



NTP

National Toxicology Program

U.S. Department of Health and Human Services

NTP TECHNICAL REPORT ON THE TOXICITY STUDIES OF

P-TOLUENESULFONAMIDE
(CASRN 70-55-3)
ADMINISTERED IN FEED TO
F344/N RATS,
F344/NTAC RATS, AND
B6C3F1/N MICE

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**NTP Technical Report on the
Toxicity Studies of *p*-Toluenesulfonamide
(CASRN 70-55-3) Administered in Feed to F344/N
Rats, F344/NTac Rats, and
B6C3F1/N Mice**

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Foreword

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

The Toxicity Study Report series began in 1991. The studies described in the Toxicity Study Report series are designed and conducted to characterize and evaluate the toxicologic potential of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in Toxicity Study Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection per se is not an indicator of a substance's toxic potential.

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

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This report has been reformatted to meet new NTP publishing requirements;
its content has not changed.

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Peer Review

The draft *NTP Technical Report on the Toxicity Studies of p-Toluenesulfonamide (CASRN 70-55-3) Administered in Feed to F344/N Rats, F344/NTac Rats, and B6C3F1/N Mice* was evaluated by the reviewers listed below. These reviewers served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determined if the design and conditions of these NTP studies were appropriate and ensured that this Toxicity Study Report presents the experimental results and conclusions fully and clearly.

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Abstract

p-Toluenesulfonamide is formed from chloramine-T, an antimicrobial agent used by the aquaculture industry to treat fish intended for human consumption. Chloramine-T is also widely used as a disinfectant in the medical, dental, veterinary, food processing, and agricultural industries. Because of its low degree of cytotoxicity, chloramine-T has been used in direct contact with tissues, including treatment for burns, in whirlpools for wounds, and as an oral mouthwash. In the agricultural industry, it is used as a broad-spectrum biocide for foot-and-mouth disease, swine vesicular disease, and poultry diseases. Chloramine-T was nominated by a private individual for toxicology studies based on its current status as an Investigational New Animal Drug for controlling proliferative gill disease and bacterial gill disease in aquaculture and the need for additional toxicology studies to support its safe use. *p*-Toluenesulfonamide was studied for toxicity by NTP because it has been shown to be the major product formed from chloramine-T. For the 2-week studies, male and female F344/N rats and B6C3F1/N mice were exposed to *p*-toluenesulfonamide (greater than 99% pure) in feed. For the 3-month studies, male and female F344/NTac rats and B6C3F1/N mice were exposed to *p*-toluenesulfonamide (greater than 99% pure) in feed. Genetic toxicology studies were conducted in *Salmonella typhimurium*, rat peripheral blood erythrocytes, and mouse peripheral blood erythrocytes.

In the 2-week studies, groups of five male and five female F344/N rats and mice were fed diets containing 0, 750, 1,500, 3,000, 10,000, or 30,000 ppm *p*-toluenesulfonamide (equivalent to average daily doses of approximately 95, 185, 370, 1,170, or 3,135 mg *p*-toluenesulfonamide/kg body weight to male F344/N rats, 80, 170, 335, 1,050, or 2,645 mg/kg to female F344/N rats, 150, 300, 700, 2,035, or 7,690 mg/kg to male mice, and 125, 280, 635, 2,410, or 6,000 mg/kg to female mice) for 15 days. All animals survived to the end of the studies. For F344/N rats, the final mean body weights of the 10,000 and 30,000 ppm groups were 91% and 71% that of male controls, respectively, and 94% and 83% that of female controls, respectively. Body weight gains were also decreased in 10,000 and 30,000 ppm rats. For mice, the final mean body weights of the 30,000 ppm groups were 86% that of male controls and 85% that of female controls. Body weight gains were also decreased in 30,000 ppm male mice and in all exposed female mice. Groups of mice exposed to 30,000 ppm lost weight during the study. In F344/N rats, feed consumption by 10,000 and 30,000 ppm males and 30,000 ppm females was less than that by the controls. In mice, feed consumption by exposed groups of mice was generally similar to that by the controls. No clinical observations or histopathologic findings were attributed to *p*-toluenesulfonamide exposure in the 2-week studies in F344/N rats or mice.

In the 2-week studies, absolute and relative kidney weights of 10,000 and 30,000 ppm female mice and relative kidney weights of 1,500 and 3,000 ppm female mice were significantly increased compared to those of the controls. There were no corresponding histologic lesions in the 2-week studies in F344/N rats or mice.

In the 3-month studies, groups of 10 male and 10 female F344/NTac rats and mice were fed diets containing 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm *p*-toluenesulfonamide (equivalent to average daily doses of approximately 50, 100, 200, 380, or 725 mg/kg to male F344/NTac rats, 30, 110, 210, 400, or 780 mg/kg to female F344/NTac rats, 120, 230, 420, 770, or 1,760 mg/kg to male mice, and 90, 210, 380, 780, or 1,890 mg/kg to female mice) for 14 weeks. Groups of 10 male and 10 female clinical pathology F344/NTac rats were exposed to the same concentrations for up to 22 days. All F344/NTac rats and male mice survived to the end of the

studies; one 10,000 ppm female mouse died during week 6. For F344/NTac rats, the final mean body weights of the 10,000 ppm groups were 93% that of male controls and 92% that of female controls. Body weight gains were also decreased in 2,500 ppm or greater male F344/NTac rats and in 5,000 and 10,000 ppm female F344/NTac rats. The mean body weight gains of 5,000 and 10,000 ppm male F344/NTac rats were significantly less than that of the controls. The final mean body weight of 1,250 ppm female F344/NTac rats was significantly greater (109%) than that of the controls; mean body weight gain was also increased in 1,250 ppm female F344/NTac rats. Feed consumption by 5,000 ppm male F344/NTac rats and 10,000 ppm male and female F344/NTac rats was less than that by controls early in the study, but generally recovered to near control values later in the study. Feed consumption by 625 and 1,250 ppm male mice was greater than that by the controls early in the study but returned to near control values later in the study. No clinical observations or histopathologic findings were attributed to *p*-toluenesulfonamide exposure in the 3-month studies in F344/NTac rats or mice.

In the 3-month studies, absolute and relative thymus weights of 10,000 ppm male F344/NTac rats were significantly less than those of the controls. Relative kidney weights of 2,500 ppm or greater male F344/NTac rats were significantly greater than those of the controls. Absolute and relative kidney weights of 10,000 ppm female mice were significantly greater than those of the controls. The relative lung weight of 10,000 ppm male mice and relative liver weight of 10,000 ppm female mice were significantly greater than those of the controls. There were no corresponding histologic lesions in the 3-month studies in F344/NTac rats or mice.

p-Toluenesulfonamide was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, or TA102 with or without exogenous metabolic activation. In vivo, no increases in micronucleated reticulocytes (polychromatic erythrocytes) or erythrocytes (normochromatic erythrocytes) were observed in peripheral blood of male or female F344/NTac rats or B6C3F1/N mice from the 3-month studies, and no biologically significant changes in the percentage of reticulocytes among total erythrocytes were seen, suggesting that *p*-toluenesulfonamide did not induce bone marrow toxicity.

Under the conditions of these 3-month feed studies, there were no treatment-related lesions in male or female F344/NTac rats or mice exposed to *p*-toluenesulfonamide in the feed at 625, 1,250, 2,500, 5,000, or 10,000 ppm. The most sensitive measures of *p*-toluenesulfonamide exposure in each species and sex were increased relative kidney weights in male F344/NTac rats [lowest observed effect level (LOEL) 2,500 ppm; 200 mg/kg], decreased body weight in female F344/NTac rats (LOEL 10,000 ppm; 780 mg/kg), increased relative lung weight in male mice (LOEL 10,000 ppm; 1,760 mg/kg), and increased relative liver weight and absolute and relative kidney weights in female mice (LOEL 10,000 ppm; 1,890 mg/kg). It is uncertain if these body weight or organ weight effects would compromise the survival or well-being of the animal after longer exposures.

Synonyms: Benzenesulfonamide, 4-methyl-; 4-methylbenzenesulfonamide; *p*-methylbenzenesulfonamide; 4-methylphenylsulfonamide; plasticizer 15; *p*-toluenesulfamide; 4-toluenesulfanamide; toluene-4-sulfonamide; 4-toluenesulfonic acid, amide; *p*-toluenesulfonylamide; toluene-*p*-sulphonamide; tolylsulfonamide; *p*-tolylsulfonamide; tosylamide; *p*-tosylamide

Trade Names: Uniplex 173, Halamid® Aqua

Summary of Findings Considered to be Toxicologically Relevant in Rats and Mice Exposed to *p*-Toluenesulfonamide in Feed for Three Months

	Male F344/NTac Rats	Female F344/NTac Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in feed	0, 625, 1,250, 2,500, 5,000, or 10,000 ppm	0, 625, 1,250, 2,500, 5,000, or 10,000 ppm	0, 625, 1,250, 2,500, 5,000, or 10,000 ppm	0, 625, 1,250, 2,500, 5,000, or 10,000 ppm
Average daily dose	0, 50, 100, 200, 380, or 725 mg/kg	0, 30, 110, 210, 400, or 780 mg/kg	0, 120, 230, 420, 770, or 1,760 mg/kg	0, 90, 210, 380, 780, or 1,890 mg/kg
Survival rates	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 9/10
Body weights	10,000 ppm group 7% less than the control group	10,000 ppm group 8% less than the control group	Exposed groups similar to the control group	Exposed groups similar to the control group
Clinical findings	None	None	None	None
Organ weights^a	↓ Absolute and relative thymus weights ↑ Relative kidney weights	None	↑ Relative lung weights	↑ Absolute and relative kidney weights ↑ Relative liver weights
Clinical pathology	None	None	None	None
Reproductive toxicity	None	Not determined	None	Not determined
Nonneoplastic effects	None	None	None	None
Genetic toxicology				
Bacterial gene mutations:		Negative in <i>S. typhimurium</i> strains TA98, TA100, and TA102 with or without S9		
Micronucleated erythrocytes				
Rat peripheral blood in vivo:		Negative in males and females		
Mouse peripheral blood in vivo:		Negative in males and females		

^aRelative organ weight = absolute organ weight/body weight.

Introduction

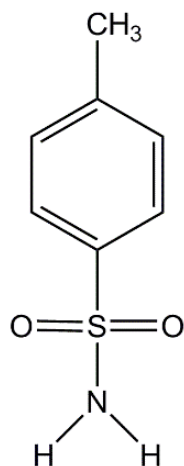


Figure 1. *p*-Toluenesulfonamide (CASRN 70-55-3; Chemical Formula: C₇H₉NO₂S; Molecular Weight: 171.23)

Synonyms: Benzenesulfonamide, 4-methyl-; 4-methylbenzenesulfonamide; *p*-methylbenzenesulfonamide; 4-methylphenylsulfonamide; plasticizer 15; *p*-toluenesulfamide; 4-toluenesulfanamide; toluene-4-sulfonamide; 4-toluenesulfonic acid, amide; *p*-toluenesulfonylamide; toluene-*p*-sulphonamide; tolylsulfonamide; *p*-tolylsulfonamide; tosylamide; *p*-tosylamide.
Trade Names: Uniplex 173, Halamid® Aqua.

Chemical and Physical Properties

p-Toluenesulfonamide is a nonvolatile chemical that exists in solid form as white flakes or crystalline powder and is stable in neutral, acidic, or alkaline conditions¹. It has a melting point of 138.5°C, boiling point of 214°C, molecular weight of 171.23, acid dissociation constant of 10.17 at 20°C, and octanol/water partition coefficient of 0.82 and is soluble in alcohol and water (3.16 × 10³ mg/L water at 25°C)².

Production, Use, and Human Exposure

p-Toluenesulfonamide is used as a plasticizer, an intermediate for pesticides and drugs, and is the primary degradation product of the disinfectant chloramine-T³⁻⁵. *p*-Toluenesulfonamide may be present as a contaminant in saccharin⁶. Local injection of *p*-toluenesulfonamide is being studied as an investigational anticancer drug in China for lung cancer in combination with other drugs⁷.

The U.S. Fish and Wildlife Service reports that chloramine-T is being tested for use in aquaculture because of the chemical's ability to kill bacterial colonies that form on fish in fish culture tanks^{8, 9}. Although chloramine-T is not licensed in the United States for use as a disinfectant in the aquaculture industry for producing fish intended for human consumption, its use has been investigated under an investigational new animal drug¹⁰ as a treatment for bacterial gill disease in freshwater or marine aquaria, garden ponds, or other aquatic systems at concentrations ranging from 6.5 to 8.5 mg/L as a 1 hour flow-through treatment¹¹. Another study reports that up to four 60-minute exposures of between 10 and 20 mg chloramine-T/L administered once daily on consecutive or alternative days was effective in reducing fish mortality associated with bacterial infections¹². A mean concentration of *p*-toluenesulfonamide

in fish after chloramine-T exposure at a concentration of 600 mg/L water was approximately 1,000 ng/g⁴.

Chloramine-T is used in Europe in aquaculture as a prevention treatment for bacterial disease using 10 mg/L in water in a flow-through basin for 1 hour¹³ and is also used in the food industry to disinfect equipment and machinery before processing. *p*-Toluenesulfonamide was found in ice cream at a range of 0.55 to 4.44 mg *p*-toluenesulfonamide/kg ice cream¹⁴. In a study conducted in Germany, *p*-toluenesulfonamide was found in wastewater (<0.02 to 50.8 µg/L), in groundwater below a former sewage farm (<0.02 to 41 µg/L), in surface water (<0.02 to 1.15 µg/L), and in drinking water (<0.02 to 0.27 µg/L)⁵.

Chloramine-T is listed as a main ingredient in whirlpool antiseptics¹⁵, in various animal husbandry procedures as a disinfectant¹⁶, and as a laboratory reagent to detect cyanide¹⁷. *p*-Toluenesulfonamide is used as an intermediate for pesticide and drug production¹.

Mean production volume for chloramine-T in the United States is reported as less than 500,000 pounds¹⁸. The National Occupational Exposure Survey reported that between 4,000 and 9,000 workers may be exposed to chloramine-T annually at 200 to 300 facilities in the United States¹⁹.

The United States Environmental Protection Agency²⁰ reports that *p*-toluenesulfonamide is not readily biodegradable in the environment.

Regulatory Status

According to the German Federal Environmental Agency, the tolerable concentration limit of *p*-toluenesulfonamide in drinking water is 0.3 µg/L³. An Investigational New Animal Drug application has been submitted to the United States Food and Drug Administration for use of chloramine-T in public fish hatcheries⁴.

Absorption, Distribution, Metabolism, Excretion, and Toxicokinetics

p-Toluenesulfonamide was well absorbed in female Wistar albino rats and excreted mainly in urine following oral administration of 29 or 200 mg/kg [¹⁴C] *p*-toluenesulfonamide⁶. Twenty-four hours after dosing, urinary excretion (combined urine and cage rinse) accounted for approximately 90% and 77% and fecal excretion accounted for approximately 4% and 1% of the 29 and 200 mg/kg doses, respectively. More than 90% of the urinary radioactivity was associated with 4-sulphamoylbenzoic acid; other minor components included *p*-toluenesulfonamide, 4-sulphamoylbzyl alcohol, 4-sulphamoylbenzaldehyde, and, at the higher dose, N-acetyltoluene-4-sulphonamide (Figure 2). In another study, following oral administration of 300 mg/kg [³⁵S] *p*-toluenesulfonamide in Wistar rats, 80% of the administered dose was found in urine with 4-sulphamoylbenzoic acid accounting for 50% of the urinary radioactivity²¹. Experiments in dogs suggested a similar metabolic pathway; 4-sulphamoylbenzoic acid was found in the urine of dogs given *p*-toluenesulfonamide²². Following intravenous administration of 33 or 198 mg/kg *p*-toluenesulfonamide to male Wistar rats for 4 days, total cytochrome P450 content in control and treated animals was similar²³. The authors demonstrated that *p*-toluenesulfonamide metabolism is likely mediated through CYP2C7, CYP2D1, and CYP3A2.

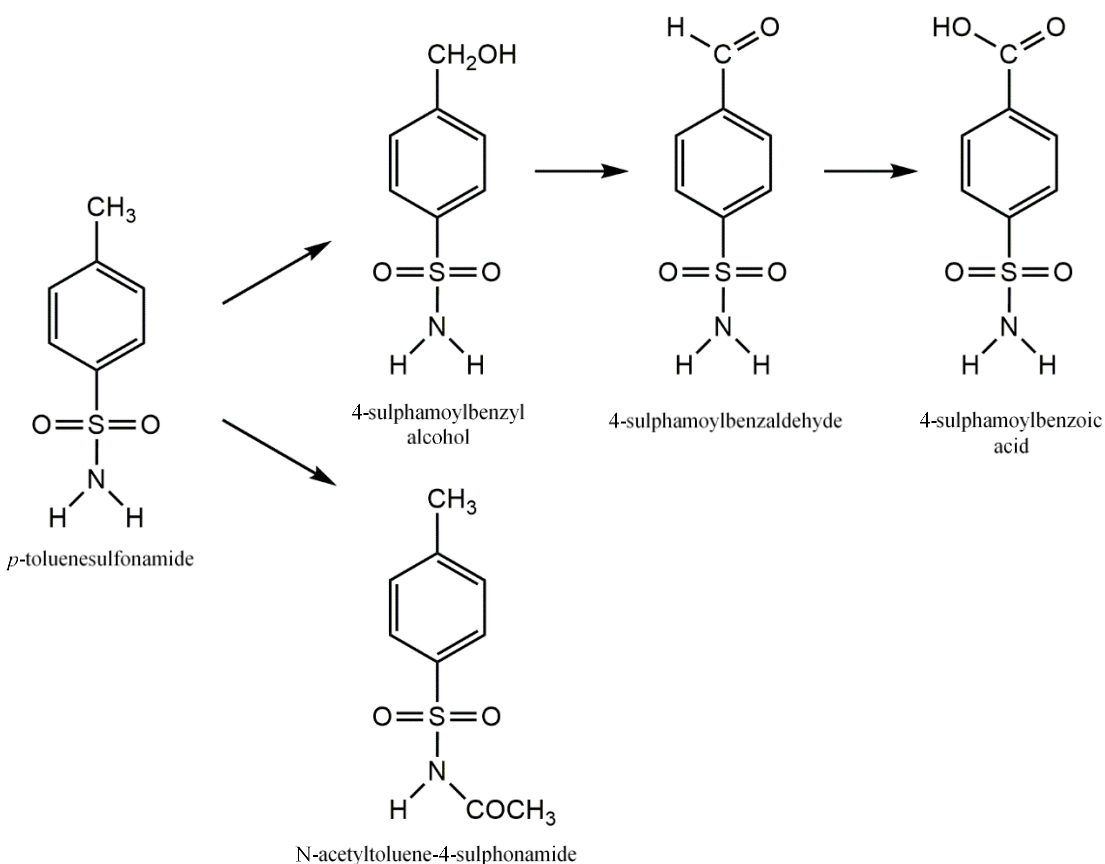


Figure 2. Metabolism of *p*-Toluenesulfonamide

Chloramine-T has been studied in fish and rats. In male Wistar rats following oral administration of 100 mg/kg or intravenous administration of 30 mg/kg, chloramine-T was rapidly distributed and eliminated with distribution and elimination half-lives of 0.42 and 1.98 hours for oral and 0.12 and 1.41 hours for intravenous administration, respectively²⁴. In another study, following oral administration of 100 mg/kg or an intraperitoneal injection of 5 mg/kg for 4 consecutive days in Wistar rats, chloramine-T was rapidly absorbed and distributed to the brain (the only tissue measured in this study)²⁵. Following exposure of adult rainbow trout to 20 mg [¹⁴C] chloramine-T, the major product detected in whole body homogenates was *p*-toluenesulfonamide; residual chloramine-T was not detected suggesting complete conversion of chloramine-T²⁶. When fingerlings or juvenile trout were exposed to 20 mg/L of [¹⁴C] chloramine-T for up to 1 hour and then transferred to fresh water for recovery, the half-lives of *p*-toluenesulfonamide estimated by radiometric analysis of whole body homogenates were 27.3 and 32.6 hours in fingerlings and juveniles, respectively²⁶.

Toxicity

Experimental Animals

LD₅₀ toxicity values reported for *p*-toluenesulfonamide are 2,330 mg/kg body weight (rat via oral gavage); 2,400 mg (mixture of 41% *ortho*- and 51% *p*-toluenesulfonamide)/kg (rat via oral

gavage); 250 mg/kg (mouse via intraperitoneal injection); and 75 mg/kg (wild bird via oral gavage)^{2, 27}.

No 14-day or 90-day rodent toxicity studies of *p*-toluenesulfonamide or chloramine-T were found in the peer-reviewed scientific literature. However, the USEPA reported that they had received toxicity study reports.

The USEPA²⁸ reports that it has received the results of a 90-day *p*-toluenesulfonamide Organisation for Economic Co-operation and Development (OECD) 408 toxicity study in rats (species of rats not specified) conducted by industry. The *p*-toluenesulfonamide was administered in the diet at concentrations of 0, 1,000, 3,000, or 10,000 ppm (approximately 0, 70, 214, and 738 mg/kg per day for males and 0, 80, 248, 795 mg/kg per day for females). Administration of 10,000 ppm resulted in a 21% reduction in body weight. Hyperplasia of the urothelium of the urinary bladder occurred in two of 10 males at 10,000 ppm. No other major treatment-related effects were reported in the summary.

Survival for different strains of fish was measured at chloramine-T concentrations of 0, 20, 60, 100, or 200 mg/L. Decreased survival of fish occurred at chloramine-T exposures of 60 mg/L or greater 96 hours after a 60 or 180 minute exposure period¹². The recommended maximum chloramine-T concentration for use in aquaculture is 20 mg/L¹².

The European Medicines Evaluation Agency²⁶ reported on a study in rats (strain not specified) exposed to chloramine-T through feeding for either 28 days or 90 days. The target doses for the 28- and 90-day exposures ranged from 150 to 1,500 mg/kg (0.533 to 5.325 mmol/kg per day) and 5 to 150 mg/kg per day (18 to 533 µmol/kg per day), respectively. Reduced body weight gains were observed in treated animals. Relative kidney weights were increased in all dosed groups in the 28-day study and in the two highest dosed groups in the 90-day study. Relative liver weights were increased in all of the dosed groups in the 90-day study. Slight increases in leukocytes and pale, discolored livers were observed in the two highest dosed groups in the 28-day study. Increased severity and frequency of calcareous deposits occurred in the kidneys of female rats receiving 50 and 150 mg/kg per day (180 and 533 µmol/kg per day) in the 90-day study.

Humans

Local injection of *p*-toluenesulfonamide was associated with mild fever, local pain, and somnolence that resolved spontaneously in a study conducted in China⁷. Local *p*-toluenesulfonamide injection did not appear to potentiate toxicity of gemcitabine plus cisplatin chemotherapy⁷.

Reproductive and Developmental Toxicity

Experimental Animals

The USEPA High Production Volume Information System contains a summary of a one generation reproductive oral gavage *p*-toluenesulfonamide study sponsored by industry²⁹. In this study, Crj:CD(SD) male rats were exposed to *p*-toluenesulfonamide 42 days prior to mating and female rats were exposed for 14 days before mating through lactation day 3 at oral doses of 0, 120, 300, and 750 mg/kg. In the high-dose group, newborn rats showed significant decreases in

body weight and survival rate. Mating performance and fertility were not affected by the test compound. Reproduction parameters were comparable among all four groups including the control. No remarkable histopathologic changes in the ovaries were observed in any of the non-pregnant females. Morphologic observations for offspring revealed no teratogenic effect of the test substance³⁰.

No studies examining the potential for developmental or prenatal toxicity in animals were found in a search of the peer-reviewed scientific literature.

Humans

No studies examining the potential for reproductive toxicity of *p*-toluenesulfonamide or chloramine-T in humans were found in a search of the peer-reviewed scientific literature.

Carcinogenicity

No studies examining the potential for carcinogenic activity of *p*-toluenesulfonamide or chloramine-T in animals or epidemiology studies in humans were found in a search of the peer-reviewed scientific literature.

Genetic Toxicity

Only one publication reporting results from genotoxicity tests with *p*-toluenesulfonamide was identified in a search of the peer-reviewed scientific literature. Eckhardt et al.³¹ tested *p*-toluenesulfonamide in assays for bacterial mutagenicity (using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538), sex-linked recessive lethal mutation induction in male *Drosophila melanogaster*, and micronucleus induction in bone marrow reticulocytes of male and female NMRI mice. The authors reported a small but significant increase in mutant colonies in *Salmonella* strain TA98 in the presence of 10% rat liver S9 when testing was conducted using a nontraditional medium, ZLM, and doses of *p*-toluenesulfonamide (9,600 to 18,000 µg/plate) that far exceeded the limit doses that are currently used (5,000 to 6,000 µg/plate). In tests using the traditional Vogel-Bonner medium, no mutagenicity was observed at any dose level, with or without S9. In the sex-linked recessive lethal mutation assay, an increase in lethals (0.67%) was observed in brood 1 following 3 days of feeding the adult male flies on a sucrose solution containing 2.5 mM *p*-toluenesulfonamide. In the mouse micronucleus test, no increase in micronucleated reticulocytes was observed in bone marrow following administration of 855 mg *p*-toluenesulfonamide/kg body weight either by intraperitoneal injection or by gavage, although the protocol was not optimal for detecting micronucleated cells in the bone marrow.

Additional, publicly available (but not published) information was found in the USEPA High Production Volume Information System database³² where results from genotoxicity tests with *p*-toluenesulfonamide using bacterial mutagenicity assays and an in vitro chromosomal aberration test are reported. *p*-Toluenesulfonamide (doses up to 5,000 µg/plate) was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537, with or without S9 and no induction of chromosomal aberrations was seen in cultured Chinese hamster lung cells. The highest concentration used in the chromosomal aberrations test in the presence of S9 was 1.7 mg/mL and the highest concentration used in the absence of S9 was 1.3 mg/mL.

p-Toluenesulfonamide was cytotoxic at a dose of 2.0 mg/mL in the absence of S9 and was cytotoxic at a concentration greater than 2.0 mg/mL in the presence of S9.

Study Rationale

Chloramine-T was nominated by a private individual for toxicologic characterization due to its status as an investigational new animal drug for controlling proliferative gill disease and bacterial gill disease in aquaculture. In response to the nomination, the FDA requested using *p*-toluenesulfonamide as the study test article because it is the primary residue in chloramine-T treated fish intended for human consumption. In addition to *p*-toluenesulfonamide 3-month toxicity studies, NTP conducted bacterial mutagenicity tests using standardized protocols.

Materials and Methods

Procurement and Characterization of *p*-Toluenesulfonamide

p-Toluenesulfonamide was obtained from Acros Organics (Geel, Belgium) in one lot (A009615201). Lot A009615201 was purified by Battelle's Organic Synthesis Group (Columbus, OH) and was renamed lot 112003. Lot 112003 was used in the 2-week and 3-month studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Operations (Columbus, OH) (Appendix F). In addition, Karl Fischer titration and elemental analyses were performed by Prevalere Life Sciences, Inc. (Whitesboro, NY). Reports on analyses performed in support of the *p*-toluenesulfonamide studies are on file at the National Institute of Environmental Health Sciences.

Lot 112003, a white crystalline chemical, was identified as *p*-toluenesulfonamide using infrared and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and by melting point analysis.

The purity of the bulk chemical was determined by elemental analyses, differential scanning calorimetry, and high-performance liquid chromatography with ultraviolet detection (HPLC/UV). Tentative impurity identification was obtained using mass spectrometry (MS) and proton and carbon-13 NMR spectroscopy.

Karl Fischer titration indicated approximately 0.1% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for *p*-toluenesulfonamide. Differential scanning calorimetry indicated a purity of 100%. HPLC/UV indicated one major peak and one reportable impurity with an individual area equal to 0.2% of the total peak area. The most probable structure for the impurity based on MS and NMR analyses was 4-methyl-*N*-phenylbenzene sulfonamide, although the impurity peak in the HPLC/UV analyses might have been composed of multiple components. The overall purity of lot 112003 was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored under a headspace of inert gas at room temperature, protected from light. Periodic reanalyses of the bulk chemical were performed at the study laboratory at BioReliance Corporation (Rockville, MD) during the 2-week and 3-month studies using HPLC/UV, and no degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared once during the 2-week studies and eight times during the 3-month studies by mixing *p*-toluenesulfonamide with feed. A premix was prepared by hand and then blended with additional feed in a Patterson-Kelly twin-shell blender. Formulations were stored in doubled polyethylene bags sealed with twist ties protected from light at room temperature for up to 42 days.

Homogeneity studies of the 750 and 30,000 ppm dose formulations and stability studies of the 750 ppm dose formulation were performed by the analytical chemistry laboratory using HPLC/UV. An additional homogeneity study of the 625 ppm dose formulation was performed by the study laboratory using HPLC/UV. Homogeneity was confirmed, and stability was confirmed

for at least 42 days for dose formulations stored in plastic zip-lock bags, protected from light, at temperatures up to room temperature, and for at least 7 days for dose formulations kept in glass feeding containers without urine and feces under simulated animal room conditions.

Periodic analyses of the dose formulations of *p*-toluenesulfonamide were conducted by the study laboratory using HPLC/UV. During the 2-week studies, the dose formulations were analyzed once; all 10 dose formulations for rats and mice were within 10% of the target concentrations (Table F-3). Animal room samples of these dose formulations were also analyzed; all 10 for male rats and two of 10 for female mice were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed (Table F-4). Of the dose formulations analyzed, all 34 for rats and mice were within 10% of the target concentrations; 15 of 30 animal room samples for rats and 13 of 30 for mice were within 10% of the target concentrations. Low recovery of *p*-toluenesulfonamide in many of the animal room samples was attributed to potential contamination of dosed feed with urine and/or feces, which may have caused irreversible binding of the test chemical to the feed. A similar behavior was observed in the simulated animal room stability studies conducted on the 750 ppm dose formulation by the analytical chemistry laboratory where a decline of formulation concentration was observed in the presence of urine and feces.

Animal Source

Male and female F344/N rats and B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc. (Germantown, NY), for the 2-week studies. For the 3-month studies, male and female F344/NTac rats were obtained from the commercial colony at Taconic Farms, Inc.; B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc. The rationale for change of rat strain from F344/N to F344/NTac was a programmatic decision. For many years, NTP used the inbred F344/N rat for its toxicity and carcinogenicity studies. Over a period of time, the F344/N rat exhibited sporadic seizures and idiopathic chylothorax, and consistently high rates of mononuclear cell leukemia and testicular neoplasia. Because of these issues in the F344/N rat and the NTP's desire to find a more fecund rat model that could be used in both reproductive and carcinogenesis studies for comparative purposes, a change in the rat model was explored. Following a workshop in 2005, the F344 rat from the Taconic commercial colony (F344/NTac) was used for a few NTP studies to allow NTP to evaluate different rat models. The F344/NTac rat was used in four subchronic, including the current 3-month study, and two chronic studies between 2005 and 2006³³.

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by the BioReliance Corporation Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Two-week Studies

The oral gavage LD₅₀ of *p*-toluenesulfonamide is 2,330 mg/kg body weight in F344/N rats. Doses selected for the 2-week feed studies were 0, 750, 1,500, 3,000, 10,000, and 30,000 ppm, with the high dose estimated to deliver approximately 3,000 mg/kg in feed.

On receipt, F344/N rats were 3 weeks old, and mice were 3 to 4 weeks old. Animals were quarantined for 13 (F344/N rats) or 14 (mice) days. F344/N rats were 5 weeks old and mice were 5 to 6 weeks old on the first day of the studies. Before the studies began, five male and five female F344/N rats were randomly selected for parasite evaluation and gross observation for evidence of disease. All results were negative.

Groups of five male and five female F344/N rats and mice were fed diets containing 0, 750, 1,500, 3,000, 10,000, or 30,000 ppm *p*-toluenesulfonamide for 15 days. Feed and water were available ad libitum. F344/N rats and female mice were housed five per cage; male mice were housed individually. Animals were observed twice daily. The animals were weighed and clinical findings were recorded initially, on day 8, and at the end of the studies; feed consumption was recorded on day 8 and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all F344/N rats and mice. The heart, right kidney, liver, lung, right testis, and thymus from each animal were weighed. Histopathologic examinations of the tissues weighed were performed on 0, 10,000, and 30,000 ppm F344/N rats and mice; also, histopathologic examinations of the right kidney were performed on 1,500 and 3,000 ppm female mice. Table 1 lists the tissues and organs examined.

Three-month Studies

On receipt, F344/NTac rats (the rat strain in use by NTP at the time of these studies) were 3 to 4 weeks old and mice were 5 to 6 weeks old. Animals were quarantined for 12 (male rats), 13 (female rats), 16 (male mice), or 17 (female mice) days. F344/NTac rats were 5 to 6 weeks old and mice were 7 to 8 weeks old on the first day of the studies. Before the studies began, five male and five female F344/NTac rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Serologic analyses were performed on five male and five female control F344/NTac rats and five male and five female sentinel mice at the end of the studies using the protocols of the NTP Sentinel Animal Program (Appendix I). All results were negative.

Groups of 10 male and 10 female F344/NTac rats and mice were fed diets containing 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm *p*-toluenesulfonamide for 14 weeks. Groups of 10 male and 10 female clinical pathology F344/NTac rats were exposed to the same concentrations for 22 days. Feed and water were available ad libitum. F344/NTac rats and female mice were housed five per cage; male mice were housed individually. Animals were observed twice daily. The animals were weighed and clinical observations were recorded initially, on day 8, weekly thereafter, and at the end of the studies. Feed consumption was recorded on day 8 and weekly thereafter. Details of the study design and animal maintenance are summarized in Table 1. Information on the feed composition and contaminants is provided in Appendix H.

Animals were anesthetized with a 70%:30% CO₂:O₂ mixture and blood was collected from the retroorbital plexus (F344/NTac rats) or retroorbital sinus (mice) of clinical pathology rats on days 3 and 22 and of core study F344/NTac rats and mice at the end of the studies for hematology and clinical chemistry (F344/NTac rats only) analyses. For hematology, blood was placed in tubes containing dipotassium EDTA as the anticoagulant. For clinical chemistry, blood was placed in tubes devoid of anticoagulant, allowed to clot, and the serum was harvested for analysis. All clinical pathology evaluations were performed at Analytics, Inc. (Gaithersburg, MD). The hematology analyses were conducted using an ABX Pentra 60 C+ Analyzer (HORIBA Instruments Inc., Irvine, CA) using reagents supplied by the manufacturer. Clinical chemistry analyses were completed using a Hitachi 717 Analyzer (Boehringer Mannheim, Indianapolis, IN) using reagents obtained from Randox Laboratories (Oceanside, CA) or Sigma Diagnostics (St. Louis, MO). All hematology and clinical chemistry parameters evaluated are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on F344/NTac rats and mice exposed to 0, 2,500, 5,000, or 10,000 ppm. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal euthanasia, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Vaginal cytology slides were assessed in accordance with the NTP Guideline for the Cytological Staging of Rat and Mouse Estrous Cycle. Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (F344/NTac rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study F344/NTac rats and mice. The heart, right kidney, liver, lung, right testis, and thymus from each animal were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all 0 and 10,000 ppm core study F344/NTac rats and mice. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the study laboratory and QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a

consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman³⁴ and Boorman et al.³⁵.

Table 1. Experimental Design and Materials and Methods in the Feed Studies of *p*-Toluenesulfonamide

Two-week Studies	Three-month Studies
Study Laboratory	
BioReliance Corporation (Rockville, MD)	BioReliance Corporation (Rockville, MD)
Strain and Species	
F344/N rats	F344/NTac rats
B6C3F1/N mice	B6C3F1/N mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
F344/N rats: 13 days	F344/NTac rats: 12 (males) or 13 (females) days
Mice: 14 days	Mice: 16 (males) or 17 (females) days
Average Age When Studies Began	
F344/N rats: 5 weeks	F344/NTac rats: 5 to 6 weeks
Mice: 5 to 6 weeks	Mice: 7 to 8 weeks
Date of First Exposure	
F344/N rats: July 5, 2006	F344/NTac rats: October 17 (males) or 18 (females), 2006
Mice: July 6, 2006	Mice: October 19 (males) or 20 (females), 2006
Duration of Exposure	
15 days	Core study F344/NTac rats and mice: 14 weeks Clinical pathology study F344/NTac rats: 22 days
Date of Last Exposure	
F344/N rats: July 19, 2006	Core study F344/NTac rats: January 16 (males) or 17 (females), 2007
Mice: July 20, 2006	Mice: January 18 (males) or 19 (females), 2007
Necropsy Dates	
F344/N rats: July 19, 2006	Core study F344/NTac rats: January 16 (males) or 17 (females), 2007
Mice: July 20, 2006	Mice: January 18 (males) or 19 (females), 2007
Average Age at Necropsy	
F344/N rats: 7 weeks	F344/NTac rats: 18 to 19 weeks
Mice: 7 to 8 weeks	Mice: 20 to 21 weeks
Size of Study Groups	
5 males and 5 females	10 males and 10 females

Two-week Studies	Three-month Studies
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies
Animals per Cage	
F344/N rats: 5	F344/NTac rats: 5
Mice: 1 (males) or 5 (females)	Mice: 1 (males) or 5 (females)
Method of Animal Identification	
Tail tattoo	Same as 2-week studies
Diet	
Irradiated NTP-2000 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available ad libitum	Same as 2-week studies
Water	
Tap water (Washington Suburban Sanitary Commission Potomac Plant) via automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as 2-week studies
Cages	
Polycarbonate (Lab Products, Inc., Seaford, DE), changed twice per week for F344/N rats and female mice and once weekly for male mice	Same as 2-week studies
Bedding	
Irradiated heat-treated Sani-Chips [®] hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed at least twice per week for F344/N rats and female mice and once weekly for male mice	Same as 2-week studies
Cage Filters	
Omnishield Paper, Remy 2024 (Dupont), Harlan Teklad, (Indianapolis, IN), changed every 2 weeks	Same as 2-week studies
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE); changed every 2 weeks	Same as 2-week studies
Animal Room Environment	
Temperature: 72° ± 3°F	Same as 2-week studies
Relative humidity: 50% ± 15%	
Room fluorescent light: 12 hours/day	
Room air changes: at least 10/hour	
Exposure Concentrations	
0, 750, 1,500, 3,000, 10,000, or 30,000 ppm in feed, available ad libitum	0, 625, 1,250, 2,500, 5,000, or 10,000 ppm in feed, available ad libitum

Two-week Studies	Three-month Studies
Type and Frequency of Observation	
Observed twice daily; animals were weighed and clinical findings were recorded initially, on day 8, and at the end of the studies. Feed consumption was recorded on day 8 and at the end of the studies.	Observed twice daily; core study animals were weighed and clinical findings were recorded initially, on day 8, weekly thereafter, and at the end of the studies; feed consumption was recorded on day 8 and weekly thereafter.
Method of Euthanasia	
Carbon dioxide asphyxiation	Same as 2-week studies
Necropsy	
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.
Clinical Pathology	
None	Blood was collected from the retroorbital sinus of clinical pathology F344/NTac rats on days 3 and 22 and from core study animals at the end of the studies for hematology and clinical chemistry (F344/NTac rats only).
	Hematology: hematocrit; hemoglobin; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte counts and differentials.
	Clinical chemistry: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids
Histopathology	
Histopathology was performed on 0, 10,000, and 30,000 ppm F344/N rats and mice. In addition to gross lesions and tissue masses the heart, right kidney, liver, lung, right testis, and thymus were examined; the right kidney was also examined in 1,500 and 3,000 ppm female mice.	Complete histopathology was performed on 0 and 10,000 ppm core study F344/NTac rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, larynx (mice), liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, pharynx (mice), ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, tongue, trachea, urinary bladder, and uterus.

Two-week Studies	Three-month Studies
Sperm Motility and Vaginal Cytology	
None	At the end of the studies, spermatid and sperm samples were collected from male animals in the 0, 2,500, 5,000, and 10,000 ppm groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from females exposed to 0, 2,500, 5,000, or 10,000 ppm for vaginal cytology evaluations.

Statistical Methods

Calculation and Analysis of Lesion Incidences

The incidences of nonneoplastic lesions are presented in Appendix A as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test³⁶, a procedure based on the overall proportion of affected animals, was used to determine significance.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett³⁷ and Williams^{38; 39}. Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley⁴⁰ (as modified by Williams⁴¹) and Dunn⁴². Jonckheere's test⁴³ was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey⁴⁴ were examined by NTP personnel, and implausible values were eliminated from the analysis.

Quality Assurance Methods

The 3-month studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations⁴⁵. In addition, as records from the 3-month studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent QA contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Toxicity Study Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Toxicity Study Report.

Genetic Toxicology

***Salmonella typhimurium* Mutagenicity Test Protocol**

Testing procedures used for *p*-toluenesulfonamide followed protocols reported by Zeiger et al.⁴⁶. *p*-Toluenesulfonamide was sent to the testing laboratory (BioReliance Corporation, Rockville, MD) as a coded aliquot. It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, and TA102 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat liver) for 20 minutes at 37°C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37°C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of five doses of *p*-toluenesulfonamide. The highest noncytotoxic dose was 3,333 µg/plate.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold-increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

Rat and Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by Witt et al.⁴⁷ and Torous et al.⁴⁸. At the end of the 3-month studies, small peripheral blood samples (60 to 120 uL) were obtained from male and female F344/NTac rats and mice, placed in tubes containing EDTA, chilled, and shipped with cold packs by overnight courier to the testing laboratory (ILS, Inc., Research Triangle Park, NC) where they were immediately fixed in ultracold methanol [MicroFlow[®] Basic Kits, Litron Laboratories, Rochester NY; Dertinger et al.⁴⁹] and stored at -80°C until analysis. Flow cytometric analyses were conducted using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA). Reticulocytes (RETs) were identified by the presence of an active transferrin receptor (CD71⁺) on the cell surface; mature erythrocytes were identified as CD71-negative (CD71⁻).

For F344/NTac rat blood samples, the analysis was restricted to the youngest RETs (i.e., the subpopulation of erythrocytes with the highest CD71 expression) to focus on the population of RETs that were least altered by the efficient action of the F344/NTac rat spleen in sequestering and destroying micronucleated red blood cells⁵⁰. Using flow cytometry, micronuclei were detected using the DNA staining dye propidium iodide (PI) in conjunction with RNase treatment. Therefore, micronucleated RETs express high levels of CD71 (CD71⁺) and PI-associated fluorescence, while micronucleated erythrocytes are negative for CD71 (CD71⁻) and show PI-associated fluorescence. Twenty thousand CD71⁺ RETs (polychromatic erythrocytes, PCEs) were scored per animal for presence of micronuclei, and approximately 1 million total erythrocytes (normochromatic erythrocytes, NCEs) were counted for the presence of micronuclei

and to determine the percentage of RETs (% PCEs) as a measure of chemical-induced bone marrow toxicity.

Based on prior experience with the large number of cells scored using flow cytometric scoring techniques⁵¹, it is reasonable to assume that the proportion of micronucleated RETs is approximately normally distributed. The statistical tests selected for trend and for pairwise comparisons with the control group depend on whether the variances among the groups are equal. Levene's test at $\alpha = 0.05$ is used to test for equal variances. In the case of equal variances, linear regression is used to test for a linear trend with dose and Williams' test is used to test for pairwise differences between each treatment group and the control group. In the case of unequal variances, Jonckheere's test is used to test for linear trend and Dunn's test is used for pairwise comparisons of each treatment group with the control group. To correct for multiple pairwise comparisons, the P value for each comparison with the control group is multiplied by the number of comparisons made. In the event that this product is greater than 1.00, it is replaced with 1.00. Trend tests and pairwise comparisons with the controls are considered statistically significant at $P \leq 0.025$, which is a Bonferroni correction to an overall 0.05 level of significance to adjust for testing for both trend and pairwise comparison. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple samples of a chemical were tested in the same assay, and different results were obtained among these samples and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The results presented in the Abstract of this Toxicity Study Report represent a scientific judgment of the overall evidence for activity of the chemical in an assay.

Results

Two-week Study in F344/N Rats

All F344/N rats survived to the end of the study (Table 2). Final mean body weights and mean body weight gains of 10,000 and 30,000 ppm males and 30,000 ppm females were significantly less than those of the controls; the mean body weight gain of 10,000 ppm females was significantly less than that of the controls. Feed consumption by 10,000 and 30,000 ppm males and 30,000 ppm females was less than that by the controls throughout the study (Table 2 and G1). Exposure concentrations of 750, 1,500, 3,000, 10,000, and 30,000 ppm resulted in average daily doses of approximately 95, 185, 370, 1,170, and 3,135 mg *p*-toluenesulfonamide/kg body weight to males and 80, 170, 335, 1,050, and 2,645 mg/kg to females. No clinical observations or histopathologic findings were attributed to *p*-toluenesulfonamide exposure.

Table 2. Survival, Body Weights, and Feed Consumption of F344/N Rats in the Two-week Feed Study of *p*-Toluenesulfonamide^a

Concentration (ppm)	Survival ^b	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)	Feed Consumption Week 1 (g)	Feed Consumption Week 2 (g)
Male							
0	5/5	90 ± 3	161 ± 5	71 ± 4		15.5	16.5
750	5/5	91 ± 2	153 ± 6	62 ± 4	95	16.4	14.2
1,500	5/5	91 ± 2	161 ± 4	70 ± 3	100	14.6	16.1
3,000	5/5	93 ± 3	159 ± 5	66 ± 3	98	15.6	15.6
10,000	5/5	91 ± 2	147 ± 3*	57 ± 2**	91	12.9	14.8
30,000	5/5	89 ± 2	114 ± 2**	25 ± 1**	71	8.3	12.8
Female							
0	5/5	90 ± 2	126 ± 3	36 ± 3		12.2	10.5
750	5/5	89 ± 2	128 ± 2	39 ± 1	102	11.9	11.9
1,500	5/5	87 ± 2	125 ± 4	38 ± 3	100	12.1	11.8
3,000	5/5	88 ± 4	121 ± 2	33 ± 2	96	11.7	11.6
10,000	5/5	90 ± 3	118 ± 3	29 ± 2*	94	11.0	10.8
30,000	5/5	90 ± 1	104 ± 1**	14 ± 2**	83	7.5	9.6

*Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** $P \leq 0.01$.

^aWeights and weight changes are given as mean ± standard error. Feed consumption is expressed as grams per animal per day.

^bNumber of animals surviving at 15 days/number initially in group.

The absolute kidney weights were decreased in 30,000 ppm males by approximately 19%; the relative kidney weights were increased in 3,000 ppm or greater males and in 10,000 and 30,000 ppm females (Table C-1). There were no histologic findings that correlated with these organ weights changes.

Other organ weight changes in male F344/N rats included decreases in the absolute heart, liver, and thymus weights at 30,000 ppm; the absolute lung weights at 1,500 ppm or greater; and the relative lung weight at 1,500 ppm (Table C-1). The relative testis weight of 30,000 ppm males was increased. Absolute heart and liver weights were decreased in 30,000 ppm female rats. There were no histologic findings that correlated with these organ weights changes, and they are considered to be primarily related to changes in body weights.

Exposure Concentration Selection Rationale: There were no treatment-related deaths, clinical toxicity, or gross or microscopic findings in male or female F344/N rats in the 2-week *p*-toluenesulfonamide feed study. The primary finding was decreased body weights at 10,000 and 30,000 ppm relative to controls. Final mean body weights at 10,000 and 30,000 ppm were 9% and 29% less than controls for males and 6% and 17% less for females, respectively. The doses selected for the 3-month feed studies in F344/NTac rats were 0, 625, 1,250, 2,500, 5,000, and 10,000 ppm.

Three-month Study in F344/NTac Rats

All core study F344/NTac rats survived to the end of the study (Table 3). The final mean body weights and the mean body weight gains of 10,000 ppm males and females were significantly less than those of the controls; in addition, the mean body weight gains of 2,500 ppm males and 5,000 ppm males and females were significantly decreased (Table 3 and Figure 3). Feed consumption by 5,000 ppm males and 10,000 ppm males and females was less than that by the controls early in the study but generally recovered to near control values later in the study (Table 3, Table G-2, Table G-3). Exposure concentrations of 625, 1,250, 2,500, 5,000, and 10,000 ppm resulted in average daily doses of approximately 50 (range 31 to 83), 100 (59 to 165), 200 (130 to 318), 380 (247 to 615), and 725 (505 to 1,553) mg *p*-toluenesulfonamide/kg body weight to males and 30 (36 to 76), 110 (68 to 154), 210 (138 to 294), 400 (275 to 555), and 780 (547 to 1,071) mg/kg to females. No clinical observations or histopathologic findings were attributed to *p*-toluenesulfonamide exposure.

Table 3. Survival, Body Weights, and Feed Consumption of F344/NTac Rats in the Three-month Feed Study of *p*-Toluenesulfonamide^a

Concentration (ppm)	Survival ^b	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)	Feed Consumption Week 1 (g)	Feed Consumption Week 14 (g)
Male							
0	10/10	87 ± 3	339 ± 7	252 ± 6		16.2	16.6
625	10/10	87 ± 4	325 ± 5	237 ± 4	96	16.4	16.1
1,250	10/10	89 ± 3	338 ± 6	249 ± 6	100	16.6	16.0
2,500	10/10	89 ± 3	323 ± 7	234 ± 6*	95	15.5	16.9
5,000	10/10	87 ± 3	320 ± 6	233 ± 6*	94	14.1	16.7
10,000	10/10	87 ± 3	315 ± 8*	228 ± 7**	93	13.2	15.9

p-Toluenesulfonamide, NTP TOX 88

Concentration (ppm)	Survival ^b	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)	Feed Consumption Week 1 (g)	Feed Consumption Week 14 (g)
Female							
0	10/10	83 ± 2	190 ± 3	107 ± 3		12.1	10.3
625	10/10	86 ± 2	190 ± 2	104 ± 2	100	12.8	10.8
1,250	10/10	85 ± 2	187 ± 4	103 ± 3	99	13.0	10.2
2,500	10/10	84 ± 2	187 ± 2	102 ± 3	98	12.3	10.3
5,000	10/10	86 ± 2	184 ± 3	98 ± 3*	97	11.6	10.1
10,000	10/10	85 ± 2	174 ± 2**	90 ± 2**	92	10.6	9.6

*Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

**Significantly different ($P \leq 0.01$) from the control group by Williams' test.

^aWeights and weight changes are given as mean ± standard error. Feed consumption is expressed as grams per animal per day.

^bNumber of animals surviving at 14 weeks/number initially in group.

p-Toluenesulfonamide, NTP TOX 88

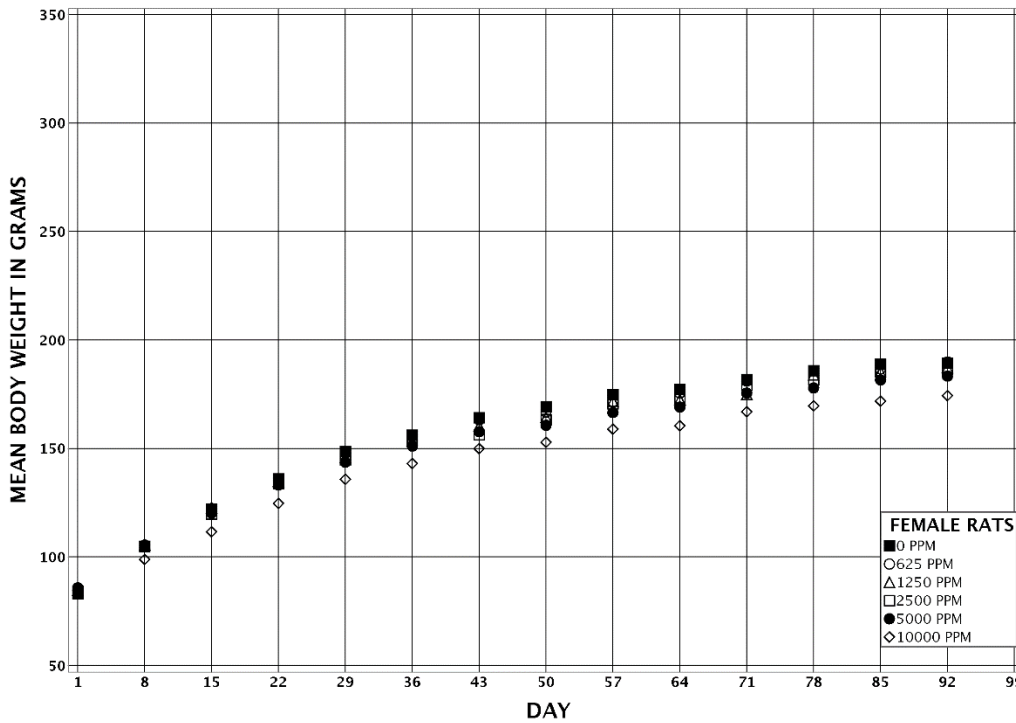
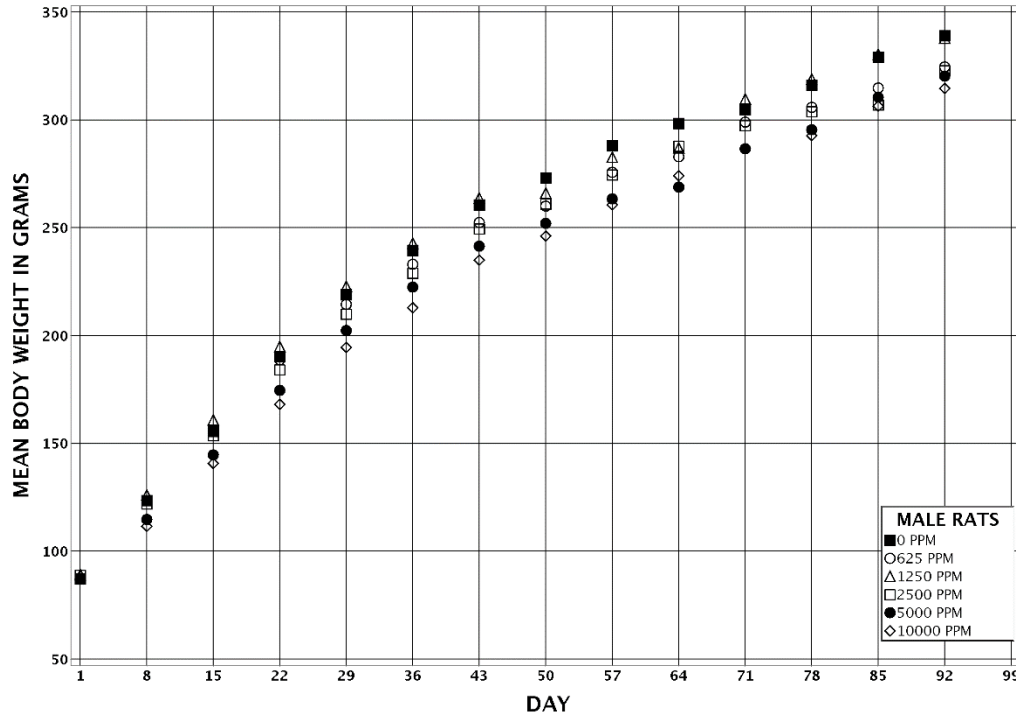


Figure 3. Growth Curves for F344/NTac Rats Exposed to *p*-Toluenesulfonamide in Feed for Three Months

A few scattered changes occurred in the hematology and clinical chemistry data for F344/NTac rats (Table B-1). These changes were minor (exposed groups were often $\leq 5\%$ different from the controls) and mostly sporadic or inconsistent between exposure concentrations, timepoints,

and/or sexes; all would have been considered within biological variability. Thus, no clinical pathology changes detected statistically for male or female F344/NTac rats were considered biologically significant or toxicologically relevant to the administration of *p*-toluenesulfonamide.

Absolute and relative thymus weights of 10,000 ppm males were significantly less by approximately 22% compared to those of the controls (Table 4, Table C-2). Relative kidney weights were increased in 2,500 ppm or greater males, and were up to approximately 14% greater than the controls in the 10,000 ppm group. Corresponding histologic lesions were not observed in the kidney or thymus. The mean absolute heart weight of 10,000 ppm females was decreased by approximately 8%, which was attributed to a similar decrease in mean body weight of that group compared to controls (Table C-2).

Male F344/NTac rats exposed to *p*-toluenesulfonamide did not display any biologically significant changes in epididymis or testis weights, epididymal sperm counts, sperm motility, or testicular spermatid counts (Table D-1). These data indicate that *p*-toluenesulfonamide exposure via dietary administration does not exhibit the potential to be a reproductive toxicant in male F344/NTac rats. Vaginal lavage slide quality precluded assessment of estrous cyclicity and statistical analyses in female F344/NTac rats.

Table 4. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male F344/NTac Rats in the Three-month Feed Study of *p*-Toluenesulfonamide^a

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Necropsy body wt	339 ± 7	325 ± 5	338 ± 6	323 ± 7	320 ± 6	315 ± 8*
R. Kidney						
Absolute	1.05 ± 0.02	1.05 ± 0.01	1.08 ± 0.02	1.10 ± 0.03	1.12 ± 0.03	1.11 ± 0.03
Relative	3.10 ± 0.04	3.25 ± 0.06	3.19 ± 0.05	3.42 ± 0.04**	3.51 ± 0.04**	3.54 ± 0.06**
Thymus						
Absolute	0.303 ± 0.012	0.270 ± 0.012	0.285 ± 0.009	0.267 ± 0.009	0.273 ± 0.009 ^b	0.237 ± 0.014**
Relative	0.893 ± 0.029	0.829 ± 0.029	0.845 ± 0.025	0.834 ± 0.044	0.850 ± 0.021 ^b	0.754 ± 0.042*

*Significantly different ($P \leq 0.05$) from the control group by Dunnett's test.

**Significantly different ($P \leq 0.01$) from the control group by Williams' test.

^aOrgan weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

^bn=9.

Two-week Study in Mice

All mice survived to the end of the study (Table 5). Final mean body weights and mean body weight gains of 30,000 ppm males and females were significantly less than those of the controls; these groups lost weight during the study. The mean body weight gains of all remaining exposed groups of females were significantly less than those of the controls. Feed consumption by exposed groups of mice was generally similar to that by the controls throughout the study (Table 5, Table G-4). Exposure concentrations of 750, 1,500, 3,000, 10,000, and 30,000 ppm resulted in average daily doses of approximately 150, 300, 700, 2,035, and 7,690 mg

p-toluenesulfonamide/kg body weight to males and 125, 280, 635, 2,410, and 6,000 mg/kg to females. No clinical observations or histopathologic findings were attributed to *p*-toluenesulfonamide exposure.

Table 5. Survival, Body Weights, and Feed Consumption of Mice in the Two-week Feed Study of *p*-Toluenesulfonamide^a

Concentration (ppm)	Survival ^b	Initial Body Weight (g)	Final Body Weight(g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)	Feed Consumption Week 1 (g)	Feed Consumption Week 2 (g)
Male							
0	5/5	22.1 ± 0.6	24.6 ± 0.5	2.5 ± 0.3	–	5.1	4.6
750	5/5	22.5 ± 0.7	24.7 ± 0.6	2.2 ± 0.4	100	5.0	4.4
1,500	5/5	22.4 ± 0.5	23.9 ± 0.9	1.5 ± 0.5	97	4.1	5.2
3,000	5/5	22.7 ± 0.5	24.8 ± 0.4	2.1 ± 0.2	101	5.8	5.3
10,000	5/5	22.3 ± 0.4	23.9 ± 0.3	1.5 ± 0.2	97	4.7	4.7
30,000	5/5	22.1 ± 0.6	21.2 ± 0.5**	-0.9 ± 0.2**	86	5.2	5.9
Female							
0	5/5	16.9 ± 0.3	19.6 ± 0.5	2.7 ± 0.2	–	3.5	3.9
750	5/5	17.2 ± 0.3	19.3 ± 0.2	2.1 ± 0.3*	98	3.0	3.1
1,500	5/5	17.3 ± 0.2	18.5 ± 0.2	1.2 ± 0.2**	94	3.3	3.4
3,000	5/5	17.0 ± 0.4	19.0 ± 0.4	2.1 ± 0.2**	97	4.0	3.6
10,000	5/5	17.4 ± 0.4	19.1 ± 0.4	1.6 ± 0.2**	97	5.1	3.7
30,000	5/5	16.9 ± 0.3	16.6 ± 0.4**	-0.3 ± 0.1**	85	3.0	3.7

*Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** $P \leq 0.01$.

^aWeights and weight changes are given as mean ± standard error. Feed consumption is expressed as grams per animal per day.

^bNumber of animals surviving at 15 days/number initially in group.

Absolute kidney weights of 10,000 and 30,000 ppm females were increased by approximately 15% and 8%, respectively, compared to those of the controls (Table C-3). Relative kidney weights were increased in 1,500 ppm or greater females and in 10,000 and 30,000 ppm males compared to those in the controls. The 30,000 ppm males and females had mean body weight decreases of approximately 14% and 15%, respectively, compared to those of the controls. There were no histological findings that correlated with these changes in kidney weights.

Other changes in organ weights included increases in relative heart weights of 10,000 ppm females and 30,000 ppm males and females; an increase in the relative lung weight of 30,000 ppm males; an increase in the relative testis weight of 30,000 ppm males; a decrease in the absolute liver weight of 30,000 ppm females; and decreases in the absolute thymus weights of 30,000 ppm males and females and relative thymus weight of 30,000 ppm males (Table C-3). There were no histologic findings that correlated with these organ weights changes, and they are considered to be primarily related to changes in body weights.

Exposure Concentration Selection Rationale: There were no treatment-related deaths, clinical toxicity, or gross or microscopic findings in male or female B6C3F1/N mice in the 2-week *p*-toluenesulfonamide feed study. The primary finding was decreased body weight at 30,000 ppm relative to controls. Body weights at 30,000 ppm were 14% less for male mice and 15% less for female mice. The doses selected for the 3-month feed studies in mice were 0, 625, 1,250, 2,500, 5,000, and 10,000 ppm.

Three-month Study in Mice

All male mice survived to the end of the study; one 10,000 ppm female mouse died during week 6 of an accidental death (Table 6). The mean body weight gains of 5,000 and 10,000 ppm males were significantly less than those of the controls; the final mean body weight and mean body weight gain of 1,250 ppm females were significantly greater than those of the controls (Table 6 and Figure 4). Feed consumption by 625 and 1,250 ppm males was greater than that by the controls early in the study but returned to near control values later in the study; feed consumption by exposed groups of females was generally similar to that by the controls throughout the study (Table 6, Table G-5, Table G-6). Exposure concentrations of 625, 1,250, 2,500, 5,000, and 10,000 ppm resulted in average daily doses of approximately 120 (range 86 to 169), 230 (158 to 312), 420 (351 to 533), 770 (666 to 1,437), and 1,760 (1,391 to 2,458) mg *p*-toluenesulfonamide/kg body weight to males and 90 (84 to 105), 210 (176 to 246), 380 (313 to 469), 780 (684 to 885), and 1,890 (1,718 to 2,215) mg/kg to females. No clinical observations or histopathologic findings were attributed to *p*-toluenesulfonamide exposure.

Table 6. Survival, Body Weights, and Feed Consumption of Mice in the Three-month Feed Study of *p*-Toluenesulfonamide^a

Concentration (ppm)	Survival ^b	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)	Feed Consumption Week 1 (g)	Feed Consumption Week 14 (g)
Male							
0	10/10	22.2 ± 0.3	32.1 ± 0.7	9.9 ± 0.5		4.3	4.3
625	10/10	22.3 ± 0.4	31.8 ± 0.9	9.6 ± 0.6	99	5.8	4.4
1,250	10/10	22.7 ± 0.4	32.4 ± 1.1	9.7 ± 0.8	101	5.8	4.1
2,500	10/10	22.7 ± 0.4	31.7 ± 0.9	9.0 ± 0.7	99	4.7	4.5
5,000	10/10	22.7 ± 0.4	30.8 ± 0.8	8.1 ± 0.5*	96	4.1	4.1
10,000	10/10	22.4 ± 0.4	30.0 ± 0.7	7.6 ± 0.5*	93	4.8	4.4
Female							
0	10/10	18.4 ± 0.2	24.8 ± 0.5	6.3 ± 0.4		3.4	3.6
625	10/10	18.7 ± 0.2	26.7 ± 0.6	8.1 ± 0.5	108	3.2	3.6
1,250	10/10	18.7 ± 0.2	27.1 ± 0.5*	8.3 ± 0.5*	109	3.8	3.8
2,500	10/10	18.7 ± 0.2	26.4 ± 0.6	7.7 ± 0.5	107	3.6	3.3
5,000	10/10	18.8 ± 0.2	25.4 ± 0.5	6.7 ± 0.6	103	3.4	3.5
10,000	9/10 ^c	18.5 ± 0.3	24.8 ± 0.7	6.2 ± 0.5	100	3.6	4.6

*Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

^aWeights and weight changes are given as mean ± standard error. Feed consumption is expressed as grams per animal per day.

^bNumber of animals surviving at 14 weeks/number initially in group.

^cWeek of death: 6.

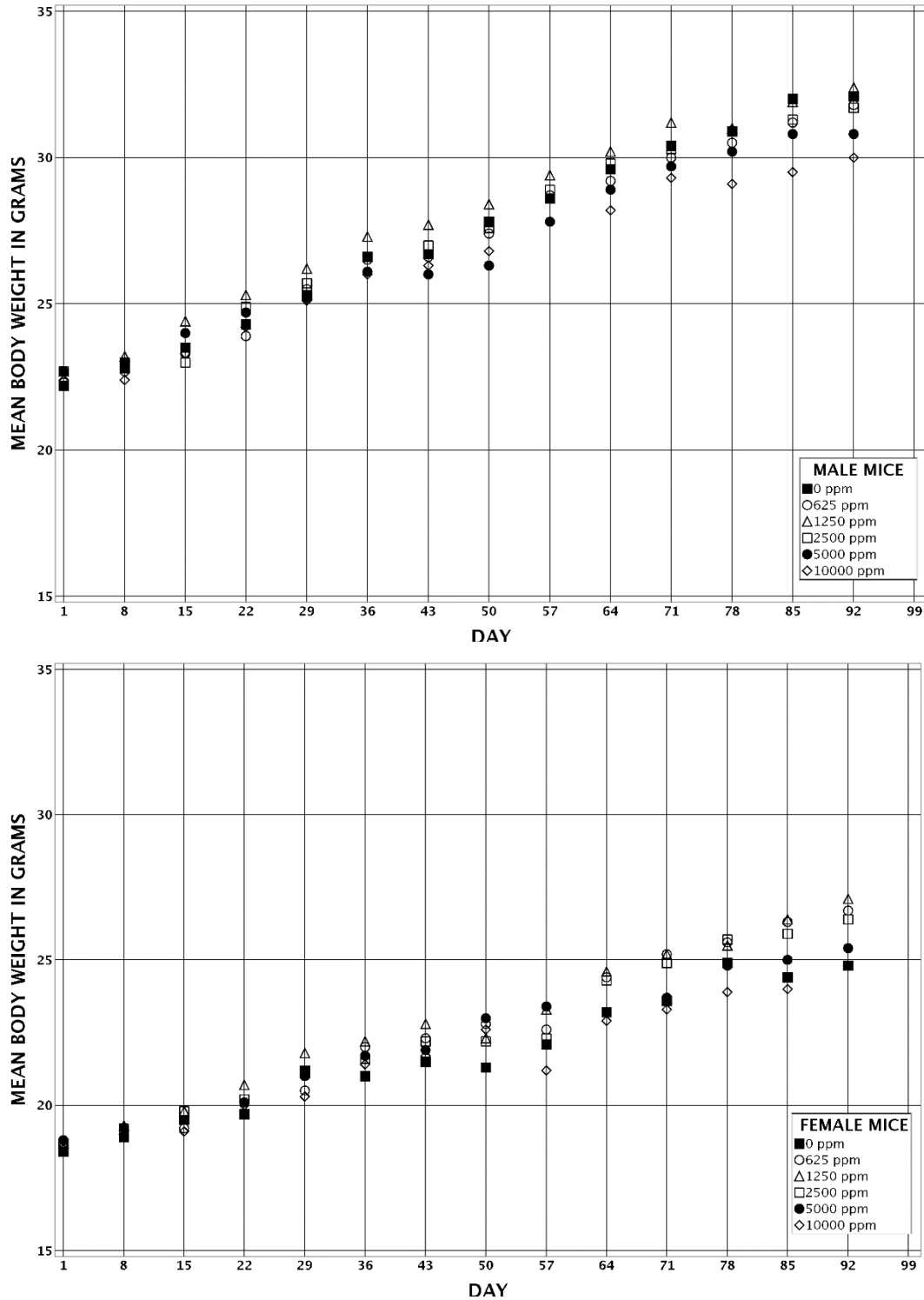


Figure 4. Growth Curves for Mice Exposed to *p*-Toluenesulfonamide in Feed for Three Months

No changes attributable to the administration of *p*-toluenesulfonamide occurred in the hematology data for male or female mice (Table B-2).

Female mice exposed to 10,000 ppm had increased absolute (approximately 13%) and relative (approximately 14%) kidney weights compared to those of the controls (Table 7, Table C-4). Relative lung weight was increased by approximately 27% in 10,000 ppm males, and relative liver weight was increased by approximately 10% in 10,000 ppm females. The mean body weight of 10,000 ppm males was approximately 7% less than that of the control group at necropsy, but there was no difference between 10,000 ppm females and the control group at necropsy. Corresponding changes were not observed histologically. Other changes in organ weights were not considered to be biologically significant.

Table 7. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Feed Study of *p*-Toluenesulfonamide^a

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	32.1 ± 0.7	31.8 ± 0.9	32.4 ± 1.1	31.7 ± 0.9	30.8 ± 0.8	30.0 ± 0.7
Lung						
Absolute	0.21 ± 0.02	0.22 ± 0.02	0.23 ± 0.01	0.20 ± 0.01	0.23 ± 0.02	0.25 ± 0.02
Relative	6.51 ± 0.52	6.80 ± 0.40	7.12 ± 0.32	6.35 ± 0.21	7.45 ± 0.48	8.24 ± 0.51*
Female						
n	10	10	10	10	10	9
Necropsy body wt	24.8 ± 0.5	26.7 ± 0.6	27.1 ± 0.5*	26.4 ± 0.6	25.4 ± 0.5	24.8 ± 0.7
R. Kidney						
Absolute	0.16 ± 0.00	0.16 ± 0.00	0.17 ± 0.00	0.16 ± 0.01	0.17 ± 0.00	0.18 ± 0.01**
Relative	6.42 ± 0.15	6.14 ± 0.12	6.23 ± 0.13	6.08 ± 0.15	6.61 ± 0.12	7.31 ± 0.16**
Liver						
Absolute	1.02 ± 0.02	1.13 ± 0.02*	1.15 ± 0.03**	1.09 ± 0.03	1.07 ± 0.03	1.12 ± 0.04
Relative	41.14 ± 0.87	42.36 ± 0.61	42.61 ± 0.63	41.44 ± 0.92	42.01 ± 0.47	45.15 ± 1.03**

*Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** $P \leq 0.01$.

^aOrgan weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Male mice exposed to *p*-toluenesulfonamide did not display any biologically significant changes in epididymis or testis weights, epididymal sperm counts, sperm motility, or testicular spermatid counts (Table D-2). These data indicate that *p*-toluenesulfonamide exposure via dietary administration does not exhibit the potential to be a reproductive toxicant in male B6C3F1/N mice. Vaginal lavage slide quality precluded assessment of estrous cyclicity and statistical analyses in female B6C3F1/N mice.

Genetic Toxicology

p-Toluenesulfonamide (33 to 10,000 µg/plate, dissolved in acetone) was tested in three strains of *Salmonella typhimurium* (TA98, TA100, and TA102), with and without 10% induced rat liver S9 enzymes; no mutagenicity was observed in any of the three bacterial strains, with or without S9 activation enzymes (Table E-1).

In vivo, no increases in micronucleated reticulocytes (polychromatic erythrocytes) or erythrocytes (normochromatic erythrocytes) were observed in peripheral blood of male or female F344/NTac rats or B6C3F1/N mice from the 3-month studies (Table E-2 and Table E-3). No biologically significant changes in the percentage of reticulocytes among total erythrocytes were seen in either of these micronucleus studies, suggesting that *p*-toluenesulfonamide did not induce bone marrow toxicity. Small but statistically significant ($P < 0.025$) increases in the percentage of reticulocytes were observed in male mice (pairwise test) and male rats (trend test), but the values in each case were within normal ranges, the increases that were observed were quite small, and the increases in percentage of reticulocytes were judged to be equivocal.

Discussion

These *p*-toluenesulfonamide studies were conducted in response to the nomination by the United States Food and Drug Administration (FDA) and a private individual. Toxicity of *p*-toluenesulfonamide was studied because it is formed from chloramine-T and found in fish when chloramine-T is used as an aquaculture disinfectant. Accordingly, there is the potential that *p*-toluenesulfonamide might be consumed when eating fish when chloramine-T is used as a disinfectant.

Aquaculture (fish farming) is the production of fish and shellfish for human consumption, for stocking sport fishing ponds and streams, and for enhancing wild populations of fish^{52; 53}. Wild harvests of fish have reached maximum sustainable yields and the aquaculture industry is one approach to producing additional fish for the food supply. In the United States aquaculture industry, there are about 6,400 farms that have combined annual revenue of 1 billion dollars⁵⁴. Catfish and trout are among the major fish produced by the aquaculture industry⁵⁵.

The United States Fish and Wildlife Service and industry sponsored studies on the use of chloramine-T in combating bacterial disease in fish in the aquaculture industry¹⁰. The FDA recently approved the use of chloramine-T to treat bacterial gill disease in freshwater-reared salmonids, external columnaris disease in walleye, and external columnaris disease in freshwater-reared warm water finfish⁵⁶.

In these NTP studies, when *p*-toluenesulfonamide was administered in the feed at concentrations up to 30,000 ppm for 2 weeks to male and female F344/N rats and B6C3F1/N mice and in the feed at concentrations up to 10,000 ppm for 3 months to male and female F344/NTac rats and B6C3F1/N mice there was no evidence for treatment-related mortality or treatment-related lesions.

In the 2-week studies, at 30,000 ppm there were treatment-related body weight effects (greater than 10% decreases in final mean body weights relative to control body weights) in male and female F344/N rats and mice. There were increased relative kidney weights in male F344/N rats (3,000 ppm or greater), female F344/N rats, and male mice (10,000 and 30,000 ppm), and female mice (1,500 ppm or greater). Decreased absolute organ weights were found in the: heart, liver, lung, and thymus of male F344/N rats; heart and liver of female F344/N rats; thymus of male and female mice; and liver of female mice. Most of these decreases in absolute organ weights occurred primarily at 30,000 ppm.

In the 3-month studies, the final mean body weights of F344/NTac rats exposed to 10,000 ppm were significantly lower than those of the controls; final mean body weights of other exposed groups of F344/NTac rats and all exposed groups of mice were generally similar to those of the controls. There were increased relative kidney weights and decreased absolute and relative thymus weights in male F344/NTac rats; biologically significant organ weight changes did not occur in female F344/NTac rats. In the mouse study, increased relative lung weights occurred in males and increased absolute and relative kidney weights and increased relative liver weights occurred in females. There was no evidence for reproductive organ toxicity in male F344/NTac rats or mice, and there was no evidence of treatment-related lesions in any organ in male or female F344/NTac rats or mice. No treatment-related lesions were seen in this 3-month NTP

study, including no urinary bladder lesions that had been reported previously in two out of 10 male rats at 10,000 ppm in a 90-day study reviewed by the USEPA²⁸.

p-Toluenesulfonamide was not mutagenic in the *Salmonella typhimurium* test nor did it induce micronuclei in reticulocytes or erythrocytes in peripheral blood of male or female F344/NTac rats or B6C3F1/N mice after 3 months of exposure.

Under the conditions of these 3-month feed studies, there were no treatment-related lesions in male or female F344/NTac rats or mice exposed to *p*-toluenesulfonamide in the feed at 625, 1,250, 2,500, 5,000, or 10,000 ppm. The most sensitive measures of *p*-toluenesulfonamide exposure in each species and sex were increased relative kidney weights in male F344/NTac rats [lowest observed effect level (LOEL) 2,500 ppm; 200 mg/kg], decreased body weight in female F344/NTac rats (LOEL 10,000 ppm; 780 mg/kg), increased relative lung weight in male mice (LOEL 10,000 ppm; 1,760 mg/kg), and increased relative liver weight and absolute and relative kidney weights in female mice (LOEL 10,000 ppm; 1,890 mg/kg). It is uncertain if these body weight or organ weight effects would compromise the survival or well-being of the animal after longer exposures.

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Appendix A. Summary of Nonneoplastic Lesions in F344/NTAC Rats and Mice

Tables

Table A-1. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Three-month Feed Study of <i>p</i> -Toluenesulfonamide.....	A-2
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Table A-1. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Three-month Feed Study of *p*-Toluenesulfonamide

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal euthanasia	10	10	10	10	10	10
Animals examined microscopically	10	–	–	–	–	10
Alimentary System						
Esophagus ^a	(10)	–	–	–	–	(10)
Intestine large, cecum	(10)	–	–	–	–	(10)
Intestine large, colon	(10)	–	–	–	–	(10)
Intestine large, rectum	(10)	–	–	–	–	(10)
Intestine small, duodenum	(10)	–	–	–	–	(10)
Intestine small, ileum	(10)	–	–	–	–	(10)
Intestine small, jejunum	(10)	–	–	–	–	(10)
Liver	(10)	–	–	–	–	(10)
Inflammation, chronic ^b	9 (1.0) ^c	–	–	–	–	9 (1.0)
Pancreas	(10)	–	–	–	–	(10)
Salivary glands	(10)	–	–	–	–	(10)
Stomach, forestomach	(10)	–	–	–	–	(10)
Stomach, glandular	(10)	–	–	–	–	(10)
Glands, ectasia, focal	1 (1.0)	–	–	–	–	1 (1.0)
Tongue	(10)	–	–	–	–	(10)
Cardiovascular System						
Blood vessel	(10)	–	–	–	–	(10)
Heart	(10)	–	–	–	–	(10)
Cardiomyopathy	4 (1.0)	–	–	–	–	4 (1.0)
Infiltration cellular, histiocyte, focal	2 (1.0)	–	–	–	–	1 (1.0)
Endocrine System						
Adrenal cortex	(10)	–	–	–	–	(10)
Adrenal medulla	(10)	–	–	–	–	(10)
Parathyroid gland	(10)	–	–	–	–	(9)
Pituitary gland	(10)	–	–	–	–	(10)
Thyroid gland	(10)	–	–	–	–	(10)

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
General Body System						
None	–	–	–	–	–	–
Genital System						
Epididymis	(10)	–	–	–	–	(10)
Preputial gland	(9)	–	–	–	–	(10)
Inflammation, chronic	2 (1.0)	–	–	–	–	–
Prostate	(10)	–	–	–	–	(10)
Seminal vesicle	(10)	–	–	–	–	(10)
Testes	(10)	–	–	–	–	(10)
Atrophy	–	–	–	–	–	1 (2.0)
Metaplasia, cartilagenous, focal	–	–	–	–	–	1 (1.0)
Hematopoietic System						
Bone marrow	(10)	–	–	–	–	(10)
Lymph node, mandibular	(10)	–	–	–	–	(10)
Lymph node, mesenteric	(10)	–	–	–	–	(10)
Infiltration cellular, histiocyte	–	–	–	–	–	1 (3.0)
Spleen	(10)	–	–	–	–	(10)
Thymus	(10)	–	–	–	–	(10)
Integumentary System						
Mammary gland	(7)	–	–	–	–	(9)
Skin	(10)	–	–	–	–	(10)
Musculoskeletal System						
Bone	(10)	–	–	–	–	(10)
Nervous System						
Brain	(10)	–	–	–	–	(10)
Respiratory System						
Lung	(10)	–	–	–	–	(10)
Hemorrhage	1 (1.0)	–	–	–	–	2 (1.0)
Infiltration cellular, histiocyte	1 (1.0)	–	–	–	–	2 (1.0)
Inflammation, chronic active	2 (1.0)	–	–	–	–	3 (1.7)
Nose	(10)	–	–	–	–	(10)
Pleura	(1)	–	–	–	–	–
Trachea	(10)	–	–	–	–	(10)

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Special Senses System						
Eye	(10)	–	–	–	–	(10)
Harderian gland	(10)	–	–	–	–	(10)
Urinary System						
Kidney	(10)	–	–	–	–	(10)
Inflammation	1 (1.0)	–	–	–	–	–
Nephropathy	4 (1.3)	–	–	–	–	4 (1.0)
Urinary bladder	(10)	–	–	–	–	(10)

^aNumber of animals with tissue examined microscopically.

^bNumber of animals with lesion.

^cAverage severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Table A-2. Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Three-month Feed Study of *p*-Toluenesulfonamide

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal euthanasia	10	10	10	10	10	10
Animals examined microscopically	10	–	–	–	–	10
Alimentary System						
Esophagus ^a	(10)	–	–	–	–	(10)
Intestine large, cecum	(10)	–	–	–	–	(10)
Intestine large, colon	(10)	–	–	–	–	(10)
Intestine large, rectum	(10)	–	–	–	–	(10)
Intestine small, duodenum	(10)	–	–	–	–	(10)
Intestine small, ileum	(10)	–	–	–	–	(10)
Intestine small, jejunum	(10)	–	–	–	–	(10)
Liver	(10)	–	–	–	–	(10)
Hepatodiaphragmatic nodule ^b	–	–	–	–	–	1
Inflammation, chronic	10 (1.2) ^c	–	–	–	–	9 (1.0)
Pancreas	(10)	–	–	–	–	(10)
Inflammation, chronic	–	–	–	–	–	1 (1.0)
Acinus, atrophy	1 (1.0)	–	–	–	–	1 (1.0)
Salivary glands	(10)	–	–	–	–	(10)
Stomach, forestomach	(10)	–	–	–	–	(10)
Stomach, glandular	(10)	–	–	–	–	(10)
Hyperplasia, basal cell	1 (1.0)	–	–	–	–	–
Tongue	(10)	–	–	–	–	(10)
Cardiovascular System						
Blood vessel	(10)	–	–	–	–	(10)
Heart	(10)	–	–	–	–	(10)
Cardiomyopathy	2 (1.0)	–	–	–	–	1 (1.0)
Endocrine System						
Adrenal cortex	(10)	–	–	–	–	(10)
Adrenal medulla	(10)	–	–	–	–	(10)
Parathyroid gland	(10)	–	–	–	–	(9)
Pituitary gland	(10)	–	–	–	–	(10)
Thyroid gland	(10)	–	–	–	–	(10)
General Body System						
None	–	–	–	–	–	–

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Genital System						
Clitoral gland	(10)	–	–	–	–	(10)
Inflammation, chronic	1 (1.0)	–	–	–	–	–
Ovary	(10)	–	–	–	–	(10)
Cyst, focal	1 (3.0)	–	–	–	–	–
Uterus	(10)	–	–	–	–	(10)
Bilateral, hydrometra	2 (2.0)	–	–	–	–	–
Hematopoietic System						
Bone marrow	(10)	–	–	–	–	(10)
Lymph node, mandibular	(10)	–	–	–	–	(10)
Lymph node, mesenteric	(10)	–	–	–	–	(10)
Spleen	(10)	–	–	–	–	(10)
Ectopic tissue	1	–	–	–	–	–
Thymus	(10)	–	–	–	–	(10)
Integumentary System						
Mammary gland	(10)	–	–	–	–	(10)
Skin	(10)	–	–	–	–	(10)
Musculoskeletal System						
Bone	(10)	–	–	–	–	(10)
Nervous System						
Brain	(10)	–	–	–	–	(10)
Respiratory System						
Lung	(10)	–	–	–	–	(10)
Infiltration cellular, histiocyte	1 (1.0)	–	–	–	–	1 (1.0)
Inflammation, chronic active	1 (1.0)	–	–	–	–	1 (2.0)
Nose	(10)	–	–	–	–	(10)
Trachea	(10)	–	–	–	–	(10)
Special Senses System						
Eye	(10)	–	–	–	–	(10)
Harderian gland	(10)	–	–	–	–	(10)
Inflammation, chronic	1 (2.0)	–	–	–	–	1 (1.0)
Urinary System						
Kidney	(10)	–	–	–	–	(10)
Nephropathy	1 (1.0)	–	–	–	–	–
Pelvis, dilatation, focal	1 (2.0)	–	–	–	–	–
Urinary bladder	(10)	–	–	–	–	(10)

^aNumber of animals with tissue examined microscopically.

^bNumber of animals with lesion.

^cAverage severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Table A-3. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Three-month Feed Study of *p*-Toluenesulfonamide

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal euthanasia	10	10	10	10	10	10
Animals examined microscopically	10	–	–	–	–	10
Alimentary System						
Esophagus ^a	(10)	–	–	–	–	(10)
Gallbladder	(9)	–	–	–	–	(10)
Intestine large, cecum	(10)	–	–	–	–	(10)
Intestine large, colon	(10)	–	–	–	–	(10)
Intestine large, rectum	(10)	–	–	–	–	(10)
Intestine small, duodenum	(10)	–	–	–	–	(10)
Intestine small, ileum	(10)	–	–	–	–	(10)
Intestine small, jejunum	(10)	–	–	–	–	(10)
Liver	(10)	–	–	–	–	(10)
Inflammation, chronic ^b	3 (1.0) ^c	–	–	–	–	3 (1.0)
Oral mucosa	(10)	–	–	–	–	(10)
Pancreas	(10)	–	–	–	–	(10)
Salivary glands	(10)	–	–	–	–	(10)
Stomach, forestomach	(10)	–	–	–	–	(10)
Stomach, glandular	(10)	–	–	–	–	(10)
Tongue	(10)	–	–	–	–	(10)
Cardiovascular System						
Blood vessel	(9)	–	–	–	–	(10)
Heart	(10)	–	–	–	–	(10)
Endocrine System						
Adrenal cortex	(10)	–	–	–	–	(10)
Subcapsular, hyperplasia	1 (1.0)	–	–	–	–	
Adrenal medulla	(10)	–	–	–	–	(10)
Parathyroid gland	(5)	–	–	–	–	(9)
Pituitary gland	(10)	–	–	–	–	(10)
Thyroid gland	(10)	–	–	–	–	(10)
Cyst	1	–	–	–	–	

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
General Body System						
None	–	–	–	–	–	–
Genital System						
Epididymis	(10)	–	–	–	–	(10)
Preputial gland	(10)	–	–	–	–	(10)
Prostate	(10)	–	–	–	–	(10)
Seminal vesicle	(10)	–	–	–	–	(10)
Testes	(10)	–	–	–	–	(10)
Hematopoietic System						
Bone marrow	(10)	–	–	–	–	(10)
Lymph node, mandibular	(10)	–	–	–	–	(10)
Lymph node, mesenteric	(10)	–	–	–	–	(10)
Spleen	(10)	–	–	–	–	(10)
Thymus	(10)	–	–	–	–	(10)
Integumentary System						
Mammary gland	(4)	–	–	–	–	(5)
Skin	(10)	–	–	–	–	(10)
Musculoskeletal System						
Bone	(10)	–	–	–	–	(10)
Nervous System						
Brain	(10)	–	–	–	–	(10)
Respiratory System						
Larynx	(10)	–	–	–	–	(10)
Lung	(10)	–	–	–	–	(10)
Nose	(10)	–	–	–	–	(10)
Trachea	(10)	–	–	–	–	(10)
Special Senses System						
Eye	(10)	–	–	–	–	(10)
Retrolbulbar, inflammation, suppurative	3 (1.3)	–	–	–	–	5 (1.4)
Harderian gland	(10)	–	–	–	–	(10)
Inflammation, suppurative	1 (2.0)	–	–	–	–	1 (2.0)

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Urinary System						
Kidney	(10)	–	–	–	–	(10)
Inflammation, chronic	2 (1.0)	–	–	–	–	–
Nephropathy	1 (1.0)	–	–	–	–	–
Urinary bladder	(10)	–	–	–	–	(10)

^aNumber of animals with tissue examined microscopically.

^bNumber of animals with lesion.

^cAverage severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Table A-4. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Three-month Feed Study of *p*-Toluenesulfonamide

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental death	–	–	–	–	–	1
Survivors						
Terminal euthanasia	10	10	10	10	10	9
Animals examined microscopically	10	–	–	–	–	10
Alimentary System						
Esophagus ^a	(10)	–	–	–	–	(10)
Gallbladder	(10)	–	–	–	–	(9)
Intestine large, cecum	(10)	–	–	–	–	(9)
Intestine large, colon	(10)	–	–	–	–	(9)
Intestine large, rectum	(10)	–	–	–	–	(9)
Intestine small, duodenum	(10)	–	–	–	–	(9)
Intestine small, ileum	(10)	–	–	–	–	(9)
Intestine small, jejunum	(10)	–	–	–	–	(9)
Liver	(10)	–	–	–	–	(9)
Inflammation, chronic ^b	2 (1.0) ^c	–	–	–	–	4 (1.0)
Necrosis	1(1.0)	–	–	–	–	–
Oral mucosa	(10)	–	–	–	–	(10)
Pancreas	(10)	–	–	–	–	(9)
Salivary glands	(10)	–	–	–	–	(10)
Stomach, forestomach	(10)	–	–	–	–	(9)
Stomach, glandular	(10)	–	–	–	–	(9)
Tongue	(10)	–	–	–	–	(10)
Cardiovascular System						
Blood vessel	(10)	–	–	–	–	(10)
Heart	(10)	–	–	–	–	(10)
Endocrine System						
Adrenal cortex	(10)	–	–	–	–	(10)
Subcapsular, hyperplasia	5 (1.8)	–	–	–	–	6 (1.8)
Adrenal medulla	(10)	–	–	–	–	(10)
Parathyroid gland	(10)	–	–	–	–	(10)

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Pituitary gland	(10)	–	–	–	–	(10)
Thyroid gland	(10)	–	–	–	–	(10)
General Body System						
None	–	–	–	–	–	–
Genital System						
Clitoral gland	(1)	–	–	–	–	(1)
Ovary	(10)	–	–	–	–	(9)
Inflammation, granulomatous, focal	–	–	–	–	–	1 (2.0)
Uterus	(10)	–	–	–	–	(9)
Hematopoietic System						
Bone marrow	(10)	–	–	–	–	(10)
Lymph node, mandibular	(10)	–	–	–	–	(10)
Lymph node, mesenteric	(10)	–	–	–	–	(8)
Spleen	(10)	–	–	–	–	(9)
Thymus	(10)	–	–	–	–	(10)
Integumentary System						
Mammary gland	(10)	–	–	–	–	(10)
Skin	(10)	–	–	–	–	(10)
Musculoskeletal System						
Bone	(10)	–	–	–	–	(10)
Nervous System						
Brain	(10)	–	–	–	–	(10)
Respiratory System						
Larynx	(10)	–	–	–	–	(10)
Lung	(10)	–	–	–	–	(10)
Hemorrhage	1 (3.0)	–	–	–	–	–
Arteriole, inflammation, focal, acute	–	–	–	–	–	1 (2.0)
Nose	(10)	–	–	–	–	(10)
Trachea	(10)	–	–	–	–	(10)
Special Senses System						
Eye	(10)	–	–	–	–	(10)
Retina, dysplasia	–	–	–	–	–	1 (2.0)
Retrolbulbar, inflammation, suppurative	1 (1.0)	–	–	–	–	1 (2.0)

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Harderian gland	(10)	–	–	–	–	(10)
Inflammation, suppurative	–	–	–	–	–	1 (2.0)
Inflammation, chronic	–	–	–	–	–	1 (1.0)
Urinary System						
Kidney	(10)	–	–	–	–	(10)
Nephropathy	2 (1.0)	–	–	–	–	–
Urinary bladder	(10)	–	–	–	–	(9)
Inflammation, chronic active	–	–	–	–	–	1 (3.0)

^aNumber of animals with tissue examined microscopically.

^bNumber of animals with lesion.

^cAverage severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Appendix B. Clinical Pathology Results

Tables

Table B-1. Hematology and Clinical Chemistry Data for F344/NTac Rats in the Three-month Feed Study of <i>p</i> -Toluenesulfonamide	B-2
Table B-2. Hematology Data for Mice in the Three-month Feed Study of <i>p</i> -Toluenesulfonamide	B-10

Table B-1. Hematology and Clinical Chemistry Data for F344/NTac Rats in the Three-month Feed Study of *p*-Toluenesulfonamide^a

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 22	10	9	10	10	10	10
Week 14	10	10	10	9	10	10
Hematocrit (auto) (%)						
Day 3	39.7 ± 0.9	40.8 ± 0.7	40.2 ± 0.7	40.4 ± 0.5	40.9 ± 0.7	40.7 ± 0.7
Day 22	46.5 ± 0.7	48.7 ± 1.8	47.6 ± 0.6	45.6 ± 0.8	45.4 ± 1.0	45.4 ± 0.7
Week 14	44.9 ± 0.4	45.7 ± 0.5	45.6 ± 0.4	44.9 ± 0.4	45.0 ± 0.4	44.2 ± 0.3
Manual hematocrit (%)						
Day 3	49.4 ± 0.6	50.4 ± 0.8	49.7 ± 0.8	50.5 ± 0.7	49.7 ± 0.4	50.7 ± 0.9
Day 22	48.7 ± 0.4	50.0 ± 1.9	49.7 ± 0.7	50.2 ± 1.1	48.7 ± 0.7	47.3 ± 0.4
Week 14	48.9 ± 0.4	50.1 ± 0.4	49.2 ± 0.5	48.7 ± 0.3	49.1 ± 0.3	48.5 ± 0.3
Hemoglobin (g/dL)						
Day 3	13.3 ± 0.3	13.6 ± 0.2	13.5 ± 0.2	13.6 ± 0.1	13.7 ± 0.2	13.8 ± 0.2
Day 22	15.5 ± 0.2	16.2 ± 0.6	15.8 ± 0.2	15.2 ± 0.2	15.1 ± 0.3	15.2 ± 0.2
Week 14	15.1 ± 0.1	15.3 ± 0.2	15.3 ± 0.1	15.0 ± 0.1	15.0 ± 0.1	14.9 ± 0.1
Erythrocytes (10⁶/μL)						
Day 3	7.03 ± 0.16	7.11 ± 0.13	7.15 ± 0.16	7.09 ± 0.10	7.29 ± 0.13	7.29 ± 0.14
Day 22	8.40 ± 0.14	8.72 ± 0.29	8.55 ± 0.12	8.18 ± 0.15	8.24 ± 0.15	8.29 ± 0.13
Week 14	9.16 ± 0.08	9.23 ± 0.08	9.11 ± 0.09	9.20 ± 0.07	9.12 ± 0.09	8.97 ± 0.05
Erythrocyte distribution width (%)						
Day 3	14.91 ± 0.31	14.98 ± 0.42	15.40 ± 0.35	14.67 ± 0.30	15.49 ± 0.28	14.41 ± 0.39
Day 22	13.19 ± 0.12	13.26 ± 0.16	12.80 ± 0.11	13.28 ± 0.19	13.30 ± 0.06	12.91 ± 0.14
Week 14	13.75 ± 0.16	13.57 ± 0.14	13.81 ± 0.13	13.61 ± 0.15	14.04 ± 0.12	13.44 ± 0.14
Reticulocytes (10³/μL)						
Day 3	211 ± 6	230 ± 10	239 ± 10	229 ± 9	234 ± 10 ^b	214 ± 9
Day 22	349 ± 14	370 ± 30	370 ± 9	362 ± 8	352 ± 16	360 ± 17
Week 14	266 ± 15	275 ± 14	293 ± 9	270 ± 12	293 ± 13	265 ± 15
Reticulocytes (%)						
Day 3	3.00 ± 0.07	3.23 ± 0.11	3.34 ± 0.11	3.23 ± 0.12	3.21 ± 0.08 ^b	2.93 ± 0.11
Day 22	4.17 ± 0.19	4.20 ± 0.21	4.34 ± 0.13	4.43 ± 0.12	4.27 ± 0.15	4.35 ± 0.23
Week 14	2.91 ± 0.17	2.98 ± 0.17	3.21 ± 0.09	2.93 ± 0.12	3.21 ± 0.13	2.96 ± 0.17

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Nucleated erythrocytes/100 leukocytes						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)						
Day 3	56.6 ± 0.3	57.4 ± 0.2	56.3 ± 0.3	57.1 ± 0.2	56.2 ± 0.3	55.9 ± 0.4
Day 22	55.3 ± 0.2	55.8 ± 0.4	55.9 ± 0.2	55.8 ± 0.2	55.1 ± 0.3	54.7 ± 0.3
Week 14	49.2 ± 0.3	49.4 ± 0.3	49.9 ± 0.5	48.8 ± 0.2	49.4 ± 0.2	49.3 ± 0.2
Mean cell hemoglobin (pg)						
Day 3	18.9 ± 0.1	19.2 ± 0.1	18.9 ± 0.1	19.2 ± 0.1	18.8 ± 0.2	18.9 ± 0.1
Day 22	18.4 ± 0.1	18.5 ± 0.1	18.6 ± 0.1	18.5 ± 0.1	18.3 ± 0.1	18.3 ± 0.1
Week 14	16.4 ± 0.1	16.6 ± 0.1	16.8 ± 0.2	16.3 ± 0.1	16.5 ± 0.0	16.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	33.5 ± 0.1	33.4 ± 0.1	33.5 ± 0.1	33.6 ± 0.1	33.4 ± 0.1	33.8 ± 0.1
Day 22	33.3 ± 0.1	33.2 ± 0.1	33.3 ± 0.1	33.3 ± 0.1	33.3 ± 0.1	33.4 ± 0.1
Week 14	33.5 ± 0.1	33.6 ± 0.1	33.5 ± 0.1	33.4 ± 0.1	33.4 ± 0.1	33.6 ± 0.1
Platelets (10 ³ /μL)						
Day 3	798.0 ± 23.0	781.0 ± 16.0	827.0 ± 21.0	814.0 ± 23.0	862.0 ± 24.0	839.0 ± 16.0
Day 22	780.0 ± 11.0	746.0 ± 24.0	769.0 ± 16.0	777.0 ± 9.0	754.0 ± 11.0	692.0 ± 29.0**
Week 14	594.0 ± 15.0	609.0 ± 12.0	600.0 ± 8.0	594.0 ± 21.0	598.0 ± 17.0	599.0 ± 14.0
Mean platelet volume (μm ³)						
Day 3	6.360 ± 0.050	6.390 ± 0.066	6.370 ± 0.058	6.440 ± 0.058	6.370 ± 0.021	6.430 ± 0.070
Day 22	6.430 ± 0.063	6.489 ± 0.093	6.440 ± 0.050	6.210 ± 0.055	6.410 ± 0.091	6.400 ± 0.116
Week 14	6.430 ± 0.072	6.360 ± 0.048	6.380 ± 0.059	6.411 ± 0.026	6.380 ± 0.066	6.330 ± 0.054
Leukocytes (10 ³ /μL)						
Day 3	7.71 ± 0.42	8.11 ± 0.48	7.64 ± 0.40	8.05 ± 0.44	8.08 ± 0.30	7.76 ± 0.32
Day 22	11.87 ± 0.35	10.82 ± 0.41	12.02 ± 0.49	12.35 ± 0.48	11.21 ± 0.36	11.50 ± 0.39
Week 14	10.45 ± 0.94	11.57 ± 0.61	9.94 ± 0.46	10.77 ± 0.44	10.95 ± 0.62	10.51 ± 0.79
Segmented neutrophils (10 ³ /μL)						
Day 3	1.84 ± 0.15	1.61 ± 0.12	1.58 ± 0.08	1.63 ± 0.08	1.53 ± 0.07	1.36 ± 0.05**
Day 22	2.57 ± 0.10	2.04 ± 0.15	2.34 ± 0.19	2.33 ± 0.11	2.09 ± 0.10*	2.21 ± 0.14
Week 14	2.46 ± 0.28	2.54 ± 0.18	2.52 ± 0.16	2.28 ± 0.12	2.46 ± 0.24	2.47 ± 0.19
Bands (10 ³ /μL)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Metamyelocyte (10³/μL)						
Day 3	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 22	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Myelocyte (10³/μL)						
Day 3	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 22	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lymphocytes (10³/μL)						
Day 3	5.49 ± 0.23	6.13 ± 0.35	5.74 ± 0.32	6.07 ± 0.35	6.19 ± 0.21	6.05 ± 0.25
Day 22	8.73 ± 0.26	8.24 ± 0.29	9.10 ± 0.37	9.42 ± 0.37	8.56 ± 0.33	8.66 ± 0.26
Week 14	7.45 ± 0.60	8.41 ± 0.40	6.84 ± 0.34	7.93 ± 0.32	7.89 ± 0.44	7.38 ± 0.55
Atypical lymphocytes (10³/μL)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10³/μL)						
Day 3	0.21 ± 0.05	0.21 ± 0.01	0.17 ± 0.02	0.20 ± 0.02	0.21 ± 0.02	0.20 ± 0.02
Day 22	0.28 ± 0.02	0.25 ± 0.01	0.30 ± 0.02	0.31 ± 0.03	0.27 ± 0.01	0.31 ± 0.01
Week 14	0.26 ± 0.05	0.33 ± 0.04	0.32 ± 0.03	0.30 ± 0.03	0.32 ± 0.03	0.33 ± 0.04
Basophils (10³/μL)						
Day 3	0.131 ± 0.018	0.135 ± 0.009	0.117 ± 0.013	0.119 ± 0.013	0.124 ± 0.010	0.122 ± 0.011
Day 22	0.234 ± 0.014	0.240 ± 0.023	0.236 ± 0.019	0.247 ± 0.025	0.232 ± 0.010	0.264 ± 0.032
Week 14	0.186 ± 0.029	0.212 ± 0.024	0.183 ± 0.016	0.189 ± 0.010	0.200 ± 0.019	0.236 ± 0.041
Eosinophils (10³/μL)						
Day 3	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01
Day 22	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
Week 14	0.10 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.02
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	13.9 ± 0.4	13.2 ± 0.7	13.3 ± 0.8	12.7 ± 0.4	11.0 ± 0.3**	11.6 ± 0.5**
Day 22	12.5 ± 0.3	12.2 ± 0.5	12.2 ± 0.3	10.6 ± 0.4*	11.3 ± 0.4	13.0 ± 0.5
Week 14	20.4 ± 0.5	18.7 ± 0.6	20.3 ± 0.7	18.3 ± 0.5*	17.0 ± 0.6**	18.3 ± 0.6**

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Creatinine (mg/dL)						
Day 3	0.60 ± 0.01	0.59 ± 0.01	0.60 ± 0.00	0.60 ± 0.00	0.60 ± 0.00	0.63 ± 0.02
Day 22	0.61 ± 0.01	0.61 ± 0.01	0.62 ± 0.01	0.61 ± 0.01	0.60 ± 0.00	0.60 ± 0.00
Week 14	0.87 ± 0.02	0.84 ± 0.02	0.87 ± 0.02	0.84 ± 0.02	0.81 ± 0.01*	0.87 ± 0.02
Total protein (g/dL)						
Day 3	5.3 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	5.3 ± 0.0	5.3 ± 0.1	5.5 ± 0.1
Day 22	6.2 ± 0.1	6.1 ± 0.1	6.2 ± 0.0	6.0 ± 0.1*	6.0 ± 0.1*	5.9 ± 0.1**
Week 14	7.5 ± 0.1	7.7 ± 0.1	7.5 ± 0.1	7.6 ± 0.1	7.5 ± 0.1	7.6 ± 0.1
Albumin (g/dL)						
Day 3	3.4 ± 0.0	3.4 ± 0.0	3.5 ± 0.1	3.4 ± 0.0	3.4 ± 0.0	3.5 ± 0.0
Day 22	3.7 ± 0.0	3.6 ± 0.0	3.7 ± 0.0	3.6 ± 0.0	3.6 ± 0.0*	3.5 ± 0.1*
Week 14	4.0 ± 0.0	4.0 ± 0.1	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.1 ± 0.0
Alanine aminotransferase (IU/L)						
Day 3	81 ± 3	74 ± 2	80 ± 2	75 ± 2	75 ± 3	77 ± 3
Day 22	67 ± 1	62 ± 2*	61 ± 1**	60 ± 1**	60 ± 2**	59 ± 1**
Week 14	110 ± 9	90 ± 5	93 ± 6	83 ± 5*	68 ± 3**	66 ± 2**
Alkaline phosphatase (IU/L)						
Day 3	839 ± 18	840 ± 15	857 ± 25	861 ± 13	892 ± 11*	891 ± 14*
Day 22	605 ± 15	613 ± 21	626 ± 13	633 ± 13	632 ± 12	638 ± 10
Week 14	305 ± 8	329 ± 8*	341 ± 10**	326 ± 8*	333 ± 9*	347 ± 7**
Creatine kinase (IU/L)						
Day 3	470 ± 54	436 ± 57	466 ± 58	455 ± 36	407 ± 35	537 ± 85
Day 22	232 ± 31	317 ± 63	234 ± 38	276 ± 36	243 ± 35	240 ± 40
Week 14	553 ± 76	701 ± 95	624 ± 76	941 ± 211	653 ± 88	826 ± 133
Sorbitol dehydrogenase (IU/L)						
Day 3	35 ± 2	39 ± 2	36 ± 2	38 ± 1	37 ± 1	32 ± 3
Day 22	31 ± 2	28 ± 3	30 ± 2	28 ± 1	29 ± 2	27 ± 2
Week 14	85 ± 9	69 ± 8	78 ± 8	63 ± 8*	60 ± 9*	60 ± 8*
Bile acids (µmol/L)						
Day 3	51.0 ± 5.2	56.7 ± 6.7	57.5 ± 8.2	46.4 ± 7.5	70.9 ± 4.7	54.5 ± 7.0
Day 22	29.8 ± 5.6	26.2 ± 3.5	37.1 ± 4.4	44.3 ± 9.5	44.5 ± 3.2	28.7 ± 4.0
Week 14	14.0 ± 2.2	12.9 ± 2.1	12.1 ± 1.2	11.6 ± 1.0	22.7 ± 3.2*	22.0 ± 2.9*

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 22	10	10	10	10	10	10
Week 14	10	10	10	9	10	10
Hematocrit (auto) (%)						
Day 3	42.3 ± 0.4	42.1 ± 0.8	42.5 ± 0.7	42.8 ± 0.6	43.1 ± 0.6	42.4 ± 0.9
Day 22	46.2 ± 0.6	46.3 ± 0.5	46.8 ± 0.4	45.2 ± 1.0	46.2 ± 0.5	47.4 ± 0.5
Week 14	44.2 ± 0.3	42.2 ± 2.2	44.1 ± 0.4	45.0 ± 0.4	43.5 ± 0.4	42.3 ± 0.6*
Manual hematocrit (%)						
Day 3	52.6 ± 0.5	51.9 ± 0.6	54.3 ± 1.1	53.6 ± 0.8	53.2 ± 0.6	53.5 ± 0.8
Day 22	50.9 ± 0.9	50.8 ± 0.5	51.2 ± 0.6	50.6 ± 0.6	50.7 ± 0.7	51.7 ± 0.4
Week 14	49.2 ± 0.4	49.7 ± 0.3	49.1 ± 0.3	49.9 ± 0.4	48.9 ± 0.4	47.6 ± 0.7
Hemoglobin (g/dL)						
Day 3	14.5 ± 0.1	14.4 ± 0.2	14.6 ± 0.2	14.7 ± 0.2	14.7 ± 0.2	14.7 ± 0.3
Day 22	16.2 ± 0.2	16.1 ± 0.2	16.4 ± 0.1	15.8 ± 0.3	16.1 ± 0.2	16.4 ± 0.1
Week 14	15.3 ± 0.1	14.7 ± 0.7	15.3 ± 0.1	15.5 ± 0.1	15.1 ± 0.1	14.6 ± 0.2**
Erythrocytes (10⁶/μL)						
Day 3	7.50 ± 0.07	7.62 ± 0.16	7.57 ± 0.13	7.65 ± 0.13	7.68 ± 0.12	7.64 ± 0.15
Day 22	8.38 ± 0.13	8.49 ± 0.08	8.50 ± 0.07	8.22 ± 0.16	8.40 ± 0.09	8.75 ± 0.10*
Week 14	8.74 ± 0.05	8.29 ± 0.41	8.62 ± 0.06	8.83 ± 0.09	8.53 ± 0.09	8.37 ± 0.13*
Erythrocyte distribution width (%)						
Day 3	13.71 ± 0.20	14.67 ± 0.49	13.99 ± 0.35	14.31 ± 0.40	14.09 ± 0.30	13.91 ± 0.37
Day 22	11.97 ± 0.12	11.96 ± 0.12	11.99 ± 0.20	12.10 ± 0.15	11.99 ± 0.18	12.00 ± 0.14
Week 14	11.55 ± 0.07	11.42 ± 0.15	11.51 ± 0.09	11.47 ± 0.10	11.38 ± 0.06	11.43 ± 0.13
Reticulocytes (10³/μL)						
Day 3	326 ± 12	330 ± 13	324 ± 10	334 ± 9	334 ± 10	335 ± 12
Day 22	176 ± 16	189 ± 12	183 ± 23	188 ± 15	192 ± 18	218 ± 25
Week 14	249 ± 17	223 ± 15	218 ± 8	243 ± 6	269 ± 15	240 ± 14
Reticulocytes (%)						
Day 3	4.33 ± 0.13	4.32 ± 0.13	4.28 ± 0.10	4.37 ± 0.13	4.34 ± 0.08	4.37 ± 0.11
Day 22	2.09 ± 0.17	2.22 ± 0.13	2.16 ± 0.28	2.31 ± 0.22	2.29 ± 0.22	2.48 ± 0.26
Week 14	2.85 ± 0.19	2.70 ± 0.14	2.53 ± 0.10	2.76 ± 0.07	3.15 ± 0.16	2.87 ± 0.17

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Nucleated erythrocytes/100 leukocytes						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)						
Day 3	56.2 ± 0.3	55.3 ± 0.3	56.2 ± 0.3	55.9 ± 0.2	56.0 ± 0.3	55.5 ± 0.3
Day 22	55.3 ± 0.2	54.4 ± 0.3	55.1 ± 0.3	55.0 ± 0.2	54.9 ± 0.3	54.2 ± 0.2*
Week 14	50.6 ± 0.2	50.8 ± 0.2	51.1 ± 0.2	51.0 ± 0.2	51.1 ± 0.1	50.4 ± 0.2
Mean cell hemoglobin (pg)						
Day 3	19.4 ± 0.1	18.9 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.2 ± 0.1
Day 22	19.3 ± 0.1	19.0 ± 0.1	19.3 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	18.8 ± 0.1*
Week 14	17.5 ± 0.1	17.7 ± 0.1	17.7 ± 0.0*	17.6 ± 0.1	17.7 ± 0.1	17.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	34.4 ± 0.1	34.3 ± 0.1	34.3 ± 0.1	34.4 ± 0.1	34.2 ± 0.1	34.6 ± 0.1
Day 22	35.0 ± 0.1	34.8 ± 0.1	35.0 ± 0.1	34.9 ± 0.2	34.9 ± 0.1	34.7 ± 0.1
Week 14	34.6 ± 0.1	34.8 ± 0.1	34.7 ± 0.1	34.5 ± 0.1	34.7 ± 0.1	34.5 ± 0.1
Platelets (10 ³ /μL)						
Day 3	695.0 ± 13.0	725.0 ± 14.0	708.0 ± 21.0	761.0 ± 16.0*	733.0 ± 16.0*	780.0 ± 20.0**
Day 22	666.0 ± 20.0	701.0 ± 16.0	679.0 ± 14.0	644.0 ± 21.0	674.0 ± 12.0	597.0 ± 20.0
Week 14	638.0 ± 11.0	592.0 ± 27.0	625.0 ± 18.0	605.0 ± 17.0	652.0 ± 10.0	654.0 ± 10.0
Mean platelet volume (μm ³)						
Day 3	6.220 ± 0.042	6.380 ± 0.084	6.300 ± 0.068	6.400 ± 0.115	6.290 ± 0.069	6.320 ± 0.080
Day 22	6.160 ± 0.048	6.370 ± 0.122	6.320 ± 0.051	6.310 ± 0.111	6.150 ± 0.064	6.230 ± 0.101
Week 14	6.820 ± 0.083	7.070 ± 0.110	6.820 ± 0.149	6.789 ± 0.181	6.940 ± 0.139	6.790 ± 0.147
Leukocytes (10 ³ /μL)						
Day 3	10.25 ± 0.39	10.48 ± 0.44	10.17 ± 0.37	10.64 ± 0.32	10.20 ± 0.32	10.13 ± 0.38
Day 22	12.60 ± 0.36	12.74 ± 0.49	12.78 ± 0.23	11.69 ± 0.38	12.11 ± 0.54	12.18 ± 0.32
Week 14	11.38 ± 0.48	12.03 ± 0.49	11.55 ± 0.66	11.37 ± 0.41	11.97 ± 0.52	10.96 ± 0.63
Segmented neutrophils (10 ³ /μL)						
Day 3	1.96 ± 0.13	1.96 ± 0.14	1.74 ± 0.07	1.84 ± 0.05	1.63 ± 0.08	1.53 ± 0.07**
Day 22	2.50 ± 0.08	2.46 ± 0.14	2.27 ± 0.09	2.09 ± 0.10*	2.06 ± 0.18*	2.16 ± 0.09*
Week 14	2.94 ± 0.15	2.86 ± 0.19	2.87 ± 0.23	2.81 ± 0.16	2.79 ± 0.22	2.47 ± 0.17
Bands (10 ³ /μL)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Metamyelocyte (10³/μL)						
Day 3	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 22	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Myelocyte (10³/μL)						
Day 3	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 22	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000 ^b
Lymphocytes (10³/μL)						
Day 3	7.87 ± 0.28	8.06 ± 0.31	8.02 ± 0.32	8.32 ± 0.29	8.12 ± 0.26	8.15 ± 0.33
Day 22	9.49 ± 0.28	9.68 ± 0.35	9.85 ± 0.17	9.03 ± 0.27	9.45 ± 0.35	9.47 ± 0.23
Week 14	7.72 ± 0.33	8.35 ± 0.36	7.84 ± 0.42	7.74 ± 0.31	8.44 ± 0.28	7.82 ± 0.42
Atypical lymphocytes (10³/μL)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 22	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10³/μL)						
Day 3	0.21 ± 0.02	0.25 ± 0.02	0.23 ± 0.01	0.26 ± 0.01	0.24 ± 0.02	0.25 ± 0.02
Day 22	0.28 ± 0.02	0.30 ± 0.02	0.33 ± 0.01	0.28 ± 0.02	0.30 ± 0.03	0.28 ± 0.02
Week 14	0.37 ± 0.04	0.45 ± 0.03	0.46 ± 0.05	0.46 ± 0.03	0.43 ± 0.05	0.37 ± 0.04
Basophils (10³/μL)						
Day 3	0.168 ± 0.011	0.179 ± 0.014	0.157 ± 0.010	0.177 ± 0.007	0.174 ± 0.014	0.161 ± 0.013
Day 22	0.264 ± 0.017	0.241 ± 0.018	0.265 ± 0.012	0.213 ± 0.015	0.242 ± 0.014	0.218 ± 0.015
Week 14	0.266 ± 0.022	0.288 ± 0.022	0.292 ± 0.034	0.260 ± 0.017	0.236 ± 0.022	0.231 ± 0.026
Eosinophils (10³/μL)						
Day 3	0.04 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00
Day 22	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.00
Week 14	0.09 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.08 ± 0.02	0.08 ± 0.01
Clinical Chemistry						
n						
Day 3	10	10	10	10	10	10
Day 22	9	10	10	10	10	10
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	12.5 ± 0.4	13.3 ± 0.3	14.2 ± 0.5	14.0 ± 0.6	13.2 ± 0.6	13.9 ± 0.5
Day 22	14.0 ± 0.3	14.6 ± 0.4	14.8 ± 0.4	15.5 ± 0.6	14.0 ± 0.5	15.1 ± 0.7

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Week 14	19.4 ± 0.3	18.1 ± 0.5	17.9 ± 0.7	18.5 ± 0.7	19.1 ± 0.9	18.6 ± 0.7
Creatinine (mg/dL)						
Day 3	0.60 ± 0.00	0.60 ± 0.00	0.61 ± 0.01	0.61 ± 0.01	0.60 ± 0.00	0.63 ± 0.02
Day 22	0.60 ± 0.00	0.60 ± 0.00	0.61 ± 0.01	0.59 ± 0.01	0.61 ± 0.01	0.60 ± 0.01
Week 14	0.80 ± 0.00	0.78 ± 0.01	0.78 ± 0.01	0.78 ± 0.01	0.79 ± 0.01	0.77 ± 0.02
Total protein (g/dL)						
Day 3	5.5 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.7 ± 0.1	5.8 ± 0.1*	5.8 ± 0.1*
Day 22	6.3 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.2 ± 0.1
Week 14	7.9 ± 0.1	7.9 ± 0.1	7.8 ± 0.1	7.6 ± 0.1	7.5 ± 0.1*	7.3 ± 0.1**
Albumin (g/dL)						
Day 3	3.6 ± 0.0	3.6 ± 0.0	3.5 ± 0.0	3.7 ± 0.0*	3.7 ± 0.0*	3.7 ± 0.1*
Day 22	3.8 ± 0.0	3.7 ± 0.0	3.8 ± 0.0	3.8 ± 0.0	3.8 ± 0.1	3.8 ± 0.0
Week 14	4.3 ± 0.0	4.3 ± 0.1	4.2 ± 0.1	4.1 ± 0.1*	4.1 ± 0.1**	4.0 ± 0.0**
Alanine aminotransferase (IU/L)						
Day 3	72 ± 2	68 ± 2	65 ± 2*	63 ± 2**	63 ± 1**	64 ± 2**
Day 22	61 ± 1	57 ± 2	59 ± 1	56 ± 2	55 ± 1*	59 ± 2
Week 14	80 ± 7	96 ± 6	74 ± 5	83 ± 6	82 ± 6	71 ± 3
Alkaline phosphatase (IU/L)						
Day 3	711 ± 12	742 ± 11	749 ± 17	760 ± 20	744 ± 15	818 ± 26**
Day 22	490 ± 13	488 ± 13	511 ± 13	538 ± 13	518 ± 12	586 ± 18**
Week 14	287 ± 6	298 ± 6	292 ± 11	315 ± 10	307 ± 9	333 ± 12**
Creatine kinase (IU/L)						
Day 3	648 ± 77	360 ± 39*	720 ± 121	504 ± 55	552 ± 90	420 ± 55
Day 22	252 ± 55	195 ± 31	223 ± 30	338 ± 127	207 ± 33	196 ± 26
Week 14	449 ± 97	276 ± 45	353 ± 57	414 ± 76	234 ± 35	302 ± 55
Sorbitol dehydrogenase (IU/L)						
Day 3	34 ± 4	36 ± 2	34 ± 4	39 ± 2	39 ± 3	39 ± 3
Day 22	33 ± 3	31 ± 2	36 ± 2	29 ± 4	29 ± 2	28 ± 2
Week 14	64 ± 4	71 ± 3	69 ± 3	72 ± 2	66 ± 3	62 ± 2
Bile acids (µmol/L)						
Day 3	40.9 ± 5.6	47.9 ± 6.8	47.5 ± 5.8	23.0 ± 3.0	32.7 ± 4.2	32.6 ± 6.6
Day 22	34.0 ± 5.2	42.6 ± 3.8	38.5 ± 3.9	27.1 ± 4.4	32.4 ± 1.6	32.1 ± 3.6
Week 14	27.9 ± 2.8	24.7 ± 3.0	33.5 ± 3.6	32.8 ± 2.8	36.0 ± 3.7	30.2 ± 4.3

*Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.

**Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

^aData are presented as mean ± standard error. Statistical tests were performed on unrounded data.

^bn = 9.

Table B-2. Hematology Data for Mice in the Three-month Feed Study of *p*-Toluenesulfonamide^a

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n	10	10	9	10	10	9
Hematocrit (auto) (%)	48.8 ± 0.9	46.0 ± 2.0	46.1 ± 2.4	49.5 ± 1.0	45.1 ± 2.2	50.8 ± 1.1
Manual hematocrit (%)	51.5 ± 0.6	50.3 ± 0.6	50.8 ± 0.3	50.9 ± 0.5	50.7 ± 0.4	51.9 ± 0.8
Hemoglobin (g/dL)	16.1 ± 0.3	15.1 ± 0.6	15.1 ± 0.8	16.2 ± 0.3	14.9 ± 0.8	16.7 ± 0.3
Erythrocytes (10 ⁶ /μL)	10.51 ± 0.21	9.89 ± 0.40	9.85 ± 0.48	10.59 ± 0.20	9.67 ± 0.46	10.87 ± 0.22
Erythrocyte distribution width (%)	12.72 ± 0.08	12.75 ± 0.09	12.76 ± 0.08	12.71 ± 0.16	12.68 ± 0.08	12.30 ± 0.10*
Reticulocytes (10 ³ /μL)	277.90 ± 18.30	248.90 ± 15.80	277.30 ± 17.70	280.10 ± 11.80	274.20 ± 19.30	316.60 ± 24.60
Reticulocytes (%)	2.63 ± 0.14	2.52 ± 0.13	2.81 ± 0.11	2.64 ± 0.09	2.83 ± 0.12	2.92 ± 0.23
Nucleated erythrocytes/100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	46.7 ± 0.3	46.5 ± 0.2	46.7 ± 0.3	46.6 ± 0.2	46.4 ± 0.2	46.7 ± 0.3
Mean cell hemoglobin (pg)	15.3 ± 0.0	15.3 ± 0.0	15.3 ± 0.1	15.3 ± 0.0	15.4 ± 0.1	15.4 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.0 ± 0.2	32.8 ± 0.1	32.8 ± 0.2	32.8 ± 0.1	33.0 ± 0.1	32.9 ± 0.1
Platelets (10 ³ /μL)	767.3 ± 27.1	772.0 ± 32.5	749.8 ± 24.8	769.0 ± 19.4	677.9 ± 28.8	704.6 ± 24.0
Mean platelet volume (μm ³)	5.770 ± 0.050	5.730 ± 0.045	5.844 ± 0.117	5.780 ± 0.103	5.890 ± 0.075	5.789 ± 0.099
Leukocytes (10 ³ /μL)	6.80 ± 0.31	6.44 ± 0.59	5.96 ± 0.67	5.74 ± 0.41	6.09 ± 0.47	5.91 ± 0.52
Segmented neutrophils (10 ³ /μL)	1.47 ± 0.09	1.45 ± 0.11	1.30 ± 0.15	1.23 ± 0.09	1.50 ± 0.22	1.25 ± 0.08
Bands (10 ³ /μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Metamyelocyte (10 ³ /μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Myelocyte (10 ³ /μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lymphocytes (10 ³ /μL)	5.24 ± 0.24	4.90 ± 0.49	4.57 ± 0.51	4.43 ± 0.33	4.51 ± 0.34	4.59 ± 0.44
Atypical lymphocytes (10 ³ /μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10 ³ /μL)	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Basophils (10 ³ /μL)	0.009 ± 0.002	0.013 ± 0.003	0.010 ± 0.002	0.010 ± 0.001	0.007 ± 0.002	0.003 ± 0.002
Eosinophils (10 ³ /μL)	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female						
n	10	9	8	9	9	8
Hematocrit (auto) (%)	44.7 ± 1.4	47.2 ± 0.5	47.4 ± 0.6	47.9 ± 0.5	45.9 ± 1.5	46.8 ± 1.0
Manual hematocrit (%)	50.6 ± 0.3	50.9 ± 0.4	50.8 ± 0.5	50.8 ± 0.4	51.0 ± 0.6	50.1 ± 0.7
Hemoglobin (g/dL)	14.7 ± 0.4	15.7 ± 0.2	15.9 ± 0.2	15.9 ± 0.2	15.2 ± 0.5	15.6 ± 0.3
Erythrocytes (10 ⁶ /μL)	9.53 ± 0.28	9.97 ± 0.10	10.10 ± 0.15	10.11 ± 0.12	9.82 ± 0.30	9.95 ± 0.21
Erythrocyte distribution width (%)	12.66 ± 0.09	12.80 ± 0.11	12.85 ± 0.12	12.53 ± 0.19	12.52 ± 0.11	12.76 ± 0.14
Reticulocytes (10 ³ /μL)	268.40 ± 15.30	267.20 ± 12.00	275.60 ± 23.60	263.70 ± 16.70	255.70 ± 13.70	259.60 ± 14.70
Reticulocytes (%)	2.82 ± 0.15	2.68 ± 0.12	2.73 ± 0.22	2.60 ± 0.15	2.61 ± 0.14	2.63 ± 0.17
Nucleated erythrocytes/100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	47.0 ± 0.2	47.3 ± 0.3	46.9 ± 0.3	47.2 ± 0.2	46.8 ± 0.1	47.0 ± 0.0
Mean cell hemoglobin (pg)	15.5 ± 0.1	15.7 ± 0.1	15.8 ± 0.1	15.8 ± 0.1	15.5 ± 0.1	15.7 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.0 ± 0.1	33.2 ± 0.1	33.6 ± 0.2	33.3 ± 0.2	33.2 ± 0.1	33.4 ± 0.2
Platelets (10 ³ /μL)	667.2 ± 23.3	688.0 ± 18.3	699.4 ± 42.4	656.8 ± 25.9	645.0 ± 24.6	612.1 ± 25.4
Mean platelet volume (μm ³)	5.430 ± 0.303	5.800 ± 0.109	5.863 ± 0.105	5.700 ± 0.094	5.622 ± 0.106	5.763 ± 0.112
Leukocytes (10 ³ /μL)	4.65 ± 0.35	4.11 ± 0.26	4.79 ± 0.31	3.73 ± 0.23	4.52 ± 0.42	4.96 ± 0.51
Segmented neutrophils (10 ³ /μL)	1.10 ± 0.05	1.03 ± 0.06	1.21 ± 0.12	0.84 ± 0.07	1.04 ± 0.09	1.24 ± 0.13
Bands (10 ³ /μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Metamyelocyte (10 ³ /μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Myelocyte (10 ³ /μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Lymphocytes (10 ³ /μL)	3.50 ± 0.31	3.03 ± 0.22	3.50 ± 0.23	2.85 ± 0.17	3.43 ± 0.34	3.66 ± 0.37
Atypical lymphocytes (10 ³ /μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes (10 ³ /μL)	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.01
Basophils (10 ³ /μL)	0.006 ± 0.002	0.004 ± 0.002	0.008 ± 0.003	0.004 ± 0.002	0.004 ± 0.002	0.010 ± 0.002
Eosinophils (10 ³ /μL)	0.01 ± 0.00	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01

*Significantly different ($P \leq 0.05$) from the control group by Dunn's test.

^aData are presented as mean ± standard error. Statistical tests were performed on unrounded data.

Appendix C. Organ Weights and Organ-Weight-to-Body-Weight Ratios

Tables

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Table C-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the Two-week Feed Study of *p*-Toluenesulfonamide^a

	0 ppm	750 ppm	1,500 ppm	3,000 ppm	10,000 ppm	30,000 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	161 ± 5	153 ± 6	161 ± 4	159 ± 5	147 ± 3*	114 ± 2**
Heart						
Absolute	0.61 ± 0.03	0.58 ± 0.03	0.64 ± 0.02	0.60 ± 0.03	0.56 ± 0.02	0.42 ± 0.01**
Relative	3.77 ± 0.15	3.75 ± 0.11	3.96 ± 0.10	3.79 ± 0.09	3.82 ± 0.08	3.72 ± 0.05
R. Kidney						
Absolute	0.68 ± 0.03	0.67 ± 0.02	0.70 ± 0.01	0.71 ± 0.03	0.71 ± 0.02	0.55 ± 0.01**
Relative	4.20 ± 0.11	4.35 ± 0.03	4.35 ± 0.06	4.44 ± 0.06*	4.82 ± 0.03**	4.82 ± 0.07**
Liver						
Absolute	8.17 ± 0.31	7.36 ± 0.35	8.06 ± 0.33	8.08 ± 0.39	7.62 ± 0.31	5.91 ± 0.10**
Relative	50.55 ± 0.65	48.00 ± 0.88	50.10 ± 0.94	50.86 ± 0.98	51.70 ± 1.20	51.90 ± 0.83
Lung						
Absolute	0.97 ± 0.04	0.90 ± 0.05	0.85 ± 0.02*	0.88 ± 0.05*	0.83 ± 0.01*	0.68 ± 0.01**
Relative	6.01 ± 0.28	5.87 ± 0.24	5.28 ± 0.22*	5.51 ± 0.12	5.66 ± 0.05	5.95 ± 0.10
R. Testis						
Absolute	0.845 ± 0.043	0.911 ± 0.041	0.907 ± 0.017	0.943 ± 0.035	0.862 ± 0.055	0.795 ± 0.050
Relative	5.232 ± 0.192	5.942 ± 0.137	5.659 ± 0.174	5.952 ± 0.196	5.836 ± 0.285	6.960 ± 0.365**
Thymus						
Absolute	0.436 ± 0.019	0.355 ± 0.025	0.372 ± 0.030	0.395 ± 0.025	0.395 ± 0.029	0.253 ± 0.006**
Relative	2.701 ± 0.100	2.316 ± 0.115	2.312 ± 0.172	2.479 ± 0.082	2.695 ± 0.239	2.220 ± 0.035
Female						
Necropsy body wt	126 ± 3	128 ± 2	125 ± 4	121 ± 2	118 ± 3	104 ± 1**
Heart						
Absolute	0.48 ± 0.01	0.49 ± 0.01	0.50 ± 0.02	0.46 ± 0.01	0.46 ± 0.01	0.42 ± 0.01**
Relative	3.83 ± 0.07	3.87 ± 0.07	4.02 ± 0.08	3.81 ± 0.09	3.91 ± 0.08	3.99 ± 0.09
R. Kidney						
Absolute	0.56 ± 0.02	0.58 ± 0.01	0.57 ± 0.02	0.55 ± 0.01	0.56 ± 0.01	0.51 ± 0.00
Relative	4.44 ± 0.11	4.52 ± 0.05	4.53 ± 0.07	4.58 ± 0.09	4.76 ± 0.08**	4.90 ± 0.03**
Liver						
Absolute	5.47 ± 0.24	5.63 ± 0.15	5.52 ± 0.22	5.38 ± 0.09	5.37 ± 0.26	4.82 ± 0.09*
Relative	43.49 ± 1.05	44.09 ± 0.97	44.07 ± 0.76	44.65 ± 1.14	45.29 ± 1.18	46.19 ± 0.82

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	750 ppm	1,500 ppm	3,000 ppm	10,000 ppm	30,000 ppm
Lung						
Absolute	0.72 ± 0.03	0.74 ± 0.02	0.74 ± 0.03	0.73 ± 0.02	0.79 ± 0.07	0.66 ± 0.01
Relative	5.75 ± 0.17	5.77 ± 0.16	5.88 ± 0.08	6.03 ± 0.11	6.68 ± 0.57	6.36 ± 0.12
Thymus						
Absolute	0.352 ± 0.020	0.354 ± 0.012	0.363 ± 0.012	0.331 ± 0.015	0.347 ± 0.019	0.294 ± 0.022
Relative	2.799 ± 0.155	2.774 ± 0.117	2.910 ± 0.093	2.744 ± 0.108	2.927 ± 0.128	2.817 ± 0.208

*Significantly different ($P \leq 0.05$) from the control group by Williams' test.

**Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

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

Table C-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/NTac Rats in the Three-month Feed Study of *p*-Toluenesulfonamide^a

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	339 ± 7	325 ± 5	338 ± 6	323 ± 7	320 ± 6	315 ± 8*
Heart						
Absolute	0.99 ± 0.02	0.98 ± 0.03	1.01 ± 0.03	0.99 ± 0.02	0.97 ± 0.02	0.96 ± 0.03
Relative	2.91 ± 0.05	3.01 ± 0.08	2.98 ± 0.04	3.06 ± 0.04	3.04 ± 0.03	3.04 ± 0.04
R. Kidney						
Absolute	1.05 ± 0.02	1.05 ± 0.01	1.08 ± 0.02	1.10 ± 0.03	1.12 ± 0.03	1.11 ± 0.03
Relative	3.10 ± 0.04	3.25 ± 0.06	3.19 ± 0.05	3.42 ± 0.04**	3.51 ± 0.04**	3.54 ± 0.06**
Liver						
Absolute	11.25 ± 0.30	10.72 ± 0.20	11.33 ± 0.30	10.97 ± 0.21	10.94 ± 0.25	10.82 ± 0.25
Relative	33.14 ± 0.48	33.03 ± 0.38	33.53 ± 0.72	34.00 ± 0.37	34.15 ± 0.39	34.39 ± 0.29
Lung						
Absolute	1.58 ± 0.08	1.47 ± 0.03	1.56 ± 0.06	1.46 ± 0.05	1.43 ± 0.06	1.50 ± 0.06
Relative	4.66 ± 0.23	4.53 ± 0.12	4.61 ± 0.19	4.52 ± 0.13	4.45 ± 0.14	4.76 ± 0.16
R. Testis						
Absolute	1.412 ± 0.016	1.408 ± 0.018	1.382 ± 0.024	1.392 ± 0.039	1.412 ± 0.019	1.364 ± 0.041
Relative	4.173 ± 0.071	4.340 ± 0.048	4.094 ± 0.076	4.315 ± 0.085	4.417 ± 0.073	4.348 ± 0.144
Thymus						
Absolute	0.303 ± 0.012	0.270 ± 0.012	0.285 ± 0.009	0.267 ± 0.009	0.273 ± 0.009 ^b	0.237 ± 0.014**
Relative	0.893 ± 0.029	0.829 ± 0.029	0.845 ± 0.025	0.834 ± 0.044	0.850 ± 0.021 ^b	0.754 ± 0.042*
Female						
Necropsy body wt	190 ± 3	190 ± 2	187 ± 4	187 ± 2	184 ± 3	174 ± 2**
Heart						
Absolute	0.64 ± 0.02	0.64 ± 0.01	0.63 ± 0.02	0.62 ± 0.01	0.62 ± 0.02	0.59 ± 0.01*
Relative	3.36 ± 0.06	3.36 ± 0.06	3.33 ± 0.05	3.32 ± 0.07	3.39 ± 0.05	3.37 ± 0.07
R. Kidney						
Absolute	0.66 ± 0.01	0.66 ± 0.01	0.67 ± 0.02	0.69 ± 0.01	0.92 ± 0.26	0.64 ± 0.01
Relative	3.49 ± 0.06	3.49 ± 0.04	3.56 ± 0.03	3.69 ± 0.03	4.87 ± 1.25	3.68 ± 0.04
Liver						
Absolute	5.98 ± 0.11	6.06 ± 0.10	6.01 ± 0.17	5.96 ± 0.11	5.90 ± 0.16	5.66 ± 0.14
Relative	31.54 ± 0.33	31.95 ± 0.45	32.04 ± 0.50	31.94 ± 0.40	32.10 ± 0.34	32.44 ± 0.77

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Lung						
Absolute	0.99 ± 0.03	1.05 ± 0.04	1.00 ± 0.04	0.99 ± 0.02	0.96 ± 0.04	0.93 ± 0.02
Relative	5.20 ± 0.08	5.52 ± 0.16	5.34 ± 0.20	5.31 ± 0.12	5.24 ± 0.16	5.33 ± 0.13
Thymus						
Absolute	0.233 ± 0.010	0.235 ± 0.008	0.229 ± 0.006	0.237 ± 0.009	0.224 ± 0.006	0.212 ± 0.009
Relative	1.230 ± 0.040	1.237 ± 0.037	1.222 ± 0.025	1.270 ± 0.042	1.225 ± 0.037	1.215 ± 0.038

*Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

**Significantly different ($P \leq 0.01$) from the control group by Williams' test.

^aOrgan weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

^bn = 9.

Table C-3. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Two-week Feed Study of *p*-Toluenesulfonamide^a

	0 ppm	750 ppm	1,500 ppm	3,000 ppm	10,000 ppm	30,000 ppm
n	5	5	5	5	5	5
Male						
Necropsy body wt	24.6 ± 0.5	24.7 ± 0.6	23.9 ± 0.9	24.8 ± 0.4	23.9 ± 0.3	21.2 ± 0.5**
Heart						
Absolute	0.12 ± 0.00	0.12 ± 0.01	0.12 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.11 ± 0.00
Relative	4.82 ± 0.04	4.96 ± 0.17	5.06 ± 0.06	4.87 ± 0.05	5.06 ± 0.07	5.33 ± 0.09**
R. Kidney						
Absolute	0.22 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.25 ± 0.01	0.22 ± 0.01
Relative	9.06 ± 0.28	9.11 ± 0.27	9.58 ± 0.23	9.39 ± 0.32	10.38 ± 0.25**	10.44 ± 0.24**
Liver						
Absolute	1.30 ± 0.02	1.22 ± 0.04	1.17 ± 0.08	1.29 ± 0.04	1.24 ± 0.02	1.12 ± 0.05
Relative	52.61 ± 0.67	49.39 ± 0.79	48.83 ± 1.51	52.00 ± 1.08	51.95 ± 0.41	52.71 ± 1.58
Lung						
Absolute	0.16 ± 0.00	0.16 ± 0.00	0.17 ± 0.01	0.16 ± 0.01	0.17 ± 0.00	0.16 ± 0.01
Relative	6.39 ± 0.22	6.31 ± 0.06	7.06 ± 0.40	6.58 ± 0.30	7.03 ± 0.14	7.40 ± 0.17**
R. Testis						
Absolute	0.100 ± 0.003	0.101 ± 0.002	0.101 ± 0.003	0.101 ± 0.002	0.100 ± 0.002	0.094 ± 0.001
Relative	4.054 ± 0.086	4.116 ± 0.076	4.231 ± 0.096	4.083 ± 0.074	4.200 ± 0.046	4.428 ± 0.107*
Thymus						
Absolute	0.046 ± 0.002	0.052 ± 0.002	0.044 ± 0.002	0.045 ± 0.002	0.044 ± 0.003	0.030 ± 0.002**
Relative	1.876 ± 0.053	2.103 ± 0.065	1.842 ± 0.089	1.797 ± 0.048	1.851 ± 0.132	1.416 ± 0.099**
Female						
Necropsy body wt	19.6 ± 0.5	19.3 ± 0.2	18.5 ± 0.2	19.0 ± 0.4	19.1 ± 0.4	16.6 ± 0.4**
Heart						
Absolute	0.09 ± 0.00	0.09 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.09 ± 0.00
Relative	4.80 ± 0.10	4.79 ± 0.17	5.15 ± 0.08	5.01 ± 0.13	5.27 ± 0.07*	5.37 ± 0.11**
R. Kidney						
Absolute	0.13 ± 0.01	0.13 ± 0.00	0.14 ± 0.00	0.14 ± 0.00	0.15 ± 0.00*	0.14 ± 0.00*
Relative	6.64 ± 0.19	6.77 ± 0.13	7.38 ± 0.13**	7.22 ± 0.07**	8.01 ± 0.08**	8.30 ± 0.14**
Liver						
Absolute	0.99 ± 0.05	0.91 ± 0.02	0.93 ± 0.02	0.99 ± 0.05	1.04 ± 0.03	0.78 ± 0.02**
Relative	50.23 ± 1.41	47.10 ± 0.90	50.06 ± 0.74	51.85 ± 1.50	54.33 ± 1.03*	47.07 ± 0.48

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	750 ppm	1,500 ppm	3,000 ppm	10,000 ppm	30,000 ppm
Lung						
Absolute	0.14 ± 0.00	0.15 ± 0.00	0.14 ± 0.00	0.13 ± 0.01	0.14 ± 0.00	0.13 ± 0.00
Relative	7.16 ± 0.14	7.56 ± 0.09	7.56 ± 0.19	6.69 ± 0.58	7.27 ± 0.06	7.85 ± 0.27
Thymus						
Absolute	0.063 ± 0.004	0.063 ± 0.003	0.066 ± 0.001	0.068 ± 0.003	0.066 ± 0.002	0.046 ± 0.002**
Relative	3.229 ± 0.232	3.279 ± 0.208	3.579 ± 0.054	3.578 ± 0.117	3.453 ± 0.049	2.788 ± 0.165

*Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** $P \leq 0.01$.

^aOrgan weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Table C-4. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Feed Study of *p*-Toluenesulfonamide^a

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	32.1 ± 0.7	31.8 ± 0.9	32.4 ± 1.1	31.7 ± 0.9	30.8 ± 0.8	30.0 ± 0.7
Heart						
Absolute	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.15 ± 0.00	0.16 ± 0.00*	0.15 ± 0.00
Relative	4.54 ± 0.10	4.75 ± 0.13	4.76 ± 0.14	4.75 ± 0.14	5.15 ± 0.13**	5.06 ± 0.12**
R. Kidney						
Absolute	0.26 ± 0.01	0.27 ± 0.01	0.28 ± 0.01	0.26 ± 0.01	0.28 ± 0.01	0.28 ± 0.01
Relative	8.06 ± 0.22	8.56 ± 0.18	8.56 ± 0.16	8.32 ± 0.26	9.17 ± 0.41**	9.26 ± 0.18**
Liver						
Absolute	1.36 ± 0.03	1.40 ± 0.03	1.42 ± 0.04	1.33 ± 0.04	1.34 ± 0.04	1.33 ± 0.03
Relative	42.26 ± 0.71	43.93 ± 0.43	43.83 ± 0.47	41.82 ± 0.48	43.48 ± 0.85	44.36 ± 0.64
Lung						
Absolute	0.21 ± 0.02	0.22 ± 0.02	0.23 ± 0.01	0.20 ± 0.01	0.23 ± 0.02	0.25 ± 0.02
Relative	6.51 ± 0.52	6.80 ± 0.40	7.12 ± 0.32	6.35 ± 0.21	7.45 ± 0.48	8.24 ± 0.51*
R. Testis						
Absolute	0.110 ± 0.002	0.112 ± 0.002	0.115 ± 0.001	0.115 ± 0.001	0.116 ± 0.002	0.113 ± 0.003
Relative	3.445 ± 0.084	3.560 ± 0.132	3.593 ± 0.113	3.637 ± 0.106	3.796 ± 0.095*	3.782 ± 0.075*
Thymus						
Absolute	0.039 ± 0.003	0.038 ± 0.003	0.036 ± 0.003	0.036 ± 0.002	0.035 ± 0.001	0.032 ± 0.003
Relative	1.199 ± 0.082	1.198 ± 0.069	1.089 ± 0.061	1.129 ± 0.062	1.155 ± 0.060	1.075 ± 0.111
Female						
n	10	10	10	10	10	9
Necropsy body wt	24.8 ± 0.5	26.7 ± 0.6	27.1 ± 0.5*	26.4 ± 0.6	25.4 ± 0.5	24.8 ± 0.7
Heart						
Absolute	0.12 ± 0.00	0.12 ± 0.00	0.13 ± 0.00	0.12 ± 0.00	0.12 ± 0.00	0.13 ± 0.00
Relative	4.88 ± 0.15	4.66 ± 0.09	4.66 ± 0.12	4.58 ± 0.10	4.69 ± 0.13	5.08 ± 0.13
R. Kidney						
Absolute	0.16 ± 0.00	0.16 ± 0.00	0.17 ± 0.00	0.16 ± 0.01	0.17 ± 0.00	0.18 ± 0.01**
Relative	6.42 ± 0.15	6.14 ± 0.12	6.23 ± 0.13	6.08 ± 0.15	6.61 ± 0.12	7.31 ± 0.16**
Liver						
Absolute	1.02 ± 0.02	1.13 ± 0.02*	1.15 ± 0.03**	1.09 ± 0.03	1.07 ± 0.03	1.12 ± 0.04
Relative	41.14 ± 0.87	42.36 ± 0.61	42.61 ± 0.63	41.44 ± 0.92	42.01 ± 0.47	45.15 ± 1.03**

p-Toluenesulfonamide, NTP TOX 88

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Lung						
Absolute	0.19 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.21 ± 0.01	0.19 ± 0.01	0.21 ± 0.01
Relative	7.68 ± 0.45	7.34 ± 0.40	7.34 ± 0.45	7.83 ± 0.43	7.30 ± 0.29	8.50 ± 0.29
Thymus						
Absolute	0.047 ± 0.002	0.046 ± 0.003	0.043 ± 0.002	0.045 ± 0.002	0.044 ± 0.003	0.039 ± 0.004
Relative	1.883 ± 0.086	1.711 ± 0.097	1.596 ± 0.068	1.696 ± 0.067	1.709 ± 0.102	1.564 ± 0.131

*Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** $P \leq 0.01$.

^aOrgan weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Appendix D. Reproductive Tissue Evaluations in Male Rats and Mice

Tables

Table D-1. Summary of Reproductive Tissue Evaluations for Male F344/NTac Rats in the Three-month Feed Study of <i>p</i> -Toluenesulfonamide.....	D-2
Table D-2. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Feed Study of <i>p</i> -Toluenesulfonamide	D-2

Table D-1. Summary of Reproductive Tissue Evaluations for Male F344/NTac Rats in the Three-month Feed Study of *p*-Toluenesulfonamide^a

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	339 ± 7	323 ± 7	320 ± 6	314 ± 8*
L. Cauda epididymis	0.1652 ± 0.0033	0.1716 ± 0.0039	0.1806 ± 0.0070	0.1754 ± 0.0054
L. Epididymis	0.4439 ± 0.0115	0.4401 ± 0.0110	0.4504 ± 0.0093	0.4437 ± 0.0100
L. Testis	1.5009 ± 0.0202	1.4818 ± 0.0291	1.4874 ± 0.0219	1.4634 ± 0.0278
Spermatid measurements				
Spermatid heads (10 ⁶ /g testis)	149.97 ± 11.34	96.99 ± 7.74**	110.66 ± 8.32	116.44 ± 7.10
Spermatid heads (10 ⁶ /testis)	225.87 ± 18.82	143.84 ± 12.03**	164.39 ± 12.33	169.60 ± 9.41
Epididymal spermatozoal measurements				
Sperm motility (%)	66.1 ± 7.1	78.8 ± 6.0	83.6 ± 2.9	78.8 ± 5.2
Sperm (10 ⁶ /g cauda epididymis)	456.3 ± 34.0	478.1 ± 31.7	454.8 ± 32.3	454.3 ± 26.1
Sperm (10 ⁶ /cauda epididymis)	75.5 ± 6.0	81.3 ± 4.2	81.8 ± 6.1	79.7 ± 5.3

*Significantly different (P ≤ 0.05) from the control group by Dunnett's test.

**Significantly different (P ≤ 0.01) from the control group by Dunn's test.

^aData are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (tissue weights) or Dunn's test (epididymal spermatozoal measurements).

Table D-2. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Feed Study of *p*-Toluenesulfonamide^a

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	32.1 ± 0.7	31.7 ± 0.9	30.8 ± 0.8	30.0 ± 0.7
L. Cauda epididymis	0.0174 ± 0.0014	0.0217 ± 0.0036	0.0207 ± 0.0019	0.0189 ± 0.0013
L. Epididymis	0.0479 ± 0.0045	0.0511 ± 0.0056	0.0504 ± 0.0035	0.0487 ± 0.0039
L. Testis	0.1083 ± 0.0021	0.1163 ± 0.0024	0.1159 ± 0.0029	0.1181 ± 0.0048
Spermatid measurements				
Spermatid heads (10 ⁶ /g testis)	207.94 ± 18.01	205.76 ± 10.65	216.08 ± 11.95	243.01 ± 19.94
Spermatid heads (10 ⁶ /testis)	22.35 ± 1.78	23.93 ± 1.34	25.04 ± 1.49	28.20 ± 2.00
Epididymal spermatozoal measurements				
Sperm motility (%)	95.4 ± 0.9	95.6 ± 0.8	93.5 ± 1.4	89.0 ± 3.9
Sperm (10 ⁶ /g cauda epididymis)	188.3 ± 13.1	146.6 ± 16.1	157.0 ± 14.8	190.8 ± 11.9
Sperm (10 ⁶ /cauda epididymis)	3.2 ± 0.2	2.8 ± 0.1	3.0 ± 0.2	3.5 ± 0.2

^aData are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

Appendix E. Genetic Toxicology

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Table E-1. Mutagenicity of *p*-Toluenesulfonamide in *Salmonella typhimurium*^a

Strain	Dose (µg/plate)	Without S9	With 10% rat S9
TA102			
	0	361 ± 25	408 ± 9
	100	396 ± 9	359 ± 6
	333	366 ± 33	414 ± 36
	1,000	353 ± 8	374 ± 7
	3,333	180 ± 21 ^c	313 ± 14 ^c
	10,000	0 ± 0 ^c	0 ± 0 ^c
Trial summary		Negative	Negative
Positive control ^b		1,410 ± 34	1,423 ± 88
TA100			
	0	121 ± 5	125 ± 2
	33	134 ± 9	139 ± 6
	100	122 ± 9	138 ± 9
	333	133 ± 10	136 ± 8
	1,000	114 ± 5	145 ± 8
	2,000	121 ± 6	
	3,333		141 ± 2
Trial summary		Negative	Negative
Positive control		595 ± 1	876 ± 65
TA98			
	0	17 ± 1	31 ± 1
	33	15 ± 2	35 ± 2
	100	20 ± 2	30 ± 2
	333	15 ± 1	27 ± 10
	1,000	17 ± 2	31 ± 2
	2,000	19 ± 3	
	3,333		0 ± 0 ^c
Trial summary		Negative	Negative
Positive control		80 ± 10	501 ± 28

^aStudy was performed at BioReliance Corporation. Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by Zeiger et al.⁴⁶ 0 µg/plate was the solvent control.

^bThe positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and mitomycin-C (TA102). The positive control for metabolic activation with all strains was 2-aminoanthracene, except 2-aminoanthracene or sterigmatocystin was used for TA102.

^cSlight toxicity.

Table E-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of F344/NTac Rats Following Administration of *p*-Toluenesulfonamide in Feed for Three Months^a

Concentration (ppm)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs ^b	P Value ^c	Micronucleated NCEs/1,000 NCEs ^b	P Value ^c	PCEs ^b (%)	P Value ^c
Male							
0	5	0.37 ± 0.07		0.12 ± 0.04		1.411 ± 0.07	
625	5	0.47 ± 0.11	0.4098	0.16 ± 0.03	0.2146	1.336 ± 0.13	1.000
1,250	5	0.57 ± 0.07	0.4823	0.29 ± 0.04	0.2575	1.504 ± 0.04	0.852
2,500	5	0.36 ± 0.05	0.5133	0.14 ± 0.02	0.2759	1.416 ± 0.11	0.894
5,000	5	0.31 ± 0.05	0.5314	0.08 ± 0.03	0.2848	1.686 ± 0.11	0.090
10,000	5	0.27 ± 0.09	0.5419	0.10 ± 0.01	0.2920	1.676 ± 0.08	0.091
		P = 0.980 ^d		P = 0.975		P = 0.010	
Female							
0	5	0.46 ± 0.09		0.20 ± 0.04		1.062 ± 0.05	
625	5	0.35 ± 0.07	0.7779	0.15 ± 0.02	0.6905	1.148 ± 0.07	0.469
1,250	5	0.32 ± 0.03	0.8551	0.20 ± 0.03	0.7758	1.288 ± 0.11	0.130
2,500	5	0.33 ± 0.08	0.8820	0.14 ± 0.03	0.8085	1.290 ± 0.10	0.139
5,000	5	0.43 ± 0.08	0.7365	0.20 ± 0.02	0.7287	1.223 ± 0.06	0.142
10,000	5	0.50 ± 0.14	0.4994	0.18 ± 0.08	0.7426	1.284 ± 0.13	0.139
		P = 0.112		P = 0.450		P = 0.251	

^aStudy was performed at ILS, Inc. The detailed protocol is presented by Witt et al.⁴⁷ and Torous et al.⁴⁸. NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte.

^bMean ± standard error.

^cPairwise comparison with the control group; exposed group values are significant at P ≤ 0.025 by Williams' test.

^dDose-related trend; significant at P ≤ 0.025 by linear regression.

Table E-3. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of *p*-Toluenesulfonamide in Feed for Three Months^a

Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs ^b	P Value ^c	Micronucleated NCEs/1,000 NCEs ^b	P Value ^c	PCEs ^b (%)	P Value ^d
Male							
0	5	2.64 ± 0.18		1.47 ± 0.03		1.538 ± 0.05	
625	5	2.48 ± 0.21	0.6100	1.49 ± 0.03	0.3650	1.663 ± 0.04	0.136
1,250	5	2.84 ± 0.25	0.3212	1.50 ± 0.05	0.3605	1.647 ± 0.06	0.162
2,500	5	2.80 ± 0.14	0.3437	1.51 ± 0.02	0.3649	1.725 ± 0.04	0.038
5,000	5	2.87 ± 0.22	0.2989	1.51 ± 0.03	0.3782	1.710 ± 0.08	0.038
10,000	5	2.85 ± 0.21	0.3070	1.48 ± 0.02	0.3887	1.759 ± 0.05	0.011
		P = 0.150 ^e		P = 0.526 ^e		P = 0.026 ^e	
Female							
0	5	1.85 ± 0.13		1.02 ± 0.03		1.351 ± 0.16	
625	5	1.77 ± 0.09	0.5748	1.03 ± 0.03	0.6186	1.289 ± 0.03	1.000
1,250	5	2.20 ± 0.18	0.3624	1.01 ± 0.02	0.7057	1.141 ± 0.12	1.000
2,500	5	1.82 ± 0.17	0.3865	1.01 ± 0.02	0.7404	1.471 ± 0.18	1.000
5,000	5	1.97 ± 0.08	0.4012	0.97 ± 0.02	0.7571	1.543 ± 0.06	1.000
10,000	5	1.86 ± 0.21	0.4121	0.98 ± 0.04	0.7704	1.525 ± 0.06	1.000
		P = 0.555 ^e		P = 0.927 ^e		P = 0.085 ^f	

^aStudy was performed at ILS, Inc. The detailed protocol is presented by Witt et al.⁴⁷ and Torous et al.⁴⁸. NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte.

^bMean ± standard error.

^cPairwise comparison with the control group; exposed group values are significant at P ≤ 0.025 by Williams' test.

^dPairwise comparison with the control group; exposed group values are significant at P ≤ 0.025 by Williams' (males) or Dunn's (females) test.

^eDose-related trend; significant at P ≤ 0.025 by linear regression.

^fDose-related trend; significant at P ≤ 0.025 by Jonckheere's test.

Appendix F. Chemical Characterization and Dose Formulation Studies

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F.1. Procurement and Characterization of *p*-Toluenesulfonamide

p-Toluenesulfonamide was obtained from Acros Organics (Geel, Belgium) in one lot (A009615201). Lot A009615201 was purified by Battelle's Organic Synthesis Group (Columbus, OH) and was renamed lot 112003. Lot 112003 was used in the 2-week and 3-month studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Operations (Columbus, OH). In addition, Karl Fischer titration and elemental analyses were performed by Prevalere Life Sciences, Inc. (Whitesboro, NY). Reports on analyses performed in support of the *p*-toluenesulfonamide studies are on file at the National Institute of Environmental Health Sciences.

Lot 112003 of the white crystalline chemical was identified as *p*-toluenesulfonamide using infrared (IR) and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and by melting point analysis. All spectra were consistent with computer calculated and/or literature spectra⁵⁷⁻⁵⁹ and the structure of *p*-toluenesulfonamide. Representative IR and proton NMR spectra are presented in Figure F-1 and Figure F-2. The melting point of the test chemical was determined to be 137.2°C, which is consistent with the literature value.

The moisture content of lot 112003 was determined using Karl Fischer titration. The purity of the bulk chemical was determined by elemental analyses, differential scanning calorimetry (DSC), and high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection by system A (Table F-1). Tentative impurity identification was obtained using mass spectrometry (MS) detection by system B and proton and carbon-13 NMR spectroscopy. DSC was conducted using a Perkin-Elmer DSC-7 scanning calorimeter (Perkin Elmer, Waltham, MA), scanning from 100°C to 150°C for the first replicate and from 120°C to 150°C for the second and third replicates, at a scanning rate of 1°C per minute under a nitrogen atmosphere.

Karl Fischer titration indicated approximately 0.1% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for *p*-toluenesulfonamide. DSC indicated a purity of 100%. HPLC/UV indicated one major peak and one reportable impurity with an individual area equal to 0.2% of the total peak area. The most probable structure for the impurity based on MS and NMR analyses was 4-methyl-*N*-phenylbenzene sulfonamide, although the impurity peak in the HPLC/UV analyses might have been composed of multiple components. The overall purity of lot 112003 was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored under a headspace of inert gas at room temperature and protected from light in sealed amber glass bottles. Periodic reanalyses of the bulk chemical were performed at the study laboratory at BioReliance Corporation (Rockville, MD) during the 2-week and 3-month studies using HPLC/UV by system A, and no degradation of the bulk chemical was detected.

F.2. Preparation and Analysis of Dose Formulations

The dose formulations were prepared once during the 2-week studies and eight times during the 3-month studies by mixing *p*-toluenesulfonamide with feed (Table F-2). A premix was prepared by hand and then blended with additional feed in a Patterson-Kelly twin-shell blender. Formulations were stored in doubled polyethylene bags sealed with twist ties protected from light at room temperature for up to 42 days.

Homogeneity studies of the 750 and 30,000 ppm dose formulations and stability studies of the 750 ppm dose formulation were performed by the analytical chemistry laboratory using HPLC/UV by system C (Table F-1). An additional homogeneity study of the 625 ppm dose formulation was performed by the study laboratory using HPLC/UV by system A. Homogeneity was confirmed, and stability was confirmed for at least 42 days for dose formulations stored in plastic zip-lock bags, protected from light, at temperatures up to room temperature, and for at least 7 days for dose formulations kept in glass feeding containers without urine and feces under simulated animal room conditions.

Periodic analyses of the dose formulations of *p*-toluenesulfonamide were conducted by the study laboratory using HPLC/UV by system A. During the 2-week studies, the dose formulations were analyzed once; all 10 dose formulations for rats and mice were within 10% of the target concentrations (Table F-3). Animal room samples of these dose formulations were also analyzed; all 10 for male rats and two of 10 for female mice were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed (Table F-4). Of the dose formulations analyzed, all 34 for rats and mice were within 10% of the target concentrations; 15 of 30 animal room samples for rats and 13 of 30 for mice were within 10% of the target concentrations. Low recovery of *p*-toluenesulfonamide in many of the animal room samples was attributed to potential contamination of dosed-feed with urine and/or feces, which may have caused irreversible binding of the test chemical to the feed. A similar behavior was observed in the simulated animal room stability studies conducted on the 750 ppm dose formulation by the analytical chemistry laboratory where a decline of formulation concentration was observed in the presence of urine and feces.

Table F-1. High-Performance Liquid Chromatography Systems Used in the Feed Studies of *p*-Toluenesulfonamide^a

Detection System	Column	Solvent System
System A		
Ultraviolet (254 nm) light	Prodigy™ ODS-3, 150 mm × 4.6 mm, 3 μm (Phenomenex, Torrance, CA)	A) 0.1% trifluoroacetic acid: 10% acetonitrile:90% water and B) 0.08% trifluoroacetic acid in acetonitrile; 100% A for 2 minutes, then linear gradient to 100% B in 13 minutes, held for 5 minutes, then linear gradient to 100% A in 0.1 minute, held for 9.9 minutes; flow rate 1.0 mL/minute
System B		
Mass spectrometry with direct infusion	Not applicable	A) 50 mM ammonium acetate:0.1% acetic acid and B) methanol; 50% A:50% B, isocratic; flow rate 0.1 mL/minute
System C		
Ultraviolet (254 nm) light	Prodigy™ ODS-3, 150 mm × 4.6 mm, 3 μm (Phenomenex)	A) 0.1% trifluoroacetic acid: 10% acetonitrile:90% water and B) 0.1% trifluoroacetic acid: 90% acetonitrile:10% water; linear gradient from 100% A to 100% B in 15 minutes, held for 5 minutes, then linear gradient to 100% A in 0.1 minute, held for 9.9 minutes.

^aThe high-performance liquid chromatographs were manufactured by Waters (Milford, MA) or Agilent (Palo Alto, CA). The mass spectrometer was manufactured by Micromass UK Limited (Manchester, England).

Table F-2. Preparation and Storage of Dose Formulations in the Feed Studies of *p*-Toluenesulfonamide

Two-week Studies	Three-month Studies
Preparation	
A premix of feed and the appropriate amount of <i>p</i> -toluenesulfonamide, previously ground into a fine powder with a mortar and pestle and sieved through a number 14 sieve until no chemical agglomerates remained, was placed in a mortar with portions of clean feed, and ground manually with a pestle. The premix was then layered into the remaining clean feed and blended in a Patterson-Kelly twin-shell blender with the intensifier bar on for 5 minutes and off for 10 minutes. The dose formulations were prepared once during the studies.	A premix of feed and the appropriate amount of <i>p</i> -toluenesulfonamide, previously ground into a fine powder with a mortar and pestle, was layered into portions of clean feed, placed in a mortar and ground manually with a pestle and sieved through a number 14 sieve until no chemical/feed agglomerates remained. The premix was then layered into the remaining clean feed and blended in a Patterson-Kelly twin-shell blender with the intensifier bar on for 5 minutes and off for 10 minutes. The dose formulations were prepared eight times during the studies.
Chemical Lot Number	
112003	112003
Maximum Storage Time	
42 days	42 days
Storage Conditions	
Stored in doubled polyethylene bags sealed with twist ties protected from light at room temperature	Stored in doubled polyethylene bags sealed with twist ties protected from light at room temperature
Study Laboratory	
BioReliance Corporation (Rockville, MD)	BioReliance Corporation (Rockville, MD)

Table F-3. Results of Analyses of Dose Formulations Administered to F344/N Rats and Mice in the Two-week Feed Studies of *p*-Toluenesulfonamide

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration ^a (ppm)	Difference from Target (%)
June 27, 2006	June 27–28, 2006	750	753	0
		750	752	0
		1,500	1,490	-1
		1,500	1,480	-1
		3,000	3,001	0
		3,000	3,001	0
		10,000	10,160	+2
		10,000	9,829	-2
		30,000	31,080	+4
		30,000	31,160	+4
	July 20–22, 2006 ^b	750	675	-10
		750	730	-3
		1,500	1,454	-3
		1,500	1,476	-2
		3,000	2,885	-4
		3,000	2,896	-3
		10,000	9,270	-7
		10,000	9,433	-6
		30,000	30,810	+3
		30,000	30,930	+3
July 20–22, 2006 ^c	750	543	-28	
	750	561	-25	
	1,500	963	-36	
	1,500	933	-38	
	3,000	2,342	-22	
	3,000	2,296	-23	
	10,000	7,860 ^d	-21	
	10,000	8,550 ^d	-15	
	30,000	30,320	+1	
	30,000	28,660	-4	

^aResults of duplicate analyses.

^bAnimal room samples for male rats.

^cAnimal room samples for female mice.

^dData taken from Table 16 of the BioReliance Dose Formulation and Purity Analysis Report dated 02/26/07.

Table F-4. Results of Analyses of Dose Formulations Administered to F344/NTac Rats and Mice in the Three-month Feed Studies of *p*-Toluenesulfonamide

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration ^a (ppm)	Difference from Target (%)
October 5, 2006	October 8 or 10, 2006	625	621 ^b	-1
		625	570 ^b	-9
		625	649 ^c	+4
		625	667 ^c	+7
		625	656 ^d	+5
		625	659 ^d	+5
		1,250	1,216	-3
		1,250	1,187	-5
		2,500	2,468	-1
		2,500	2,494	0
		5,000	4,884	-2
		5,000	4,814	-4
		10,000	9,710	-3
		10,000	9,747	-3
	October 31, 2006 ^e	625	525	-16
		625	550	-12
		1,250	954	-24
		1,250	1,026	-18
		2,500	2,019	-19
		2,500	2,129	-15
5,000		3,968	-21	
5,000		4,140	-17	
10,000		8,225	-18	
10,000		8,460	-15	
October 31, 2006 ^f	625	302	-52	
	625	288	-54	
	1,250	759	-39	
	1,250	850	-32	
	2,500	1,562	-38	
	2,500	1,554	-38	
	5,000	2,793	-44	
	5,000	2,835	-43	

p-Toluenesulfonamide, NTP TOX 88

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration ^a (ppm)	Difference from Target (%)
		10,000	7,362	-26
		10,000	7,338	-27
November 27, 2006	November 27, 2006	625	611	-2
		625	572	-8
		1,250	1,211	-3
		1,250	1,279	+2
		2,500	2,705	+8
		2,500	2,736	+9
		5,000	5,383	+8
		5,000	5,070	+1
		10,000	10,700	+7
		10,000	10,620	+6
	December 18, 2006 ^e	625	502	-20
		625	548	-12
		1,250	1,123	-10
		1,250	1,148	-8
		2,500	2,371	-5
		2,500	2,249	-10
		5,000	4,919	-2
		5,000	4,749	-5
		10,000	9,502	-5
		10,000	9,745	-3
	December 18, 2006 ^f	625	585	-6
		625	594	-5
		1,250	1,260	+1
		1,250	1,194	-4
		2,500	2,545	+2
		2,500	2,369	-5
		5,000	4,535	-9
		5,000	4,843	-3
		10,000	9,787	-2
		10,000	9,833	-2
December 19, 2006	December 19, 2006	625	619	-1
		625	617	-1

p-Toluenesulfonamide, NTP TOX 88

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration ^a (ppm)	Difference from Target (%)
		1,250	1,212	-3
		1,250	1,227	-2
		2,500	2,401	-4
		2,500	2,397	-4
		5,000	4,812	-4
		5,000	4,998	0
		10,000	9,739	-3
		10,000	9,839	-2
	March 2, 2007 ^e	625	586	-6
		625	528	-16
		1,250	1,012	-19
		1,250	1,006	-20
		2,500	2,373	-5
		2,500	2,326	-7
		5,000	4,733	-5
		5,000	4,550	-9
		10,000	9,356	-6
		10,000	9,272	-7
	March 2, 2007 ^f	625	574	-8
		625	522	-16
		1,250	1,145	-8
		1,250	1,105	-12
		2,500	2,177	-13
		2,500	2,136	-15
		5,000	4,728	-5
		5,000	4,402	-12
		10,000	8,676	-13
		10,000	8,723	-13

^aResults of duplicate analyses.

^bLeft blender position.

^cRight blender position.

^dBottom blender position.

^eAnimal room samples for rats.

^fAnimal room samples for mice.

p-Toluenesulfonamide, NTP TOX 88

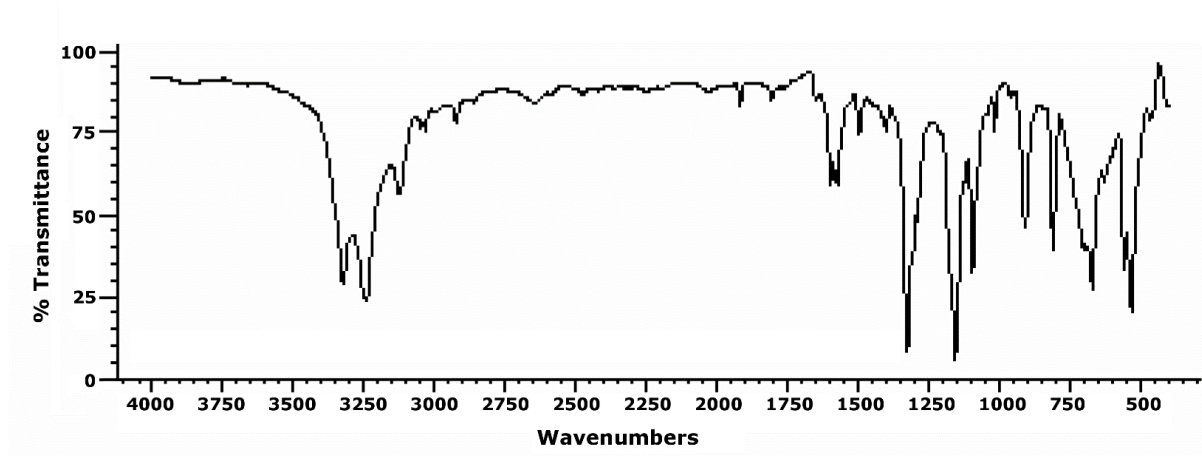


Figure F-1. Infrared Absorption Spectrum of *p*-Toluenesulfonamide

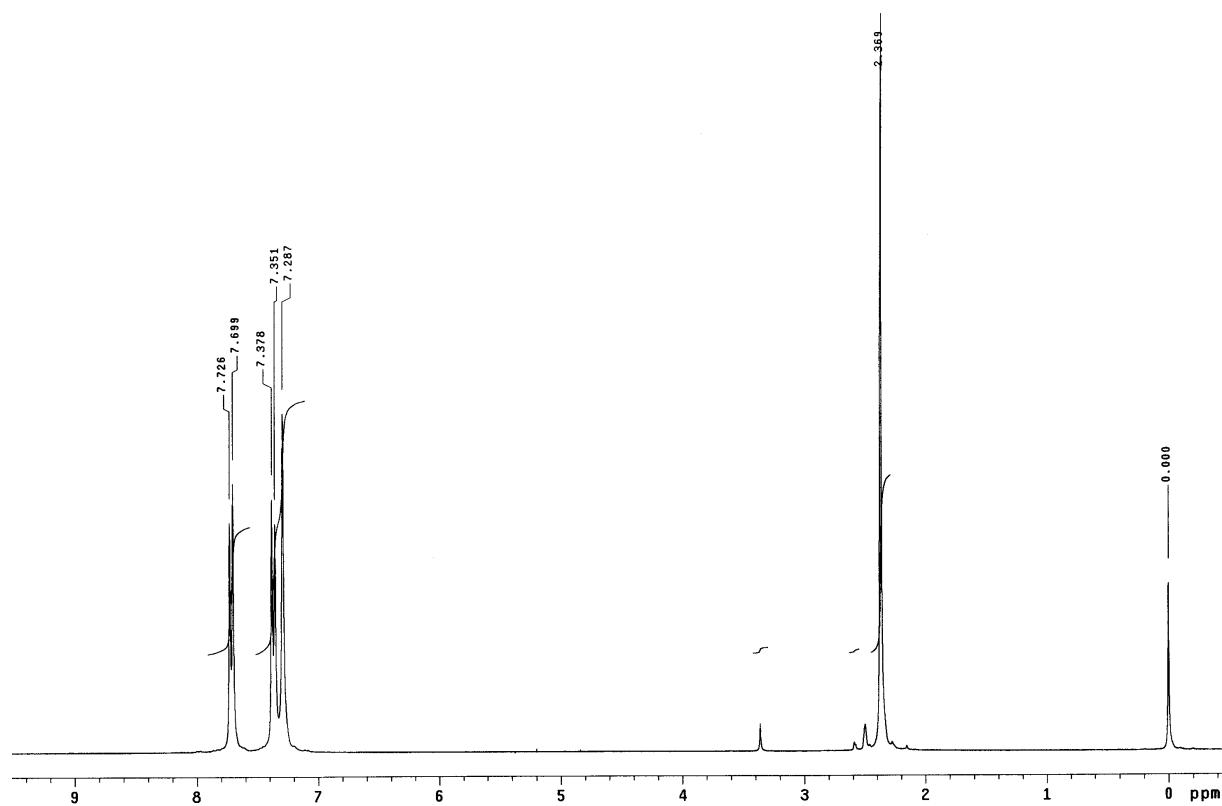


Figure F-2. Proton Nuclear Magnetic Resonance Spectrum of *p*-Toluenesulfonamide

Appendix G. Feed and Compound Consumption in the Feed Studies of *p*-Toluenesulfonamide

Tables

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Table G-1. Feed and Compound Consumption by F344/N Rats in the Two-week Feed Study of *p*-Toluenesulfonamide

Week	0 ppm		750 ppm			1,500 ppm			3,000 ppm		
	Feed ^a (g/day)	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose ^b (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
Male											
1	–	90	–	91	–	–	91	–	–	93	–
2	15.5	127	16.4	126	98	14.6	124	177	15.6	128	366
3	16.5	161	14.2	153	70	16.1	161	150	15.6	159	295
Female											
1	–	90	–	89	–	–	87	–	–	88	–
2	12.2	108	11.9	110	81	12.1	108	168	11.7	103	340
3	10.5	126	11.9	128	70	11.8	125	142	11.6	121	288
						10,000 ppm			30,000 ppm		
						Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
Male											
1						–	91	–	–	89	–
2						12.9	116	1,110	8.3	92	2,698
3						14.8	147	1,005	12.8	114	3,369
Female											
1						–	90	–	–	90	–
2						11.0	104	1,061	7.5	92	2,449
3						10.8	118	913	9.6	104	2,761

^aGrams of feed consumed per animal per day.

^bMilligrams of *p*-toluenesulfonamide consumed per kilogram body weight per day.

Table G-2. Feed and Compound Consumption by Male F344/NTac Rats in the Three-month Feed Study of *p*-Toluenesulfonamide

Week	0 ppm		625 ppm			1,250 ppm			2,500 ppm		
	Feed ^a (g/day)	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose ^b (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	–	87	–	87	–	–	89	–	–	89	–
2	16.2	124	16.4	123	83	16.6	126	165	15.5	122	318
3	16.5	156	16.3	156	65	17.0	161	132	15.8	154	257
4	18.1	190	17.5	189	58	18.3	195	117	17.1	184	232
5	17.9	219	17.8	215	52	18.3	223	103	17.4	210	207
6	16.2	240	16.7	233	45	17.9	243	92	16.9	229	185
7	18.5	261	17.2	253	43	18.0	264	85	17.9	250	179
8	17.5	273	16.0	260	39	16.7	266	78	17.0	261	163
9	17.3	288	16.6	276	38	16.9	283	75	16.9	275	154
10	16.7	298	15.3	283	34	18.8	287	82	16.5	288	143
11	15.0	305	17.0	299	36	19.2	310	78	16.2	298	136
12	16.9	316	16.4	306	34	17.5	319	69	15.9	304	131
13	16.6	329	15.8	315	31	16.5	331	62	15.9	307	130
14	16.6	339	16.1	325	31	16.0	338	59	16.9	323	131

	5,000 ppm			10,000 ppm		
	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	–	87	–	–	87	–
2	14.1	115	615	13.2	112	1,182
3	15.4	145	532	14.2	141	1,009
4	16.7	175	478	15.5	168	922
5	17.1	202	422	30.2	195	1,553
6	17.2	223	386	16.6	213	780
7	17.4	242	360	16.8	235	715
8	17.8	252	353	15.9	246	646
9	16.2	263	308	16.0	261	614
10	13.3	269	247	16.0	274	584
11	17.1	287	298	16.4	287	572
12	16.5	295	279	15.8	293	540
13	16.3	311	262	16.0	307	522
14	16.7	320	261	15.9	315	505

^aGrams of feed consumed per animal per day.

^bMilligrams of *p*-toluenesulfonamide consumed per kilogram body weight per day.

Table G-3. Feed and Compound Consumption by Female F344/NTac Rats in the Three-month Feed Study of *p*-Toluenesulfonamide

Week	0 ppm		625 ppm			1,250 ppm			2,500 ppm		
	Feed ^a (g/day)	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose ^b (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	–	83	–	86	–	–	85	–	–	84	–
2	12.1	105	12.8	106	76	13.0	106	154	12.3	105	294
3	12.1	122	12.5	122	64	12.3	123	125	12.3	120	257
4	12.4	136	12.7	136	59	12.2	135	113	12.4	134	232
5	12.1	149	12.5	148	53	11.9	146	102	12.2	145	211
6	11.8	156	12.0	154	49	12.0	153	98	12.0	154	195
7	12.1	164	12.3	163	47	11.6	160	90	11.6	156	185
8	11.8	169	11.3	167	42	11.2	164	85	11.6	164	177
9	11.4	175	11.3	172	41	11.4	172	83	11.7	171	171
10	11.1	177	11.3	176	40	10.4	173	75	11.1	173	161
11	11.1	182	11.5	181	40	11.0	175	79	11.6	179	162
12	10.5	186	11.1	186	37	11.1	184	75	10.9	182	150
13	10.4	189	10.6	187	36	10.5	185	71	10.8	185	146
14	10.3	190	10.8	190	36	10.2	187	68	10.3	187	138
						5,000 ppm			10,000 ppm		
						Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1						–	86	–	–	85	–
2						11.6	105	555	10.6	99	1,071
3						11.5	120	478	10.4	112	932
4						12.0	133	451	11.1	125	890
5						11.6	144	403	10.5	136	773
6						11.6	151	384	11.2	143	783
7						11.1	158	352	10.6	150	707
8						10.8	161	336	10.5	153	687
9						11.1	167	333	10.5	159	660
10						10.6	169	313	9.7	161	604
11						10.8	176	308	10.4	167	623
12						10.4	178	292	9.8	170	577
13						10.1	182	278	9.4	172	547
14						10.1	184	275	9.6	174	550

^aGrams of feed consumed per animal per day.

^bMilligrams of *p*-toluenesulfonamide consumed per kilogram body weight per day.

Table G-4. Feed and Compound Consumption by Mice in the Two-week Feed Study of *p*-Toluenesulfonamide

Week	0 ppm		750 ppm			1,500 ppm			3,000 ppm		
	Feed ^a (g/day)	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose ^b (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
Male											
1	–	22.1	–	22.5	–	–	22.4	–	–	22.7	–
2	5.1	23.3	5.0	23.4	160	4.1	23.4	263	5.8	23.0	756
3	4.6	24.6	4.4	24.7	134	5.2	23.9	326	5.3	24.8	641
Female											
1	–	16.9	–	17.2	–	–	17.3	–	–	17.0	–
2	3.5	18.2	3.0	17.9	125	3.3	17.6	281	4.0	17.8	673
3	3.9	19.6	3.1	19.3	120	3.4	18.5	276	3.6	19.0	567
						10,000 ppm			30,000 ppm		
						Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
Male											
						–	22.3	–	–	22.1	–
						4.7	22.9	2,054	5.2	20.6	7,573
						4.7	23.9	1,970	5.9	21.2	8,341
Female											
						–	17.4	–	–	16.9	–
						5.1	17.3	2,951	3.0	16.1	5,590
						3.7	19.1	1,941	3.7	16.6	6,679

^aGrams of feed consumed per animal per day.

^bMilligrams of *p*-toluenesulfonamide consumed per kilogram body weight per day.

Table G-5. Feed and Compound Consumption by Male Mice in the Three-month Feed Study of *p*-Toluenesulfonamide

Week	0 ppm		625 ppm			1,250 ppm			2,500 ppm		
	Feed ^a (g/day)	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose ^b (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	–	22.2	–	22.2	–	–	22.7	–	–	22.7	–
2	4.3	22.8	5.8	22.7	160	5.8	23.2	312	4.7	23.0	511
3	4.6	23.5	6.3	23.3	169	5.0	24.4	256	4.9	23.0	533
4	4.4	24.3	4.9	23.9	128	4.2	25.2	208	4.4	24.9	442
5	4.4	25.3	5.1	25.5	125	5.7	26.2	272	5.1	25.6	497
6	5.6	26.6	6.3	26.5	149	6.0	27.3	275	5.4	26.6	508
7	5.6	26.7	5.5	26.6	129	5.6	27.7	253	5.1	27.0	472
8	5.3	27.8	4.6	27.4	105	4.4	28.4	194	5.1	27.6	462
9	4.6	28.6	5.2	28.7	113	4.9	29.4	208	4.7	28.9	407
10	5.0	29.6	4.7	29.2	101	5.4	30.2	223	5.0	29.8	420
11	4.3	30.4	4.5	30.0	94	4.8	31.2	193	4.4	30.3	364
12	4.6	30.9	4.6	30.5	94	4.2	31.0	169	4.5	30.9	364
13	4.8	32.0	4.7	31.2	94	4.8	31.9	188	4.4	31.3	351
14	4.3	32.1	4.4	31.8	86	4.1	32.4	158	4.5	31.7	355
						5,000 ppm			10,000 ppm		
						Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1						–	22.7	–	–	22.4	–
2						4.1	23.0	893	4.8	22.4	2,144
3						4.3	24.0	896	4.9	23.3	2,108
4						4.1	24.7	831	4.2	24.2	1,734
5						4.4	25.2	873	5.5	25.1	2,193
6						7.5	26.1	1,437	6.4	26.0	2,458
7						6.5	26.0	1,248	5.3	26.3	2,017
8						5.3	26.3	1,006	5.2	26.8	1,940
9						5.3	27.8	954	4.7	27.8	1,694
10						5.9	28.9	1,022	4.9	28.2	1,737
11						4.9	29.7	826	4.5	29.2	1,539
12						4.7	30.2	779	4.1	29.1	1,409
13						4.8	30.8	779	4.1	29.5	1,391
14						4.1	30.8	666	4.4	30.0	1,466

^aGrams of feed consumed per animal per day.

^bMilligrams of *p*-toluenesulfonamide consumed per kilogram body weight per day.

Table G-6. Feed and Compound Consumption by Female Mice in the Three-month Feed Study of *p*-Toluenesulfonamide

Week	0 ppm		625 ppm			1,250 ppm			2,500 ppm		
	Feed ^a (g/day)	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose ^b (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1	–	18.4	–	18.7	–	–	18.7	–	–	18.7	–
2	3.4	18.9	3.2	19.1	105	3.8	19.3	246	3.6	19.2	469
3	3.0	19.5	3.1	19.2	101	3.3	19.8	208	3.4	19.8	430
4	3.1	19.7	3.2	20.1	100	3.4	20.7	205	3.3	20.2	409
5	3.1	21.2	3.4	20.5	104	3.3	21.8	190	3.3	21.1	392
6	3.4	21.0	3.5	22.0	99	3.4	22.2	192	3.4	21.6	394
7	3.4	21.5	3.5	22.3	98	3.8	22.8	208	3.3	22.2	371
8	3.6	21.3	3.5	22.8	96	3.5	22.3	197	3.0	22.2	338
9	3.7	22.0	3.8	22.6	105	3.5	23.3	187	3.6	22.3	403
10	3.6	23.2	3.7	24.4	95	3.5	24.6	178	3.7	24.3	380
11	3.4	23.6	3.8	25.2	94	3.7	25.2	184	3.7	24.9	372
12	3.6	24.9	4.0	25.6	98	3.7	25.5	181	4.1	25.7	399
13	3.5	24.4	3.9	26.3	93	3.9	26.4	185	4.0	25.9	387
14	3.6	24.8	3.6	26.7	84	3.8	27.1	176	3.3	26.4	313
						5,000 ppm			10,000 ppm		
						Feed (g/day)	Body Weight (g)	Dose (mg/kg)	Feed (g/day)	Body Weight (g)	Dose (mg/kg)
1						–	18.8	–	–	18.5	–
2						3.4	19.2	885	3.6	19.0	1,898
3						3.1	19.5	793	3.5	19.1	1,830
4						3.4	20.1	846	4.2	20.0	2,100
5						3.0	21.0	713	4.2	20.1	2,094
6						3.3	21.7	762	4.1	21.4	1,920
7						3.0	21.9	684	4.2	21.7	1,940
8						3.9	23.0	848	5.0	22.6	2,215
9						3.8	23.4	811	4.3	21.2	2,030
10						3.6	23.2	777	4.4	22.9	1,924
11						3.3	23.7	696	4.0	23.3	1,718
12						3.6	24.8	727	4.6	23.9	1,921
13						3.5	25.0	699	4.8	24.0	2,003
14						3.5	25.4	689	4.6	24.8	1,857

^aGrams of feed consumed per animal per day.

^bMilligrams of *p*-toluenesulfonamide consumed per kilogram body weight per day.

Appendix H. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration

Tables

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Table H-1. Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix ^a	0.5
Mineral premix ^b	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

^aWheat middlings as carrier.

^bCalcium carbonate as carrier.

Table H-2. Vitamins and Minerals in NTP-2000 Rat and Mouse Ration^a

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α -Tocopheryl acetate	100 IU	—
Niacin	23 mg	—
Folic acid	1.1 mg	—
<i>d</i> -Pantothenic acid	10 mg	<i>d</i> -Calcium pantothenate
Riboflavin	3.3 mg	—
Thiamine	4 mg	Thiamine mononitrate
B ₁₂	52 μ g	—
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	d-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

^aPer kg of finished product.

Table H-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.4 ± 0.85	14–15.2	2
Crude fat (% by weight)	8.4 ± 0.35	8.1–8.6	2
Crude fiber (% by weight)	9 ± 0.78	8.4–9.5	2
Ash (% by weight)	4.9 ± 0.14	4.8–5.0	2
Amino Acids (% of total diet)			
Arginine	0.786 ± 0.070	0.67–0.97	23
Cystine	0.220 ± 0.024	0.15–0.25	23
Glycine	0.700 ± 0.040	0.62–0.80	23
Histidine	0.351 ± 0.076	0.27–0.68	23
Isoleucine	0.546 ± 0.043	0.43–0.66	23
Leucine	1.095 ± 0.066	0.96–1.24	23
Lysine	0.705 ± 0.116	0.31–0.86	23
Methionine	0.409 ± 0.045	0.26–0.49	23
Phenylalanine	0.628 ± 0.039	0.54–0.72	23
Threonine	0.506 ± 0.042	0.43–0.61	23
Tryptophan	0.150 ± 0.028	0.11–0.20	23
Tyrosine	0.405 ± 0.063	0.28–0.54	23
Valine	0.664 ± 0.042	0.55–0.73	23
Essential Fatty Acids (% of total diet)			
Linoleic	3.96 ± 0.254	3.49–4.55	23
Linolenic	0.30 ± 0.031	0.21–0.35	23
Vitamins			
Vitamin A (IU/kg)	3,720 ± 41	3,430–4,010	2
Vitamin D (IU/kg)	1,000 ^a	–	–
α-Tocopherol (ppm)	80.26 ± 21.5603	27.0–124.0	23
Thiamine (ppm) ^b	7.0 ± 0.35	6.7–7.2	2
Riboflavin (ppm)	7.7 ± 2.87	4.20–17.50	23
Niacin (ppm)	79.2 ± 8.97	66.4–98.2	23
Pantothenic acid (ppm)	27 ± 12.35	17.4–81.0	23
Pyridoxine (ppm) ^b	9.54 ± 1.94	6.44–13.7	23
Folic acid (ppm)	1.61 ± 0.47	1.15–3.27	23
Biotin (ppm)	0.32 ± 0.10	0.20–0.704	23
Vitamin B ₁₂ (ppb)	53.4 ± 38.7	18.3–174.0	23
Choline (ppm) ^b	2,773 ± 590	1,160–3,790	23

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Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.922 ± 0.015	0.911–0.932	2
Phosphorus (%)	0.535 ± 0.006	0.531–0.539	2
Potassium (%)	0.667 ± 0.030	0.626–0.733	23
Chloride (%)	0.385 ± 0.038	0.300–0.474	23
Sodium (%)	0.189 ± 0.016	0.160–0.222	23
Magnesium (%)	0.216 ± 0.060	0.185–0.490	23
Sulfur (%)	0.170 ± 0.029	0.116–0.209	14
Iron (ppm)	187 ± 38.6	135–311	23
Manganese (ppm)	51 ± 10.19	21.0–73.1	23
Zinc (ppm)	53.6 ± 8.37	43.3–78.5	23
Copper (ppm)	7.1 ± 2.540	3.21–16.3	23
Iodine (ppm)	0.503 ± 0.201	0.158–0.972	23
Chromium (ppm)	0.696 ± 0.269	0.330–1.380	23
Cobalt (ppm)	0.248 ± 0.163	0.094–0.864	21

^aFrom formulation.

^bAs hydrochloride (thiamine and pyridoxine) or chloride (choline).

Table H-4. Contaminant Levels in NTP-2000 Rat and Mouse Ration^a

	Mean ± Standard Deviation ^b	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.28 ± 0.025	0.27–0.30	2
Cadmium (ppm)	0.06 ± 0.001	0.06–0.06	2
Lead (ppm)	0.13 ± 0.068	0.08–0.18	2
Mercury (ppm)	<0.02	–	2
Selenium (ppm)	0.19 ± 0.016	0.18–0.20	2
Aflatoxins (ppb)	<5.00	–	2
Nitrate nitrogen (ppm) ^c	20.4 ± 14.7	10.0–30.8	2
Nitrite nitrogen (ppm) ^c	<0.61	–	2
BHA (ppm) ^d	<1.0	–	2
BHT (ppm) ^d	<1.0	–	2
Aerobic plate count (CFU/g)	10 ± 0.0	10.0	2
Coliform (MPN/g)	3.0 ± 0.0	3.0	2
<i>Escherichia coli</i> (MPN/g)	<10	–	2
<i>Salmonella</i> (MPN/g)	Negative	–	2
Total nitrosoamines (ppb) ^e	3.8 ± 1.91	2.4–5.1	2
<i>N</i> -Nitrosodimethylamine (ppb) ^e	1.5 ± 0.14	1.4–1.6	2
<i>N</i> -Nitrosopyrrolidine (ppb) ^e	2.3 ± 1.77	1.0–3.5	2
Pesticides (ppm)			
α-BHC	<0.01	–	24
β-BHC	<0.02	–	24
γ-BHC	<0.01	–	24
δ-BHC	<0.01	–	24
Heptachlor	<0.01	–	24
Aldrin	<0.01	–	24
Heptachlor epoxide	<0.01	–	24
DDE	<0.01	–	24
DDD	<0.01	–	24
DDT	<0.01	–	24
HCB	<0.01	–	24
Mirex	<0.01	–	24
Methoxychlor	<0.05	–	24
Dieldrin	<0.01	–	24
Endrin	<0.01	–	24

p-Toluenesulfonamide, NTP TOX 88

	Mean ± Standard Deviation ^b	Range	Number of Samples
Telodrin	<0.01	–	24
Chlordane	<0.05	–	24
Toxaphene	<0.10	–	24
Estimated PCBs	<0.20	–	24
Ronnel	<0.01	–	24
Ethion	<0.02	–	24
Trithion	<0.05	–	24
Diazinon	<0.10	–	24
Methyl chlorpyrifos	0.103 ± 0.067	0.055–0.15	2
Methyl parathion	<0.02	–	24
Ethyl parathion	<0.02	–	24
Malathion	0.119 ± 0.140	0.020–0.218	2
Endosulfan I	<0.01	–	24
Endosulfan II	<0.01	–	24
Endosulfan sulfate	<0.03	–	24

^aAll samples were irradiated. CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride.

^bFor values less than the limit of detection, the detection limit is given as the mean.

^cSources of contamination: alfalfa, grains, and fish meal.

^dSources of contamination: soy oil and fish meal.

^eAll values were corrected for percent recovery.

Appendix I. Sentinel Animal Program

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I.1. Methods

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

Blood samples were collected, allowed to clot, and the serum was separated. All samples were processed appropriately and tested at BioReliance Corporation (Rockville, MD) for determination of the presence of pathogens. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Table I-1. Laboratory Methods and Agents Tested for in the Sentinel Animal Program

Method and Test	Time of Collection
Rats	
Three-month Study	
ELISA	
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
RCV/SDA	Study termination
Mice	
Three-month Study	
ELISA	
Ectromelia virus	Study termination
EDIM (epizootic diarrhea of infant mice)	Study termination
GDVII (Theiler's murine encephalomyelitis virus)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
Mouse adenovirus (MAAd-1)	Study termination
MHV (mouse hepatitis virus)	Study termination
MMV, VP2 (mouse minute virus, viral protein 2)	Study termination
MPV, VP2 (mouse parvovirus, viral protein 2)	Study termination
PVM	Study termination
Reovirus	Study termination
Sendai	Study termination

I.2. Results

All test results were negative.



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