postnatal parameters that were calculated for this study are presented in Text Table B. The indices include those for F0 males and females to produce F1 litters.

Calculation of Standard Reproductive Toxicity Parameters

The calculations used to summarize standard reproductive toxicity parameters for this study are presented in Text Table C.

Text Table B
Reproductive and Offspring Indices

Females:	Mating index (%)	=	$\frac{\text{No. females sperm positive}}{\text{No. females paired}} \times 100$
	Fertility index (%)	=	$\frac{\text{No. females pregnant}}{\text{No. females sperm positive}} \times 100$
	Gestational index (%)	=	$\frac{\text{No. females with live litters}}{\text{No. females pregnant}} \times 100$
Males:	Mating index (%)	=	$\frac{\text{No. males impregnating females}}{\text{No. males paired}} \times 100$
	Fertility index (%)	=	$\frac{\text{No. males siring litters}}{\text{No. males impregnating females}} \times 100$
	Pregnancy index (%)	=	$\frac{\text{No. pregnant females}}{\text{No. males impregnating females}} \times 100$

(continued)

Text Table B (continued)

Offspring:	Live birth index (%)	=	$\frac{\text{No. live pups at birth}}{\text{Total no. pups born}} \times 100$
	4-Day survival index (%)	=	$\frac{\text{No. pups surviving 4 days (precul)} }{\text{Total no. live pups at birth}} \times 100$
	7-Day survival index (%)	=	$\frac{\text{No. pups surviving 7 days}}{\text{Total no. live pups at 4 days (postcull)}} \times 100$
	14-Day survival index (%)	=	$\frac{\text{No. pups surviving 14 days}}{\text{Total no. live pups at 7 days}} \times 100$
	21-Day survival index (%)	=	$\frac{\text{No. pups surviving 21 days}}{\text{Total no. live pups at 14 days}} \times 100$
	Lactation index (%)	=	$\frac{\text{No. pups surviving 21 days}}{\text{Total no. live pups at 4 days (postcull)}} \times 100$

Text Table C

Formulas for Calculating Standard Reproductive Toxicity Study Parameters^a

The following endpoints are calculated for each animal, and then the mean is calculated using the animal values.

1. Body Weight Change

body weight at end of measurement period – body weight at beginning of measurement period

2. Feed Consumption in Grams per Day

((feed weight at beginning of measurement period) – (feed weight at end of measurement period)) /
number of days in measurement period

3. Feed Consumption in Grams per Day per Kilogram Body Weight

feed consumption in grams per day / average of all body weights taken during measurement period
in kilograms

4. Relative Organ Weight

(organ weight / sacrifice body weight) x 100

5. Relative Organ to Brain Weight

(organ weight / brain weight) x 100

The following endpoints are calculated for each litter (dam), and then the mean is calculated using the litter (dam) values.

1. Percent Postimplantation Loss per Dam and Arcsine Root Transformation

100 x ((no. implantation sites – no. live pups) / no. of implantation sites)
arcsine (square root ((no. implantation sites – no. live pups) / no. of implantation sites))

2. Stillbirth Index per Dam and Arcsine Root Transformation

100 x (no. dead pups delivered / total number of pups delivered)
arcsine (square root (no. dead pups delivered / total number of pups delivered))

3. Live Birth Index per Dam and Arcsine Root Transformation

100 x (no. live pups delivered / total number of pups delivered)
arcsine (square root (no. live pups delivered / total number of pups delivered))

4. Four-Day Survival Index per Dam and Arcsine Root Transformation

100 x (no. pups alive on pnd 4 / no. of pups alive on pnd 0)
arcsine (square root (no. pups alive on pnd 4 / no. of pups alive on pnd 0))

(continued)

Text Table C (continued)

5. Percent Males per Litter and Arcsine Root Transformation

$$100 \times (\text{no. males in litter for given pnd} / \text{no. sexed in litter for given pnd})$$
$$\text{arcsine} (\text{square root} (\text{no. males in litter for given pnd} / \text{no. sexed in litter for given pnd}))$$

6. Average Pup Body Weight per Litter

$$\text{sum of all individual pup weights in litter for given pnd} / \text{no. pups weighed in litter for given pnd}$$

7. Average Male Pup Body Weight per Litter

$$\text{sum of all individual male pup weights in litter for given pnd} / \text{no. male pups weighed in litter for given pnd}$$

8. Average Female Pup Body Weight per Litter

$$\text{sum of all individual female pup weights in litter for given pnd} / \text{no. female pups weighed in litter for given pnd}$$

^a As required for FDA Good Laboratory Practices (subpart J, paragraph 58.185, no. 11), FIFRA Good Laboratory Practice Standards (subpart J, paragraph 160.185, no. 11), and TSCA Good Laboratory Practice Standards (subpart J, paragraph 792.185, no. 11).

Statistical Analyses

The unit of comparison was the male, the female, the pregnant female, or the litter, as appropriate. Treatment groups were compared to the concurrent control group using either parametric ANOVA under the standard assumptions or robust regression method (Zeger and Liang, 1986; Royall, 1986; Huber, 1967) that do not assume homogeneity of variance or normality. The homogeneity of variance assumption was examined via Levene's Test (Levene, 1960), which is much more robust to the underlying distribution of the data than the traditional Bartlett's Test. If Levene's Test indicated lack of homogeneity of variance ($p < 0.05$), robust regression methods were used to test all treatment effects. The robust regression methods used variance estimators that made no assumptions regarding homogeneity of variance or normality of the data. They were used to test for overall treatment group differences, followed by individual tests for exposed vs. control group comparisons (via Wald chi-square tests), if the

overall treatment effect was significant. The presence of linear trends was analyzed by GLM procedures for homogenous data or by robust regression methods for nonhomogenous data (SAS Institute Inc., 1989a,b, 1990a,b,c, 1996a,b, 1997). Standard ANOVA methods, as well as Levene's Test, are available in the GLM procedure of SAS® Release 6.12 (SAS Institute Inc., 1989a,b, 1996a,b, 1997), and the robust regression methods are available in the REGRESS procedure of SUDAAN® Release 7.5.3 (Shah et al., 1997).

If Levene's Test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® 6.12 was used to evaluate the overall effect of treatment and when a significant treatment effect was present, to compare each exposed group to control via Dunnett's Test (Dunnett, 1955, 1964). For the litter-derived percentage data (e.g., pnd 0-4 pup survival indices), the ANOVA was weighted according to litter size. A one-tailed test (i.e., Dunnett's Test) was used for all pairwise comparisons to the vehicle control group, except that a two-tailed test was used for parental and pup body weight and organ weight parameters, feed consumption, percent males per litter, and AGD. For endpoints which involved only two groups (e.g., for F0 recovery males and for certain F1 male andrological sessments), Student's t-test was used, available in SAS® Release 6.12 (SAS Institute Inc., 1997).

Frequency data such as reproductive indices (e.g., mating and fertility indices) were not transformed. All indices were analyzed by Chi-Square Test for Independence for differences among treatment groups (Snedecor and Cochran, 1967) and by the Cochran-Armitage Test for Linear Trend on Proportions (Cochran, 1954; Armitage, 1955; Agresti et al., 1990). If Chi-Square results revealed significant ($p < 0.05$) differences among groups, then a Fisher's Exact Probability Test, with appropriate adjustments for multiple comparisons, was used for pairwise comparisons between each treatment group and the control group. Acquisition of developmental landmarks (e.g., vaginal patency and preputial separation), as well as AGD, was also analyzed by Analysis of Covariance (ANCOVA; in addition to ANOVA analysis) using body weight at acquisition or measurement as the covariate. For correlated data (e.g., body and organ weights at necropsy of weanlings, with more than one pup/sex/litter), SUDAAN® software (Shah et al., 1997) was used for analysis of overall significance, presence of trend, and pairwise comparisons to the control group values.

A test for statistical outliers (SAS Institute, Inc., 1990b) was performed on parental body weights, feed consumption (in g/day), and F0 adult and F1 weanling and adult organ weights. If examination of pertinent study data did not provide a plausible, biologically-sound reason for inclusion of the data flagged as "outlier," the data were excluded from summarization and analysis and were designated as outliers. If feed consumption data for a given animal for a given observational interval (e.g., sd 0-7 or 7-14 during the prebreed exposure period) were designated outliers or unrealistic, then summarized data for that animal encompassing this period (e.g., sd 0-14 for the prebreed exposure period) also did not include this value.

Personnel

This study was conducted at RTI, Research Triangle Park, NC, under contract to General Electric Company. Dr. R.L. Joiner, Manager, Global Toxicology, GE, was the Sponsor's Representative. Dr. R.W. Tyl served as Study Director. Reproductive and Developmental Toxicology personnel included Ms. M.C. Marr (Laboratory Supervisor), Ms. C.B. Myers (Reproductive Toxicity Study Supervisor and Data Analyst), Mr. W.P. Ross and Ms. M.C. Rieth (Study Team), Ms. V.I. Wilson, Ms. L.B. Pelletier, Ms. D.A. Wenzel, Ms. C.N. Bauman, Ms. M.P. Gower, and Mr. T.W. Wiley. Bulk chemical handling and dose formulations were provided by Mr. M.M. Veselica (Supervisor, RTI Materials Handling Facility), Mr. D.L. Hubbard, Mr. R.A. Price, and Mr. J.E. Larson. Analyses of dose formulations were performed by Ms. N.P. Castillo (Analytical Task Leader). Analyses of the bulk test material were provided by the Sponsor. Animal care was provided by Dr. D.B. Feldman, DVM, ACLAM, Veterinarian, and Mr. F.N. Ali, Manager of RTI Animal Research Facility. Andrological assessments were performed by Dr. P.A. Fail (Manager), Ms. C.D. Sloan, Ms. K.D. Vick, and Mr. T.W. Wiley. Histologic preparations were supervised by Ms. T.-Y. Chang (HT-ASCP). Pathologic evaluation of histologic slides was performed by Dr. J.C. Seely, DVM, ACVP, EPL, Inc., Research Triangle Park, NC. RTI Quality Assurance personnel were Mr. D.L. Brodish, (Manager through December 31, 2001), Ms. D.J. Smith (Manager from January 1, 2002), Ms. C.D. Keller, Ms. P.D. Hall, Ms. M.D. Phillips, Ms. E.D. Shinauld, Ms. T.M. Kenney, and Ms. D.D. Rowe.

The final report was prepared by Dr. R.W. Tyl, Ms. B.T. Hamby, and Ms. M.C. Marr, with assistance from Ms. C.B. Myers on data compilation and statistical analyses, and by Mr.

T.W. Wiley on data entry. Ms. M.C. Marr was responsible for all transfer of custody procedures for histology and pathology, study records, and for archiving the study records.

Individual Scientist Reports and Protocol/Amendment

The individual scientist reports were prepared and signed by the author(s) and included as appendices to the final report.

The protocol and two amendments detailing the design and conduct of the study are presented in Appendix IV of this final report.

Storage of Records

All original data sheets, records, biological specimens, blocks, and slides for the present study will be stored in the RTI Archives, under the control of the RTI Chemistry and Life Sciences Archivist, along with all biological samples collected during the course of the study, which remain the responsibility of RTI. Worksheets and computer printouts, which were generated in the statistical analysis of data, are stored in the RTI Archives. Copies of this report are filed with the RTI Archives and with GE Plastics. Records and samples from this study in RTI Archives may be released to the Sponsor upon written request.

Compliance

All records, data, biological specimens, and reports will be maintained in storage for the time period specified by the appropriate testing guidelines or for as long as the quality of the preparation affords evaluation, whichever is less. This study was performed, to the extent possible, in compliance with OECD Guideline for the Testing of Chemicals (OECD 421; 1995) and under TSCA (U.S. EPA, 1989) and OECD (OECD, 1998) Good Laboratory Practice principles and regulations (with one exception: The individual who performed the gross trimming and embedding for the F0 male organs did not annotate or sign the data forms for these procedures; the data forms were completed by the individual's supervisor). This study exceeded the OECD 421 study design by following the offspring to adulthood, with continued exposure and assessments of reproductive structures and functions. The RTI Animal Research Facility is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International.

RESULTS

Dose Formulations

Prior to and during the study, dose formulations (5.0 and 200.0 mg/ml) encompassing the range of concentrations employed in the study (actual concentrations used were 10, 40, and 200 mg/ml) were found to be homogeneous, stable for four hours under room temperature (to simulate daily dosing periods), and stable for at least 34 days in amber bottles under refrigerated conditions (approximately 5°C), so formulations were used within the stability limits established and were stored under refrigeration. Dose formulations were 94.3-110% of target concentrations, and nominal values were therefore used in the report. No TNPP was detected in the vehicle control formulations with an estimated limit of detection of 0.2 mg/ml (Table 1 and Appendix D).

Fate of F0 Animals

No parental males died on study (Table 2). Therefore, there were 10, 10, 10, and 10 F0 males that were evaluated at scheduled sacrifice at 0, 50, 200, and 1000 mg/kg/day, respectively. Unscheduled deaths occurred in F0 females at 50 and 1000 mg/kg/day (1 and 4, respectively). Therefore, there were 10, 9, 10, and 6 F0 females that were evaluated at scheduled sacrifice at 0, 50, 200, and 1000 mg/kg/day. The unscheduled deaths of the low dose F0 female during gestation and one of the high dose F0 females during lactation were attributed to dosing errors and were not considered treatment related. Of the three remaining unscheduled F0 female deaths, all were found dead on gd 22, likely due to dystocia; their demise was considered treatment related. Five and four recovery males at 0 and 1000 mg/kg/day, respectively, were evaluated at scheduled sacrifice. The unscheduled death in the high dose recovery male was attributed to a dosing error and was not considered treatment related.

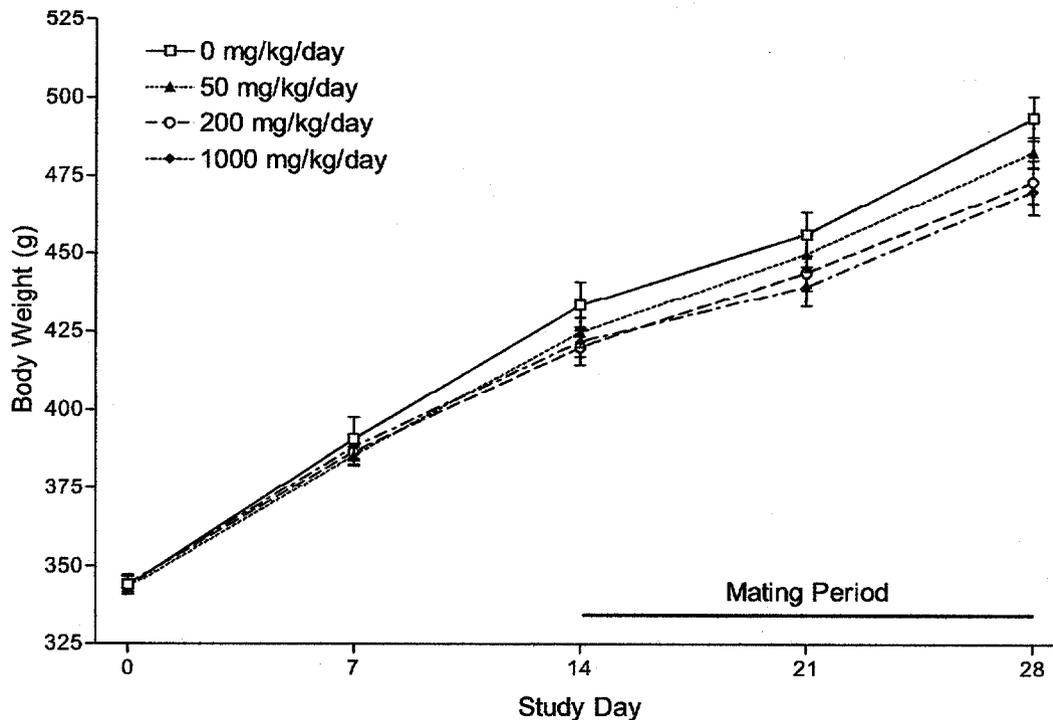
F0 Prebreed

F0 Males

There were no statistically significant differences among groups for F0 male body weights for any time point during the prebreed and mating periods (sd 0 to 28) (Table 3 and Figure 2). The only significant difference in body weight change was a significant decrease at 1000 mg/kg/day from sd 0 to 28 (from the beginning of prebreeding to the end of mating). There were no significant effects on F0 male feed consumption expressed as g/day, except during the

first week of prebreeding (sd 0-7) when it was significantly decreased at 50 mg/kg/day. Feed consumption values expressed as g/kg/day were significantly increased at 1000 mg/kg/day during the second week of prebreeding (sd 7-14) (Table 4).

Figure 2. F0 Male Body Weights



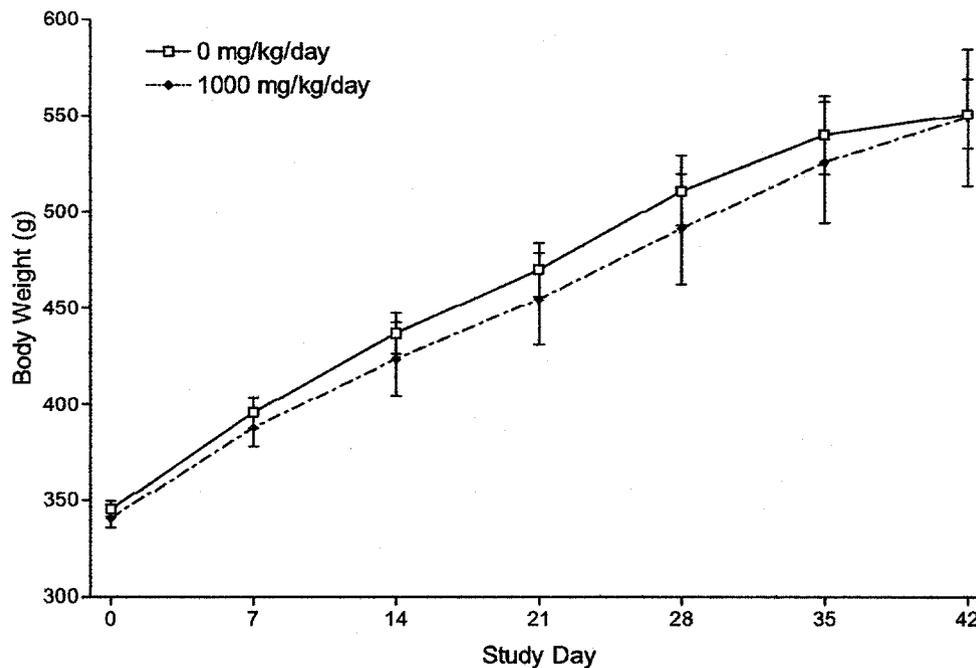
Clinical observations of F0 males during this period included alopecia in four males, rust-colored fur in two males, thickened area in the skin around the neck in one male, and rooting postdosing in one male each at 0 mg/kg/day; efflux of dosing solution in four males, rooting postdosing in three males, and alopecia, audible breathing postdosing, rooting prior to dosing, and rust-colored fur in one male each at 50 mg/kg/day; efflux of the dosing solution in four males, alopecia in two males, and rooting postdosing in one male at 200 mg/kg/day; rooting postdosing in ten males, efflux of the dosing solution in two males, and rust-colored fur in one male at 1000 mg/kg/day (Table 5). Rooting postdosing was noted across all groups, including the control group (with highest incidence at 1000 mg/kg/day). This behavior was most likely

due to the administration of the test material; it is not considered indicative of toxicity, per se. None of the other findings exhibited a dose-response pattern of incidence or severity.

F0 Recovery Males

There were no statistically significant differences between control and high-dose males for body weight or body weight change during the four-week treatment period or during the two-week recovery period. The lower but not statistically different body weights in the parental groups appeared to recover to (or near) normal by the end of the second week of recovery (Table 6 and Figure 3). Clinical signs were minimal and included rust-colored fur in two males and alopecia and rooting postdosing in one male each at 0 mg/kg/day; and rooting postdosing in two males and efflux of the dosing solution and piloerection in one male each at 1000 mg/kg/day. One male assigned to the recovery group at 1000 mg/kg/day was found dead on sd 8 during the dosing period (Table 7).

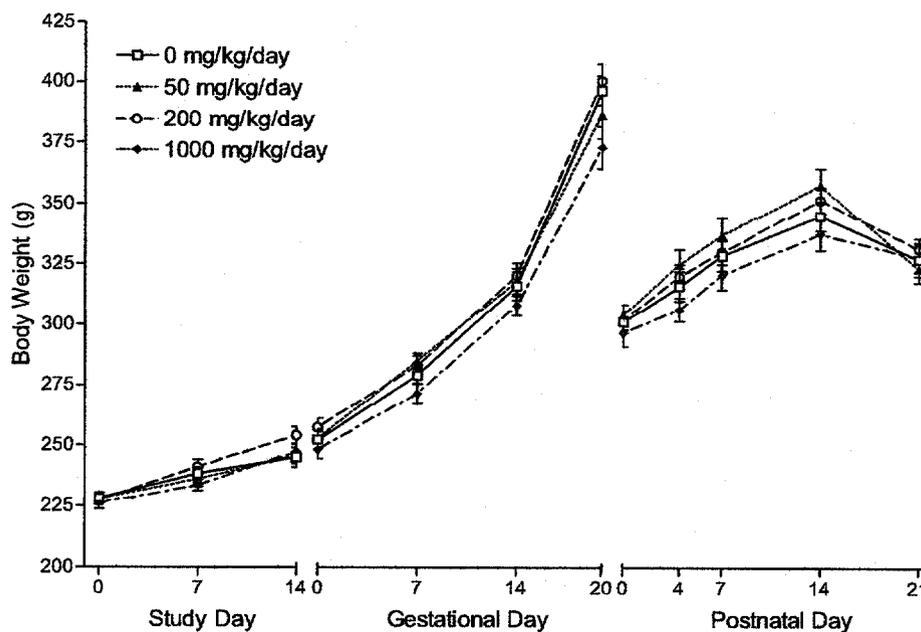
Figure 3. Recovery Male Body Weights



F0 Females

There were no significant differences among groups for F0 female body weights or body weight changes during the prebreed, mating, or postmating period (sd 0 to 35) (Table 8 and Figure 4). There were no significant effects of treatment on F0 female feed consumption expressed as g/day or g/kg/day for the first week of the prebreed period (Table 9). Feed consumption expressed as g/day for the second week of prebreed was significantly increased at 1000 mg/kg/day. Feed consumption expressed as g/kg/day was significantly increased at 1000 mg/kg/day for the second week of prebreeding and for the entire prebreed period. Clinical observations of the F0 females included rooting postdosing in four females, efflux of the dosing solution in three females, and alopecia in one female at 0 mg/kg/day; audible breathing, difficulty in handling, efflux of the dosing solution, hyperactivity, and rooting postdosing in one female each at 50 mg/kg/day; efflux of dosing solution, and rooting postdosing in four females each, alopecia in two females, and rooting prior to dosing in one female at 200 mg/kg/day; alopecia and efflux of the dosing solution in two females each and audible breathing and rooting postdosing in one female each at 1000 mg/kg/day (Table 10).

Figure 4. F0 Female Body Weights



F0 Gestation

There were no significant differences in the F0 maternal body weights or body weight change during gestation (Table 11 and Figure 4). There were no treatment-related effects across groups for maternal feed consumption for gd 0 to 7, 7 to 14, or 0 to 20 when expressed as g/day, and there were no treatment-related effects across groups for maternal feed consumption for gd 0 to 7 when expressed as g/kg/day (Table 12). Feed consumption values for gd 14 to 20, expressed as g/day, were significantly increased at 1000 mg/kg/day, and feed consumption values for gd 7 to 14, 14 to 20, and 0 to 20, when expressed as g/kg/day, were significantly increased at 1000 mg/kg/day. Clinical observations during gestation included alopecia and rust-colored fur in one female each at 0 mg/kg/day; rust-colored fur in two females (one found dead on gd 20) and efflux of dosing solution in one female at 50 mg/kg/day; alopecia in five females and rooting postdosing in three females at 200 mg/kg/day; and rooting postdosing in seven females, piloerection in two females, and audible breathing, dehydration, efflux of the dosing solution, rooting prior to dosing, salivating postdosing, and sore(s) in one female each at 1000 mg/kg/day; three females were found dead on gd 22 (Table 13). The three dams that died on gd 22 were in the midst of delivery, and their demise may have been due to dystocia.

F0 Lactation

There were no significant differences in F0 maternal lactational body weights or body weight change (Table 14 and Figure 4). F0 maternal lactational feed consumption, expressed as g/day and g/kg/day, was unaffected at all TNPP doses (Table 15). Maternal clinical observations during lactation included alopecia in three females and efflux of the dosing solution, piloerection, and rust-colored fur in one female each at 0 mg/kg/day; thin fur in one female at 50 mg/kg/day; alopecia in seven females and piloerection and rust-colored fur in one female each at 200 mg/kg/day; and rooting postdosing in seven females, and alopecia, efflux of the dosing solution, and rust-colored fur in one female each at 1000 mg/kg/day; one female was found dead on pnd 15 (Table 16).

F0 Reproductive Indices

There were no effects of exposure to TNPP on any F0 reproductive indices during the production of F1 offspring. Mating, fertility, pregnancy, and gestational indices were equivalent

across groups (Table 17), and gestational length was equivalent across all groups. There were also no differences across groups for the number of total implantation sites per litter, percent postimplantation loss per litter, or number of total, live, or dead pups per litter at birth (pnd 0) (Table 17).

F1 Lactation

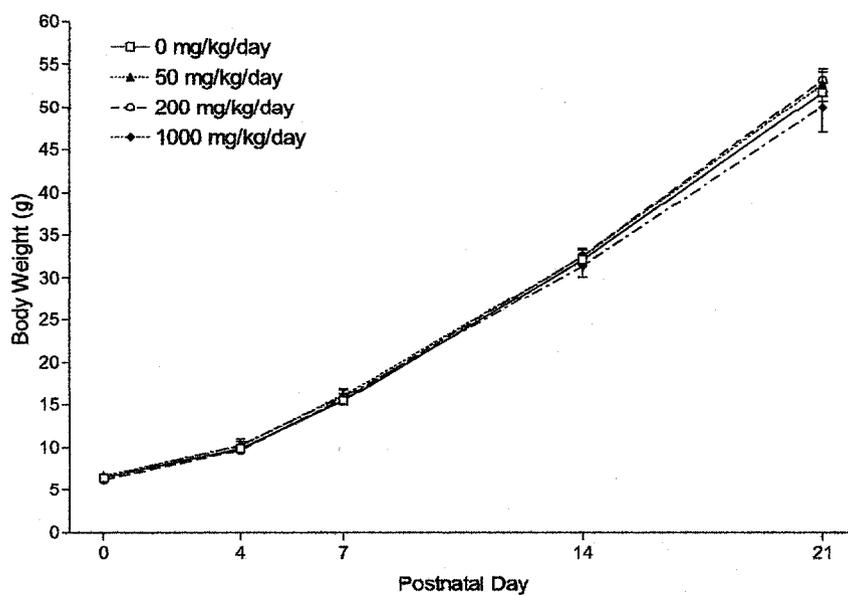
There were 10, 8, 10, and 7 live litters on pnd 0 at 0, 50, 200, and 1000 mg/kg/day, respectively. Live birth and stillbirth indices were unaffected across all groups, as were the survival indices for pnd 0-4, 4-7, 7-14, and 14-21 (Table 17). The mean number of live pups per litter for pnd 0, 7, 14, and 21 was unaffected across all groups (Table 18). However, the mean number of live pups per litter for pnd 4 (pre-cull) was significantly reduced at 1000 mg/kg/day. Mean F1 female and male AGDs (absolute or adjusted for body weight) per litter on pnd 0 were equivalent across all groups. Mean F1 pup body weights per litter (sexes combined or separately) were unaffected by treatment on pnd 0, 4, 7, 14, and 21 across all groups (Table 18 and Figure 5A for F1 males and Figure 5B for F1 females). The sex ratio (percent male pups/litter) was also unaffected at any dose for any interval (Table 18). Average number of nipples/areolae per male pup and the percent male pups with one or more nipples/areolae were equivalent across all groups. F1 pup clinical observations during lactation indicated that F1 pup mortality for pnd 0-21 was 5, 10, 7, and 1 (plus four euthanized since the dam was found dead on pnd 15) pups at 0, 50, 200, and 1000 mg/kg/day, respectively. Thread-like tail, missing tail, and tail $\frac{3}{4}$ brown or missing was present in four pups at 0 mg/kg/day. At 200 mg/kg/day, one dead male pup (on pnd 0) exhibited exencephaly, and alopecia was observed in five males and five females on pnd 12. Alopecia and thin fur in the same five males and five females were observed on pnd 14 (Table 19).

Necropsy findings of F1 pups found dead or euthanized moribund on pnd 0-21 included the usual findings: pups that died on pnd 0 exhibited open (fetal state) or closed ductus arteriosus (postnatal state), no air (fetal state) or air (postnatal state) in lungs, no or little milk in stomach, and autolysis of abdominal organs. One dead male pup at 200 mg/kg/day exhibited exencephaly on pnd 0 (see above). Pups that died or were sacrificed moribund on pnd 1-7 usually exhibited closed ductus arteriosus and milk in stomach or were too autolyzed to evaluate (Table 20). By weaning of the F1 litters on pnd 21, there were 10, 7, 10, and 6 litters at 0, 50,

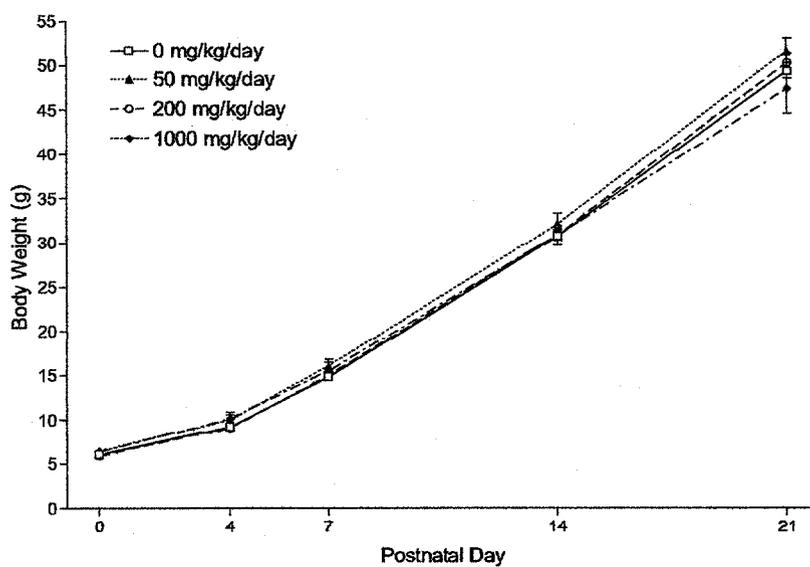
200, and 1000 mg/kg/day, respectively (Table 17). Necropsy findings for F1 pups culled on pnd 4 included only one female at 50 mg/kg/day with bilateral dilated ureters (Table 21).

Figure 5. F1 Offspring Body Weights by Sex by Litter

5A. Males



5B. Females



There were 38, 23, 40, and 21 F1 male offspring and 40, 27, 40, and 19 F1 female offspring evaluated at scheduled sacrifice on pnd 21 at 0, 50, 200, and 1000 mg/kg/day, respectively. There were no effects on F1 male and female body weights at sacrifice on pnd 21 at any dose (Table 22). Absolute weights for brain, spleen, paired testes, and paired epididymides were equivalent across all groups for the F1 males on pnd 21. Absolute thymus weight was significantly increased at 50 mg/kg/day, significantly decreased at 200 mg/kg/day, and unaffected at 1000 mg/kg/day. Thymus and brain weights, relative to terminal body weight, were unaffected. Thymus weight, relative to brain weight, was significantly increased at 50 mg/kg/day and unaffected at 200 and 1000 mg/kg/day. Spleen, paired testes, and paired epididymides weights, relative to terminal body and brain weight, were unaffected by treatment with TNPP across all groups for the F1 males. There were no significant differences in absolute or weights relative to terminal body weights for the brain, thymus, spleen, paired ovaries, or uterus weights for the F1 females on pnd 21 across all groups. Also, no significant differences were noted at any dose for organ weights, relative to terminal body or brain weights, for the thymus, spleen, paired ovaries, or the uterus of the F1 females on pnd 21 (Table 22). Necropsy findings for F1 pups on pnd 21 included hydronephrosis of the right kidney in 3, 1, and 2 males at 0, 50, and 1000 mg/kg/day, respectively (Table 23).

Necropsy of F0 Parental Animals

Males

At scheduled sacrifice, mean body weights were equivalent across all groups. Absolute weights of the brain, thymus, heart, liver, spleen, paired adrenal glands, paired testes, paired epididymides, prostate, and paired seminal vesicles with coagulating glands were equivalent across all groups (Table 24). Absolute paired kidney weights were significantly increased at 1000 mg/kg/day. The weights of the brain, thymus, heart, liver, spleen, paired adrenal glands, paired testes, paired epididymides, prostate, and seminal vesicles with coagulating glands, relative to terminal body or brain weights, were unaffected across all groups. Paired kidney weights, relative to terminal body and brain weights, were significantly increased at 1000 mg/kg/day (Table 24). Necropsy findings included alopecia at 0, 50, and 200 mg/kg/day (4, 1, and 1 males, respectively) and rust-colored fur at 0, 50, and 1000 mg/kg/day (2, 1, and 1 males, respectively). Gross necropsy findings of F0 parental males exhibited no treatment- or dose-

related pattern of incidence or severity at scheduled sacrifice (Table 25). Histological findings included minimal corticomedullary junction mineralization of the kidney in three males at 1000 mg/kg/day (with no males with this finding at 0 mg/kg/day), which was considered a treatment-related lesion. A variety of other histopathologic changes were observed in the F0 males at both 0 and 1000 mg/kg/day (e.g., cyst on renal medulla, necrosis of renal tubule epithelium, and nephropathy). These changes were typical of the spontaneous microscopic renal pathology that can be observed at this age and in this strain of rat and were not considered treatment related (Table 25 and Histopathology Report, Appendix III).

Recovery Males

There were no differences found for terminal body weight or absolute or relative (to body weight or brain) organ weights between the control and high-dose animals (Table 26). Alopecia, hydronephrosis of the right kidney, and rust-colored fur at 0 mg/kg/day were the only gross necropsy findings for scheduled necropsies (Table 27). For the male in the 1000 mg/kg/day recovery group found dead on sd 8, gross necropsy findings included severely congested lungs, probably due to aspiration of dosing solution. Therefore, this finding was attributed to dosing error and was not considered treatment related.

Females

At scheduled sacrifice, mean body weights of F0 females were equivalent across all groups (Table 28). Absolute weights of the brain, thymus, heart, liver, spleen, paired adrenal glands, and uterus with cervix and vagina were equivalent across all groups (Table 28). The weights, relative to terminal body weights, of the brain, thymus, heart, liver, spleen, paired adrenal glands, and uterus with cervix and vagina were unaffected across all groups. The weights, relative to terminal brain weights, of the thymus, heart, liver, spleen, paired adrenal glands, and uterus with cervix and vagina were also unaffected across all groups. Paired kidney weights, absolute and relative to both terminal body and brain weights, were significantly increased at 200 mg/kg/day (but not at 1000 mg/kg/day). Absolute paired ovary weights and ovary weights, relative to both terminal body and brain weights, were significantly decreased at 1000 mg/kg/day. Gross necropsy findings of F0 parental females included alopecia in three females, and hydronephrosis of the right kidney and rust-colored fur in one female each at 0

mg/kg/day; thin fur in one female and a fluid-filled uterus in one female at 50 mg/kg/day; alopecia in five females and rust-colored fur in one female at 200 mg/kg/day; and alopecia in one female and rust-colored fur in one female at 1000 mg/kg/day (Table 29). Gross necropsy findings of F0 parental females exhibited no treatment- or dose-related pattern of incidence or severity of findings at scheduled sacrifice.

Necropsy findings for unscheduled deaths included large intestine with feces present, congested lungs with dark 5-8 mm areas on all lobes, and a small amount of oil droplets present, probably due to aspiration of dosing solution, ingesta present in small intestine, stomach full of food, and a small amount of oil droplets present in the trachea (due to dosing error) in one female at 50 mg/kg/day; pale liver in three females, kidneys that appeared reduced in size bilaterally, and thin-walled uterus in two females each, and chromodacryorrhea, one resorbed conceptus in the right horn (all other implant sites normal), perioral wetness, small amount of blood at vaginal opening, and 2-4mm brown areas in glandular portion of the stomach in one female each at 1000 mg/kg/day. For the unscheduled necropsy findings, the F0 female at 50 mg/kg/day found dead during gestation and one F0 female at 1000 mg/kg/day found dead during lactation exhibited findings consistent with dosing error. These deaths are not attributed to treatment (Table 29).

For three additional high dose F0 females, the gross necropsy findings appeared to be treatment related. All were found dead on gd 22, likely due to dystocia and probably treatment related, based predominantly on the necropsy findings (all had pale livers, two had thin-walled uteruses, two had reduced kidney size bilaterally, one had blood at the vaginal opening, and fetuses/pups were present). A variety of other histopathologic changes were observed in the F0 females at 0 and 1000 mg/kg/day, including renal medullary cyst, chronic renal inflammation, and nephropathy (Table 29 and Histopathology Report, Appendix III). These changes were typical of the spontaneous microscopic pathology that can be observed at this age and in this strain of rat and were not considered treatment related.

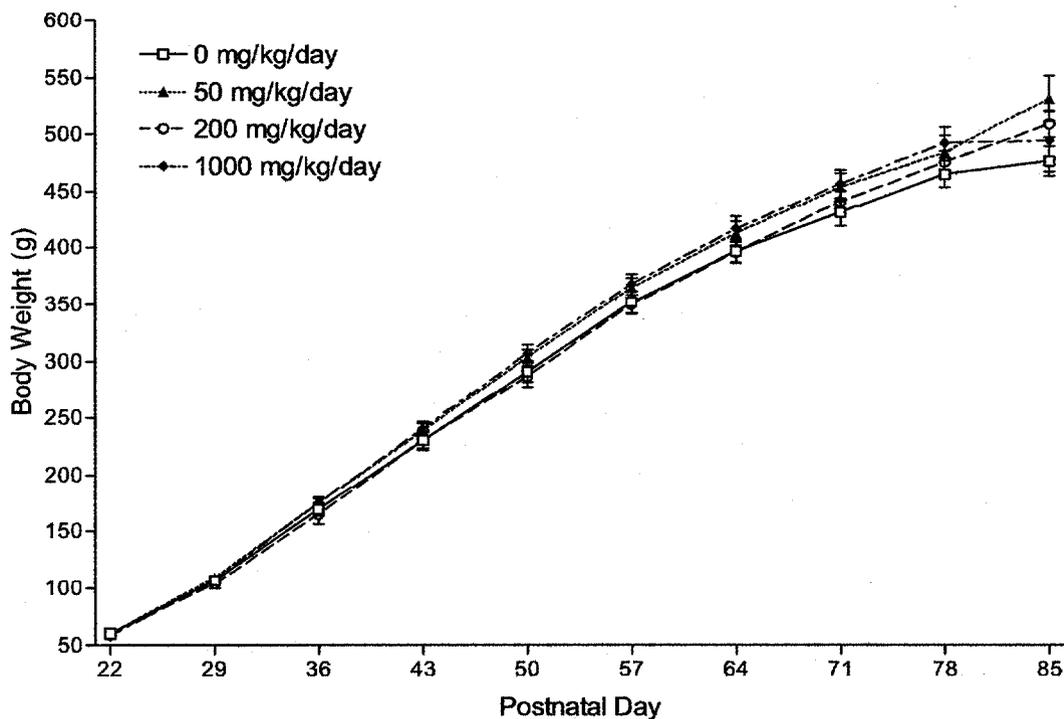
Fate of F1 Animals

There were 10, 10, 10, and 10 F1 males and females each at 0, 50, 200, and 1000 mg/kg/day, respectively, evaluated at scheduled necropsy (Table 30).

Males

F1 male age at acquisition of preputial separation was equivalent across all groups (Table 31). There were no significant differences among groups for F1 male body weights or body weight changes during the postweaning period (pnd 22 to 85) (Table 32 and Figure 6). There were no differences in feed consumption expressed as g/day or g/kg/day at 0, 50, or 200 mg/kg/day during the postweaning period. However, feed consumption expressed as g/day was significantly increased from pnd 43 to 50 and from pnd 64 to 71 at 1000 mg/kg/day. Feed consumption expressed as g/kg/day was significantly increased from pnd 29 to 36, from pnd 43 to 50, from pnd 64 to 71, and from pnd 22 to 78 at 1000 mg/kg/day (Table 33). Clinical observations included efflux of the dosing solution in eight males, sore(s) in four males, alopecia and piloerection in two males each, and dehydration, soft feces, and rust-colored fur in one male each at 0 mg/kg/day; efflux of the dosing solution in five males, and piloerection, and broken toenail in one male each at 50 mg/kg/day; efflux of the dosing solution in seven males, and alopecia, chromodacryorrhea, dehydration, piloerection, sore(s), and broken toenail in one male each at 200 mg/kg/day; and efflux of the dosing solution in four males, chromodacryorrhea in two males, and alopecia, lethargic, and sore(s) in one male each at 1000 mg/kg/day. Rooting postdosing occurred in 1, 1, 3, and 9 males at 0, 50, 200, and 1000 mg/kg/day, respectively, most likely due to the dosing regimen. Efflux of the dosing solution occurred in all groups, including the control group, with no dose-related pattern (Table 34). This is typically observed at the onset of dosing young animals and toward the end of a prolonged dosing period.

Figure 6. F1 Male Body Weights



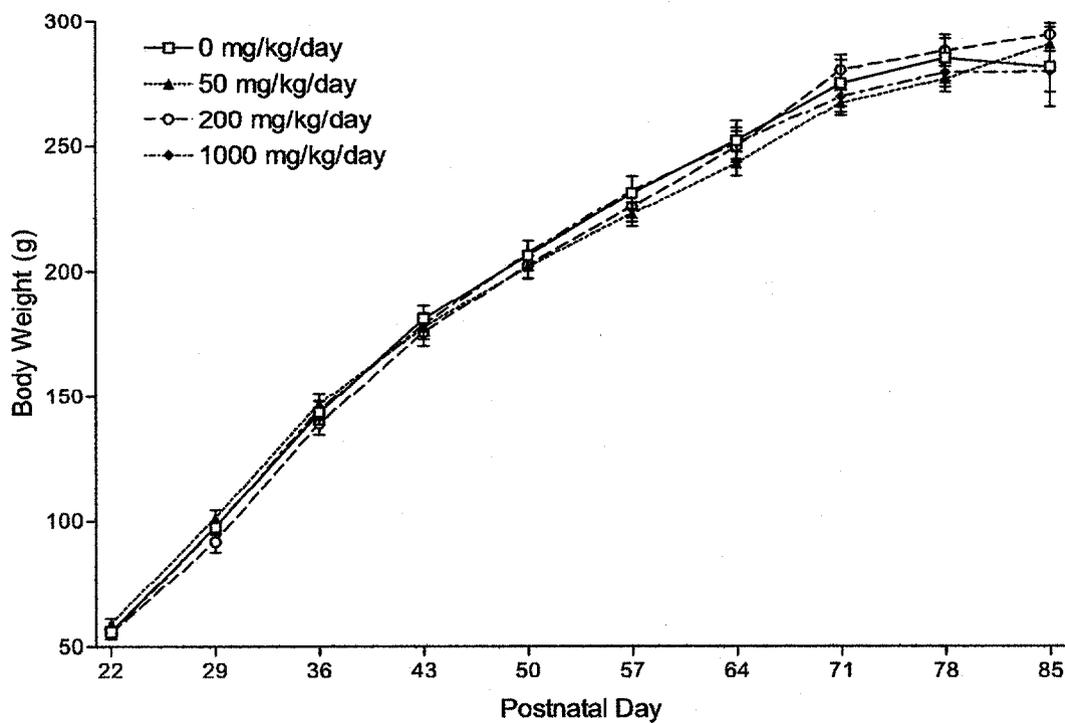
Females

F1 female age at acquisition of vaginal patency was equivalent across all groups (Table 31). There were no significant differences in body weight or body weight change from pnd 22 to pnd 85 across all groups (Table 35 and Figure 7). Feed consumption values, expressed as g/day, were also equivalent across all dose groups for the F1 females from pnd 22 to 85. There were no significant differences in feed consumption expressed as g/kg/day at 0 or 50 mg/kg/day. However, feed consumption values expressed as g/kg/day were significantly increased at 200 mg/kg/day for pnd 29 to 36, and also were significantly increased at 1000 mg/kg/day for pnd 43 to 50, pnd 50 to 57, and pnd 22 to 78 (Table 36). Clinical observations included alopecia and efflux of the dosing solution in three females each, and rooting postdosing, rust-colored fur, salivating prior to dosing, sore(s), and pink-tinged vaginal smear in one female each at 0 mg/kg/day; efflux of the dosing solution in three females, rooting postdosing in two females and alopecia, salivating prior to dosing, and broken toenail in one female each at 50 mg/kg/day; efflux of the dosing solution in five females and alopecia, rooting postdosing, and brown clot in

vaginal smear in one female each at 200 mg/kg/day; and rooting postdosing in eight females, efflux of dosing solution in three females, salivating prior to dosing in two females, and scab(s) in one female at 1000 mg/kg/day (Table 37).

There were no significant differences in estrous cycle lengths for the F1 females across all groups. All ten females in each group were cycling. The number (percent) of females with abnormal cycles were 1 (10.0%), 2 (20.0%), 5 (50.0%), and 2 (20.0%), with mean cycle lengths of 4.97, 4.39, 6.23, and 4.78 days at 0, 50, 200, and 1000 mg/kg/day, respectively (Table 38). The F1 females with abnormal cycles were most likely because of biological variability and were not considered treatment-related effects.

Figure 7. F1 Female Body Weights



Necropsy of Adult F1 Animals

Males

At scheduled sacrifice, at approximately 85 days of age, mean body weights were equivalent across all groups (Table 39). Absolute weights of the brain, thymus, heart, liver, spleen, paired kidneys, paired adrenals, paired testes, paired epididymides, prostate, and seminal vesicles with coagulating glands were equivalent across all groups. The weights (relative to terminal body weights) of the brain, thymus, heart, liver, spleen, paired kidneys, paired adrenal glands, paired testes, prostate, and paired seminal vesicles with coagulating glands were also unaffected across all groups. Paired epididymides weights, relative to terminal body weights, were significantly decreased at 1000 mg/kg/day. The weights (relative to brain weights) of the thymus, heart, liver, spleen, paired kidneys, paired adrenal glands, paired testes, paired epididymides, prostate, and paired seminal vesicles with coagulating glands were unaffected across all groups (Table 39).

For the F1 males, there were no significant differences for percent motile sperm or progressively motile sperm evaluated in all groups, or for epididymal sperm concentration, testicular homogenization-resistant spermatid head concentration, daily sperm production per testis, efficiency of daily sperm production, or percent abnormal sperm between the 0 and 1000 mg/kg/day groups (Table 39). Gross necropsy findings included hydronephrosis of the right kidney in one and three males at 0 and 200 mg/kg/day, respectively; alopecia in one male at 0 mg/kg/day, and right seminal vesicle reduced in size in one male at 50 mg/kg/day at scheduled sacrifice (Table 40). Gross necropsy findings of F1 males exhibited no treatment- or dose-related pattern of incidence or severity at scheduled sacrifice. Histopathologic findings included minimal (one male) and moderate (one male) corticomedullary junction mineralization of the kidney in two males at 1000 mg/kg/day versus none at 0 mg/kg/day (Table 40); this finding was considered treatment related, since this lesion is rarely, if ever, observed in control males (although it is a common finding in control females). A variety of other histopathologic changes were observed in the F1 males at 0 and 1000 mg/kg/day (Table 40 and Histopathology Report, Appendix III). These latter changes were typical of the spontaneous microscopic pathology that can be observed at this age and in this strain of rat and were not considered treatment related.

Females

At scheduled sacrifice, at approximately 85 days of age, mean body weights were equivalent across all groups (Table 41). Absolute weights of the brain, heart, liver, spleen, paired kidneys, paired adrenals, paired ovaries, and uterus with cervix and vagina were equivalent across all groups. Absolute thymus weights were significantly increased at 1000 mg/kg/day. The weights (relative to terminal body weights) of the thymus, heart, liver, spleen, paired kidneys, paired adrenal glands, paired ovaries, and uterus with cervix and vagina were unaffected across all groups. Brain weights, relative to terminal body weight, were significantly decreased at 200 mg/kg/day (but not at 50 or 1000 mg/kg/day). The weights (relative to brain weights) of the heart, spleen, paired adrenal glands, paired ovaries, and uterus with cervix and vagina were unaffected across all groups. Thymus and liver weights, relative to brain weight, were significantly increased at 1000 mg/kg/day, and paired kidney weights, relative to brain weight, were significantly increased at 200 and 1000 mg/kg/day in pairwise comparisons to the control values (Table 41). Gross necropsy findings included alopecia in 3, 1, and 1 females at 0, 50, and 200 mg/kg/day, respectively; rust-colored fur in one female at 0 mg/kg/day and bilateral hydronephrosis in one female at 200 mg/kg/day (Table 42). Gross necropsy findings of F1 females exhibited no treatment- or dose-related pattern of incidence or severity at scheduled sacrifice. Histopathologic findings included minimal corticomedullary junction mineralization of the kidney in three females and mild corticomedullary junction mineralization of the kidney in one female (total of four) at 0 mg/kg/day, and minimal corticomedullary junction mineralization of the kidney in three females and mild corticomedullary junction mineralization of the kidney in one female (total of four) at 1000 mg/kg/day. These findings were not considered related to treatment and are typically observed in females. A variety of other histopathologic changes were observed in the F0 females at 0 and 1000 mg/kg/day (Table 42). These changes were typical of the spontaneous microscopic pathology that can be observed at this age and in this strain of rat and were not considered treatment related (see Histopathology Report in Appendix III).

DISCUSSION

F0 and F1 Adult Systemic Toxicity

The following discussion focuses on treatment-related effects. Other changes noted in the text tables were considered random, due to biological variation, and not treatment related. Text Table D provides a summary of F0 adult systemic toxicity. There were no treatment-related deaths for the F0 males. For parental males, minor systemic toxicity was present at 1000 mg/kg/day, expressed as a trend toward decreased body weights and reduced body weight gains. Feed consumption expressed as g/kg/day was significantly increased at 1000 mg/kg/day during mating. This finding was considered most likely because of the excessive rooting behavior observed during the dosing period. Paired kidney weights, both absolute and relative to terminal body and brain weight, were significantly increased at 1000 mg/kg/day. There were no treatment-related effects for the gross necropsy findings. However, histological findings included minimal corticomedullary junction mineralization in the kidneys in three of ten males (with no males exhibiting this finding at 0 mg/kg) at 1000 mg/kg/day, which correlated with the increased kidney weights, both absolute and relative to body and brain weight, at this dose. In recovery males, there was a trend toward increasing body weights in the high dose group, so that at the end of the two-week recovery period, the body weights were similar to the control group values. There was no effect on kidney weights in the recovery group.

Three F0 females at 1000 mg/kg/day were found dead on gd 22, possibly attributable to dystocia. Dystocia was evident due to the inability of the dams to deliver their pups. Examination of the pups at maternal necropsy indicated that they were full term and normal in external appearance. F0 parental females did not exhibit any overt adult systemic toxicity at any dose, as evidenced by a lack of statistically significantly different body weights or weight changes during prebreed, gestation or lactation, changes in feed consumption, or gross necropsy findings. However, trends toward increased feed consumption in females from the high-dose group (except during lactation) were noted. As with the males, because of the clinical signs, particularly of rooting behavior following dosing, the feed consumption differences were considered likely related to this observation. Paired ovary weights (absolute and relative to terminal body and brain weights) were significantly decreased at 1000 mg/kg/day. Gross necropsy and histological findings of F0 parental females exhibited no treatment- or dose-related pattern of incidence or severity at scheduled sacrifice.

Text Table E presents a summary of F1 adult systemic toxicity. There were no unscheduled deaths for the adult F1 males. There were no significant differences in body weight or weight gain for the F1 males during the postweaning period (from pnd 22 to 85) at any dose. Increased feed consumption as g/day and g/kg/day at 1000 mg/kg/day, considered related to increased rooting behavior, was observed. Paired epididymides weights, relative to terminal body weights, were significantly decreased at 1000 mg/kg/day. This finding is of uncertain toxicological significance, since there were no changes in epididymal weight in the F0 generation or in absolute organ weight in the F1 generation. There were no treatment-related effects for gross necropsy findings. Histological findings included corticomedullary junction mineralization in the kidneys of two of ten males (with no males exhibiting this finding at 0 mg/kg/day) at 1000 mg/kg/day and were considered treatment related.

There were no treatment-related deaths for the adult F1 females. There were no significant differences in body weight or weight gain for the F1 females during the postweaning period (from pnd 22 to 85). Feed consumption values (as g/kg/day), presumably associated with excessive rooting behavior, were increased at 1000 mg/kg/day. Also, there were no significant differences in the F1 female estrous cycles across all groups. Ovary weights (absolute and relative) were decreased in the high-dose group. There were no treatment-related effects for the gross necropsy or histopathological findings.

Text Table D. Summary of F0 Adult Systemic Toxicity

Trisnonylphenyl Phosphite (mg/kg/day)	F0			
	0	50	200	1000
PARENTAL MALES				
Deaths	0	0	0	0
<u>Prebreed Exposure</u>				
Body Weights	---	---	---	---
Weight Change	---	---	---	↓
Clinical Observations	---	---	---	---
Feed Consumption: g/day	---	↓	---	---
g/kg/day	---	---	---	↑↑
<u>Necropsy</u>				
Body Weight	---	---	---	---
Organ Weights:				
Liver	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Paired Kidneys	A	---	---	↑
	R - Body	---	---	↑↑
	R - Brain	---	---	↑↑
Brain	A	---	---	---
	R - Body	---	---	---
Thymus	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Heart	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Spleen	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Paired Adrenal Glands	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---

(continued)

Text Table D (continued)

Trisnonylphenyl Phosphite (mg/kg/day)		F0			
		0	50	200	1000
Paired Testes	A	---	---	---	---
	R - Body	---	---	---	---
	R - Brain	---	---	---	---
Paired Epididymides	A	---	---	---	---
	R - Body	---	---	---	---
	R - Brain	---	---	---	---
Prostate	A	---	---	---	---
	R - Body	---	---	---	---
	R - Brain	---	---	---	---
Seminal Vesicles	A	---	---	---	---
	R - Body	---	---	---	---
	R - Brain	---	---	---	---
Gross Findings		---	---	---	---
Histopathology: ^a					
Kidneys – Corticomedullary junction mineralization					
	Minimal	0			3
F0 FEMALES					
Deaths		0	1	0	4
<u>Prebreed Exposure</u>					
Body Weights		---	---	---	---
Weight Change		---	---	---	---
Clinical Observations		---	---	---	---
Feed Consumption:	g/day	---	---	---	↑↑
	g/kg/day	---	---	---	↑↑ ^b
<u>Gestation</u>					
Body Weights		---	---	---	---
Weight Change		---	---	---	---
Clinical Observations		---	---	---	---
Feed Consumption:	g/day	---	---	---	↑
	g/kg/day	---	---	---	↑↑

(continued)

Text Table D (continued)

Trisnonylphenyl Phosphite (mg/kg/day)	F0			
	0	50	200	1000
<u>Lactation (pnd 0-21)</u>				
Body Weights	---	---	---	---
Weight Change	---	---	---	---
Clinical Observations	---	---	---	---
Feed Consumption: g/day	---	---	---	---
g/kg/day	---	---	---	---
<u>Necropsy</u>				
Body Weights	---	---	---	---
Organ Weights:				
Brain	A	---	---	---
	R - Body	---	---	---
Thymus	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Heart	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Liver	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Spleen	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Paired Kidneys	A	---	↑	---
	R - Body	---	↑	---
	R - Brain	---	↑	---
Paired Adrenal Glands	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Uterus with Vagina and Cervix	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---

(continued)

Text Table D (continued)

Trisnonylphenyl Phosphite (mg/kg/day)	F0			
	0	50	200	1000
Paired Ovaries A	---	---	---	↓↓
R - Body	---	---	---	↓
R - Brain	---	---	---	↓
Gross Findings	---	---	---	---
Histopathology: ^a				
Kidneys – Corticomedullary junction mineralization				
Minimal	5			1

^a Histopathology was performed on control and high dose animals only

↑, ↑↑ = statistically significant increase; one symbol – p<0.05; two symbols – p<0.01

↓, ↓↓ = statistically significant decrease; one symbol – p<0.05; two symbols – p<0.01

^b Change in one or more intervals

--- = no statistically significant difference

A = absolute organ weight

R - Body = organ weight relative to terminal body weight (%)

R - Brain = organ weight relative to terminal brain weight (%)

Text Table E. Summary of F1 Adult Systemic Toxicity

Trisnonylphenyl Phosphite (mg/kg/day)	F1			
	0	50	200	1000
F1 ADULT MALES				
Deaths	0	0	0	0
<u>Postweaning Period (pnd 22 to 85)</u>				
Body Weights	---	---	---	---
Weight Change	---	---	---	---
Clinical Observations	---	---	---	---
Feed Consumption: g/day	---	---	---	↑
g/kg/day	---	---	---	↑, ↑↑
<u>Necropsy</u>				
Body Weight	---	---	---	---
Organ Weights:				
Liver	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Paired Kidneys	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Brain	A	---	---	---
	R - Body	---	---	---
Thymus	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Heart	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Spleen	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Paired Adrenal Glands	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---

(continued)

Text Table E (continued)

Trisnonylphenyl Phosphite (mg/kg/day)		F1			
		0	50	200	1000
Paired Testes	A	---	---	---	---
	R - Body	---	---	---	---
	R - Brain	---	---	---	---
Paired Epididymides	A	---	---	---	---
	R - Body	---	---	---	↓
	R - Brain	---	---	---	---
Prostate	A	---	---	---	---
	R - Body	---	---	---	---
	R - Brain	---	---	---	---
Seminal Vesicles	A	---	---	---	---
	R - Body	---	---	---	---
	R - Brain	---	---	---	---
Gross Findings		---	---	---	---
Histopathology. ^a					
Kidneys – Corticomedullary junction mineralization					
	Minimal	0			1
	Moderate	0			1
F1 ADULT FEMALES					
Deaths		0	0	0	0
<u>Postweaning Exposure (pnd 22 to 85)</u>					
Body Weights		---	---	---	---
Weight Change		---	---	---	---
Clinical Observations		---	---	---	---
Feed Consumption:	g/day	---	---	---	---
	g/kg/day	---	---	↑	↑

(continued)

Text Table E (continued)

Trisnonylphenyl Phosphite (mg/kg/day)	F1			
	0	50	200	1000
<u>Necropsy</u>				
Body Weights	---	---	---	---
Organ Weights:				
Brain	A	---	---	---
	R - Body	---	↓	---
Thymus	A	---	---	↑↑
	R - Body	---	---	---
	R - Brain	---	---	↑↑
Heart	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Liver	A	---	---	---
	R - Body	---	---	↑↑↑
	R - Brain	---	---	---
Spleen	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Paired Kidneys	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	↑↑	↑
Paired Adrenal Glands	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Uterus with Vagina and Cervix	A	---	---	---
	R - Body	---	---	---
	R - Brain	---	---	---
Paired Ovaries	A	---	---	↓
	R - Body	---	---	---
	R - Brain	---	---	---
Gross Findings	---	---	---	---

(continued)

Text Table E (continued)

Trisnonylphenyl Phosphite (mg/kg/day)	F1			
	0	50	200	1000
Histopathology: ^a				
Kidneys – Corticomedullary junction mineralization				
Minimal	3			3
Mild	1			1

^a Histopathology performed on control and high dose animals only

↑, ↑↑, ↑↑↑ = statistically significant increase; one symbol = $p < 0.05$; two symbols = $p < 0.01$; three symbols = $p < 0.001$

↓, ↓↓ = statistically significant decrease; one symbol = $p < 0.05$; two symbols = $p < 0.01$

--- = no statistically significant difference

A = absolute organ weight in grams

R - Body = organ weight relative to terminal body weight (%)

R - Brain = organ weight relative to terminal brain weight (%)

F0 Parental Reproductive Toxicity

Text Table F presents a summary of the F0 parental reproductive toxicity parameters. The increased percent postimplantation loss at 50 mg/kg/day (15.48% versus the control value of 5.58%) was accompanied by a high variance term and is considered due to biologic variation and not treatment (or dose) related.

Text Table F
Summary of F0 Parental Male and Female Reproductive Toxicity

Trisnonylphenyl Phosphite (mg/kg/day)	F0			
	0	50	200	1000
FEMALES				
Precoital interval, days	3.1	2.5	2.3	2.2
Indices: Mating	---	---	---	---
Fertility	---	---	---	---
Gestational	---	---	---	---
Gestational length, days	22.2	22.4	22.1	22.3
Death due to dystocia on gd 22	0	0	0	3
No. implant sites/litter	15.80	14.67	16.90	13.50
% postimplantation loss/litter	5.58	15.48	5.68	9.04
No. total pups/litter, pnd 0	15.1	13.8	16.3	12.1
No. live pups/litter, pnd 0	14.9	12.8	15.9	12.0
No. dead pups/litter, pnd 0	0.2	1.0	0.4	0.1
No. females pregnant	10	9	10	10
No. litters on pnd 0	10	8	10	7
No. litters on pnd 21	10	7	10	6
MALES				
Indices: Mating	---	---	---	---
Fertility	---	---	---	---
Pregnancy	---	---	---	---

--- = no statistically significant difference

F1 Offspring Toxicity

Text Table G presents a summary of F1 offspring toxicity parameters. There was evidence of F1 offspring toxicity at 1000 mg/kg/day, expressed as reduced live litter size on pnd 4. The value on pnd 0 at 1000 mg/kg/day was not statistically significantly different from the control group value. There were no other indications of F1 offspring toxicity either pre- or postnatally through lactation, with no effects on offspring survival, AGD, sex ratio (% males) per litter, average number of nipples/areolae for male pups, or body weights per litter. Age at acquisition of F1 male preputial separation and F1 female vaginal opening was equivalent across all groups. There were no effects on body weights for the F1 males and females at sacrifice on pnd 21 at any dose.

Text Table G
Summary of F1 Offspring Toxicity

Trisnonylphenyl Phosphite (mg/kg/day)	F1			
	0	50	200	1000
Stillbirth index	---	---	---	---
Live birth index	---	---	---	---
Survival index (pnd 0-4, precull)	---	---	---	---
Survival index (pnd 4-7, postcull)	---	---	---	---
Survival index (pnd 7-14)	---	---	---	---
Survival index (pnd 14-21)	---	---	---	---
Lactational survival index (pnd 4 postcull - 21)	---	---	---	---
No. live pups/litter, pnd 0	14.9	12.8	15.9	12.0
No. live pups/litter, pnd 4 (precull)	14.8	14.3	15.6	12.0*
No. live pups/litter, pnd 7 (postcull)	9.8	10.0	10.0	9.1
No. live pups/litter, pnd 14 (postcull)	9.8	10.0	10.0	9.1
No. live pups/litter, pnd 21 (postcull)	9.8	10.0	10.0	10.0
Sex ratio (% males/litter)	---	---	---	---

(continued)

Text Table G (continued)

Trisnonylphenyl Phosphite (mg/kg/day)		F1			
		0	50	200	1000
AGD/litter, pnd 0:	Males (mm)	2.04	2.14	2.09	2.10
	Females (mm)	0.96	1.00	0.93	0.95
Pup body weight/litter, pnd 0 (g):	Males	---	---	---	---
	Females	---	---	---	---
	All pups	---	---	---	---
Pup body weight/litter, pnd 4 (g):	Males	---	---	---	---
	Females	---	---	---	---
	All pups	---	---	---	---
Pup body weight/litter, pnd 7 (g):	Males	---	---	---	---
	Females	---	---	---	---
	All pups	---	---	---	---
Pup body weight/litter, pnd 14 (g):	Males	---	---	---	---
	Females	---	---	---	---
	All pups	---	---	---	---
Pup body weight/litter, pnd 21 (g):	Males	---	---	---	---
	Females	---	---	---	---
	All pups	---	---	---	---
Average number of nipples per male pup		0	0	0	0
Average number of areolae per male pup		0	0	0	0
Age at vaginal opening for F1 females (days)		29.4	29.6	30.4	28.7
Age at preputial separation for F1 males (days)		42.4	42.1	42.5	41.0
Estrous cycle length (days)		4.97	4.37	6.23	4.78
Histopathology:					
Reproductive organs:	Males ^a	---	---	---	---
	Females ^a	---	---	---	---
Systemic organs:	Males ^a	---	---	---	↑ ^b
	Females ^a	---	---	---	---

(continued)

Text Table G (continued)

Trisnonylphenyl Phosphite (mg/kg/day)	F1			
	0	50	200	1000
Andrology				
% Motile sperm	---	---	---	---
% Progressively motile sperm	---	---	---	---
Epididymal sperm concentration ^a	---	---	---	---
Testicular spermatid head count ^a	---	---	---	---
Daily sperm production ^a	---	---	---	---
Efficiency of daily sperm production ^a	---	---	---	---

^a Indicated parameters were assessed in control and high dose animals only

^{↑b} Presence of renal corticomedullary mineralization in two F1 males at 1000 mg/kg/day versus 0 at 0 mg/kg/day. F1 females exhibited the same findings with the same incidence and severity at both 0 and 1000 mg/kg/day.

--- = No statistically significant difference

* = Statistically significantly different from control group value at $p < 0.05$

CONCLUSIONS

Trisnonylphenyl phosphite (TNPP, CAS No. 26523-78-4), administered by gavage once daily at 0, 50, 200, and 1000 mg/kg/day to parental F0 CD® (SD) rats, ten/sex/group, through prebreed, mating, gestation, and lactation and direct dosing to F1 offspring from weaning to scheduled sacrifice, resulted in adult F0 parental systemic toxicity at 1000 mg/kg/day in males, expressed as reduced body weight gain, increased kidney weights, and mineralization of the renal corticomedullary junction. Three of ten pregnant F0 females at 1000 mg/kg/day died in late pregnancy, possibly due to dystocia. In addition, ovary weights were decreased at 1000 mg/kg/day in F0 but not F1 adult females. There was F1 offspring toxicity observed postnatally at 1000 mg/kg/day, expressed as reduced litter size on pnd 4 precull (but not on pnd 0) and corticomedullary mineralization in the adult F1 male kidneys at 1000 mg/kg/day. No effects on reproductive parameters, developmental landmarks, F1 estrous cycles, or F1 andrology were observed. Therefore, under the conditions of this study in rats, the NOAELs for systemic parental and for reproductive and offspring toxicity were 200 mg/kg/day.

REFERENCES

- Agresti, A., C.R. Mehta, and N.R. Patel (1990). Exact inference for contingency tables with ordered categories. *J. Amer. Statist. Assoc.* **85**, 453-458.
- Armitage, P. (1955). Test for linear trends in proportions and frequencies. *Biometrics* **11**, 375-386.
- Cochran, W. (1954). Some methods for strengthening the common χ^2 tests. *Biometrics* **10**, 417-451.
- Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.
- Dunnett, C.W. (1964). New tables for multiple comparisons with a control. *Biometrics* **20**, 482-491.
- Hafez, E.S.E. (ed.) (1970). *Reproduction and Breeding Techniques for Laboratory Animals*. Lea and Febiger, Philadelphia, PA.
- Huber, P.J. (1967). The behavior of maximum likelihood estimates under nonstandard conditions. In: *Proceedings of the Fifth Berkeley Symposium on Mathematical Statistics and Probability* **1**, 221-233.
- Levene, H. (1960). Robust tests for the equality of variance. In: *Contributions to Probability and Statistics* (I. Olkin, S.G. Ghurye, W. Hoeffding, W.G. Madow, and H.B. Mann, Eds.), Palo Alto, CA, Stanford University Press, pp. 278-292.
- NRC (1996). *Guide for the Care and Use of Laboratory Animals*. Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council. National Academy Press, National Institutes of Health. Revised 1996.
- Organization for Economic Cooperation and Development (OECD) (1995). Guideline for Testing of Chemicals, No. 421. "Reproductive/ Developmental Toxicity Screening Test," pages 1-10; adopted July 27, 1995.
- Organization for Economic Cooperation and Development (OECD) (1998). OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, No. 1, OECD Principles of Good Laboratory Practice (as revised in 1997).
- Royall, R.M. (1986). Model robust confidence intervals using maximum likelihood estimators. *International Statistical Review* **54**, 221-226.
- SAS Institute Inc. (1989a). *SAS® Language and Procedures: Usage*, Version 6, First Edition, SAS Institute Inc., Cary, NC, 638 pp.
- SAS Institute Inc. (1989b). *SAS/STAT® Users' Guide*, Version 6, Fourth Edition, Volumes 1 and 2, SAS Institute Inc., Cary, NC, 1686 pp.

SAS Institute Inc. (1990a). *SAS® Language: Reference*, Version 6, First Edition, SAS Institute Inc., Cary, NC, 1042 pp.

SAS Institute Inc. (1990b). *SAS® Language: Procedures Guide*, Version 6, Third Edition, SAS Institute Inc., Cary, NC, 705 pp.

SAS Institute Inc. (1990c). *SAS® Companion for the VMS™ Environment*, Version 6, First Edition, SAS Institute Inc., Cary, NC, 457 pp.

SAS Institute Inc. (1996a). *SAS® Companion for the Microsoft Windows Environment*, SAS Institute Inc., Cary, NC, 302 pp.

SAS Institute Inc. (1996b). *SAS/STAT® Software: Changes and Enhancements Through Release 6.11*, SAS Institute Inc., Cary, NC, 1104 pp.

SAS Institute Inc. (1997). *SAS/STAT® Software: Changes and Enhancements Through Release 6.12*, SAS Institute Inc., Cary, NC, 1167 pp.

Shah, B.V., B.G. Barnwell, and G.S. Bieler (1997). *SUDAAN® Software for the Statistical Analysis of Correlated Data. User's Manual*. Release 7.5, Volume 1, Research Triangle Institute, Research Triangle Park, NC.

Snedecor, G.W. and W.G. Cochran (1967). *Statistical Methods*. Sixth Edition, Iowa State University Press, Ames, IA.

U.S. Environmental Protection Agency (EPA) (1989). Toxic Substances Control Act, EPA (TSCA); Good Laboratory Practice Standards; Final Rule. *Federal Register* **54(158)**, 34034-34050 (August 17, 1989).

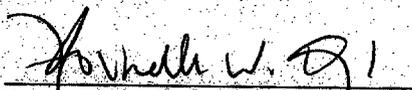
Zeger, S. and K. Liang (1986). Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* **42**, 121-130.

PROTOCOL DEVIATIONS

1. The protocol specifies that F0 maternal feed consumption will be measured on pnd 0-4 and pnd 4-7. The preprinted forms inadvertently did not have a column for feed measurement on pnd 4. Therefore, feed consumption could only be calculated for pnd 0-7. This inadvertent error was not caught until the end of the study.
2. The protocol specifies that for females, which do not deliver by gd 26, the organs will be saved but no weights taken. Because of the small number of animals on study, organs were weighed and included in the statistical analysis for all dams. This is consistent with other reproductive toxicity studies conducted in this laboratory.
3. The protocol specifies that all animals will have access to feed *ad libitum*. During lactation, when pups begin self-feeding, the technician at the afternoon check misjudged the amount of feed necessary to allow the female *ad libitum* access until the following morning. Therefore, for Female 38, Rx Code 07706, after a weight loss noted on pnd 14, the female was reweighed the following day to allow the most accurate dose to be administered. Female 64, Rx Code 46719, was not reweighed the day after *ad libitum* was resupplied, for reasons unknown, but was receiving over 90% of the dose she should have. Female 76, Rx Code 07706, did not lose weight, so it was assumed that although there was no feed to weigh on pnd 14, that the food had not run out during the peak of the night feeding cycle. Feed consumption data were not entered, summarized, or analyzed; they remain in the study data. The Study Director sees no need to remove the body weight data.
4. The protocol specified that all animals would be dosed at 5 ml/kg. Animal No. 74 received 1.35 ml on October 15, 2001 to October 18, 2001. She should have received 1.4 ml; she received 96% of the dose she should have. Animal No. 120 received 1.20 ml instead of 0.81 ml on November 27-28, 2001; this was 148% of the dose she should have received. Animal No. 168 received 0.87 ml on November 27 to December 3, 2001. She should have received 0.88 ml, which was 98.9% of what she should have received. Female No. 68 had her weight recorded incorrectly on gd 14, so she received 1.35 ml (correct for that weight). The following day, No. 68 was reweighed and received 1.60 ml (correct for her weight). The percent error for the dose volume on gd 14 cannot be calculated since we do not know her correct weight on that day.
5. The protocol states that for F1 postwean observations and procedures for mortality that observations for mortality will be made twice daily (a.m. and p.m.). A snowstorm on the night of January 2 and the day of January 3, 2002, caused RTI to close due to unsafe driving conditions. RTI was closed Thursday, January 3 and Friday, January 4, 2002. All morning activities were conducted for this study. However, it was deemed unnecessary, because of the road conditions, to require a technician to come to RTI for the afternoon mortality check. No animals were found dead during the morning check on either Thursday or Friday nor on the following Saturday.

6. The protocol states that the temperature range for the animal rooms will be 66-77°F and the relative humidity (RH) will be 30-70% (NRC, 1996). On September 24, 2001, the RH was 92.1% in animal room 305 for one hour. The RH excursion was brief (maximum of one hour) and occurred only once.

In the Study Director's professional opinion, these protocol deviations did not affect the study design, performance, or interpretation and are presented for completeness.



Rochelle W. Tyl, Ph.D., DABT
Study Director

04/09/02
Date



Quality Assurance Statement

Study Title: Combined Reproductive/Developmental Toxicity Screening Test of Trisnonylphenyl Phosphite (TNPP) Administered via Oral Gavage to CD® (Sprague-Dawley) Rats (Modified OECD 421)

Sponsor: General Electric Company

Study Code: Rt01-GE4

Protocol Number: RTI-810

This study was audited by the Chemistry and Life Sciences Quality Assurance Unit and the results of the inspections and audits were reported to the study director and management as identified below. To the best of our knowledge, the reported results of this Final Study Report accurately describes the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director And Management
Protocol Review	7/18/01	7/18/01
Dose Formulation	8/30/01	8/31/01
Sample Preparation	8/31/01	8/31/01
PND 0*	9/6/01	9/6/01
Necropsy-F0 Males	10/4/01	10/4/01
Necropsy	11/2/01	11/2/01
Dose Formulation	11/29/01	11/30/01
Standard Preparation	12/20/01	12/20/01
Histology	1/22/01	1/24/01
Data Audit/Report Tables	2/28; 3/1, 4-8, 11-16, 18-22, 25-26/02	3/26/02
Analytical Data/Report Audit	3/11, 12, 15, 18-20/02	3/20/02
Report Audit	2/28; 3/1; 4/1-5/02	4/5/02

*Process inspection conducted on a related study Rt01-GE2, RTI-804

Celia D. Keller
Celia D. Keller
Quality Assurance Assistant Manager

04/10/02
Date

Approval:

Doris J. Smith
Doris J. Smith
Quality Assurance Manager

04/10/2002
Date

07895.300

62

Table 1. Analyses of Dose Formulations

Group No.	Rx Code	Color Code	Date(s) of Formulation	Nominal Concentration (mg/ml) ^a	Analytical Concentration (mg/ml)	Mean % of Nominal \pm SD (RSD)
1	46719	Green	08-30-01	0	ND ^b	NA ^c
			09-14-01		ND	NA
			10-03-01		ND	NA
			11-01-01		ND	NA
			11-29-01 ^d		—	—
			12-19-01		ND	NA
2	92042	Red	08-30-01	10	10.6	106 \pm 3.18 (3.0%) ^e
			09-14-01		10.6	106 \pm 2.52 (2.4%)
			10-03-01		10.8	98.2 \pm 3.1 (3.2%)
			11-01-01		9.4	94.4 \pm 1.75 (1.9%)
			11-29-01 ^d		—	—
			12-19-01		10.9 ^e	109 \pm 5.9 (5.4%) ^f
3	07706	Blue	08-30-01	40	43.6	109 \pm 0.58 (0.5%)
			09-14-01		41.0	102 \pm 4.37 (4.3%)
			10-03-01		43.1	105 \pm 1.5 (1.5%)
			11-01-01		44.0	110 \pm 1.73 (1.6%)
			11-29-01 ^d		—	—
			12-19-01		43.5 ^e	109 \pm 2.3 (2.1%) ^f
4	33749	Yellow	08-30-01	200	203.3	102 \pm 2.08 (2.0%)
			09-14-01		194.3	96.9 \pm 1.88 (1.9%)
			10-03-01		189.6	94.3 \pm 0.76 (0.8%)
			11-01-01		191.0	95.5 \pm 0.0 (0.0)
			11-29-01 ^d		—	—
			12-19-01		198.7	99.3 \pm 0.58 (0.6%)

^a Dosing solutions were formulated in corn oil vehicle for administration at 5.0 ml/kg. The doses were therefore 0, 50, 200, and 1000 mg/kg/day.

^b ND = not detected; estimated limit of detection is 0.2 mg/ml.

^c NA = not applicable.

^d Formulation date for which no analysis was scheduled. Analyses were required for the first four consecutive formulation dates and then for every other formulation date (if the first four formulations resulted in analytical values within the protocol-mandated range).

^e Data are presented as mean (% relative standard deviation), n=3

^f Analysis for the 10 and 40 mg/ml formulations on December 20, 2001, resulted in values 114% and 111% of nominal, respectively (outside the protocol-mandated range of within \pm 10% of nominal). The archive samples were submitted for verification of analysis. When the values from six samples (three original plus three archive) were combined, the formulations were within the acceptable range (109% of nominal).