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Number 23

NTP Technical Report on Toxicity Studies of

o-, *m*-, and *p*-Nitrotoluenes

(CAS Nos.: 88-72-2, 99-08-1, 99-99-0)

Administered in Dosed Feed to F344/N Rats and B6C3F$_1$ Mice

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The NTP Report on the toxicity studies of *o*-, *m*-, and *p*-nitrotoluenes is based primarily on 2-week and 13-week studies that began February 9, 1988, and ended August 17, 1989, at Hazleton Laboratories of America, Rockville, MD.

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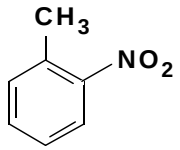

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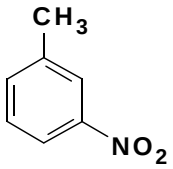
CAS No. 88-72-2



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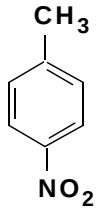

***p*-Nitrotoluene**
CAS No. 99-99-0

Molecular Formula: $C_7H_7NO_2$
Molecular Weight: 137.13

Synonyms: *o*-NT, 2NT, 2-nitrotoluene, 2-methylnitrobenzene, 2-nitrotoluol;
m-NT, 3NT, 3-nitrotoluene, 3-methylnitrobenzene, 3-nitrotoluol
p-NT, 4NT, 4-nitrotoluene, 4-methylnitrobenzene, 4-nitrotoluol

Nitrotoluenes are high production volume chemicals used in the synthesis of agricultural and rubber chemicals and in various dyes. Because of differences in the metabolism of the 3 isomers and their capability to bind to DNA, comparative toxicity studies of *o*-, *m*-, or *p*-nitrotoluene were conducted in F344 rats and B6C3F$_1$ mice. Animals were evaluated for histopathology, clinical pathology, and toxicity to the reproductive system. The nitrotoluenes were also studied in several *in vitro* and *in vivo* assays for genetic toxicity.

In 14-day studies, *o*-nitrotoluene, *m*-nitrotoluene, or *p*-nitrotoluene was administered in the feed to male and female rats and mice at concentrations ranging from 388 to 20000 ppm (5 animals/chemical/species/sex/dose). There were no effects on survival or clinical signs of toxicity in these studies, although animals at the higher doses showed decreases in body weight gains relative to controls.

In the 13-week studies, *o*-, *m*-, or *p*-nitrotoluene was given to male and female rats and mice (10 animals/chemical/species/sex/dose) in the feed at concentrations between 625 and 10000 ppm. The estimated daily doses based on measures of feed consumption were 40 to 900 mg nitrotoluene/kg body weight/day for rats and 100 to 2000 mg/kg/day for mice and were similar for each of the 3 isomers when compared for each dietary level/sex/species. There were no effects on survival in any of the studies, and clinical signs of toxicity were limited to decreases in feed consumption. Decreased body weight gains occurred in dosed rats and mice in all studies at the higher dose levels and were most pronounced in rats receiving *o*-nitrotoluene.

In rats, histopathologic analyses after 13 weeks of dosing showed toxicity to kidney, spleen, and testis in animals receiving any of the 3 isomers, and toxicity to the liver and mesothelium in male rats given *o*-nitrotoluene. Kidney toxicity observed in male rats was characterized by the presence of hyaline droplets in tubular epithelial cells, attributed to an increase in the level of α-2u globulin. Pigment, possibly lipofuscin, and karyomegaly in the *p*-nitrotoluene study were

present in the renal tubular epithelium of dosed male and female rats. In the spleen of treated male and female rats, there was a mild increase in hematopoiesis, hemosiderin deposition, and/or congestion; this effect was most severe with the para-isomer, followed by the ortho- and then the meta-isomer. Administration of *o*-, *m*-, or *p*-nitrotoluene impaired testicular function of the rat, shown by degeneration of the testis and reduction in sperm concentration, motility, and spermatid number. All 3 isomers increased the length of the estrous cycle in rats. Hepatic toxicity was characterized by cytoplasmic vacuolization and oval cell hyperplasia and by an increase in the level of serum bile acids, SDH, and ALT activities in male rats given *o*-nitrotoluene. There was no histopathologic evidence for liver toxicity in male or female rats with the *m*- or *p*-isomers, or in female rats with the *o*-isomer; but evidence of liver injury was observed in these groups, indicated by increases in relative liver weights and elevations in bile acids and liver enzymes in serum. Mesotheliomas of the tunica vaginalis were observed in 3/10 male rats receiving *o*-nitrotoluene at 5000 ppm, and mesothelial cell hyperplasia was observed in 2/10 male rats receiving *o*-nitrotoluene at 10000 ppm.

The only histopathologic evidence for toxicity in mice in the 13-week studies occurred in the olfactory epithelium in mice receiving *o*-nitrotoluene, where the chemical caused degeneration and metaplasia. No liver lesions were noted in mice, but the 3 isomers caused increases in relative liver weights. There was no toxicity to the reproductive system in male or female mice treated with any of the nitrotoluene isomers.

The 3 nitrotoluene isomers were not mutagenic in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98. Only *p*-nitrotoluene induced chromosomal aberrations in cultured Chinese hamster ovary (CHO) cells, and this required metabolic activation. Sister-chromatid exchanges were increased in CHO cells following exposure to each isomer; the requirement for metabolic activation varied. Only *p*-nitrotoluene was studied in the mouse lymphoma L5178Y test; it caused mutations with metabolic activation. Unscheduled DNA synthesis (UDS) was increased in *in vitro* incubations of hepatocytes isolated from both sexes of rats and mice after receiving a single *in vivo* oral dose of *o*-nitrotoluene. UDS was not increased in a similar study with male rats given *m*- or *p*-nitrotoluene. *o*-Nitrotoluene also induced s-phase DNA synthesis in hepatocytes of rats but not in those of mice.

In summary, the 3 nitrotoluene isomers were toxic to the kidney, spleen and/or reproductive system in rats; *o*-nitrotoluene also caused lesions in the liver of male rats. No treatment-related lesions were noted in mice except with *o*-nitrotoluene where olfactory epithelium degeneration occurred. The increase in relative liver weights and the increase in UDS in liver indicate that all 3 isomers affected the liver of female rats and of male and female mice, even though histopathologic lesions were not observed. In general, the extent of the toxicity was most severe with the *o*-isomer in both rats and mice. *o*-Nitrotoluene was carcinogenic in male rats in 13-week studies, based on the occurrence of mesothelioma and mesothelial cell hyperplasia in dosed groups.

Summary of Selected Treatment-Related Effects in the 13-Week Nitrotoluene Studies

	o-Nitrotoluene		*m*-Nitrotoluene		*p*-Nitrotoluene	
	Male	Female	Male	Female	Male	Female
RATS						
Final Body Weight (90% or less than control)	↓(3) [a]	↓(3)	↓(5)	↓(5)	↓(5)	↓(5)
Liver						
Relative weight	↑(1)	↑(1)	↑(5)	↑(5)	↑(4)	↑(5)
ALT	↑(4)	–	–	↑ (4)	–	↑ (5)
SDH	↑(3)	–	–	–	–	–
Bile Acids	↑(4)	↑(5)	↑(4)	↑(5)	↑(5)	–
Nonneoplastic lesions	+(3)	–	–	–	–	–
Kidney						
Relative weight	↑(3)	↑(2)	↑(5)	↑(4)	↑(4)	↑(5)
Nonneoplastic lesions	+(2)	+(3)	+(1)	–	+(1)	+(1)
Spleen						
Hematology	(3)	(3)	(4)	(4)	(3)	(3)
Nonneoplastic lesions	+(2)	+(3)	+(3)	+(3)	+(1)	+(1)
Testis						
Spermatid count	↓(4)		↓(5)		↓(5)	
Nonneoplastic lesions	+(4)		+(5)		+(5)	
Mesothelium						
Neoplastic and preneoplastic lesions	+(4)		–		–	
Estrous cycle length		↑(5)		↑(4)		↑(5)
MICE						
Final Body Weight (90% or less than control)	↓(3)	↓(3)	↓(5)	↓(5)	↓(5)	↓(5)
Nose						
Nonneoplastic lesions	+(2)	+(2)	–	–	–	–
Liver						
Relative Weight	↑(3)	↑(2)	↑(1)	↑(1)	↑(1)	↑(1)

[a] Lowest dose group in which an effect was seen; 1 = 625 ppm; 2 = 1250; 3 = 2500; 4 = 5000; 5 = 10000.
\+ Presence of treatment-related histopathology.

PEER REVIEW

Peer Review Panel

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of *o*-, *m*-, and *p*-nitrotoluenes on November 21, 1991, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members act to determine if the design and conditions of the NTP studies were appropriate and to ensure that the toxicity study report fully and clearly presents the experimental results and conclusions.

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Summary of Peer Review Comments

Dr. J.K. Dunnick, NIEHS, introduced the short-term toxicity studies of *o*-, *m*-, and *p*-nitrotoluenes by reviewing the uses and rationale for study, the experimental design, and the results.

Dr. Goodman, a principal reviewer, said the report was written well and the results clearly presented. He stated that the rationale behind the use of each of the genetic toxicology tests employed should be presented and there should be some discussion regarding results. He suggested that a specific subsection of the Discussion could be devoted to genetic toxicology. Dr. Dunnick reported that in collaboration with Dr. E. Zeiger, NIEHS, the genetic toxicology section would be upgraded and expanded.

Dr. Davidson, a second principal reviewer, said the report did a good job of presenting background information and summarizing the results. She commented that although the degree of toxicity of the *ortho*-isomer is compared with the other 2 isomers, the *meta* and *para* isomers are not compared with each other regarding relative toxicity. Dr. Dunnick agreed that such a comparison should be added to the Abstract. Dr. Davidson noted that considering that the main uses of nitrotoluenes are in the agricultural, rubber and dye industries, it would relevant to state how occupational groups (machine operators, welders, cutters, etc.) are exposed to the chemicals. Dr. Janet Haartz, NIOSH, said the only isomer for which occupational data is available is the *para*-isomer. There were no listings for the *meta*- and *orth*-isomers.

Seeing no objections, Dr. Klaassen accepted the report, with the suggested editorial and other changes, on behalf of the panel.

INTRODUCTION

Physical Properties, Environmental Occurrence, and Exposures to Nitrotoluenes

Ortho- and *para*-nitrotoluenes are important commercial chemicals used to synthesize agricultural and rubber chemicals, azo and sulfur dyes, and dyes for cotton, wool, silk, leather, and paper. An estimated 29 million pounds (10.8 x 10^6 kg) of the *ortho*-isomer and 15 million pounds (5.6 x 10^6 kg) of the *para*-isomer are used annually in the United States. The third isomer, *meta*-nitrotoluene, is produced in "negligible" quantities. (Dunlap, 1981; Abshire and Hughes, 1982).

Nitrotoluenes are yellow liquids at room temperature. They are produced by the nitration of toluene with an aqueous acidic mixture of H_2SO_4 and HNO_3 at a temperature that starts at 25°C and is slowly raised to 37°C. The resulting product contains 55-60% *o*-nitrotoluene, 3-4% *m*-nitrotoluene, and 35-40% *p*-nitrotoluene. The isomers may be separated by a combination of fractional distillation and crystallization (Dunlap, 1981). Isomers of nitrotoluene differ in the position of the nitro group in relation to the methyl group on the benzene ring. While the chemical formula is the same for all isomers, their chemical and physical properties vary (Table 1).

TABLE 1 Physical Properties of the Nitrotoluenes

	o-nitrotoluene	*m*-nitrotoluene	*p*-nitrotoluene
Boiling point	220.4°C	232.6°C	238.3°C
Melting point	-9.3°C	15°C	51.7°C
Density (20°C)	1.163	1.157	1.286
Solubility (H_20, 30°C)	652 mg/L	498 mg/L	442 mg/L
Volatility (20°C)	0.1 mm Hg	0.1 mm Hg	0.1 mm Hg
Volatility (30°C)	0.25 mm Hg	0.25 mm Hg	0.25 mm Hg
Log partition coef.	2.30	2.40	2.37

Environmental surveys have detected *o*-nitrotoluene in rivers and drinking water (Shackelford and Keith, 1976); all 3 isomers of nitrotoluene have been found in waste streams and atmospheric emissions from industrial plants (Shackelford and Keith, 1976; Forsten, 1973). The Federal Occupational Safety and Health Administration (OSHA) set an 8-hour, time-weighted average (TWA) permissible exposure limit of 5 ppm (30 mg/m^3) for nitrotoluenes, while the American Conference of Governmental Industrial Hygienists has recommended an 8-hour, TWA threshold limit value of 2 ppm (11 mg/m^3) (ACGIH, 1988).

The National Occupational Exposure Survey found exposure to *p*-nitrotoluene among workers in 5 different occupational groups: biological technicians; painting and paint-spraying machine operators; machine operators; welders and cutters; and operators of separating, filtering, and clarifying machines. The latter group accounted for approximately 60 % of potential exposures. An estimated 4350 people in the United States potentially could be exposed to *p*-nitrotoluene in the workplace. Data on exposure potential in the workplace for the *m-* and *o-* isomers was not available (J.A. Seta, 1991, personal communication).

Acute Toxicity

Oral LD_{50} values for rats and mice are: *o*-Nitrotoluene: 891 mg/kg (rats), 2463 mg/kg (mice); *m*-Nitrotoluene: 1072 mg/kg (rats), 330 mg/kg (mice); *p*-nitrotoluene: 2144 mg/kg (rats), 1231 mg/kg (mice). These acute toxicity studies did not include a histopathologic examination of tissues (RTECS, 1990; Ciss *et al.*, 1980a, b).

Chronic Toxicity and Carcinogenicity

There have been no reports of rodent chronic toxicity or carcinogenicity studies on the nitrotoluenes. Interest in the carcinogenicity of mononitrotoluenes stems from the results of long-term rodent studies using technical-grade dinitrotoluene (DNT), 2,6-dinitrotoluene, or 2,4-dinitrotoluene. Results of these studies suggest that 2,6-DNT is a potent carcinogen in rat liver (Rickert *et al.*, 1984).

The results of Weisburger *et al.* (1978) have also suggested that *ortho*-substituted aromatic compounds are more potent carcinogens than corresponding isomers with *meta*- or *para*-substitutions. This was seen with *o-, m-, and p*–toluidine in rats and mice, as well as with other compounds. The toluidine studies are of interest because reduction of the nitro group of the nitrotoluenes yields the corresponding toluidine. The NCI has reported that *o*-toluidine was positive in 2-year studies in male rats (mesotheliomas, splenic sarcomas, subcutaneous fibromas), in female rats (splenic sarcomas, urinary bladder transitional cell tumors, tumors of the mammary gland), in male mice (hemangioma or hemangiosarcoma), and in female mice (liver tumors) (NCI, 1979).

Metabolism

The comparative metabolism of *o*-, *m*-, and *p*-nitrotoluenes administered orally was studied in F344 rats (deBethizy and Rickert, 1984; Chism and Rickert, 1985; Rickert *et al.*, 1984b; Chism *et al.*, 1984). Following an oral dose of all 3 radiolabeled compounds as individual chemicals, 73 to 86% of the dose was excreted in the urine within 72 hours. Fecal excretion accounted for 5 to 13% of the dose, and minimal amounts of label were captured in expired breath (Chism *et al.,* 1984). The major urinary metabolites are shown in Table 2.

TABLE 2 Urinary Metabolites of *o-, m-,* and *p*-Nitrotoluene (Chism *et al.,* 1984)

o-Nitrotoluene	*m*-Nitrotoluene	*p*-Nitrotoluene
o-nitrobenzoic acid (29%)*	*m*-nitrobenzoic acid (21%)	*p*-nitrobenzoic acid (28%)
o-nitrobenzyl glucuronide (14%)	*m*-nitrohippuric acid (24%)	*p*-nitrohippuric acid (13%)
S-(*o*-nitrobenzyl)-N-acetylcysteine (12%)	*m*-acetamidobenzoic acid (12%)	*p*-acetamidobenzoic acid (27%)
unidentified, contains sulfur (16%)		

*Percent of administered label appearing in urine.

All 3 isomers apparently were converted to the corresponding benzyl alcohol and to benzoic acid in the liver; the *m*- and *p*-isomers underwent conjugation with glycine to form the hippuric acid, or nitro reduction and acylation. For *o*-nitrotoluene, formation of the *o*-nitrobenzyl alcohol glucuronide was a major metabolic pathway. Conjugation with glucuronide was not a major metabolic route for the *m*- and *p*-isomers. The *o*-nitrobenzyl glucuronide was excreted via the bile into the intestine, where bacterial enzymes reduced the nitro group to form aminobenzyl alcohol. The aminobenzyl alcohol was reabsorbed and further metabolized by hepatic enzymes to a species capable of covalent binding to hepatic DNA. Recent studies by Chism and Rickert (1989) suggested that *o*-aminobenzylsulfate was the metabolite of *o*-nitrotoluene responsible for binding covalently to DNA (see Figure 1).

An analogous metabolic pathway was followed by 2,6-DNT, which was oxidized in the liver to 2,6-dinitrobenzyl alcohol, then conjugated with glucuronic acid and excreted in the bile. Intestinal microflora hydrolyzed the glucuronide and reduced the nitro group to form 2-amino-6-nitrobenzyl alcohol. A portion of this metabolite was reabsorbed from the intestine and oxidized to a hydroxylamine by hepatic enzymes. The hydroxylamine was then conjugated with sulfate by hepatic sulfotransferase. The unstable N-sulfate decomposed to form an electrophilic nitrenium ion which could react with cellular nucleophiles such as DNA. This electrophilic ion was formed in the liver, hence the high carcinogenic activity of 2,6-DNT for rodent liver (Kedderis *et al.*, 1984). 2,6-DNT was more active than 2,4-DNT in the *in vivo/in vitro* hepatocyte unscheduled DNA synthesis assay (Mirsalis and Butterworth, 1982).

The metabolic profiles for 2,6-dinitrotoluene and *o*-nitrotoluene were similar. Both were excreted as glucuronides into the intestine where bacterial enzymes reduce nitro groups; the aminobenzyl alcohols were reabsorbed and further metabolized in the liver to electrophilic compounds which presumably could interact with DNA. Binding of 2,6-DNT and *o*-nitrotoluene to rat hepatic DNA was decreased by pretreatment with sulfotransferase inhibitors, suggesting that the final step in the activation of both chemicals was formation of an unstable N-O-sulfate which decomposed to yield an electrophilic nitrenium ion. It was suggested that the carcinogenic potential for 2,6-DNT and *o*-nitrotoluene would be similar (Rickert *et al.*, 1984b).

Genetic Toxicity

The testing of the mononitrotoluenes *in vitro* for mutagenicity has generally yielded negative results, although occasional positive responses in various assays have been reported. The aromatic nitro group of the nitrotoluenes is considered a structural alert to potential DNA reactivity (Tennant and Ashby, 1991), but such activity would presumably be dependent upon the metabolic activity of the test system. For example, it is likely that reduction of the nitro group to produce an aromatic amine would be necessary for a positive response in the *Salmonella* assay. The *o*- and *m*-nitrotoluene isomers demonstrated no mutagenic activity in any of several strains of *Salmonella typhimurium*, with or without S9 metabolic activation; isolated positive responses were reported for *p*-nitrotoluene in strain TA100, with or without S9 (Chiu *et al.*, 1978; Miyata *et al.*, 1981; Spanggord *et al.*, 1982; Haworth *et al.,* 1983; Suzuki *et al.*, 1983; Shimizu and Yano, 1986; Kawai *et al.,* 1987). *p*-Nitrotoluene also induced cell

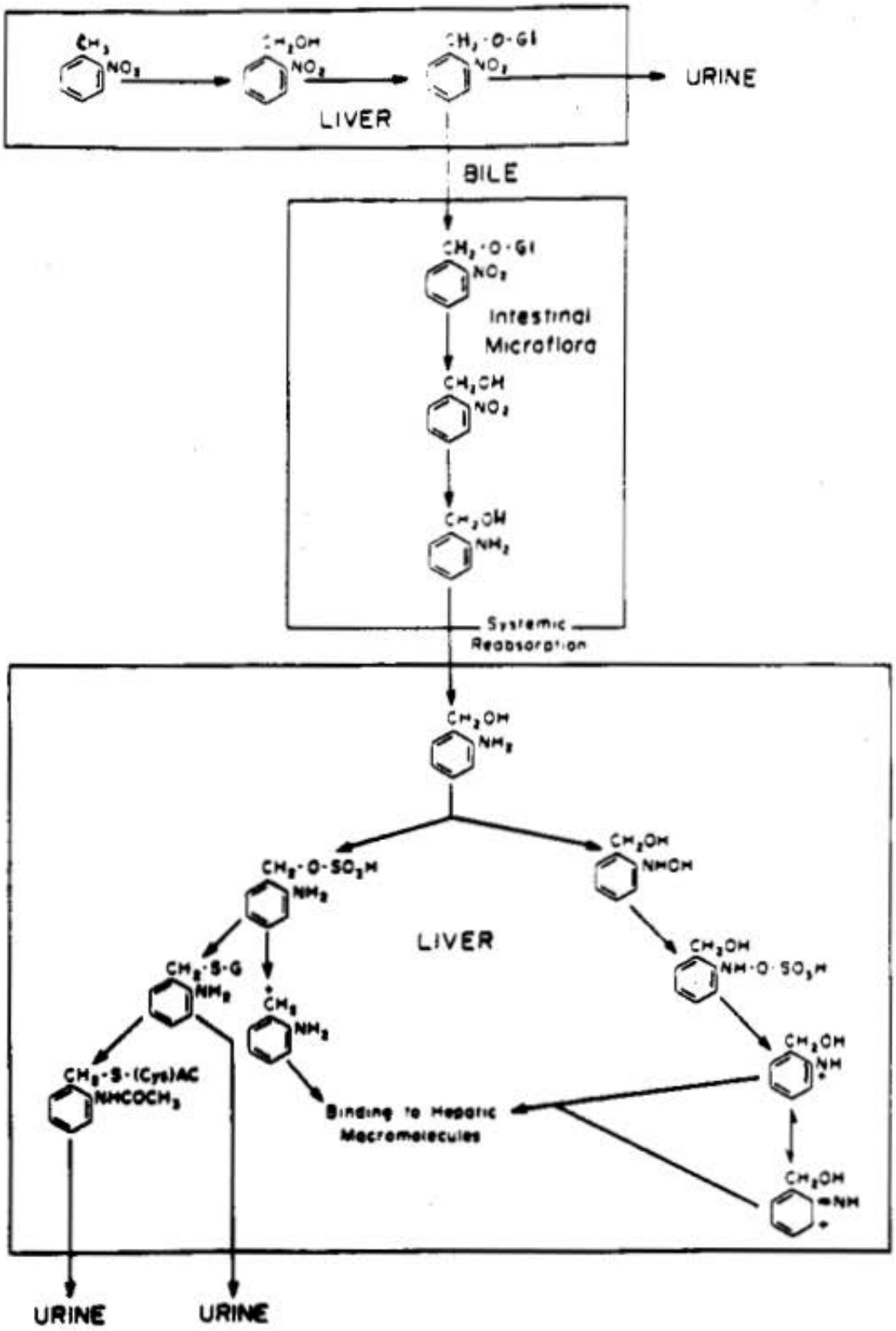

Figure 1 Proposed Pathway for Bioactivation of *o*-Nitrotoluene (Chism and Rickert, 1985)

growth inhibition, a measure of DNA damage, in *B. subtilis* M45/H17 in the absence of S9 (Shimizu and Yano, 1986); a weakly positive response was reported in this assay for *o*-nitrotoluene, and results with the *m*-isomer were negative.

All 3 mononitrotoluene isomers induced sister chromatid exchanges (SCE) in Chinese hamster ovary (CHO) cells; only *m*-nitrotoluene required S9 for a positive response (Galloway *et al.*, 1987). Only *p*-nitrotoluene induced chromosomal aberrations in CHO cells in the presence of S9 (Galloway *et al.*, 1987); however, no increases in micronuclei (sampled 24 hr post-treatment) or chromosomal aberrations (sampled 6, 24, and 48 hours post-treatment) were observed in bone marrow erythrocytes of male B6C3F$_1$ mice given a single i.p. injection of *p*-nitrotoluene (Ohuchida *et al.*, 1989; Furukawa *et al.*, 1989).

No induction of unscheduled DNA synthesis (UDS) was observed in male F344 rat hepatocytes or spermatocytes treated with *m*- or *p*-nitrotoluene *in vitro* (Doolittle *et al.*, 1983; Working and Butterworth, 1984) or *in vivo* (Doolittle *et al.*, 1983; Mirsalis *et al.*, 1989; Butterworth *et al.*, 1989). *o*-Nitrotoluene was also unable to induce UDS in rat hepatocytes *in vitro*, but a strong positive response was observed in hepatocytes of male F344 rats treated *in vivo* (Doolittle *et al.*, 1983). No induction of UDS by *o*-nitrotoluene was noted in germ-free male rats, indicating that activation of *o*-nitrotoluene, or an intermediate metabolic conjugate by intestinal bacteria, is necessary to the process. No induction of UDS was observed in hepatocytes of female rats treated *in vivo*, possibly the result of differences in hepatic metabolism or disposition of *o*-nitrotoluene between the sexes. Differences between males and females have been attributed to the fact that males excrete more of the glucuronic conjugates of the nitrotoluenes into the bile and, subsequently, into the intestine where they are metabolized further by bacterial systems. Sex-related differences in metabolism have also been observed with the dinitrotoluenes in rats (Long and Rickert, 1983).

In another series of experiments, male F344 rats were given a single oral dose of either (ring-U-^{14}C) *o*-, *m*-, or *p*-nitrotoluene. Covalent binding to hepatic macromolecules and to DNA was measured. *o*-Nitrotoluene was observed to bind at higher concentrations to the hepatic macromolecules and was the only isomer shown to bind to DNA. Binding was inhibited by sulfotransferase inhibitors (Rickert *et al.*, 1984b).

Study Rationale and Design

The National Institute for Occupational Safety and Health (NIOSH) and the NTP nominated the nitrotoluenes for rodent toxicity and carcinogenicity studies, based on the considerable human exposure to these chemicals, as well as the absence of long-term studies for carcinogenicity in rodents. Because of the known differences in the metabolism of the 3 nitrotoluene isomers and in the capability of their metabolites to bind to DNA, a comparative study of the toxicity and carcinogenicity of all 3 isomers was recommended. Studies were performed to evaluate the toxicity (histopathology, clinical pathology, reproductive system evaluations) of the 3 nitrotoluene isomers in F344 rats and B6C3F$_1$ mice by administering the isomers orally in the feed. Additional studies included *in vitro* genetic toxicity studies of mutagenesis in *Salmonella* and mouse lymphoma L5178Y cells, chromosome aberration and sister-chromatid exchange

studies in Chinese hamster ovary cells, and UDS studies in hepatocytes isolated from animals given a single oral dose of the nitrotoluenes.

MATERIALS AND METHODS

Procurement and Characterization of *o*-, *m*-, and *p*-Nitrotoluene

o-Nitrotoluene and *p*-nitrotoluene were obtained from Aldrich Chemical Co. (Milwaukee, WI), and *m*-nitrotoluene was obtained from Eastman Kodak Co. (Rochester, NY). Cumulative analytical data for each isomer indicated a purity of greater than 96%. Infrared, ultraviolet, and nuclear magnetic resonance spectra were consistent with the structure of the chemicals and with available literature references. The elemental analyses results for carbon, hydrogen and nitrogen agreed with theoretical values. No impurities greater than 1% relative to the major peak (either *o*-, *m*- or *p*-nitrotoluene) were observed by high performance liquid chromatography (HPLC). The bulk chemicals were stored at room temperature, protected from light. Quantitative reanalysis was undertaken at approximately 4-month intervals. Results from these HPLC analyses revealed no degradation of the bulk chemical during the course of the studies.

Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms, Inc. (Germantown, NY), and were placed on study at 6-8 weeks of age, following acclimation periods of 10-15 days. The animals were offspring of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. At the end of the acclimation period, selected animals were killed and evaluated for evidence of disease. Blood samples were collected and the sera analyzed for viral titers from 5 animals per sex per species at study start and at termination in the 13-week studies. No positive antibody titers were detected in 5 viral screens performed in rats and 12 viral screens performed in mice (Boorman *et al.*, 1986; Rao *et al.*, 1989a, 1989b).

Study Design

Rats were housed 5 per cage; mice were housed individually in all studies. Polycarbonate cages were used for both species. Animals were maintained in rooms at 66–79°F in 32-90% relative humidity with approximately 16-29 room air changes per hour. Fluorescent light was provided for 12 hours per day (See Table 3).

Control groups received NIH-07 feed (Zeigler Brothers, Gardners, PA) while treated groups received NIH-07 feed mixed with the appropriate concentration of *o*-, *m*-, or *p*-nitrotoluene. Dosed feed formulations for 13-week studies were measured regularly during the study period and found to be acceptable (± 10 percent of the target concentration) with the following exception: in the 13-week study in mice, a 1250 ppm mixture of *m*-nitrotoluene was analyzed at -10.4% of target concentration; because this feed/chemical mixture was to be given to animals for only 2 days prior to necropsy, the mixture was retained and used.

Prior to beginning the 14-day study, animals were assigned randomly to each dosage group using a computer-generated randomization procedure. Groups of 5 animals/sex/species received diets containing *o*-, m-, or *p*-nitrotoluene for 14 consecutive days. Rats received diets of *o*- or *m*-nitrotoluene at 0, 625[1] , 1250, 2500, 5000, or 10000 ppm; mice were given diets with *o*- or *m*-nitrotoluenes at 0, 388, 625[1], 1250, 2500, or 5000 ppm. Other groups of rats were given *p*-nitrotoluene at 0, 1250, 2500, 5000, 10000, or 20000 ppm; mice were given the *p*-isomer at 0, 675, 1250, 2500, 5000, or 10000 ppm.

Mortality/morbidity checks were performed twice a day; body weights were recorded at study start, after 1 week, and just before necropsy. All animals were examined for gross lesions at necropsy, and the liver and representative portions of gross lesions were examined microscopically from all dose groups and controls. Additionally, the testis/epididymis and uterus were examined in rats given *m*-nitrotoluene; the kidney, spleen, thymus, and stomach were evaluated microscopically in rats and mice given *p*-nitrotoluene.

Based on the results of the 14-day studies, a high dose of 10000 ppm was selected for the 13-week study in rats and mice. The 3 nitrotoluene isomers were given to groups of 10 animals/sex/dose/species, over an identical dose range in the 13-week studies (625, 1250, 2500, 5000, and 10000 ppm) to allow for comparison of toxic effects. In the 13-week studies, mortality/morbidity checks were performed twice a day; body weights were recorded at study start and weekly thereafter.

At the end of the 13-week studies, complete necropsies were performed on all animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed; organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all control animals, all early death animals, and all animals in the highest dose groups with at least 60% survivors. Target tissues were examined in animals from lower dose groups until a no-effect level was determined. All lesions observed at necropsy also were examined microscopically. Tissues and groups examined for both sexes of rats and mice are listed in Table 3. Additional sections of spleen from both dosed and control rats were stained for iron (Perl's stain); additional sections of kidney were stained with Periodic acid Schiff (PAS), with and without diastase; Hall's stain (bile); acid fast (lipofuscin); and Perl's stain. Sections of kidney from all dose and control groups of male rats were stained by the Mallory-Heidenhain method to evaluate the morphology of the protein droplets ("hyaline droplets") in the tubular epithelium and lumen.

Upon completion of the histologic evaluation by the laboratory pathologist, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. The results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

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[1] Study protocols called for a concentration of 625 ppm, but occasional batches were formulated to 675 ppm and were considered acceptable. This concentration group is designated as 625 ppm for the purposes of this report.

Kidney Total Protein and α–2u-Globulin Determination

The kidneys of male rats in the 13-week studies with *o*- and *p*-nitrotoluene were evaluated for α-2u globulin accumulation. To determine total protein and α-2u-globulin in the kidney, the entire right kidney from each male rat was homogenized with twice the organ volume of buffered saline (pH 7.2) at 4°C, then stored at -20°C until analysis. At that time, kidney homogenates were thawed, then centrifuged at 2000 RPM for 10 minutes. Total protein content in the supernatant was determined by the bicinchoninic acid assay (Kit No. BCA-1, Sigma, St. Louis, MO; Smith *et al.*, 1985). The amount of α-2u-globulin in the supernatant was determined by an enzyme-linked immunosorbant assay (ELISA) as described by Charbonneau *et al.* (1987). The standard α-2u-globulin and the antibody (a mouse immunoglobulin G raised toward rat α-2u-globulin) for ELISA were provided by Dr. S. Borghoff (Chemical Industry Institute of Toxicology, Research Triangle Park, NC). The second antibody (anti-mouse IgG), conjugated with alkaline phosphatase, was obtained from Sigma Co. (St. Louis, MO). Results were expressed as the ratio of α-2u-globulin to total protein in the supernatant. These assays were run parallel with the α-2u-globulin assays previously reported for *p*-chloro-α,α,α-trifluorotoluene (NTP, 1991).

Reproductive System Evaluations

Sperm morphology and vaginal cytology (SMVC) evaluations were performed in the 13-week studies for rats and mice given diets containing 1 of the 3 nitrotoluene isomers in doses of 0, 2500, 5000, or 10000 ppm. Procedures were according to methods described by Morrissey *et al.* (1988). For the 12 days prior to sacrifice, females were subject to vaginal lavage with saline. The relative preponderance of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were used to identify the stages of the estrual cycle.

Sperm motility was evaluated at necropsy as follows: the sperm that extruded from a small cut in the distal caudal epididymis were dispersed in solution, cover slipped, and counted. Two independent observers counted the number of moving and non-moving sperm in 5 fields of 30 sperm or less per field. After sperm-sampling for motility evaluation, the cauda was placed in phosphate buffered saline (PBS), and minced; the solution was mixed gently and heat-fixed at 65°C. Sperm density subsequently was determined using a hemocytometer.

To quantify spermatogenesis, the left testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the testis in PBS containing 10% DMSO. Homogenization-resistant spermatid nuclei were enumerated using a hemocytometer.

Clinical Chemistry and Hematology

For each isomer, clinical chemistry and hematology evaluations were conducted on special study male and female rats after 1 and 3 weeks of treatment and on core animals at termination. Animals were anesthetized with 70% CO_2/30% O_2 and bled from the retroorbital sinus using heparinized microcapillary tubes. For hematologic analyses, samples were collected in plastic

tubes containing potassium EDTA. Automated analyses were performed using a Coulter S-Plus IV (Hialeah, FL) and included erythrocyte, leukocyte, and platelet counts, hematocrit (HCT), hemoglobin (HGB) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Leukocyte differentials and morphologic evaluations of blood cells were determined from blood smears stained with Wright's stain. Reticulocytes were stained by mixing equal amounts of blood and new methylene blue and incubating the preparation for 20 minutes. Smears made from these preparations were examined microscopically for determination of reticulocyte counts. Methemoglobin concentrations were measured using a Co-Oximeter 482 (Instrumentation Laboratories, Lexington MA).

For clinical chemistry determinations, blood was collected in tubes devoid of an anticoagulant, allowed to clot, and centrifuged for the collection of serum. Assays for activities of alanine aminotransferase (ALT), alkaline phosphatase (AP), creatine kinase (CK), and concentrations of total protein, albumin, urea nitrogen (UN), and creatinine were performed using an Hitachi 737 chemistry analyzer (Indianapolis, IN) and reagents and methods from the manufacturer. Activities of SDH and concentrations of total bile acids were determined using a Baker Centrifichem 500 (Allentown, PA) and reagent kits obtained from Sigma Chemical Co. (St. Louis, MO).

Genetic Toxicity Studies

Mutagenicity Studies

Mutagenicity studies of the nitrotoluenes in *Salmonella typhimurium* were conducted as described in Haworth *et al.* (1983). Briefly, the nitrotoluenes were tested in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537, using a preincubation assay in both the absence or presence of Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. *o*- and *p*-Nitrotoluenes were tested at concentrations up to 1000 µg/plate, and m-nitrotoluene at up to 333 µg/plate; higher concentrations were toxic. A positive response was defined in this assay as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any single strain/activation combination. An equivocal response was defined as an increase in revertants which was not dose-related, not reproducible, or was of insufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies is observed following chemical treatment.

Induction of Trifluorothymidine (TFT) Resistance in Mouse Lymphoma L5178Y Cells

The experimental protocols and statistical methods were presented by McGregor *et al.* (1988). Mouse lymphoma L5178Y/TK$^{+/-}$ cells were maintained at 37°C as suspension cultures in supplemented Fischer's medium; normal cycling time was about 10 hours.

All treatment levels and controls within an experiment were replicated. Treated cultures contained 6 x 10^6 cells in a 10 ml volume of medium. Incubation with study chemical continued for 4 hours, at which time the medium plus chemical was removed, and the cells were

resuspended in 20 ml of fresh medium and incubated for an additional 2 days to express the mutant phenotype. Log phase growth was maintained. After the 48 hour expression period, cells were plated in medium and soft agar supplemented with TFT for selection of TFT-resistant cells ($TK^{-/-}$), and in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37°C in 5% CO_2 for 10-12 days.

Chinese Hamster Ovary Cytogenetics Assays

Testing was performed as reported by Loveday *et al.* (1989). Briefly, Chinese hamster ovary cells (CHO) were incubated with the nitrotoluenes or solvent (dimethyl sulfoxide) for induction of sister chromatid exchanges (SCE) and chromosomal aberrations (ABS) both in the presence and absence of Aroclor 1254-induced male Sprague Dawley rat liver S9 and cofactor mix. Cultures were incubated for sufficient time to reach first metaphase for chromosomal aberration analysis or second metaphase division for the sister chromatid exchange assay. Additional details are provided in Appendix D.

Unscheduled DNA Synthesis

The induction of unscheduled DNA synthesis (UDS) was evaluated in an *in vivo/in vitro* protocol according to the methods of Mirsalis *et al.* (1985). A summary of the methods and results of these studies is presented in Appendix E.

Statistical Methods

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971; 1972) and Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose-response (Dunnett, Dunn). If the P-value from Jonckheere's test was greater than or equal to 0.10, Dunn's or Dunnett's test was used rather than Shirley's or Williams' test.

The outlier test of Dixon and Massey (1951) was employed to detect extreme values. No value selected by the outlier test was eliminated unless it was at least twice the next largest value or at most half of the next smallest value.

Analysis of Vaginal Cytology Data

Since the data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for the simultaneous equality of measurements across dose levels.

Analysis of Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells

All data were evaluated statistically for both trend and peak response. Both responses had to be significant ($P < 0.05$) for a chemical to be positive for induction of TFT-resistance; a single significant response led to a "questionable" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call. Minimum criteria for accepting an experiment as valid, and a detailed description of the statistical analysis and data evaluation are presented in Caspary *et al.* (1988).

Analysis of CHO Cytogenetics Assays

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value has been shown to be a statistically conservative positive response in this assay (Margolin *et al.*, 1986). The probability of this level of difference occurring by chance at 1 dose point is less than 0.01; the probability for such a chance occurrence at 2 dose points is less than 0.001. A single increased dose was considered weak evidence of a positive response (+W); two increased doses were sufficient to evaluate the trial as positive (+).

Chromosomal aberration data is presented as percentage of cells with aberrations. Both the dose-response curve and individual dose points were statistically analyzed. For a single trial, a statistically significant ($P<.05$) difference for 1 dose point and a significant trend ($P<0.015$) was considered weak evidence for a positive response (W+); significant differences for two or more doses indicated the trial was positive (+) (Galloway *et al.*, 1987).

Quality Assurance

The studies of *o-*, *m-*, and *p*-nitrotoluenes were performed in compliance with FDA Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of Hazleton Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies. The operations of the Quality Assurance Unit were monitored by the NTP.

TABLE 3 Experimental Design of the Dosed Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes in F344/N Rats and B6C3F$_1$ Mice

EXPERIMENTAL DESIGN	Each nitrotoluene isomer was studied with both male and female F344/N rats and B6C3F$_1$ mice in separate 14-day and 13-week dosed-feed studies.
Size of Study Groups	14-Day Studies: 5 males and 5 females of each species /dose group/isomer. 13-Week Studies: 10 males and 10 females of each species/dose group/isomer (core study).
Doses/Duration of Access to Dosed Feed	14-Day Studies, *o*- and *m*-Nitrotoluene: Rats--0, 625*, 1250, 2500, 5000, or 10000 ppm *ad libitum*. Mice--0, 388, 625*, 1250, 2500, or 5000 ppm *ad libitum*. * Study protocols called for a concentration of 625 ppm, but occasional batches were formulated to 675 ppm and were considered acceptable. This concentration group is designated as 625 ppm for the purposes of this report. 14-Day Studies, *p*-Nitrotoluene: Rats--0,1250, 2500, 5000, 10000, or 20000 ppm *ad libitum* Mice--0, 675, 1250, 2500, 5000, or 10000 ppm *ad libitum*. 13 Week Studies, *o*-, *m*-, and *p*-Nitrotoluenes: All animals--0, 625, 1250, 2500, 5000, or 10000 ppm *ad libitum.*
Type and Frequency of Observation	14-Day Studies: observed 2x/day for mortality/moribundity; 1x/week for clinical signs of toxicity; weighed initially, after 1 week, and at necropsy. Feed consumption was measured weekly. 13-Week Studies: observed 2x/day for mortality/moribundity; body weight and clinical observations recorded weekly and at necropsy. Feed consumption measured weekly.
Necropsy and Histologic Examinations	13-Week Studies: Complete necropsy performed on all animals. Protocol-required tissues examined in all control animals, all early death animals, and all animals in the highest dose group with 60% survivors. The following tissues were examined: gross lesions, tissue masses or suspect tumors and regional lymph nodes, skin, mandibular and mesenteric lymph nodes, mammary glands with adjacent skin, salivary glands, thigh muscle, ileum, colon, cecum, rectum, liver, femur (to include diaphysis with marrow cavity and epiphysis), thymus, trachea, lungs and bronchi, heart, thyroid, parathyroids, esophagus, stomach, duodenum, jejunum, pancreas, spleen, kidneys, adrenal glands, urinary bladder, seminal vesicles, prostate, testes, epididymides, ovaries, uterus, nasal cavity and nasal turbinates, brain with stem, pituitary, preputial or clitoral glands. The following organs were weighed at termination of the study: Heart, liver (with gallbladder in mice), lungs, right kidney, thymus, and right testicle.
Clinical Chemistry/Hematology	13-Week Studies: Blood and serum samples analyzed from rats at 1 week, 3 weeks, and at the end of the 13-week studies.
Reproductive System Evaluations	13-Week Studies: Male and female rats and mice evaluated from the 0, 2500, 5000, and 10000 ppm dose groups.
ANIMALS AND ANIMAL MAINTENANCE	
Strain, Species, Source	F344/N rats; B6C3F$_1$ mice; Taconic Farms, Germantown, NY
Study Laboratory	Hazleton Laboratories, Rockville, Md
Time Held Before Study	14-Day Studies: rats--13-15 days; mice--13-14 days 13-Week Studies: rats--10-15 days; mice--12-14 days
Age When Placed on Study	14-Day Studies: rats--42 days; mice--42-49 days 13-Week Studies: rats--6 weeks; mice--6 weeks
Age When Killed	14-Day Studies: rats--56 days; mice--56-63 days 13-Week Studies: rats--19 weeks; mice--19 weeks
Method of Animal Distribution	Animals were weighed and randomized using a computer program

TABLE 3 Experimental Design of the Dosed Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes in F344/N Rats and B6C3F$_1$ Mice

ANIMALS AND ANIMAL MAINTENANC	
Diet	NIH-07 Open Formula (Ziegler Bros., Inc., Gardners, PA) *ad libitum*
Animal Room Environment	Rats housed 5/cage; mice housed individually; 66-79° F; 32-90% humidity; 12 hours fluorescent light/day; 16-29 air changes/hour.

RESULTS

In-Life Findings with *o*-, *m*-, and *p*-Nitrotoluenes in the 14-Day Studies in F344/N Rats

All rats given diets containing *o*-, *m*-, or *p*-nitrotoluene, and controls, survived to the end of the studies. Body weight gains of male rats (Table 4) were reduced in groups receiving the various isomers at 5000 ppm and higher concentrations. In addition, male rats receiving *m*-nitrotoluene at 2500 ppm gained less weight than controls, and males receiving *p*-nitrotoluene at 20000 ppm lost weight. Female weight gain was generally affected at higher nitrotoluene concentrations than males, with clear reductions in gain seen in females given *o*-nitrotoluene at 10000 ppm, *m*-nitrotoluene at 5000 ppm and above, and *p*-nitrotoluene at 10000 ppm; *p*-nitrotoluene at 20000 ppm caused weight loss (Table 5). Feed consumption was generally decreased at the higher dietary nitrotoluene concentrations, and was reduced in the *p*-nitrotoluene study at 20000 ppm to the extent that the estimated compound consumed did not differ markedly from that consumed by the 10000 ppm groups. This suggested poor palatability of the diets at the higher nitrotoluene concentrations. Other than the reduced body weight gains, there were no clear chemically related clinical signs in any of the studies.

Post-Life Findings with *o*-, *m*-, and *p*-Nitrotoluenes in the 14-Day Studies in F344/N Rats

o-Nitrotoluene

There were no gross lesions related to treatment. The only tissue examined microscopically was liver. In 4/5 male rats in the 10000 ppm group, there was minimal oval cell hyperplasia in the liver consisting of a proliferation of small cells with pale staining oval-shaped nuclei. These cells were dispersed between hepatocytes in the portal areas. No lesions were observed in the livers of female rats.

m-Nitrotoluene

At necropsy, chemically-related gross lesions were described as a reduction in size of the testis and uterus in rats from the 10000 ppm groups. The testis, epididymis, uterus, and liver from all rats were examined microscopically. All males in the highest dose group had a mild to moderate degeneration of the testis characterized by a loss of germinal epithelium and the presence of abnormal (syncytia) spermatids in the lumen of seminiferous tubules and ducts of the epididymis. One of 5 males in the 5000 ppm group had moderate testicular degeneration, but the lesion was unilateral, and thus the relationship of testicular degeneration to treatment at this exposure level was uncertain. By microscopic examination, the uteri of female rats in the highest-dose group were small; this was the result of thinner muscular walls and less development of the endometrium compared to controls and lower groups.

TABLE 4 Survival, Weight Gain, and Feed and Compound Consumption of Male F344/N Rats in the 14-Day Dosed Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes*

Dose (ppm) in Feed	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]	Average Feed Consumption[d]	Estimated Chemical Consumed[e]
***o*-NITROTOLUENE**							
0	5/5	142	217	75		15.6	0
625	5/5	149	219	70	100	16.7	56
1250	5/5	139	211	72	98	15.1	98
2500	5/5	132	205	73	95	13.6	178
5000	5/5	145	201	56	93	14.3	383
10000	5/5	141	174	33	80	11.4	696
***m*-NITROTOLUENE**							
0	5/5	140	227	87		17.5	0
625	5/5	138	218	79	96	17.5	61
1250	5/5	143	222	79	98	17.4	108
2500	5/5	135	206	71	91	16.0	259
5000	5/5	136	200	64	88	15.7	431
10000	5/5	133	171	38	75	14.1	881
***p*-NITROTOLUENE**							
0	5/5	162	235	73		16.9	0
1250	5/5	156	223	67	95	16.1	106
2500	5/5	160	229	69	97	16.4	211
5000	5/5	153	208	55	89	16.1	446
10000	5/5	162	176	14	75	12.2	723
20000	5/5	160	136	-24	58	6.4	869

* See bottom of page for key.

TABLE 5 Survival, Weight Gain, and Feed and Compound Consumption of Female F344/N Rats in the 14-Day Dosed Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes

Dose (ppm) in Feed	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]	Average Feed Consumption[d]	Estimated Chemical Consumed[e]
***o*-NITROTOLUENE**							
0	5/5	116	147	31		10.2	0
625	5/5	110	144	34	98	11.0	55
1250	5/5	114	144	30	98	11.0	102
2500	5/5	113	143	30	97	10.2	190
5000	5/5	113	144	31	98	10.4	382
10000	5/5	113	139	26	94	10.2	779
***m*-NITROTOLUENE**							
0	5/5	107	146	39		11.0	0
625	5/5	109	148	39	101	11.7	58
1250	5/5	106	146	40	100	12.4	114
2500	5/5	111	148	37	101	12.0	215
5000	5/5	110	136	26	93	10.8	420
10000	5/5	106	125	19	86	8.8	754
***p*-NITROTOLUENE**							
0	5/5	128	157	29		11.6	0
1250	5/5	126	158	32	101	11.9	105
2500	5/5	125	155	30	99	11.3	203
5000	5/5	125	147	32	94	11.0	404
10000	5/5	119	124	5	79	7.4	610
20000	5/5	126	97	-29	62	3.4	611

Key to Tables 4 and 5:

a Number surviving to study termination/number of animals per group.

b Mean weight change of the animals in each dosage group.

c (Mean weight of dose group/mean weight of control) X 100.

d 6 consumption measurements/14 days, compiled weekly, units of g/day.

e Expressed as mg/kg/day

p-Nitrotoluene

There were no treatment-related gross observations at necropsy. The liver, spleen, and stomach were examined microscopically from all rats, and the thymus was examined from all controls and the 3 highest dose groups. Increased congestion and extramedullary hematopoiesis were seen in the spleen of 1 male rat at 5000 ppm and in most males and females at 10000 and 20000 ppm. Lymphoid depletion occurred in the thymus and spleen of a few rats in the 10000 and 20000 ppm groups; this was attributed to the marked reduction in body weight gain and/or body weight loss during the study.

In-Life Findings with *o*-, *m*-, and *p*-Nitrotoluenes in the 13-Week Studies with F344/N Rats

All animals survived to the end of the studies with the 3 isomers (Tables 6, 7). Body weight gains of males and females given diets containing *o*-nitrotoluene were reduced in a dose-related fashion. Body weight gains of rats receiving *m*- or *p*-nitrotoluene were reduced in groups given diet containing 5000 or 10000 ppm. Feed consumption was less in the groups receiving the 10000 ppm of the nitrotoluenes compared to controls, and this effect was most evident in male rats receiving feed containing *o*-nitrotoluene. There were no other clinical signs of toxicity attributed to the nitrotoluenes.

Clinical Pathology and Post-Life Findings with *o*-, *m*-, and *p*-Nitrotoluenes in the 13-Week Studies with F344/N Rats

o-Nitrotoluene

After 1 week of treatment with *o*-nitrotoluene, increases in erythrocyte, leukocyte (lymphocyte), and platelet counts, HGB and methemoglobin concentrations, were detected in male rats primarily in the 10000 ppm dose group; methemoglobin, platelets, and leukocytes [lymphocytes] also were increased at 5000 ppm (Appendix B). (HCT was increased in males in the 10000 ppm dose group, but the increase was not statistically significant.) Reticulocyte counts were decreased in male rats in the 2 highest dose groups. In female rats, effects were confined to increases in methemoglobin concentration and platelet counts in animals in the highest and 2 highest dose groups, respectively. Biochemical changes in serum at this time included decreases in concentrations of total protein and albumin in male rats in all treatment groups and in female rats in the 2 highest dose groups.

In male and female rats, 3 weeks of treatment with *o*-nitrotoluene diets produced mild decreases in erythrocyte count, hemoglobin concentration, MCHC, and HCT (effects in highest [male] or 2 highest [female] dose groups) and moderate increases in platelet, nucleated erythrocyte (male only), and leukocyte (lymphocyte) counts and methemoglobin concentrations (3 to 4 highest dose groups). Biochemical findings in serum included decreases in concentrations of total protein and albumin and activities of AP in animals in multiple dose

TABLE 6 Survival, Weight Gain, and Feed and Compound Consumption of Male F344/N Rats in the 13-Week Dosed Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes

Dose (ppm) In Feed	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]	Average Feed Consumption[d]	Estimated Chemical Consumed[e]
***o*-NITROTOLUENE**							
0	10/10	113	353	240		16.5	0
625	10/10	108	339	231	96	16.1	45
1250	10/10	111	329	218	93	16.1	89
2500	10/10	113	309	196	88	15.8	179
5000	10/10	107	254	147	72	13.7	353
10000	10/10	101	198	97	56	11.4	694
***m*-NITROTOLUENE**							
0	10/10	119	346	227		16.3	0
625	10/10	123	354	231	103	16.5	46
1250	10/10	119	342	223	99	16.1	86
2500	10/10	123	353	230	102	16.3	171
5000	10/10	125	338	213	98	15.8	342
10000	10/10	123	281	158	81	13.4	661
***p*-NITROTOLUENE**							
0	10/10	136	350	214		16.4	0
625	10/10	145	349	204	100	15.9	42
1250	10/10	130	342	212	98	15.6	82
2500	10/10	133	341	207	97	15.8	165
5000	10/10	137	315	178	90	15.4	342
10000	10/10	138	253	115	72	14.1	723

* See bottom of page for key.

TABLE 7 Survival, Weight Gain, and Feed and Compound Consumption of Female F344/N Rats in the 13-Week Dosed Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes

Dose (ppm) In Feed	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]	Average Feed Consumption[d]	Estimated Chemical Consumed[e]
***o*-NITROTOLUENE**							
0	10/10	93	205	112		11.0	0
625	10/10	90	197	107	96	10.4	44
1250	10/10	93	192	99	94	10.3	87
2500	10/10	88	179	91	87	9.9	178
5000	10/10	90	170	80	83	9.5	340
10000	10/10	88	158	70	77	9.0	675
***m*-NITROTOLUENE**							
0	10/10	98	194	96		10.8	0
625	10/10	95	199	104	103	10.7	48
1250	10/10	96	194	98	100	10.3	87
2500	10/10	94	195	101	101	10.2	172
5000	10/10	95	177	82	91	9.4	336
10000	10/10	97	166	69	86	8.4	638
***p*-NITROTOLUENE**							
0	10/10	110	199	89		10.9	0
625	10/10	108	199	91	100	10.6	44
1250	10/10	116	200	84	101	10.7	82
2500	10/10	111	193	82	97	10.5	164
5000	10/10	106	182	76	91	10.0	335
10000	10/10	109	173	64	87	9.7	680

Key to Tables 6 and 7:

a Number surviving to study termination / number of animals per group.(

b Mean weight change of the animals in each dosage group.

c (Mean weight of dose group/mean weight of control) X 100.

d Based on 80 total consumption measurements/13 weeks, units of g/day.

e Expressed as mg/kg/day.

groups. Serum concentrations of bile acids were increased in male rats in the highest dose group.

After 13 weeks of treatment, mild decreases in erythrocyte count, HGB concentration, MCHC, and HCT were present in male and female rats in the highest 2 or 3 dose groups (Appendix B). Mild increases in MCV, MCH, platelet, reticulocyte, and leukocyte (lymphocyte) counts, and methemoglobin concentrations were associated with these changes in animals in many of the same dose groups. In male rats in the highest treatment group, there were mild increases in concentrations of total protein and albumin, while in female rats in the 2 highest treatment groups, concentrations of the same analytes were decreased. In animals in both sexes, mild to moderate increases occurred in concentrations of bile acids in the highest 1 or 2 dose groups. Additionally, in male rats only, there were mild increases in activities of ALT and SDH.

At necropsy, chemically-related gross lesions were observed in the liver, testis, and spleen of male rats. In all males from the 10000 ppm exposure group, the liver appeared larger and the testes smaller than in controls; alterations in the color (pale, mottled focus) of the liver and testis were also observed. In 5 males from the highest exposure group, the spleen appeared darker and/or slightly thicker than in controls. Absolute and relative liver weights were increased with increasing doses of *o*-nitrotoluene in male and female rats (Appendix A). There was a decrease in relative testis weight in the 10000 ppm dose group, and absolute epididymal weights were markedly lower than controls in animals in the 2500, 5000, and 10000 ppm groups (Appendix C). The organ-to-body-weight ratios of several organs including heart, lungs, and thymus were higher than controls in several dosed groups, which was attributed to the lower body weights of animals in these groups, and the normally higher organ-weight-to-body-weight ratios in smaller animals. The relative weight of the kidney was increased in dosed animals of both sexes, more so in males than females.

Treatment-related histopathologic effects were identified in the liver, kidney, spleen, testis, and epididymis of males and in the kidney and spleen of females (Table 8). Liver lesions occurred only in males at dose levels of 2500 ppm and higher; these consisted of cytoplasmic vacuolization, oval cell hyperplasia, and inflammation (Plates 1-3). Cytoplasmic vacuolization was characterized by multiple rounded to oval shaped clear spaces of varying size in hepatocytes throughout the liver lobule. This was slightly more prominent in the portal areas. Oval cell hyperplasia consisted of an increased number of small cells with pale staining cytoplasm and round to oval-shaped nuclei. These cells were generally interspersed between hepatocytes in single or double rows but sometimes formed small nodules or ductular structures in the portal area of the liver lobule. An accentuated lobular pattern was evident when the proliferation of oval cells extended between portal areas of adjacent lobules (Plate 2). Inflammation consisted of a minimal to mild focal infiltration of inflammatory cells in the liver. This was similar to the focal aggregates of mononuclear inflammatory cells which are occasionally seen in portal areas of control and treated rats. Focal inflammation was sometimes associated with areas of oval cell hyperplasia but was also seen as foci that were randomly scattered throughout the liver. The relationship of the inflammation to treatment was less clear than for the other hepatic lesions. Although the incidence of inflammation was slightly increased in male rats at the higher exposures, the severity was similar among all dosed and control groups.

TABLE 8 Lesions in F344/N Rats Receiving *o*-Nitrotoluene for 13 Weeks[a]

Dose (ppm)	0	625	1250	2500	5000	10000
MALE						
Liver						
oval cell hyperplasia	0	0	0	2 (1.0)	10 (1.2)	10 (2.2)
vacuolization	0	0	0	6 (1.3)	9 (1.8)	10 (3.0)
inflammation	5 (1.8)	5 (1.0)	5 (1.6)	10 (1.5)	10 (1.8)	8 (1.8)
Kidney						
nephropathy, hyaline droplet	0	0	6 (1.0)	10 (1.6)	10 (2.8)	9 (2.6)
regeneration	2 (1.0)	6 (1.0)	2 (1.0)	2 (1.0)	5 (1.0)	6 (1.1)
pigment	0	0	0	0	1 (1.0)	10 (1.0)
Spleen						
hematopoiesis	0	0	0	6 (1.3)	10 (2.0)	10 (2.0)
hemosiderin pigment	0	0	0	7 (1.3)	10 (2.0)	10 (2.0)
capsular fibrosis	0	0	1 (1.0)	1 (2.0)	1 (1.0)	9 (1.9)
Testis						
degeneration	0	0	0	0	10 (2.3)	10 (4.0)
Epididymis						
mesothelial hyperplasia	0	0	0	0	0	2
mesothelioma	0	0	0	0	3	0
FEMALE						
Kidney						
pigment	0	0	0	3 (1.0)	10 (1.1)	10 (1.8)
Spleen						
hematopoiesis	0	0	0	0	1 (1.0)	10 (1.0)
hemosiderin pigment	0	0	0	5 (1.0)	9 (2.0)	10 (2.0)
capsular fibrosis	0	0	0	0	1 (1.0)	2 (1.0)

[a] Incidence and severity score ( ) based on a scale of 1 to 4; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 10.

Treatment-related lesions occurred in the kidney of male and female rats. In both sexes of rats, there was an accumulation of brown pigment globules in the cytoplasm of the tubular epithelium of the renal cortex (Plate 4). This pigment, possibly lipofuscin, stained positive with PAS but did not stain with iron or bile stains. In addition to the presence of pigment, in the kidney of male rats there was a hyaline droplet nephropathy in groups fed diets containing 1250 ppm or more *o*-nitrotoluene. Hyaline droplet nephropathy was characterized by an accumulation of eosinophilic, crystalline-shaped, or amorphous to spherical, proteinaceous droplets (globules) in the cytoplasm or lumen of the renal tubules. Using the Mallory-Heidenhain stain for protein, these droplets in dosed rats appeared intensely eosinophilic, similar to the staining observed for the smaller, more uniform (size and shape) protein "resorption droplets" typically present in the kidney of control male rats (Plates 5-6). Microscopic features of more severe hyaline droplet nephropathy, such as necrosis and regeneration of renal tubular epithelium, the presence of granular casts in tubule lumen at the junction of the inner and outer stripe of the outer medulla, and focal mineralization, did not occur in this study. Although there was a slight increase in the incidence of tubular cell regeneration in the kidney of some groups of dosed male rats, the severity was not increased, and the increased incidence was not dose-related (Table 8).

To confirm that hyaline droplets represented an accumulation of α–2u globulin, this protein was measured by an ELISA technique in homogenates of kidneys of male rats from the 13-week *o*-

and *p*-nitrotoluene studies (Table 9). Increased levels of α–2u globulin were measured in the kidney of male rats from 3 highest *o*-nitrotoluene exposure groups (Table 9).

TABLE 9 α–2u Globulin Concentrations in Kidneys of Male Rats[a]

Dietary Concentration (ppm)	*o*-Nitrotoluene	*p*-Nitrotoluene
0	6.7	7.2
625	6.4	16.6
1250	7.6	14.1
2500	14.3	13.7
5000	15.0	15.1
10000	32.0	20.3

[a] Percent of supernatant protein.

Treatment-related microscopic lesions also were identified in the spleen of male and female rats from groups receiving 2500 ppm or greater *o*-nitrotoluene (Table 8). These were characterized by an increase in hematopoiesis and accumulation of hemosiderin (iron-positive) pigment in the red pulp to a greater extent than normally occurs in the spleen of rats. Both hematopoiesis and hemosiderin pigment increased in incidence and/or severity with increasing dose. In addition, there was capsular fibrosis of the spleen in 9/10 male rats and 2/10 female rats in the 10000 ppm group. This lesion consisted of a focal, irregular thickening of the splenic capsule as result of fibrosis. Associated with these focal areas of fibrosis was a minimal focal hypertrophy and hyperplasia of the mesothelial cells on the serosal surface of the spleen.

Degeneration of the testis occurred in all male rats from the 5000 and 10000 ppm groups (Plates 7-8). In high dose rats there was almost complete absence of germinal epithelium in the seminiferous tubules; the few remaining cells lining the basal lamina of seminiferous tubules were primarily Sertoli cells. Focal mineralization of cell debris was present in a few seminiferous tubules of the more severely affected males in the high dose group. In rats with testicular degeneration, the ducts of the epididymis contained cellular debris and the staining intensity of the prostatic and seminal vesicular secretions was reduced. For sperm evaluations, a decrease in epididymal sperm motility and concentration and in testicular spermatid count was seen in animals in the 5000 and 10000 ppm groups (Appendix C). In females, there was a increase in estrous cycle length and, among the highest dose group (10000 ppm), only 4/10 animals had a measurable estrous cycle (Appendix C). There were no histopathologic, treatment-related effects in the uterus or ovaries.

Two male rats of the 10000 ppm *o*-nitrotoluene group had mesothelial cell hyperplasia of the tunica vaginalis on the surface of the epididymis, and mesotheliomas occurred at the same anatomic location in 3 male rats from the 5000 ppm group. Hyperplasia consisted of relatively small, focal proliferation of mesothelial cells on the surface of the epididymis. In these focal areas, there was minimal or no development of a supporting fibrovascular stroma for the proliferation of mesothelial cells. The mesotheliomas were of a relatively larger size and consisted of prominent, densely cellular focal proliferations arising from the tunica vaginalis (Plates 9-10). The cuboidal to polyhedral-shaped, neoplastic mesothelial cells formed solid sheets of cells as well as prominent papillary or villous projections on the surface of the tunica

vaginalis. A distinct fibrous stroma was present in the villous projections; mitotic figures were present in the tumor cells, and there was evidence of local invasion/infiltration into the stroma and fat of the tunica vaginalis. Metastatic foci were not observed microscopically. Mesotheliomas have not been previously identified in dosed or control rats from any of the 13-week toxicity studies conducted by the National Toxicology Program.

***m*-Nitrotoluene**

One week of treatment with *m*-nitrotoluene produced mild increases in erythrocyte count, HGB concentration (males only), and HCT in male and female rats in the highest dose group (Appendix B). Other relevant findings were mild decreases in reticulocyte and platelet counts and activity of AP and mild increases in concentrations of UN, creatinine, and albumin.

After 3 weeks of treatment with *m*-nitrotoluene, erythrocyte count, HGB concentration, and HCT were decreased in male rats in most dose groups and in female rats in the highest dose group. Increases in reticulocyte, nucleated erythrocyte, and platelet (males only) counts and methemoglobin concentrations also were present in the highest dose groups of both sexes. Mild increases in lymphocyte counts were seen in high dose male and female rats; high dose females had increases in leukocyte counts. Serum biochemical effects in male rats consisted of minimal decreases in concentrations of UN; increases in concentrations of creatinine were noted in male and female rats. Additionally, a mild increase in activity of ALT occurred in female rats in the 3 highest dose groups.

At the end of 13 weeks, erythrocyte counts, HGB concentration, and HCT were decreased in female rats in the highest 1 or 2 dose groups; in male rats, only erythrocyte counts were decreased in the highest treatment group. Increases occurred in MCV, MCH, reticulocyte and platelet counts, and methemoglobin concentrations in rats of both sexes in high dose groups (5000 and 10000 ppm). Biochemical changes in serum were limited to mild to moderate increases in bile acid concentrations in male and female rats in the 5000 and/or 10000 ppm groups.

At necropsy, the only treatment-related gross lesions were seen in the 10000 ppm group of male rats. In 4/10 rats, the testes and epididymes were smaller than in controls. Relative liver weights were moderately increased in males and females in the highest dose group (Appendix A), and relative kidney weights were increased in the top 2 dose groups in both sexes. The relative testis weight was substantially less than controls in the 10000 ppm group.

There was a treatment-related hyaline droplet nephropathy in the kidney of male rats. This was characterized by the presence of eosinophilic protein droplets in the renal tubular epithelium and tubule lumen. The droplets were irregular-shaped and increased in size and number as compared to the protein "resorption droplets" typically present in the kidney of control male rats. Hyaline droplet nephropathy was graded a minimal severity in all dosed groups, but the number of protein droplets increased with dose. Necrosis and increased regeneration of the renal tubular epithelium, granular casts, and focal mineralization were not associated with the hyaline droplet nephropathy in this study. A no-effect-level for the hyaline droplet nephropathy

was not achieved. The amount of α–2u globulin in the kidneys of animals in the *m*-nitrotoluene study was not measured.

An increase in hemosiderin pigment and congestion of the spleen was observed in treated male and female rats when compared to controls; both were of minimal to mild severity (Table 10). Congestion was diagnosed when the vascular spaces of the red pulp were distended with erythrocytes; increased hemosiderin pigment was diagnosed when the brown (iron-positive) pigment in macrophages of the red pulp exceeded the amount normally seen in the spleen of control rats.

TABLE 10 Lesions in F344/N Rats Receiving *m*-Nitrotoluene for 13 Weeks[a]

Dose (ppm)	0	625	1250	2500	5000	10000
MALE						
Kidney						
nephropathy, hyaline droplet	0	9 (1.0)	10 (1.0)	10 (1.0)	10 (1.0)	10 (1.0)
Spleen						
hemosiderin pigment	0	1 (1.0)	0	2 (1.0)	5 (1.0)	10 (1.4)
congestion	1 (1.0)	0	0	1 (1.0)	0	9 (1.0)
Testis						
degeneration	0	0	0	0	0	10 (2.2)
FEMALE						
Spleen						
hemosiderin pigment	1 (1.0)	9 (1.1)	10 (1.1)	10 (1.2)	8 (1.5)	10 (1.2)
congestion	0	0	0	0	2 (1.0)	9 (1.0)

[a] Incidence and severity score ( ) based on a scale of 1 to 4; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 10.

All male rats from the 10000 ppm group displayed mild to moderate degeneration of the testis, characterized by a reduction of germ cells and mature spermatids in the seminiferous tubules; cellular debris was present in the ducts of the epididymis. This testicular lesion was accompanied by a reduction in epididymal sperm count and concentration (Appendix C). Among females there was a dose-related decrease in the length of estrous and an increase in the period of diestrus. The length of the estrous cycle increased while the number of cycling animals diminished (Appendix C). There were no gross or histopathologic effects on the uterus or ovaries in this 13-week study.

p-Nitrotoluene

One week of treatment with *p*-nitrotoluene produced mild increases in erythrocyte count, HGB concentration, and HCT in male and female rats in the 5000 and/or 10000 ppm dose groups (Appendix B). Reticulocyte counts were decreased in rats in the highest dose groups of both sexes; platelet counts were decreased in the 3 highest dose groups of female rats; methemoglobin concentrations were not affected. Activity of ALT in serum was increased in female rats in the 2 highest dose groups.

After 3 weeks of treatment, there were mild decreases in erythrocyte counts in male rats and in HGB concentrations in female rats, both in high dose groups. In male rats there were increases in MCV and MCH and, in animals of both sexes, in counts of nucleated erythrocytes. Biochemical findings were limited to animals in the top dose groups, and included mild increases in activity of ALT in male and female rats and in concentrations of bile acids in female rats.

At the end of 13 weeks, erythrocyte count, HGB concentration, and HCT (females only) were decreased in rats in multiple dose groups. Additionally, there were increases in MCV, reticulocyte count (female rats only), methemoglobin concentration, and numbers of nucleated erythrocytes. Biochemically, there were minimal increases in serum UN and creatinine and mild increases in concentrations of bile acids in male rats in the 10000 ppm dose group. Total protein concentrations decreased in male and female rats in multiple dose groups.

At necropsy, the relative liver and kidney weights of males at the 2 highest doses, and females at the highest dose, were mildly increased compared to controls (Appendix A). Unlike with the *o*- and *m*-nitrotoluenes, relative testis weights were unchanged in dosed animals in the *p*-nitrotoluene studies. Potential treatment-related gross lesions were limited to 2 males from the 10000 ppm exposure group which had small testes.

Microscopically, treatment-related lesions were found in the kidney, spleen, and testis of rats (Table 11). In male rats, there was a dose-related increase in hyaline droplet nephropathy, karyomegaly, and pigment in the kidney. As described in previous sections, hyaline droplet nephropathy was characterized by the presence of an increase in the number and size of abnormally shaped eosinophilic protein droplets within the cytoplasm and lumen of renal tubules. These droplets stained intensely eosinophilic with the Mallory-Heidenhain stain for protein. Measurement of α-2u-globulin content of the kidney confirmed an increased accumulation of this protein in dosed male rats (Table 9). Karyomegaly was present in the proximal tubular epithelium of dosed male and female rats. This minimal to mild nuclear enlargement was most prominent in the 5000 ppm and 10000 ppm groups. A yellow to brown pigment also was present in the cytoplasm of renal tubule cells of dosed male and female rats; this pigment, possibly lipofuscin, was PAS-positive; it did not stain with acid-fast, iron, or bile stains.

A retrospective examination was performed on kidney sections of male and female rats from all dose groups in the 14-day study of *p*-nitrotoluene. Hyaline droplet nephropathy was present in all dose groups of male rats but was most prominent in the 1250, 2500, and 5000 ppm groups. The reason for the decreased prominence of hyaline droplets in the 10000 and the 20000 ppm groups relative to that seen in the lower doses could not be determined. It is possible the marked decreased body weight gain or actual body weight loss which occurred during 14-day study in these 2 high-dose groups may have resulted in decreased production of α-2u globulin by the liver and a reduction in the amount available for resorption by the kidney. There was no evidence of necrosis and regeneration of tubular epithelium; granular casts or focal mineralization were not present in the 14-day study. Karyomegaly and pigment observed in the 13-week study were not present in the kidney of male or female rats from the 14-day study.

Also in the 13-week study for male and female rats, there was an increase in hematopoiesis, hemosiderin pigment, and congestion compared to that which is typically seen in the spleen of control rats (Table 11). Degeneration of the testis (minimal to mild) was seen in high-dose male rats; this was characterized by the absence of spermatogenesis, decreased number of germinal epithelial cells, and the presence of syncytial giant cells (degenerate spermatids) in a few seminiferous tubules, usually at the periphery of the testis. Epididymal sperm concentration and testicular spermatid head count were reduced in high dose males but to a lesser extent than with the *o*- or *m*-nitrotoluenes (Appendix C). Among females, 9/10 in the 10000 ppm group did not have a discernible estrous cycle. There were no gross or histopathologic changes in the uterus or ovaries at the end of this 13-week study.

Table 11 Lesions in F344/N Rats Receiving p-Nitrotoluene for 13 Weeks[a]

Dose (ppm)	0	625	1250	2500	5000	10000
MALE						
Kidney						
nephropathy, hyaline droplet	0	10 (1.0)	10 (1.0)	10 (1.0)	10 (2.0)	10 (2.0)
karyomegaly	0	0	3 (1.0)	5 (1.0)	10 (2.0)	10 (2.0)
pigment	0	0	0	0	0	10 (1.0)
Spleen						
hematopoiesis	0	6 (1.0)	9 (1.0)	10 (1.1)	10 (1.2)	10 (2.2)
hemosiderin pigment	0	6 (1.0)	8 (1.0)	10 (1.1)	9 (1.3)	10 (2.4)
congestion	0	8 (1.0)	10 (1.0)	9 (1.0)	10 (1.0)	10 (2.0)
Testis						
degeneration	0	0	1 (2.0)	0	1 (2.0)	4 (1.8)
FEMALE						
Kidney						
karyomegaly	0	10 (1.0)	10 (1.0)	10 (2.0)	10 (2.0)	10 (2.0)
pigment	0	10 (1.0)	10 (1.0)	10 (1.0)	10 (2.0)	10 (2.0)
Spleen						
hematopoiesis	0	4 (1.0)	4 (1.7)	5 (1.2)	9 (1.2)	10 (1.8)
hemosiderin pigment	0	5 (1.0)	6 (1.0)	10 (1.6)	10 (1.9)	10 (2.0)
congestion	0	4 (1.0)	6 (1.0)	10 (1.0)	10 (1.2)	10 (2.0)

[a] Incidence and severity score ( ) based on a scale of 1 to 4; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 10.

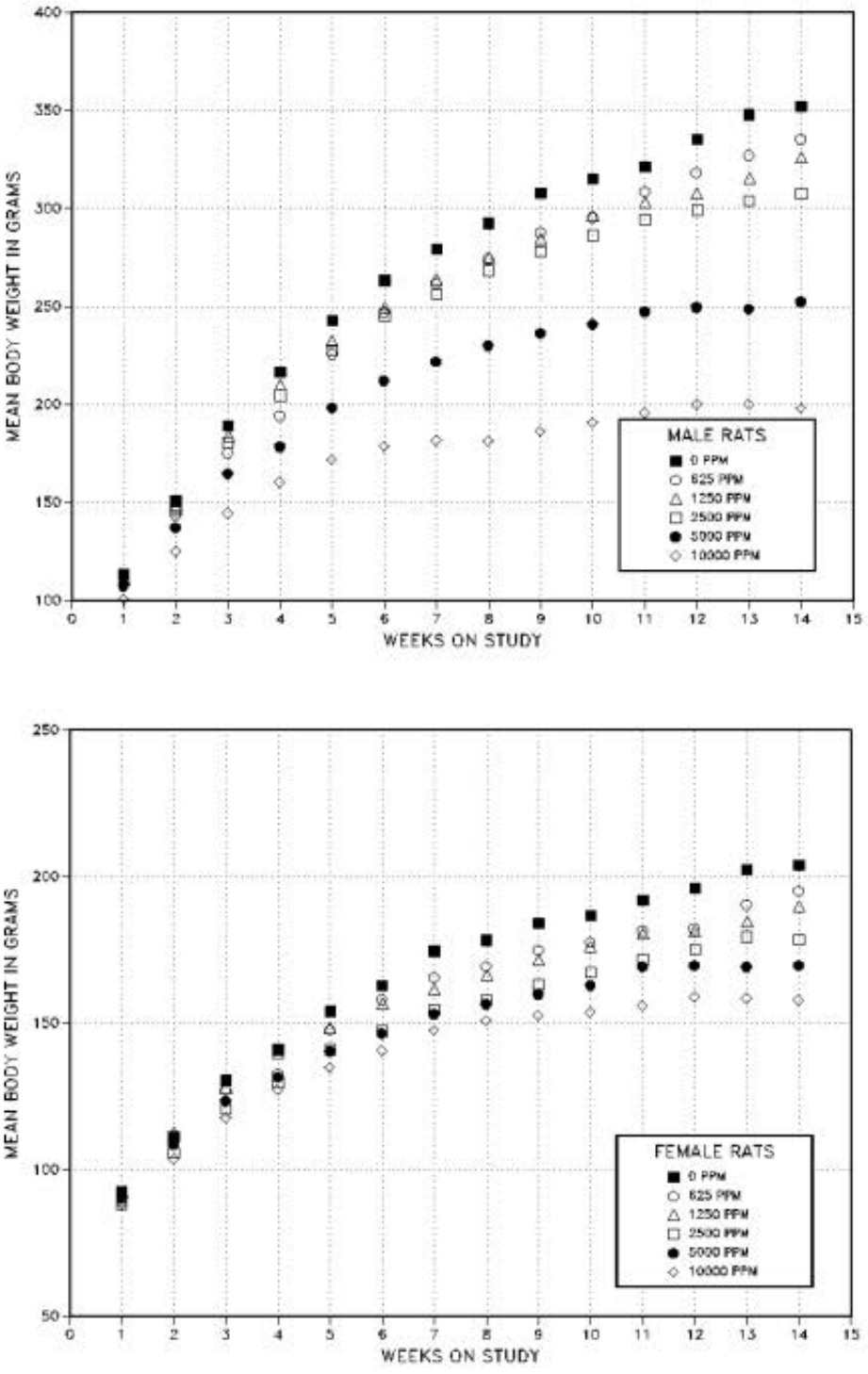


Figure 2 Body Weights of F344/N Rats Exposed to *o*-Nitrotoluene by Dosed Feed for 13 Weeks

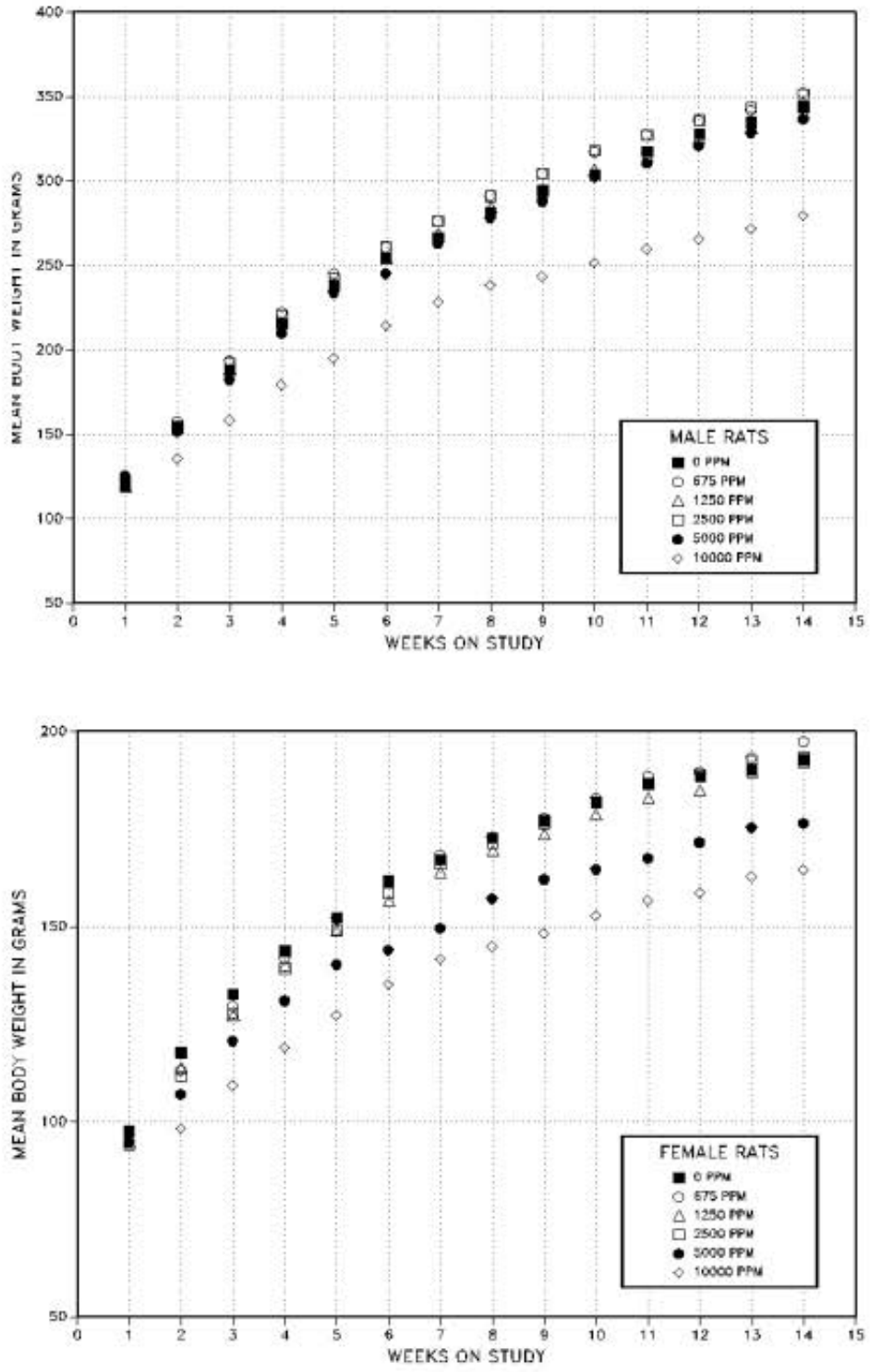


Figure 3 Body Weights of F344/N Rats Exposed to *m*-Nitrotoluene by Dosed Feed for 13 Weeks

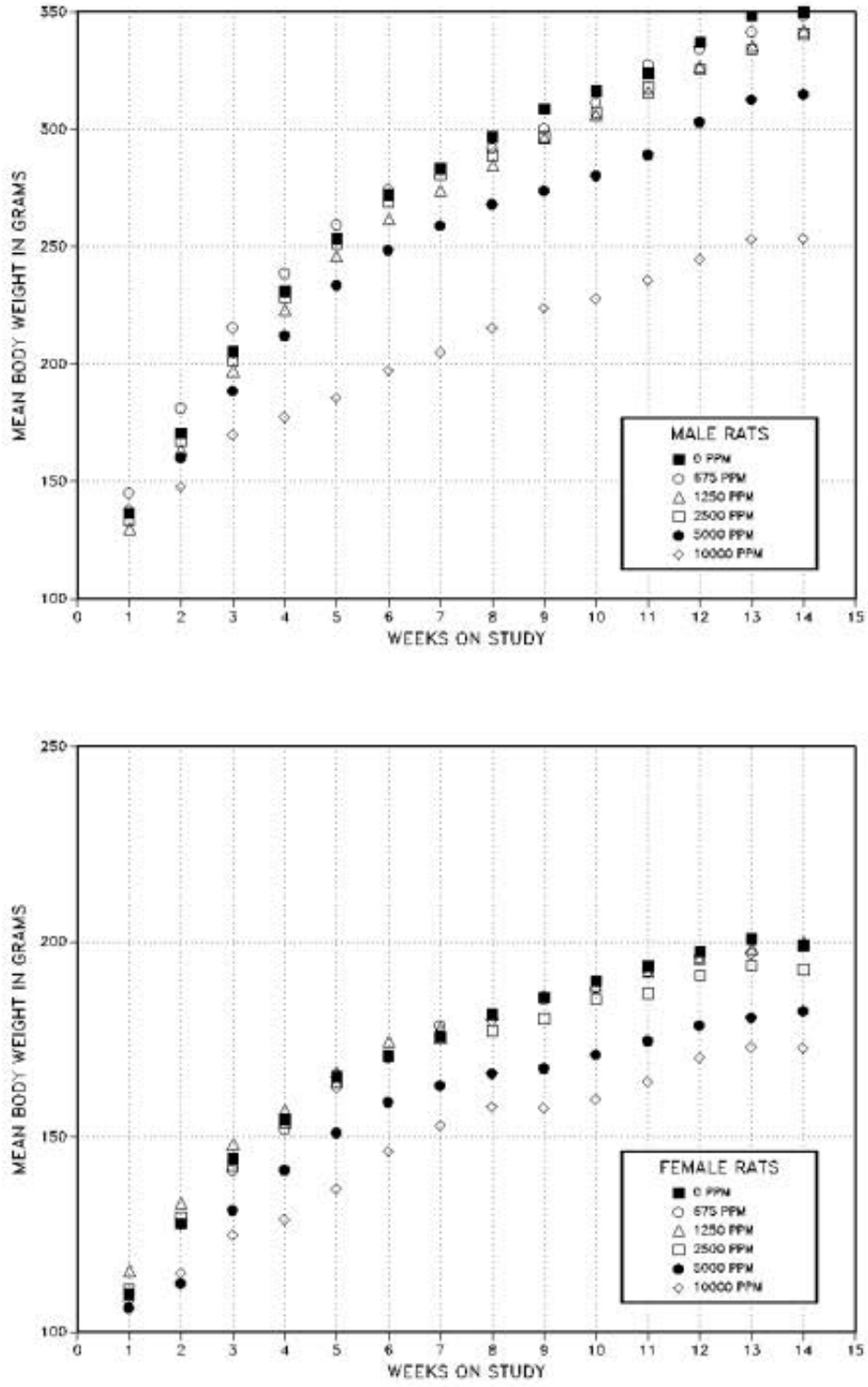


Figure 4 Body Weights of F344/N Rats Exposed to *p*-Nitrotoluene by Dosed Feed for 13 Weeks

PLATES

Plate 1. Liver from control male rat; compare with Plate 2. (H&E 35X)

Plate 2. Liver from male rat exposed to 10000 ppm *o*-nitrotoluene. Note the pale vacuolated appearance of hepatocytes and the accentuated lobular pattern (arrows) resulting from oval cell proliferation. (H&E 35X)

Plate 3. Detail of the [ ] area from Plate 2 showing cytoplasmic vacuolization of hepatocytes (arrows) and proliferation of oval cells in the periportal area. (H&E 170X)

Plate 4. Kidney from female rat exposed to 10000 ppm *o*-nitrotoluene. Note intensely positive staining pigment droplets (arrows) in the cytoplasm of the tubular epithelial cells. (PAS 170X)

Plate 5. Kidney from control male rat showing normal appearance of protein droplets (arrows) in the tubular epithelial cells; compare with appearance of protein droplets in kidney from dosed male rat in Plate 6. (Mallory-Heidenhain 170X)

Plate 6. Kidney from male rat exposed to 10000 ppm *o*-nitrotoluene. Large, rounded to irregular-shaped protein droplets (arrows) are present in tubule lumina and the cytoplasm of the tubular epithelial cells. (Mallory-Heidenhain 170X)

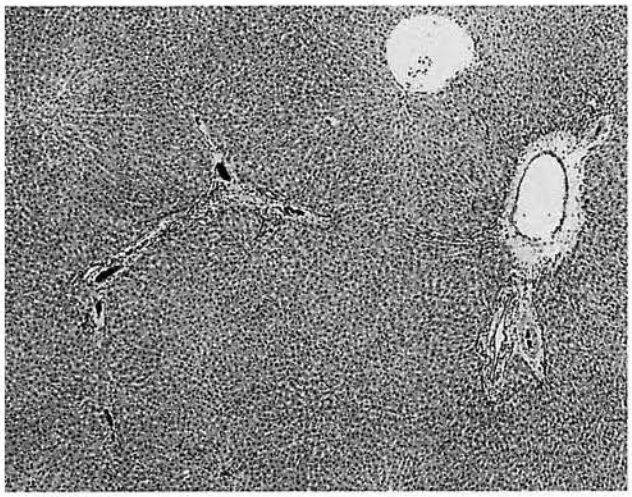


PLATE 1

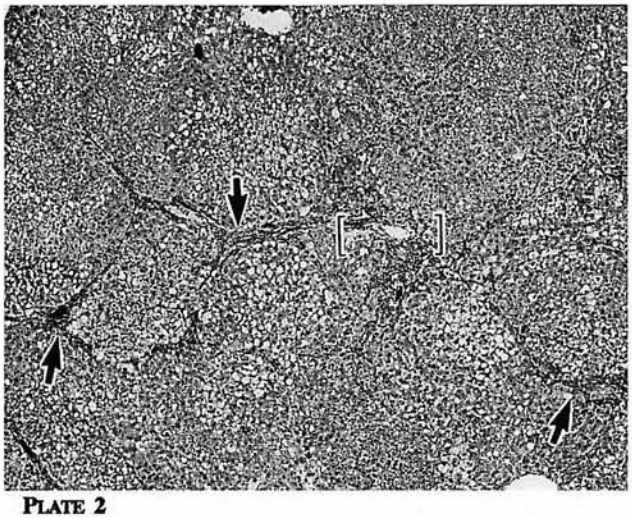
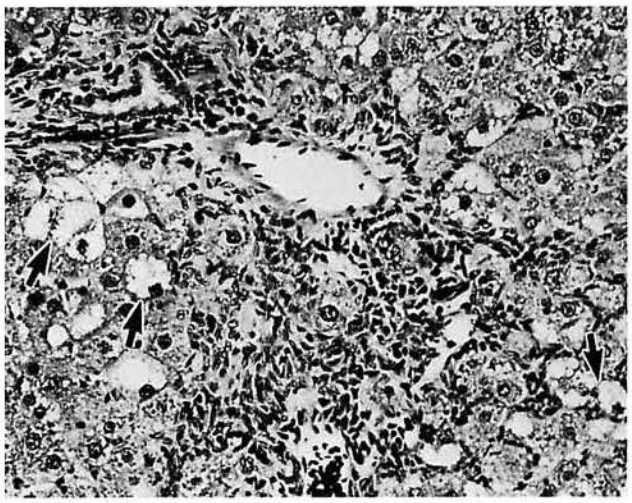


PLATE 2



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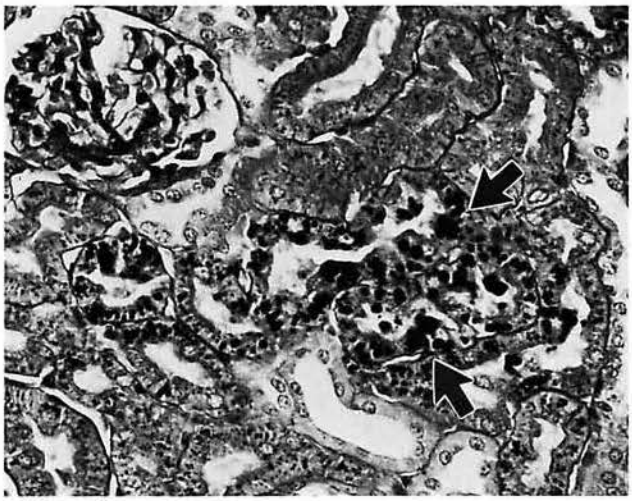

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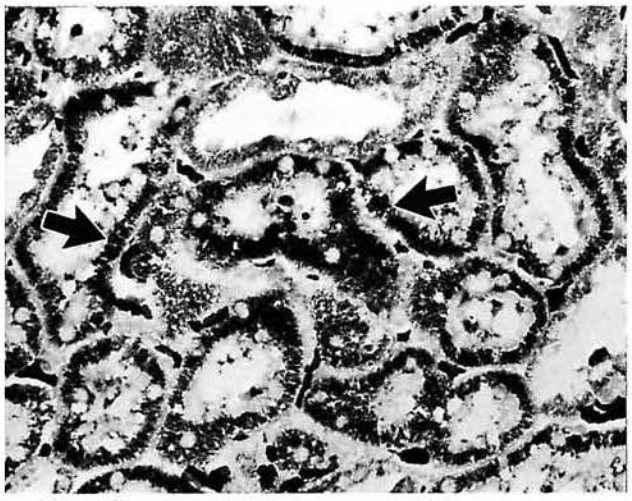


PLATE 5

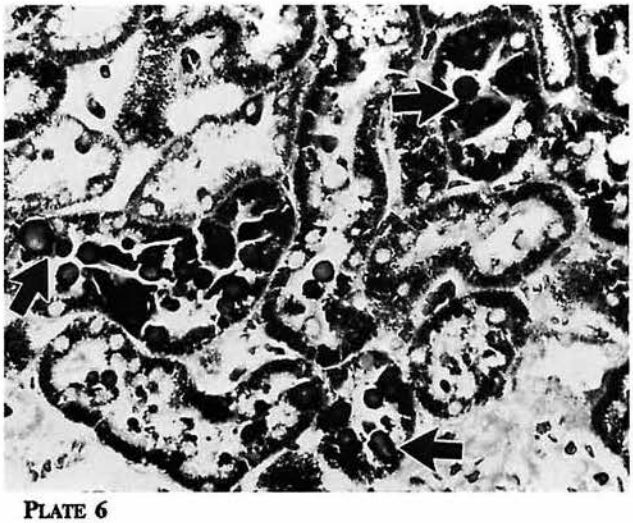


PLATE 6

PLATES

Plate 7. Testis from control male rat showing normal appearance of seminiferous tubule; compare with Plate 8. (H&E 170X)

Plate 8. Testis from male rat exposed to 10000 ppm *o*-nitrotoluene showing marked degeneration of the germinal epithelium. Seminiferous tubules are lined by a few vacuolated Sertoli cells and there is no evidence of spermatogenesis. (H&E 170X)

Plate 9. Epididymis (E) from male rat exposed to 5000 ppm *o*-nitrotoluene showing mesothelioma as a densely cellular mass with a papillary/villous surface arising from tunica vaginalis. See Plate 10 for detail of the [ ] area. (H&E 55X)

Plate 10. Detail of [ ] area from Plate 9 showing typical cuboidal to polyhedral shaped cells along the surface of the tumor (top of photo) and the solid sheet of neoplastic cells surrounding lipocytes of the epididymal fat. Note mitotic figures (arrows). (H&E 230X)

Plate 11. Olfactory mucosa along the dorsal meatus in control male mouse. Note the normal appearance of the olfactory epithelium (arrows) and prominent nerve (N) bundles in the lamina propria. (H&E 170X)

Plate 12. Olfactory mucosa along dorsal meatus in male mouse exposed to 10000 ppm *o*-nitrotoluene. As compared with control in Plate 11, there is marked degeneration and loss of the olfactory epithelium. There is hyper-plasia and dilatation of Bowman's glands (arrows) in the lamina propria and atrophy of the nerve bundles. (H&E 170X)

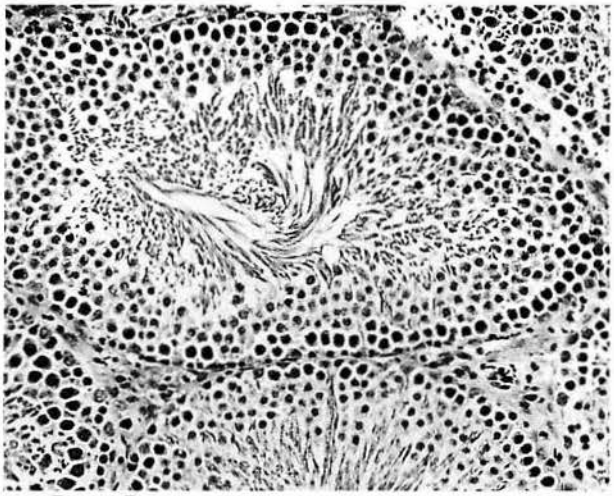
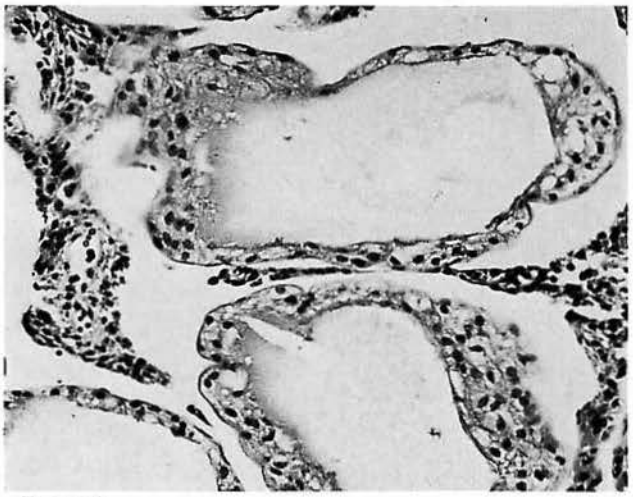


PLATE 7



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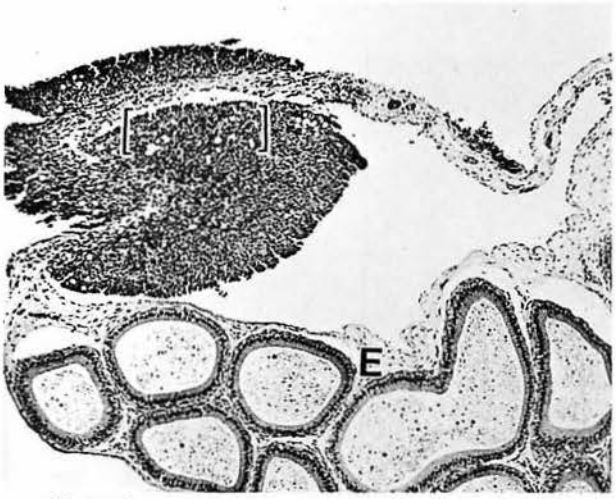

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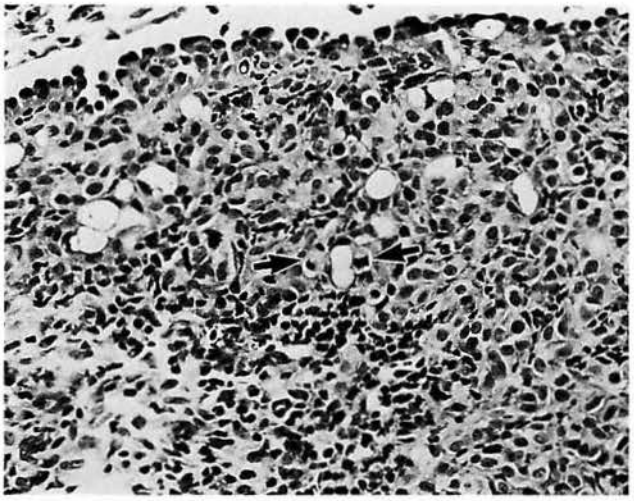

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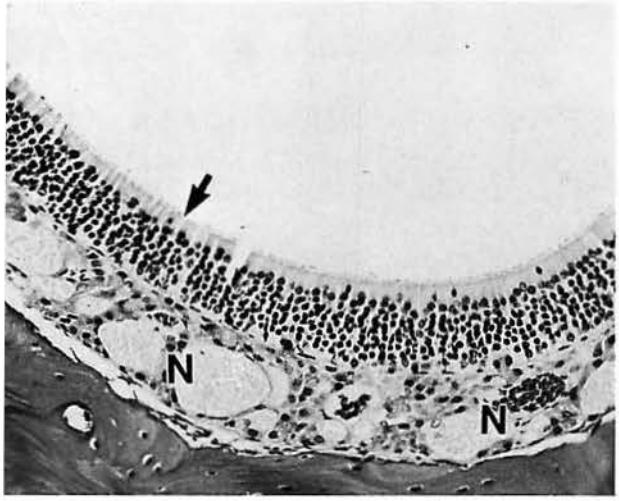


PLATE 11

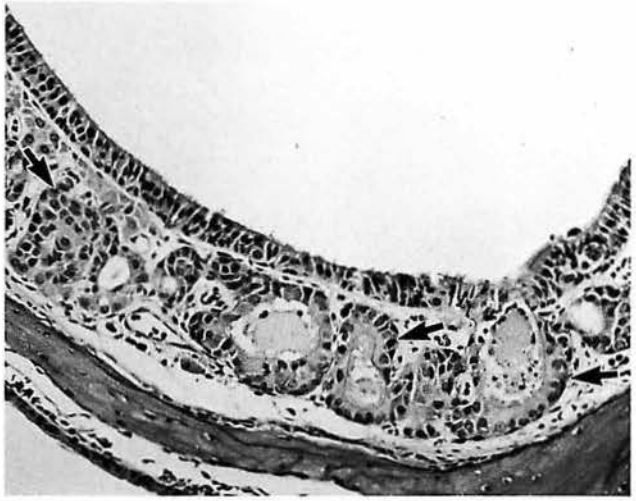


PLATE 12

In-Life Findings with *o*-, *m*-, and *p*-Nitrotoluenes in the 14-Day Studies in B6C3F$_1$ Mice

All mice survived to the end of the 14-day studies with the nitrotoluene isomers (Tables 12,13). Body weight gains of mice were variable but appeared less than controls in mice given the diet with the highest concentration of each isomer (5000 ppm for *o*- and *m*-nitrotoluene, and 10000 ppm for *p*-nitrotoluene). Other than effects on body weight, there were no clear chemically related, clinical signs of toxicity. Feed consumption was quite high for certain groups. This typically indicates the presence of mice within these groups that habitually scatter feed.

Post-Life Findings with *o*-, *m*-, and *p*-Nitrotoluenes in the 14-Day Studies in B6C3F$_1$ Mice

At necropsy, liver weights were increased somewhat in the top 3 dose groups of males receiving *o*-nitrotoluene. No other gross or microscopic findings were attributed to chemical administration.

Relative liver weights were increased in females given feed containing all but the lowest concentration of *m*-nitrotoluene and in males given 2500 or 5000 ppm. However, no chemically related gross or microscopic lesions were observed.

The relative liver weights were increased in a dose-related fashion in all groups of males and in all but the low dose group of females receiving *p*-nitrotoluene. Increased hematopoiesis of the spleen was seen in a few mice from several groups, including controls; however, it was not dose-related in severity or incidence and was not considered to be related to chemical administration.

TABLE 12 Survival, Weight Gain, and Feed and Compound Consumption of Male B6C3F$_1$ Mice in the 14-Day Dosed Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes*

Dose (ppm) In Feed	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]	Average Feed Consumption[d]	Estimated Chemical Consumed[e]
***o*-NITROTOLUENE**							
0	5/5	23.0	23.4	0.4		4.6	0
388	5/5	23.2	25.0	1.8	107	4.0	63
675	5/5	23.8	25.1	1.3	107	3.8	106
1250	5/5	23.0	24.7	1.7	105	4.0	204
2500	5/5	23.1	24.3	1.2	104	3.9	405
5000	5/5	23.8	24.1	0.3	103	4.0	854
***m*-NITROTOLUENE**							
0	5/5	24.5	26.3	1.8		5.0	0
388	5/5	25.4	26.8	1.4	102	4.5	66
675	5/5	25.2	27.0	1.8	103	4.4	113
1250	5/5	24.8	25.6	0.8	97	4.3	212
2500	5/5	24.7	25.8	1.1	98	4.2	409
5000	5/5	25.5	25.8	0.3	98	3.9	779
***p*-NITROTOLUENE**							
0	5/5	24.9	26.1	1.2		13.8	0
675	5/5	24.8	26.7	1.9	102	7.7	202
1250	5/5	24.3	26.1	1.8	100	8.0	397
2500	5/5	24.5	26.5	2.0	102	6.0	588
5000	5/5	25.0	26.1	1.1	100	4.7	920
10000	5/5	24.6	24.5	-0.1	94	3.8	1548

* See key below.

TABLE 13 Survival, Weight Gain, and Feed and Compound Consumption of Female B6C3F$_1$ Mice in the 14-Day Dosed Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes

Dose (ppm) In Feed	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]	Average Feed Consumption[d]	Estimated Chemical Consumed[e]
***o*-NITROTOLUENE**							
0	5/5	17.6	20.5	2.9		6.2	0
388	5/5	18.1	20.9	2.8	102	7.1	134
675	5/5	18.3	20.8	2.5	101	6.5	217
1250	5/5	17.5	20.5	3.0	100	6.3	397
2500	5/5	17.9	20.5	2.6	100	5.0	631
5000	5/5	18.3	19.8	1.5	97	4.7	1224
***m*-NITROTOLUENE**							
0	5/5	19.5	22.1	2.6		5.2	0
388	5/5	20.3	21.9	1.6	99	5.1	92
675	5/5	20.3	23.0	2.7	104	5.5	164
1250	5/5	19.6	21.2	1.6	96	5.0	297
2500	5/5	19.5	22.3	2.8	101	4.7	543
5000	5/5	19.9	21.3	1.4	96	3.8	901
***p*-NITROTOLUENE**							
0	5/5	20.3	22.0	1.7		14.7	0
675	5/5	19.3	22.0	2.7	100	11.9	388
1250	5/5	19.0	21.9	2.9	100	10.6	647
2500	5/5	19.8	22.5	2.7	102	6.4	755
5000	5/5	19.3	21.1	1.8	96	5.1	1262
10000	5/5	19.4	20.4	1.0	93	4.0	2010

Key to Tables 12 and 13:

a Number surviving to study termination / number of animals per group.

b Mean weight change of the animals in each dosage group.

c (Mean weight of dose group/mean weight of control) X 100.

d 6 consumption measurements/14 days, compiled weekly, units of g/day.

e Expressed as mg/kg/day.

In-Life Findings with *o*-, *m*-, and *p*-Nitrotoluenes in the 13-Week Studies in B6C3F$_1$ Mice

All animals survived to the end of the studies (Tables 14, 15). Body weight gains of dosed male and female mice were decreased relative to controls in the 2 highest dose groups with each isomer (Figures 5, 6, and 7); feed consumption also was decreased in these groups. There were no clinical signs attributed to administration of the nitrotoluenes.

Clinical Pathology and Post-Life Findings with *o*-, *m*-, and *p*-Nitrotoluenes in the 13-Week Studies in B6C3F$_1$ Mice

o-Nitrotoluene

At necropsy, no gross lesions related to treatment were found; relative liver weights were increased in males and females in the top 3 and 4 dose groups respectively (Appendix A). Relative kidney weights were somewhat decreased in males and increased in females with increasing dose. Relative lung weights were increased in females in the 5000 and 10000 ppm groups.

Microscopically, nasal lesions were seen in the olfactory epithelium of male and female mice, primarily in the 5000 and 10000 ppm groups; lesions were present in a few mice in the 2500 and 1250 ppm groups. This olfactory epithelial lesion was most commonly present in the region of the dorsal meatus (Level II); however, in high dose mice with more severe lesions, the olfactory epithelium in Level III also was affected. The lesion, diagnosed as degeneration/ metaplasia, had multiple components (Plates 11-12). There was moderate thinning of the olfactory epithelial nuclear layer; frequently ciliated columnar cells (respiratory metaplasia) had replaced the olfactory epithelium in this location. Marked decrease (atrophy) was noted in the size and number of nerve bundles in the lamina propria in areas of olfactory degeneration. In the areas of olfactory epithelial degeneration, the underlying Bowman's glands in the lamina propria were dilated and the lumen often contained eosinophilic cellular debris and a few inflammatory cells. In dosed female mice there was a slight increase compared to controls in the number of eosinophilic protein globules within the cytoplasm of the respiratory epithelium adjacent to the areas of olfactory degeneration.

Sperm motility was significantly decreased in mice from the 10000 ppm group compared to controls. No other parameters in males or females were affected in evaluations of the reproductive system (Appendix C).

TABLE 14 Survival, Weight Gain, and Feed and Compound Consumption of Male B6C3F$_1$ Mice in the 13-Week Dosed Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes

Dose (ppm) In Feed	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]	Average Feed Consumption[d]	Estimated Chemical Consumed[e]
***o*-NITROTOLUENE**							
0	10/10	21.0	33.3.	13.3		4.5	0
625	10/10	21.6	34.5	12.9	104	4.5	104
1250	10/10	21.1	32.9	11.8	99	4.7	223
2500	10/10	20.9	32.8	11.9	99	4.3	415
5000	10/10	21.1	29.2	8.1	88	3.8	773
10000	10/10	20.7	25.9	5.2	78	3.4	1536
***m*-NITROTOLUENE**							
0	10/10	22.2	36.3	14.1		4.7	0
0625	10/10	22.0	34.8	13.8	96	4.7	114
1250	10/10	21.8	34.6	12.8	96	4.6	208
2500	10/10	22.1	34.7	12.6	96	4.4	398
5000	10/10	21.8	31.0	9.2	88	3.8	743
10000	10/10	21.6	27.7	6.1	76	3.4	1422
***p*-NITROTOLUENE**							
0	10/10	20.9	33.5	12.6	100	5.3	0
625	10/10	20.8	33.1	12.3	99	5.1	131
1250	10/10	21.3	33.8	12.5	101	4.5	212
2500	10/10	20.6	32.3	11.7	97	4.5	439
5000	10/10	21.3	31.1	9.8	93	4.1	813
10000	10/10	21.1	29.9	8.8	89	3.7	1491

* See key below.

TABLE 15 Survival, Weight Gain, and Feed and Compound Consumption of Female B6C3F$_1$ Mice in the 13-Week Dosed Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes

Dose (ppm) In Feed	Survival[a]	Mean Body Weight (grams) Initial	Final	Change[b]	Final Weight Relative to Controls (%)[c]	Average Feed Consumption[d]	Estimated Chemical Consumed[e]
***o*-NITROTOLUENE**							
0	10/10	18.1	32.8	14.7		5.3	0
625	10/10	18.3	34.0	15.7	104	5.1	132
1250	10/10	17.9	33.4	15.5	101	5.2	268
2500	10/10	18.1	32.1	14.0	98	5.3	542
5000	10/10	18.1	29.5	11.4	90	4.7	1007
10000	10/10	18.1	22.9	4.8	70	3.4	1712
***m*-NITROTOLUENE**							
0	10/10	19.2	33.4	14.2		5.1	0
625	10/10	19.1	34.3	15.2	103	5.2	139
1250	10/10	18.8	34.2	15.4	102	5.1	254
2500	10/10	19.1	32.6	13.5	98	4.9	493
5000	10/10	18.5	29.6	11.1	88	4.2	884
10000	10/10	18.7	24.3	5.6	72	3.4	1550
***p*-NITROTOLUENE**							
0	10/10	17.6	29.6	12.0		6.5	0
625	10/10	18.2	33.0	14.8	112	5.8	164
1250	10/10	18.4	33.3	14.9	113	6.2	320
2500	10/10	17.2	27.9	10.7	94	5.6	625
5000	10/10	17.3	27.1	9.8	92	4.8	1075
10000	10/10	17.6	24.9	7.3	84	3.5	1634

Key to Tables 14 and 15

a Number surviving to study termination / number of animals per group.
b Mean weight change of the animals in each dosage group.
c (Mean weight of dose group/mean weight of control) X 100.
d Based on 80 total consumption measurements/13 weeks, g/day.
e Expressed as mg/kg/day.

m-Nitrotoluene

At necropsy, dose-related increases in relative liver weights were noted in both males and females (Appendix A), and relative lung weights were increased in the top dose groups of each sex. Despite these organ weight changes, no gross or microscopic lesions related to treatment with *m*-nitrotoluene were seen in either male or female mice. Also, no changes were noted in reproductive system evaluations in male or female mice (Appendix C).

p-Nitrotoluene

At necropsy, relative liver weights showed dose-related increases in all groups of males and females (Appendix A). There were no gross or microscopic treatment-related lesions seen in either males or females given *p*-nitrotoluene. *p*-Nitrotoluene had no adverse effects on measured reproductive parameters (Appendix C).

TABLE 16 Lesions in B6C3F$_1$ Mice Receiving *o*-Nitrotoluene for 13 Weeks[a]

Dose (ppm)	0	625	1250	2500	5000	10000
MALE						
Nose						
olfactory epithelium, degeneration/metaplasia	0	0	1 (1.0)	2 (1.0)	10 (2.0)	10 (3.0)
FEMALE						
Nose						
olfactory epithelium, degeneration/metaplasia	0	0	2 (1.5)	9 (1.0)	10 (1.9)	10 (2.9)

[a] Incidence and severity score ( ) based on a scale of 1 to 4; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked. Severity scores are averages based on the number of animals with lesions from groups of 10.

Genetic Toxicity

The 3 nitrotoluene isomers were tested for induction of gene mutations in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 in a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat and Syrian hamster liver S9. There was no observed increase in mutant colonies following treatment with any of the isomers (Appendix D, Tables D1, D2, D3; Haworth *et al.*, 1983). Test concentrations were limited by toxicity to 1000 µg/plate for *o*- and *p*- isomers, and to 333 µg/plate for the *m*-isomer. Only *p*-nitrotoluene was tested for induction of gene mutations in mouse lymphoma L5178Y/tk$^{+/-}$ cells; a positive response was obtained in trials conducted with Aroclor 1254-induced male Fisher rat liver S9 (Appendix D, Table D4).

The 3 isomers were tested for induction of sister-chromatid exchanges (SCE) (Appendix D, Table D5, D6, D7) and chromosomal aberrations (ABS) (Appendix D, Table D8, D9, D10) in Chinese hamster ovary cells with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Galloway *et al.*, 1987). All 3 nitrotoluene isomers induced SCE, although requirements for S9

varied; only the *p*-isomer induced chromosomal aberrations. The positive ABS response occurred at the highest dose tested in each of 2 trials conducted with S9; a delayed harvest protocol was used to offset chemical-induced cell-cycle delay. The cytotoxicity of *p*-nitrotoluene may be a factor to consider in the interpretation of the positive ABS response; however, cytotoxicity was also evident in the absence of S9, and no increase in ABS was observed under those conditions.

In an *in vivo/in vitro* unscheduled DNA synthesis assay (Appendix E), positive results were found with hepatocytes isolated from both sexes of rats and female mice after receiving a single *in vivo* oral gavage dose of *o*-nitrotoluene. UDS was not increased in a similar study with male rats given *m*- or *p*-nitrotoluene. S-phase DNA synthesis was increased in hepatocytes of female rats given single injections of *o*-nitrotoluene at doses ranging from 200 to 750 mg/kg, and in male rats at the highest dose tested (500 mg/kg, data not shown). No increases in S-phase synthesis were seen in mice.

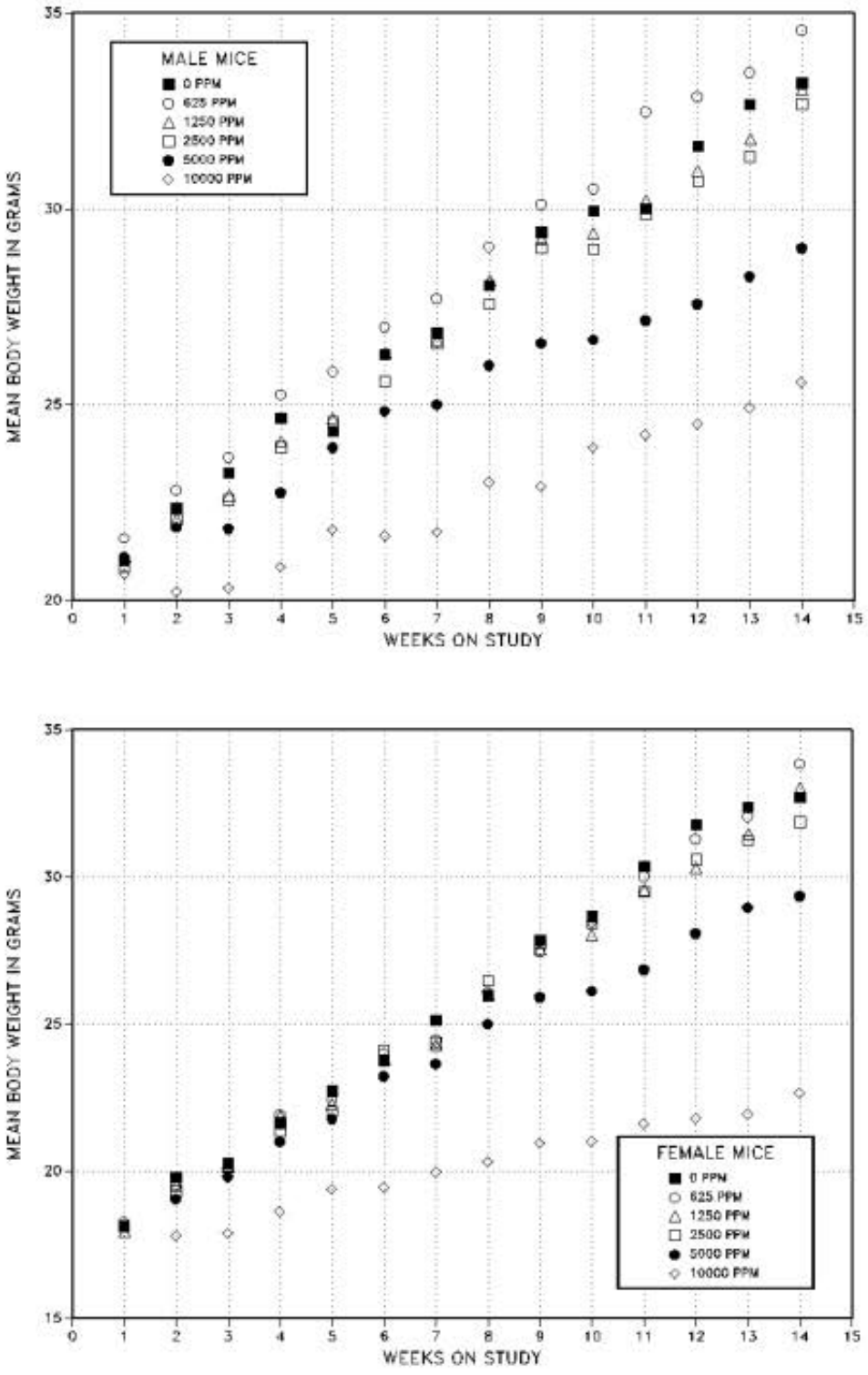


Figure 5 Body Weights of B6C3F$_1$ Mice Exposed to *o*-Nitrotoluene by Dosed Feed for 13 Weeks

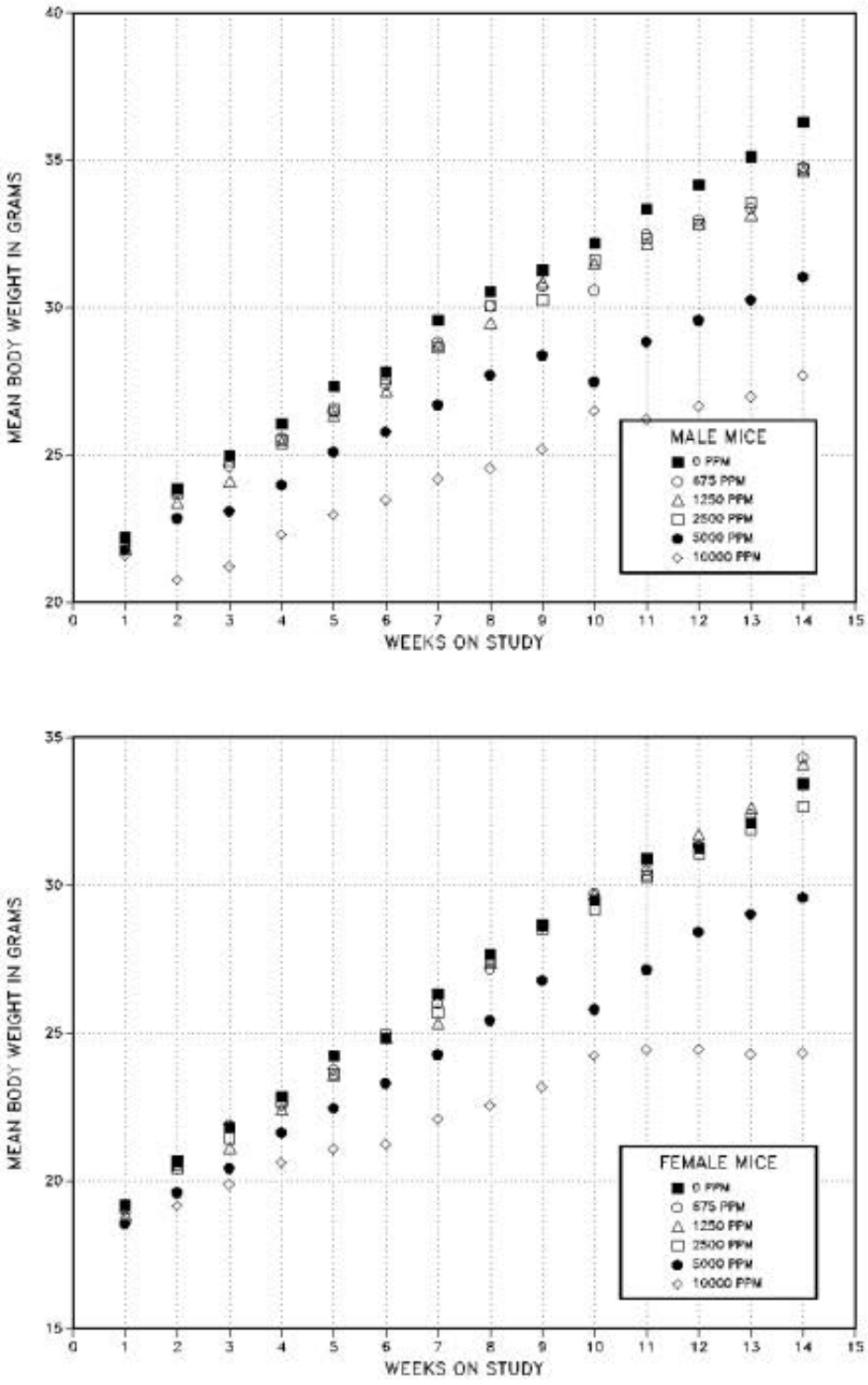


Figure 6 Body Weights of B6C3F$_1$ Mice Exposed to *m*-Nitrotoluene by Dosed Feed for 13 Weeks

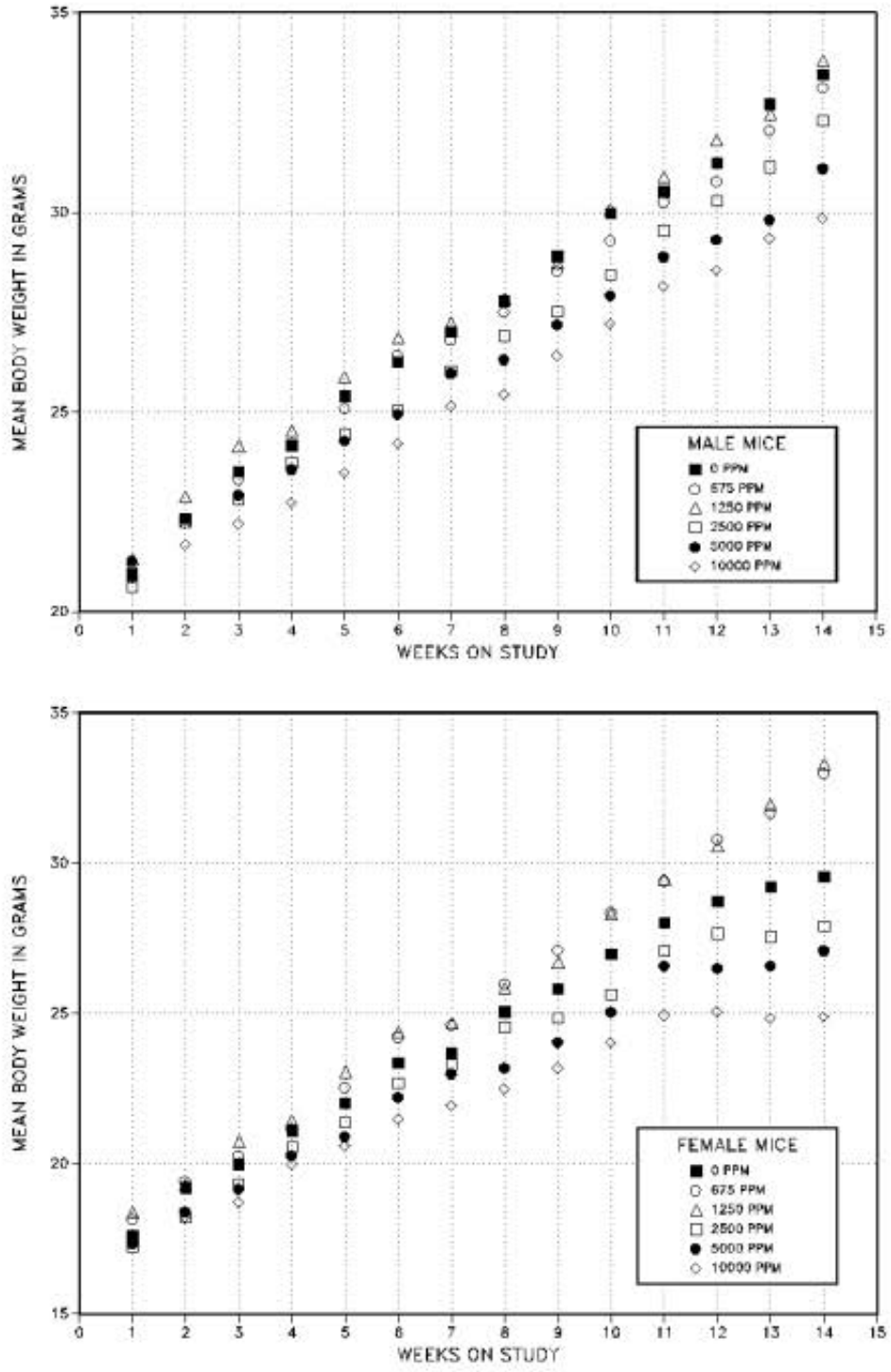


Figure 7 Body Weights of B6C3F$_1$ Mice Exposed to *p*-Nitrotoluene by Dosed Feed for 13 Weeks

DISCUSSION

The studies described in this report were conducted to obtain further information on the comparative toxicity of *o*-, *m*-, and *p*-nitrotoluene in the F344/N rat and B6C3F$_1$ mouse. Previous studies with isomers of nitrotoluene have shown that there are differences in their metabolism; it was hypothesized that the *o*-nitrotoluene isomer might be more toxic and/or carcinogenic than the other isomers because through its metabolism, an electrophile can be formed that can interact with DNA and protein (Butterworth *et al.*, 1989; Rickert *et al.*, 1984b).

In the 14-day and 13-week studies with all isomers, there was no evidence of mortality, or clinical signs of toxicity other than effects on body weight and feed consumption. In the 13-week studies, all isomers were evaluated in rats and mice over the same concentration range (625-10000 ppm) to obtain comparative toxicity data. Estimated amounts of the *o*-, *m*-, or *p*-isomers consumed ranged from approximately 40 to 900 mg/kg body weight/day for rats and 100 to 2000 mg/kg/day for mice; all animals received 625 to 10000 ppm of the chemical mixed with feed. The amount of chemical consumed was similar for each of the 3 isomers when compared for each dose level/sex/species. Decreased body weight gain was a common finding with all 3 isomers, but was most pronounced in rats receiving *o*-nitrotoluene (Table 17) and in males more than females. Differential palatability of the feed mixes could have contributed to the observed reduction in feed consumption and weight gain.

Hepatic Toxicity

After 1 and 3 weeks' treatment of rats with *o*-nitrotoluene, decreased concentrations of total serum protein were due to moderate decreases in albumin. Because of the absence of evidence for other hepatic effects at 1 week, it is unlikely that decreased hepatic function or increased catabolism were involved. The probable explanation for the decrease in albumin is a reduction in absorption of amino acids caused by malabsorption or malnutrition (decreased feed intake). Decreased activities of AP, which generally accompany reductions in feed intake, did not occur until 3 weeks. In male rats given 10000 ppm *o*-nitrotoluene, a mild increase in serum bile acid concentration was noted without evidence of hepatocellular necrosis or enzyme leakage (no change in activities of ALT or SDH). This was consistent with cholestasis and decreased hepatocellular function. In female rats, there were minimal increases in serum activities of ALT in animals given *m* - and *p* -nitrotoluenes and mild increases in bile acids in animals females given *p*-nitrotoluene. Although minimal hepatocellular enzyme leakage was detected in female rats, the predominant finding, as in male rats, remained one of decreased function and/or cholestasis.

By the end of 13 weeks, the predominant biochemical effect in the serum of male and female rats was mild to moderate increases in bile acid concentrations in animals in the top 1 or 2 dose groups with all 3 nitrotoluene isomers (except *p*-nitrotoluene in female rats). These changes were associated with minimal increases in ALT, only, in male rats given *o*-nitrotoluene. As at 3

weeks, these findings are consistent with a primary lesion of cholestasis and/or decreased hepatocellular function. No morphologic change in hepatocytes was seen in rats

TABLE 17 Summary of Selected Treatment-Related Effects in the 13-Week Nitrotoluene Studies

	o-NITROTOLUENE		*m*-NITROTOLUENE		*p*-NITROTOLUENE	
	Male	Female	Male	Female	Male	Female
RATS						
Final Body Weight (90% or less than control)	↓(3) a	↓(3)	↓(5)	↓(5)	↓(5)	↓(5)
Liver						
Relative weight	↑(1)	↑(1)	↑(5)	↑(5)	↑(4)	↑(5)
ALT	↑(4)	–	–	↑ (4)	–	↑ (5)
SDH	↑(3)	–	–	–	–	–
Bile Acids	↑(4)	↑(5)	↑(4)	↑(5)	↑(5)	–
Nonneoplastic lesions	+(3)	–	–	–	–	–
Kidney						
Relative weight	↑(3)	↑(2)	↑(5)	↑(4)	↑(4)	↑(5)
Nonneoplastic lesions	+(2)	+(3)	+(1)	–	+(1)	+(1)
Spleen						
Hematology	(3)	(3)	(4)	(4)	(3)	(3)
Nonneoplastic lesions	+(2)	+(3)	+(3)	+(3)	+(1)	+(1)
Testis						
Sperm count	↓(4)		↓(5)		↓(5)	
Nonneoplastic lesions	+(4)		+(5)		+(5)	
Mesothelium						
Neoplastic and preneoplastic lesions	+(4)		–		–	
Estrous cycle length		↑(5)		↑(4)		↑(5)
MICE						
Final Body Weight (90% or less than control)	↓(3)	↓(3)	↓(5)	↓(5)	↓(5)	↓(5)
Nose						
Nonneoplastic lesions	+(2)	+(2)	–	–	–	–
Liver						
Relative Weight	↑(3)	↑(2)	↑(1)	↑(1)	↑(1)	↑(1)

a Lowest dose group in which an effect was seen, 1 = 625 ppm; 2 = 1250; 3 = 2500; 4 = 5000; 5 = 10000.
+ Presence of treatment-related histopathology.

given the *m-* or *p*-nitrotoluenes or in mice given any of the nitrotoluene isomers, although liver weights of rats and mice were increased in all studies.

Treatment-related microscopic lesions in the liver were seen only in male rats receiving *o*-nitrotoluene at dietary levels of 2500 ppm and above (Table 17) and consisted primarily of cytoplasmic vacuolization and oval cell hyperplasia. The cytoplasmic vacuolization may have been related to accumulation of water or lipid in the cytoplasmic matrix or endoplasmic reticulum of hepatocytes. Oval cell hyperplasia increased in incidence and severity with dose in the 13-week study. Based upon the minimal hyperplasia present at 14 days in only the highest dose animals, it was apparent the lesion progressed in severity with increased time of chemical

administration. Although there is some controversy as to its origin, the oval cell generally is considered to be a stem cell capable of differentiating to a hepatocyte and/or biliary epithelial cell (Evarts *et al.*, 1987, 1990; Popp and Cattley, 1991). It has been shown that chemically induced oval cell hyperplasia may regress following cessation of treatment (Tatematsu *et al.*, 1984). Oval cell hyperplasia occurs in the liver of rats following exposure to a number of hepatocarcinogens including methapyrilene (Reznik-Schuller and Gregg, 1983), 2-acetylaminofluorene (Tatematsu *et al.*, 1984), 3-methyl-4-dimethylamino-azobenzene (Dempo *et al.*, 1975) and furan (Maronpot, *et al.*, 1991). It may be present with bile duct hyperplasia and cholangiofibrosis (Popp and Cattley, 1991; Maronpot 1991; Maronpot *et al.*, 1991) and is considered a preneoplastic change.

Renal Toxicity

Treatment-related kidney lesions were seen in female and/or male rats with all 3 nitrotoluene isomers; the extent and nature of the lesions was more severe in males. Kidney-weight-to-body-weight ratios were elevated in treated rats in all 3 studies, especially with *o*-nitrotoluene. Small increases in relative kidney weights were seen among female mice, but these were attributed to the decreased body weight in the high dose groups.

Hyaline droplet nephropathy was a consistent finding in the kidney of male rats given *o*-, *m*-, or *p*-nitrotoluene. The histopathological change was attributed to an increased concentration of α–2u globulin in the kidney (measured only for *o*- and *p*-isomers). Based upon the microscopic examination of the kidney, the severity of the hyaline droplet nephropathy was similar for the *o*-, *m*-, and *p*-nitrotoluene isomers. However, the presence of pigment (lipofuscin or possibly the test compound or its metabolite) in the kidney was seen in both male and female rats exposed to only the *o*- and *p*-isomers, and karyomegaly of renal tubular epithelium was seen consistently with the *p*-isomer in male and female rats. These additional histopathological findings suggest the *o*- and *p*- isomers have potential for greater renal toxicity than the *m*-isomer. Previous studies with *m*- and *p*-nitrobenzoic acids, major metabolites of 2 of the isomers reported here, had similar histopathologic effects on the kidney. Both produced a hyaline droplet nephropathy in male rats; *p*-nitrobenzoic acid also caused pigment accumulation and karyomegaly in the kidney of male and female rats.

Hyaline droplet (α–2u globulin) nephropathy is a well recognized, chemically inducible renal lesion which occurs in male rats (U.S. EPA, 1991; Swenberg, 1989). α–2u Globulin is formed in the liver of male rats and excreted in the urine. Approximately 40% of the α–2u globulin in the urine remains in the filtrate, and approximately 60% is reabsorbed, particularly by the proximal convoluted tubular cells. The absorbed protein is normally visible microscopically as small granules or droplets ("resorption droplets") in the cytoplasm of the tubule cells; this protein is subsequently hydrolyzed by lysosomal enzymes. Chemicals, such as unleaded gasoline, trimethylpentane, and dimethyl methyl phosphonate, can bind to the α–2u globulin and interfere with the breakdown of this protein within the tubular epithelium. The reabsorbed protein accumulates in the tubular epithelium and appears microscopically as an increased number of abnormally large crystalline or globular deposits ("hyaline droplets"). It has been shown that excessive hyaline droplet accumulation can result in tubular epithelial cell necrosis, and further

sequela, including tubular cell regeneration, formation of granular casts, inflammation, mineralization in the papilla, and, in some cases, tubular cell neoplasms (Alden *et al.*, 1991; Eurell *et al.*, 1990; Swenberg, 1989; U.S. EPA, 1991). In the nitrotoluene studies reported here, there was no evidence of treatment-related necrosis or proliferative lesions in the kidney.

Hematopoietic/Splenic Effects

The predominant effect of nitrotoluene administration on the hematopoietic system of rats was a rather slow onset of a mild regenerative anemia, with hemosiderin accumulation and evidence of increased hematopoiesis in the spleen. Increases in platelet and lymphocyte counts were occasionally seen in male and female rats given *o*- and *m*-nitrotoluene. Review of hematology slides from male and female rats treated with *o*-nitrotoluene revealed that increases in WBC counts were produced by lymphocytes and mature neutrophils. A common mechanism for leukocytosis and thrombocytosis is a physiologic response secondary to effects of the chemical. In this case, changes in WBC and platelet counts could be due to endogenous release of epinephrine in response to fear, excitement, or increased physical activity. Treatment for 1 week with *o*-, *m*-, or *p*-nitrotoluene produced effects consistent with mild hemoconcentration in male and female rats. Increases in methemoglobin concentrations were mild (approximately 2-fold in the 10000 ppm dose group) and occurred only with *o*-nitrotoluene. Reticulocyte counts were generally decreased by all 3 chemicals in the top 1 or 2 dose groups (5000 or 1000 ppm). It is likely that this represented a direct effect of the nitrotoluenes to suppress hematopoiesis in the bone marrow. These blood changes were transient, however, because increased reticulocyte counts occurred at subsequent collections, possibly resulting from an increase in splenic hematopoiesis. Selective suppression of bone marrow hematopoiesis with maintenance of splenic hematopoiesis could occur through differences in tissue susceptibilities due to local microenvironments. Bone marrow stromal cells are sensitive to a variety of chemotherapeutic agents and chemicals (Luster *et al.*, 1989). For example, mice treated with AZT (3'-azido-2',3'-didexoythymidine) had marked bone marrow hypoplasia but had increased levels of splenic hematopoiesis (Thompson *et al.*, 1991). A similar effect may be occurring with the nitrotoluenes.

By 3 weeks of administration, methemoglobin concentrations were increased in male and female rats receiving *o*- and *m*-nitrotoluenes. The mechanism of the anemia is assumed to involve oxidative damage to hemoglobin leading to Heinz body formation and decreased erythrocyte survival. This effect is similar to methemoglobin-induced anemias in animals and humans associated with exposure to many aniline and nitroaromatic compounds (Finch, 1948; Smith, 1986).

At 13 weeks, mild, regenerative anemias still were evident in male and female rats given the nitrotoluenes. The anemia in male rats receiving *m*-nitrotoluene was minimal. Methemoglobin concentrations remained increased in animals in multiple dose groups.

In the spleen of dosed male and female rats, there was generally a minimal to mild increase in hematopoiesis, hemosiderin deposition and/or congestion. The mildest histopathologic effect observed in the spleen was seen with *m*-nitrotoluene; although hemosiderin and congestion were increased in most dosed groups, there was no increase in hematopoiesis (Table 17).

Evidence of somewhat more severe splenic effects was seen with exposure to the *o*- and *p*-isomers, which resulted in greater accumulation of hemosiderin pigment, and increased hematopoiesis. Furthermore, in the *o*-isomer study, there was minimal to mild fibrosis of the spleen capsule. In studies of *m*- and *p*-nitrobenzoic acid (metabolites of *m*- and *p*-nitrotoluene) increased hemosiderin pigment and congestion of the spleen were seen only with *p*-nitrobenzoic acid. In all studies, there was no microscopic evidence of hyperplasia or cellular depletion in sections of bone marrow from dosed animals.

A number of aromatic amines, such as *p*-chloroaniline, have been shown to affect the hematopoietic system of rats, resulting in increased levels of methemoglobin and anemia as well as increased hemosiderin, congestion, and hematopoiesis of the spleen (Chhabra *et al.*, 1990). Chronic exposure of rats to this group of chemicals, which includes *o*-toluidine, has resulted in splenic sarcomas (Chhabra *et al.*, 1991; Goodman *et al.*, 1984). Because of the structural similarity of *o*-nitrotoluene and *o*-toluidine, the spleen should be considered as a potential target site for a carcinogenic effect in a 2-year study.

Reproductive System Toxicity

The nitrotoluene isomers adversely affected the reproductive system in both male and female rats, while mice showed no evidence of reproductive effects from exposure to these chemicals. The *o*-isomer appeared generally more toxic than the other isomers. Caudal weight, testicular weight, and epididymis weight were less in relation to body weight in male rats receiving *o*-nitrotoluene at the 5000 and 10000 ppm levels, or *m*-nitrotoluene at the highest level (10000 ppm) than in the other groups where reductions in absolute weight were seen. There were virtually no sperm present in the epididymides of rats receiving *o*-nitrotoluene at 10000 ppm. In addition, there was a smaller number of sperm as well as fewer motile sperm and morphologically intact sperm, relative to controls, among rats given the 10000 ppm dose of *m*- and *p*-isomers. Sperm counts were also diminished in the 5000 ppm group of rats receiving *o*-nitrotoluene.

The testis in rats showed marked degeneration of the seminiferous tubules in animals receiving *o*-nitrotoluene at the 5000 and 10000 ppm dose levels. Degeneration of the testis also occurred in rats receiving 10000 ppm of the *m*- and *p*-isomers, although the severity of this lesion was less than that observed with the *o*-isomer at this dose level. Testicular degeneration occurred in previous NTP 13-week studies of *m*- and *p*-nitrobenzoic acid, metabolites of *m*- and *p*-nitrotoluene, respectively. For both *p*- and *m*-nitrobenzoic acid, degeneration was present only at the 10000 ppm dietary dose level. Testicular degeneration was seen in mice given *p*-nitrobenzoic acid; however, this only occurred in animals given 20000 ppm, twice the highest dose level used in the nitrotoluene studies.

The *o*- and *m*-nitrotoluenes increased the length of the estrous cycle in rats receiving the 10000 ppm dose. The *p*-isomer had no effect on the length of the estrous cycle. The proportion of rats in diestrus increased with all 3 isomers.

Olfactory Toxicity

The only chemically-related histopathologic lesion observed in mice receiving the nitrotoluene isomers was degeneration and metaplasia of the olfactory epithelium in the region of the dorsal meatus in the nasal cavity of males and females given *o*-nitrotoluene. This effect was not seen with the other isomers and did not occur in rats. It is possible the olfactory changes reflected an irritant effect related to inhalation of the chemical; however, all 3 isomers have the same volatility (Verschueren, 1983). Olfactory changes in this study may be related to a unique local irritant effect of the *o*-isomer or one of its metabolites produced in the olfactory epithelium of mice. With direct irritant substances, the olfactory epithelium along the dorsal meatus is commonly affected resulting in atrophy or thinning, and the respiratory epithelium can be affected as well (Gaskell, 1990). Conversely, with specific olfactory toxins which are activated at a distant site and transported to the olfactory mucosa via the circulation, there may be large areas of affected olfactory mucosa throughout the posterior nasal cavity. The mechanism for the development of the olfactory epithelium degeneration and metaplasia in this study is unknown. The respiratory metaplasia and Bowman's gland changes are consistent with reparative or regenerative attempts. There was little evidence of an irritant effect on the respiratory mucosa. There were no changes in the respiratory epithelium of male mice and only a slight increase in intracytoplasmic protein globules in the respiratory epithelium of female mice. These protein globules, which are commonly present in control mice, may be increased with chemical exposure and have been described as adaptive or non specific changes (Monticello *et al.*, 1990; Gopinath *et. al.*, 1987).

Carcinogenicity

The occurrence of neoplasia after only 13 weeks of chemical administration is an unusual finding in rodent studies; however, in the 13-week study in male rats, *o*-nitrotoluene caused mesothelial cell hyperplasia at 10000 ppm, and in the 5000 ppm group, 3/10 animals developed mesothelioma. The mesothelial cell hyperplasia at 10000 ppm was considered to be a preneoplastic lesion. It is not known why mesothelioma occurred in the 5000 ppm dose group, and not at 10000 ppm, but this may have been related to the generally more severe toxicity and lower body weights in animals given 10000 ppm. Studies by Rao *et al.* (1987), have shown a decrease in neoplasia in animals that weigh significantly less than control animals, and Lok *et al.* (1988, 1990), have shown reduced cell proliferation and carcinogenesis in calorie restricted animals.

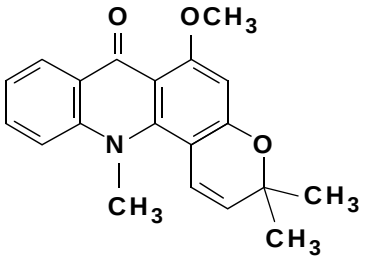
Chemically induced mesotheliomas are relatively rare in rats in NTP carcinogenicity studies and have not been associated with treatment in mice (Huff *et al.*, 1991). A survey of 379 chemicals subjected to long-term studies in rodents showed only 8 compounds that caused mesotheliomas in the male rat: acronycine, cytembena, 1,2-dibromoethane, 3,3-dimethoxy-benzidine dihydrochloride, 3,3'-dimethylbenzidine dihydrochloride, glycidol, and *o*-toluidine (Figure 8). *o*-Toluidine and the benzidine compounds have structural similarities to *o*-nitrotoluene. The historical incidence of mesotheliomas in control male rats is 2.7% and is 0.1% in females (Haseman *et al.*, 1990). *o*-Toluidine has been shown to cause a high incidence of mesothelioma in rats in a 2-year study, although these tumors were not detected as early as those in the *o*-

nitrotoluene study (NCI, 1979). The structural similarity between *o*-toluidine and *o*-nitrotoluene (both contain a benzene ring with a methyl and a nitrogen-containing group on adjacent carbons) suggests that there may be a common intermediate responsible for the observed carcinogenicity. For example, the hydroxylamine formed from reduction of the nitro group or oxidation of the amino group might be such an intermediate; however, there are other possibilities. *o*-Toluidine was also shown to produce an increased incidence of splenic, urinary bladder, and mammary gland tumors in rats, as well as hemangiosarcomas and liver tumors in mice (NCI, 1979). *o*-Toluidine has been associated with bladder tumor formation in humans (Ward *et al.*, 1991).

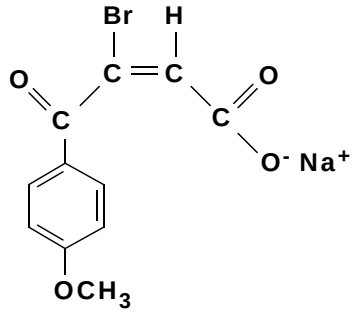
Neoplasms occurred only in male rats dosed with *o*-nitrotoluene in these studies. The development of neoplasia in the *o*-nitrotoluene-dosed male rats supports previous observations (Doolittle *et al.*, 1983) that an electrophilic metabolite capable of interacting with DNA is formed preferentially from the *o*-nitrotoluene isomer in the male rat and with the current findings of substantially increased UDS in the liver of male rats given *o*-nitrotoluene. It is not known if this "electrophilic ion" forms at low levels in the other groups, which might also lead to the development of neoplasia after a longer period of dosing.

The lack of positive mutagenic responses in *Salmonella* for these chemicals could be due to the lack of metabolic reduction produced by intestinal flora *in vivo*, and necessary to form the DNA-reactive product of *o*-nitrotoluene. The *Salmonella* test protocol did not incorporate reductive metabolism, or cecal flora metabolism, and, thus, this test system may not accurately predict the *in vivo* mutagenic activity of these compounds.

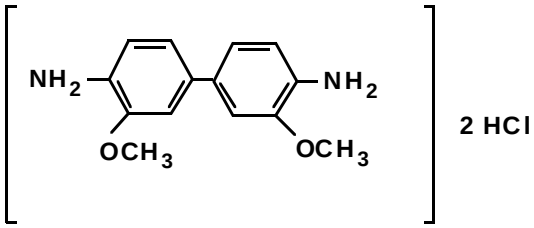
It is estimated there are 7-13 new cases of mesotheliomas per million people per year in the United States. Most of these cases have been associated with exposure to asbestos (Craighead, 1987). In humans, chromosomal abnormalities have been found in malignant pleural mesotheliomas (Tiainen *et al.*, 1988; Hagemeijer *et al.*, 1990; Pelin-Enlund *et al.*, 1990). Genetic alterations of the p53 gene are a common feature of human malignant mesotheliomas (Cote *et al.*, 1991). Additional studies will be needed to determine if the *o*-nitrotoluene-induced mesotheliomas contain genetic alterations similar to those reported in humans.



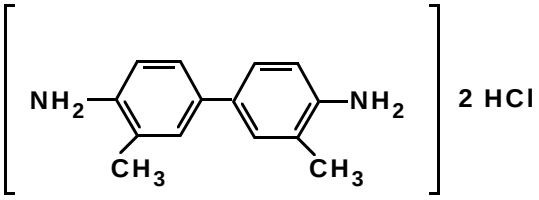
ACRONYCINE



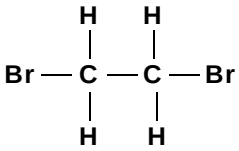
CYTEMBENA



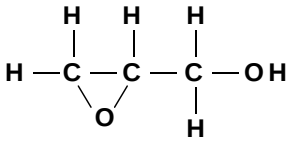
3,3'-DIMETHOXYBENZIDINE
DIHYDROCHLORIDE



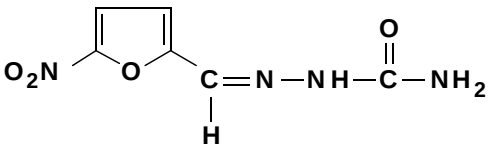
3,3'-DIMETHYLBENZIDINE
DIHYDROCHLORIDE



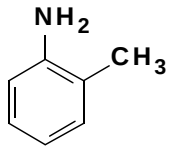
1,2-DIBROMOETHANE



GLYCIDOL



NITROFURAZONE



o-TOLUIDINE

Figure 8 Structures of Chemicals Causing Mesotheliomas

Summary

A summary of findings in the 13-week nitrotoluene studies appears in Table 17. Toxic effects were most severe with *o*-nitrotoluene in male rats. The chemical caused depressed body weight gain at doses of 2500 ppm and above, and toxic effects to the liver, spleen, and kidney at dietary concentrations of 1250 ppm and above. Even at the lower dose of 625 ppm, liver weights were increased. Changes in clinical chemistry parameters, indicating liver toxicity, were observed in male rats at dosage levels of 2500 ppm and above. More importantly, *o*-nitrotoluene caused chemically-related tumors in male rats after only 13 weeks of dosing, as demonstrated by the occurrence of mesothelial cell hyperplasia and mesothelioma.

Among female rats, *o*-nitrotoluene caused body weight effects at dosage levels of 2500 ppm and above, and kidney and spleen lesions at levels of 2500 ppm and above. In mice, lesions in liver, kidney, and spleen were not observed after administration of *o*-nitrotoluene, but significant lesions of the nasal cavity were observed at dosage levels of 1250 ppm and above, and body weight effects were noted at levels of 2500 ppm and above.

m-Nitrotoluene caused body weight effects in rats and mice at 10000 ppm. Lesions were observed in the kidneys of male rats dosed with this isomer at levels of 625 ppm and above; at 10000 ppm histopathologic effects were observed in the testis. *m*-Nitrotoluene also caused significant histopathologic effects in the spleen of male and female rats at the 2500 ppm and higher doses. No treatment-related lesions were observed in mice.

p-Nitrotoluene caused body weight effects in rats and mice at 10000 ppm. Significant lesions were observed in the kidney and spleen of rats at all dose levels for this isomer, but no histopathologic lesions were observed in mice.

o-, *m*-, and *p*-Nitrotoluene impaired testicular function of the rat, as shown microscopically and by measurement of sperm density, motility, and number; the 3 isomers also increased the length of the estrual cycle in rats. Although some of these effects may have been due in small part to decreased body weight gains, the severity of the effects indicates that the nitrotoluenes are likely directly toxic to the reproductive system.

These comparative toxicity studies of *o*-, *m*-, and *p*-nitrotoluene show that the chemicals have the potential to cause injury to the kidney, spleen and/or hematopoietic, and reproductive system in rodents, with the severity of effects generally greater with the *o*-isomer, compared to the *m*- and *p*-nitrotoluenes. *o*-Nitrotoluene was carcinogenic in male rats in 13-week studies, based on the occurrence of mesotheliomas and mesothelial hyperplasia in dosed groups.

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APPENDIX A

Organ Weights and Organ-Weight-to-Body-Weight Ratios

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Necropsy body wt						
o-Nitrotoluene	353 ± 5	339 ± 6	329 ± 9*	309 ± 6**	254 ± 4**	198 ± 3**
m-Nitrotoluene	346 ± 8	354 ± 4	342 ± 5	353 ± 6	338 ± 6	281 ± 5**
p-Nitrotoluene	350 ± 6	349 ± 7	342 ± 6	341 ± 11	315 ± 6**	253 ± 6**
Heart						
o-Nitrotoluene						
Absolute	1.039 ± 0.019	1.019 ± 0.016	1.021 ± 0.029	1.003 ± 0.026	0.985 ± 0.011	0.881 ± 0.016**
Relative	2.94 ± 0.05	3.01 ± 0.04	3.11 ± 0.04*	3.24 ± 0.06**	3.88 ± 0.07**	4.44 ± 0.04**
m-Nitrotoluene						
Absolute	0.983 ± 0.022	1.037 ± 0.027	0.992 ± 0.019	0.999 ± 0.026	0.958 ± 0.020	0.851 ± 0.024**
Relative	2.84 ± 0.03	2.92 ± 0.05	2.90 ± 0.03	2.83 ± 0.05	2.83 ± 0.05	3.03 ± 0.06
p-Nitrotoluene						
Absolute	1.121 ± 0.015	1.089 ± 0.022	1.050 ± 0.025*	1.045 ± 0.035*	0.966 ± 0.023**	0.858 ± 0.022**
Relative	3.18 ± 0.06	3.09 ± 0.04	3.04 ± 0.04	3.04 ± 0.05	3.02 ± 0.04	3.42 ± 0.05
Right Kidney						
o-Nitrotoluene						
Absolute	1.176 ± 0.021	1.167 ± 0.026	1.130 ± 0.035	1.137 ± 0.031	1.058 ± 0.015**	1.017 ± 0.027**
Relative	3.33 ± 0.04	3.45 ± 0.04	3.44 ± 0.05	3.67 ± 0.05**	4.17 ± 0.08**	5.12 ± 0.09**
m-Nitrotoluene						
Absolute	1.118 ± 0.023	1.177 ± 0.020	1.128 ± 0.030	1.209 ± 0.024	1.203 ± 0.035	1.126 ± 0.030
Relative	3.24 ± 0.05	3.32 ± 0.05	3.30 ± 0.07	3.43 ± 0.06*	3.55 ± 0.06**	4.00 ± 0.07**
p-Nitrotoluene						
Absolute	1.133 ± 0.026	1.150 ± 0.033	1.118 ± 0.026	1.149 ± 0.073	1.114 ± 0.029	0.926 ± 0.022**
Relative	3.21 ± 0.04	3.25 ± 0.03	3.24 ± 0.04	3.32 ± 0.11	3.49 ± 0.06**	3.69 ± 0.05**
Liver						
o-Nitrotoluene						
Absolute	12.00 ± 0.35	12.50 ± 0.25	12.88 ± 0.38	13.78 ± 0.39**	14.62 ± 0.32**	15.07 ± 0.38**
Relative	33.9 ± 0.7	36.9 ± 0.5**	39.2 ± 0.6**	44.5 ± 0.8**	57.6 ± 1.4**	76.0 ± 1.5**
m-Nitrotoluene						
Absolute	11.47 ± 0.48	12.08 ± 0.26	10.92 ± 0.35	12.13 ± 0.34	11.15 ± 0.30	10.96 ± 0.32
Relative	33.1 ± 0.8	34.1 ± 0.5	31.9 ± 0.7	34.3 ± 0.6	32.9 ± 0.4	39.0 ± 0.9**
p-Nitrotoluene						
Absolute	11.35 ± 0.25	11.34 ± 0.36	11.20 ± 0.24	10.86 ± 0.47	11.28 ± 0.27	9.60 ± 0.30**
Relative	32.2 ± 0.4	32.1 ± 0.5	32.4 ± 0.3	31.5 ± 0.4	35.3 ± 0.4**	38.3 ± 1.0**
Lungs						
o-Nitrotoluene						
Absolute	1.676 ± 0.056	1.898 ± 0.079	1.578 ± 0.070	1.528 ± 0.053	1.445 ± 0.058*	1.214 ± 0.043**
Relative	4.74 ± 0.15	5.62 ± 0.26*	4.80 ± 0.17	4.94 ± 0.13	5.69 ± 0.22**	6.11 ± 0.18**
m-Nitrotoluene						
Absolute	1.575 ± 0.077	1.820 ± 0.182	1.798 ± 0.118	1.582 ± 0.058	1.675 ± 0.111	1.285 ± 0.040**
Relative	4.56 ± 0.22	5.12 ± 0.48	5.25 ± 0.31	4.48 ± 0.15	4.94 ± 0.29	4.57 ± 0.11
p-Nitrotoluene						
Absolute	1.509 ± 0.031	1.589 ± 0.054	1.568 ± 0.046	1.525 ± 0.053	1.541 ± 0.063	1.220 ± 0.035**
Relative	4.28 ± 0.08	4.51 ± 0.17	4.55 ± 0.13	4.45 ± 0.16	4.83 ± 0.19*	4.87 ± 0.14**

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Right Testis						
o-Nitrotoluene						
Absolute	1.409 ± 0.031	1.386 ± 0.024	1.361 ± 0.042	1.346 ± 0.020	1.011 ± 0.046**	0.517 ± 0.024**
Relative	3.99 ± 0.07	4.09 ± 0.04	4.14 ± 0.06	4.36 ± 0.06	4.00 ± 0.23	2.61 ± 0.12**
m-Nitrotoluene						
Absolute	1.372 ± 0.021	1.432 ± 0.027	1.356 ± 0.034	1.412 ± 0.025	1.392 ± 0.020	0.759 ± 0.071**
Relative	3.98 ± 0.06	4.04 ± 0.06	3.97 ± 0.10	4.01 ± 0.07	4.12 ± 0.05	2.69 ± 0.24**
p-Nitrotoluene						
Absolute	1.447 ± 0.025	1.431 ± 0.036	1.347 ± 0.043	1.410 ± 0.045	1.348 ± 0.029*	1.030 ± 0.076**
Relative	4.10 ± 0.06	4.05 ± 0.05	3.90 ± 0.12	4.10 ± 0.04	4.22 ± 0.06	4.10 ± 0.27
Thymus						
o-Nitrotoluene						
Absolute	0.321 ± 0.012	0.315 ± 0.012	0.301 ± 0.016	0.298 ± 0.017	0.327 ± 0.024	0.307 ± 0.020
Relative	0.91 ± 0.04	0.94 ± 0.05	0.92 ± 0.05	0.97 ± 0.05	1.29 ± 0.10**	1.55 ± 0.10**
m-Nitrotoluene						
Absolute	0.333 ± 0.017	0.337 ± 0.023	0.352 ± 0.015	0.332 ± 0.014	0.341 ± 0.020	0.266 ± 0.015*
Relative	9.59 ± 0.33	9.49 ± 0.59	10.32 ± 0.44	9.42 ± 0.43	10.09 ± 0.56	9.48 ± 0.57
p-Nitrotoluene						
Absolute	0.338 ± 0.014	0.347 ± 0.023	0.308 ± 0.018	0.342 ± 0.020	0.310 ± 0.017	0.222 ± 0.018**
Relative	9.62 ± 0.49	9.79 ± 0.52	8.93 ± 0.51	9.94 ± 0.51	9.67 ± 0.42	8.83 ± 0.66

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

[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*- or *p*-nitrotoluene.

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

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

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Necropsy body wt						
o-Nitrotoluene	205 ± 3	197 ± 3	192 ± 4*	179 ± 3**	170 ± 2**	158 ± 3**
m-Nitrotoluene	194 ± 3	199 ± 3	194 ± 3	195 ± 3	177 ± 2**	166 ± 3**
p-Nitrotoluene	199 ± 2	199 ± 3	200 ± 3	193 ± 3	182 ± 3**	173 ± 3**
Heart						
o-Nitrotoluene						
Absolute	0.673 ± 0.011	0.670 ± 0.014	0.648 ± 0.015	0.617 ± 0.015*	0.628 ± 0.007**	0.634 ± 0.011*
Relative	3.29 ± 0.04	3.40 ± 0.05	3.37 ± 0.04	3.44 ± 0.06*	3.70 ± 0.03**	4.01 ± 0.05**
m-Nitrotoluene						
Absolute	0.681 ± 0.011	0.669 ± 0.013	0.720 ± 0.022	0.658 ± 0.015	0.633 ± 0.012*	0.608 ± 0.027*
Relative	3.51 ± 0.07	3.35 ± 0.04	3.72 ± 0.10	3.38 ± 0.06	3.58 ± 0.06	3.66 ± 0.14
p-Nitrotoluene						
Absolute	0.710 ± 0.012	0.704 ± 0.022	0.700 ± 0.013	0.684 ± 0.014	0.669 ± 0.020	0.591 ± 0.009**
Relative	3.52 ± 0.08	3.45 ± 0.10	3.48 ± 0.08	3.49 ± 0.08	3.61 ± 0.08	3.40 ± 0.04
Right Kidney						
o-Nitrotoluene						
Absolute	0.684 ± 0.013	0.692 ± 0.009	0.680 ± 0.016	0.632 ± 0.013*	0.650 ± 0.011*	0.633 ± 0.008**
Relative	3.34 ± 0.05	3.52 ± 0.08	3.54 ± 0.07*	3.53 ± 0.05*	3.83 ± 0.07**	4.01 ± 0.06**
m-Nitrotoluene						
Absolute	0.687 ± 0.011	0.705 ± 0.018	0.714 ± 0.015	0.709 ± 0.020	0.679 ± 0.008	0.663 ± 0.016
Relative	3.54 ± 0.04	3.53 ± 0.06	3.69 ± 0.06	3.64 ± 0.07	3.84 ± 0.06**	4.00 ± 0.06**
p-Nitrotoluene						
Absolute	0.696 ± 0.014	0.685 ± 0.010	0.700 ± 0.013	0.664 ± 0.014	0.632 ± 0.017**	0.637 ± 0.014**
Relative	3.44 ± 0.06	3.36 ± 0.03	3.47 ± 0.03	3.38 ± 0.05	3.41 ± 0.07	3.66 ± 0.07*
Liver						
o-Nitrotoluene						
Absolute	6.10 ± 0.12	6.39 ± 0.14	6.63 ± 0.23	5.66 ± 0.13	6.10 ± 0.15	6.67 ± 0.12
Relative	29.8 ± 0.7	32.5 ± 0.6*	34.5 ± 1.0**	31.6 ± 0.5*	36.0 ± 1.1**	42.2 ± 0.5**
m-Nitrotoluene						
Absolute	6.41 ± 0.14	6.33 ± 0.14	6.44 ± 0.16	6.30 ± 0.14	5.67 ± 0.17**	5.99 ± 0.14*
Relative	33.0 ± 0.6	31.7 ± 0.6	33.2 ± 0.6	32.4 ± 0.5	32.0 ± 0.8	36.2 ± 0.8*
p-Nitrotoluene						
Absolute	5.92 ± 0.10	6.29 ± 0.10	6.03 ± 0.13	5.57 ± 0.12	5.51 ± 0.09	6.28 ± 0.14
Relative	29.3 ± 0.4	30.8 ± 0.4*	29.9 ± 0.5	28.4 ± 0.3	29.8 ± 0.6	36.1 ± 0.7**
Lungs						
o-Nitrotoluene						
Absolute	1.126 ± 0.040	1.046 ± 0.028	1.067 ± 0.044[3]	0.988 ± 0.024**	1.065 ± 0.045	0.995 ± 0.047**
Relative	5.50 ± 0.19	5.31 ± 0.11	5.53 ± 0.17[3]	5.52 ± 0.11	6.26 ± 0.25*	6.28 ± 0.25*
m-Nitrotoluene						
Absolute	1.039 ± 0.043	1.042 ± 0.036	1.057 ± 0.050	1.091 ± 0.026	0.841 ± 0.027**	0.830 ± 0.017**
Relative	5.36 ± 0.23	5.22 ± 0.15	5.49 ± 0.34	5.62 ± 0.15	4.76 ± 0.16*	5.01 ± 0.06
p-Nitrotoluene						
Absolute	1.081 ± 0.016	1.095 ± 0.027	1.238 ± 0.111	1.088 ± 0.028	1.028 ± 0.027	0.970 ± 0.031*
Relative	5.36 ± 0.11	5.37 ± 0.11	6.12 ± 0.49	5.55 ± 0.13	5.54 ± 0.10	5.57 ± 0.14

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Thymus						
o-Nitrotoluene						
Absolute	0.242 ± 0.009	0.234 ± 0.011	0.224 ± 0.009	0.222 ± 0.010	0.206 ± 0.014*	0.231 ± 0.014
Relative	1.18 ± 0.04	1.19 ± 0.05	1.17 ± 0.05	1.24 ± 0.06	1.21 ± 0.08	1.46 ± 0.09*
m-Nitrotoluene						
Absolute	0.257 ± 0.012	0.260 ± 0.010	0.262 ± 0.009	0.274 ± 0.007	0.223 ± 0.006*	0.222 ± 0.007*
Relative	1.32 ± 0.05	1.30 ± 0.04	1.36 ± 0.05	1.41 ± 0.04	1.26 ± 0.02	1.35 ± 0.05
p-Nitrotoluene						
Absolute	0.287 ± 0.012	0.260 ± 0.008*	0.266 ± 0.010	0.280 ± 0.012	0.244 ± 0.011**	0.240 ± 0.008**
Relative	1.42 ± 0.06	1.27 ± 0.04	1.32 ± 0.05	1.43 ± 0.06	1.31 ± 0.05	1.39 ± 0.06

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).
[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*- or *p*-nitrotoluene.
[3] n=9.
* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test.
** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test.

TABLE A3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Mice In the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Necropsy body wt						
o-Nitrotoluene	33.3 ± 0.4	34.5 ± 0.9	32.9 ± 0.6	32.8 ± 0.5	29.2 ± 0.3**	25.9 ± 0.3**
m-Nitrotoluene	36.5 ± 0.8	35.1 ± 0.5	34.9 ± 1.0	34.9 ± 0.7	31.3 ± 0.8**	27.7 ± 0.4**
p-Nitrotoluene	33.5 ± 0.4	33.1 ± 0.8	33.8 ± 0.7	32.3 ± 0.5	31.1 ± 0.4**	29.9 ± 0.7**
Heart						
o-Nitrotoluene						
Absolute	0.152 ± 0.002	0.149 ± 0.003	0.150 ± 0.003	0.160 ± 0.007	0.136 ± 0.003**	0.121 ± 0.002**
Relative	4.57 ± 0.10	4.34 ± 0.12	4.56 ± 0.09	4.86 ± 0.17	4.65 ± 0.10	4.68 ± 0.09
m-Nitrotoluene						
Absolute	0.200 ± 0.014	0.170 ± 0.010	0.169 ± 0.007	0.163 ± 0.006*	0.163 ± 0.009*	0.156 ± 0.006*
Relative	5.52 ± 0.45	4.88 ± 0.35	4.85 ± 0.16	4.67 ± 0.12	5.19 ± 0.24	5.63 ± 0.22
p-Nitrotoluene						
Absolute	0.171 ± 0.005	0.175 ± 0.007	0.162 ± 0.006	0.155 ± 0.008	0.147 ± 0.005**	0.149 ± 0.006**
Relative	5.19 ± 0.18	5.35 ± 0.22	4.89 ± 0.22	4.81 ± 0.27	4.72 ± 0.13	4.99 ± 0.14
Right Kidney						
o-Nitrotoluene						
Absolute	0.304 ± 0.006	0.306 ± 0.005	0.303 ± 0.005	0.290 ± 0.004	0.249 ± 0.005**	0.207 ± 0.004**
Relative	9.14 ± 0.23	8.91 ± 0.17	9.21 ± 0.16	8.85 ± 0.17	8.51 ± 0.15*	7.99 ± 0.15**
m-Nitrotoluene						
Absolute	0.325 ± 0.006	0.321 ± 0.007	0.319 ± 0.008	0.314 ± 0.008	0.282 ± 0.008**	0.254 ± 0.005**
Relative	8.94 ± 0.28	9.15 ± 0.19	9.18 ± 0.24	9.01 ± 0.21	9.01 ± 0.18	9.17 ± 0.17
p-Nitrotoluene						
Absolute	0.273 ± 0.007	0.281 ± 0.007	0.300 ± 0.010	0.271 ± 0.006	0.262 ± 0.009	0.267 ± 0.007
Relative	8.28 ± 0.22	8.61 ± 0.36	9.00 ± 0.16	8.39 ± 0.14	8.44 ± 0.33	8.95 ± 0.15
Liver						
o-Nitrotoluene						
Absolute	1.49 ± 0.03	1.50 ± 0.03	1.52 ± 0.04	1.61 ± 0.03*	1.57 ± 0.03*	1.52 ± 0.03
Relative	44.6 ± 0.7	43.6 ± 0.5	46.0 ± 0.8	49.0 ± 0.5**	53.5 ± 0.8**	58.8 ± 0.9**
m-Nitrotoluene						
Absolute	1.65 ± 0.04	1.70 ± 0.04	1.74 ± 0.04	1.79 ± 0.03	1.69 ± 0.04	1.60 ± 0.03
Relative	45.2 ± 0.8	48.5 ± 1.0*	50.0 ± 0.9**	51.5 ± 0.7**	53.9 ± 0.6**	57.9 ± 0.9**
p-Nitrotoluene						
Absolute	1.42 ± 0.02	1.50 ± 0.03*	1.65 ± 0.03**	1.49 ± 0.02**	1.62 ± 0.04**	1.74 ± 0.05**
Relative	43.2 ± 0.7	45.7 ± 0.9*	49.6 ± 0.6**	46.3 ± 0.6**	52.1 ± 0.7**	58.3 ± 0.9**
Lungs						
o-Nitrotoluene						
Absolute	0.198 ± 0.008	0.214 ± 0.013	0.183 ± 0.008	0.219 ± 0.010	0.196 ± 0.011	0.171 ± 0.007*
Relative	5.96 ± 0.28	6.21 ± 0.36	5.58 ± 0.27	6.65 ± 0.24	6.71 ± 0.41	6.61 ± 0.28
m-Nitrotoluene						
Absolute	0.245 ± 0.017	0.266 ± 0.018	0.258 ± 0.018	0.226 ± 0.015	0.250 ± 0.016	0.260 ± 0.019
Relative	6.78 ± 0.54	7.59 ± 0.53	7.47 ± 0.57	6.47 ± 0.38	7.96 ± 0.43	9.35 ± 0.63**
p-Nitrotoluene						
Absolute	0.205 ± 0.010	0.229 ± 0.008	0.207 ± 0.009	0.210 ± 0.013	0.214 ± 0.009	0.241 ± 0.015
Relative	6.23 ± 0.34	7.01 ± 0.32	6.26 ± 0.34	6.51 ± 0.39	6.88 ± 0.28	8.08 ± 0.49*

TABLE A3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Mice in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Right Testis						
o-Nitrotoluene						
Absolute	0.122 ± 0.002	0.119 ± 0.003	0.127 ± 0.002	0.123 ± 0.001	0.119 ± 0.002	0.110 ± 0.002**
Relative	3.66 ± 0.06	3.46 ± 0.10	3.85 ± 0.06	3.74 ± 0.05	4.06 ± 0.08**	4.26 ± 0.05**
m-Nitrotoluene						
Absolute	0.128 ± 0.003	0.123 ± 0.002	0.121 ± 0.003	0.123 ± 0.003	0.122 ± 0.002	0.117 ± 0.002**
Relative	3.51 ± 0.10	3.50 ± 0.07	3.49 ± 0.10	3.52 ± 0.09	3.90 ± 0.09**	4.20 ± 0.07**
p-Nitrotoluene						
Absolute	0.121 ± 0.004	0.119 ± 0.002	0.118 ± 0.002	0.118 ± 0.002	0.116 ± 0.003	0.117 ± 0.002
Relative	3.69 ± 0.14	3.64 ± 0.09	3.55 ± 0.08	3.67 ± 0.08	3.73 ± 0.09	3.93 ± 0.10
Thymus						
o-Nitrotoluene						
Absolute	0.043 ± 0.002	0.043 ± 0.003	0.043 ± 0.002	0.040 ± 0.002	0.036 ± 0.002*	0.037 ± 0.002
Relative	1.29 ± 0.06	1.25 ± 0.08	1.30 ± 0.07	1.22 ± 0.05	1.24 ± 0.07	1.44 ± 0.07
m-Nitrotoluene						
Absolute	0.050 ± 0.003	0.052 ± 0.003	0.055 ± 0.003	0.051 ± 0.004	0.049 ± 0.003	0.046 ± 0.002
Relative	1.37 ± 0.08	1.47 ± 0.07	1.56 ± 0.07	1.47 ± 0.10	1.55 ± 0.09	1.64 ± 0.07*
p-Nitrotoluene						
Absolute	0.051 ± 0.002	0.048 ± 0.002	0.050 ± 0.003	0.050 ± 0.003	0.043 ± 0.002	0.050 ± 0.004
Relative	1.54 ± 0.06	1.46 ± 0.07	1.50 ± 0.09	1.56 ± 0.09	1.39 ± 0.08	1.68 ± 0.14

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

[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*- or *p*-nitrotoluene.

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

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

TABLE A4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Mice in the 13-Week Feed Studies of *o-*, *m-*, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Necropsy body wt						
o-Nitrotoluene	32.8 ± 0.7	34.0 ± 0.5	33.4 ± 0.7	32.1 ± 0.8	29.5 ± 0.6**	22.9 ± 0.3**
m-Nitrotoluene	33.8 ± 0.8	34.7 ± 0.6	34.6 ± 1.1	33.2 ± 0.4	29.8 ± 0.8**	24.4 ± 0.5**
p-Nitrotoluene	29.6 ± 0.7	33.0 ± 0.5	33.3 ± 0.6	27.9 ± 0.6	27.1 ± 0.4	24.9 ± 0.5**
Heart						
o-Nitrotoluene						
Absolute	0.134 ± 0.005	0.139 ± 0.005	0.135 ± 0.003	0.131 ± 0.002	0.144 ± 0.010	0.103 ± 0.003**
Relative	4.09 ± 0.12	4.09 ± 0.13	4.05 ± 0.10	4.09 ± 0.08	4.84 ± 0.25*	4.51 ± 0.15*
m-Nitrotoluene						
Absolute	0.153 ± 0.007	0.162 ± 0.007	0.154 ± 0.006	0.157 ± 0.008	0.158 ± 0.008	0.126 ± 0.005**
Relative	4.55 ± 0.22	4.69 ± 0.24	4.48 ± 0.22	4.74 ± 0.25	5.33 ± 0.29*	5.18 ± 0.21
p-Nitrotoluene						
Absolute	0.144 ± 0.004	0.141 ± 0.005	0.144 ± 0.005	0.139 ± 0.005	0.139 ± 0.005	0.124 ± 0.005**
Relative	4.96 ± 0.14	4.33 ± 0.17	4.44 ± 0.18	4.98 ± 0.14	5.14 ± 0.22	4.99 ± 0.19
Right Kidney						
o-Nitrotoluene						
Absolute	0.201 ± 0.006	0.215 ± 0.005	0.217 ± 0.005	0.217 ± 0.005	0.207 ± 0.005	0.162 ± 0.003**
Relative	6.12 ± 0.12	6.33 ± 0.13	6.50 ± 0.11*	6.79 ± 0.21**	7.02 ± 0.17**	7.09 ± 0.13**
m-Nitrotoluene						
Absolute	0.215 ± 0.005	0.232 ± 0.003	0.238 ± 0.003*	0.252 ± 0.012**	0.230 ± 0.010	0.197 ± 0.004
Relative	6.38 ± 0.21	6.71 ± 0.12	6.92 ± 0.19	7.61 ± 0.40**	7.79 ± 0.46**	8.09 ± 0.15**
p-Nitrotoluene						
Absolute	0.201 ± 0.005	0.220 ± 0.004	0.219 ± 0.004	0.199 ± 0.004	0.201 ± 0.003	0.190 ± 0.006
Relative	6.93 ± 0.17	6.75 ± 0.13	6.74 ± 0.13	7.15 ± 0.17	7.43 ± 0.13*	7.66 ± 0.28*
Liver						
o-Nitrotoluene						
Absolute	1.37 ± 0.04	1.46 ± 0.04	1.51 ± 0.04	1.49 ± 0.03	1.52 ± 0.04	1.28 ± 0.02
Relative	41.7 ± 0.6	43.0 ± 0.8	45.1 ± 0.7**	46.5 ± 1.1**	51.4 ± 0.9**	55.8 ± 1.2**
m-Nitrotoluene						
Absolute	1.43 ± 0.04	1.63 ± 0.03	1.82 ± 0.06**	1.83 ± 0.03**	1.61 ± 0.04	1.36 ± 0.03
Relative	42.2 ± 0.7	46.9 ± 0.7**	52.6 ± 0.9**	55.0 ± 0.7**	54.1 ± 0.9**	55.9 ± 1.0**
p-Nitrotoluene						
Absolute	1.31 ± 0.03	1.57 ± 0.05**	1.61 ± 0.03**	1.39 ± 0.04	1.43 ± 0.03	1.41 ± 0.04
Relative	45.1 ± 0.8	48.0 ± 0.8*	49.4 ± 1.0**	49.7 ± 1.0**	52.7 ± 0.9**	56.6 ± 0.8**
Lungs						
o-Nitrotoluene						
Absolute	0.194 ± 0.008	0.221 ± 0.016[3]	0.192 ± 0.009	0.189 ± 0.005	0.237 ± 0.017	0.193 ± 0.016
Relative	5.95 ± 0.33	6.59 ± 0.53[3]	5.76 ± 0.25	5.92 ± 0.20	7.96 ± 0.47**	8.47 ± 0.72**
m-Nitrotoluene						
Absolute	0.232 ± 0.014	0.254 ± 0.018	0.234 ± 0.011	0.236 ± 0.016	0.268 ± 0.020	0.229 ± 0.015
Relative	6.95 ± 0.55	7.33 ± 0.51	6.76 ± 0.23	7.12 ± 0.51	9.06 ± 0.76*	9.45 ± 0.68**
p-Nitrotoluene						
Absolute	0.242 ± 0.011	0.226 ± 0.010	0.216 ± 0.011	0.215 ± 0.013	0.228 ± 0.012	0.205 ± 0.014*
Relative	8.34 ± 0.38	6.92 ± 0.28	6.67 ± 0.40*	7.71 ± 0.44	8.41 ± 0.39	8.26 ± 0.57

TABLE A4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Mice In the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Thymus						
o-Nitrotoluene						
Absolute	0.059 ± 0.002	0.056 ± 0.003	0.059 ± 0.002	0.056 ± 0.003	0.053 ± 0.002	0.045 ± 0.002**
Relative	1.81 ± 0.06	1.67 ± 0.10	1.76 ± 0.08	1.76 ± 0.09	1.80 ± 0.09	1.97 ± 0.11
m-Nitrotoluene						
Absolute	0.062 ± 0.004	0.064 ± 0.004	0.063 ± 0.004	0.066 ± 0.003	0.059 ± 0.003	0.050 ± 0.002*
Relative	1.84 ± 0.10	1.83 ± 0.12	1.83 ± 0.09	1.98 ± 0.11	1.98 ± 0.13	2.05 ± 0.07
p-Nitrotoluene						
Absolute	0.056 ± 0.002	0.068 ± 0.003	0.064 ± 0.003	0.055 ± 0.003	0.054 ± 0.005	0.051 ± 0.002
Relative	1.94 ± 0.08	2.09 ± 0.11	1.96 ± 0.08	1.97 ± 0.11	1.97 ± 0.17	2.03 ± 0.07

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).
[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*- or *p*-nitrotnoluene.
[3] n=9.
* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test.
** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test.

APPENDIX B

Hematology and Clinical Chemistry Data

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology						
Hematocrit (%)						
o-Nitrotoluene						
Week 1	44.3 ± 0.6	43.0 ± 0.5	43.5 ± 0.5	42.4 ± 0.6	43.3 ± 0.4	46.5 ± 0.5
Week 3	46.5 ± 0.4	45.7 ± 0.5	46.4 ± 0.4	46.2 ± 0.3	46.9 ± 0.3[3]	44.0 ± 0.5**[3]
Week 13	45.8 ± 0.4	45.8 ± 0.4	45.4 ± 0.3	44.6 ± 0.6	43.3 ± 0.3**	42.2 ± 0.5**
m-Nitrotoluene						
Week 1	42.2 ± 0.5	43.2 ± 0.4	43.2 ± 0.4[3]	43.8 ± 0.4*	43.0 ± 0.4	45.7 ± 0.4**
Week 3	47.9 ± 0.7	45.6 ± 0.4*[3]	45.6 ± 0.4*	45.3 ± 0.3**	47.0 ± 0.3[3]	44.9 ± 0.4**
Week 13	45.3 ± 0.3	46.6 ± 0.5	45.9 ± 0.3	46.7 ± 0.3	44.1 ± 0.5	45.7 ± 0.4
p-Nitrotoluene						
Week 1	44.8 ± 0.9	45.2 ± 0.5	45.3 ± 0.6	45.7 ± 0.6	46.4 ± 0.9	48.1 ± 0.4**
Week 3	47.2 ± 0.4	48.4 ± 0.7[3]	48.2 ± 0.5[3]	47.4 ± 0.3	47.7 ± 0.3	47.5 ± 0.4[4]
Week 13	45.8 ± 0.4	44.6 ± 0.6	45.1 ± 0.3[3]	44.0 ± 0.6	44.1 ± 0.3*	45.4 ± 0.6
Hemoglobin (g/dL)						
o-Nitrotoluene						
Week 1	14.8 ± 0.2	14.3 ± 0.2	14.6 ± 0.2	14.1 ± 0.2	14.4 ± 0.2	15.7 ± 0.2*
Week 3	16.0 ± 0.2	15.7 ± 0.3	15.9 ± 0.2	15.8 ± 0.1	16.0 ± 0.1[3]	14.7 ± 0.2**[3]
Week 13	15.9 ± 0.2	15.9 ± 0.1	15.9 ± 0.1	15.4 ± 0.2	14.7 ± 0.1**	14.3 ± 0.2**
m-Nitrotoluene						
Week 1	14.3 ± 0.2	14.7 ± 0.2	14.7 ± 0.2[3]	14.9 ± 0.1*	14.5 ± 0.1	15.4 ± 0.2**
Week 3	16.3 ± 0.3	15.4 ± 0.1*[3]	15.3 ± 0.1*	15.3 ± 0.1**	15.8 ± 0.1[3]	14.8 ± 0.2**
Week 13	15.6 ± 0.1	16.1 ± 0.2	15.8 ± 0.1	16.0 ± 0.1	14.9 ± 0.2	15.2 ± 0.1
p-Nitrotoluene						
Week 1	15.7 ± 0.3	15.8 ± 0.2	15.7 ± 0.2	16.0 ± 0.3	16.3 ± 0.4	17.0 ± 0.2**
Week 3	16.1 ± 0.2	16.7 ± 0.3[3]	16.7 ± 0.2[3]	16.5 ± 0.1	16.5 ± 0.1	16.4 ± 0.2[3]
Week 13	15.9 ± 0.1	15.4 ± 0.2	15.7 ± 0.1	15.4 ± 0.2	15.2 ± 0.1**	15.0 ± 0.2**
Erythrocytes (10^6/μL)						
o-Nitrotoluene						
Week 1	7.26 ± 0.11	7.04 ± 0.11	7.16 ± 0.11	6.98 ± 0.10	7.18 ± 0.08	7.78 ± 0.09*
Week 3	7.85 ± 0.10	7.70 ± 0.12	7.84 ± 0.09	7.78 ± 0.09	7.95 ± 0.05[3]	7.32 ± 0.10*[3]
Week 13	9.06 ± 0.06	9.11 ± 0.08	9.03 ± 0.06	8.85 ± 0.11	8.39 ± 0.06**	7.89 ± 0.07**
m-Nitrotoluene						
Week 1	7.08 ± 0.12	7.23 ± 0.07	7.22 ± 0.08[3]	7.36 ± 0.07*	7.26 ± 0.09	7.76 ± 0.08**
Week 3	8.10 ± 0.13	7.64 ± 0.08*[3]	7.61 ± 0.07*	7.56 ± 0.07**	7.95 ± 0.08*[3]	7.37 ± 0.09**
Week 13	8.84 ± 0.07	9.09 ± 0.08	8.95 ± 0.06	9.07 ± 0.05	8.48 ± 0.09	8.32 ± 0.08**
p-Nitrotoluene						
Week 1	7.73 ± 0.14	7.77 ± 0.10	7.86 ± 0.10	7.87 ± 0.12	8.04 ± 0.16	8.33 ± 0.06**
Week 3	8.31 ± 0.11	8.39 ± 0.12[3]	8.36 ± 0.10[3]	8.17 ± 0.03	8.21 ± 0.10	7.88 ± 0.10*[3]
Week 13	8.97 ± 0.08	8.74 ± 0.11	8.86 ± 0.06	8.66 ± 0.12	8.56 ± 0.05**	8.33 ± 0.10**

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Mean cell volume (fL)						
o-Nitrotoluene						
Week 1	61.1 ± 0.3	61.1 ± 0.3	60.8 ± 0.3	60.7 ± 0.2	60.4 ± 0.2	59.8 ± 0.3**
Week 3	59.3 ± 0.3	59.4 ± 0.3	59.3 ± 0.3	59.4 ± 0.3	59.0 ± 0.4[3]	60.1 ± 0.3[3]
Week 13	50.5 ± 0.1	50.3 ± 0.2	50.2 ± 0.1	50.4 ± 0.2	51.6 ± 0.1**	53.4 ± 0.4**
m-Nitrotoluene						
Week 1	59.7 ± 0.3	59.7 ± 0.3	59.8 ± 0.3[3]	59.6 ± 0.1	59.2 ± 0.3	59.0 ± 0.2
Week 3	59.2 ± 0.4	59.7 ± 0.3[3]	59.9 ± 0.3	59.9 ± 0.3	59.2 ± 0.3[3]	60.9 ± 0.5
Week 13	51.2 ± 0.2	51.3 ± 0.2	51.3 ± 0.1	51.4 ± 0.2	52.1 ± 0.1**	55.0 ± 0.3**
p-Nitrotoluene						
Week 1	57.9 ± 0.4	58.2 ± 0.3	57.6 ± 0.3	58.2 ± 0.2	57.8 ± 0.3	57.7 ± 0.2
Week 3	56.8 ± 0.4	57.7 ± 0.2*[3]	57.6 ± 0.2[3]	58.0 ± 0.3**	58.1 ± 0.4**	60.3 ± 0.5**[4]
Week 13	51.1 ± 0.1	51.0 ± 0.2	50.9 ± 0.1	50.9 ± 0.1	51.5 ± 0.2	54.5 ± 0.2**
Mean cell hemoglobin (pg)						
o-Nitrotoluene						
Week 1	20.4 ± 0.1	20.4 ± 0.1	20.5 ± 0.1	20.2 ± 0.1	20.1 ± 0.1*	20.2 ± 0.1
Week 3	20.4 ± 0.1	20.4 ± 0.1	20.3 ± 0.1	20.3 ± 0.1	20.1 ± 0.1*[3]	20.1 ± 0.1[3]
Week 13	17.6 ± 0.1	17.5 ± 0.1	17.6 ± 0.1	17.5 ± 0.1	17.6 ± 0.1	18.1 ± 0.1**
m-Nitrotoluene						
Week 1	20.2 ± 0.1	20.3 ± 0.1	20.3 ± 0.1[3]	20.2 ± 0.1	19.9 ± 0.1	19.8 ± 0.1*
Week 3	20.1 ± 0.1	20.1 ± 0.1[3]	20.2 ± 0.1	20.2 ± 0.1	19.9 ± 0.1[3]	20.0 ± 0.1
Week 13	17.6 ± 0.1	17.7 ± 0.1	17.6 ± 0.1	17.7 ± 0.1	17.6 ± 0.1	18.3 ± 0.1**
p-Nitrotoluene						
Week 1	20.3 ± 0.1	20.4 ± 0.1	20.0 ± 0.1	20.4 ± 0.1	20.2 ± 0.2	20.4 ± 0.1
Week 3	19.4 ± 0.2	19.9 ± 0.1**[3]	20.0 ± 0.1**[3]	20.3 ± 0.2**	20.1 ± 0.1**	20.8 ± 0.2**[4]
Week 13	17.8 ± 0.1	17.6 ± 0.1	17.7 ± 0.1[3]	17.8 ± 0.2	17.7 ± 0.1	18.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
o-Nitrotoluene						
Week 1	33.4 ± 0.1	33.3 ± 0.2	33.6 ± 0.1	33.4 ± 0.1	33.3 ± 0.1	33.7 ± 0.1
Week 3	34.3 ± 0.1	34.3 ± 0.2	34.3 ± 0.1	34.1 ± 0.1	34.1 ± 0.1[3]	33.5 ± 0.2**[3]
Week 13	34.7 ± 0.1	34.8 ± 0.1	34.9 ± 0.1	34.6 ± 0.1	34.0 ± 0.1**	33.9 ± 0.1**
m-Nitrotoluene						
Week 1	33.8 ± 0.2	34.0 ± 0.2	34.0 ± 0.1[3]	33.9 ± 0.2	33.7 ± 0.1	33.6 ± 0.2
Week 3	34.0 ± 0.2	33.8 ± 0.1[3]	33.6 ± 0.1	33.7 ± 0.1[3]	33.7 ± 0.1	32.9 ± 0.1**
Week 13	34.4 ± 0.2	34.6 ± 0.1	34.3 ± 0.2	34.3 ± 0.2	33.8 ± 0.1**	33.3 ± 0.2**
p-Nitrotoluene						
Week 1	35.1 ± 0.1	35.0 ± 0.1	34.7 ± 0.2	35.0 ± 0.2	35.0 ± 0.2	35.3 ± 0.1
Week 3	34.2 ± 0.2	34.6 ± 0.1[3]	34.6 ± 0.1[3]	34.9 ± 0.2**	34.7 ± 0.1*	34.6 ± 0.2*[4]
Week 13	34.8 ± 0.1	34.6 ± 0.1	34.7 ± 0.2[3]	35.1 ± 0.5	34.5 ± 0.1	33.1 ± 0.2**

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Platelets (10^3/μL)						
o-Nitrotoluene						
Week 1	1,153.3 ± 14.0	1,095.9 ± 32.0	1,198.6 ± 26.1	1,202.9 ± 19.0	1,260.9 ± 19.3**	1,318.6 ± 76.8**
Week 3	934.4 ± 49.9	1,037.9 ± 15.5	1,062.3 ± 18.7*	1,172.3 ± 33.2**	994.6 ± 59.2[3]	1,460.8 ± 42.4**[3]
Week 13	717.5 ± 4.1	813.3 ± 19.4**	761.9 ± 34.9**	939.3 ± 64.8**	913.4 ± 27.4**	949.6 ± 35.0**
m-Nitrotoluene						
Week 1	1,031.6 ± 46.1	1,047.8 ± 22.5	1,044.3 ± 17.4[3]	1,045.2 ± 19.8	941.8 ± 41.9*	931.5 ± 22.1**
Week 3	942.2 ± 21.1	976.2 ± 26.5[3]	1,008.2 ± 18.3*	1,009.8 ± 19.0*	952.3 ± 19.7[3]	1,051.3 ± 19.5**
Week 13	739.9 ± 32.3	740.9 ± 12.7	751.2 ± 10.3	751.3 ± 22.4	789.9 ± 13.1	882.5 ± 24.2**
p-Nitrotoluene						
Week 1	1,073.4 ± 30.3	1,065.0 ± 28.9	1,036.9 ± 23.4	1,043.3 ± 17.9[3]	1,013.2 ± 18.6[3]	1,021.0 ± 27.0
Week 3	971.7 ± 17.3	938.3 ± 7.3[3]	935.4 ± 13.6[3]	1,002.3 ± 16.3	966.4 ± 19.8	1,051.8 ± 37.4[4]
Week 13	733.3 ± 16.4	722.7 ± 16.7	662.3 ± 36.2[3]	683.8 ± 47.2	766.0 ± 11.6	719.0 ± 40.6
Reticulocytes (10^6/μL)						
o-Nitrotoluene						
Week 1	0.84 ± 0.05	0.83 ± 0.06	0.76 ± 0.06	0.77 ± 0.03	0.66 ± 0.05**	0.62 ± 0.05**
Week 3	0.38 ± 0.03	0.43 ± 0.04	0.34 ± 0.03	0.39 ± 0.04	0.19 ± 0.05[3]	0.53 ± 0.06[3]
Week 13	0.25 ± 0.03	0.25 ± 0.03	0.26 ± 0.01[3]	0.26 ± 0.02	0.40 ± 0.05*	0.46 ± 0.03**
m-Nitrotoluene						
Week 1	0.25 ± 0.03	0.29 ± 0.03	0.29 ± 0.02[3]	0.27 ± 0.02	0.25 ± 0.01	0.12 ± 0.01**
Week 3	0.15 ± 0.01	0.18 ± 0.02[3]	0.16 ± 0.02	0.18 ± 0.02	0.17 ± 0.02[3]	0.29 ± 0.03**
Week 13	0.32 ± 0.02	0.36 ± 0.04	0.32 ± 0.02	0.32 ± 0.03	0.35 ± 0.03	0.44 ± 0.03**
p-Nitrotoluene						
Week 1	0.21 ± 0.01	0.22 ± 0.02	0.23 ± 0.03	0.25 ± 0.02	0.22 ± 0.03	0.08 ± 0.01**
Week 3	0.41 ± 0.03	0.37 ± 0.03[3]	0.32 ± 0.04[3]	0.34 ± 0.04	0.41 ± 0.04	0.35 ± 0.05[4]
Week 13	0.13 ± 0.01	0.12 ± 0.02	0.09 ± 0.02[3]	0.09 ± 0.02	0.16 ± 0.02	0.18 ± 0.03
Leukocytes (10^3/μL)						
o-Nitrotoluene						
Week 1	7.55 ± 0.26	7.28 ± 0.46	8.15 ± 0.34	8.19 ± 0.41	9.08 ± 0.42*	9.95 ± 0.37**
Week 3	11.74 ± 0.57	10.53 ± 0.66	10.99 ± 0.47	11.03 ± 0.60	11.50 ± 1.12[3]	18.04 ± 1.12**[3]
Week 13	10.70 ± 0.27	11.08 ± 0.33	12.38 ± 0.54*	14.49 ± 1.08**	15.50 ± 0.63**	17.98 ± 0.83**
m-Nitrotoluene						
Week 1	9.43 ± 0.62	9.99 ± 0.65	10.30 ± 0.36[3]	10.53 ± 0.30	9.37 ± 0.43	10.01 ± 0.71
Week 3	11.12 ± 0.56	9.89 ± 0.61[3]	10.45 ± 0.43	10.52 ± 0.55	11.27 ± 0.59[3]	12.38 ± 0.23
Week 13	10.87 ± 0.55	11.96 ± 0.56	11.24 ± 0.31	11.18 ± 0.46	11.74 ± 0.46	10.09 ± 0.59
p-Nitrotoluene						
Week 1	11.14 ± 0.46	9.70 ± 0.61	9.90 ± 0.62	10.01 ± 0.50	10.12 ± 0.67	11.03 ± 0.51
Week 3	11.47 ± 0.46	11.46 ± 0.50[3]	11.31 ± 0.59[3]	11.73 ± 0.36	11.22 ± 0.41	11.05 ± 0.50[4]
Week 13	11.00 ± 0.40	10.26 ± 0.25	9.51 ± 0.61[3]	8.72 ± 0.56**	9.80 ± 0.38	10.33 ± 0.29

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Segmented neutrophils ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	0.93 ± 0.13	0.84 ± 0.13	0.71 ± 0.10	0.84 ± 0.12	1.02 ± 0.09	0.95 ± 0.12
Week 3	0.94 ± 0.08	0.76 ± 0.08	0.80 ± 0.07	0.70 ± 0.09	0.89 ± 0.18[3]	1.52 ± 0.23[3]
Week 13	1.44 ± 0.17	1.41 ± 0.12	1.78 ± 0.23	1.66 ± 0.18[3]	2.11 ± 0.26*	1.91 ± 0.25
m-Nitrotoluene						
Week 1	0.78 ± 0.10	1.00 ± 0.21	0.93 ± 0.14[3]	1.27 ± 0.16*	0.96 ± 0.15	1.06 ± 0.10
Week 3	1.00 ± 0.09	0.93 ± 0.15[3]	1.23 ± 0.17	0.82 ± 0.05	1.04 ± 0.12[3]	0.92 ± 0.12
Week 13	1.61 ± 0.19	1.62 ± 0.25	1.91 ± 0.38	1.51 ± 0.18	1.82 ± 0.22	1.34 ± 0.14
p-Nitrotoluene						
Week 1	1.17 ± 0.18	0.77 ± 0.07	1.17 ± 0.18	1.25 ± 0.16	1.22 ± 0.11	1.47 ± 0.13
Week 3	1.48 ± 0.19	1.57 ± 0.26[3]	1.47 ± 0.15[3]	1.70 ± 0.14	1.33 ± 0.12	1.41 ± 0.19[4]
Week 13	1.47 ± 0.152	1.11 ± 0.102	1.37 ± 0.30[3]	1.30 ± 0.12	1.33 ± 0.18	1.51 ± 0.15
Lymphocytes ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	6.57 ± 0.15	6.31 ± 0.39	7.35 ± 0.29	7.28 ± 0.34	7.97 ± 0.38**	8.93 ± 0.40**
Week 3	10.67 ± 0.56	9.65 ± 0.60	10.08 ± 0.46	10.16 ± 0.68	10.50 ± 1.07[3]	16.33 ± 1.06**[3]
Week 13	9.14 ± 0.20	9.56 ± 0.43	10.48 ± 0.64	11.54 ± 0.62**	13.23 ± 0.75**	15.90 ± 0.77**
m-Nitrotoluene						
Week 1	8.40 ± 0.56	8.92 ± 0.61	9.20 ± 0.45[3]	9.06 ± 0.24	8.18 ± 0.42	8.71 ± 0.64
Week 3	9.90 ± 0.60	8.77 ± 0.49[3]	9.04 ± 0.51	9.52 ± 0.52	9.96 ± 0.61[3]	11.27 ± 0.25*
Week 13	9.10 ± 0.43	10.16 ± 0.59	9.06 ± 0.40	9.47 ± 0.45	9.68 ± 0.52	8.55 ± 0.52
p-Nitrotoluene						
Week 1	9.81 ± 0.50	8.90 ± 0.60	8.69 ± 0.58	8.68 ± 0.48	8.78 ± 0.59	9.52 ± 0.52
Week 3	9.87 ± 0.45	9.77 ± 0.27[3]	9.70 ± 0.51[3]	9.77 ± 0.33	9.81 ± 0.42	9.54 ± 0.55[4]
Week 13	9.42 ± 0.36	9.05 ± 0.23	8.11 ± 0.70[3]	7.36 ± 0.47*	8.43 ± 0.30	8.75 ± 0.25
Monocytes ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	0.07 ± 0.03	0.13 ± 0.04	0.06 ± 0.02	0.06 ± 0.02	0.08 ± 0.04	0.05 ± 0.02
Week 3	0.09 ± 0.03	0.09 ± 0.03	0.10 ± 0.05	0.06 ± 0.02	0.11 ± 0.04[3]	0.11 ± 0.05[3]
Week 13	0.04 ± 0.02	0.04 ± 0.03	0.08 ± 0.05	0.09 ± 0.04	0.12 ± 0.04	0.11 ± 0.05
m-Nitrotoluene						
Week 1	0.22 ± 0.04	0.06 ± 0.02*	0.06 ± 0.02*[3]	0.14 ± 0.04	0.18 ± 0.04	0.18 ± 0.05
Week 3	0.17 ± 0.05	0.13 ± 0.05[3]	0.13 ± 0.05	0.16 ± 0.04	0.19 ± 0.06[3]	0.18 ± 0.04
Week 13	0.08 ± 0.04	0.10 ± 0.05	0.15 ± 0.06	0.12 ± 0.06	0.16 ± 0.07	0.07 ± 0.03
p-Nitrotoluene						
Week 1	0.11 ± 0.04	0.02 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.06 ± 0.03	0.02 ± 0.01
Week 3	0.11 ± 0.03	0.08 ± 0.03[3]	0.12 ± 0.05[3]	0.22 ± 0.05	0.08 ± 0.02	0.09 ± 0.04[4]
Week 13	0.05 ± 0.02	0.04 ± 0.02	0.02 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.06 ± 0.03

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Eosinophils ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.01
Week 3	0.05 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	0.09 ± 0.03	0.00 ± 0.00[3]	0.09 ± 0.05[3]
Week 13	0.08 ± 0.03	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.03	0.06 ± 0.03	0.06 ± 0.03
m-Nitrotoluene						
Week 1	0.04 ± 0.02	0.02 ± 0.01	0.08 ± 0.03	0.04 ± 0.02	0.04 ± 0.02	0.06 ± 0.02
Week 3	0.05 ± 0.03	0.02 ± 0.02	0.06 ± 0.02	0.01 ± 0.01	0.06 ± 0.03	0.01 ± 0.01
Week 13	0.08 ± 0.03	0.08 ± 0.03	0.10 ± 0.04	0.07 ± 0.03	0.07 ± 0.03	0.14 ± 0.04
p-Nitrotoluene						
Week 1	0.04 ± 0.02	0.02 ± 0.01	0.00 ± 0.00	0.05 ± 0.02	0.05 ± 0.02	0.02 ± 0.01
Week 3	0.01 ± 0.01	0.02 ± 0.02[3]	0.01 ± 0.01[3]	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01[4]
Week 13	0.05 ± 0.02	0.05 ± 0.02	0.01 ± 0.01[3]	0.06 ± 0.03	0.03 ± 0.02	0.01 ± 0.01
Nucleated erythrocytes/100 leukocytes						
o-Nitrotoluene						
Week 1	0.80 ± 0.33	0.70 ± 0.30	0.60 ± 0.34	1.00 ± 0.39	0.80 ± 0.39	0.80 ± 0.29
Week 3	0.20 ± 0.13	0.10 ± 0.10	0.20 ± 0.13	0.60 ± 0.22	0.20 ± 0.13	1.44 ± 0.63*c
Week 13	0.20 ± 0.13	0.10 ± 0.10	0.30 ± 0.15	0.20 ± 0.20	0.50 ± 0.22	0.40 ± 0.22
m-Nitrotoluene						
Week 1	0.60 ± 0.22	1.10 ± 0.35	0.67 ± 0.37[3]	0.20 ± 0.13	1.30 ± 0.40	0.50 ± 0.22
Week 3	0.30 ± 0.21	0.00 ± 0.00[3]	0.60 ± 0.31	0.40 ± 0.22	0.44 ± 0.34[3]	2.80 ± 0.65**
Week 13	0.50 ± 0.22	0.50 ± 0.22	0.30 ± 0.21	0.20 ± 0.13	0.50 ± 0.22	0.80 ± 0.29
p-Nitrotoluene						
Week 1	0.50 ± 0.22	0.70 ± 0.26	0.90 ± 0.41	0.70 ± 0.34	0.70 ± 0.42	0.70 ± 0.30
Week 3	0.30 ± 0.21	0.56 ± 0.34[3]	0.11 ± 0.11[3]	0.00 ± 0.00	0.60 ± 0.31	2.00 ± 0.46**[4]
Week 13	0.40 ± 0.16	0.20 ± 0.20	0.78 ± 0.43[3]	0.70 ± 0.34	0.50 ± 0.22	2.40 ± 0.67**
Chemistry						
Urea nitrogen (mg/dL)						
o-Nitrotoluene						
Week 1	20.0 ± 0.5	18.8 ± 0.5	19.1 ± 0.4	19.2 ± 0.6	20.7 ± 0.5	20.5 ± 0.4
Week 3	21.0 ± 0.4	18.3 ± 0.7**	20.6 ± 0.7	17.4 ± 0.5**	16.9 ± 0.4**	20.4 ± 0.4**[3]
Week 13	19.9 ± 0.5	20.5 ± 0.6	21.6 ± 0.6	20.5 ± 0.8	19.1 ± 0.7	19.5 ± 0.2
m-Nitrotoluene						
Week 1	17.6 ± 0.9	21.4 ± 1.5*	17.8 ± 0.7	19.3 ± 0.6	19.9 ± 0.6	21.0 ± 0.4**
Week 3	23.5 ± 0.8	23.3 ± 1.5	21.4 ± 0.3*	20.1 ± 0.5**	21.5 ± 0.5*	20.4 ± 0.2**
Week 13	17.3 ± 0.4	18.4 ± 0.6	15.2 ± 0.8	16.4 ± 0.5	13.5 ± 0.5**	17.3 ± 0.5
p-Nitrotoluene						
Week 1	20.3 ± 2.0	17.9 ± 1.0	19.7 ± 0.8	16.8 ± 0.9	16.4 ± 0.6*	17.7 ± 1.1
Week 3	18.5 ± 0.8	18.9 ± 0.9[3]	18.6 ± 0.7[3]	19.2 ± 0.6	13.8 ± 0.6**	14.9 ± 0.9**[4]
Week 13	20.3 ± 0.9	22.5 ± 1.0	22.5 ± 0.9	22.5 ± 0.7	20.8 ± 0.9	23.7 ± 0.7*

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Creatinine (mg/dL)						
o-Nitrotoluene						
Week 1	0.55 ± 0.02	0.55 ± 0.02	0.54 ± 0.02	0.53 ± 0.02	0.56 ± 0.02	0.53 ± 0.02
Week 3	0.60 ± 0.00	0.59 ± 0.01	0.60 ± 0.00	0.58 ± 0.01	0.53 ± 0.02**	0.56 ± 0.02**[3]
Week 13	0.70 ± 0.00	0.70 ± 0.00	0.70 ± 0.00	0.70 ± 0.02	0.70 ± 0.00	0.68 ± 0.01
m-Nitrotoluene						
Week 1	0.51 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.54 ± 0.02	0.57 ± 0.02**	0.63 ± 0.02**
Week 3	0.57 ± 0.02	0.57 ± 0.02	0.60 ± 0.00	0.57 ± 0.02	0.60 ± 0.00	0.65 ± 0.02**
Week 13	0.65 ± 0.02	0.68 ± 0.01	0.66 ± 0.02	0.69 ± 0.01	0.71 ± 0.02*	0.77 ± 0.02**
p-Nitrotoluene						
Week 1	0.68 ± 0.01	0.59 ± 0.03*	0.61 ± 0.02	0.58 ± 0.02*	0.61 ± 0.03	0.64 ± 0.03
Week 3	0.57 ± 0.03	0.62 ± 0.03[3]	0.64 ± 0.04[3]	0.64 ± 0.02	0.65 ± 0.02	0.59 ± 0.02[4]
Week 13	0.72 ± 0.02	0.69 ± 0.02	0.74 ± 0.02	0.81 ± 0.02**	0.83 ± 0.03**	0.94 ± 0.03**
Total protein (g/dL)						
o-Nitrotoluene						
Week 1	6.5 ± 0.1	6.1 ± 0.1**	6.1 ± 0.1*[3]	5.7 ± 0.1**	5.7 ± 0.1**	5.3 ± 0.1**[4]
Week 3	6.4 ± 0.1	6.1 ± 0.1*	5.8 ± 0.1**	5.5 ± 0.1**	5.2 ± 0.1**	5.0 ± 0.1**[3]
Week 13	6.9 ± 0.0	7.1 ± 0.1	7.1 ± 0.1	6.9 ± 0.3	7.1 ± 0.1	7.3 ± 0.0**
m-Nitrotoluene						
Week 1	5.7 ± 0.1	5.8 ± 0.0	5.8 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1*
Week 3	6.2 ± 0.1	6.0 ± 0.1	6.1 ± 0.0	5.9 ± 0.1	6.2 ± 0.0	6.0 ± 0.1
Week 13	6.8 ± 0.1	6.9 ± 0.1	6.7 ± 0.1	6.9 ± 0.0	6.6 ± 0.1	6.8 ± 0.1
p-Nitrotoluene						
Week 1	6.4 ± 0.1	6.2 ± 0.1*	6.2 ± 0.1*	6.0 ± 0.1**	6.0 ± 0.1**	6.0 ± 0.1**[3]
Week 3	6.0 ± 0.1	6.3 ± 0.2[3]	6.3 ± 0.2[3]	5.9 ± 0.1	6.0 ± 0.2	6.0 ± 0.1[4]
Week 13	7.0 ± 0.1	6.8 ± 0.1	6.6 ± 0.1*	6.6 ± 0.1**	6.6 ± 0.1**	6.4 ± 0.1**
Albumin (g/dL)						
o-Nitrotoluene						
Week 1	5.0 ± 0.1	4.7 ± 0.1**	4.7 ± 0.1*[3]	4.4 ± 0.1**	4.4 ± 0.0**	4.1 ± 0.0**[4]
Week 3	5.1 ± 0.0	4.9 ± 0.1	4.7 ± 0.0**	4.5 ± 0.1**	4.3 ± 0.1**	4.1 ± 0.1**[3]
Week 13	5.0 ± 0.0	5.2 ± 0.1*	5.3 ± 0.1**	5.1 ± 0.2**	5.4 ± 0.1**	5.5 ± 0.1**
m-Nitrotoluene						
Week 1	4.6 ± 0.0	4.7 ± 0.0	4.7 ± 0.0	4.7 ± 0.0	4.8 ± 0.0**	5.0 ± 0.0**
Week 3	5.0 ± 0.1	4.8 ± 0.0	4.9 ± 0.0	4.8 ± 0.1	5.1 ± 0.0	5.1 ± 0.1
Week 13	5.1 ± 0.1	5.2 ± 0.1	5.0 ± 0.0	5.3 ± 0.1	5.1 ± 0.1	5.3 ± 0.1*
p-Nitrotoluene						
Week 1	4.0 ± 0.0	3.9 ± 0.0	3.9 ± 0.0	3.9 ± 0.0	4.0 ± 0.1	4.0 ± 0.1
Week 3	3.9 ± 0.1	4.0 ± 0.1[3]	4.0 ± 0.1[3]	4.0 ± 0.1	4.1 ± 0.1*	4.2 ± 0.1**[4]
Week 13	4.3 ± 0.1	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.1	4.4 ± 0.0	4.4 ± 0.1

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Methemoglobin (%)						
o-Nitrotoluene						
Week 1	1.39 ± 0.23	1.22 ± 0.19	1.21 ± 0.16	1.64 ± 0.25	2.17 ± 0.12**	3.09 ± 0.19**
Week 3	1.41 ± 0.14[4]	1.98 ± 0.24	2.41 ± 0.31*	2.99 ± 0.23**[3]	4.28 ± 0.32**	8.41 ± 0.34**[5]
Week 13	2.46 ± 0.35	3.21 ± 0.68	3.73 ± 0.46[4]	4.87 ± 0.42**	6.87 ± 0.39**[3]	11.11 ± 0.60**[4]
m-Nitrotoluene						
Week 1	–[6]	–	–	–	–	–
Week 3	2.00 ± 0.23	2.04 ± 0.19[4]	1.98 ± 0.24	2.22 ± 0.21	2.31 ± 0.16	3.28 ± 0.22**[4]
Week 13	3.19 ± 0.14	3.64 ± 0.31	3.14 ± 0.18	3.71 ± 0.18*	3.89 ± 0.24*	4.56 ± 0.36 **
p-Nitrotoluene						
Week 1	3.68 ± 0.61	3.07 ± 0.53	3.49 ± 0.38	3.37 ± 0.63	2.56 ± 0.26	3.57 ± 0.55
Week 3	5.65 ± 0.44	6.26 ± 0.26[3]	5.88 ± 0.31[3]	5.88 ± 0.42	6.59 ± 0.41	7.10 ± 0.52**[4]
Week 13	6.54 ± 0.18	5.43 ± 0.26	6.13 ± 0.20[4]	5.74 ± 0.26	7.05 ± 0.21	8.08 ± 0.33*
Alkaline phosphatase (IU/L)						
o-Nitrotoluene						
Week 1	749 ± 12	743 ± 22	738 ± 13	740 ± 21	770 ± 20	682 ± 17[3]
Week 3	557 ± 5	522 ± 10**	536 ± 12*	480 ± 10**	405 ± 10**	381 ± 7**[3]
Week 13	238 ± 4	240 ± 6	241 ± 9[3]	214 ± 5*	216 ± 6	235 ± 4
m-Nitrotoluene						
Week 1	612 ± 15	620 ± 14	600 ± 13	601 ± 10	551 ± 14**	527 ± 12**
Week 3	494 ± 13	513 ± 11	488 ± 6	488 ± 9	459 ± 14	430 ± 10**
Week 13	249 ± 3	259 ± 6	224 ± 3**	255 ± 6	207 ± 7**	207 ± 7**
p-Nitrotoluene						
Week 1	367 ± 11	371 ± 10	348 ± 9	371 ± 8	345 ± 10	336 ± 13
Week 3	299 ± 7	321 ± 9[3]	280 ± 7[3]	288 ± 6	265 ± 4**	246 ± 7**[4]
Week 13	206 ± 5	223 ± 4	203 ± 8	202 ± 6	207 ± 7	216 ± 12
Alanine aminotransferase (IU/L)						
o-Nitrotoluene						
Week 1	40 ± 1	44 ± 1	42 ± 1	47 ± 4	48 ± 2**	41 ± 2[3]
Week 3	48 ± 1	45 ± 1	48 ± 1	47 ± 2	49 ± 1	42 ± 1**[3]
Week 13	55 ± 2	50 ± 3	55 ± 1	56 ± 4	63 ± 4	68 ± 3**
m-Nitrotoluene						
Week 1	42 ± 1	40 ± 1	42 ± 1	42 ± 1	41 ± 2	49 ± 3
Week 3	45 ± 1	45 ± 1	49 ± 4	41 ± 1	46 ± 1	48 ± 2
Week 13	64 ± 6	84 ± 6	53 ± 5	67 ± 3	48 ± 1**	51 ± 2*
p-Nitrotoluene						
Week 1	34 ± 1	33 ± 1	32 ± 1	31 ± 1	34 ± 2	35 ± 1
Week 3	33 ± 1	31 ± 1[3]	31 ± 1[3]	32 ± 1	34 ± 1	43 ± 5**[4]
Week 13	46 ± 1	43 ± 2	49 ± 3	47 ± 3[3]	36 ± 1**	35 ± 1**

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Creatine kinase (IU/L)						
o-Nitrotoluene						
Week 1	337.7 ± 32.5	320.2 ± 43.2	256.0 ± 24.2[3]	347.3 ± 53.2	284.6 ± 22.1[3]	245.1 ± 12.5[4]
Week 3	278.7 ± 14.5	243.4 ± 22.9	212.5 ± 17.1	285.6 ± 25.2	211.8 ± 18.5	301.8 ± 40.3[3]
Week 13	114.8 ± 14.9	94.1 ± 3.8	97.2 ± 5.5	113.9 ± 14.2	128.4 ± 23.9	141.0 ± 19.0
m-Nitrotoluene						
Week 1	336.4 ± 33.1[3]	340.6 ± 51.9	437.8 ± 65.4	361.0 ± 58.4	319.8 ± 48.9	308.2 ± 33.5
Week 3	265.0 ± 42.8	205.6 ± 12.1	297.2 ± 53.9	214.8 ± 30.4	177.9 ± 16.2[3]	219.4 ± 21.8
Week 13	112.1 ± 8.8	124.4 ± 18.1	118.2 ± 12.5	98.9 ± 7.2	146.6 ± 29.1	144.9 ± 19.7
p-Nitrotoluene						
Week 1	183.5 ± 21.5	179.5 ± 29.6	146.6 ± 22.3	176.9 ± 22.7	166.5 ± 46.3	302.0 ± 128.4
Week 3	166.1 ± 17.5	159.2 ± 24.5[3]	162.2 ± 18.8[3]	143.3 ± 11.5	135.7 ± 14.9	238.6 ± 66.2[4]
Week 13	55.8 ± 3.6[3]	75.0 ± 19.2	92.6 ± 23.8	80.2 ± 10.9	71.2 ± 16.0	76.4 ± 13.0
Sorbitol dehydrogenase (IU/L)						
o-Nitrotoluene						
Week 1	8 ± 1	7 ± 1	9 ± 1	8 ± 1	9 ± 1	9 ± 1
Week 3	5 ± 1	6 ± 0	7 ± 1	6 ± 0	6 ± 0	6 ± 1[3]
Week 13	13 ± 1	14 ± 1	14 ± 1	17 ± 1**	16 ± 1**	18 ± 1**
m-Nitrotoluene						
Week 1	6 ± 1	5 ± 1	5 ± 1	5 ± 0	4 ± 0	6 ± 1
Week 3	9 ± 1	10 ± 1	10 ± 1	9 ± 1	8 ± 1	11 ± 1
Week 13	10 ± 0	12 ± 1	10 ± 1	12 ± 0	11 ± 1	10 ± 1
p-Nitrotoluene						
Week 1	7 ± 0	6 ± 0	6 ± 0*	6 ± 0*	6 ± 0**	5 ± 0**
Week 3	5 ± 0	5 ± 0[3]	6 ± 0[3]	5 ± 0	5 ± 0	5 ± 1[4]
Week 13	10 ± 1	10 ± 0	13 ± 1	13 ± 1[3]	7 ± 0**	7 ± 1**
Bile acids (μmol/L)						
o-Nitrotoluene						
Week 1	14.10 ± 1.61	10.10 ± 1.25	14.20 ± 3.72	34.30 ± 8.64	35.60 ± 7.07	22.50 ± 4.24
Week 3	14.60 ± 5.52	6.80 ± 1.85	8.80 ± 2.52	9.00 ± 2.47	15.30 ± 3.25	35.89 ± 5.18**[3]
Week 13	7.40 ± 0.86	10.20 ± 1.67	7.60 ± 1.49	11.10 ± 1.84	23.50 ± 4.20**	28.50 ± 3.60**
m-Nitrotoluene						
Week 1	11.67 ± 2.40[3]	14.20 ± 5.52	19.10 ± 4.75	16.90 ± 4.42	19.30 ± 7.03	22.44 ± 3.81[3]
Week 3	9.60 ± 1.85	6.10 ± 1.39	5.40 ± 0.73	6.30 ± 1.76	6.90 ± 2.10	37.00 ± 6.80
Week 13	4.00 ± 0.99	4.60 ± 1.33	5.40 ± 1.44	4.50 ± 1.39	15.00 ± 1.59**	23.90 ± 5.14**
p-Nitrotoluene						
Week 1	11.80 ± 1.23	11.20 ± 2.67	7.50 ± 1.01	10.90 ± 2.47	8.80 ± 0.83	16.10 ± 2.23
Week 3	9.40 ± 1.18	8.56 ± 1.83[3]	7.89 ± 1.47[3]	10.30 ± 1.49	6.70 ± 1.14	16.88 ± 4.17[4]
Week 13	6.10 ± 0.98	5.80 ± 0.90	5.50 ± 0.48	7.30 ± 1.71	9.00 ± 2.25[3]	16.20 ± 2.03**

[1] Mean ± standard error for groups of 10 animals unless otherwise specified.
[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*-nitrotoluene or *p*-nitrotoluene.
[3] n=9.
[4] n=8.
[5] n=7.
[6] Inadequate data for analysis.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology						
Hematocrit (%)						
o-Nitrotoluene						
Week 1	43.3 ± 0.2	43.1 ± 0.4	43.6 ± 0.5	43.3 ± 0.5[3]	43.0 ± 0.6[3]	44.1 ± 0.6
Week 3	46.5 ± 0.3	47.2 ± 0.5	47.6 ± 0.5	46.9 ± 0.4	44.4 ± 0.5[3]*	44.4 ± 0.7*
Week 13	44.3 ± 0.4	44.3 ± 0.6[3]	43.2 ± 0.4	43.1 ± 0.5[3]	42.5 ± 0.6*	41.3 ± 0.3**
m-Nitrotoluene						
Week 1	44.4 ± 0.8[4]	43.8 ± 0.5	43.8 ± 0.4	44.2 ± 0.5[4]	45.6 ± 0.7	46.5 ± 0.4*
Week 3	46.5 ± 0.8	46.5 ± 0.5[5]	46.7 ± 0.3[3]	47.2 ± 0.3[3]	47.3 ± 0.4[4]	43.6 ± 0.7*[5]
Week 13	45.6 ± 0.4	46.3 ± 0.3	45.3 ± 0.3	44.7 ± 0.4	44.6 ± 0.3	43.6 ± 0.5**[3]
p-Nitrotoluene						
Week 1	44.3 ± 0.3[4]	44.5 ± 0.5[3]	44.8 ± 0.6	45.0 ± 0.8	46.5 ± 0.5*	46.8 ± 0.6**[3]
Week 3	48.2 ± 0.5[3]	48.1 ± 0.6	48.4 ± 0.4	46.6 ± 0.4[3]	48.3 ± 0.6[3]	47.1 ± 0.7
Week 13	45.7 ± 0.3[3]	44.6 ± 0.2**	44.4 ± 0.5*	45.1 ± 0.3*	45.4 ± 0.5[3]	43.7 ± 0.4**[3]
Hemoglobin (g/dL)						
o-Nitrotoluene						
Week 1	15.0 ± 0.1	15.0 ± 0.1	15.1 ± 0.2	15.0 ± 0.2[3]	14.9 ± 0.2[3]	15.3 ± 0.2
Week 3	16.4 ± 0.1	16.6 ± 0.2	16.6 ± 0.2	16.4 ± 0.1	15.5 ± 0.2**[3]	15.4 ± 0.2**
Week 13	15.7 ± 0.2	15.8 ± 0.2[3]	15.2 ± 0.2	15.2 ± 0.2[3]	15.0 ± 0.2**	14.4 ± 0.1**
m-Nitrotoluene						
Week 1	15.4 ± 0.3[4]	15.2 ± 0.2	15.3 ± 0.2	15.4 ± 0.2[4]	15.6 ± 0.3	15.8 ± 0.2
Week 3	15.9 ± 0.3	16.0 ± 0.2[5]	16.1 ± 0.1[3]	16.1 ± 0.1[3]	16.0 ± 0.1[4]	14.4 ± 0.2**[5]
Week 13	15.6 ± 0.2	15.9 ± 0.1	15.6 ± 0.1	15.4 ± 0.1	15.1 ± 0.1*	14.4 ± 0.2**[3]
p-Nitrotoluene						
Week 1	16.0 ± 0.1[4]	15.9 ± 0.2[3]	16.1 ± 0.2	16.0 ± 0.3	16.7 ± 0.2*	16.7 ± 0.2*[3]
Week 3	17.4 ± 0.2[3]	17.2 ± 0.2	17.4 ± 0.2	16.7 ± 0.2*[3]	17.5 ± 0.3[3]	16.6 ± 0.3*
Week 13	15.9 ± 0.1[3]	15.4 ± 0.1**	15.4 ± 0.2*	15.6 ± 0.1*	15.4 ± 0.3[3]	14.7 ± 0.1**[3]
Erythrocytes (10^6/μL)						
o-Nitrotoluene						
Week 1	7.40 ± 0.04	7.35 ± 0.07	7.43 ± 0.09	7.38 ± 0.12[3]	7.34 ± 0.10[3]	7.57 ± 0.12
Week 3	8.03 ± 0.07	8.12 ± 0.11	8.20 ± 0.09	8.10 ± 0.09	7.63 ± 0.08*[3]	7.52 ± 0.12**
Week 13	8.38 ± 0.07	8.40 ± 0.09[3]	8.19 ± 0.08	8.08 ± 0.10*[3]	7.84 ± 0.10**	7.37 ± 0.05**
m-Nitrotoluene						
Week 1	7.68 ± 0.14[4]	7.50 ± 0.10	7.49 ± 0.06	7.61 ± 0.09[4]	7.79 ± 0.13	8.02 ± 0.08*
Week 3	7.79 ± 0.16	7.77 ± 0.11[5]	7.77 ± 0.06[3]	7.90 ± 0.08[3]	7.97 ± 0.06[4]	7.23 ± 0.10*[5]
Week 13	8.42 ± 0.08	8.54 ± 0.06	8.40 ± 0.05	8.27 ± 0.07	8.06 ± 0.06**	7.53 ± 0.06**[3]
p-Nitrotoluene						
Week 1	7.75 ± 0.04[4]	7.78 ± 0.08[3]	7.87 ± 0.09	7.91 ± 0.15	8.17 ± 0.09**	8.15 ± 0.10**[3]
Week 3	8.33 ± 0.08[3]	8.30 ± 0.11	8.38 ± 0.08	8.10 ± 0.09[3]	8.47 ± 0.14[3]	8.03 ± 0.13
Week 13	8.50 ± 0.04[3]	8.35 ± 0.04*	8.31 ± 0.09*	8.45 ± 0.05	8.40 ± 0.09[3]	8.00 ± 0.07**[3]

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Creatine kinase (IU/L)						
o-Nitrotoluene						
Week 1	337.7 ± 32.5	320.2 ± 43.2	256.0 ± 24.2[3]	347.3 ± 53.2	284.6 ± 22.1[3]	245.1 ± 12.5[4]
Week 3	278.7 ± 14.5	243.4 ± 22.9	212.5 ± 17.1	285.6 ± 25.2	211.8 ± 18.5	301.8 ± 40.3[3]
Week 13	114.8 ± 14.9	94.1 ± 3.8	97.2 ± 5.5	113.9 ± 14.2	128.4 ± 23.9	141.0 ± 19.0
m-Nitrotoluene						
Week 1	336.4 ± 33.1[3]	340.6 ± 51.9	437.8 ± 65.4	361.0 ± 58.4	319.8 ± 48.9	308.2 ± 33.5
Week 3	265.0 ± 42.8	205.6 ± 12.1	297.2 ± 53.9	214.8 ± 30.4	177.9 ± 16.2[3]	219.4 ± 21.8
Week 13	112.1 ± 8.8	124.4 ± 18.1	118.2 ± 12.5	98.9 ± 7.2	146.6 ± 29.1	144.9 ± 19.7
p-Nitrotoluene						
Week 1	183.5 ± 21.5	179.5 ± 29.6	146.6 ± 22.3	176.9 ± 22.7	166.5 ± 46.3	302.0 ± 128.4
Week 3	166.1 ± 17.5	159.2 ± 24.5[3]	162.2 ± 18.8[3]	143.3 ± 11.5	135.7 ± 14.9	238.6 ± 66.2[4]
Week 13	55.8 ± 3.6[3]	75.0 ± 19.2	92.6 ± 23.8	80.2 ± 10.9	71.2 ± 16.0	76.4 ± 13.0
Sorbitol dehydrogenase (IU/L)						
o-Nitrotoluene						
Week 1	8 ± 1	7 ± 1	9 ± 1	8 ± 1	9 ± 1	9 ± 1
Week 3	5 ± 1	6 ± 0	7 ± 1	6 ± 0	6 ± 0	6 ± 1[3]
Week 13	13 ± 1	14 ± 1	14 ± 1	17 ± 1**	16 ± 1**	18 ± 1**
m-Nitrotoluene						
Week 1	6 ± 1	5 ± 1	5 ± 1	5 ± 0	4 ± 0	6 ± 1
Week 3	9 ± 1	10 ± 1	10 ± 1	9 ± 1	8 ± 1	11 ± 1
Week 13	10 ± 0	12 ± 1	10 ± 1	12 ± 0	11 ± 1	10 ± 1
p-Nitrotoluene						
Week 1	7 ± 0	6 ± 0	6 ± 0*	6 ± 0*	6 ± 0**	5 ± 0**
Week 3	5 ± 0	5 ± 0[3]	6 ± 0[3]	5 ± 0	5 ± 0	5 ± 1[4]
Week 13	10 ± 1	10 ± 0	13 ± 1	13 ± 1[3]	7 ± 0**	7 ± 1**
Bile acids (µmol/L)						
o-Nitrotoluene						
Week 1	14.10 ± 1.61	10.10 ± 1.25	14.20 ± 3.72	34.30 ± 8.64	35.60 ± 7.07	22.50 ± 4.24
Week 3	14.60 ± 5.52	6.80 ± 1.85	8.80 ± 2.52	9.00 ± 2.47	15.30 ± 3.25	35.89 ± 5.18**[3]
Week 13	7.40 ± 0.86	10.20 ± 1.67	7.60 ± 1.49	11.10 ± 1.84	23.50 ± 4.20**	28.50 ± 3.60**
m-Nitrotoluene						
Week 1	11.67 ± 2.40[3]	14.20 ± 5.52	19.10 ± 4.75	16.90 ± 4.42	19.30 ± 7.03	22.44 ± 3.81[3]
Week 3	9.60 ± 1.85	6.10 ± 1.39	5.40 ± 0.73	6.30 ± 1.76	6.90 ± 2.10	37.00 ± 6.80
Week 13	4.00 ± 0.99	4.60 ± 1.33	5.40 ± 1.44	4.50 ± 1.39	15.00 ± 1.59**	23.90 ± 5.14**
p-Nitrotoluene						
Week 1	11.80 ± 1.23	11.20 ± 2.67	7.50 ± 1.01	10.90 ± 2.47	8.80 ± 0.83	16.10 ± 2.23
Week 3	9.40 ± 1.18	8.56 ± 1.83[3]	7.89 ± 1.47[3]	10.30 ± 1.49	6.70 ± 1.14	16.88 ± 4.17[4]
Week 13	6.10 ± 0.98	5.80 ± 0.90	5.50 ± 0.48	7.30 ± 1.71	9.00 ± 2.25[3]	16.20 ± 2.03**

[1] Mean ± standard error for groups of 10 animals unless otherwise specified.
[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*-nitrotoluene or *p*-nitrotoluene.
[3] n=9.
[4] n=8.
[5] n=7.
[6] Inadequate data for analysis.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology						
Hematocrit (%)						
o-Nitrotoluene						
Week 1	43.3 ± 0.2	43.1 ± 0.4	43.6 ± 0.5	43.3 ± 0.5[3]	43.0 ± 0.6[3]	44.1 ± 0.6
Week 3	46.5 ± 0.3	47.2 ± 0.5	47.6 ± 0.5	46.9 ± 0.4	44.4 ± 0.5[3*]	44.4 ± 0.7*
Week 13	44.3 ± 0.4	44.3 ± 0.6[3]	43.2 ± 0.4	43.1 ± 0.5[3]	42.5 ± 0.6*	41.3 ± 0.3**
m-Nitrotoluene						
Week 1	44.4 ± 0.8[4]	43.8 ± 0.5	43.8 ± 0.4	44.2 ± 0.5[4]	45.6 ± 0.7	46.5 ± 0.4*
Week 3	46.5 ± 0.8	46.5 ± 0.5[5]	46.7 ± 0.3[3]	47.2 ± 0.3[3]	47.3 ± 0.4[4]	43.6 ± 0.7*[5]
Week 13	45.6 ± 0.4	46.3 ± 0.3	45.3 ± 0.3	44.7 ± 0.4	44.6 ± 0.3	43.6 ± 0.5**[3]
p-Nitrotoluene						
Week 1	44.3 ± 0.3[4]	44.5 ± 0.5[3]	44.8 ± 0.6	45.0 ± 0.8	46.5 ± 0.5*	46.8 ± 0.6**[3]
Week 3	48.2 ± 0.5[3]	48.1 ± 0.6	48.4 ± 0.4	46.6 ± 0.4[3]	48.3 ± 0.6[3]	47.1 ± 0.7
Week 13	45.7 ± 0.3[3]	44.6 ± 0.2**	44.4 ± 0.5*	45.1 ± 0.3*	45.4 ± 0.5[3]	43.7 ± 0.4**[3]
Hemoglobin (g/dL)						
o-Nitrotoluene						
Week 1	15.0 ± 0.1	15.0 ± 0.1	15.1 ± 0.2	15.0 ± 0.2[3]	14.9 ± 0.2[3]	15.3 ± 0.2
Week 3	16.4 ± 0.1	16.6 ± 0.2	16.6 ± 0.2	16.4 ± 0.1	15.5 ± 0.2**[3]	15.4 ± 0.2**
Week 13	15.7 ± 0.2	15.8 ± 0.2[3]	15.2 ± 0.2	15.2 ± 0.2[3]	15.0 ± 0.2**	14.4 ± 0.1**
m-Nitrotoluene						
Week 1	15.4 ± 0.3[4]	15.2 ± 0.2	15.3 ± 0.2	15.4 ± 0.2[4]	15.6 ± 0.3	15.8 ± 0.2
Week 3	15.9 ± 0.3	16.0 ± 0.2[5]	16.1 ± 0.1[3]	16.1 ± 0.1[3]	16.0 ± 0.1[4]	14.4 ± 0.2**[5]
Week 13	15.6 ± 0.2	15.9 ± 0.1	15.6 ± 0.1	15.4 ± 0.1	15.1 ± 0.1*	14.4 ± 0.2**[3]
p-Nitrotoluene						
Week 1	16.0 ± 0.1[4]	15.9 ± 0.2[3]	16.1 ± 0.2	16.0 ± 0.3	16.7 ± 0.2*	16.7 ± 0.2*[3]
Week 3	17.4 ± 0.2[3]	17.2 ± 0.2	17.4 ± 0.2	16.7 ± 0.2*[3]	17.5 ± 0.3[3]	16.6 ± 0.3*
Week 13	15.9 ± 0.1[3]	15.4 ± 0.1**	15.4 ± 0.2*	15.6 ± 0.1*	15.4 ± 0.3[3]	14.7 ± 0.1**[3]
Erythrocytes (10^6/μL)						
o-Nitrotoluene						
Week 1	7.40 ± 0.04	7.35 ± 0.07	7.43 ± 0.09	7.38 ± 0.12[3]	7.34 ± 0.10[3]	7.57 ± 0.12
Week 3	8.03 ± 0.07	8.12 ± 0.11	8.20 ± 0.09	8.10 ± 0.09	7.63 ± 0.08*[3]	7.52 ± 0.12**
Week 13	8.38 ± 0.07	8.40 ± 0.09[3]	8.19 ± 0.08	8.08 ± 0.10*[3]	7.84 ± 0.10**	7.37 ± 0.05**
m-Nitrotoluene						
Week 1	7.68 ± 0.14[4]	7.50 ± 0.10	7.49 ± 0.06	7.61 ± 0.09[4]	7.79 ± 0.13	8.02 ± 0.08*
Week 3	7.79 ± 0.16	7.77 ± 0.11[5]	7.77 ± 0.06[3]	7.90 ± 0.08[3]	7.97 ± 0.06[4]	7.23 ± 0.10*[5]
Week 13	8.42 ± 0.08	8.54 ± 0.06	8.40 ± 0.05	8.27 ± 0.07	8.06 ± 0.06**	7.53 ± 0.06**[3]
p-Nitrotoluene						
Week 1	7.75 ± 0.04[4]	7.78 ± 0.08[3]	7.87 ± 0.09	7.97 ± 0.15	8.17 ± 0.09**	8.15 ± 0.10**[3]
Week 3	8.33 ± 0.08[3]	8.30 ± 0.11	8.38 ± 0.08	8.10 ± 0.09[3]	8.47 ± 0.14[3]	8.03 ± 0.13
Week 13	8.50 ± 0.04[3]	8.35 ± 0.04*	8.31 ± 0.09*	8.45 ± 0.05	8.40 ± 0.09[3]	8.00 ± 0.07**[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Mean cell volume (fL)						
o-Nitrotoluene						
Week 1	58.5 ± 0.3	58.7 ± 0.2	58.6 ± 0.3	58.7 ± 0.3[3]	58.6 ± 0.1[3]	58.3 ± 0.1
Week 3	58.0 ± 0.3	58.1 ± 0.3	58.0 ± 0.4	57.9 ± 0.4	58.2 ± 0.3[3]	59.1 ± 0.3*
Week 13	52.9 ± 0.1	52.8 ± 0.2[3]	52.8 ± 0.1	53.4 ± 0.1**[3]	54.2 ± 0.1**	56.1 ± 0.1**
m-Nitrotoluene						
Week 1	57.8 ± 0.2[4]	58.5 ± 0.3	58.4 ± 0.2	58.1 ± 0.3[4]	58.5 ± 0.2	58.0 ± 0.2
Week 3	59.7 ± 0.3	59.8 ± 0.3[5]	60.0 ± 0.2[3]	59.8 ± 0.2[3]	59.3 ± 0.1[4]	60.3 ± 0.3[5]
Week 13	54.1 ± 0.2	54.2 ± 0.1	54.0 ± 0.1	54.0 ± 0.1	55.3 ± 0.2**	57.9 ± 0.2**[3]
p-Nitrotoluene						
Week 1	57.2 ± 0.4[4]	57.1 ± 0.2[3]	57.0 ± 0.3	56.9 ± 0.3	56.9 ± 0.2	57.3 ± 0.2[3]
Week 3	57.8 ± 0.3[3]	58.0 ± 0.2	57.5 ± 0.2	57.5 ± 0.3[3]	57.1 ± 0.4[3]	58.6 ± 0.4
Week 13	53.7 ± 0.1[3]	53.4 ± 0.1	53.4 ± 0.1	53.3 ± 0.1	54.1 ± 0.2[3]	54.7 ± 0.2*[3]
MCH (pg)						
o-Nitrotoluene						
Week 1	20.2 ± 0.1	20.4 ± 0.0	20.3 ± 0.1	20.3 ± 0.1[3]	20.3 ± 0.1[3]	20.2 ± 0.1
Week 3	20.4 ± 0.1	20.5 ± 0.1	20.2 ± 0.2	20.3 ± 0.2	20.2 ± 0.1[3]	20.5 ± 0.1
Week 13	18.8 ± 0.1	18.8 ± 0.1[3]	18.6 ± 0.1	18.8 ± 0.1[3]	19.1 ± 0.1*	19.6 ± 0.1**
m-Nitrotoluene						
Week 1	20.1 ± 0.1[4]	20.3 ± 0.1	20.4 ± 0.1	20.2 ± 0.1[4]	20.1 ± 0.1	19.7 ± 0.1*
Week 3	20.4 ± 0.1	20.6 ± 0.1[5]	20.7 ± 0.1[3]	20.4 ± 0.1[3]	20.1 ± 0.1[4]	20.0 ± 0.2[5]
Week 13	18.6 ± 0.1	18.6 ± 0.0	18.6 ± 0.1	18.6 ± 0.1	18.8 ± 0.1	19.1 ± 0.1**[3]
p-Nitrotoluene						
Week 1	20.7 ± 0.1[4]	20.5 ± 0.1[3]	20.5 ± 0.1	20.2 ± 0.1*	20.5 ± 0.1	20.5 ± 0.1[3]
Week 3	20.9 ± 0.2[3]	20.7 ± 0.1	20.8 ± 0.1	20.6 ± 0.2[3]	20.7 ± 0.1[3]	20.7 ± 0.1
Week 13	18.7 ± 0.1[3]	18.5 ± 0.1	18.5 ± 0.1	18.5 ± 0.1	18.3 ± 0.3[3]	18.4 ± 0.1[3]
Mean cell hemoglobin concentration (g/dL)						
o-Nitrotoluene						
Week 1	34.5 ± 0.2	34.7 ± 0.1	34.6 ± 0.1	34.6 ± 0.1[3]	34.6 ± 0.2[3]	34.6 ± 0.1
Week 3	35.2 ± 0.1	35.2 ± 0.1	34.8 ± 0.2	35.0 ± 0.2	34.8 ± 0.1[3]	34.6 ± 0.1**
Week 13	35.5 ± 0.1	35.6 ± 0.2[3]	35.3 ± 0.2	35.2 ± 0.1[3]	35.2 ± 0.2	34.9 ± 0.2**
m-Nitrotoluene						
Week 1	34.8 ± 0.1[4]	34.7 ± 0.1	34.9 ± 0.2	34.7 ± 0.3[4]	34.3 ± 0.2	34.0 ± 0.2**
Week 3	34.1 ± 0.1	34.5 ± 0.2[5]	34.4 ± 0.1[3]	34.1 ± 0.1[3]	33.8 ± 0.2[4]	33.1 ± 0.2**[5]
Week 13	34.3 ± 0.1	34.4 ± 0.1	34.5 ± 0.1	34.5 ± 0.1	33.9 ± 0.1*	33.0 ± 0.1**[3]
p-Nitrotoluene						
Week 1	36.1 ± 0.1[4]	35.8 ± 0.2[3]	35.9 ± 0.2	35.6 ± 0.1	36.0 ± 0.1	35.8 ± 0.2[3]
Week 3	36.2 ± 0.2[3]	35.8 ± 0.3	36.2 ± 0.1	35.9 ± 0.2[3]	36.2 ± 0.2[3]	35.3 ± 0.2**
Week 13	34.8 ± 0.2[3]	34.6 ± 0.1	34.7 ± 0.2	34.7 ± 0.1	33.8 ± 0.5[3]*	33.7 ± 0.1**[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Platelets ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	1,014.5 ± 89.1	1,107.5 ± 41.3	1,144.6 ± 28.0	1,227.4 ± 51.2[3]	1,219.9 ± 20.7*[3]	1,275.7 ± 17.3**
Week 3	934.8 ± 47.3	901.9 ± 53.0	915.8 ± 46.1	820.2 ± 80.3	1,021.4 ± 22.9[3]	1,125.0 ± 44.4*
Week 13	683.7 ± 42.2	743.0 ± 19.2[3]	777.9 ± 35.3	751.4 ± 57.0	816.6 ± 41.7**	790.8 ± 60.0*
m-Nitrotoluene						
Week 1	1,045.3 ± 20.2[4]	972.6 ± 22.6*	981.3 ± 46.3	909.3 ± 98.3[4]	953.6 ± 22.2*	885.6 ± 23.0**
Week 3	934.6 ± 51.4	959.7 ± 24.9[5]	958.7 ± 12.7[3]	846.6 ± 78.6[3]	918.6 ± 24.2[4]	941.6 ± 86.9[5]
Week 13	792.4 ± 23.8	762.0 ± 14.9	758.8 ± 10.3	747.7 ± 25.9	785.6 ± 11.9	851.6 ± 20.0*[3]
p-Nitrotoluene						
Week 1	1,013.8 ± 28.3[4]	985.0 ± 33.0[3]	1,005.5 ± 21.0	882.2 ± 34.9**	931.4 ± 16.7*	965.8 ± 19.7*[3]
Week 3	999.3 ± 20.3[3]	976.7 ± 17.1	1,022.4 ± 19.4	964.4 ± 15.1[3]	1,022.6 ± 24.7[3]	1,035.8 ± 17.5
Week 13	791.7 ± 13.0[3]	752.2 ± 7.2	711.4 ± 30.0	565.4 ± 76.8*	677.6 ± 34.2*[3]	785.0 ± 16.6[3]
Reticulocytes ($10^6/\mu L$)						
o-Nitrotoluene						
Week 1	0.57 ± 0.03	0.54 ± 0.02	0.63 ± 0.07	0.62 ± 0.05[3]	0.58 ± 0.05[3]	0.51 ± 0.04
Week 3	0.24 ± 0.04	0.26 ± 0.02	0.25 ± 0.03	0.21 ± 0.02	0.31 ± 0.06[3]	0.34 ± 0.04**
Week 13	0.23 ± 0.02	0.21 ± 0.02[3]	0.27 ± 0.03	0.29 ± 0.04[3]	0.32 ± 0.04*	0.35 ± 0.04**
m-Nitrotoluene						
Week 1	0.20 ± 0.02[4]	0.23 ± 0.03	0.21 ± 0.01	0.20 ± 0.03[4]	0.18 ± 0.02	0.08 ± 0.01**
Week 3	0.11 ± 0.01[3]	0.13 ± 0.02[5]	0.09 ± 0.01[3]	0.10 ± 0.01[3]	0.11 ± 0.01[4]	0.25 ± 0.04**[5]
Week 13	0.20 ± 0.03	0.21 ± 0.02	0.21 ± 0.02	0.23 ± 0.02	0.29 ± 0.03*	0.35 ± 0.03**[3]
p-Nitrotoluene						
Week 1	0.13 ± 0.02[4]	0.14 ± 0.01[3]	0.14 ± 0.01	0.13 ± 0.01	0.11 ± 0.02	0.08 ± 0.01**[3]
Week 3	0.20 ± 0.02[3]	0.13 ± 0.01	0.17 ± 0.03	0.17 ± 0.02[3]	0.18 ± 0.02[3]	0.24 ± 0.03
Week 13	0.06 ± 0.01[3]	0.09 ± 0.01	0.10 ± 0.02*	0.11 ± 0.01**	0.15 ± 0.02**[3]	0.21 ± 0.04**[3]
Leukocytes ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	10.64 ± 0.40	10.35 ± 0.69	10.62 ± 0.34	9.82 ± 0.43[3]	11.11 ± 0.71[3]	12.04 ± 0.53
Week 3	9.30 ± 0.36	10.51 ± 0.61	10.70 ± 0.76	11.41 ± 0.62*	12.24 ± 0.64**[3]	13.82 ± 0.93**
Week 13	9.32 ± 0.43	9.69 ± 0.49[3]	10.56 ± 0.66	12.87 ± 0.90**[3]	14.21 ± 0.83**	14.55 ± 0.63**
m-Nitrotoluene						
Week 1	11.95 ± 0.45[4]	10.29 ± 0.70	10.03 ± 0.46*	11.00 ± 0.68[4]	10.26 ± 0.40	10.53 ± 0.36
Week 3	10.91 ± 0.55	11.91 ± 0.65[5]	11.28 ± 0.46[3]	10.71 ± 0.61[3]	11.00 ± 0.80[4]	13.39 ± 0.42*[5]
Week 13	10.54 ± 0.66	9.45 ± 0.68	10.00 ± 0.30	11.02 ± 0.38	10.10 ± 0.50	11.32 ± 0.58[3]
p-Nitrotoluene						
Week 1	10.18 ± 0.22[4]	10.82 ± 0.39[3]	10.70 ± 0.40	10.17 ± 0.84	10.78 ± 0.55	9.81 ± 0.49[3]
Week 3	13.73 ± 0.95[3]	11.25 ± 0.69	11.27 ± 0.82	10.64 ± 0.89[3]	12.96 ± 0.78[3]	11.22 ± 0.63
Week 13	11.20 ± 0.31[3]	9.46 ± 0.25*	11.85 ± 1.12	11.57 ± 0.74	10.63 ± 0.22[3]	12.10 ± 1.12[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Segmented neutrophils ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	0.87 ± 0.10	0.79 ± 0.15	0.79 ± 0.11	0.77 ± 0.11[3]	0.84 ± 0.16[3]	0.76 ± 0.09
Week 3	0.69 ± 0.09	0.73 ± 0.10	0.78 ± 0.12	0.80 ± 0.11	1.02 ± 0.10*[3]	1.06 ± 0.20
Week 13	1.35 ± 0.26	1.32 ± 0.09[3]	1.32 ± 0.18	1.46 ± 0.24[3]	1.67 ± 0.16	1.16 ± 0.13
m-Nitrotoluene						
Week 1	1.18 ± 0.39[4]	0.81 ± 0.15	0.72 ± 0.09	1.36 ± 0.24[4]	0.91 ± 0.12	0.86 ± 0.07
Week 3	1.52 ± 0.34	0.90 ± 0.10[5]	1.04 ± 0.15[3]	1.10 ± 0.15[3]	1.05 ± 0.18[4]	1.07 ± 0.11[5]
Week 13	2.09 ± 0.63	1.75 ± 0.29	1.39 ± 0.12	2.31 ± 0.27	1.24 ± 0.15	1.66 ± 0.22[3]
p-Nitrotoluene						
Week 1	0.95 ± 0.15[4]	1.20 ± 0.20[3]	1.25 ± 0.14	1.07 ± 0.11	1.04 ± 0.09	1.39 ± 0.12[3]
Week 3	1.53 ± 0.33[3]	1.41 ± 0.15	1.30 ± 0.18	1.39 ± 0.20[3]	1.87 ± 0.27[3]	2.24 ± 0.48
Week 13	2.23 ± 0.31[3]	1.64 ± 0.23	1.97 ± 0.29[3]	2.16 ± 0.47	1.44 ± 0.14[3]	1.67 ± 0.23[3]
Lymphocytes ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	9.65 ± 0.40	9.43 ± 0.62	9.68 ± 0.26	8.94 ± 0.40[3]	10.07 ± 0.61[3]	11.12 ± 0.55
Week 3	8.48 ± 0.33	9.70 ± 0.62	9.88 ± 0.73	10.48 ± 0.59*	11.18 ± 0.62**[3]	12.68 ± 0.83**
Week 13	7.88 ± 0.46	8.28 ± 0.43[3]	9.18 ± 0.58	11.31 ± 0.71**[3]	12.41 ± 0.77**	13.39 ± 0.59**
m-Nitrotoluene						
Week 1	10.58 ± 0.31[4]	9.25 ± 0.67	9.11 ± 0.39	9.45 ± 0.49[4]	9.17 ± 0.42	9.46 ± 0.31
Week 3	9.09 ± 0.54	10.69 ± 0.62[5]	10.00 ± 0.46[3]	9.18 ± 0.56[3]	9.64 ± 0.71[4]	12.09 ± 0.47**[5]
Week 13	8.37 ± 0.45	7.46 ± 0.48	8.50 ± 0.26	8.49 ± 0.37	8.71 ± 0.42	9.47 ± 0.66[3]
p-Nitrotoluene						
Week 1	9.13 ± 0.24[4]	9.51 ± 0.45[3]	9.31 ± 0.39	8.98 ± 0.84	9.62 ± 0.48	8.27 ± 0.43[3]
Week 3	12.06 ± 0.80[3]	9.78 ± 0.59*	9.12 ± 1.07*	9.10 ± 0.83*[3]	10.91 ± 0.607[3]	8.76 ± 0.70**
Week 13	8.67 ± 0.18[3]	7.40 ± 0.21	8.56 ± 0.43	9.09 ± 0.43	8.97 ± 0.26[3]	10.31 ± 1.04[3]
Monocytes ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	0.07 ± 0.03	0.10 ± 0.03	0.09 ± 0.03	0.10 ± 0.03[3]	0.14 ± 0.04[3]	0.12 ± 0.04
Week 3	0.08 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.07 ± 0.03	0.01 ± 0.01[3]	0.05 ± 0.03
Week 13	0.05 ± 0.02	0.06 ± 0.02[3]	0.04 ± 0.02	0.02 ± 0.02[3]	0.10 ± 0.04	0.00 ± 0.00
m-Nitrotoluene						
Week 1	0.09 ± 0.05[4]	0.15 ± 0.04	0.17 ± 0.05	0.15 ± 0.05[4]	0.11 ± 0.04	0.12 ± 0.04
Week 3	0.17 ± 0.06	0.24 ± 0.07[5]	0.21 ± 0.06[3]	0.20 ± 0.05[3]	0.18 ± 0.09[4]	0.19 ± 0.08[5]
Week 13	0.02 ± 0.01	0.10 ± 0.03	0.03 ± 0.02	0.10 ± 0.04	0.08 ± 0.05	0.07 ± 0.03[3]
p-Nitrotoluene						
Week 1	0.05 ± 0.03[4]	0.06 ± 0.02[3]	0.08 ± 0.04	0.08 ± 0.03	0.08 ± 0.03	0.08 ± 0.06[3]
Week 3	0.12 ± 0.04[3]	0.04 ± 0.03	0.09 ± 0.05[3]	0.11 ± 0.05[3]	0.16 ± 0.06[3]	0.07 ± 0.03
Week 13	0.23 ± 0.06[3]	0.32 ± 0.05	0.16 ± 0.06	0.19 ± 0.05	0.18 ± 0.04[3]	0.10 ± 0.04[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Eosinophils ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	0.03 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.02 ± 0.02[3]	0.02 ± 0.02[3]	0.03 ± 0.02
Week 3	0.06 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.04 ± 0.02[3]	0.05 ± 0.03
Week 13	0.04 ± 0.02	0.03 ± 0.02[3]	0.01 ± 0.01	0.07 ± 0.03[3]	0.05 ± 0.02	0.00 ± 0.00
m-Nitrotoluene						
Week 1	0.08 ± 0.03[4]	0.08 ± 0.03	0.01 ± 0.01	0.03 ± 0.02[4]	0.05 ± 0.02	0.07 ± 0.02
Week 3	0.13 ± 0.05	0.07 ± 0.03[5]	0.08 ± 0.02[3]	0.18 ± 0.03[3]	0.13 ± 0.05[4]	0.01 ± 0.01[5]
Week 13	0.05 ± 0.03	0.13 ± 0.03	0.08 ± 0.02	0.11 ± 0.04	0.07 ± 0.02	0.12 ± 0.04[3]
p-Nitrotoluene						
Week 1	0.05 ± 0.02[4]	0.04 ± 0.02[3]	0.06 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	0.07 ± 0.03[3]
Week 3	0.03 ± 0.02[3]	0.02 ± 0.01	0.05 ± 0.03	0.04 ± 0.02[3]	0.01 ± 0.01[3]	0.04 ± 0.02
Week 13	0.06 ± 0.02[3]	0.11 ± 0.02	0.12 ± 0.05	0.10 ± 0.04	0.03 ± 0.02[3]	0.01 ± 0.01[3]
Nucleated erythrocytes/100 leukocytes						
o-Nitrotoluene						
Week 1	0.20 ± 0.20	0.40 ± 0.22	0.40 ± 0.22	0.40 ± 0.22	0.44 ± 0.24[3]	0.30 ± 0.15
Week 3	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.22	0.00 ± 0.00	0.11 ± 0.11[3]	0.10 ± 0.10
Week 13	0.10 ± 0.10	0.22 ± 0.15[3]	0.60 ± 0.22	0.11 ± 0.11[3]	0.30 ± 0.21	0.30 ± 0.15
m-Nitrotoluene						
Week 1	0.50 ± 0.17	1.50 ± 0.89	1.10 ± 0.38	0.40 ± 0.16	0.70 ± 0.30	0.30 ± 0.21
Week 3	0.30 ± 0.21	0.57 ± 0.57[5]	0.00 ± 0.00[3]	0.22 ± 0.15[3]	0.50 ± 0.27[4]	1.29 ± 0.36*[5]
Week 13	0.00 ± 0.00	0.40 ± 0.22	0.00 ± 0.00	0.30 ± 0.30	0.60 ± 0.34	2.11 ± 0.61**[3]
p-Nitrotoluene						
Week 1	0.25 ± 0.16[4]	0.33 ± 0.17[3]	0.30 ± 0.21	0.20 ± 0.13	0.40 ± 0.22	0.11 ± 0.11[3]
Week 3	0.00 ± 0.00[3]	0.10 ± 0.10	0.00 ± 0.00	0.44 ± 0.29[3]	0.22 ± 0.22[3]	1.10 ± 0.38**
Week 13	0.22 ± 0.15[3]	0.30 ± 0.15	0.30 ± 0.21	0.40 ± 0.22	1.00 ± 0.33*[3]	5.22 ± 2.01**[3]
Chemistry						
Urea nitrogen (mg/dL)						
o-Nitrotoluene						
Week 1	20.6 ± 0.8[5]	20.8 ± 0.5	20.3 ± 0.7[4]	19.9 ± 0.8[3]	20.4 ± 0.8	20.0 ± 0.6
Week 3	22.3 ± 0.5	20.1 ± 0.6*	20.9 ± 0.8	17.6 ± 0.3**	16.7 ± 0.4**	21.2 ± 0.6**
Week 13	18.5 ± 0.6	20.5 ± 0.7	20.3 ± 0.8	18.4 ± 1.0	19.0 ± 0.9	17.8 ± 0.6
m-Nitrotoluene						
Week 1	18.1 ± 0.8	20.2 ± 0.8	20.1 ± 0.7	20.0 ± 0.9	21.7 ± 0.9**	22.1 ± 0.5**
Week 3	21.0 ± 0.6	22.3 ± 0.5[5]	21.3 ± 0.5[3]	22.8 ± 0.7	22.1 ± 0.5[3]	22.6 ± 0.7[5]
Week 13	16.5 ± 0.6	17.9 ± 0.9	16.8 ± 0.6	16.5 ± 0.4	15.3 ± 0.7	18.2 ± 0.9
p-Nitrotoluene						
Week 1	18.6 ± 0.5[3]	19.6 ± 0.8	19.7 ± 0.9	20.0 ± 1.5	20.7 ± 1.9	19.3 ± 0.7
Week 3	14.5 ± 0.8[4]	13.8 ± 0.6	14.4 ± 0.3	13.7 ± 0.6[3]	12.4 ± 0.4[3]	17.1 ± 0.8
Week 13	21.7 ± 0.6	21.3 ± 0.5	20.2 ± 0.6	21.8 ± 0.9	24.3 ± 1.1	22.6 ± 0.5[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Creatinine (mg/dL)						
o-Nitrotoluene						
Week 1	0.59 ± 0.01[5]	0.59 ± 0.01	0.58 ± 0.02[4]	0.57 ± 0.02[3]	0.58 ± 0.01	0.55 ± 0.02
Week 3	0.61 ± 0.01	0.57 ± 0.02*	0.57 ± 0.02	0.57 ± 0.02	0.52 ± 0.01**	0.52 ± 0.01**
Week 13	0.68 ± 0.01	0.68 ± 0.02	0.67 ± 0.02	0.65 ± 0.02	0.60 ± 0.02**	0.62 ± 0.01**
m-Nitrotoluene						
Week 1	0.50 ± 0.00	0.55 ± 0.02*	0.54 ± 0.02*	0.56 ± 0.02**	0.58 ± 0.01**	0.64 ± 0.02**
Week 3	0.57 ± 0.03	0.57 ± 0.02[5]	0.60 ± 0.0[3]	0.60 ± 0.00	0.60 ± 0.02[3]	0.63 ± 0.02*[5]
Week 13	0.69 ± 0.01	0.72 ± 0.01	0.73 ± 0.02	0.73 ± 0.02	0.70 ± 0.02	0.74 ± 0.02
p-Nitrotoluene						
Week 1	0.51 ± 0.05[3]	0.57 ± 0.02	0.60 ± 0.03	0.52 ± 0.05	0.60 ± 0.03[3]	0.57 ± 0.02[3]
Week 3	0.64 ± 0.03[4]	0.62 ± 0.02	0.69 ± 0.02	0.61 ± 0.04[3]	0.68 ± 0.02[3]	0.69 ± 0.02
Week 13	0.73 ± 0.03	0.79 ± 0.02	0.76 ± 0.03	0.83 ± 0.02**	0.81 ± 0.02*	0.83 ± 0.02**[3]
Total protein (g/dL)						
o-Nitrotoluene						
Week 1	5.8 ± 0.1[5]	5.7 ± 0.1[3]	5.6 ± 0.1[4]	5.7 ± 0.1[4]	5.4 ± 0.0**[3]	5.1 ± 0.1**
Week 3	6.1 ± 0.1	6.0 ± 0.0	5.9 ± 0.1	5.8 ± 0.0**	5.4 ± 0.1**	5.1 ± 0.0**
Week 13	7.1 ± 0.1	7.3 ± 0.1	7.1 ± 0.1	6.8 ± 0.1	6.5 ± 0.1**	6.3 ± 0.1**
m-Nitrotoluene						
Week 1	5.6 ± 0.0	5.6 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.6 ± 0.1
Week 3	6.0 ± 0.1	6.0 ± 0.0[5]	5.9 ± 0.1[3]	6.0 ± 0.1	5.9 ± 0.0[3]	6.0 ± 0.0[5]
Week 13	6.6 ± 0.1	7.0 ± 0.1	6.8 ± 0.1	6.6 ± 0.1	6.2 ± 0.0**	6.1 ± 0.1**
p-Nitrotoluene						
Week 1	5.7 ± 0.1[3]	5.5 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1
Week 3	5.8 ± 0.1[4]	5.8 ± 0.1	5.8 ± 0.1	5.6 ± 0.1[3]	5.6 ± 0.1[3]	6.0 ± 0.1
Week 13	6.7 ± 0.1	7.1 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.4 ± 0.0*	6.3 ± 0.1*[3]
Albumin (g/dL)						
o-Nitrotoluene						
Week 1	4.6 ± 0.1[5]	4.6 ± 0.1[3]	4.5 ± 0.1[4]	4.5 ± 0.1[4]	4.3 ± 0.1**[3]	4.1 ± 0.1**
Week 3	4.8 ± 0.0	4.7 ± 0.0	4.7 ± 0.1	4.5 ± 0.0**	4.3 ± 0.1**	4.1 ± 0.0**
Week 13	5.3 ± 0.1	5.4 ± 0.1	5.3 ± 0.1	5.2 ± 0.1	4.9 ± 0.1**	4.8 ± 0.0**
m-Nitrotoluene						
Week 1	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.8 ± 0.0*	4.9 ± 0.1**
Week 3	4.8 ± 0.1	4.8 ± 0.0[5]	4.7 ± 0.0[3]	4.8 ± 0.1	4.7 ± 0.0[3]	4.9 ± 0.1[5]
Week 13	5.1 ± 0.1	5.5 ± 0.1	5.4 ± 0.0	5.1 ± 0.1	4.9 ± 0.0	4.9 ± 0.1
p-Nitrotoluene						
Week 1	3.1 ± 0.6[3]	3.8 ± 0.1	3.8 ± 0.0	3.8 ± 0.0	4.0 ± 0.0*[3]	3.9 ± 0.0
Week 3	3.9 ± 0.1[4]	3.9 ± 0.0	3.9 ± 0.0	3.8 ± 0.0[3]	3.8 ± 0.0[3]	4.1 ± 0.0*
Week 13	4.2 ± 0.1	4.4 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.1 ± 0.0	4.2 ± 0.0[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Methemoglobin (%)						
o-Nitrotoluene						
Week 1	1.71 ± 0.13[3]	1.89 ± 0.19[3]	2.14 ± 0.40[3]	1.92 ± 0.26[3]	2.35 ± 0.17[4]	2.94 ± 0.18**
Week 3	1.46 ± 0.12	2.10 ± 0.27*[3]	1.77 ± 0.24[3]	2.36 ± 0.22**[4]	4.24 ± 0.24**	5.45 ± 0.21**
Week 13	2.01 ± 0.37[4]	2.88 ± 0.69[5]	3.35 ± 0.38*	2.90 ± 0.25	4.06 ± 0.40**[4]	4.31 ± 0.48**
m-Nitrotoluene						
Week 1	–[6]	–	–	–	–	–
Week 3	1.66 ± 0.20[3]	2.50 ± 0.33[5]	1.97 ± 0.24[3]	2.14 ± 0.25	2.09 ± 0.23[3]	3.00 ± 0.21**[5]
Week 13	3.09 ± 0.28	3.15 ± 0.27	3.48 ± 0.242	3.46 ± 0.39	3.82 ± 0.22[3]	5.00 ± 0.35**[3]
p-Nitrotoluene						
Week 1	2.68 ± 0.24[4]	2.64 ± 0.31[3]	2.61 ± 0.34	2.52 ± 0.24	2.18 ± 0.42	2.00 ± 0.16[3]
Week 3	6.92 ± 0.35[3]	5.94 ± 0.38	6.56 ± 0.47	5.84 ± 0.59[3]	6.06 ± 0.37[3]	6.52 ± 0.54
Week 13	6.36 ± 0.28[3]	5.94 ± 0.28	6.17 ± 0.54	5.91 ± 0.26	6.29 ± 0.17[3]	9.02 ± 0.20**[3]
Alkaline phosphatase (IU/L)						
o-Nitrotoluene						
Week 1	600 ± 14[5]	599 ± 14[3]	600 ± 13[4]	588 ± 13[3]	563 ± 10	561 ± 12
Week 3	449 ± 11	397 ± 12*	426 ± 15*	372 ± 6**	314 ± 9**	321 ± 10**
Week 13	186 ± 5	203 ± 10	201 ± 6	173 ± 10	150 ± 7*	166 ± 4*
m-Nitrotoluene						
Week 1	511 ± 20	535 ± 14	523 ± 13	524 ± 13	515 ± 14	458 ± 13*
Week 3	402 ± 15	398 ± 10[5]	381 ± 7[3]	412 ± 12	391 ± 12[3]	372 ± 8[5]
Week 13	196 ± 7	191 ± 6	192 ± 4	184 ± 7	196 ± 4	210 ± 9
p-Nitrotoluene						
Week 1	291 ± 7[7]	277 ± 10	269 ± 17[3]	331 ± 18[4]	277 ± 13[5]	269 ± 10[4]
Week 3	192 ± 4[4]	188 ± 8	186 ± 6	178 ± 4[3]	174 ± 3[3]	190 ± 6
Week 13	195 ± 10	195 ± 6	179 ± 8	195 ± 4	231 ± 10**	218 ± 11*[3]
Alanine aminotransferase (IU/L)						
o-Nitrotoluene						
Week 1	41 ± 2[5]	39 ± 1[3]	39 ± 1[4]	39 ± 1[3]	38 ± 1	38 ± 1
Week 3	44 ± 1	44 ± 1	44 ± 1	41 ± 2	41 ± 1	38 ± 1**
Week 13	45 ± 2	49 ± 3	49 ± 2	40 ± 1*	41 ± 1*	42 ± 5**
m-Nitrotoluene						
Week 1	38 ± 1	37 ± 1	38 ± 1	40 ± 1	46 ± 1**	48 ± 2**
Week 3	36 ± 1	37 ± 1[5]	36 ± 1[3]	41 ± 1**	42 ± 1**[3]	45 ± 1**[5]
Week 13	50 ± 2	59 ± 3	48 ± 4	53 ± 4	42 ± 1	54 ± 2
p-Nitrotoluene						
Week 1	27 ± 3[5]	25 ± 1	28 ± 1	29 ± 1	31 ± 1**[4]	37 ± 1**
Week 3	29 ± 1[4]	28 ± 1	30 ± 2	28 ± 1[3]	29 ± 1[3]	35 ± 2*
Week 13	53 ± 4	45 ± 3	41 ± 3**	46 ± 3*	44 ± 2*	36 ± 1**[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Creatine kinase (IU/L)						
o-Nitrotoluene						
Week 1	291.0 ± 29.3[5]	274.7 ± 23.2[5]	274.6 ± 28.2[5]	361.6 ± 49.6[5]	398.8 ± 37.5[3]	354.2 ± 111.9
Week 3	290.0 ± 37.5	201.1 ± 22.3	268.0 ± 24.0	303.3 ± 59.0	226.8 ± 28.4	350.0 ± 61.7
Week 13	89.9 ± 5.3	140.4 ± 35.8	83.0 ± 5.9	99.9 ± 9.3	96.6 ± 6.7	120.4 ± 39.4
m-Nitrotoluene						
Week 1	329.4 ± 40.9[3]	481.1 ± 99.3	325.0 ± 43.0	493.4 ± 88.1	358.1 ± 59.3	385.3 ± 53.9
Week 3	241.1 ± 32.3	202.3 ± 23.7[5]	218.2 ± 26.4[3]	248.1 ± 20.5	228.0 ± 58.3[3]	271.4 ± 22.7
Week 13	139.0 ± 17.6	125.9 ± 10.7	197.8 ± 34.6	146.3 ± 30.1	153.6 ± 22.6	160.3 ± 21.7
p-Nitrotoluene						
Week 1	176.6 ± 36.9[5]	141.5 ± 19.2	139.0 ± 14.1	218.5 ± 54.1	159.9 ± 9.4[3]	184.0 ± 29.0
Week 3	201.9 ± 64.0[4]	140.6 ± 24.1	166.3 ± 20.6	157.1 ± 29.6[3]	154.2 ± 31.4[3]	351.2 ± 115.5
Week 13	51.00 ± 7.8[3]	93.7 ± 22.6	91.2 ± 33.2	80.9 ± 9.4	126.9 ± 44.4	61.33 ± 5.5[3]
Sorbitol dehydrogenase (IU/L)						
o-Nitrotoluene						
Week 1	10 ± 1	10 ± 1	10 ± 1	9 ± 1	10 ± 1	10 ± 1
Week 3	10 ± 1	11 ± 1	10 ± 1	10 ± 1	11 ± 1	9 ± 1
Week 13	12 ± 1	11 ± 1	12 ± 0	12 ± 1	11 ± 0	13 ± 1
m-Nitrotoluene						
Week 1	5 ± 1	4 ± 0	5 ± 1	5 ± 1[3]	6 ± 1	4 ± 1[3]
Week 3	7 ± 1	8 ± 1[5]	8 ± 1[3]	7 ± 1	9 ± 1[3]	7 ± 1[5]
Week 13	11 ± 1	13 ± 1	11 ± 1	12 ± 1	10 ± 1	11 ± 1
p-Nitrotoluene						
Week 1	6 ± 1[8]	5 ± 0	6 ± 0[4]	6 ± 0[4]	5 ± 0[5]	6 ± 1[3]
Week 3	13 ± 1[4]	10 ± 1*	11 ± 1*	9 ± 1**[3]	9 ± 1**[3]	7 ± 1**
Week 13	10 ± 1	10 ± 1	9 ± 0	10 ± 1	10 ± 0	8 ± 0[9]
Bile acids (μmol/L)						
o-Nitrotoluene						
Week 1	20.20 ± 3.31	20.70 ± 6.46	12.40 ± 3.76	13.10 ± 2.15	16.80 ± 3.39	14.80 ± 3.08
Week 3	9.70 ± 3.11	7.10 ± 1.89	9.60 ± 2.30	5.40 ± 1.16	8.50 ± 1.49	8.80 ± 1.40
Week 13	11.56 ± 2.24[3]	16.90 ± 2.89	14.10 ± 2.71	13.20 ± 2.39	17.30 ± 3.79	21.20 ± 1.91**
m-Nitrotoluene						
Week 1	9.11 ± 1.39[3]	19.33 ± 7.85[3]	14.89 ± 6.88[3]	10.57 ± 2.92[5]	17.33 ± 2.09*[3]	37.88 ± 6.37**[4]
Week 3	20.22 ± 3.64[3]	11.17 ± 5.24[8]	11.33 ± 1.69[3]	16.50 ± 5.83	15.33 ± 3.27[3]	49.17 ± 4.83[8]
Week 13	9.00 ± 2.31	6.80 ± 1.02	17.20 ± 3.04*	12.70 ± 1.78	16.30 ± 3.71	49.30 ± 9.65**
p-Nitrotoluene						
Week 1	10.89 ± 1.55[3]	10.70 ± 2.37	9.60 ± 0.85	8.70 ± 0.75	13.56 ± 2.59[3]	10.30 ± 1.01
Week 3	16.13 ± 2.07[4]	14.90 ± 2.48	13.60 ± 1.18	15.33 ± 2.32[3]	16.33 ± 1.73[3]	41.60 ± 9.35*
Week 13	12.90 ± 1.33	18.70 ± 2.99	16.00 ± 3.76	22.90 ± 3.74	17.80 ± 2.57	19.33 ± 2.38[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

[1] Mean ± standard error for groups of 10 animals unless otherwise specified.
[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*-nitrotoluene or *p*-nitrotoluene.
[3] n=9.
[4] n=8.
[5] n=7.
[6] Insufficient data for analysis.
[7] n=4.
[8] n=6.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

APPENDIX C

Reproductive Tissue Evaluations and Estrous Cycle Length

TABLE C1 Summary of Reproductive Tissue Evaluations in Male Rats in the 13-Week Feed Study of *o*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	353 ± 5	309 ± 6**	254 ± 4**	198 ± 3**
Left testicle	1.48 ± 0.028	1.39 ± 0.025*	1.07 ± 0.045**	0.54 ± 0.024**
Left epididymis	0.50 ± 0.011	0.40 ± 0.014**	0.28 ± 0.012**	0.12 ± 0.008**
Left epididymal tail	0.20 ± 0.010	0.16 ± 0.008*	0.12 ± 0.007**	0.05 ± 0.003**
Spermatid measurements				
Spermatid heads (10^7/g testis)	12.40 ± 0.44	12.58 ± 0.48	13.17 ± 0.85	4.41 ± 1.19**
Spermatid heads (10^7/testis)	18.30 ± 0.65	17.39 ± 0.62	14.02 ± 0.97**	2.35 ± 0.63**
Spermatid count (mean/10^4 mL suspension)	91.48 ± 3.23	86.93 ± 3.11	70.08 ± 4.83**	11.88 ± 3.10**
Spermatozoal measurements				
Motility (%)	78 ± 1	76 ± 2	73 ± 1*	6.5 ± 5.5**
Concentration (10^6/g cet)[2]	417 ± 28	433 ± 38	179 ± 22**	14 ± 5**

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] g cet = grams of caudal epididymal tissue.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C2 Summary of Estrous Cycle Characterization in Female Rats in the 13-Week Feed Study of *o*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Necropsy body weight (g)	205 ± 3	179 ± 3**	170 ± 2**	158 ± 3**
Estrous cycle length (days)	4.90 ± 0.10	4.70 ± 0.17	5.30 ± 0.21	6.63 ± 0.80**[2]
Estrous stages as % of cycle[3]				
diestrus	36.7	39.2	40.8	62.5
proestrus	7.5	15.8	15.0	10.8
estrus	32.5	25.0	25.0	15.8
metestrus	23.3	20.0	19.2	10.8

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=4, estrous cycle length longer than 12 days or unclear in 6 of 10 animals.
[3] Evidence by multivariate analysis of variance (MANOVA) suggests that females in all dose groups differ from controls in the relative frequency of time spent in the estrous stages; P=0.06 for the 2,500 ppm group, P=0.04 for the 5,000 ppm group, and P≤0.01 for the 10,000 ppm group.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C3 Summary of Reproductive Tissue Evaluations in Male Rats in the 13-Week Feed Study of *m*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	346 ± 8	353 ± 6	338 ± 6	281 ± 5**
Left testicle	1.41 ± 0.026	1.46 ± 0.028	1.47 ± 0.024	0.79 ± 0.087**
Left epididymis	0.46 ± 0.009	0.47 ± 0.010	0.46 ± 0.012	0.29 ± 0.017**
Left epididymal tail	0.16 ± 0.007	0.16 ± 0.008	0.15 ± 0.007[2]	0.08 ± 0.005**
Spermatid measurements				
Spermatid heads (10^7/g testis)	11.38 ± 0.58	9.81 ± 0.49	11.07 ± 0.63	3.54 ± 1.50**
Spermatid heads (10^7/testis)	15.99 ± 0.74	14.26 ± 0.70	16.18 ± 0.88	3.79 ± 1.71**
Spermatid count (mean/10^4 mL suspension)	79.93 ± 3.70	71.30 ± 3.50	80.88 ± 4.40	18.95 ± 8.56**
Spermatozoal measurements				
Motility (%)	70 ± 4	73 ± 3	76 ± 3	20 ± 13[3]
Concentration (10^6/g cet)[4]	581 ± 31	496 ± 28*	629 ± 41[2]	143 ± 52**

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=9.
[3] n=8.
[4] g cet = grams of caudal epididymal tissue.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C4 Summary of Estrous Cycle Characterization in Female Rats in the 13-Week Feed Study of *m*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Necropsy body weight (g)	194 ± 3	195 ± 3	177 ± 2**	166 ± 3**
Estrous cycle length (days)	5.00 ± 0.13	5.00 ± 0.00	6.50 ± 0.38**[2]	6.00 ± 0.58*[3]
Estrous stages as % of cycle[4]				
diestrus	37.5	40.8	49.2	75.8
proestrus	10.0	15.0	15.8	4.2
estrus	36.7	29.2	19.2	11.7
metestrus	15.8	15.0	15.8	7.5
uncertain diagnosis	0.0	0.0	0.0	0.8

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=9; estrous cycle length longer than 12 days or unclear in 1 of 10 animals.
[3] n=3; estrous cycle length longer than 12 days or unclear in 7 of 10 animals.
[4] Evidence by multivariate analysis of variance (MANOVA) suggests that females in the 10,000 ppm group differ significantly (P≤0.01) from controls in the relative frequency of time spent in the estrous stages. Females in this group spent more time in diestrus and less time in other stages than did controls.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C5 Summary of Reproductive Tissue Evaluations in Male Rats in the 13-Week Feed Study of *p*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	353 ± 6	344 ± 11	320 ± 6**	251 ± 6**
Left testicle	1.51 ± 0.025	1.47 ± 0.045	1.42 ± 0.028	1.09 ± 0.079**
Left epididymis	0.46 ± 0.011	0.45 ± 0.020	0.44 ± 0.012	0.33 ± 0.027**
Left epididymal tail	0.18 ± 0.007	0.18 ± 0.009	0.20 ± 0.018	0.13 ± 0.016*
Spermatid measurements				
Spermatid heads (10^7/g testis)	10.13 ± 0.42	9.81 ± 0.41	9.73 ± 0.49	8.71 ± 0.82
Spermatid heads (10^7/testis)	15.29 ± 0.71	14.31 ± 0.55	13.78 ± 0.64	10.03 ± 1.34**
Spermatid count (mean/10^4 mL suspension)	76.43 ± 3.55	71.55 ± 2.74	68.90 ± 3.22	50.15 ± 6.70**
Spermatozoal measurements				
Motility (%)	79 ± 2	77 ± 3	81 ± 2	59 ± 11
Concentration (10^6/g cet)[2]	501 ± 45	604 ± 112[3]	451 ± 40	325 ± 60*[3]

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] g cet = grams of caudal epididymal tissue.
[3] n=9.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C6 Summary of Estrous Cycle Characterization in Female Rats in the 13-Week Feed Study of *p*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Necropsy body weight (g)	202 ± 3	196 ± 3	185 ± 3**	174 ± 3**
Estrous cycle length (days)	5.15 ± 0.13	5.15 ± 0.08	6.05 ± 0.51	5.00[2]
Estrous stages as % of cycle[3]				
diestrus	45.8	45.0	55.0	78.3
proestrus	15.0	14.2	12.5	4.2
estrus	24.2	25.8	20.0	11.7
metestrus	15.0	15.0	12.5	5.8

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=1 (standard error not calculated); estrous cycle length longer than 12 days or unclear in 9 of 10 animals.
[3] Evidence by multivariate analysis of variance (MANOVA) suggests that females in the 10,000 ppm group differ significantly (P≤0.01) from controls in the relative frequency of time spent in the estrous stages. Females in this group spent less time in proestrus and more time in diestrus than controls and other dosed groups.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C7 Summary of Reproductive Tissue Evaluations in Male Mice in the 13-Week Feed Study of *o*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	33.3 ± 0.4	32.8 ± 0.5	29.2 ± 0.3**	25.9 ± 0.3**
Left testicle	0.120 ± 0.002	0.117 ± 0.001	0.113 ± 0.002*	0.107 ± 0.003**
Left epididymis	0.053 ± 0.002	0.051 ± 0.002	0.047 ± 0.002*	0.039 ± 0.001**
Left epididymal tail	0.022 ± 0.001	0.021 ± 0.001	0.018 ± 0.001**	0.015 ± 0.001**
Spermatid measurements				
Spermatid heads (10^7/g testis)	22.49 ± 1.20	21.38 ± 0.82	24.28 ± 0.82	25.28 ± 1.00
Spermatid heads (10^7/testis)	2.69 ± 0.14	2.50 ± 0.10	2.73 ± 0.08	2.70 ± 0.09
Spermatid count (mean/10^4 mL suspension)	83.88 ± 4.25	78.28 ± 3.01	85.30 ± 2.56	84.28 ± 2.94
Spermatozoal measurements				
Motility (%)	77 ± 2	78 ± 2	72 ± 3	48 ± 3**
Concentration (10^6/g cet)[2]	606 ± 37[3]	640 ± 59	447 ± 81[3]	472 ± 76

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] g cet = grams of caudal epididymal tissue.
[3] n=9.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C8 Summary of Estrous Cycle Characterization in Female Mice in the 13-Week Feed Study of *o*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Necropsy body weight (g)	32.8 ± 0.7	32.1 ± 0.8	29.5 ± 0.6**	22.9 ± 0.3**
Estrous cycle length (days)	4.55 ± 0.16	4.40 ± 0.15	4.20 ± 0.13	4.75 ± 0.09[2]
Estrous stages as % of cycle[3]				
diestrus	28.3	28.3	33.3	36.7
proestrus	22.5	19.2	22.5	20.8
estrus	31.7	32.5	26.7	29.2
metestrus	17.5	20.0	16.7	13.3

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=8; estrous cycle length longer than 12 days or unclear in 2 of 10 animals.
[3] Evidence by multivariate analysis of variance (MANOVA) suggests no significant differences from controls in the relative frequency of time that dosed females spent in the estrous stages.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C9 Summary of Reproductive Tissue Evaluations in Male Mice in the 13-Week Feed Study of *m*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	36.5 ± 0.8	34.9 ± 0.7	31.3 ± 0.8**	27.7 ± 0.4**
Left testicle	0.122 ± 0.003	0.117 ± 0.003	0.116 ± 0.003	0.112 ± 0.002*
Left epididymis	0.053 ± 0.002	0.047 ± 0.001*	0.048 ± 0.001*	0.043 ± 0.001**
Left epididymal tail	0.022 ± 0.001	0.020 ± 0.001[2]	0.019 ± 0.001	0.017 ± 0.001**
Spermatid measurements				
Spermatid heads (10^7/g testis)	17.67 ± 1.43	15.99 ± 1.21	19.43 ± 1.62	19.86 ± 1.95
Spermatid heads (10^7/testis)	2.16 ± 0.18	1.88 ± 0.16	2.26 ± 0.21	2.22 ± 0.22
Spermatid count (mean/10^4 mL suspension)	67.33 ± 5.59	58.80 ± 5.05	70.68 ± 6.54	69.25 ± 6.88
Spermatozoal measurements				
Motility (%)	76 ± 1	78 ± 1	73 ± 4	77 ± 2
Concentration (10^6/g cet)[3]	844 ± 54	937 ± 89[2]	792 ± 77	1,052 ± 75

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=9.
[3] *g cet = grams of caudal epididymal tissue.*
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C10 Summary of Estrous Cycle Characterization in Female Mice in the 13-Week Feed Study of *m*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Necropsy body weight (g)	33.8 ± 0.8	33.2 ± 0.4	29.8 ± 0.8**	24.4 ± 0.5**
Estrous cycle length (days)	4.50 ± 0.15	4.38 ± 0.16[2]	4.22 ± 0.09[3]	4.20 ± 0.13
Estrous stages as % of cycle[3]				
diestrus	25.0	30.8	30.0	25.8
proestrus	25.0	21.7	20.0	20.0
estrus	34.2	30.8	31.7	30.8
metestrus	15.8	16.7	18.3	23.3

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=8; estrous cycle length longer than 12 days or unclear in 2 of 10 animals.
[3] n=9; estrous cycle length longer than 12 days or unclear in 1 of 10 animals.
[4] Evidence by multivariate analysis of variance (MANOVA) suggests no significant differences from controls in the relative frequency of time that dosed females spent in the estrous stages.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C11 Summary of Reproductive Tissue Evaluations in Male Mice in the 13-Week Feed Study of *p*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	33.0 ± 0.5	32.3 ± 0.5	31.1 ± 0.4*	29.9 ±0.7**
Left testicle	0.117 ± 0.003	0.115 ± 0.002	0.112 ± 0.002	0.115 ± 0.002
Left epididymis	0.048 ± 0.002	0.046 ± 0.002	0.046 ± 0.002	0.044 ± 0.001
Left epididymal tail	0.017 ± 0.001	0.017 ± 0.001	0.018 ± 0.001	0.015 ± 0.001
Spermatid measurements				
Spermatid heads (10^7/g testis)	18.26 ± 0.63	19.96 ± 0.79	21.06 ± 0.93*	21.21 ± 0.73**
Spermatid heads (10^7/testis)	2.12 ± 0.07	2.29 ± 0.08	2.37 ± 0.12	2.43 ± 0.10*
Spermatid count (mean/10^4 mL suspension)	66.15 ± 2.13	71.65 ± 2.60	74.00 ± 3.74	75.93 ± 3.24*
Spermatozoal measurements				
Motility (%)	72 ± 7	75 ± 2	78 ± 3	74 ± 2
Concentration (10^6/g cet)[2]	1,143 ± 57	1,361 ± 96	1,173 ± 118[3]	1,421 ± 367

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] g cet = grams of caudal epididymal tissue.
[3] n=9.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C12 Summary of Estrous Cycle Characterization in Female Mice in the 13-Week Feed Study of *p*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Necropsy body weight (g)	29.1 ± 0.6	27.9 ± 0.6	27.1 ± 0.4	24.9 ± 0.5**
Estrous cycle length (days)	4.35 ± 0.13	4.10 ± 0.07	4.20 ± 0.11	4.20 ± 0.11
Estrous stages as % of cycle[2]				
diestrus	30.8	28.3	31.7	33.3
proestrus	20.0	24.2	22.5	20.8
estrus	29.2	28.3	30.8	30.8
metestrus	20.0	19.2	15.0	15.0

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] Evidence by multivariate analysis of variance (MANOVA) suggests no significant differences from controls in the relative frequency of time that dosed females spent in the estrous stages.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

APPENDIX D

Genetic Toxicology

TABLE D1 Mutagenicity of *o*-Nitrotoluene in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at SRI, International							
TA100	0.0	145 ± 1.5	120 ± 4.7	160 ± 11.8	126 ± 9.1	154 ± 16.5	117 ± 7.8
	3.0	151 ± 6.1	118 ± 8.5				
	10.0	142 ± 7.8	105 ± 4.7	149 ± 5.4	133 ± 4.7	150 ± 7.6	130 ± 3.8
	33.0	139 ± 12.7	110 ± 7.5	148 ± 15.8	125 ± 0.9	126 ± 12.7	133 ± 7.5
	100.0	146 ± 12.4	104 ± 11.2	154 ± 5.5	125 ± 13.9	134 ± 7.4	147 ± 5.3
	333.0	45 ± 5.9[3]	Toxic	156 ± 7.4	125 ± 7.0	111 ± 6.3	114 ± 1.9
	666.0				137 ± 5.7		119 ± 2.5
	1,000.0			Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[4]		321 ± 11.7	424 ± 16.2	2,113 ± 4.8	1,895 ± 83.7	1,055 ± 61.4	900 ± 15.3
TA1535	0.0	21 ± 3.7	15 ± 1.7	10 ± 1.7	12 ± 2.1	7 ± 2.5	10 ± 1.9
	3.0	22 ± 3.7	10 ± 1.5				
	10.0	22 ± 3.0	11 ± 1.2	7 ± 2.0	6 ± 1.7	9 ± 1.5	10 ± 2.8
	33.0	20 ± 5.5	14 ± 1.5	9 ± 3.2	10 ± 2.6	8 ± 2.5	10 ± 2.1
	100.0	20 ± 3.9	18 ± 5.2	11 ± 3.5	10 ± 1.5	7 ± 2.3	7 ± 2.9
	333.0	2 ± 1.2[3]	0 ± 0.0[3]	7 ± 0.9	8 ± 0.7	11 ± 1.9	11 ± 1.5
	666.0				Toxic		9 ± 3.0
	1,000.0			3 ± 1.8[3]		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		384 ± 17.9	396 ± 2.3	429 ± 31.8	507 ± 35.4	255 ± 18.4	313 ± 77.0
TA1537	0.0	8 ± 0.6	5 ± 2.6	5 ± 1.9	8 ± 2.6	13 ± 2.9	6 ± 0.9
	3.0	12 ± 3.0	4 ± 0.6				
	10.0	9 ± 2.6	3 ± 0.7	7 ± 2.5	9 ± 2.3	11 ± 2.5	5 ± 1.3
	33.0	9 ± 0.0	4 ± 0.3	6 ± 0.9	6 ± 1.2	9 ± 1.5	6 ± 0.7
	100.0	5 ± 0.6	3 ± 0.9	7 ± 1.0	8 ± 2.6	8 ± 0.7	8 ± 0.7
	333.0	0 ± 0.0[3]	Toxic	8 ± 2.6	6 ± 1.5	6 ± 1.5	6 ± 1.0
	666.0				Toxic		6 ± 1.3
	1,000.0			Toxic		0 ± 0.0[3]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		99 ± 3.5	100 ± 18.2	385 ± 63.6	353 ± 32.0	238 ± 4.4	283 ± 14.2
TA98	0.0	25 ± 0.9	22 ± 2.7	28 ± 1.9	28 ± 4.6	38 ± 6.7	24 ± 2.6
	3.0	25 ± 0.6	24 ± 1.9				
	10.0	24 ± 3.7	16 ± 0.9	22 ± 2.2	35 ± 1.7	28 ± 4.3	29 ± 2.3
	33.0	24 ± 3.3	17 ± 1.5	25 ± 4.1	29 ± 1.8	26 ± 4.1	30 ± 0.9
	100.0	19 ± 3.5	36 ± 22.3	25 ± 2.1	27 ± 1.3	27 ± 1.2	34 ± 2.7
	333.0	Toxic	0 ± 0.0[3]	27 ± 0.7	28 ± 2.6	21 ± 3.7	31 ± 3.8
	666.0				0 ± 0.0[3]		32 ± 0.5
	1,000.0			10 ± 5.4[3]		0 ± 0.0[3]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		830 ± 33.4	760 ± 8.0	1,845 ± 75.2	1,761 ± 147.7	613 ± 12.5	640 ± 8.7

TABLE D1 Mutagenicity of *o*-Nitrotoluene in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at EG&G Mason Research Institute							
TA100	0.0	122 ± 9.1	138 ± 16.3	79 ± 2.6	134 ± 6.2	86 ± 10.1	123 ± 7.9
	3.3		135 ± 4.3		127 ± 2.3		149 ± 7.8
	10.0	125 ± 3.5	139 ± 5.8	89 ± 3.5	118 ± 7.6	78 ± 3.2	128 ± 3.2
	33.0	104 ± 1.2	122 ± 6.9	76 ± 3.7	134 ± 2.5	109 ± 6.2	147 ± 21.7
	100.0	113 ± 3.7	121 ± 11.1	89 ± 3.5	135 ± 9.3	118 ± 6.1	142 ± 5.2
	333.0	83 ± 1.9[3]	132 ± 9.2[3]	90 ± 7.3[3]	148 ± 3.3	111 ± 7.2[3]	115 ± 11.0
	666.0	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		2,037 ± 65.0	2,103 ± 44.5	1,741 ± 95.2	1,900 ± 92.3	1,045 ± 81.2	991 ± 44.5
TA1535	0.0	22 ± 2.0	25 ± 3.8	9 ± 1.3	14 ± 1.7	15 ± 1.5	10 ± 0.6
	3.3		26 ± 2.5		15 ± 3.2		10 ± 1.2
	10.0	37 ± 3.6	23 ± 3.3	10 ± 1.8	17 ± 2.5	12 ± 2.3	9 ± 1.7
	33.0	28 ± 3.2	24 ± 1.7	10 ± 1.9	20 ± 1.5	12 ± 0.6	10 ± 3.3
	100.0	28 ± 1.7	22 ± 4.9	8 ± 3.0	16 ± 1.5	13 ± 1.5	19 ± 2.0
	333.0	29 ± 2.9[3]	23 ± 2.8[3]	11 ± 2.0[3]	16 ± 2.5	12 ± 3.8[3]	12 ± 1.2
	666.0	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Equivocal
Positive control		1,380 ± 77.8	1,320 ± 43.1	74 ± 5.6	128 ± 6.3	110 ± 7.0	108 ± 5.5
TA1537	0.0	6 ± 0.7	8 ± 1.5	6 ± 0.6	13 ± 1.2	8 ± 1.0	10 ± 1.5
	3.3	8 ± 2.1	9 ± 2.1	8 ± 0.9			
	10.0	5 ± 1.2	5 ± 1.8	6 ± 0.6	12 ± 0.0	8 ± 0.3	6 ± 1.2
	33.0	6 ± 1.0	6 ± 1.2	13 ± 0.9	9 ± 0.6	8 ± 1.3	7 ± 2.1
	100.0	6 ± 1.3	8 ± 1.3	9 ± 3.1	8 ± 1.2	6 ± 1.2	6 ± 0.9
	333.0	7 ± 1.7[3]	6 ± 0.3[3]	10 ± 2.7[3]	11 ± 0.9	5 ± 0.9[3]	7 ± 0.9
	666.0	Toxic		6 ± 2.1[3]		6 ± 0.3[3]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		361 ± 85.0	901 ± 105.9	118 ± 12.0	173 ± 7.2	123 ± 2.3	71 ± 4.9
TA98	0.0	16 ± 1.2	14 ± 2.7	31 ± 1.5	28 ± 1.2	20 ± 2.3	25 ± 0.7
	3.3		20 ± 3.8		31 ± 1.9		29 ± 1.2
	10.0	20 ± 2.7	16 ± 1.9	29 ± 2.6	31 ± 7.1	22 ± 3.5	27 ± 0.3
	33.0	18 ± 3.2	22 ± 1.2	32 ± 3.1	32 ± 1.9	23 ± 1.2	27 ± 5.2
	100.0	18 ± 4.4	20 ± 1.2	26 ± 6.4	34 ± 2.3	24 ± 4.0	25 ± 1.9
	333.0	14 ± 0.6[3]	12 ± 1.8[3]	27 ± 2.3	36 ± 3.5	27 ± 3.5[3]	27 ± 3.7
	666.0	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,661 ± 63.7	2,119 ± 51.4	1,189 ± 54.6	1,454 ± 95.4	1,173 ± 31.0	874 ± 29.2

TABLE D1 Mutagenicity of *o*-Nitrotoluene in *Salmonella typhimurium* (continued)

[1] The detailed protocol and these data are presented in Haworth *et al.* (1983). Cells and *o*-nitrotoluene or solvent (dimethylsulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. The high dose was limited by toxicity; 0 μg/plate dose is the solvent control.

[2] Revertants are presented as mean ± standard error from 3 plates.

[3] Slight toxicity.

[4] Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE D2 Mutagenicity of *m*-Nitrotoluene in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0.0	88 ± 7.5	142 ± 4.6	81 ± 3.3	106 ± 5.9	128 ± 14.0	105 ± 8.5
	3.3	119 ± 7.0	135 ± 6.0	113 ± 5.8	110 ± 5.7	116 ± 3.5	126 ± 4.5
	10.0	114 ± 8.1	136 ± 2.5	112 ± 7.4	110 ± 3.8	109 ± 4.8	115 ± 9.6
	33.0	118 ± 5.9	151 ± 6.1	106 ± 11.0	105 ± 7.7	123 ± 7.9	128 ± 3.7
	100.0	121 ± 4.0	143 ± 10.4	113 ± 8.5	116 ± 5.0	122 ± 3.2	125 ± 1.3
	333.0	103 ± 13.0[3]	120 ± 8.4[3]	80 ± 1.8[3]	106 ± 9.5[3]	119 ± 11.3[3]	133 ± 12.3[3]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[4]		1,620 ± 26.0	2,080 ± 58.4	1,559 ± 67.3	2,190 ± 78.4	893 ± 37.0	994 ± 38.4
TA1535	0.0	27 ± 1.3	29 ± 4.4	10 ± 0.6	13 ± 1.5	12 ± 1.7	10 ± 3.2
	3.3	30 ± 2.6	35 ± 2.2	11 ± 0.3	12 ± 0.9	13 ± 1.7	10 ± 2.0
	10.0	29 ± 1.5	29 ± 6.0	7 ± 1.2	11 ± 1.3	10 ± 0.9	11 ± 2.1
	33.0	32 ± 2.6	33 ± 1.2	8 ± 0.9	8 ± 0.9	11 ± 2.3	9 ± 0.7
	100.0	36 ± 1.5	30 ± 1.5	7 ± 1.7	8 ± 0.3	12 ± 1.8	11 ± 0.6
	333.0	31 ± 1.8[3]	26 ± 0.7[3]	11 ± 2.3[3]	13 ± 1.7	11 ± 0.3[3]	10 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,214 ± 55.5	1,426 ± 28.3	105 ± 0.7	167 ± 9.0	118 ± 8.3	58 ± 2.2
TA1537	0.0	5 ± 0.6	5 ± 0.9	10 ± 0.6	9 ± 2.3	10 ± 0.7	6 ± 1.5
	3.3	6 ± 1.3	8 ± 0.3	8 ± 0.9	11 ± 1.8	11 ± 0.9	6 ± 0.3
	10.0	5 ± 1.7	5 ± 1.2	9 ± 1.8	10 ± 1.9	12 ± 0.6	7 ± 2.4
	33.0	8 ± 1.2	7 ± 1.2	10 ± 1.0	6 ± 1.7	10 ± 0.6	7 ± 1.2
	100.0	8 ± 2.6	3 ± 1.0	10 ± 1.2	13 ± 2.5	12 ± 2.1	8 ± 2.3
	333.0	5 ± 0.3[3]	2 ± 0.9[3]	10 ± 2.1[3]	9 ± 0.3	10 ± 1.0[3]	6 ± 2.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		852 ± 128.6	469 ± 107.7	172 ± 14.2	262 ± 12.5	122 ± 12.0	60 ± 6.2
TA98	0.0	16 ± 3.5	21 ± 3.6	27 ± 6.2	29 ± 2.8	33 ± 1.8	25 ± 3.5
	3.3	16 ± 1.5	15 ± 0.3	31 ± 2.9	25 ± 1.5	29 ± 1.9	29 ± 0.9
	10.0	16 ± 1.0	20 ± 3.1	29 ± 4.0	26 ± 1.5	28 ± 3.8	24 ± 1.9
	33.0	19 ± 3.5	19 ± 1.8	33 ± 4.5	25 ± 3.4	30 ± 2.6	32 ± 2.2
	100.0	18 ± 2.0	22 ± 1.8	34 ± 1.8	28 ± 1.2	25 ± 2.1	24 ± 4.6
	333.0	18 ± 2.5[3]	13 ± 1.7[3]	31 ± 0.6[3]	27 ± 3.3[3]	29 ± 4.8[3]	23 ± 4.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,303 ± 41.7	1,707 ± 61.0	1,894 ± 156.8	2,059 ± 84.9	1,113 ± 15.9	779 ± 32.7

[1] Study performed at EG&G Mason Research Institute. The detailed protocol and these data are presented in Haworth *et al.* (1983). Cells and *m*-nitrotoluene or solvent (dimethylsulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. High dose was limited by toxicity or solubility, but did not exceed 10,000 µg/plate; 0 µg/plate dose is the solvent control.

[2] Revertants are presented as mean ± standard error from 3 plates.

[3] Slight toxicity.

[4] Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE D3 Mutagenicity of *p*-Nitrotoluene in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0.0	154 ± 10.8	127 ± 13.3	125 ± 4.4	145 ± 8.7	134 ± 6.3	128 ± 15.3
	3.3		125 ± 10.5				
	10.0	164 ± 8.7	141 ± 5.3		131 ± 3.8		118 ± 9.5
	33.0	155 ± 9.6	109 ± 6.5	131 ± 7.8	134 ± 6.9	145 ± 10.8	131 ± 3.9
	100.0	169 ± 0.9	124 ± 9.8	129 ± 4.5	136 ± 5.7	134 ± 10.1	137 ± 6.2
	333.0	177 ± 7.1[3]	147 ± 3.8[3]	141 ± 12.5	164 ± 5.5	156 ± 9.4	137 ± 4.5
	500.0				143 ± 11.9[3]		136 ± 5.0[3]
	667.0	Toxic		155 ± 3.5[3]		Toxic	
	1,000.0			Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[4]		2,263 ± 120.4	1,343 ± 30.9	1,391 ± 136.2	1,191 ± 27.7	813 ± 60.8	1,061 ± 52.5
TA1535	0.0	30 ± 2.1	22 ± 2.0	12 ± 2.0	11 ± 2.1	14 ± 0.6	9 ± 0.3
	3.0		24 ± 0.9				
	10.0	39 ± 3.3	20 ± 1.3		15 ± 0.7		9 ± 1.9
	33.0	30 ± 1.9	19 ± 1.2	9 ± 0.9	10 ± 1.0	12 ± 0.3	14 ± 2.5
	100.0	35 ± 2.3	19 ± 3.0	19 ± 2.2	12 ± 2.2	13 ± 0.9	11 ± 1.7
	333.0	24 ± 0.9[3]	21 ± 2.7[3]	14 ± 2.9	17 ± 1.9	18 ± 3.5	16 ± 1.5
	500.0				11 ± 2.6[3]		10 ± 1.5[3]
	667.0	Toxic		Toxic		Toxic	
	1,000.0			Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,469 ± 33.8	980 ± 33.6	121 ± 14.2	49 ± 2.5	56 ± 2.7	42 ± 3.5
TA1537	0.0	6 ± 1.5	5 ± 1.9	6 ± 0.3	4 ± 1.5	11 ± 1.5	7 ± 1.7
	3.3		4 ± 2.6				
	10.0	7 ± 0.3	5 ± 0.9		7 ± 0.7		7 ± 1.9
	33.0	5 ± 0.9	4 ± 1.5	6 ± 1.0	6 ± 0.3	13 ± 2.5	5 ± 2.0
	100.0	8 ± 1.5	3 ± 1.2	10 ± 2.2	7 ± 0.0	10 ± 2.4	6 ± 1.0
	333.0	7 ± 2.7[3]	4 ± 0.7	7 ± 0.9	5 ± 2.5	12 ± 3.5	6 ± 2.7
	500.0				6 ± 1.0[3]		6 ± 1.0[3]
	667.0	Toxic		Toxic		Toxic	
	1,000.0			Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		557 ± 68.4	379 ± 61.2	130 ± 11.5	133 ± 3.8	46 ± 7.1	77 ± 6.1
TA98	0.0	25 ± 2.7	13 ± 1.7	30 ± 3.6	23 ± 2.1	31 ± 2.3	23 ± 5.6
	3.3		11 ± 0.6				
	10.0	16 ± 2.4	15 ± 1.7		23 ± 2.5		23 ± 2.3
	33.0	19 ± 4.0	15 ± 1.2	36 ± 3.3	22 ± 0.3	35 ± 3.4	20 ± 1.2
	100.0	18 ± 1.9	14 ± 1.3	31 ± 2.3	27 ± 4.5	31 ± 2.0	24 ± 4.1
	333.0	20 ± 1.9[3]	11 ± 2.0	36 ± 4.2	27 ± 1.2	27 ± 2.8	22 ± 0.9
	500.0				21 ± 5.0[3]		16 ± 2.0[3]
	667.0	Toxic		Toxic		17 ± 0.6[3]	
	1,000.0			Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		904 ± 87.8	1,347 ± 43.7	1,468 ± 63.0	1,045 ± 12.6	515 ± 10.9	752 ± 10.7

TABLE D3 Mutagenicity of *p*-Nitrotoluene in *Salmonella typhimurium* (continued)

[1] Study performed at EG&G Mason Research Institute. The detailed protocol and these data are presented in Haworth *et al.* (1983). Cells and *p*-nitrotoluene or solvent (dimethylsulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. High dose was limited by toxicity. 0 µg/plate dose is the solvent control.

[2] Revertants are presented as mean ± standard error from 3 plates.

[3] Slight toxicity.

[4] Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE D4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitrotoluene[1]

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[2]	Average Mutant Fraction
-S9						
Trial 1						
Ethanol						
		68	89	60	29	
		90	104	92	34	
		100	108	89	30	31
Methyl methanesulfonate						
	5	37	24	534	483	
		47	28	511	361	
		47	30	492	349	398[3]
p-Nitrotoluene						
	75	56	61	45	27	
		90	66	73	27	
		84	79	50	20	25
	100	81	72	53	22	
		58	51	51	30	
		61	44	50	27	26
	150	67	52	68	34	
		81	81	69	28	31
	180	85	48	70	27	
		72	38	82	38	
		86	41	124	48	38
	200	59	29	81	46	
		52	22	79	50	
		58	32	53	30	42
	240	30	14	42	47	
		46	21	77	56	
		51	19	68	45	49[3]

TABLE D4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitrotoluene (continued)

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 2						
Ethanol						
		81	77	44	18	
		83	102	48	19	
		103	121	54	17	18
Methyl methanesulfonate						
	5	96	55	345	120	
		68	42	405	198	159[3]
p-Nitrotoluene						
	25	109	81	73	22	
		113	82	71	21	
		109	87	58	18	20
	50	116	72	77	22	
	75	104	56	49	16	
		109	73	45	14	15
	100	114	50	58	17	
		103	56	60	19	
		59	38	70	40	25
	150	95	43	79	28	
		76	39	79	35	
		94	38	67	24	29[3]
	250	36	11	50	47	
		60	13	66	37	
		80	24	53	22	35[3]

TABLE D4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitrotoluene (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9[4]						
Trial 1						
Acetone						
		105	101	77	24	
		88	81	70	26	
		97	105	52	18	
		105	114	59	19	22
Methylcholanthrene						
	2.5	81	50	337	140	
		91	48	328	120	
		75	42	319	143	134[3]
p-Nitrotoluene						
	50	87	76	96	37	
		83	93	63	25	
		84	77	105	42	35[3]
	75	82	68	100	41	
		68	63	77	38	
		94	74	78	28	35[3]
	100	102	71	130	42	
		87	57	120	46	
		86	61	87	34	41[3]
	150	77	60	111	48	
		80	63	85	35	
		68	57	85	42	42[3]
	200	103	42	170	55	
		97	53	109	38	
		80	40	129	54	49[3]
	300[5]	108	41	141	44	
		91	43	71	26	35
	500	Lethal				
		Lethal				

TABLE D4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitrotoluene (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 2						
Acetone						
		91	107	69	25	
		90	84	66	24	
		104	115	71	23	
		94	94	72	26	25
Methylcholanthrene						
	2.5	56	51	484	286	
		98	109	363	124	
		85	68	501	198	203[3]
p-Nitrotoluene						
	50	101	81	86	28	
		72	102	61	28	
		86	83	64	25	27
	75	72	77	60	28	
		105	88	65	21	
		104	80	68	22	23
	100	108	86	82	25	
		117	79	91	26	
		109	89	113	35	29
	150	111	69	113	34	
		108	98	112	34	
		104	78	87	28	32
	200	90	72	89	33	
		109	71	120	37	
		112	67	123	36	35
	300[5]	99	47	149	50	
		106	57	122	38	
		110	45	106	32	40[3]
	500	Lethal				
		Lethal				

TABLE D4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitrotoluene (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 3						
Acetone						
		79	115	85	36	
		102	108	139	45	
		104	90	128	41	
		101	87	122	40	41
Methylcholanthrene						
	2.5	51	21	842	549	
		55	21	1,031	623	
		67	34	995	497	556[3]
p-Nitrotoluene						
	50	107	70	219	69	
		110	68	185	56	
		120	65	178	49	58
	75	84	61	181	72	
		103	69	263	85	
		112	48	175	52	70[3]
	100	113	62	263	78	
		111	44	234	70	
		86	37	162	63	70[3]
	150	93	35	251	90	
		105	39	380	120	
		87	31	205	79	96[3]
	200	86	17	436	170	
		90	22	353	131	150[3]
	300[5]	75	8	418	186	
		84	13	472	187	
		38	4	479	424	266[3]
	500	Lethal				
		Lethal				
		Lethal				

TABLE D4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitrotoluene (continued)

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 4						
Ethanol						
		88	91	108	41	
		106	101	93	29	
		118	108	118	33	34
Methylcholanthrene						
	2.5	96	65	785	272	
		92	41	952	346	
		91	43	726	266	294[3]
p-Nitrotoluene						
	50	77	52	160	70	
		109	82	144	44	
		108	61	139	43	52[3]
	100	108	59	204	63	
		97	52	180	62	62[3]
	150	97	45	189	65	
		111	50	124	37	51
	200	98	40	205	70	
		118	39	233	66	
		88	31	316	119	85[3]
	250[5]	103	28	194	63	
		94	29	300	107	
		105	32	249	79	83[3]
	300[5]	92	19	272	98	
		83	13	388	156	
		94	29	192	68	108[3]

[1] Study performed at Litton Bionetics, Inc. The experimental protocol is presented in detail by Myhr *et al.* (1985). The highest dose of *p*-nitrotoluene was determined by solubility and toxicity. All doses are tested in triplicate. The average of the three tests is presented in the table. Cells (6 x 10^5/mL) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3 x 10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency.

[2] Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF/1 x 10^6 cells treated); MF=mutant fraction.

[3] Significant positive response.

[4] Tests conducted with metabolic activation were performed as described in [1] except that S9, prepared from the livers of Aroclor 1254-induced Fischer 344 rats, was added at the same time as the *p*-nitrotoluene and/or solvent.

[5] Precipitate of *p*-nitrotoluene formed at this concentration.

TABLE D5 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *o*-Nitrotoluene[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)[2]
-S9[3]								
Trial 1								
Summary: Questionable								
Dimethylsulfoxide		50	1,035	485	0.46	9.7	25.5	
Mitomycin-C	0.005	50	1,037	2,236	2.15	44.7	25.5	360.15
o-Nitrotoluene	117.000	50	1,039	569	0.54	11.4	34.0[4]	16.87
	176.000	50	1,021	557	0.54	11.1	34.0[4]	16.42
	218.000	50	1,035	576	0.55	11.5	34.0[4]	18.76
	282.000	0						
								P=0.005[5]
+S9[6]								
Trial 1								
Summary: Positive								
Dimethylsulfoxide		50	1,050	380	0.36	7.6	25.5	
Cyclophosphamide	1.50	50	1,049	1,651	1.57	33.0	25.5	334.89
o-Nitrotoluene	354.83	50	1,050	499	0.47	10.0	25.5	31.32*
	380.95	50	1,050	467	0.44	9.3	25.5	22.90*
	423.28	50	1,047	488	0.46	9.8	25.5	28.79*
								P=0.001

TABLE D5 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *o*-Nitrotoluene (continued)

[1] Study performed at Litton Bionetics, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the SCE protocol and these data are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *o*-nitrotoluene or solvent (dimethylsulfoxide) as described in [3] and [5] below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.

[2] SCEs/chromosome of culture exposed to *o*-nitrotoluene relative to those of culture exposed to solvent.

[3] In the absence of S9, cells were incubated with *o*-nitrotoluene or solvent for 2 hours at 37° C. Then BrdU was added and incubation was continued for 23.5 hours. Cells were washed, fresh medium containing BrdU and Colcemid was added, and incubation was continued for 2 to 3 hours.

[4] Because of chemical-induced delay in the cell division cycle, harvest time was extended to maximize the proportion of second division cells available for analysis.

[5] Significance of relative SCEs/chromosome tested by linear regression vs. log of the dose.

[6] In the presence of S9, cells were incubated with *o*-nitrotoluene or solvent for 2 hours at 37° C. The cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 25.5 hours, with Colcemid present for the final 2 to 3 hours. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

* Positive (≥20% increase over solvent control).

TABLE D6 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *m*-Nitrotoluene[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)[2]
-S9[3]								
Trial 1								
Summary: Weak positive								
Dimethylsulfoxide		50	1,031	434	0.42	8.7	25.5	
Mitomycin-C	0.005	25	511	851	1.66	34.0	25.5	295.62
m-Nitrotoluene	3.840	50	1,020	488	0.47	9.8	25.5	13.65
	11.500	50	1,029	442	0.42	8.8	25.5	2.04
	38.400	50	986	475	0.48	9.5	25.5	14.44
	115.000	28	556	297	0.53	10.6	25.5	26.90*
								P=0.003[4]
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,045	477	0.45	9.5	25.5	
Mitomycin-C	0.005	25	519	866	1.66	34.6	25.5	265.56
m-Nitrotoluene	150.000	50	1,035	616	0.59	12.3	33.3[5]	30.39*
	196.000	50	1,033	576	0.55	11.5	33.3[5]	22.16*
	253.000	50	1,017	585	0.57	11.7	33.3[5]	26.02*
								P=0.001

TABLE D6 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *m*-Nitrotoluene (continued)

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)
+S9[6]								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,003	343	0.34	6.9	25.5	
Cyclophosphamide	1.5	25	505	522	1.03	20.9	25.5	202.27
m-Nitrotoluene	38.4	50	1,021	307	0.30	6.1	25.5	-12.08
	115.0	50	1,009	356	0.35	7.1	25.5	3.17
	384.0	50	989	313	0.31	6.3	25.5	-7.45
								P=0.625

[1] Study performed at Litton Bionetics, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the SCE protocol and these data are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *m*-nitrotoluene or solvent (dimethylsulfoxide) as described in [3] and [5] below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.

[2] SCEs/chromosome of culture exposed to *m*-nitrotoluene relative to those of culture exposed to solvent.

[3] In the absence of S9, cells were incubated with *m*-nitrotoluene or solvent for 2 hours at 37° C. Then BrdU was added and incubation was continued for 23.5 hours. Cells were washed, fresh medium containing BrdU and Colcemid was added, and incubation was continued for 2 to 3 hours.

[4] Significance of relative SCEs/chromosome tested by regression vs. log of the dose.

[5] Because of chemical-induced delay in the cell division cycle, harvest time was extended to maximize the proportion of second division cells available for analysis.

[6] In the presence of S9, cells were incubated with *m*-nitrotoluene or solvent for 2 hours at 37° C. The cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 25.5 hours, with Colcemid present for the final 2 to 3 hours. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

* Positive (≥20% increase over solvent control).

TABLE D7 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *p*-Nitrotoluene[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)[2]
-S9[3]								
Trial 1								
Summary: Weak positive								
Dimethylsulfoxide		50	1,038	487	0.46	9.7	25.5	
Mitomycin-C	0.005	25	520	798	1.53	31.9	25.5	227.09
p-Nitrotoluene	50.000	43	889	382	0.42	8.9	25.5	-8.42
	167.000	50	1,024	576	0.56	11.5	32.8[4]	19.89
	500.000[5]	50	1,018	637	0.62	12.7	32.8[4]	33.37*
								P=0.000[6]
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,036	532	0.51	10.6	25.5	
Mitomycin-C	0.005	25	522	596	1.14	23.8	25.5	122.34
p-Nitrotoluene	200.000[5]	50	1,035	732	0.70	14.6	35.8[4]	37.73*
	300.000[5]	50	1,032	698	0.67	14.0	35.8[4]	31.71*
	400.000[5]	50	1,034	803	0.77	16.1	35.8[4]	51.23*
	500.000[5]	0					35.8[4]	
								P=0.000

TABLE D7 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *p*-Nitrotoluene (continued)

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)
+S9[7]								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,042	467	0.44	9.3	25.5	
Cyclophosphamide	1.5	25	525	1,082	2.06	43.3	25.5	359.86
p-Nitrotoluene	50.0	50	1,043	464	0.44	9.3	25.5	-0.74
	167.0	50	1,044	480	0.45	9.6	25.5	2.59
	500.0[5]	50	1,031	510	0.49	10.2	25.5	10.37
								P=0.052
Trial 2								
Summary: Weak positive								
Dimethylsulfoxide		50	1,041	467	0.44	9.3	25.5	
Cyclophosphamide	1.5	25	517	654	1.26	26.2	25.5	181.99
p-Nitrotoluene	600.0[5]	50	1,027	535	0.52	10.7	25.5	16.12
	700.0[5]	50	1,030	657	0.63	13.1	35.5[4]	42.19*
								P=0.000
Trial 3								
Summary: Positive								
Dimethylsulfoxide		50	1,047	451	0.43	9.0	25.5	
Cyclophosphamide	1.5	25	521	872	1.67	34.9	25.5	288.55
p-Nitrotoluene	550.0[5]	50	1,038	905	0.87	18.1	25.5	102.41*
	600.0[5]	50	1,032	787	0.76	15.7	25.5	77.04*
	650.0[5]	50	1,039	886	0.85	17.7	25.5	97.97*
								P=0.000

TABLE D7 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *p*-Nitrotoluene (continued)

[1] Study performed at Litton Bionetics, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the SCE protocol and these data are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *p*-nitrotoluene or solvent (dimethylsulfoxide) as described in [3] and [6] below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.

[2] SCEs/chromosome of culture exposed to *p*-nitrotoluene relative to those of culture exposed to solvent.

[3] In the absence of S9, cells were incubated with *p*-nitrotoluene or solvent for 2 hours at 37° C. Then BrdU was added and incubation was continued for 23.5 hours. Cells were washed, fresh medium containing BrdU and Colcemid was added, and incubation was continued for 2 to 3 hours.

[4] Because of chemical-induced delay in the cell division cycle, harvest time was extended to maximize the proportion of second division cells available for analysis.

[5] Precipitate of *p*-nitrotoluene formed.

[6] Significance of relative SCEs/chromosome tested by linear regression vs. log of the dose.

[7] In the presence of S9, cells were incubated with *p*-nitrotoluene or solvent for 2 hours at 37° C. The cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 25.5 hours, with Colcemid present for the final 2 to 3 hours. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

* Positive (≥20% increase over solvent control).

TABLE D8 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *o*-Nitrotoluene[1]

-S9[2]					+S9[3]				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 – Harvest time: 10.5 hours Summary: Negative					**Trial 1** – Harvest time: 10.5 hours Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	3	0.03	3.0		100	5	0.05	4.0
Mitomycin-C					Cyclophosphamide				
0.5	100	20	0.20	16.0	50	100	30	0.30	20.0
o-Nitrotoluene					*o*-Nitrotoluene				
200.7	100	2	0.02	2.0	375.36	100	3	0.03	2.0
252.5	100	1	0.01	1.0	398.82	100	9	0.09	8.0
393.6	100	1	0.01	1.0	422.28	100	5	0.05	5.0
				P=0.873[4]					P=0.168

[1] Study performed at Litton Bionetics, Inc. Abs=aberrations. A detailed presentation of the protocol and these data are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *o*-nitrotoluene or solvent (dimethylsulfoxide) as indicated in [2] and [3]. Cells were arrested in first metaphase by addition of Colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.

[2] In the absence of S9, cells were incubated with *o*-nitrotoluene or solvent for 8.5 hours at 37° C. Cells were then washed and fresh medium containing Colcemid was added for an additional 2 hours followed by harvest.

[3] In the presence of S9, cells were incubated with *o*-nitrotoluene or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8.5 hours. Colcemid was added for the last 2 to 3 hours of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

[4] Significance of percent cells with aberrations tested by linear regression trend test vs. log of the dose.

TABLE D9 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *m*-Nitrotoluene[1]

-S9[2]					+S9[3]				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 – Harvest time: 10.6 hours Summary: Negative					**Trial 1** – Harvest time: 10.4 hours Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	5	0.05	5.0		100	2	0.02	2.0
Mitomycin-C					Cyclophosphamide				
0.5	50	21	0.42	22.0	50	50	50	1.00	42.0
m-Nitrotoluene					*m*-Nitrotoluene				
150	100	0	0.00	0.0	150	100	4	0.04	4.0
300	100	2	0.02	2.0	300	100	4	0.04	4.0
398	50	2	0.04	4.0	398	100	3	0.03	3.0
				P=0.706[4]					P=0.332
Trial 2 – Harvest time: 20.0 hours[5] Summary: Negative					**Trial 2** – Harvest time: 20.0 hours[5] Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	4	0.04	3.0		100	2	0.02	2.0
Mitomycin-C					Cyclophosphamide				
0.063	50	25	0.50	34.0	10	50	20	0.40	18.0
m-Nitrotoluene					*m*-Nitrotoluene				
248	100	6	0.06	6.0	437	100	4	0.04	4.0
299	100	5	0.05	5.0	460	100	2	0.02	2.0
345	100	3	0.03	3.0	483	100	6	0.06	6.0
				P=0.535					P=0.114

[1] Study performed at Litton Bionetics, Inc. Abs=aberrations. A detailed presentation of the protocol and these data are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *m*-nitrotoluene or solvent (dimethylsulfoxide) as indicated in [2] and [4]. Cells were arrested in first metaphase by addition of Colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.

[2] In the absence of S9, cells were incubated with *m*-nitrotoluene or solvent for 8 hours at 37° C. Cells were then washed and fresh medium containing Colcemid was added for an additional 2 to 3 hours followed by harvest.

[3] In the presence of S9, cells were incubated with *m*-nitrotoluene or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8.4 hours. Colcemid was added for the last 2 to 3 hours of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

[4] Significance of percent cells with aberrations tested by linear regression trend test vs. log of the dose.

[5] Because of significant chemical-induced cell cycle delay, incubation time prior to addition of Colcemid was lengthened to provide sufficient metaphases at harvest.

TABLE D10 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *p*-Nitrotoluene[1]

-S9[2]					+S9[3]				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 – Harvest time: 20.0 hours Summary: Negative					**Trial 1** – Harvest time: 20.3 hours Summary: Weak positive				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	3	0.03	3.0		100	9	0.09	8.0
Mitomycin-C					Cyclophosphamide				
0.062	50	24	0.48	32.0	10	50	40	0.80	44.0
p-Nitrotoluene					*p*-Nitrotoluene				
300	100	7	0.07	7.0	500[5]	100	12	0.12	7.0
400	100	10	0.10	10.0	550[5]	100	10	0.10	8.0
500	100	9	0.09	9.0	600[5]	100	30	0.30	24.0*
				P=0.032[4]					P<0.001
Trial 2 – Harvest time: 21.0 hours Summary: Negative					**Trial 2** – Harvest time: 21.0 hours Weak positive				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	7	0.07	6.0		100	7	0.07	7.0
Mitomycin-C					Cyclophosphamide				
0.062	50	30	0.60	44.0	10	50	14	0.28	26.0
p-Nitrotoluene					*p*-Nitrotoluene				
300	100	9	0.09	8.0	400	100	3	0.03	3.0
400	100	0	0.00	0.0	500	100	2	0.02	2.0
500	100	6	0.06	5.0	550	100	23	0.23	21.0*
				P=0.897					P=0.003

[1] Study performed at Litton Bionetics, Inc. Abs=aberrations. A detailed presentation of the protocol and these data are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *p*-nitrotoluene or solvent (dimethylsulfoxide) as indicated in [2] and [3]. Cells were arrested in first metaphase by addition of Colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa. Because of significant chemical-induced cell cycle delay, incubation time prior to addition of Colcemid was lengthened from the usual 8- to 10-hour period to provide sufficient metaphases at harvest.

[2] In the absence of S9, cells were incubated with *p*-nitrotoluene or solvent for 18 hours at 37° C. Cells were then washed and fresh medium containing Colcemid was added for an additional 2 to 3 hours followed by harvest.

[3] In the presence of S9, cells were incubated with *p*-nitrotoluene or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 18 to 19 hours. Colcemid was added for the last 2 to 3 hours of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

[4] Significance of percent cells with aberrations tested by linear regression trend test vs. log of the dose.

[5] Precipitate of *p*-nitrotoluene formed at this concentration.

* Positive (≥20% increase over solvent control).

APPENDIX A

Organ Weights and Organ-Weight-to-Body-Weight Ratios

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats in the 13-Week Feed Studies of *o-*, *m-*, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Necropsy body wt						
o-Nitrotoluene	353 ± 5	339 ± 6	329 ± 9*	309 ± 6**	254 ± 4**	198 ± 3**
m-Nitrotoluene	346 ± 8	354 ± 4	342 ± 5	353 ± 6	338 ± 6	281 ± 5**
p-Nitrotoluene	350 ± 6	349 ± 7	342 ± 6	341 ± 11	315 ± 6**	253 ± 6**
Heart						
o-Nitrotoluene						
Absolute	1.039 ± 0.019	1.019 ± 0.016	1.021 ± 0.029	1.003 ± 0.026	0.985 ± 0.011	0.881 ± 0.016**
Relative	2.94 ± 0.05	3.01 ± 0.04	3.11 ± 0.04*	3.24 ± 0.06**	3.88 ± 0.07**	4.44 ± 0.04**
m-Nitrotoluene						
Absolute	0.983 ± 0.022	1.037 ± 0.027	0.992 ± 0.019	0.999 ± 0.026	0.958 ± 0.020	0.851 ± 0.024**
Relative	2.84 ± 0.03	2.92 ± 0.05	2.90 ± 0.03	2.83 ± 0.05	2.83 ± 0.05	3.03 ± 0.06
p-Nitrotoluene						
Absolute	1.121 ± 0.015	1.089 ± 0.022	1.050 ± 0.025*	1.045 ± 0.035*	0.966 ± 0.023**	0.858 ± 0.022**
Relative	3.18 ± 0.06	3.09 ± 0.04	3.04 ± 0.04	3.04 ± 0.05	3.02 ± 0.04	3.42 ± 0.05
Right Kidney						
o-Nitrotoluene						
Absolute	1.176 ± 0.021	1.167 ± 0.026	1.130 ± 0.035	1.137 ± 0.031	1.058 ± 0.015**	1.017 ± 0.027**
Relative	3.33 ± 0.04	3.45 ± 0.04	3.44 ± 0.05	3.67 ± 0.05**	4.17 ± 0.08**	5.12 ± 0.09**
m-Nitrotoluene						
Absolute	1.118 ± 0.023	1.177 ± 0.020	1.128 ± 0.030	1.209 ± 0.024	1.203 ± 0.035	1.126 ± 0.030
Relative	3.24 ± 0.05	3.32 ± 0.05	3.30 ± 0.07	3.43 ± 0.06*	3.55 ± 0.06**	4.00 ± 0.07**
p-Nitrotoluene						
Absolute	1.133 ± 0.026	1.150 ± 0.033	1.118 ± 0.026	1.149 ± 0.073	1.114 ± 0.029	0.926 ± 0.022**
Relative	3.21 ± 0.04	3.25 ± 0.03	3.24 ± 0.04	3.32 ± 0.11	3.49 ± 0.06**	3.69 ± 0.05**
Liver						
o-Nitrotoluene						
Absolute	12.00 ± 0.35	12.50 ± 0.25	12.88 ± 0.38	13.78 ± 0.39**	14.62 ± 0.32**	15.07 ± 0.38**
Relative	33.9 ± 0.7	36.9 ± 0.5**	39.2 ± 0.6**	44.5 ± 0.8**	57.6 ± 1.4**	76.0 ± 1.5**
m-Nitrotoluene						
Absolute	11.47 ± 0.48	12.08 ± 0.26	10.92 ± 0.35	12.13 ± 0.34	11.15 ± 0.30	10.96 ± 0.32
Relative	33.1 ± 0.8	34.1 ± 0.5	31.9 ± 0.7	34.3 ± 0.6	32.9 ± 0.4	39.0 ± 0.9**
p-Nitrotoluene						
Absolute	11.35 ± 0.25	11.34 ± 0.36	11.20 ± 0.24	10.86 ± 0.47	11.28 ± 0.27	9.60 ± 0.30**
Relative	32.2 ± 0.4	32.1 ± 0.5	32.4 ± 0.3	31.5 ± 0.4	35.3 ± 0.4**	38.3 ± 1.0**
Lungs						
o-Nitrotoluene						
Absolute	1.676 ± 0.056	1.898 ± 0.079	1.578 ± 0.070	1.528 ± 0.053	1.445 ± 0.058*	1.214 ± 0.043**
Relative	4.74 ± 0.15	5.62 ± 0.26*	4.80 ± 0.17	4.94 ± 0.13	5.69 ± 0.22**	6.11 ± 0.18**
m-Nitrotoluene						
Absolute	1.575 ± 0.077	1.820 ± 0.182	1.798 ± 0.118	1.582 ± 0.058	1.675 ± 0.111	1.285 ± 0.040**
Relative	4.56 ± 0.22	5.12 ± 0.48	5.25 ± 0.31	4.48 ± 0.15	4.94 ± 0.29	4.57 ± 0.11
p-Nitrotoluene						
Absolute	1.509 ± 0.031	1.589 ± 0.054	1.568 ± 0.046	1.525 ± 0.053	1.541 ± 0.063	1.220 ± 0.035**
Relative	4.28 ± 0.08	4.51 ± 0.17	4.55 ± 0.13	4.45 ± 0.16	4.83 ± 0.19*	4.87 ± 0.14**

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Right Testis						
o-Nitrotoluene						
Absolute	1.409 ± 0.031	1.386 ± 0.024	1.361 ± 0.042	1.346 ± 0.020	1.011 ± 0.046**	0.517 ± 0.024**
Relative	3.99 ± 0.07	4.09 ± 0.04	4.14 ± 0.06	4.36 ± 0.06	4.00 ± 0.23	2.61 ± 0.12**
m-Nitrotoluene						
Absolute	1.372 ± 0.021	1.432 ± 0.027	1.356 ± 0.034	1.412 ± 0.025	1.392 ± 0.020	0.759 ± 0.071**
Relative	3.98 ± 0.06	4.04 ± 0.06	3.97 ± 0.10	4.01 ± 0.07	4.12 ± 0.05	2.69 ± 0.24**
p-Nitrotoluene						
Absolute	1.447 ± 0.025	1.431 ± 0.036	1.347 ± 0.043	1.410 ± 0.045	1.348 ± 0.029*	1.030 ± 0.076**
Relative	4.10 ± 0.06	4.05 ± 0.05	3.90 ± 0.12	4.10 ± 0.04	4.22 ± 0.06	4.10 ± 0.27
Thymus						
o-Nitrotoluene						
Absolute	0.321 ± 0.012	0.315 ± 0.012	0.301 ± 0.016	0.298 ± 0.017	0.327 ± 0.024	0.307 ± 0.020
Relative	0.91 ± 0.04	0.94 ± 0.05	0.92 ± 0.05	0.97 ± 0.05	1.29 ± 0.10**	1.55 ± 0.10**
m-Nitrotoluene						
Absolute	0.333 ± 0.017	0.337 ± 0.023	0.352 ± 0.015	0.332 ± 0.014	0.341 ± 0.020	0.266 ± 0.015*
Relative	9.59 ± 0.33	9.49 ± 0.59	10.32 ± 0.44	9.42 ± 0.43	10.09 ± 0.56	9.48 ± 0.57
p-Nitrotoluene						
Absolute	0.338 ± 0.014	0.347 ± 0.023	0.308 ± 0.018	0.342 ± 0.020	0.310 ± 0.017	0.222 ± 0.018**
Relative	9.62 ± 0.49	9.79 ± 0.52	8.93 ± 0.51	9.94 ± 0.51	9.67 ± 0.42	8.83 ± 0.66

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

[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*- or *p*-nitrotoluene.

* 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' or Dunnett's test.

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Necropsy body wt						
o-Nitrotoluene	205 ± 3	197 ± 3	192 ± 4*	179 ± 3**	170 ± 2**	158 ± 3**
m-Nitrotoluene	194 ± 3	199 ± 3	194 ± 3	195 ± 3	177 ± 2**	166 ± 3**
p-Nitrotoluene	199 ± 2	199 ± 3	200 ± 3	193 ± 3	182 ± 3**	173 ± 3**
Heart						
o-Nitrotoluene						
Absolute	0.673 ± 0.011	0.670 ± 0.014	0.648 ± 0.015	0.617 ± 0.015*	0.628 ± 0.007**	0.634 ± 0.011*
Relative	3.29 ± 0.04	3.40 ± 0.05	3.37 ± 0.04	3.44 ± 0.06*	3.70 ± 0.03**	4.01 ± 0.05**
m-Nitrotoluene						
Absolute	0.681 ± 0.011	0.669 ± 0.013	0.720 ± 0.022	0.658 ± 0.015	0.633 ± 0.012*	0.608 ± 0.027*
Relative	3.51 ± 0.07	3.35 ± 0.04	3.72 ± 0.10	3.38 ± 0.06	3.58 ± 0.06	3.66 ± 0.14
p-Nitrotoluene						
Absolute	0.710 ± 0.012	0.704 ± 0.022	0.700 ± 0.013	0.684 ± 0.014	0.669 ± 0.020	0.591 ± 0.009**
Relative	3.52 ± 0.08	3.45 ± 0.10	3.48 ± 0.08	3.49 ± 0.08	3.61 ± 0.08	3.40 ± 0.04
Right Kidney						
o-Nitrotoluene						
Absolute	0.684 ± 0.013	0.692 ± 0.009	0.680 ± 0.016	0.632 ± 0.013*	0.650 ± 0.011*	0.633 ± 0.008**
Relative	3.34 ± 0.05	3.52 ± 0.08	3.54 ± 0.07*	3.53 ± 0.05*	3.83 ± 0.07**	4.01 ± 0.06**
m-Nitrotoluene						
Absolute	0.687 ± 0.011	0.705 ± 0.018	0.714 ± 0.015	0.709 ± 0.020	0.679 ± 0.008	0.663 ± 0.016
Relative	3.54 ± 0.04	3.53 ± 0.06	3.69 ± 0.06	3.64 ± 0.07	3.84 ± 0.06**	4.00 ± 0.06**
p-Nitrotoluene						
Absolute	0.696 ± 0.014	0.685 ± 0.010	0.700 ± 0.013	0.664 ± 0.014	0.632 ± 0.017**	0.637 ± 0.014**
Relative	3.44 ± 0.06	3.36 ± 0.03	3.47 ± 0.03	3.38 ± 0.05	3.41 ± 0.07	3.66 ± 0.07*
Liver						
o-Nitrotoluene						
Absolute	6.10 ± 0.12	6.39 ± 0.14	6.63 ± 0.23	5.66 ± 0.13	6.10 ± 0.15	6.67 ± 0.12
Relative	29.8 ± 0.7	32.5 ± 0.6*	34.5 ± 1.0**	31.6 ± 0.5*	36.0 ± 1.1**	42.2 ± 0.5**
m-Nitrotoluene						
Absolute	6.41 ± 0.14	6.33 ± 0.14	6.44 ± 0.16	6.30 ± 0.14	5.67 ± 0.17**	5.99 ± 0.14*
Relative	33.0 ± 0.6	31.7 ± 0.6	33.2 ± 0.6	32.4 ± 0.5	32.0 ± 0.8	36.2 ± 0.8*
p-Nitrotoluene						
Absolute	5.92 ± 0.10	6.29 ± 0.10	6.03 ± 0.13	5.57 ± 0.12	5.51 ± 0.09	6.28 ± 0.14
Relative	29.3 ± 0.4	30.8 ± 0.4*	29.9 ± 0.5	28.4 ± 0.3	29.8 ± 0.6	36.1 ± 0.7**
Lungs						
o-Nitrotoluene						
Absolute	1.126 ± 0.040	1.046 ± 0.028	1.067 ± 0.044[3]	0.988 ± 0.024**	1.065 ± 0.045	0.995 ± 0.047**
Relative	5.50 ± 0.19	5.31 ± 0.11	5.53 ± 0.17[3]	5.52 ± 0.11	6.26 ± 0.25*	6.28 ± 0.25*
m-Nitrotoluene						
Absolute	1.039 ± 0.043	1.042 ± 0.036	1.057 ± 0.050	1.091 ± 0.026	0.841 ± 0.027**	0.830 ± 0.017**
Relative	5.36 ± 0.23	5.22 ± 0.15	5.49 ± 0.34	5.62 ± 0.15	4.76 ± 0.16*	5.01 ± 0.06
p-Nitrotoluene						
Absolute	1.081 ± 0.016	1.095 ± 0.027	1.238 ± 0.111	1.088 ± 0.028	1.028 ± 0.027	0.970 ± 0.031*
Relative	5.36 ± 0.11	5.37 ± 0.11	6.12 ± 0.49	5.55 ± 0.13	5.54 ± 0.10	5.57 ± 0.14

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rats In the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Thymus						
o-Nitrotoluene						
Absolute	0.242 ± 0.009	0.234 ± 0.011	0.224 ± 0.009	0.222 ± 0.010	0.206 ± 0.014*	0.231 ± 0.014
Relative	1.18 ± 0.04	1.19 ± 0.05	1.17 ± 0.05	1.24 ± 0.06	1.21 ± 0.08	1.46 ± 0.09*
m-Nitrotoluene						
Absolute	0.257 ± 0.012	0.260 ± 0.010	0.262 ± 0.009	0.274 ± 0.007	0.223 ± 0.006*	0.222 ± 0.007*
Relative	1.32 ± 0.05	1.30 ± 0.04	1.36 ± 0.05	1.41 ± 0.04	1.26 ± 0.02	1.35 ± 0.05
p-Nitrotoluene						
Absolute	0.287 ± 0.012	0.260 ± 0.008*	0.266 ± 0.010	0.280 ± 0.012	0.244 ± 0.011**	0.240 ± 0.008**
Relative	1.42 ± 0.06	1.27 ± 0.04	1.32 ± 0.05	1.43 ± 0.06	1.31 ± 0.05	1.39 ± 0.06

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).
[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*- or *p*-nitrotoluene.
[3] n=9.
* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test.
** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test.

TABLE A3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Mice In the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Necropsy body wt						
o-Nitrotoluene	33.3 ± 0.4	34.5 ± 0.9	32.9 ± 0.6	32.8 ± 0.5	29.2 ± 0.3**	25.9 ± 0.3**
m-Nitrotoluene	36.5 ± 0.8	35.1 ± 0.5	34.9 ± 1.0	34.9 ± 0.7	31.3 ± 0.8**	27.7 ± 0.4**
p-Nitrotoluene	33.5 ± 0.4	33.1 ± 0.8	33.8 ± 0.7	32.3 ± 0.5	31.1 ± 0.4**	29.9 ± 0.7**
Heart						
o-Nitrotoluene						
Absolute	0.152 ± 0.002	0.149 ± 0.003	0.150 ± 0.003	0.160 ± 0.007	0.136 ± 0.003**	0.121 ± 0.002**
Relative	4.57 ± 0.10	4.34 ± 0.12	4.56 ± 0.09	4.86 ± 0.17	4.65 ± 0.10	4.68 ± 0.09
m-Nitrotoluene						
Absolute	0.200 ± 0.014	0.170 ± 0.010	0.169 ± 0.007	0.163 ± 0.006*	0.163 ± 0.009*	0.156 ± 0.006*
Relative	5.52 ± 0.45	4.88 ± 0.35	4.85 ± 0.16	4.67 ± 0.12	5.19 ± 0.24	5.63 ± 0.22
p-Nitrotoluene						
Absolute	0.171 ± 0.005	0.175 ± 0.007	0.162 ± 0.006	0.155 ± 0.008	0.147 ± 0.005**	0.149 ± 0.006**
Relative	5.19 ± 0.18	5.35 ± 0.22	4.89 ± 0.22	4.81 ± 0.27	4.72 ± 0.13	4.99 ± 0.14
Right Kidney						
o-Nitrotoluene						
Absolute	0.304 ± 0.006	0.306 ± 0.005	0.303 ± 0.005	0.290 ± 0.004	0.249 ± 0.005**	0.207 ± 0.004**
Relative	9.14 ± 0.23	8.91 ± 0.17	9.21 ± 0.16	8.85 ± 0.17	8.51 ± 0.15*	7.99 ± 0.15**
m-Nitrotoluene						
Absolute	0.325 ± 0.006	0.321 ± 0.007	0.319 ± 0.008	0.314 ± 0.008	0.282 ± 0.008**	0.254 ± 0.005**
Relative	8.94 ± 0.28	9.15 ± 0.19	9.18 ± 0.24	9.01 ± 0.21	9.01 ± 0.18	9.17 ± 0.17
p-Nitrotoluene						
Absolute	0.273 ± 0.007	0.281 ± 0.007	0.300 ± 0.010	0.271 ± 0.006	0.262 ± 0.009	0.267 ± 0.007
Relative	8.28 ± 0.22	8.61 ± 0.36	9.00 ± 0.16	8.39 ± 0.14	8.44 ± 0.33	8.95 ± 0.15
Liver						
o-Nitrotoluene						
Absolute	1.49 ± 0.03	1.50 ± 0.03	1.52 ± 0.04	1.61 ± 0.03*	1.57 ± 0.03*	1.52 ± 0.03
Relative	44.6 ± 0.7	43.6 ± 0.5	46.0 ± 0.8	49.0 ± 0.5**	53.5 ± 0.8**	58.8 ± 0.9**
m-Nitrotoluene						
Absolute	1.65 ± 0.04	1.70 ± 0.04	1.74 ± 0.04	1.79 ± 0.03	1.69 ± 0.04	1.60 ± 0.03
Relative	45.2 ± 0.8	48.5 ± 1.0*	50.0 ± 0.9**	51.5 ± 0.7**	53.9 ± 0.6**	57.9 ± 0.9**
p-Nitrotoluene						
Absolute	1.42 ± 0.02	1.50 ± 0.03*	1.65 ± 0.03**	1.49 ± 0.02**	1.62 ± 0.04**	1.74 ± 0.05**
Relative	43.2 ± 0.7	45.7 ± 0.9*	49.6 ± 0.6**	46.3 ± 0.6**	52.1 ± 0.7**	58.3 ± 0.9**
Lungs						
o-Nitrotoluene						
Absolute	0.198 ± 0.008	0.214 ± 0.013	0.183 ± 0.008	0.219 ± 0.010	0.196 ± 0.011	0.171 ± 0.007*
Relative	5.96 ± 0.28	6.21 ± 0.36	5.58 ± 0.27	6.65 ± 0.24	6.71 ± 0.41	6.61 ± 0.28
m-Nitrotoluene						
Absolute	0.245 ± 0.017	0.266 ± 0.018	0.258 ± 0.018	0.226 ± 0.015	0.250 ± 0.016	0.260 ± 0.019
Relative	6.78 ± 0.54	7.59 ± 0.53	7.47 ± 0.57	6.47 ± 0.38	7.96 ± 0.43	9.35 ± 0.63**
p-Nitrotoluene						
Absolute	0.205 ± 0.010	0.229 ± 0.008	0.207 ± 0.009	0.210 ± 0.013	0.214 ± 0.009	0.241 ± 0.015
Relative	6.23 ± 0.34	7.01 ± 0.32	6.26 ± 0.34	6.51 ± 0.39	6.88 ± 0.28	8.08 ± 0.49*

TABLE A3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Mice in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Right Testis						
o-Nitrotoluene						
Absolute	0.122 ± 0.002	0.119 ± 0.003	0.127 ± 0.002	0.123 ± 0.001	0.119 ± 0.002	0.110 ± 0.002**
Relative	3.66 ± 0.06	3.46 ± 0.10	3.85 ± 0.06	3.74 ± 0.05	4.06 ± 0.08**	4.26 ± 0.05**
m-Nitrotoluene						
Absolute	0.128 ± 0.003	0.123 ± 0.002	0.121 ± 0.003	0.123 ± 0.003	0.122 ± 0.002	0.117 ± 0.002**
Relative	3.51 ± 0.10	3.50 ± 0.07	3.49 ± 0.10	3.52 ± 0.09	3.90 ± 0.09**	4.20 ± 0.07**
p-Nitrotoluene						
Absolute	0.121 ± 0.004	0.119 ± 0.002	0.118 ± 0.002	0.118 ± 0.002	0.116 ± 0.003	0.117 ± 0.002
Relative	3.69 ± 0.14	3.64 ± 0.09	3.55 ± 0.08	3.67 ± 0.08	3.73 ± 0.09	3.93 ± 0.10
Thymus						
o-Nitrotoluene						
Absolute	0.043 ± 0.002	0.043 ± 0.003	0.043 ± 0.002	0.040 ± 0.002	0.036 ± 0.002*	0.037 ± 0.002
Relative	1.29 ± 0.06	1.25 ± 0.08	1.30 ± 0.07	1.22 ± 0.05	1.24 ± 0.07	1.44 ± 0.07
m-Nitrotoluene						
Absolute	0.050 ± 0.003	0.052 ± 0.003	0.055 ± 0.003	0.051 ± 0.004	0.049 ± 0.003	0.046 ± 0.002
Relative	1.37 ± 0.08	1.47 ± 0.07	1.56 ± 0.07	1.47 ± 0.10	1.55 ± 0.09	1.64 ± 0.07*
p-Nitrotoluene						
Absolute	0.051 ± 0.002	0.048 ± 0.002	0.050 ± 0.003	0.050 ± 0.003	0.043 ± 0.002	0.050 ± 0.004
Relative	1.54 ± 0.06	1.46 ± 0.07	1.50 ± 0.09	1.56 ± 0.09	1.39 ± 0.08	1.68 ± 0.14

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

[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*- or *p*-nitrotoluene.

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

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

TABLE A4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Mice in the 13-Week Feed Studies of *o-*, *m-*, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
n	10	10	10	10	10	10
Necropsy body wt						
o-Nitrotoluene	32.8 ± 0.7	34.0 ± 0.5	33.4 ± 0.7	32.1 ± 0.8	29.5 ± 0.6**	22.9 ± 0.3**
m-Nitrotoluene	33.8 ± 0.8	34.7 ± 0.6	34.6 ± 1.1	33.2 ± 0.4	29.8 ± 0.8**	24.4 ± 0.5**
p-Nitrotoluene	29.6 ± 0.7	33.0 ± 0.5	33.3 ± 0.6	27.9 ± 0.6	27.1 ± 0.4	24.9 ± 0.5**
Heart						
o-Nitrotoluene						
Absolute	0.134 ± 0.005	0.139 ± 0.005	0.135 ± 0.003	0.131 ± 0.002	0.144 ± 0.010	0.103 ± 0.003**
Relative	4.09 ± 0.12	4.09 ± 0.13	4.05 ± 0.10	4.09 ± 0.08	4.84 ± 0.25*	4.51 ± 0.15*
m-Nitrotoluene						
Absolute	0.153 ± 0.007	0.162 ± 0.007	0.154 ± 0.006	0.157 ± 0.008	0.158 ± 0.008	0.126 ± 0.005**
Relative	4.55 ± 0.22	4.69 ± 0.24	4.48 ± 0.22	4.74 ± 0.25	5.33 ± 0.29*	5.18 ± 0.21
p-Nitrotoluene						
Absolute	0.144 ± 0.004	0.141 ± 0.005	0.144 ± 0.005	0.139 ± 0.005	0.139 ± 0.005	0.124 ± 0.005**
Relative	4.96 ± 0.14	4.33 ± 0.17	4.44 ± 0.18	4.98 ± 0.14	5.14 ± 0.22	4.99 ± 0.19
Right Kidney						
o-Nitrotoluene						
Absolute	0.201 ± 0.006	0.215 ± 0.005	0.217 ± 0.005	0.217 ± 0.005	0.207 ± 0.005	0.162 ± 0.003**
Relative	6.12 ± 0.12	6.33 ± 0.13	6.50 ± 0.11*	6.79 ± 0.21**	7.02 ± 0.17**	7.09 ± 0.13**
m-Nitrotoluene						
Absolute	0.215 ± 0.005	0.232 ± 0.003	0.238 ± 0.003*	0.252 ± 0.012**	0.230 ± 0.010	0.197 ± 0.004
Relative	6.38 ± 0.21	6.71 ± 0.12	6.92 ± 0.19	7.61 ± 0.40**	7.79 ± 0.46**	8.09 ± 0.15**
p-Nitrotoluene						
Absolute	0.201 ± 0.005	0.220 ± 0.004	0.219 ± 0.004	0.199 ± 0.004	0.201 ± 0.003	0.190 ± 0.006
Relative	6.93 ± 0.17	6.75 ± 0.13	6.74 ± 0.13	7.15 ± 0.17	7.43 ± 0.13*	7.66 ± 0.28*
Liver						
o-Nitrotoluene						
Absolute	1.37 ± 0.04	1.46 ± 0.04	1.51 ± 0.04	1.49 ± 0.03	1.52 ± 0.04	1.28 ± 0.02
Relative	41.7 ± 0.6	43.0 ± 0.8	45.1 ± 0.7**	46.5 ± 1.1**	51.4 ± 0.9**	55.8 ± 1.2**
m-Nitrotoluene						
Absolute	1.43 ± 0.04	1.63 ± 0.03	1.82 ± 0.06**	1.83 ± 0.03**	1.61 ± 0.04	1.36 ± 0.03
Relative	42.2 ± 0.7	46.9 ± 0.7**	52.6 ± 0.9**	55.0 ± 0.7**	54.1 ± 0.9**	55.9 ± 1.0**
p-Nitrotoluene						
Absolute	1.31 ± 0.03	1.57 ± 0.05**	1.61 ± 0.03**	1.39 ± 0.04	1.43 ± 0.03	1.41 ± 0.04
Relative	45.1 ± 0.8	48.0 ± 0.8*	49.4 ± 1.0**	49.7 ± 1.0**	52.7 ± 0.9**	56.6 ± 0.8**
Lungs						
o-Nitrotoluene						
Absolute	0.194 ± 0.008	0.221 ± 0.016[3]	0.192 ± 0.009	0.189 ± 0.005	0.237 ± 0.017	0.193 ± 0.016
Relative	5.95 ± 0.33	6.59 ± 0.53[3]	5.76 ± 0.25	5.92 ± 0.20	7.96 ± 0.47**	8.47 ± 0.72**
m-Nitrotoluene						
Absolute	0.232 ± 0.014	0.254 ± 0.018	0.234 ± 0.011	0.236 ± 0.016	0.268 ± 0.020	0.229 ± 0.015
Relative	6.95 ± 0.55	7.33 ± 0.51	6.76 ± 0.23	7.12 ± 0.51	9.06 ± 0.76*	9.45 ± 0.68**
p-Nitrotoluene						
Absolute	0.242 ± 0.011	0.226 ± 0.010	0.216 ± 0.011	0.215 ± 0.013	0.228 ± 0.012	0.205 ± 0.014*
Relative	8.34 ± 0.38	6.92 ± 0.28	6.67 ± 0.40*	7.71 ± 0.44	8.41 ± 0.39	8.26 ± 0.57

TABLE A4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Mice In the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Thymus						
o-Nitrotoluene						
Absolute	0.059 ± 0.002	0.056 ± 0.003	0.059 ± 0.002	0.056 ± 0.003	0.053 ± 0.002	0.045 ± 0.002**
Relative	1.81 ± 0.06	1.67 ± 0.10	1.76 ± 0.08	1.76 ± 0.09	1.80 ± 0.09	1.97 ± 0.11
m-Nitrotoluene						
Absolute	0.062 ± 0.004	0.064 ± 0.004	0.063 ± 0.004	0.066 ± 0.003	0.059 ± 0.003	0.050 ± 0.002*
Relative	1.84 ± 0.10	1.83 ± 0.12	1.83 ± 0.09	1.98 ± 0.11	1.98 ± 0.13	2.05 ± 0.07
p-Nitrotoluene						
Absolute	0.056 ± 0.002	0.068 ± 0.003	0.064 ± 0.003	0.055 ± 0.003	0.054 ± 0.005	0.051 ± 0.002
Relative	1.94 ± 0.08	2.09 ± 0.11	1.96 ± 0.08	1.97 ± 0.11	1.97 ± 0.17	2.03 ± 0.07

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).
[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*- or *p*-nitrotnoluene.
[3] n=9.
* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test.
** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test.

APPENDIX B

Hematology and Clinical Chemistry Data

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology						
Hematocrit (%)						
o-Nitrotoluene						
Week 1	44.3 ± 0.6	43.0 ± 0.5	43.5 ± 0.5	42.4 ± 0.6	43.3 ± 0.4	46.5 ± 0.5
Week 3	46.5 ± 0.4	45.7 ± 0.5	46.4 ± 0.4	46.2 ± 0.3	46.9 ± 0.3[3]	44.0 ± 0.5**[3]
Week 13	45.8 ± 0.4	45.8 ± 0.4	45.4 ± 0.3	44.6 ± 0.6	43.3 ± 0.3**	42.2 ± 0.5**
m-Nitrotoluene						
Week 1	42.2 ± 0.5	43.2 ± 0.4	43.2 ± 0.4[3]	43.8 ± 0.4*	43.0 ± 0.4	45.7 ± 0.4**
Week 3	47.9 ± 0.7	45.6 ± 0.4*[3]	45.6 ± 0.4*	45.3 ± 0.3**	47.0 ± 0.3[3]	44.9 ± 0.4**
Week 13	45.3 ± 0.3	46.6 ± 0.5	45.9 ± 0.3	46.7 ± 0.3	44.1 ± 0.5	45.7 ± 0.4
p-Nitrotoluene						
Week 1	44.8 ± 0.9	45.2 ± 0.5	45.3 ± 0.6	45.7 ± 0.6	46.4 ± 0.9	48.1 ± 0.4**
Week 3	47.2 ± 0.4	48.4 ± 0.7[3]	48.2 ± 0.5[3]	47.4 ± 0.3	47.7 ± 0.3	47.5 ± 0.4[4]
Week 13	45.8 ± 0.4	44.6 ± 0.6	45.1 ± 0.3[3]	44.0 ± 0.6	44.1 ± 0.3*	45.4 ± 0.6
Hemoglobin (g/dL)						
o-Nitrotoluene						
Week 1	14.8 ± 0.2	14.3 ± 0.2	14.6 ± 0.2	14.1 ± 0.2	14.4 ± 0.2	15.7 ± 0.2*
Week 3	16.0 ± 0.2	15.7 ± 0.3	15.9 ± 0.2	15.8 ± 0.1	16.0 ± 0.1[3]	14.7 ± 0.2**[3]
Week 13	15.9 ± 0.2	15.9 ± 0.1	15.9 ± 0.1	15.4 ± 0.2	14.7 ± 0.1**	14.3 ± 0.2**
m-Nitrotoluene						
Week 1	14.3 ± 0.2	14.7 ± 0.2	14.7 ± 0.2[3]	14.9 ± 0.1*	14.5 ± 0.1	15.4 ± 0.2**
Week 3	16.3 ± 0.3	15.4 ± 0.1*[3]	15.3 ± 0.1*	15.3 ± 0.1**	15.8 ± 0.1[3]	14.8 ± 0.2**
Week 13	15.6 ± 0.1	16.1 ± 0.2	15.8 ± 0.1	16.0 ± 0.1	14.9 ± 0.2	15.2 ± 0.1
p-Nitrotoluene						
Week 1	15.7 ± 0.3	15.8 ± 0.2	15.7 ± 0.2	16.0 ± 0.3	16.3 ± 0.4	17.0 ± 0.2**
Week 3	16.1 ± 0.2	16.7 ± 0.3[3]	16.7 ± 0.2[3]	16.5 ± 0.1	16.5 ± 0.1	16.4 ± 0.2[3]
Week 13	15.9 ± 0.1	15.4 ± 0.2	15.7 ± 0.1	15.4 ± 0.2	15.2 ± 0.1**	15.0 ± 0.2**
Erythrocytes (10^6/μL)						
o-Nitrotoluene						
Week 1	7.26 ± 0.11	7.04 ± 0.11	7.16 ± 0.11	6.98 ± 0.10	7.18 ± 0.08	7.78 ± 0.09*
Week 3	7.85 ± 0.10	7.70 ± 0.12	7.84 ± 0.09	7.78 ± 0.09	7.95 ± 0.05[3]	7.32 ± 0.10*[3]
Week 13	9.06 ± 0.06	9.11 ± 0.08	9.03 ± 0.06	8.85 ± 0.11	8.39 ± 0.06**	7.89 ± 0.07**
m-Nitrotoluene						
Week 1	7.08 ± 0.12	7.23 ± 0.07	7.22 ± 0.08[3]	7.36 ± 0.07*	7.26 ± 0.09	7.76 ± 0.08**
Week 3	8.10 ± 0.13	7.64 ± 0.08*[3]	7.61 ± 0.07*	7.56 ± 0.07**	7.95 ± 0.08*[3]	7.37 ± 0.09**
Week 13	8.84 ± 0.07	9.09 ± 0.08	8.95 ± 0.06	9.07 ± 0.05	8.48 ± 0.09	8.32 ± 0.08**
p-Nitrotoluene						
Week 1	7.73 ± 0.14	7.77 ± 0.10	7.86 ± 0.10	7.87 ± 0.12	8.04 ± 0.16	8.33 ± 0.06**
Week 3	8.31 ± 0.11	8.39 ± 0.12[3]	8.36 ± 0.10[3]	8.17 ± 0.03	8.21 ± 0.10	7.88 ± 0.10*[3]
Week 13	8.97 ± 0.08	8.74 ± 0.11	8.86 ± 0.06	8.66 ± 0.12	8.56 ± 0.05**	8.33 ± 0.10**

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Mean cell volume (fL)						
o-Nitrotoluene						
Week 1	61.1 ± 0.3	61.1 ± 0.3	60.8 ± 0.3	60.7 ± 0.2	60.4 ± 0.2	59.8 ± 0.3**
Week 3	59.3 ± 0.3	59.4 ± 0.3	59.3 ± 0.3	59.4 ± 0.3	59.0 ± 0.4[3]	60.1 ± 0.3[3]
Week 13	50.5 ± 0.1	50.3 ± 0.2	50.2 ± 0.1	50.4 ± 0.2	51.6 ± 0.1**	53.4 ± 0.4**
m-Nitrotoluene						
Week 1	59.7 ± 0.3	59.7 ± 0.3	59.8 ± 0.3[3]	59.6 ± 0.1	59.2 ± 0.3	59.0 ± 0.2
Week 3	59.2 ± 0.4	59.7 ± 0.3[3]	59.9 ± 0.3	59.9 ± 0.3	59.2 ± 0.3[3]	60.9 ± 0.5
Week 13	51.2 ± 0.2	51.3 ± 0.2	51.3 ± 0.1	51.4 ± 0.2	52.1 ± 0.1**	55.0 ± 0.3**
p-Nitrotoluene						
Week 1	57.9 ± 0.4	58.2 ± 0.3	57.6 ± 0.3	58.2 ± 0.2	57.8 ± 0.3	57.7 ± 0.2
Week 3	56.8 ± 0.4	57.7 ± 0.2*[3]	57.6 ± 0.2[3]	58.0 ± 0.3**	58.1 ± 0.4**	60.3 ± 0.5**[4]
Week 13	51.1 ± 0.1	51.0 ± 0.2	50.9 ± 0.1	50.9 ± 0.1	51.5 ± 0.2	54.5 ± 0.2**
Mean cell hemoglobin (pg)						
o-Nitrotoluene						
Week 1	20.4 ± 0.1	20.4 ± 0.1	20.5 ± 0.1	20.2 ± 0.1	20.1 ± 0.1*	20.2 ± 0.1
Week 3	20.4 ± 0.1	20.4 ± 0.1	20.3 ± 0.1	20.3 ± 0.1	20.1 ± 0.1*[3]	20.1 ± 0.1[3]
Week 13	17.6 ± 0.1	17.5 ± 0.1	17.6 ± 0.1	17.5 ± 0.1	17.6 ± 0.1	18.1 ± 0.1**
m-Nitrotoluene						
Week 1	20.2 ± 0.1	20.3 ± 0.1	20.3 ± 0.1[3]	20.2 ± 0.1	19.9 ± 0.1	19.8 ± 0.1*
Week 3	20.1 ± 0.1	20.1 ± 0.1[3]	20.2 ± 0.1	20.2 ± 0.1	19.9 ± 0.1[3]	20.0 ± 0.1
Week 13	17.6 ± 0.1	17.7 ± 0.1	17.6 ± 0.1	17.7 ± 0.1	17.6 ± 0.1	18.3 ± 0.1**
p-Nitrotoluene						
Week 1	20.3 ± 0.1	20.4 ± 0.1	20.0 ± 0.1	20.4 ± 0.1	20.2 ± 0.2	20.4 ± 0.1
Week 3	19.4 ± 0.2	19.9 ± 0.1**[3]	20.0 ± 0.1**[3]	20.3 ± 0.2**	20.1 ± 0.1**	20.8 ± 0.2**[4]
Week 13	17.8 ± 0.1	17.6 ± 0.1	17.7 ± 0.1[3]	17.8 ± 0.2	17.7 ± 0.1	18.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
o-Nitrotoluene						
Week 1	33.4 ± 0.1	33.3 ± 0.2	33.6 ± 0.1	33.4 ± 0.1	33.3 ± 0.1	33.7 ± 0.1
Week 3	34.3 ± 0.1	34.3 ± 0.2	34.3 ± 0.1	34.1 ± 0.1	34.1 ± 0.1[3]	33.5 ± 0.2**[3]
Week 13	34.7 ± 0.1	34.8 ± 0.1	34.9 ± 0.1	34.6 ± 0.1	34.0 ± 0.1**	33.9 ± 0.1**
m-Nitrotoluene						
Week 1	33.8 ± 0.2	34.0 ± 0.2	34.0 ± 0.1[3]	33.9 ± 0.2	33.7 ± 0.1	33.6 ± 0.2
Week 3	34.0 ± 0.2	33.8 ± 0.1[3]	33.6 ± 0.1	33.7 ± 0.1[3]	33.7 ± 0.1	32.9 ± 0.1**
Week 13	34.4 ± 0.2	34.6 ± 0.1	34.3 ± 0.2	34.3 ± 0.2	33.8 ± 0.1**	33.3 ± 0.2**
p-Nitrotoluene						
Week 1	35.1 ± 0.1	35.0 ± 0.1	34.7 ± 0.2	35.0 ± 0.2	35.0 ± 0.2	35.3 ± 0.1
Week 3	34.2 ± 0.2	34.6 ± 0.1[3]	34.6 ± 0.1[3]	34.9 ± 0.2**	34.7 ± 0.1*	34.6 ± 0.2*[4]
Week 13	34.8 ± 0.1	34.6 ± 0.1	34.7 ± 0.2[3]	35.1 ± 0.5	34.5 ± 0.1	33.1 ± 0.2**

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Platelets (10^3/μL)						
o-Nitrotoluene						
Week 1	1,153.3 ± 14.0	1,095.9 ± 32.0	1,198.6 ± 26.1	1,202.9 ± 19.0	1,260.9 ± 19.3**	1,318.6 ± 76.8**
Week 3	934.4 ± 49.9	1,037.9 ± 15.5	1,062.3 ± 18.7*	1,172.3 ± 33.2**	994.6 ± 59.2*[3]	1,460.8 ± 42.4**[3]
Week 13	717.5 ± 4.1	813.3 ± 19.4**	761.9 ± 34.9**	939.3 ± 64.8**	913.4 ± 27.4**	949.6 ± 35.0**
m-Nitrotoluene						
Week 1	1,031.6 ± 46.1	1,047.8 ± 22.5	1,044.3 ± 17.4[3]	1,045.2 ± 19.8	941.8 ± 41.9*	931.5 ± 22.1**
Week 3	942.2 ± 21.1	976.2 ± 26.5[3]	1,008.2 ± 18.3*	1,009.8 ± 19.0*	952.3 ± 19.7[3]	1,051.3 ± 19.5**
Week 13	739.9 ± 32.3	740.9 ± 12.7	751.2 ± 10.3	751.3 ± 22.4	789.9 ± 13.1	882.5 ± 24.2**
p-Nitrotoluene						
Week 1	1,073.4 ± 30.3	1,065.0 ± 28.9	1,036.9 ± 23.4	1,043.3 ± 17.9[3]	1,013.2 ± 18.6[3]	1,021.0 ± 27.0
Week 3	971.7 ± 17.3	938.3 ± 7.3[3]	935.4 ± 13.6[3]	1,002.3 ± 16.3	966.4 ± 19.8	1,051.8 ± 37.4[4]
Week 13	733.3 ± 16.4	722.7 ± 16.7	662.3 ± 36.2[3]	683.8 ± 47.2	766.0 ± 11.6	719.0 ± 40.6
Reticulocytes (10^6/μL)						
o-Nitrotoluene						
Week 1	0.84 ± 0.05	0.83 ± 0.06	0.76 ± 0.06	0.77 ± 0.03	0.66 ± 0.05**	0.62 ± 0.05**
Week 3	0.38 ± 0.03	0.43 ± 0.04	0.34 ± 0.03	0.39 ± 0.04	0.19 ± 0.05[3]	0.53 ± 0.06[3]
Week 13	0.25 ± 0.03	0.25 ± 0.03	0.26 ± 0.01[3]	0.26 ± 0.02	0.40 ± 0.05*	0.46 ± 0.03**
m-Nitrotoluene						
Week 1	0.25 ± 0.03	0.29 ± 0.03	0.29 ± 0.02[3]	0.27 ± 0.02	0.25 ± 0.01	0.12 ± 0.01**
Week 3	0.15 ± 0.01	0.18 ± 0.02[3]	0.16 ± 0.02	0.18 ± 0.02	0.17 ± 0.02[3]	0.29 ± 0.03**
Week 13	0.32 ± 0.02	0.36 ± 0.04	0.32 ± 0.02	0.32 ± 0.03	0.35 ± 0.03	0.44 ± 0.03**
p-Nitrotoluene						
Week 1	0.21 ± 0.01	0.22 ± 0.02	0.23 ± 0.03	0.25 ± 0.02	0.22 ± 0.03	0.08 ± 0.01**
Week 3	0.41 ± 0.03	0.37 ± 0.03[3]	0.32 ± 0.04[3]	0.34 ± 0.04	0.41 ± 0.04	0.35 ± 0.05[4]
Week 13	0.13 ± 0.01	0.12 ± 0.02	0.09 ± 0.02[3]	0.09 ± 0.02	0.16 ± 0.02	0.18 ± 0.03
Leukocytes (10^3/μL)						
o-Nitrotoluene						
Week 1	7.55 ± 0.26	7.28 ± 0.46	8.15 ± 0.34	8.19 ± 0.41	9.08 ± 0.42*	9.95 ± 0.37**
Week 3	11.74 ± 0.57	10.53 ± 0.66	10.99 ± 0.47	11.03 ± 0.60	11.50 ± 1.12[3]	18.04 ± 1.12**[3]
Week 13	10.70 ± 0.27	11.08 ± 0.33	12.38 ± 0.54*	14.49 ± 1.08**	15.50 ± 0.63**	17.98 ± 0.83**
m-Nitrotoluene						
Week 1	9.43 ± 0.62	9.99 ± 0.65	10.30 ± 0.36[3]	10.53 ± 0.30	9.37 ± 0.43	10.01 ± 0.71
Week 3	11.12 ± 0.56	9.89 ± 0.61[3]	10.45 ± 0.43	10.52 ± 0.55	11.27 ± 0.59[3]	12.38 ± 0.23
Week 13	10.87 ± 0.55	11.96 ± 0.56	11.24 ± 0.31	11.18 ± 0.46	11.74 ± 0.46	10.09 ± 0.59
p-Nitrotoluene						
Week 1	11.14 ± 0.46	9.70 ± 0.61	9.90 ± 0.62	10.01 ± 0.50	10.12 ± 0.67	11.03 ± 0.51
Week 3	11.47 ± 0.46	11.46 ± 0.50[3]	11.31 ± 0.59[3]	11.73 ± 0.36	11.22 ± 0.41	11.05 ± 0.50[4]
Week 13	11.00 ± 0.40	10.26 ± 0.25	9.51 ± 0.61[3]	8.72 ± 0.56**	9.80 ± 0.38	10.33 ± 0.29

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Segmented neutrophils ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	0.93 ± 0.13	0.84 ± 0.13	0.71 ± 0.10	0.84 ± 0.12	1.02 ± 0.09	0.95 ± 0.12
Week 3	0.94 ± 0.08	0.76 ± 0.08	0.80 ± 0.07	0.70 ± 0.09	0.89 ± 0.18[3]	1.52 ± 0.23[3]
Week 13	1.44 ± 0.17	1.41 ± 0.12	1.78 ± 0.23	1.66 ± 0.18[3]	2.11 ± 0.26*	1.91 ± 0.25
m-Nitrotoluene						
Week 1	0.78 ± 0.10	1.00 ± 0.21	0.93 ± 0.14[3]	1.27 ± 0.16*	0.96 ± 0.15	1.06 ± 0.10
Week 3	1.00 ± 0.09	0.93 ± 0.15[3]	1.23 ± 0.17	0.82 ± 0.05	1.04 ± 0.12[3]	0.92 ± 0.12
Week 13	1.61 ± 0.19	1.62 ± 0.25	1.91 ± 0.38	1.51 ± 0.18	1.82 ± 0.22	1.34 ± 0.14
p-Nitrotoluene						
Week 1	1.17 ± 0.18	0.77 ± 0.07	1.17 ± 0.18	1.25 ± 0.16	1.22 ± 0.11	1.47 ± 0.13
Week 3	1.48 ± 0.19	1.57 ± 0.26[3]	1.47 ± 0.15[3]	1.70 ± 0.14	1.33 ± 0.12	1.41 ± 0.19[4]
Week 13	1.47 ± 0.152	1.11 ± 0.102	1.37 ± 0.30[3]	1.30 ± 0.12	1.33 ± 0.18	1.51 ± 0.15
Lymphocytes ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	6.57 ± 0.15	6.31 ± 0.39	7.35 ± 0.29	7.28 ± 0.34	7.97 ± 0.38**	8.93 ± 0.40**
Week 3	10.67 ± 0.56	9.65 ± 0.60	10.08 ± 0.46	10.16 ± 0.68	10.50 ± 1.07[3]	16.33 ± 1.06**[3]
Week 13	9.14 ± 0.20	9.56 ± 0.43	10.48 ± 0.64	11.54 ± 0.62**	13.23 ± 0.75**	15.90 ± 0.77**
m-Nitrotoluene						
Week 1	8.40 ± 0.56	8.92 ± 0.61	9.20 ± 0.45[3]	9.06 ± 0.24	8.18 ± 0.42	8.71 ± 0.64
Week 3	9.90 ± 0.60	8.77 ± 0.49[3]	9.04 ± 0.51	9.52 ± 0.52	9.96 ± 0.61[3]	11.27 ± 0.25*
Week 13	9.10 ± 0.43	10.16 ± 0.59	9.06 ± 0.40	9.47 ± 0.45	9.68 ± 0.52	8.55 ± 0.52
p-Nitrotoluene						
Week 1	9.81 ± 0.50	8.90 ± 0.60	8.69 ± 0.58	8.68 ± 0.48	8.78 ± 0.59	9.52 ± 0.52
Week 3	9.87 ± 0.45	9.77 ± 0.27[3]	9.70 ± 0.51[3]	9.77 ± 0.33	9.81 ± 0.42	9.54 ± 0.55[4]
Week 13	9.42 ± 0.36	9.05 ± 0.23	8.11 ± 0.70[3]	7.36 ± 0.47*	8.43 ± 0.30	8.75 ± 0.25
Monocytes ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	0.07 ± 0.03	0.13 ± 0.04	0.06 ± 0.02	0.06 ± 0.02	0.08 ± 0.04	0.05 ± 0.02
Week 3	0.09 ± 0.03	0.09 ± 0.03	0.10 ± 0.05	0.06 ± 0.02	0.11 ± 0.04[3]	0.11 ± 0.05[3]
Week 13	0.04 ± 0.02	0.04 ± 0.03	0.08 ± 0.05	0.09 ± 0.04	0.12 ± 0.04	0.11 ± 0.05
m-Nitrotoluene						
Week 1	0.22 ± 0.04	0.06 ± 0.02*	0.06 ± 0.02*[3]	0.14 ± 0.04	0.18 ± 0.04	0.18 ± 0.05
Week 3	0.17 ± 0.05	0.13 ± 0.05[3]	0.13 ± 0.05	0.16 ± 0.04	0.19 ± 0.06[3]	0.18 ± 0.04
Week 13	0.08 ± 0.04	0.10 ± 0.05	0.15 ± 0.06	0.12 ± 0.06	0.16 ± 0.07	0.07 ± 0.03
p-Nitrotoluene						
Week 1	0.11 ± 0.04	0.02 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.06 ± 0.03	0.02 ± 0.01
Week 3	0.11 ± 0.03	0.08 ± 0.03[3]	0.12 ± 0.05[3]	0.22 ± 0.05	0.08 ± 0.02	0.09 ± 0.04[4]
Week 13	0.05 ± 0.02	0.04 ± 0.02	0.02 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.06 ± 0.03

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Eosinophils (10^3/μL)						
o-Nitrotoluene						
Week 1	0.02 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	0.02 ± 0.01
Week 3	0.05 ± 0.03	0.02 ± 0.01	0.02 ± 0.01	0.09 ± 0.03	0.00 ± 0.00[3]	0.09 ± 0.05[3]
Week 13	0.08 ± 0.03	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.03	0.06 ± 0.03	0.06 ± 0.03
m-Nitrotoluene						
Week 1	0.04 ± 0.02	0.02 ± 0.01	0.08 ± 0.03	0.04 ± 0.02	0.04 ± 0.02	0.06 ± 0.02
Week 3	0.05 ± 0.03	0.02 ± 0.02	0.06 ± 0.02	0.01 ± 0.01	0.06 ± 0.03	0.01 ± 0.01
Week 13	0.08 ± 0.03	0.08 ± 0.03	0.10 ± 0.04	0.07 ± 0.03	0.07 ± 0.03	0.14 ± 0.04
p-Nitrotoluene						
Week 1	0.04 ± 0.02	0.02 ± 0.01	0.00 ± 0.00	0.05 ± 0.02	0.05 ± 0.02	0.02 ± 0.01
Week 3	0.01 ± 0.01	0.02 ± 0.02[3]	0.01 ± 0.01[3]	0.02 ± 0.01	0.00 ± 0.00	0.01 ± 0.01[4]
Week 13	0.05 ± 0.02	0.05 ± 0.02	0.01 ± 0.01[3]	0.06 ± 0.03	0.03 ± 0.02	0.01 ± 0.01
Nucleated erythrocytes/100 leukocytes						
o-Nitrotoluene						
Week 1	0.80 ± 0.33	0.70 ± 0.30	0.60 ± 0.34	1.00 ± 0.39	0.80 ± 0.39	0.80 ± 0.29
Week 3	0.20 ± 0.13	0.10 ± 0.10	0.20 ± 0.13	0.60 ± 0.22	0.20 ± 0.13	1.44 ± 0.63*c
Week 13	0.20 ± 0.13	0.10 ± 0.10	0.30 ± 0.15	0.20 ± 0.20	0.50 ± 0.22	0.40 ± 0.22
m-Nitrotoluene						
Week 1	0.60 ± 0.22	1.10 ± 0.35	0.67 ± 0.37[3]	0.20 ± 0.13	1.30 ± 0.40	0.50 ± 0.22
Week 3	0.30 ± 0.21	0.00 ± 0.00[3]	0.60 ± 0.31	0.40 ± 0.22	0.44 ± 0.34[3]	2.80 ± 0.65**
Week 13	0.50 ± 0.22	0.50 ± 0.22	0.30 ± 0.21	0.20 ± 0.13	0.50 ± 0.22	0.80 ± 0.29
p-Nitrotoluene						
Week 1	0.50 ± 0.22	0.70 ± 0.26	0.90 ± 0.41	0.70 ± 0.34	0.70 ± 0.42	0.70 ± 0.30
Week 3	0.30 ± 0.21	0.56 ± 0.34[3]	0.11 ± 0.11[3]	0.00 ± 0.00	0.60 ± 0.31	2.00 ± 0.46**[4]
Week 13	0.40 ± 0.16	0.20 ± 0.20	0.78 ± 0.43[3]	0.70 ± 0.34	0.50 ± 0.22	2.40 ± 0.67**
Chemistry						
Urea nitrogen (mg/dL)						
o-Nitrotoluene						
Week 1	20.0 ± 0.5	18.8 ± 0.5	19.1 ± 0.4	19.2 ± 0.6	20.7 ± 0.5	20.5 ± 0.4
Week 3	21.0 ± 0.4	18.3 ± 0.7**	20.6 ± 0.7	17.4 ± 0.5**	16.9 ± 0.4**	20.4 ± 0.4**[3]
Week 13	19.9 ± 0.5	20.5 ± 0.6	21.6 ± 0.6	20.5 ± 0.8	19.1 ± 0.7	19.5 ± 0.2
m-Nitrotoluene						
Week 1	17.6 ± 0.9	21.4 ± 1.5*	17.8 ± 0.7	19.3 ± 0.6	19.9 ± 0.6	21.0 ± 0.4**
Week 3	23.5 ± 0.8	23.3 ± 1.5	21.4 ± 0.3*	20.1 ± 0.5**	21.5 ± 0.5*	20.4 ± 0.2**
Week 13	17.3 ± 0.4	18.4 ± 0.6	15.2 ± 0.8	16.4 ± 0.5	13.5 ± 0.5**	17.3 ± 0.5
p-Nitrotoluene						
Week 1	20.3 ± 2.0	17.9 ± 1.0	19.7 ± 0.8	16.8 ± 0.9	16.4 ± 0.6*	17.7 ± 1.1
Week 3	18.5 ± 0.8	18.9 ± 0.9[3]	18.6 ± 0.7[3]	19.2 ± 0.6	13.8 ± 0.6**	14.9 ± 0.9**[4]
Week 13	20.3 ± 0.9	22.5 ± 1.0	22.5 ± 0.9	22.5 ± 0.7	20.8 ± 0.9	23.7 ± 0.7*

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Creatinine (mg/dL)						
o-Nitrotoluene						
Week 1	0.55 ± 0.02	0.55 ± 0.02	0.54 ± 0.02	0.53 ± 0.02	0.56 ± 0.02	0.53 ± 0.02
Week 3	0.60 ± 0.00	0.59 ± 0.01	0.60 ± 0.00	0.58 ± 0.01	0.53 ± 0.02**	0.56 ± 0.02**[3]
Week 13	0.70 ± 0.00	0.70 ± 0.00	0.70 ± 0.00	0.70 ± 0.02	0.70 ± 0.00	0.68 ± 0.01
m-Nitrotoluene						
Week 1	0.51 ± 0.01	0.52 ± 0.01	0.52 ± 0.01	0.54 ± 0.02	0.57 ± 0.02**	0.63 ± 0.02**
Week 3	0.57 ± 0.02	0.57 ± 0.02	0.60 ± 0.00	0.57 ± 0.02	0.60 ± 0.00	0.65 ± 0.02**
Week 13	0.65 ± 0.02	0.68 ± 0.01	0.66 ± 0.02	0.69 ± 0.01	0.71 ± 0.02*	0.77 ± 0.02**
p-Nitrotoluene						
Week 1	0.68 ± 0.01	0.59 ± 0.03*	0.61 ± 0.02	0.58 ± 0.02*	0.61 ± 0.03	0.64 ± 0.03
Week 3	0.57 ± 0.03	0.62 ± 0.03[3]	0.64 ± 0.04[3]	0.64 ± 0.02	0.65 ± 0.02	0.59 ± 0.02[4]
Week 13	0.72 ± 0.02	0.69 ± 0.02	0.74 ± 0.02	0.81 ± 0.02**	0.83 ± 0.03**	0.94 ± 0.03**
Total protein (g/dL)						
o-Nitrotoluene						
Week 1	6.5 ± 0.1	6.1 ± 0.1**	6.1 ± 0.1*[3]	5.7 ± 0.1**	5.7 ± 0.1**	5.3 ± 0.1**[4]
Week 3	6.4 ± 0.1	6.1 ± 0.1*	5.8 ± 0.1**	5.5 ± 0.1**	5.2 ± 0.1**	5.0 ± 0.1**[3]
Week 13	6.9 ± 0.0	7.1 ± 0.1	7.1 ± 0.1	6.9 ± 0.3	7.1 ± 0.1	7.3 ± 0.0**
m-Nitrotoluene						
Week 1	5.7 ± 0.1	5.8 ± 0.0	5.8 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1*
Week 3	6.2 ± 0.1	6.0 ± 0.1	6.1 ± 0.0	5.9 ± 0.1	6.2 ± 0.0	6.0 ± 0.1
Week 13	6.8 ± 0.1	6.9 ± 0.1	6.7 ± 0.1	6.9 ± 0.0	6.6 ± 0.1	6.8 ± 0.1
p-Nitrotoluene						
Week 1	6.4 ± 0.1	6.2 ± 0.1*	6.2 ± 0.1*	6.0 ± 0.1**	6.0 ± 0.1**	6.0 ± 0.1**[3]
Week 3	6.0 ± 0.1	6.3 ± 0.2[3]	6.3 ± 0.2[3]	5.9 ± 0.1	6.0 ± 0.2	6.0 ± 0.1[4]
Week 13	7.0 ± 0.1	6.8 ± 0.1	6.6 ± 0.1*	6.6 ± 0.1**	6.6 ± 0.1**	6.4 ± 0.1**
Albumin (g/dL)						
o-Nitrotoluene						
Week 1	5.0 ± 0.1	4.7 ± 0.1**	4.7 ± 0.1*[3]	4.4 ± 0.1**	4.4 ± 0.0**	4.1 ± 0.0**[4]
Week 3	5.1 ± 0.0	4.9 ± 0.1	4.7 ± 0.0**	4.5 ± 0.1**	4.3 ± 0.1**	4.1 ± 0.1**[3]
Week 13	5.0 ± 0.0	5.2 ± 0.1*	5.3 ± 0.1**	5.1 ± 0.2**	5.4 ± 0.1**	5.5 ± 0.1**
m-Nitrotoluene						
Week 1	4.6 ± 0.0	4.7 ± 0.0	4.7 ± 0.0	4.7 ± 0.0	4.8 ± 0.0**	5.0 ± 0.0**
Week 3	5.0 ± 0.1	4.8 ± 0.0	4.9 ± 0.0	4.8 ± 0.1	5.1 ± 0.0	5.1 ± 0.1
Week 13	5.1 ± 0.1	5.2 ± 0.1	5.0 ± 0.0	5.3 ± 0.1	5.1 ± 0.1	5.3 ± 0.1*
p-Nitrotoluene						
Week 1	4.0 ± 0.0	3.9 ± 0.0	3.9 ± 0.0	3.9 ± 0.0	4.0 ± 0.1	4.0 ± 0.1
Week 3	3.9 ± 0.1	4.0 ± 0.1[3]	4.0 ± 0.1[3]	4.0 ± 0.1	4.1 ± 0.1*	4.2 ± 0.1**[4]
Week 13	4.3 ± 0.1	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.1	4.4 ± 0.0	4.4 ± 0.1

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Methemoglobin (%)						
o-Nitrotoluene						
Week 1	1.39 ± 0.23	1.22 ± 0.19	1.21 ± 0.16	1.64 ± 0.25	2.17 ± 0.12**	3.09 ± 0.19**
Week 3	1.41 ± 0.14[4]	1.98 ± 0.24	2.41 ± 0.31*	2.99 ± 0.23**[3]	4.28 ± 0.32**	8.41 ± 0.34**[5]
Week 13	2.46 ± 0.35	3.21 ± 0.68	3.73 ± 0.46[4]	4.87 ± 0.42**	6.87 ± 0.39**[3]	11.11 ± 0.60**[4]
m-Nitrotoluene						
Week 1	–[6]	–	–	–	–	–
Week 3	2.00 ± 0.23	2.04 ± 0.19[4]	1.98 ± 0.24	2.22 ± 0.21	2.31 ± 0.16	3.28 ± 0.22**[4]
Week 13	3.19 ± 0.14	3.64 ± 0.31	3.14 ± 0.18	3.71 ± 0.18*	3.89 ± 0.24*	4.56 ± 0.36 **
p-Nitrotoluene						
Week 1	3.68 ± 0.61	3.07 ± 0.53	3.49 ± 0.38	3.37 ± 0.63	2.56 ± 0.26	3.57 ± 0.55
Week 3	5.65 ± 0.44	6.26 ± 0.26[3]	5.88 ± 0.31[3]	5.88 ± 0.42	6.59 ± 0.41	7.10 ± 0.52**[4]
Week 13	6.54 ± 0.18	5.43 ± 0.26	6.13 ± 0.20[4]	5.74 ± 0.26	7.05 ± 0.21	8.08 ± 0.33*
Alkaline phosphatase (IU/L)						
o-Nitrotoluene						
Week 1	749 ± 12	743 ± 22	738 ± 13	740 ± 21	770 ± 20	682 ± 17[3]
Week 3	557 ± 5	522 ± 10**	536 ± 12*	480 ± 10**	405 ± 10**	381 ± 7**[3]
Week 13	238 ± 4	240 ± 6	241 ± 9[3]	214 ± 5*	216 ± 6	235 ± 4
m-Nitrotoluene						
Week 1	612 ± 15	620 ± 14	600 ± 13	601 ± 10	551 ± 14**	527 ± 12**
Week 3	494 ± 13	513 ± 11	488 ± 6	488 ± 9	459 ± 14	430 ± 10**
Week 13	249 ± 3	259 ± 6	224 ± 3**	255 ± 6	207 ± 7**	207 ± 7**
p-Nitrotoluene						
Week 1	367 ± 11	371 ± 10	348 ± 9	371 ± 8	345 ± 10	336 ± 13
Week 3	299 ± 7	321 ± 9[3]	280 ± 7[3]	288 ± 6	265 ± 4**	246 ± 7**[4]
Week 13	206 ± 5	223 ± 4	203 ± 8	202 ± 6	207 ± 7	216 ± 12
Alanine aminotransferase (IU/L)						
o-Nitrotoluene						
Week 1	40 ± 1	44 ± 1	42 ± 1	47 ± 4	48 ± 2**	41 ± 2[3]
Week 3	48 ± 1	45 ± 1	48 ± 1	47 ± 2	49 ± 1	42 ± 1**[3]
Week 13	55 ± 2	50 ± 3	55 ± 1	56 ± 4	63 ± 4	68 ± 3**
m-Nitrotoluene						
Week 1	42 ± 1	40 ± 1	42 ± 1	42 ± 1	41 ± 2	49 ± 3
Week 3	45 ± 1	45 ± 1	49 ± 4	41 ± 1	46 ± 1	48 ± 2
Week 13	64 ± 6	84 ± 6	53 ± 5	67 ± 3	48 ± 1**	51 ± 2*
p-Nitrotoluene						
Week 1	34 ± 1	33 ± 1	32 ± 1	31 ± 1	34 ± 2	35 ± 1
Week 3	33 ± 1	31 ± 1[3]	31 ± 1[3]	32 ± 1	34 ± 1	43 ± 5**[4]
Week 13	46 ± 1	43 ± 2	49 ± 3	47 ± 3[3]	36 ± 1**	35 ± 1**

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Creatine kinase (IU/L)						
o-Nitrotoluene						
Week 1	337.7 ± 32.5	320.2 ± 43.2	256.0 ± 24.2[3]	347.3 ± 53.2	284.6 ± 22.1[3]	245.1 ± 12.5[4]
Week 3	278.7 ± 14.5	243.4 ± 22.9	212.5 ± 17.1	285.6 ± 25.2	211.8 ± 18.5	301.8 ± 40.3[3]
Week 13	114.8 ± 14.9	94.1 ± 3.8	97.2 ± 5.5	113.9 ± 14.2	128.4 ± 23.9	141.0 ± 19.0
m-Nitrotoluene						
Week 1	336.4 ± 33.1[3]	340.6 ± 51.9	437.8 ± 65.4	361.0 ± 58.4	319.8 ± 48.9	308.2 ± 33.5
Week 3	265.0 ± 42.8	205.6 ± 12.1	297.2 ± 53.9	214.8 ± 30.4	177.9 ± 16.2[3]	219.4 ± 21.8
Week 13	112.1 ± 8.8	124.4 ± 18.1	118.2 ± 12.5	98.9 ± 7.2	146.6 ± 29.1	144.9 ± 19.7
p-Nitrotoluene						
Week 1	183.5 ± 21.5	179.5 ± 29.6	146.6 ± 22.3	176.9 ± 22.7	166.5 ± 46.3	302.0 ± 128.4
Week 3	166.1 ± 17.5	159.2 ± 24.5[3]	162.2 ± 18.8[3]	143.3 ± 11.5	135.7 ± 14.9	238.6 ± 66.2[4]
Week 13	55.8 ± 3.6[3]	75.0 ± 19.2	92.6 ± 23.8	80.2 ± 10.9	71.2 ± 16.0	76.4 ± 13.0
Sorbitol dehydrogenase (IU/L)						
o-Nitrotoluene						
Week 1	8 ± 1	7 ± 1	9 ± 1	8 ± 1	9 ± 1	9 ± 1
Week 3	5 ± 1	6 ± 0	7 ± 1	6 ± 0	6 ± 0	6 ± 1[3]
Week 13	13 ± 1	14 ± 1	14 ± 1	17 ± 1**	16 ± 1**	18 ± 1**
m-Nitrotoluene						
Week 1	6 ± 1	5 ± 1	5 ± 1	5 ± 0	4 ± 0	6 ± 1
Week 3	9 ± 1	10 ± 1	10 ± 1	9 ± 1	8 ± 1	11 ± 1
Week 13	10 ± 0	12 ± 1	10 ± 1	12 ± 0	11 ± 1	10 ± 1
p-Nitrotoluene						
Week 1	7 ± 0	6 ± 0	6 ± 0*	6 ± 0*	6 ± 0**	5 ± 0**
Week 3	5 ± 0	5 ± 0[3]	6 ± 0[3]	5 ± 0	5 ± 0	5 ± 1[4]
Week 13	10 ± 1	10 ± 0	13 ± 1	13 ± 1[3]	7 ± 0**	7 ± 1**
Bile acids (μmol/L)						
o-Nitrotoluene						
Week 1	14.10 ± 1.61	10.10 ± 1.25	14.20 ± 3.72	34.30 ± 8.64	35.60 ± 7.07	22.50 ± 4.24
Week 3	14.60 ± 5.52	6.80 ± 1.85	8.80 ± 2.52	9.00 ± 2.47	15.30 ± 3.25	35.89 ± 5.18**[3]
Week 13	7.40 ± 0.86	10.20 ± 1.67	7.60 ± 1.49	11.10 ± 1.84	23.50 ± 4.20**	28.50 ± 3.60**
m-Nitrotoluene						
Week 1	11.67 ± 2.40[3]	14.20 ± 5.52	19.10 ± 4.75	16.90 ± 4.42	19.30 ± 7.03	22.44 ± 3.81[3]
Week 3	9.60 ± 1.85	6.10 ± 1.39	5.40 ± 0.73	6.30 ± 1.76	6.90 ± 2.10	37.00 ± 6.80
Week 13	4.00 ± 0.99	4.60 ± 1.33	5.40 ± 1.44	4.50 ± 1.39	15.00 ± 1.59**	23.90 ± 5.14**
p-Nitrotoluene						
Week 1	11.80 ± 1.23	11.20 ± 2.67	7.50 ± 1.01	10.90 ± 2.47	8.80 ± 0.83	16.10 ± 2.23
Week 3	9.40 ± 1.18	8.56 ± 1.83[3]	7.89 ± 1.47[3]	10.30 ± 1.49	6.70 ± 1.14	16.88 ± 4.17[4]
Week 13	6.10 ± 0.98	5.80 ± 0.90	5.50 ± 0.48	7.30 ± 1.71	9.00 ± 2.25[3]	16.20 ± 2.03**

[1] Mean ± standard error for groups of 10 animals unless otherwise specified.
[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*-nitrotoluene or *p*-nitrotoluene.
[3] n=9.
[4] n=8.
[5] n=7.
[6] Inadequate data for analysis.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology						
Hematocrit (%)						
o-Nitrotoluene						
Week 1	43.3 ± 0.2	43.1 ± 0.4	43.6 ± 0.5	43.3 ± 0.5[3]	43.0 ± 0.6[3]	44.1 ± 0.6
Week 3	46.5 ± 0.3	47.2 ± 0.5	47.6 ± 0.5	46.9 ± 0.4	44.4 ± 0.5[3*]	44.4 ± 0.7*
Week 13	44.3 ± 0.4	44.3 ± 0.6[3]	43.2 ± 0.4	43.1 ± 0.5[3]	42.5 ± 0.6*	41.3 ± 0.3**
m-Nitrotoluene						
Week 1	44.4 ± 0.8[4]	43.8 ± 0.5	43.8 ± 0.4	44.2 ± 0.5[4]	45.6 ± 0.7	46.5 ± 0.4*
Week 3	46.5 ± 0.8	46.5 ± 0.5[5]	46.7 ± 0.3[3]	47.2 ± 0.3[3]	47.3 ± 0.4[4]	43.6 ± 0.7*[5]
Week 13	45.6 ± 0.4	46.3 ± 0.3	45.3 ± 0.3	44.7 ± 0.4	44.6 ± 0.3	43.6 ± 0.5**[3]
p-Nitrotoluene						
Week 1	44.3 ± 0.3[4]	44.5 ± 0.5[3]	44.8 ± 0.6	45.0 ± 0.8	46.5 ± 0.5*	46.8 ± 0.6**[3]
Week 3	48.2 ± 0.5[3]	48.1 ± 0.6	48.4 ± 0.4	46.6 ± 0.4[3]	48.3 ± 0.6[3]	47.1 ± 0.7
Week 13	45.7 ± 0.3[3]	44.6 ± 0.2**	44.4 ± 0.5*	45.1 ± 0.3*	45.4 ± 0.5[3]	43.7 ± 0.4**[3]
Hemoglobin (g/dL)						
o-Nitrotoluene						
Week 1	15.0 ± 0.1	15.0 ± 0.1	15.1 ± 0.2	15.0 ± 0.2[3]	14.9 ± 0.2[3]	15.3 ± 0.2
Week 3	16.4 ± 0.1	16.6 ± 0.2	16.6 ± 0.2	16.4 ± 0.1	15.5 ± 0.2**[3]	15.4 ± 0.2**
Week 13	15.7 ± 0.2	15.8 ± 0.2[3]	15.2 ± 0.2	15.2 ± 0.2[3]	15.0 ± 0.2**	14.4 ± 0.1**
m-Nitrotoluene						
Week 1	15.4 ± 0.3[4]	15.2 ± 0.2	15.3 ± 0.2	15.4 ± 0.2[4]	15.6 ± 0.3	15.8 ± 0.2
Week 3	15.9 ± 0.3	16.0 ± 0.2[5]	16.1 ± 0.1[3]	16.1 ± 0.1[3]	16.0 ± 0.1[4]	14.4 ± 0.2**[5]
Week 13	15.6 ± 0.2	15.9 ± 0.1	15.6 ± 0.1	15.4 ± 0.1	15.1 ± 0.1*	14.4 ± 0.2**[3]
p-Nitrotoluene						
Week 1	16.0 ± 0.1[4]	15.9 ± 0.2[3]	16.1 ± 0.2	16.0 ± 0.3	16.7 ± 0.2*	16.7 ± 0.2*[3]
Week 3	17.4 ± 0.2[3]	17.2 ± 0.2	17.4 ± 0.2	16.7 ± 0.2*[3]	17.5 ± 0.3[3]	16.6 ± 0.3*
Week 13	15.9 ± 0.1[3]	15.4 ± 0.1**	15.4 ± 0.2*	15.6 ± 0.1*	15.4 ± 0.3[3]	14.7 ± 0.1**[3]
Erythrocytes (10^6/μL)						
o-Nitrotoluene						
Week 1	7.40 ± 0.04	7.35 ± 0.07	7.43 ± 0.09	7.38 ± 0.12[3]	7.34 ± 0.10[3]	7.57 ± 0.12
Week 3	8.03 ± 0.07	8.12 ± 0.11	8.20 ± 0.09	8.10 ± 0.09	7.63 ± 0.08*[3]	7.52 ± 0.12**
Week 13	8.38 ± 0.07	8.40 ± 0.09[3]	8.19 ± 0.08	8.08 ± 0.10*[3]	7.84 ± 0.10**	7.37 ± 0.05**
m-Nitrotoluene						
Week 1	7.68 ± 0.14[4]	7.50 ± 0.10	7.49 ± 0.06	7.61 ± 0.09[4]	7.79 ± 0.13	8.02 ± 0.08*
Week 3	7.79 ± 0.16	7.77 ± 0.11[5]	7.77 ± 0.06[3]	7.90 ± 0.08[3]	7.97 ± 0.06[4]	7.23 ± 0.10*[5]
Week 13	8.42 ± 0.08	8.54 ± 0.06	8.40 ± 0.05	8.27 ± 0.07	8.06 ± 0.06**	7.53 ± 0.06**[3]
p-Nitrotoluene						
Week 1	7.75 ± 0.04[4]	7.78 ± 0.08[3]	7.87 ± 0.09	7.91 ± 0.15	8.17 ± 0.09**	8.15 ± 0.10**[3]
Week 3	8.33 ± 0.08[3]	8.30 ± 0.11	8.38 ± 0.08	8.10 ± 0.09[3]	8.47 ± 0.14[3]	8.03 ± 0.13
Week 13	8.50 ± 0.04[3]	8.35 ± 0.04*	8.31 ± 0.09*	8.45 ± 0.05	8.40 ± 0.09[3]	8.00 ± 0.07**[3]

TABLE B1 Hematology and Clinical Chemistry Data for Male Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Creatine kinase (IU/L)						
o-Nitrotoluene						
Week 1	337.7 ± 32.5	320.2 ± 43.2	256.0 ± 24.2[3]	347.3 ± 53.2	284.6 ± 22.1[3]	245.1 ± 12.5[4]
Week 3	278.7 ± 14.5	243.4 ± 22.9	212.5 ± 17.1	285.6 ± 25.2	211.8 ± 18.5	301.8 ± 40.3[3]
Week 13	114.8 ± 14.9	94.1 ± 3.8	97.2 ± 5.5	113.9 ± 14.2	128.4 ± 23.9	141.0 ± 19.0
m-Nitrotoluene						
Week 1	336.4 ± 33.1[3]	340.6 ± 51.9	437.8 ± 65.4	361.0 ± 58.4	319.8 ± 48.9	308.2 ± 33.5
Week 3	265.0 ± 42.8	205.6 ± 12.1	297.2 ± 53.9	214.8 ± 30.4	177.9 ± 16.2[3]	219.4 ± 21.8
Week 13	112.1 ± 8.8	124.4 ± 18.1	118.2 ± 12.5	98.9 ± 7.2	146.6 ± 29.1	144.9 ± 19.7
p-Nitrotoluene						
Week 1	183.5 ± 21.5	179.5 ± 29.6	146.6 ± 22.3	176.9 ± 22.7	166.5 ± 46.3	302.0 ± 128.4
Week 3	166.1 ± 17.5	159.2 ± 24.5[3]	162.2 ± 18.8[3]	143.3 ± 11.5	135.7 ± 14.9	238.6 ± 66.2[4]
Week 13	55.8 ± 3.6[3]	75.0 ± 19.2	92.6 ± 23.8	80.2 ± 10.9	71.2 ± 16.0	76.4 ± 13.0
Sorbitol dehydrogenase (IU/L)						
o-Nitrotoluene						
Week 1	8 ± 1	7 ± 1	9 ± 1	8 ± 1	9 ± 1	9 ± 1
Week 3	5 ± 1	6 ± 0	7 ± 1	6 ± 0	6 ± 0	6 ± 1[3]
Week 13	13 ± 1	14 ± 1	14 ± 1	17 ± 1**	16 ± 1**	18 ± 1**
m-Nitrotoluene						
Week 1	6 ± 1	5 ± 1	5 ± 1	5 ± 0	4 ± 0	6 ± 1
Week 3	9 ± 1	10 ± 1	10 ± 1	9 ± 1	8 ± 1	11 ± 1
Week 13	10 ± 0	12 ± 1	10 ± 1	12 ± 0	11 ± 1	10 ± 1
p-Nitrotoluene						
Week 1	7 ± 0	6 ± 0	6 ± 0*	6 ± 0*	6 ± 0**	5 ± 0**
Week 3	5 ± 0	5 ± 0[3]	6 ± 0[3]	5 ± 0	5 ± 0	5 ± 1[4]
Week 13	10 ± 1	10 ± 0	13 ± 1	13 ± 1[3]	7 ± 0**	7 ± 1**
Bile acids (μmol/L)						
o-Nitrotoluene						
Week 1	14.10 ± 1.61	10.10 ± 1.25	14.20 ± 3.72	34.30 ± 8.64	35.60 ± 7.07	22.50 ± 4.24
Week 3	14.60 ± 5.52	6.80 ± 1.85	8.80 ± 2.52	9.00 ± 2.47	15.30 ± 3.25	35.89 ± 5.18**[3]
Week 13	7.40 ± 0.86	10.20 ± 1.67	7.60 ± 1.49	11.10 ± 1.84	23.50 ± 4.20**	28.50 ± 3.60**
m-Nitrotoluene						
Week 1	11.67 ± 2.40[3]	14.20 ± 5.52	19.10 ± 4.75	16.90 ± 4.42	19.30 ± 7.03	22.44 ± 3.81[3]
Week 3	9.60 ± 1.85	6.10 ± 1.39	5.40 ± 0.73	6.30 ± 1.76	6.90 ± 2.10	37.00 ± 6.80
Week 13	4.00 ± 0.99	4.60 ± 1.33	5.40 ± 1.44	4.50 ± 1.39	15.00 ± 1.59**	23.90 ± 5.14**
p-Nitrotoluene						
Week 1	11.80 ± 1.23	11.20 ± 2.57	7.50 ± 1.01	10.90 ± 2.47	8.80 ± 0.83	16.10 ± 2.23
Week 3	9.40 ± 1.18	8.56 ± 1.83[3]	7.89 ± 1.47[3]	10.30 ± 1.49	6.70 ± 1.14	16.88 ± 4.17[4]
Week 13	6.10 ± 0.98	5.80 ± 0.90	5.50 ± 0.48	7.30 ± 1.71	9.00 ± 2.25[3]	16.20 ± 2.03**

[1] Mean ± standard error for groups of 10 animals unless otherwise specified.
[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*-nitrotoluene or *p*-nitrotoluene.
[3] n=9.
[4] n=8.
[5] n=7.
[6] Inadequate data for analysis.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes[1]

	0 ppm	625/675 ppm[2]	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology						
Hematocrit (%)						
o-Nitrotoluene						
Week 1	43.3 ± 0.2	43.1 ± 0.4	43.6 ± 0.5	43.3 ± 0.5[3]	43.0 ± 0.6[3]	44.1 ± 0.6
Week 3	46.5 ± 0.3	47.2 ± 0.5	47.6 ± 0.5	46.9 ± 0.4	44.4 ± 0.5[3]*	44.4 ± 0.7*
Week 13	44.3 ± 0.4	44.3 ± 0.6[3]	43.2 ± 0.4	43.1 ± 0.5[3]	42.5 ± 0.6*	41.3 ± 0.3**
m-Nitrotoluene						
Week 1	44.4 ± 0.8[4]	43.8 ± 0.5	43.8 ± 0.4	44.2 ± 0.5[4]	45.6 ± 0.7	46.5 ± 0.4*
Week 3	46.5 ± 0.8	46.5 ± 0.5[5]	46.7 ± 0.3[3]	47.2 ± 0.3[3]	47.3 ± 0.4[4]	43.6 ± 0.7*[5]
Week 13	45.6 ± 0.4	46.3 ± 0.3	45.3 ± 0.3	44.7 ± 0.4	44.6 ± 0.3	43.6 ± 0.5**[3]
p-Nitrotoluene						
Week 1	44.3 ± 0.3[4]	44.5 ± 0.5[3]	44.8 ± 0.6	45.0 ± 0.8	46.5 ± 0.5*	46.8 ± 0.6**[3]
Week 3	48.2 ± 0.5[3]	48.1 ± 0.6	48.4 ± 0.4	46.6 ± 0.4[3]	48.3 ± 0.6[3]	47.1 ± 0.7
Week 13	45.7 ± 0.3[3]	44.6 ± 0.2**	44.4 ± 0.5*	45.1 ± 0.3*	45.4 ± 0.5[3]	43.7 ± 0.4**[3]
Hemoglobin (g/dL)						
o-Nitrotoluene						
Week 1	15.0 ± 0.1	15.0 ± 0.1	15.1 ± 0.2	15.0 ± 0.2[3]	14.9 ± 0.2[3]	15.3 ± 0.2
Week 3	16.4 ± 0.1	16.6 ± 0.2	16.6 ± 0.2	16.4 ± 0.1	15.5 ± 0.2**[3]	15.4 ± 0.2**
Week 13	15.7 ± 0.2	15.8 ± 0.2[3]	15.2 ± 0.2	15.2 ± 0.2[3]	15.0 ± 0.2**	14.4 ± 0.1**
m-Nitrotoluene						
Week 1	15.4 ± 0.3[4]	15.2 ± 0.2	15.3 ± 0.2	15.4 ± 0.2[4]	15.6 ± 0.3	15.8 ± 0.2
Week 3	15.9 ± 0.3	16.0 ± 0.2[5]	16.1 ± 0.1[3]	16.1 ± 0.1[3]	16.0 ± 0.1[4]	14.4 ± 0.2**[5]
Week 13	15.6 ± 0.2	15.9 ± 0.1	15.6 ± 0.1	15.4 ± 0.1	15.1 ± 0.1*	14.4 ± 0.2**[3]
p-Nitrotoluene						
Week 1	16.0 ± 0.1[4]	15.9 ± 0.2[3]	16.1 ± 0.2	16.0 ± 0.3	16.7 ± 0.2*	16.7 ± 0.2*[3]
Week 3	17.4 ± 0.2[3]	17.2 ± 0.2	17.4 ± 0.2	16.7 ± 0.2*[3]	17.5 ± 0.3[3]	16.6 ± 0.3*
Week 13	15.9 ± 0.1[3]	15.4 ± 0.1**	15.4 ± 0.2*	15.6 ± 0.1*	15.4 ± 0.3[3]	14.7 ± 0.1**[3]
Erythrocytes (10^6/μL)						
o-Nitrotoluene						
Week 1	7.40 ± 0.04	7.35 ± 0.07	7.43 ± 0.09	7.38 ± 0.12[3]	7.34 ± 0.10[3]	7.57 ± 0.12
Week 3	8.03 ± 0.07	8.12 ± 0.11	8.20 ± 0.09	8.10 ± 0.09	7.63 ± 0.08*[3]	7.52 ± 0.12**
Week 13	8.38 ± 0.07	8.40 ± 0.09[3]	8.19 ± 0.08	8.08 ± 0.10*[3]	7.84 ± 0.10**	7.37 ± 0.05**
m-Nitrotoluene						
Week 1	7.68 ± 0.14[4]	7.50 ± 0.10	7.49 ± 0.06	7.61 ± 0.09[4]	7.79 ± 0.13	8.02 ± 0.08*
Week 3	7.79 ± 0.16	7.77 ± 0.11[5]	7.77 ± 0.06[3]	7.90 ± 0.08[3]	7.97 ± 0.06[4]	7.23 ± 0.10*[5]
Week 13	8.42 ± 0.08	8.54 ± 0.06	8.40 ± 0.05	8.27 ± 0.07	8.06 ± 0.06**	7.53 ± 0.06**[3]
p-Nitrotoluene						
Week 1	7.75 ± 0.04[4]	7.78 ± 0.08[3]	7.87 ± 0.09	7.91 ± 0.15	8.17 ± 0.09**	8.15 ± 0.10**[3]
Week 3	8.33 ± 0.08[3]	8.30 ± 0.11	8.38 ± 0.08	8.13 ± 0.09[3]	8.47 ± 0.14[3]	8.03 ± 0.13
Week 13	8.50 ± 0.04[3]	8.35 ± 0.04*	8.31 ± 0.09*	8.45 ± 0.05	8.40 ± 0.09[3]	8.00 ± 0.07**[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Mean cell volume (fL)						
o-Nitrotoluene						
Week 1	58.5 ± 0.3	58.7 ± 0.2	58.6 ± 0.3	58.7 ± 0.3[3]	58.6 ± 0.1[3]	58.3 ± 0.1
Week 3	58.0 ± 0.3	58.1 ± 0.3	58.0 ± 0.4	57.9 ± 0.4	58.2 ± 0.3[3]	59.1 ± 0.3*
Week 13	52.9 ± 0.1	52.8 ± 0.2[3]	52.8 ± 0.1	53.4 ± 0.1**[3]	54.2 ± 0.1**	56.1 ± 0.1**
m-Nitrotoluene						
Week 1	57.8 ± 0.2[4]	58.5 ± 0.3	58.4 ± 0.2	58.1 ± 0.3[4]	58.5 ± 0.2	58.0 ± 0.2
Week 3	59.7 ± 0.3	59.8 ± 0.3[5]	60.0 ± 0.2[3]	59.8 ± 0.2[3]	59.3 ± 0.1[4]	60.3 ± 0.3[5]
Week 13	54.1 ± 0.2	54.2 ± 0.1	54.0 ± 0.1	54.0 ± 0.1	55.3 ± 0.2**	57.9 ± 0.2**[3]
p-Nitrotoluene						
Week 1	57.2 ± 0.4[4]	57.1 ± 0.2[3]	57.0 ± 0.3	56.9 ± 0.3	56.9 ± 0.2	57.3 ± 0.2[3]
Week 3	57.8 ± 0.3[3]	58.0 ± 0.2	57.5 ± 0.2	57.5 ± 0.3[3]	57.1 ± 0.4[3]	58.6 ± 0.4
Week 13	53.7 ± 0.1[3]	53.4 ± 0.1	53.4 ± 0.1	53.3 ± 0.1	54.1 ± 0.2[3]	54.7 ± 0.2*[3]
MCH (pg)						
o-Nitrotoluene						
Week 1	20.2 ± 0.1	20.4 ± 0.0	20.3 ± 0.1	20.3 ± 0.1[3]	20.3 ± 0.1[3]	20.2 ± 0.1
Week 3	20.4 ± 0.1	20.5 ± 0.1	20.2 ± 0.2	20.3 ± 0.2	20.2 ± 0.1[3]	20.5 ± 0.1
Week 13	18.8 ± 0.1	18.8 ± 0.1[3]	18.6 ± 0.1	18.8 ± 0.1[3]	19.1 ± 0.1*	19.6 ± 0.1**
m-Nitrotoluene						
Week 1	20.1 ± 0.1[4]	20.3 ± 0.1	20.4 ± 0.1	20.2 ± 0.1[4]	20.1 ± 0.1	19.7 ± 0.1*
Week 3	20.4 ± 0.1	20.6 ± 0.1[5]	20.7 ± 0.1[3]	20.4 ± 0.1[3]	20.1 ± 0.1[4]	20.0 ± 0.2[5]
Week 13	18.6 ± 0.1	18.6 ± 0.0	18.6 ± 0.1	18.6 ± 0.1	18.8 ± 0.1	19.1 ± 0.1**[3]
p-Nitrotoluene						
Week 1	20.7 ± 0.1[4]	20.5 ± 0.1[3]	20.5 ± 0.1	20.2 ± 0.1*	20.5 ± 0.1	20.5 ± 0.1[3]
Week 3	20.9 ± 0.2[3]	20.7 ± 0.1	20.8 ± 0.1	20.6 ± 0.2[3]	20.7 ± 0.1[3]	20.7 ± 0.1
Week 13	18.7 ± 0.1[3]	18.5 ± 0.1	18.5 ± 0.1	18.5 ± 0.1	18.3 ± 0.3[3]	18.4 ± 0.1[3]
Mean cell hemoglobin concentration (g/dL)						
o-Nitrotoluene						
Week 1	34.5 ± 0.2	34.7 ± 0.1	34.6 ± 0.1	34.6 ± 0.1[3]	34.6 ± 0.2[3]	34.6 ± 0.1
Week 3	35.2 ± 0.1	35.2 ± 0.1	34.8 ± 0.2	35.0 ± 0.2	34.8 ± 0.1[3]	34.6 ± 0.1**
Week 13	35.5 ± 0.1	35.6 ± 0.2[3]	35.3 ± 0.2	35.2 ± 0.1[3]	35.2 ± 0.2	34.9 ± 0.2**
m-Nitrotoluene						
Week 1	34.8 ± 0.1[4]	34.7 ± 0.1	34.9 ± 0.2	34.7 ± 0.3[4]	34.3 ± 0.2	34.0 ± 0.2**
Week 3	34.1 ± 0.1	34.5 ± 0.2[5]	34.4 ± 0.1[3]	34.1 ± 0.1[3]	33.8 ± 0.2[4]	33.1 ± 0.2**[5]
Week 13	34.3 ± 0.1	34.4 ± 0.1	34.5 ± 0.1	34.5 ± 0.1	33.9 ± 0.1*	33.0 ± 0.1**[3]
p-Nitrotoluene						
Week 1	36.1 ± 0.1[4]	35.8 ± 0.2[3]	35.9 ± 0.2	35.6 ± 0.1	36.0 ± 0.1	35.8 ± 0.2[3]
Week 3	36.2 ± 0.2[3]	35.8 ± 0.3	36.2 ± 0.1	35.9 ± 0.2[3]	36.2 ± 0.2[3]	35.3 ± 0.2**
Week 13	34.8 ± 0.2[3]	34.6 ± 0.1	34.7 ± 0.2	34.7 ± 0.1	33.8 ± 0.5[3]*	33.7 ± 0.1**[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Platelets (10^3/μL)						
o-Nitrotoluene						
Week 1	1,014.5 ± 89.1	1,107.5 ± 41.3	1,144.6 ± 28.0	1,227.4 ± 51.2[3]	1,219.9 ± 20.7*[3]	1,275.7 ± 17.3**
Week 3	934.8 ± 47.3	901.9 ± 53.0	915.8 ± 46.1	820.2 ± 80.3	1,021.4 ± 22.9[3]	1,125.0 ± 44.4*
Week 13	683.7 ± 42.2	743.0 ± 19.2[3]	777.9 ± 35.3	751.4 ± 57.0	816.6 ± 41.7**	790.8 ± 60.0*
m-Nitrotoluene						
Week 1	1,045.3 ± 20.2[4]	972.6 ± 22.6*	981.3 ± 46.3	909.3 ± 98.3[4]	953.6 ± 22.2*	885.6 ± 23.0**
Week 3	934.6 ± 51.4	959.7 ± 24.9[5]	958.7 ± 12.7[3]	846.6 ± 78.6[3]	918.6 ± 24.2[4]	941.6 ± 86.9[5]
Week 13	792.4 ± 23.8	762.0 ± 14.9	758.8 ± 10.3	747.7 ± 25.9	785.6 ± 11.9	851.6 ± 20.0*[3]
p-Nitrotoluene						
Week 1	1,013.8 ± 28.3[4]	985.0 ± 33.0[3]	1,005.5 ± 21.0	882.2 ± 34.9**	931.4 ± 16.7*	965.8 ± 19.7*[3]
Week 3	999.3 ± 20.3[3]	976.7 ± 17.1	1,022.4 ± 19.4	964.4 ± 15.1[3]	1,022.6 ± 24.7[3]	1,035.8 ± 17.5
Week 13	791.7 ± 13.0[3]	752.2 ± 7.2	711.4 ± 30.0	565.4 ± 76.8*	677.6 ± 34.2*[3]	785.0 ± 16.6[3]
Reticulocytes (10^6/μL)						
o-Nitrotoluene						
Week 1	0.57 ± 0.03	0.54 ± 0.02	0.63 ± 0.07	0.62 ± 0.05[3]	0.58 ± 0.05[3]	0.51 ± 0.04
Week 3	0.24 ± 0.04	0.26 ± 0.02	0.25 ± 0.03	0.21 ± 0.02	0.31 ± 0.06[3]	0.34 ± 0.04**
Week 13	0.23 ± 0.02	0.21 ± 0.02[3]	0.27 ± 0.03	0.29 ± 0.04[3]	0.32 ± 0.04*	0.35 ± 0.04**
m-Nitrotoluene						
Week 1	0.20 ± 0.02[4]	0.23 ± 0.03	0.21 ± 0.01	0.20 ± 0.03[4]	0.18 ± 0.02	0.08 ± 0.01**
Week 3	0.11 ± 0.01[3]	0.13 ± 0.02[5]	0.09 ± 0.01[3]	0.10 ± 0.01[3]	0.11 ± 0.01[4]	0.25 ± 0.04**[5]
Week 13	0.20 ± 0.03	0.21 ± 0.02	0.21 ± 0.02	0.23 ± 0.02	0.29 ± 0.03*	0.35 ± 0.03**[3]
p-Nitrotoluene						
Week 1	0.13 ± 0.02[4]	0.14 ± 0.01[3]	0.14 ± 0.01	0.13 ± 0.01	0.11 ± 0.02	0.08 ± 0.01**[3]
Week 3	0.20 ± 0.02[3]	0.13 ± 0.01	0.17 ± 0.03	0.17 ± 0.02[3]	0.18 ± 0.02[3]	0.24 ± 0.03
Week 13	0.06 ± 0.01[3]	0.09 ± 0.01	0.10 ± 0.02*	0.11 ± 0.01**	0.15 ± 0.02**[3]	0.21 ± 0.04**[3]
Leukocytes (10^3/μL)						
o-Nitrotoluene						
Week 1	10.64 ± 0.40	10.35 ± 0.69	10.62 ± 0.34	9.82 ± 0.43[3]	11.11 ± 0.71[3]	12.04 ± 0.53
Week 3	9.30 ± 0.36	10.51 ± 0.61	10.70 ± 0.76	11.41 ± 0.62*	12.24 ± 0.64**[3]	13.82 ± 0.93**
Week 13	9.32 ± 0.43	9.69 ± 0.49[3]	10.56 ± 0.66	12.87 ± 0.90**[3]	14.21 ± 0.83**	14.55 ± 0.63**
m-Nitrotoluene						
Week 1	11.95 ± 0.45[4]	10.29 ± 0.70	10.03 ± 0.46*	11.00 ± 0.68[4]	10.26 ± 0.40	10.53 ± 0.36
Week 3	10.91 ± 0.55	11.91 ± 0.65[5]	11.28 ± 0.46[3]	10.71 ± 0.61[3]	11.00 ± 0.80[4]	13.39 ± 0.42**[5]
Week 13	10.54 ± 0.66	9.45 ± 0.68	10.00 ± 0.30	11.02 ± 0.38	10.10 ± 0.50	11.32 ± 0.58[3]
p-Nitrotoluene						
Week 1	10.18 ± 0.22[4]	10.82 ± 0.39[3]	10.70 ± 0.40	10.17 ± 0.84	10.78 ± 0.55	9.81 ± 0.49[3]
Week 3	13.73 ± 0.95[3]	11.25 ± 0.69	11.27 ± 0.82	10.64 ± 0.89[3]	12.96 ± 0.78[3]	11.22 ± 0.63
Week 13	11.20 ± 0.31[3]	9.46 ± 0.25*	11.85 ± 1.12	11.57 ± 0.74	10.63 ± 0.22[3]	12.10 ± 1.12[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Segmented neutrophils (10^3/μL)						
o-Nitrotoluene						
Week 1	0.87 ± 0.10	0.79 ± 0.15	0.79 ± 0.11	0.77 ± 0.11[3]	0.84 ± 0.16[3]	0.76 ± 0.09
Week 3	0.69 ± 0.09	0.73 ± 0.10	0.78 ± 0.12	0.80 ± 0.11	1.02 ± 0.10*[3]	1.06 ± 0.20
Week 13	1.35 ± 0.26	1.32 ± 0.09[3]	1.32 ± 0.18	1.46 ± 0.24[3]	1.67 ± 0.16	1.16 ± 0.13
m-Nitrotoluene						
Week 1	1.18 ± 0.39[4]	0.81 ± 0.15	0.72 ± 0.09	1.36 ± 0.24[4]	0.91 ± 0.12	0.86 ± 0.07
Week 3	1.52 ± 0.34	0.90 ± 0.10[5]	1.04 ± 0.15[3]	1.10 ± 0.15[3]	1.05 ± 0.18[4]	1.07 ± 0.11[5]
Week 13	2.09 ± 0.63	1.75 ± 0.29	1.39 ± 0.12	2.31 ± 0.27	1.24 ± 0.15	1.66 ± 0.22[3]
p-Nitrotoluene						
Week 1	0.95 ± 0.15[4]	1.20 ± 0.20[3]	1.25 ± 0.14	1.07 ± 0.11	1.04 ± 0.09	1.39 ± 0.12[3]
Week 3	1.53 ± 0.33[3]	1.41 ± 0.15	1.30 ± 0.18	1.39 ± 0.20[3]	1.87 ± 0.27[3]	2.24 ± 0.48
Week 13	2.23 ± 0.31[3]	1.64 ± 0.23	1.97 ± 0.29[3]	2.16 ± 0.47	1.44 ± 0.14[3]	1.67 ± 0.23[3]
Lymphocytes (10^3/μL)						
o-Nitrotoluene						
Week 1	9.65 ± 0.40	9.43 ± 0.62	9.68 ± 0.26	8.94 ± 0.40[3]	10.07 ± 0.61[3]	11.12 ± 0.55
Week 3	8.48 ± 0.33	9.70 ± 0.62	9.88 ± 0.73	10.48 ± 0.59*	11.18 ± 0.62**[3]	12.68 ± 0.83**
Week 13	7.88 ± 0.46	8.28 ± 0.43[3]	9.18 ± 0.58	11.31 ± 0.71**[3]	12.41 ± 0.77**	13.39 ± 0.59**
m-Nitrotoluene						
Week 1	10.58 ± 0.31[4]	9.25 ± 0.67	9.11 ± 0.39	9.45 ± 0.49[4]	9.17 ± 0.42	9.46 ± 0.31
Week 3	9.09 ± 0.54	10.69 ± 0.62[5]	10.00 ± 0.46[3]	9.18 ± 0.56[3]	9.64 ± 0.71[4]	12.09 ± 0.47**[5]
Week 13	8.37 ± 0.45	7.46 ± 0.48	8.50 ± 0.26	8.49 ± 0.37	8.71 ± 0.42	9.47 ± 0.66[3]
p-Nitrotoluene						
Week 1	9.13 ± 0.24[4]	9.51 ± 0.45[3]	9.31 ± 0.39	8.98 ± 0.84	9.62 ± 0.48	8.27 ± 0.43[3]
Week 3	12.06 ± 0.80[3]	9.78 ± 0.59*	9.12 ± 1.07*	9.10 ± 0.83*[3]	10.91 ± 0.607[3]	8.76 ± 0.70**
Week 13	8.67 ± 0.18[3]	7.40 ± 0.21	8.56 ± 0.43	9.09 ± 0.43	8.97 ± 0.26[3]	10.31 ± 1.04[3]
Monocytes (10^3/μL)						
o-Nitrotoluene						
Week 1	0.07 ± 0.03	0.10 ± 0.03	0.09 ± 0.03	0.10 ± 0.03[3]	0.14 ± 0.04[3]	0.12 ± 0.04
Week 3	0.08 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.07 ± 0.03	0.01 ± 0.01[3]	0.05 ± 0.03
Week 13	0.05 ± 0.02	0.06 ± 0.02[3]	0.04 ± 0.02	0.02 ± 0.02[3]	0.10 ± 0.04	0.00 ± 0.00
m-Nitrotoluene						
Week 1	0.09 ± 0.05[4]	0.15 ± 0.04	0.17 ± 0.05	0.15 ± 0.05[4]	0.11 ± 0.04	0.12 ± 0.04
Week 3	0.17 ± 0.06	0.24 ± 0.07[5]	0.21 ± 0.06[3]	0.20 ± 0.05[3]	0.18 ± 0.09[4]	0.19 ± 0.08[5]
Week 13	0.02 ± 0.01	0.10 ± 0.03	0.03 ± 0.02	0.10 ± 0.04	0.08 ± 0.05	0.07 ± 0.03[3]
p-Nitrotoluene						
Week 1	0.05 ± 0.03[4]	0.06 ± 0.02[3]	0.08 ± 0.04	0.08 ± 0.03	0.08 ± 0.03	0.08 ± 0.06[3]
Week 3	0.12 ± 0.04[3]	0.04 ± 0.03	0.09 ± 0.05[3]	0.11 ± 0.05[3]	0.16 ± 0.06[3]	0.07 ± 0.03
Week 13	0.23 ± 0.06[3]	0.32 ± 0.05	0.16 ± 0.06	0.19 ± 0.05	0.18 ± 0.04[3]	0.10 ± 0.04[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of o-, m-, and p-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Hematology (continued)						
Eosinophils ($10^3/\mu L$)						
o-Nitrotoluene						
Week 1	0.03 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.02 ± 0.02[3]	0.02 ± 0.02[3]	0.03 ± 0.02
Week 3	0.06 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.04 ± 0.02[3]	0.05 ± 0.03
Week 13	0.04 ± 0.02	0.03 ± 0.02[3]	0.01 ± 0.01	0.07 ± 0.03[3]	0.05 ± 0.02	0.00 ± 0.00
m-Nitrotoluene						
Week 1	0.08 ± 0.03[4]	0.08 ± 0.03	0.01 ± 0.01	0.03 ± 0.02[4]	0.05 ± 0.02	0.07 ± 0.02
Week 3	0.13 ± 0.05	0.07 ± 0.03[5]	0.08 ± 0.02[3]	0.18 ± 0.03[3]	0.13 ± 0.05[4]	0.01 ± 0.01[5]
Week 13	0.05 ± 0.03	0.13 ± 0.03	0.08 ± 0.02	0.11 ± 0.04	0.07 ± 0.02	0.12 ± 0.04[3]
p-Nitrotoluene						
Week 1	0.05 ± 0.02[4]	0.04 ± 0.02[3]	0.06 ± 0.02	0.05 ± 0.02	0.04 ± 0.02	0.07 ± 0.03[3]
Week 3	0.03 ± 0.02[3]	0.02 ± 0.01	0.05 ± 0.03	0.04 ± 0.02[3]	0.01 ± 0.01[3]	0.04 ± 0.02
Week 13	0.06 ± 0.02[3]	0.11 ± 0.02	0.12 ± 0.05	0.10 ± 0.04	0.03 ± 0.02[3]	0.01 ± 0.01[3]
Nucleated erythrocytes/100 leukocytes						
o-Nitrotoluene						
Week 1	0.20 ± 0.20	0.40 ± 0.22	0.40 ± 0.22	0.40 ± 0.22	0.44 ± 0.24[3]	0.30 ± 0.15
Week 3	0.00 ± 0.00	0.00 ± 0.00	0.40 ± 0.22	0.00 ± 0.00	0.11 ± 0.11[3]	0.10 ± 0.10
Week 13	0.10 ± 0.10	0.22 ± 0.15[3]	0.60 ± 0.22	0.11 ± 0.11[3]	0.30 ± 0.21	0.30 ± 0.15
m-Nitrotoluene						
Week 1	0.50 ± 0.17	1.50 ± 0.89	1.10 ± 0.38	0.40 ± 0.16	0.70 ± 0.30	0.30 ± 0.21
Week 3	0.30 ± 0.21	0.57 ± 0.57[5]	0.00 ± 0.00[3]	0.22 ± 0.15[3]	0.50 ± 0.27[4]	1.29 ± 0.36*[5]
Week 13	0.00 ± 0.00	0.40 ± 0.22	0.00 ± 0.00	0.30 ± 0.30	0.60 ± 0.34	2.11 ± 0.61**[3]
p-Nitrotoluene						
Week 1	0.25 ± 0.16[4]	0.33 ± 0.17[3]	0.30 ± 0.21	0.20 ± 0.13	0.40 ± 0.22	0.11 ± 0.11[3]
Week 3	0.00 ± 0.00[3]	0.10 ± 0.10	0.00 ± 0.00	0.44 ± 0.29[3]	0.22 ± 0.22[3]	1.10 ± 0.38**
Week 13	0.22 ± 0.15[3]	0.30 ± 0.15	0.30 ± 0.21	0.40 ± 0.22	1.00 ± 0.33*[3]	5.22 ± 2.01**[3]
Chemistry						
Urea nitrogen (mg/dL)						
o-Nitrotoluene						
Week 1	20.6 ± 0.8[5]	20.8 ± 0.5	20.3 ± 0.7[4]	19.9 ± 0.8[3]	20.4 ± 0.8	20.0 ± 0.6
Week 3	22.3 ± 0.5	20.1 ± 0.6*	20.9 ± 0.8	17.6 ± 0.3**	16.7 ± 0.4**	21.2 ± 0.6**
Week 13	18.5 ± 0.6	20.5 ± 0.7	20.3 ± 0.8	18.4 ± 1.0	19.0 ± 0.9	17.8 ± 0.6
m-Nitrotoluene						
Week 1	18.1 ± 0.8	20.2 ± 0.8	20.1 ± 0.7	20.0 ± 0.9	21.7 ± 0.9**	22.1 ± 0.5**
Week 3	21.0 ± 0.6	22.3 ± 0.5[5]	21.3 ± 0.5[3]	22.8 ± 0.7	22.1 ± 0.5[3]	22.6 ± 0.7[5]
Week 13	16.5 ± 0.6	17.9 ± 0.9	16.8 ± 0.6	16.5 ± 0.4	15.3 ± 0.7	18.2 ± 0.9
p-Nitrotoluene						
Week 1	18.6 ± 0.5[3]	19.6 ± 0.8	19.7 ± 0.9	20.0 ± 1.5	20.7 ± 1.9	19.3 ± 0.7
Week 3	14.5 ± 0.8[4]	13.8 ± 0.6	14.4 ± 0.3	13.7 ± 0.6[3]	12.4 ± 0.4[3]	17.1 ± 0.8
Week 13	21.7 ± 0.6	21.3 ± 0.5	20.2 ± 0.6	21.8 ± 0.9	24.3 ± 1.1	22.6 ± 0.5[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Creatinine (mg/dL)						
o-Nitrotoluene						
Week 1	0.59 ± 0.01[5]	0.59 ± 0.01	0.58 ± 0.02[4]	0.57 ± 0.02[3]	0.58 ± 0.01	0.55 ± 0.02
Week 3	0.61 ± 0.01	0.57 ± 0.02*	0.57 ± 0.02	0.57 ± 0.02	0.52 ± 0.01**	0.52 ± 0.01**
Week 13	0.68 ± 0.01	0.68 ± 0.02	0.67 ± 0.02	0.65 ± 0.02	0.60 ± 0.02**	0.62 ± 0.01**
m-Nitrotoluene						
Week 1	0.50 ± 0.00	0.55 ± 0.02*	0.54 ± 0.02*	0.56 ± 0.02**	0.58 ± 0.01**	0.64 ± 0.02**
Week 3	0.57 ± 0.03	0.57 ± 0.02[5]	0.60 ± 0.0[3]	0.60 ± 0.00	0.60 ± 0.02[3]	0.63 ± 0.02*[5]
Week 13	0.69 ± 0.01	0.72 ± 0.01	0.73 ± 0.02	0.73 ± 0.02	0.70 ± 0.02	0.74 ± 0.02
p-Nitrotoluene						
Week 1	0.51 ± 0.05[3]	0.57 ± 0.02	0.60 ± 0.03	0.52 ± 0.05	0.60 ± 0.03[3]	0.57 ± 0.02[3]
Week 3	0.64 ± 0.03[4]	0.62 ± 0.02	0.69 ± 0.02	0.61 ± 0.04[3]	0.68 ± 0.02[3]	0.69 ± 0.02
Week 13	0.73 ± 0.03	0.79 ± 0.02	0.76 ± 0.03	0.83 ± 0.02**	0.81 ± 0.02*	0.83 ± 0.02**[3]
Total protein (g/dL)						
o-Nitrotoluene						
Week 1	5.8 ± 0.1[5]	5.7 ± 0.1[3]	5.6 ± 0.1[4]	5.7 ± 0.1[4]	5.4 ± 0.0**[3]	5.1 ± 0.1**
Week 3	6.1 ± 0.1	6.0 ± 0.0	5.9 ± 0.1	5.8 ± 0.0**	5.4 ± 0.1**	5.1 ± 0.0**
Week 13	7.1 ± 0.1	7.3 ± 0.1	7.1 ± 0.1	6.8 ± 0.1	6.5 ± 0.1**	6.3 ± 0.1**
m-Nitrotoluene						
Week 1	5.6 ± 0.0	5.6 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.6 ± 0.1
Week 3	6.0 ± 0.1	6.0 ± 0.0[5]	5.9 ± 0.1[3]	6.0 ± 0.1	5.9 ± 0.0[3]	6.0 ± 0.0[5]
Week 13	6.6 ± 0.1	7.0 ± 0.1	6.8 ± 0.1	6.6 ± 0.1	6.2 ± 0.0**	6.1 ± 0.1**
p-Nitrotoluene						
Week 1	5.7 ± 0.1[3]	5.5 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1
Week 3	5.8 ± 0.1[4]	5.8 ± 0.1	5.8 ± 0.1	5.6 ± 0.1[3]	5.6 ± 0.1[3]	6.0 ± 0.1
Week 13	6.7 ± 0.1	7.1 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.4 ± 0.0*	6.3 ± 0.1*[3]
Albumin (g/dL)						
o-Nitrotoluene						
Week 1	4.6 ± 0.1[5]	4.6 ± 0.1[3]	4.5 ± 0.1[4]	4.5 ± 0.1[4]	4.3 ± 0.1**[3]	4.1 ± 0.1**
Week 3	4.8 ± 0.0	4.7 ± 0.0	4.7 ± 0.1	4.5 ± 0.0**	4.3 ± 0.1**	4.1 ± 0.0**
Week 13	5.3 ± 0.1	5.4 ± 0.1	5.3 ± 0.1	5.2 ± 0.1	4.9 ± 0.1**	4.8 ± 0.0**
m-Nitrotoluene						
Week 1	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.8 ± 0.0*	4.9 ± 0.1**
Week 3	4.8 ± 0.1	4.8 ± 0.0[5]	4.7 ± 0.0[3]	4.8 ± 0.1	4.7 ± 0.0[3]	4.9 ± 0.1[5]
Week 13	5.1 ± 0.1	5.5 ± 0.1	5.4 ± 0.0	5.1 ± 0.1	4.9 ± 0.0	4.9 ± 0.1
p-Nitrotoluene						
Week 1	3.1 ± 0.6[3]	3.8 ± 0.1	3.8 ± 0.0	3.8 ± 0.0	4.0 ± 0.0*[3]	3.9 ± 0.0
Week 3	3.9 ± 0.1[4]	3.9 ± 0.0	3.9 ± 0.0	3.8 ± 0.0[3]	3.8 ± 0.0[3]	4.1 ± 0.0*
Week 13	4.2 ± 0.1	4.4 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.1 ± 0.0	4.2 ± 0.0[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Methemoglobin (%)						
o-Nitrotoluene						
Week 1	1.71 ± 0.13[3]	1.89 ± 0.19[3]	2.14 ± 0.40[3]	1.92 ± 0.26[3]	2.35 ± 0.17[4]	2.94 ± 0.18**
Week 3	1.46 ± 0.12	2.10 ± 0.27*[3]	1.77 ± 0.24[3]	2.36 ± 0.22**[4]	4.24 ± 0.24**	5.45 ± 0.21**
Week 13	2.01 ± 0.37[4]	2.88 ± 0.69[5]	3.35 ± 0.38*	2.90 ± 0.25	4.06 ± 0.40**[4]	4.31 ± 0.48**
m-Nitrotoluene						
Week 1	–[6]	–	–	–	–	–
Week 3	1.66 ± 0.20[3]	2.50 ± 0.33[5]	1.97 ± 0.24[3]	2.14 ± 0.25	2.09 ± 0.23[3]	3.00 ± 0.21**[5]
Week 13	3.09 ± 0.28	3.15 ± 0.27	3.48 ± 0.242	3.46 ± 0.39	3.82 ± 0.22[3]	5.00 ± 0.35**[3]
p-Nitrotoluene						
Week 1	2.68 ± 0.24[4]	2.64 ± 0.31[3]	2.61 ± 0.34	2.52 ± 0.24	2.18 ± 0.42	2.00 ± 0.16[3]
Week 3	6.92 ± 0.35[3]	5.94 ± 0.38	6.56 ± 0.47	5.84 ± 0.59[3]	6.06 ± 0.37[3]	6.52 ± 0.54
Week 13	6.36 ± 0.28[3]	5.94 ± 0.28	6.17 ± 0.54	5.91 ± 0.26	6.29 ± 0.17[3]	9.02 ± 0.20**[3]
Alkaline phosphatase (IU/L)						
o-Nitrotoluene						
Week 1	600 ± 14[5]	599 ± 14[3]	600 ± 13[4]	588 ± 13[3]	563 ± 10	561 ± 12
Week 3	449 ± 11	397 ± 12*	426 ± 15*	372 ± 6**	314 ± 9**	321 ± 10**
Week 13	186 ± 5	203 ± 10	201 ± 6	173 ± 10	150 ± 7*	166 ± 4*
m-Nitrotoluene						
Week 1	511 ± 20	535 ± 14	523 ± 13	524 ± 13	515 ± 14	458 ± 13*
Week 3	402 ± 15	398 ± 10[5]	381 ± 7[6]	412 ± 12	391 ± 12[3]	372 ± 8[5]
Week 13	196 ± 7	191 ± 6	192 ± 4	184 ± 7	196 ± 4	210 ± 9
p-Nitrotoluene						
Week 1	291 ± 7[7]	277 ± 10	269 ± 17[3]	331 ± 18[4]	277 ± 13[5]	269 ± 10[4]
Week 3	192 ± 4[4]	188 ± 8	186 ± 6	178 ± 4[3]	174 ± 3[3]	190 ± 6
Week 13	195 ± 10	195 ± 6	179 ± 8	195 ± 4	231 ± 10**	218 ± 11*[3]
Alanine aminotransferase (IU/L)						
o-Nitrotoluene						
Week 1	41 ± 2[5]	39 ± 1[3]	39 ± 1[4]	39 ± 1[3]	38 ± 1	38 ± 1
Week 3	44 ± 1	44 ± 1	44 ± 1	41 ± 2	41 ± 1	38 ± 1**
Week 13	45 ± 2	49 ± 3	49 ± 2	40 ± 1*	41 ± 1*	42 ± 5**
m-Nitrotoluene						
Week 1	38 ± 1	37 ± 1	38 ± 1	40 ± 1	46 ± 1**	48 ± 2**
Week 3	36 ± 1	37 ± 1[5]	36 ± 1[3]	41 ± 1**	42 ± 1**[3]	45 ± 1**[5]
Week 13	50 ± 2	59 ± 3	48 ± 4	53 ± 4	42 ± 1	54 ± 2
p-Nitrotoluene						
Week 1	27 ± 3[5]	25 ± 1	28 ± 1	29 ± 1	31 ± 1**[4]	37 ± 1**
Week 3	29 ± 1[4]	28 ± 1	30 ± 2	28 ± 1[3]	29 ± 1[3]	35 ± 2*
Week 13	53 ± 4	45 ± 3	41 ± 3**	46 ± 3*	44 ± 2*	36 ± 1**[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

	0 ppm	625/675 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Chemistry (continued)						
Creatine kinase (IU/L)						
o-Nitrotoluene						
Week 1	291.0 ± 29.3[5]	274.7 ± 23.2[5]	274.6 ± 28.2[5]	361.6 ± 49.6[5]	398.8 ± 37.5[3]	354.2 ± 111.9
Week 3	290.0 ± 37.5	201.1 ± 22.3	268.0 ± 24.0	303.3 ± 59.0	226.8 ± 28.4	350.0 ± 61.7
Week 13	89.9 ± 5.3	140.4 ± 35.8	83.0 ± 5.9	99.9 ± 9.3	96.6 ± 6.7	120.4 ± 39.4
m-Nitrotoluene						
Week 1	329.4 ± 40.9[3]	481.1 ± 99.3	325.0 ± 43.0	493.4 ± 88.1	358.1 ± 59.3	385.3 ± 53.9
Week 3	241.1 ± 32.3	202.3 ± 23.7[5]	218.2 ± 26.4[3]	248.1 ± 20.5	228.0 ± 58.3[3]	271.4 ± 22.7
Week 13	139.0 ± 17.6	125.9 ± 10.7	197.8 ± 34.6	146.3 ± 30.1	153.6 ± 22.6	160.3 ± 21.7
p-Nitrotoluene						
Week 1	176.6 ± 36.9[5]	141.5 ± 19.2	139.0 ± 14.1	218.5 ± 54.1	159.9 ± 9.4[3]	184.0 ± 29.0
Week 3	201.9 ± 64.0[4]	140.6 ± 24.1	166.3 ± 20.6	157.1 ± 29.6[3]	154.2 ± 31.4[3]	351.2 ± 115.5
Week 13	51.00 ± 7.8[3]	93.7 ± 22.6	91.2 ± 33.2	80.9 ± 9.4	126.9 ± 44.4	61.33 ± 5.5[3]
Sorbitol dehydrogenase (IU/L)						
o-Nitrotoluene						
Week 1	10 ± 1	10 ± 1	10 ± 1	9 ± 1	10 ± 1	10 ± 1
Week 3	10 ± 1	11 ± 1	10 ± 1	10 ± 1	11 ± 1	9 ± 1
Week 13	12 ± 1	11 ± 1	12 ± 0	12 ± 1	11 ± 0	13 ± 1
m-Nitrotoluene						
Week 1	5 ± 1	4 ± 0	5 ± 1	5 ± 1[3]	6 ± 1	4 ± 1[3]
Week 3	7 ± 1	8 ± 1[5]	8 ± 1[3]	7 ± 1	9 ± 1[3]	7 ± 1[5]
Week 13	11 ± 1	13 ± 1	11 ± 1	12 ± 1	10 ± 1	11 ± 1
p-Nitrotoluene						
Week 1	6 ± 1[8]	5 ± 0	6 ± 0[4]	6 ± 0[4]	5 ± 0[5]	6 ± 1[3]
Week 3	13 ± 1[4]	10 ± 1*	11 ± 1*	9 ± 1**[3]	9 ± 1**[3]	7 ± 1**
Week 13	10 ± 1	10 ± 1	9 ± 0	10 ± 1	10 ± 0	8 ± 0[3]
Bile acids (μmol/L)						
o-Nitrotoluene						
Week 1	20.20 ± 3.31	20.70 ± 6.46	12.40 ± 3.76	13.10 ± 2.15	16.80 ± 3.39	14.80 ± 3.08
Week 3	9.70 ± 3.11	7.10 ± 1.89	9.60 ± 2.30	5.40 ± 1.16	8.50 ± 1.49	8.80 ± 1.40
Week 13	11.56 ± 2.24[3]	16.90 ± 2.89	14.10 ± 2.71	13.20 ± 2.39	17.30 ± 3.79	21.20 ± 1.91**
m-Nitrotoluene						
Week 1	9.11 ± 1.39[3]	19.33 ± 7.85[3]	14.89 ± 6.88[3]	10.57 ± 2.92[5]	17.33 ± 2.09*[3]	37.88 ± 6.37***[4]
Week 3	20.22 ± 3.64[3]	11.17 ± 5.24[8]	11.33 ± 1.69[3]	16.50 ± 5.83	15.33 ± 3.27[3]	49.17 ± 4.83[8]
Week 13	9.00 ± 2.31	6.80 ± 1.02	17.20 ± 3.04*	12.70 ± 1.78	16.30 ± 3.71	49.30 ± 9.65**
p-Nitrotoluene						
Week 1	10.89 ± 1.55[3]	10.70 ± 2.37	9.60 ± 0.85	8.70 ± 0.75	13.56 ± 2.59[3]	10.30 ± 1.01
Week 3	16.13 ± 2.07[4]	14.90 ± 2.48	13.60 ± 1.18	15.33 ± 2.32[3]	16.33 ± 1.73[3]	41.60 ± 9.35*
Week 13	12.90 ± 1.33	18.70 ± 2.99	16.00 ± 3.76	22.90 ± 3.74	17.80 ± 2.57	19.33 ± 2.38[3]

TABLE B2 Hematology and Clinical Chemistry Data for Female Rats in the 13-Week Feed Studies of *o*-, *m*-, and *p*-Nitrotoluenes (continued)

[1] Mean ± standard error for groups of 10 animals unless otherwise specified.
[2] Animals tested received 625 ppm *o*-nitrotoluene or 675 ppm *m*-nitrotoluene or *p*-nitrotoluene.
[3] n=9.
[4] n=8.
[5] n=7.
[6] Insufficient data for analysis.
[7] n=4.
[8] n=6.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

APPENDIX C

Reproductive Tissue Evaluations and Estrous Cycle Length

TABLE C1 Summary of Reproductive Tissue Evaluations in Male Rats in the 13-Week Feed Study of *o*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	353 ± 5	309 ± 6**	254 ± 4**	198 ± 3**
Left testicle	1.48 ± 0.028	1.39 ± 0.025*	1.07 ± 0.045**	0.54 ± 0.024**
Left epididymis	0.50 ± 0.011	0.40 ± 0.014**	0.28 ± 0.012**	0.12 ± 0.008**
Left epididymal tail	0.20 ± 0.010	0.16 ± 0.008*	0.12 ± 0.007**	0.05 ± 0.003**
Spermatid measurements				
Spermatid heads (10^7/g testis)	12.40 ± 0.44	12.58 ± 0.48	13.17 ± 0.85	4.41 ± 1.19**
Spermatid heads (10^7/testis)	18.30 ± 0.65	17.39 ± 0.62	14.02 ± 0.97**	2.35 ± 0.63**
Spermatid count (mean/10^4 mL suspension)	91.48 ± 3.23	86.93 ± 3.11	70.08 ± 4.83**	11.88 ± 3.10**
Spermatozoal measurements				
Motility (%)	78 ± 1	76 ± 2	73 ± 1*	6.5 ± 5.5**
Concentration (10^6/g cet)[2]	417 ± 28	433 ± 38	179 ± 22**	14 ± 5**

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] g cet = grams of caudal epididymal tissue.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C2 Summary of Estrous Cycle Characterization in Female Rats in the 13-Week Feed Study of *o*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Necropsy body weight (g)	205 ± 3	179 ± 3**	170 ± 2**	158 ± 3**
Estrous cycle length (days)	4.90 ± 0.10	4.70 ± 0.17	5.30 ± 0.21	6.63 ± 0.80**[2]
Estrous stages as % of cycle[3]				
diestrus	36.7	39.2	40.8	62.5
proestrus	7.5	15.8	15.0	10.8
estrus	32.5	25.0	25.0	15.8
metestrus	23.3	20.0	19.2	10.8

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=4, estrous cycle length longer than 12 days or unclear in 6 of 10 animals.
[3] Evidence by multivariate analysis of variance (MANOVA) suggests that females in all dose groups differ from controls in the relative frequency of time spent in the estrous stages; P=0.06 for the 2,500 ppm group, P=0.04 for the 5,000 ppm group, and P≤0.01 for the 10,000 ppm group.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C3 Summary of Reproductive Tissue Evaluations in Male Rats in the 13-Week Feed Study of *m*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	346 ± 8	353 ± 6	338 ± 6	281 ± 5**
Left testicle	1.41 ± 0.026	1.46 ± 0.028	1.47 ± 0.024	0.79 ± 0.087**
Left epididymis	0.46 ± 0.009	0.47 ± 0.010	0.46 ± 0.012	0.29 ± 0.017**
Left epididymal tail	0.16 ± 0.007	0.16 ± 0.008	0.15 ± 0.007[2]	0.08 ± 0.005**
Spermatid measurements				
Spermatid heads (10^7/g testis)	11.38 ± 0.58	9.81 ± 0.49	11.07 ± 0.63	3.54 ± 1.50**
Spermatid heads (10^7/testis)	15.99 ± 0.74	14.26 ± 0.70	16.18 ± 0.88	3.79 ± 1.71**
Spermatid count (mean/10^4 mL suspension)	79.93 ± 3.70	71.30 ± 3.50	80.88 ± 4.40	18.95 ± 8.56**
Spermatozoal measurements				
Motility (%)	70 ± 4	73 ± 3	76 ± 3	20 ± 13[3]
Concentration (10^6/g cet)[4]	581 ± 31	496 ± 28*	629 ± 41[2]	143 ± 52**

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=9.
[3] n=8.
[4] g cet = grams of caudal epididymal tissue.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C4 Summary of Estrous Cycle Characterization in Female Rats in the 13-Week Feed Study of *m*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Necropsy body weight (g)	194 ± 3	195 ± 3	177 ± 2**	166 ± 3**
Estrous cycle length (days)	5.00 ± 0.13	5.00 ± 0.00	6.50 ± 0.38**[2]	6.00 ± 0.58*[3]
Estrous stages as % of cycle[4]				
diestrus	37.5	40.8	49.2	75.8
proestrus	10.0	15.0	15.8	4.2
estrus	36.7	29.2	19.2	11.7
metestrus	15.8	15.0	15.8	7.5
uncertain diagnosis	0.0	0.0	0.0	0.8

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=9; estrous cycle length longer than 12 days or unclear in 1 of 10 animals.
[3] n=3; estrous cycle length longer than 12 days or unclear in 7 of 10 animals.
[4] Evidence by multivariate analysis of variance (MANOVA) suggests that females in the 10,000 ppm group differ significantly (P≤0.01) from controls in the relative frequency of time spent in the estrous stages. Females in this group spent more time in diestrus and less time in other stages than did controls.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C5 Summary of Reproductive Tissue Evaluations in Male Rats in the 13-Week Feed Study of *p*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	353 ± 6	344 ± 11	320 ± 6**	251 ± 6**
Left testicle	1.51 ± 0.025	1.47 ± 0.045	1.42 ± 0.028	1.09 ± 0.079**
Left epididymis	0.46 ± 0.011	0.45 ± 0.020	0.44 ± 0.012	0.33 ± 0.027**
Left epididymal tail	0.18 ± 0.007	0.18 ± 0.009	0.20 ± 0.018	0.13 ± 0.016*
Spermatid measurements				
Spermatid heads (10^7/g testis)	10.13 ± 0.42	9.81 ± 0.41	9.73 ± 0.49	8.71 ± 0.82
Spermatid heads (10^7/testis)	15.29 ± 0.71	14.31 ± 0.55	13.78 ± 0.64	10.03 ± 1.34**
Spermatid count (mean/10^4 mL suspension)	76.43 ± 3.55	71.55 ± 2.74	68.90 ± 3.22	50.15 ± 6.70**
Spermatozoal measurements				
Motility (%)	79 ± 2	77 ± 3	81 ± 2	59 ± 11
Concentration (10^6/g cet)[2]	501 ± 45	604 ± 112[3]	451 ± 40	325 ± 60*[3]

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] g cet = grams of caudal epididymal tissue.
[3] n=9.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C6 Summary of Estrous Cycle Characterization in Female Rats in the 13-Week Feed Study of *p*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Necropsy body weight (g)	202 ± 3	196 ± 3	185 ± 3**	174 ± 3**
Estrous cycle length (days)	5.15 ± 0.13	5.15 ± 0.08	6.05 ± 0.51	5.00[2]
Estrous stages as % of cycle[3]				
diestrus	45.8	45.0	55.0	78.3
proestrus	15.0	14.2	12.5	4.2
estrus	24.2	25.8	20.0	11.7
metestrus	15.0	15.0	12.5	5.8

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=1 (standard error not calculated); estrous cycle length longer than 12 days or unclear in 9 of 10 animals.
[3] Evidence by multivariate analysis of variance (MANOVA) suggests that females in the 10,000 ppm group differ significantly (P≤0.01) from controls in the relative frequency of time spent in the estrous stages. Females in this group spent less time in proestrus and more time in diestrus than controls and other dosed groups.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C7 Summary of Reproductive Tissue Evaluations in Male Mice in the 13-Week Feed Study of *o*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	33.3 ± 0.4	32.8 ± 0.5	29.2 ± 0.3**	25.9 ± 0.3**
Left testicle	0.120 ± 0.002	0.117 ± 0.001	0.113 ± 0.002*	0.107 ± 0.003**
Left epididymis	0.053 ± 0.002	0.051 ± 0.002	0.047 ± 0.002*	0.039 ± 0.001**
Left epididymal tail	0.022 ± 0.001	0.021 ± 0.001	0.018 ± 0.001**	0.015 ± 0.001**
Spermatid measurements				
Spermatid heads (10^7/g testis)	22.49 ± 1.20	21.38 ± 0.82	24.28 ± 0.82	25.28 ± 1.00
Spermatid heads (10^7/testis)	2.69 ± 0.14	2.50 ± 0.10	2.73 ± 0.08	2.70 ± 0.09
Spermatid count (mean/10^4 mL suspension)	83.88 ± 4.25	78.28 ± 3.01	85.30 ± 2.56	84.28 ± 2.94
Spermatozoal measurements				
Motility (%)	77 ± 2	78 ± 2	72 ± 3	48 ± 3**
Concentration (10^6/g cet)[2]	606 ± 37[3]	640 ± 59	447 ± 81[3]	472 ± 76

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] g cet = grams of caudal epididymal tissue.
[3] n=9.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C8 Summary of Estrous Cycle Characterization in Female Mice in the 13-Week Feed Study of *o*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Necropsy body weight (g)	32.8 ± 0.7	32.1 ± 0.8	29.5 ± 0.6**	22.9 ± 0.3**
Estrous cycle length (days)	4.55 ± 0.16	4.40 ± 0.15	4.20 ± 0.13	4.75 ± 0.09[2]
Estrous stages as % of cycle[3]				
diestrus	28.3	28.3	33.3	36.7
proestrus	22.5	19.2	22.5	20.8
estrus	31.7	32.5	26.7	29.2
metestrus	17.5	20.0	16.7	13.3

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=8; estrous cycle length longer than 12 days or unclear in 2 of 10 animals.
[3] Evidence by multivariate analysis of variance (MANOVA) suggests no significant differences from controls in the relative frequency of time that dosed females spent in the estrous stages.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C9 Summary of Reproductive Tissue Evaluations in Male Mice in the 13-Week Feed Study of *m*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	36.5 ± 0.8	34.9 ± 0.7	31.3 ± 0.8**	27.7 ± 0.4**
Left testicle	0.122 ± 0.003	0.117 ± 0.003	0.116 ± 0.003	0.112 ± 0.002*
Left epididymis	0.053 ± 0.002	0.047 ± 0.001*	0.048 ± 0.001*	0.043 ± 0.001**
Left epididymal tail	0.022 ± 0.001	0.020 ± 0.001[2]	0.019 ± 0.001	0.017 ± 0.001**
Spermatid measurements				
Spermatid heads (10^7/g testis)	17.67 ± 1.43	15.99 ± 1.21	19.43 ± 1.62	19.86 ± 1.95
Spermatid heads (10^7/testis)	2.16 ± 0.18	1.88 ± 0.16	2.26 ± 0.21	2.22 ± 0.22
Spermatid count (mean/10^4 mL suspension)	67.33 ± 5.59	58.80 ± 5.05	70.68 ± 6.54	69.25 ± 6.88
Spermatozoal measurements				
Motility (%)	76 ± 1	78 ± 1	73 ± 4	77 ± 2
Concentration (10^6/g cet)[3]	844 ± 54	937 ± 89[2]	792 ± 77	1,052 ± 75

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=9.
[3] g cet = grams of caudal epididymal tissue.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C10 Summary of Estrous Cycle Characterization in Female Mice in the 13-Week Feed Study of *m*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Necropsy body weight (g)	33.8 ± 0.8	33.2 ± 0.4	29.8 ± 0.8**	24.4 ± 0.5**
Estrous cycle length (days)	4.50 ± 0.15	4.38 ± 0.16[2]	4.22 ± 0.09[3]	4.20 ± 0.13
Estrous stages as % of cycle[3]				
diestrus	25.0	30.8	30.0	25.8
proestrus	25.0	21.7	20.0	20.0
estrus	34.2	30.8	31.7	30.8
metestrus	15.8	16.7	18.3	23.3

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] n=8; estrous cycle length longer than 12 days or unclear in 2 of 10 animals.
[3] n=9; estrous cycle length longer than 12 days or unclear in 1 of 10 animals.
[4] Evidence by multivariate analysis of variance (MANOVA) suggests no significant differences from controls in the relative frequency of time that dosed females spent in the estrous stages.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C11 Summary of Reproductive Tissue Evaluations in Male Mice in the 13-Week Feed Study of *p*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Weights (g)				
Necropsy body weight	33.0 ± 0.5	32.3 ± 0.5	31.1 ± 0.4*	29.9 ±0.7**
Left testicle	0.117 ± 0.003	0.115 ± 0.002	0.112 ± 0.002	0.115 ± 0.002
Left epididymis	0.048 ± 0.002	0.046 ± 0.002	0.046 ± 0.002	0.044 ± 0.001
Left epididymal tail	0.017 ± 0.001	0.017 ± 0.001	0.018 ± 0.001	0.015 ± 0.001
Spermatid measurements				
Spermatid heads (10^7/g testis)	18.26 ± 0.63	19.96 ± 0.79	21.06 ± 0.93*	21.21 ± 0.73**
Spermatid heads (10^7/testis)	2.12 ± 0.07	2.29 ± 0.08	2.37 ± 0.12	2.43 ± 0.10*
Spermatid count (mean/10^4 mL suspension)	66.15 ± 2.13	71.65 ± 2.60	74.00 ± 3.74	75.93 ± 3.24*
Spermatozoal measurements				
Motility (%)	72 ± 7	75 ± 2	78 ± 3	74 ± 2
Concentration (10^6/g cet)[2]	1,143 ± 57	1,361 ± 96	1,173 ± 118[3]	1,421 ± 367

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] g cet = grams of caudal epididymal tissue.
[3] n=9.
* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

TABLE C12 Summary of Estrous Cycle Characterization in Female Mice in the 13-Week Feed Study of *p*-Nitrotoluene[1]

Study Parameters	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Necropsy body weight (g)	29.1 ± 0.6	27.9 ± 0.6	27.1 ± 0.4	24.9 ± 0.5**
Estrous cycle length (days)	4.35 ± 0.13	4.10 ± 0.07	4.20 ± 0.11	4.20 ± 0.11
Estrous stages as % of cycle[2]				
diestrus	30.8	28.3	31.7	33.3
proestrus	20.0	24.2	22.5	20.8
estrus	29.2	28.3	30.8	30.8
metestrus	20.0	19.2	15.0	15.0

[1] Data presented as mean ± standard error; n=10, except where noted.
[2] Evidence by multivariate analysis of variance (MANOVA) suggests no significant differences from controls in the relative frequency of time that dosed females spent in the estrous stages.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

APPENDIX D

Genetic Toxicology

TABLE D1 Mutagenicity of *o*-Nitrotoluene in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at SRI, International							
TA100	0.0	145 ± 1.5	120 ± 4.7	160 ± 11.8	126 ± 9.1	154 ± 16.5	117 ± 7.8
	3.0	151 ± 6.1	118 ± 8.5				
	10.0	142 ± 7.8	105 ± 4.7	149 ± 5.4	133 ± 4.7	150 ± 7.6	130 ± 3.8
	33.0	139 ± 12.7	110 ± 7.5	148 ± 15.8	125 ± 0.9	126 ± 12.7	133 ± 7.5
	100.0	146 ± 12.4	104 ± 11.2	154 ± 5.5	125 ± 13.9	134 ± 7.4	147 ± 5.3
	333.0	45 ± 5.9[3]	Toxic	156 ± 7.4	125 ± 7.0	111 ± 6.3	114 ± 1.9
	666.0				137 ± 5.7		119 ± 2.5
	1,000.0			Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[4]		321 ± 11.7	424 ± 16.2	2,113 ± 4.8	1,895 ± 83.7	1,055 ± 61.4	900 ± 15.3
TA1535	0.0	21 ± 3.7	15 ± 1.7	10 ± 1.7	12 ± 2.1	7 ± 2.5	10 ± 1.9
	3.0	22 ± 3.7	10 ± 1.5				
	10.0	22 ± 3.0	11 ± 1.2	7 ± 2.0	6 ± 1.7	9 ± 1.5	10 ± 2.8
	33.0	20 ± 5.5	14 ± 1.5	9 ± 3.2	10 ± 2.6	8 ± 2.5	10 ± 2.1
	100.0	20 ± 3.9	18 ± 5.2	11 ± 3.5	10 ± 1.5	7 ± 2.3	7 ± 2.9
	333.0	2 ± 1.2[3]	0 ± 0.0[3]	7 ± 0.9	8 ± 0.7	11 ± 1.9	11 ± 1.5
	666.0				Toxic		9 ± 3.0
	1,000.0			3 ± 1.8[3]		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		384 ± 17.9	396 ± 2.3	429 ± 31.8	507 ± 35.4	255 ± 18.4	313 ± 77.0
TA1537	0.0	8 ± 0.6	5 ± 2.6	5 ± 1.9	8 ± 2.6	13 ± 2.9	6 ± 0.9
	3.0	12 ± 3.0	4 ± 0.6				
	10.0	9 ± 2.6	3 ± 0.7	7 ± 2.5	9 ± 2.3	11 ± 2.5	5 ± 1.3
	33.0	9 ± 0.0	4 ± 0.3	6 ± 0.9	6 ± 1.2	9 ± 1.5	6 ± 0.7
	100.0	5 ± 0.6	3 ± 0.9	7 ± 1.0	8 ± 2.6	8 ± 0.7	8 ± 0.7
	333.0	0 ± 0.0[3]	Toxic	8 ± 2.6	6 ± 1.5	6 ± 1.5	6 ± 1.0
	666.0				Toxic		6 ± 1.3
	1,000.0			Toxic		0 ± 0.0[3]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		99 ± 3.5	100 ± 18.2	385 ± 63.6	353 ± 32.0	238 ± 4.4	283 ± 14.2
TA98	0.0	25 ± 0.9	22 ± 2.7	28 ± 1.9	28 ± 4.6	38 ± 6.7	24 ± 2.6
	3.0	25 ± 0.6	24 ± 1.9				
	10.0	24 ± 3.7	16 ± 0.9	22 ± 2.2	35 ± 1.7	28 ± 4.3	29 ± 2.3
	33.0	24 ± 3.3	17 ± 1.5	25 ± 4.1	29 ± 1.8	26 ± 4.1	30 ± 0.9
	100.0	19 ± 3.5	36 ± 22.3	25 ± 2.1	27 ± 1.3	27 ± 1.2	34 ± 2.7
	333.0	Toxic	0 ± 0.0[3]	27 ± 0.7	28 ± 2.6	21 ± 3.7	31 ± 3.8
	666.0				0 ± 0.0[3]		32 ± 0.5
	1,000.0			10 ± 5.4[3]		0 ± 0.0[3]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		830 ± 33.4	760 ± 8.0	1,845 ± 75.2	1,761 ± 147.7	613 ± 12.5	640 ± 8.7

TABLE D1 Mutagenicity of *o*-Nitrotoluene in *Salmonella typhimurium* (continued)

Strain	Dose (µg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at EG&G Mason Research Institute							
TA100	0.0	122 ± 9.1	138 ± 16.3	79 ± 2.6	134 ± 6.2	86 ± 10.1	123 ± 7.9
	3.3		135 ± 4.3		127 ± 2.3		149 ± 7.8
	10.0	125 ± 3.5	139 ± 5.8	89 ± 3.5	118 ± 7.6	78 ± 3.2	128 ± 3.2
	33.0	104 ± 1.2	122 ± 6.9	76 ± 3.7	134 ± 2.5	109 ± 6.2	147 ± 21.7
	100.0	113 ± 3.7	121 ± 11.1	89 ± 3.5	135 ± 9.3	118 ± 6.1	142 ± 5.2
	333.0	83 ± 1.9[3]	132 ± 9.2[3]	90 ± 7.3[3]	148 ± 3.3	111 ± 7.2[3]	115 ± 11.0
	666.0	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		2,037 ± 65.0	2,103 ± 44.5	1,741 ± 95.2	1,900 ± 92.3	1,045 ± 81.2	991 ± 44.5
TA1535	0.0	22 ± 2.0	25 ± 3.8	9 ± 1.3	14 ± 1.7	15 ± 1.5	10 ± 0.6
	3.3		26 ± 2.5		15 ± 3.2		10 ± 1.2
	10.0	37 ± 3.6	23 ± 3.3	10 ± 1.8	17 ± 2.5	12 ± 2.3	9 ± 1.7
	33.0	28 ± 3.2	24 ± 1.7	10 ± 1.9	20 ± 1.5	12 ± 0.6	10 ± 3.3
	100.0	28 ± 1.7	22 ± 4.9	8 ± 3.0	16 ± 1.5	13 ± 1.5	19 ± 2.0
	333.0	29 ± 2.9[3]	23 ± 2.8[3]	11 ± 2.0[3]	16 ± 2.5	12 ± 3.8[3]	12 ± 1.2
	666.0	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Equivocal
Positive control		1,380 ± 77.8	1,320 ± 43.1	74 ± 5.6	128 ± 6.3	110 ± 7.0	108 ± 5.5
TA1537	0.0	6 ± 0.7	8 ± 1.5	6 ± 0.6	13 ± 1.2	8 ± 1.0	10 ± 1.5
	3.3	8 ± 2.1	9 ± 2.1	8 ± 0.9			
	10.0	5 ± 1.2	5 ± 1.8	6 ± 0.6	12 ± 0.0	8 ± 0.3	6 ± 1.2
	33.0	6 ± 1.0	6 ± 1.2	13 ± 0.9	9 ± 0.6	8 ± 1.3	7 ± 2.1
	100.0	6 ± 1.3	8 ± 1.3	9 ± 3.1	8 ± 1.2	6 ± 1.2	6 ± 0.9
	333.0	7 ± 1.7[3]	6 ± 0.3[3]	10 ± 2.7[3]	11 ± 0.9	5 ± 0.9[3]	7 ± 0.9
	666.0	Toxic		6 ± 2.1[3]		6 ± 0.3[3]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		361 ± 85.0	901 ± 105.9	118 ± 12.0	173 ± 7.2	123 ± 2.3	71 ± 4.9
TA98	0.0	16 ± 1.2	14 ± 2.7	31 ± 1.5	28 ± 1.2	20 ± 2.3	25 ± 0.7
	3.3		20 ± 3.8		31 ± 1.9		29 ± 1.2
	10.0	20 ± 2.7	16 ± 1.9	29 ± 2.6	31 ± 7.1	22 ± 3.5	27 ± 0.3
	33.0	18 ± 3.2	22 ± 1.2	32 ± 3.1	32 ± 1.9	23 ± 1.2	27 ± 5.2
	100.0	18 ± 4.4	20 ± 1.2	26 ± 6.4	34 ± 2.3	24 ± 4.0	25 ± 1.9
	333.0	14 ± 0.6[3]	12 ± 1.8[3]	27 ± 2.3	36 ± 3.5	27 ± 3.5[3]	27 ± 3.7
	666.0	Toxic		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,661 ± 63.7	2,119 ± 51.4	1,189 ± 54.6	1,454 ± 95.4	1,173 ± 31.0	874 ± 29.2

TABLE D1 Mutagenicity of *o*-Nitrotoluene in *Salmonella typhimurium* (continued)

[1] The detailed protocol and these data are presented in Haworth *et al.* (1983). Cells and *o*-nitrotoluene or solvent (dimethylsulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. The high dose was limited by toxicity; 0 μg/plate dose is the solvent control.

[2] Revertants are presented as mean ± standard error from 3 plates.

[3] Slight toxicity.

[4] Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE D2 Mutagenicity of *m*-Nitrotoluene in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0.0	88 ± 7.5	142 ± 4.6	81 ± 3.3	106 ± 5.9	128 ± 14.0	105 ± 8.5
	3.3	119 ± 7.0	135 ± 6.0	113 ± 5.8	110 ± 5.7	116 ± 3.5	126 ± 4.5
	10.0	114 ± 8.1	136 ± 2.5	112 ± 7.4	110 ± 3.8	109 ± 4.8	115 ± 9.6
	33.0	118 ± 5.9	151 ± 6.1	106 ± 11.0	105 ± 7.7	123 ± 7.9	128 ± 3.7
	100.0	121 ± 4.0	143 ± 10.4	113 ± 8.5	116 ± 5.0	122 ± 3.2	125 ± 1.3
	333.0	103 ± 13.0[3]	120 ± 8.4[3]	80 ± 1.8[3]	106 ± 9.5[3]	119 ± 11.3[3]	133 ± 12.3[3]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[4]		1,620 ± 26.0	2,080 ± 58.4	1,559 ± 67.3	2,190 ± 78.4	893 ± 37.0	994 ± 38.4
TA1535	0.0	27 ± 1.3	29 ± 4.4	10 ± 0.6	13 ± 1.5	12 ± 1.7	10 ± 3.2
	3.3	30 ± 2.6	35 ± 2.2	11 ± 0.3	12 ± 0.9	13 ± 1.7	10 ± 2.0
	10.0	29 ± 1.5	29 ± 6.0	7 ± 1.2	11 ± 1.3	10 ± 0.9	11 ± 2.1
	33.0	32 ± 2.6	33 ± 1.2	8 ± 0.9	8 ± 0.9	11 ± 2.3	9 ± 0.7
	100.0	36 ± 1.5	30 ± 1.5	7 ± 1.7	8 ± 0.3	12 ± 1.8	11 ± 0.6
	333.0	31 ± 1.8[3]	26 ± 0.7[3]	11 ± 2.3[3]	13 ± 1.7	11 ± 0.3[3]	10 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,214 ± 55.5	1,426 ± 28.3	105 ± 0.7	167 ± 9.0	118 ± 8.3	58 ± 2.2
TA1537	0.0	5 ± 0.6	5 ± 0.9	10 ± 0.6	9 ± 2.3	10 ± 0.7	6 ± 1.5
	3.3	6 ± 1.3	8 ± 0.3	8 ± 0.9	11 ± 1.8	11 ± 0.9	6 ± 0.3
	10.0	5 ± 1.7	5 ± 1.2	9 ± 1.8	10 ± 1.9	12 ± 0.6	7 ± 2.4
	33.0	8 ± 1.2	7 ± 1.2	10 ± 1.0	6 ± 1.7	10 ± 0.6	7 ± 1.2
	100.0	8 ± 2.6	3 ± 1.0	10 ± 1.2	13 ± 2.5	12 ± 2.1	8 ± 2.3
	333.0	5 ± 0.3[3]	2 ± 0.9[3]	10 ± 2.1[3]	9 ± 0.3	10 ± 1.0[3]	6 ± 2.3
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		852 ± 128.6	469 ± 107.7	172 ± 14.2	262 ± 12.5	122 ± 12.0	60 ± 6.2
TA98	0.0	16 ± 3.5	21 ± 3.6	27 ± 6.2	29 ± 2.8	33 ± 1.8	25 ± 3.5
	3.3	16 ± 1.5	15 ± 0.3	31 ± 2.9	25 ± 1.5	29 ± 1.9	29 ± 0.9
	10.0	16 ± 1.0	20 ± 3.1	29 ± 4.0	26 ± 1.5	28 ± 3.8	24 ± 1.9
	33.0	19 ± 3.5	19 ± 1.8	33 ± 4.5	25 ± 3.4	30 ± 2.6	32 ± 2.2
	100.0	18 ± 2.0	22 ± 1.8	34 ± 1.8	28 ± 1.2	25 ± 2.1	24 ± 4.6
	333.0	18 ± 2.5[3]	13 ± 1.7[3]	31 ± 0.6[3]	27 ± 3.3[3]	29 ± 4.8[3]	23 ± 4.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,303 ± 41.7	1,707 ± 61.0	1,894 ± 156.8	2,059 ± 84.9	1,113 ± 15.9	779 ± 32.7

[1] Study performed at EG&G Mason Research Institute. The detailed protocol and these data are presented in Haworth *et al.* (1983). Cells and *m*-nitrotoluene or solvent (dimethylsulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. High dose was limited by toxicity or solubility, but did not exceed 10,000 µg/plate; 0 µg/plate dose is the solvent control.

[2] Revertants are presented as mean ± standard error from 3 plates.

[3] Slight toxicity.

[4] Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE D3 Mutagenicity of *p*-Nitrotoluene in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0.0	154 ± 10.8	127 ± 13.3	125 ± 4.4	145 ± 8.7	134 ± 6.3	128 ± 15.3
	3.3		125 ± 10.5				
	10.0	164 ± 8.7	141 ± 5.3		131 ± 3.8		118 ± 9.5
	33.0	155 ± 9.6	109 ± 6.5	131 ± 7.8	134 ± 6.9	145 ± 10.8	131 ± 3.9
	100.0	169 ± 0.9	124 ± 9.8	129 ± 4.5	136 ± 5.7	134 ± 10.1	137 ± 6.2
	333.0	177 ± 7.1[3]	147 ± 3.8[3]	141 ± 12.5	164 ± 5.5	156 ± 9.4	137 ± 4.5
	500.0				143 ± 11.9[3]		136 ± 5.0[3]
	667.0	Toxic		155 ± 3.5[3]		Toxic	
	1,000.0			Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[4]		2,263 ± 120.4	1,343 ± 30.9	1,391 ± 136.2	1,191 ± 27.7	813 ± 60.8	1,061 ± 52.5
TA1535	0.0	30 ± 2.1	22 ± 2.0	12 ± 2.0	11 ± 2.1	14 ± 0.6	9 ± 0.3
	3.0		24 ± 0.9				
	10.0	39 ± 3.3	20 ± 1.3		15 ± 0.7		9 ± 1.9
	33.0	30 ± 1.9	19 ± 1.2	9 ± 0.9	10 ± 1.0	12 ± 0.3	14 ± 2.5
	100.0	35 ± 2.3	19 ± 3.0	19 ± 2.2	12 ± 2.2	13 ± 0.9	11 ± 1.7
	333.0	24 ± 0.9[3]	21 ± 2.7[3]	14 ± 2.9	17 ± 1.9	18 ± 3.5	16 ± 1.5
	500.0				11 ± 2.6[3]		10 ± 1.5[3]
	667.0	Toxic		Toxic		Toxic	
	1,000.0			Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,469 ± 33.8	980 ± 33.6	121 ± 14.2	49 ± 2.5	56 ± 2.7	42 ± 3.5
TA1537	0.0	6 ± 1.5	5 ± 1.9	6 ± 0.3	4 ± 1.5	11 ± 1.5	7 ± 1.7
	3.3		4 ± 2.6				
	10.0	7 ± 0.3	5 ± 0.9		7 ± 0.7		7 ± 1.9
	33.0	5 ± 0.9	4 ± 1.5	6 ± 1.0	6 ± 0.3	13 ± 2.5	5 ± 2.0
	100.0	8 ± 1.5	3 ± 1.2	10 ± 2.2	7 ± 0.0	10 ± 2.4	6 ± 1.0
	333.0	7 ± 2.7[3]	4 ± 0.7	7 ± 0.9	5 ± 2.5	12 ± 3.5	6 ± 2.7
	500.0				6 ± 1.0[3]		6 ± 1.0[3]
	667.0	Toxic		Toxic		Toxic	
	1,000.0			Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		557 ± 68.4	379 ± 61.2	130 ± 11.5	133 ± 3.8	46 ± 7.1	77 ± 6.1
TA98	0.0	25 ± 2.7	13 ± 1.7	30 ± 3.6	23 ± 2.1	31 ± 2.3	23 ± 5.6
	3.3		11 ± 0.6				
	10.0	16 ± 2.4	15 ± 1.7		23 ± 2.5		23 ± 2.3
	33.0	19 ± 4.0	15 ± 1.2	36 ± 3.3	22 ± 0.3	35 ± 3.4	20 ± 1.2
	100.0	18 ± 1.9	14 ± 1.3	31 ± 2.3	27 ± 4.5	31 ± 2.0	24 ± 4.1
	333.0	20 ± 1.9[3]	11 ± 2.0	36 ± 4.2	27 ± 1.2	27 ± 2.8	22 ± 0.9
	500.0				21 ± 5.0[3]		16 ± 2.0[3]
	667.0	Toxic		Toxic		17 ± 0.6[3]	
	1,000.0			Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		904 ± 87.8	1,347 ± 43.7	1,468 ± 63.0	1,045 ± 12.6	515 ± 10.9	752 ± 10.7

TABLE D3 Mutagenicity of *p*-Nitrotoluene in *Salmonella typhimurium* (continued)

[1] Study performed at EG&G Mason Research Institute. The detailed protocol and these data are presented in Haworth *et al.* (1983). Cells and *p*-nitrotoluene or solvent (dimethylsulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. High dose was limited by toxicity. 0 µg/plate dose is the solvent control.

[2] Revertants are presented as mean ± standard error from 3 plates.

[3] Slight toxicity.

[4] Positive control; 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE D4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitrotoluene[1]

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[2]	Average Mutant Fraction
-S9						
Trial 1						
Ethanol						
		68	89	60	29	
		90	104	92	34	
		100	108	89	30	31
Methyl methanesulfonate						
	5	37	24	534	483	
		47	28	511	361	
		47	30	492	349	398[3]
p-Nitrotoluene						
	75	56	61	45	27	
		90	66	73	27	
		84	79	50	20	25
	100	81	72	53	22	
		58	51	51	30	
		61	44	50	27	26
	150	67	52	68	34	
		81	81	69	28	31
	180	85	48	70	27	
		72	38	82	38	
		86	41	124	48	38
	200	59	29	81	46	
		52	22	79	50	
		58	32	53	30	42
	240	30	14	42	47	
		46	21	77	56	
		51	19	68	45	49[3]

TABLE D4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitrotoluene (continued)

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 2						
Ethanol						
		81	77	44	18	
		83	102	48	19	
		103	121	54	17	18
Methyl methanesulfonate						
	5	96	55	345	120	
		68	42	405	198	159[3]
p-Nitrotoluene						
	25	109	81	73	22	
		113	82	71	21	
		109	87	58	18	20
	50	116	72	77	22	
	75	104	56	49	16	
		109	73	45	14	15
	100	114	50	58	17	
		103	56	60	19	
		59	38	70	40	25
	150	95	43	79	28	
		76	39	79	35	
		94	38	67	24	29[3]
	250	36	11	50	47	
		60	13	66	37	
		80	24	53	22	35[3]

TABLE D4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitrotoluene (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9[4]						
Trial 1						
Acetone						
		105	101	77	24	
		88	81	70	26	
		97	105	52	18	
		105	114	59	19	22
Methylcholanthrene						
	2.5	81	50	337	140	
		91	48	328	120	
		75	42	319	143	134[3]
p-Nitrotoluene						
	50	87	76	96	37	
		83	93	63	25	
		84	77	105	42	35[3]
	75	82	68	100	41	
		68	63	77	38	
		94	74	78	28	35[3]
	100	102	71	130	42	
		87	57	120	46	
		86	61	87	34	41[3]
	150	77	60	111	48	
		80	63	85	35	
		68	57	85	42	42[3]
	200	103	42	170	55	
		97	53	109	38	
		80	40	129	54	49[3]
	300[5]	108	41	141	44	
		91	43	71	26	35
	500	Lethal				
		Lethal				

TABLE D4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitrotoluene (continued)

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 2						
Acetone						
		91	107	69	25	
		90	84	66	24	
		104	115	71	23	
		94	94	72	26	25
Methylcholanthrene						
	2.5	56	51	484	286	
		98	109	363	124	
		85	68	501	198	203[3]
p-Nitrotoluene						
	50	101	81	86	28	
		72	102	61	28	
		86	83	64	25	27
	75	72	77	60	28	
		105	88	65	21	
		104	80	68	22	23
	100	108	86	82	25	
		117	79	91	26	
		109	89	113	35	29
	150	111	69	113	34	
		108	98	112	34	
		104	78	87	28	32
	200	90	72	89	33	
		109	71	120	37	
		112	67	123	36	35
	300[5]	99	47	149	50	
		106	57	122	38	
		110	45	106	32	40[3]
	500	Lethal				
		Lethal				

TABLE D4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitrotoluene (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 3						
Acetone						
		79	115	85	36	
		102	108	139	45	
		104	90	128	41	
		101	87	122	40	41
Methylcholanthrene						
	2.5	51	21	842	549	
		55	21	1,031	623	
		67	34	995	497	556[3]
p-Nitrotoluene						
	50	107	70	219	69	
		110	68	185	56	
		120	65	178	49	58
	75	84	61	181	72	
		103	69	263	85	
		112	48	175	52	70[3]
	100	113	62	263	78	
		111	44	234	70	
		86	37	162	63	70[3]
	150	93	35	251	90	
		105	39	380	120	
		87	31	205	79	96[3]
	200	86	17	436	170	
		90	22	353	131	150[3]
	300[5]	75	8	418	186	
		84	13	472	187	
		38	4	479	424	266[3]
	500	Lethal				
		Lethal				
		Lethal				

TABLE D4 Induction of Trifluorothymidine Resistance in Mouse Lymphoma L5178Y Cells by *p*-Nitrotoluene (continued)

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
Trial 4						
Ethanol						
		88	91	108	41	
		106	101	93	29	
		118	108	118	33	34
Methylcholanthrene						
	2.5	96	65	785	272	
		92	41	952	346	
		91	43	726	266	294[3]
p-Nitrotoluene						
	50	77	52	160	70	
		109	82	144	44	
		108	61	139	43	52[3]
	100	108	59	204	63	
		97	52	180	62	62[3]
	150	97	45	189	65	
		111	50	124	37	51
	200	98	40	205	70	
		118	39	233	66	
		88	31	316	119	85[3]
	250[5]	103	28	194	63	
		94	29	300	107	
		105	32	249	79	83[3]
	300[5]	92	19	272	98	
		83	13	388	156	
		94	29	192	68	108[3]

[1] Study performed at Litton Bionetics, Inc. The experimental protocol is presented in detail by Myhr *et al.* (1985). The highest dose of *p*-nitrotoluene was determined by solubility and toxicity. All doses are tested in triplicate. The average of the three tests is presented in the table. Cells (6 x 10^5/mL) were treated for 4 hours at 37° C in medium, washed, resuspended in medium, and incubated for 48 hours at 37° C. After expression, 3 x 10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium and soft agar to determine the cloning efficiency.

[2] Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF/1 x 10^6 cells treated); MF=mutant fraction.

[3] Significant positive response.

[4] Tests conducted with metabolic activation were performed as described in [1] except that S9, prepared from the livers of Aroclor 1254-induced Fischer 344 rats, was added at the same time as the *p*-nitrotoluene and/or solvent.

[5] Precipitate of *p*-nitrotoluene formed at this concentration.

TABLE D5 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *o*-Nitrotoluene[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)[2]
-S9[3]								
Trial 1								
Summary: Questionable								
Dimethylsulfoxide		50	1,035	485	0.46	9.7	25.5	
Mitomycin-C	0.005	50	1,037	2,236	2.15	44.7	25.5	360.15
o-Nitrotoluene	117.000	50	1,039	569	0.54	11.4	34.0[4]	16.87
	176.000	50	1,021	557	0.54	11.1	34.0[4]	16.42
	218.000	50	1,035	576	0.55	11.5	34.0[4]	18.76
	282.000	0						
								P=0.005[5]
+S9[6]								
Trial 1								
Summary: Positive								
Dimethylsulfoxide		50	1,050	380	0.36	7.6	25.5	
Cyclophosphamide	1.50	50	1,049	1,651	1.57	33.0	25.5	334.89
o-Nitrotoluene	354.83	50	1,050	499	0.47	10.0	25.5	31.32*
	380.95	50	1,050	467	0.44	9.3	25.5	22.90*
	423.28	50	1,047	488	0.46	9.8	25.5	28.79*
								P=0.001

TABLE D5 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *o*-Nitrotoluene (continued)

[1] Study performed at Litton Bionetics, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the SCE protocol and these data are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *o*-nitrotoluene or solvent (dimethylsulfoxide) as described in [3] and [5] below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.

[2] SCEs/chromosome of culture exposed to *o*-nitrotoluene relative to those of culture exposed to solvent.

[3] In the absence of S9, cells were incubated with *o*-nitrotoluene or solvent for 2 hours at 37° C. Then BrdU was added and incubation was continued for 23.5 hours. Cells were washed, fresh medium containing BrdU and Colcemid was added, and incubation was continued for 2 to 3 hours.

[4] Because of chemical-induced delay in the cell division cycle, harvest time was extended to maximize the proportion of second division cells available for analysis.

[5] Significance of relative SCEs/chromosome tested by linear regression vs. log of the dose.

[6] In the presence of S9, cells were incubated with *o*-nitrotoluene or solvent for 2 hours at 37° C. The cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 25.5 hours, with Colcemid present for the final 2 to 3 hours. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

* Positive (≥20% increase over solvent control).

TABLE D6 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *m*-Nitrotoluene[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)[2]
-S9[3]								
Trial 1								
Summary: Weak positive								
Dimethylsulfoxide		50	1,031	434	0.42	8.7	25.5	
Mitomycin-C	0.005	25	511	851	1.66	34.0	25.5	295.62
m-Nitrotoluene	3.840	50	1,020	488	0.47	9.8	25.5	13.65
	11.500	50	1,029	442	0.42	8.8	25.5	2.04
	38.400	50	986	475	0.48	9.5	25.5	14.44
	115.000	28	556	297	0.53	10.6	25.5	26.90*
								P=0.003[4]
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,045	477	0.45	9.5	25.5	
Mitomycin-C	0.005	25	519	866	1.66	34.6	25.5	265.56
m-Nitrotoluene	150.000	50	1,035	616	0.59	12.3	33.3[5]	30.39*
	196.000	50	1,033	576	0.55	11.5	33.3[5]	22.16*
	253.000	50	1,017	585	0.57	11.7	33.3[5]	26.02*
								P=0.001

TABLE D6 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *m*-Nitrotoluene (continued)

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)
+S9[6]								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,003	343	0.34	6.9	25.5	
Cyclophosphamide	1.5	25	505	522	1.03	20.9	25.5	202.27
m-Nitrotoluene	38.4	50	1,021	307	0.30	6.1	25.5	-12.08
	115.0	50	1,009	356	0.35	7.1	25.5	3.17
	384.0	50	989	313	0.31	6.3	25.5	-7.45
								P=0.625

[1] Study performed at Litton Bionetics, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the SCE protocol and these data are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *m*-nitrotoluene or solvent (dimethylsulfoxide) as described in [3] and [5] below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.

[2] SCEs/chromosome of culture exposed to *m*-nitrotoluene relative to those of culture exposed to solvent.

[3] In the absence of S9, cells were incubated with *m*-nitrotoluene or solvent for 2 hours at 37° C. Then BrdU was added and incubation was continued for 23.5 hours. Cells were washed, fresh medium containing BrdU and Colcemid was added, and incubation was continued for 2 to 3 hours.

[4] Significance of relative SCEs/chromosome tested by regression vs. log of the dose.

[5] Because of chemical-induced delay in the cell division cycle, harvest time was extended to maximize the proportion of second division cells available for analysis.

[6] In the presence of S9, cells were incubated with *m*-nitrotoluene or solvent for 2 hours at 37° C. The cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 25.5 hours, with Colcemid present for the final 2 to 3 hours. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

* Positive (≥20% increase over solvent control).

TABLE D7 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *p*-Nitrotoluene[1]

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)[2]
-S9[3]								
Trial 1								
Summary: Weak positive								
Dimethylsulfoxide		50	1,038	487	0.46	9.7	25.5	
Mitomycin-C	0.005	25	520	798	1.53	31.9	25.5	227.09
p-Nitrotoluene	50.000	43	889	382	0.42	8.9	25.5	-8.42
	167.000	50	1,024	576	0.56	11.5	32.8[4]	19.89
	500.000[5]	50	1,018	637	0.62	12.7	32.8[4]	33.37*
								P=0.000[6]
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,036	532	0.51	10.6	25.5	
Mitomycin-C	0.005	25	522	596	1.14	23.8	25.5	122.34
p-Nitrotoluene	200.000[5]	50	1,035	732	0.70	14.6	35.8[4]	37.73*
	300.000[5]	50	1,032	698	0.67	14.0	35.8[4]	31.71*
	400.000[5]	50	1,034	803	0.77	16.1	35.8[4]	51.23*
	500.000[5]	0					35.8[4]	
								P=0.000

TABLE D7 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *p*-Nitrotoluene (continued)

Compound	Dose (µg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/ Chromosome (%)
+S9[7]								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,042	467	0.44	9.3	25.5	
Cyclophosphamide	1.5	25	525	1,082	2.06	43.3	25.5	359.86
p-Nitrotoluene	50.0	50	1,043	464	0.44	9.3	25.5	-0.74
	167.0	50	1,044	480	0.45	9.6	25.5	2.59
	500.0[5]	50	1,031	510	0.49	10.2	25.5	10.37
								P=0.052
Trial 2								
Summary: Weak positive								
Dimethylsulfoxide		50	1,041	467	0.44	9.3	25.5	
Cyclophosphamide	1.5	25	517	654	1.26	26.2	25.5	181.99
p-Nitrotoluene	600.0[5]	50	1,027	535	0.52	10.7	25.5	16.12
	700.0[5]	50	1,030	657	0.63	13.1	35.5[4]	42.19*
								P=0.000
Trial 3								
Summary: Positive								
Dimethylsulfoxide		50	1,047	451	0.43	9.0	25.5	
Cyclophosphamide	1.5	25	521	872	1.67	34.9	25.5	288.55
p-Nitrotoluene	550.0[5]	50	1,038	905	0.87	18.1	25.5	102.41*
	600.0[5]	50	1,032	787	0.76	15.7	25.5	77.04*
	650.0[5]	50	1,039	886	0.85	17.7	25.5	97.97*
								P=0.000

TABLE D7 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *p*-Nitrotoluene (continued)

[1] Study performed at Litton Bionetics, Inc. SCE=sister chromatid exchange; BrdU=bromodeoxyuridine. A detailed description of the SCE protocol and these data are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *p*-nitrotoluene or solvent (dimethylsulfoxide) as described in [3] and [6] below and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.

[2] SCEs/chromosome of culture exposed to *p*-nitrotoluene relative to those of culture exposed to solvent.

[3] In the absence of S9, cells were incubated with *p*-nitrotoluene or solvent for 2 hours at 37° C. Then BrdU was added and incubation was continued for 23.5 hours. Cells were washed, fresh medium containing BrdU and Colcemid was added, and incubation was continued for 2 to 3 hours.

[4] Because of chemical-induced delay in the cell division cycle, harvest time was extended to maximize the proportion of second division cells available for analysis.

[5] Precipitate of *p*-nitrotoluene formed.

[6] Significance of relative SCEs/chromosome tested by linear regression vs. log of the dose.

[7] In the presence of S9, cells were incubated with *p*-nitrotoluene or solvent for 2 hours at 37° C. The cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 25.5 hours, with Colcemid present for the final 2 to 3 hours. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

* Positive (≥20% increase over solvent control).

TABLE D8 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *o*-Nitrotoluene[1]

-S9[2]					+S9[3]				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 – Harvest time: 10.5 hours Summary: Negative					**Trial 1** – Harvest time: 10.5 hours Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	3	0.03	3.0		100	5	0.05	4.0
Mitomycin-C					Cyclophosphamide				
0.5	100	20	0.20	16.0	50	100	30	0.30	20.0
o-Nitrotoluene					*o*-Nitrotoluene				
200.7	100	2	0.02	2.0	375.36	100	3	0.03	2.0
252.5	100	1	0.01	1.0	398.82	100	9	0.09	8.0
393.6	100	1	0.01	1.0	422.28	100	5	0.05	5.0
				P=0.873[4]					P=0.168

[1] Study performed at Litton Bionetics, Inc. Abs=aberrations. A detailed presentation of the protocol and these data are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *o*-nitrotoluene or solvent (dimethylsulfoxide) as indicated in [2] and [3]. Cells were arrested in first metaphase by addition of Colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.

[2] In the absence of S9, cells were incubated with *o*-nitrotoluene or solvent for 8.5 hours at 37° C. Cells were then washed and fresh medium containing Colcemid was added for an additional 2 hours followed by harvest.

[3] In the presence of S9, cells were incubated with *o*-nitrotoluene or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8.5 hours. Colcemid was added for the last 2 to 3 hours of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

[4] Significance of percent cells with aberrations tested by linear regression trend test vs. log of the dose.

TABLE D9 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *m*-Nitrotoluene[1]

-S9[2]					+S9[3]				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 – Harvest time: 10.6 hours Summary: Negative					**Trial 1** – Harvest time: 10.4 hours Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	5	0.05	5.0		100	2	0.02	2.0
Mitomycin-C					Cyclophosphamide				
0.5	50	21	0.42	22.0	50	50	50	1.00	42.0
m-Nitrotoluene					*m*-Nitrotoluene				
150	100	0	0.00	0.0	150	100	4	0.04	4.0
300	100	2	0.02	2.0	300	100	4	0.04	4.0
398	50	2	0.04	4.0	398	100	3	0.03	3.0
				P=0.706[4]					P=0.332
Trial 2 – Harvest time: 20.0 hours[5] Summary: Negative					**Trial 2** – Harvest time: 20.0 hours[5] Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	4	0.04	3.0		100	2	0.02	2.0
Mitomycin-C					Cyclophosphamide				
0.063	50	25	0.50	34.0	10	50	20	0.40	18.0
m-Nitrotoluene					*m*-Nitrotoluene				
248	100	6	0.06	6.0	437	100	4	0.04	4.0
299	100	5	0.05	5.0	460	100	2	0.02	2.0
345	100	3	0.03	3.0	483	100	6	0.06	6.0
				P=0.535					P=0.114

[1] Study performed at Litton Bionetics, Inc. Abs=aberrations. A detailed presentation of the protocol and these data are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *m*-nitrotoluene or solvent (dimethylsulfoxide) as indicated in [2] and [4]. Cells were arrested in first metaphase by addition of Colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.

[2] In the absence of S9, cells were incubated with *m*-nitrotoluene or solvent for 8 hours at 37° C. Cells were then washed and fresh medium containing Colcemid was added for an additional 2 to 3 hours followed by harvest.

[3] In the presence of S9, cells were incubated with *m*-nitrotoluene or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 8.4 hours. Colcemid was added for the last 2 to 3 hours of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

[4] Significance of percent cells with aberrations tested by linear regression trend test vs. log of the dose.

[5] Because of significant chemical-induced cell cycle delay, incubation time prior to addition of Colcemid was lengthened to provide sufficient metaphases at harvest.

TABLE D10 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *p*-Nitrotoluene[1]

-S9[2]					+S9[3]				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 – Harvest time: 20.0 hours Summary: Negative					**Trial 1** – Harvest time: 20.3 hours Summary: Weak positive				
Dimethylsulfoxide					Dimethylsulfoxide				
100	3	0.03	3.0		100	9	0.09	8.0	
Mitomycin-C					Cyclophosphamide				
0.062	50	24	0.48	32.0	10	50	40	0.80	44.0
p-Nitrotoluene					*p*-Nitrotoluene				
300	100	7	0.07	7.0	500[5]	100	12	0.12	7.0
400	100	10	0.10	10.0	550[5]	100	10	0.10	8.0
500	100	9	0.09	9.0	600[5]	100	30	0.30	24.0*
				P=0.032[4]					P<0.001
Trial 2 – Harvest time: 21.0 hours Summary: Negative					**Trial 2** – Harvest time: 21.0 hours Weak positive				
Dimethylsulfoxide					Dimethylsulfoxide				
100	7	0.07	6.0		100	7	0.07	7.0	
Mitomycin-C					Cyclophosphamide				
0.062	50	30	0.60	44.0	10	50	14	0.28	26.0
p-Nitrotoluene					*p*-Nitrotoluene				
300	100	9	0.09	8.0	400	100	3	0.03	3.0
400	100	0	0.00	0.0	500	100	2	0.02	2.0
500	100	6	0.06	5.0	550	100	23	0.23	21.0*
				P=0.897					P=0.003

[1] Study performed at Litton Bionetics, Inc. Abs=aberrations. A detailed presentation of the protocol and these data are presented by Galloway *et al.* (1987). Briefly, Chinese hamster ovary cells were incubated with *p*-nitrotoluene or solvent (dimethylsulfoxide) as indicated in [2] and [3]. Cells were arrested in first metaphase by addition of Colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa. Because of significant chemical-induced cell cycle delay, incubation time prior to addition of Colcemid was lengthened from the usual 8- to 10-hour period to provide sufficient metaphases at harvest.

[2] In the absence of S9, cells were incubated with *p*-nitrotoluene or solvent for 18 hours at 37° C. Cells were then washed and fresh medium containing Colcemid was added for an additional 2 to 3 hours followed by harvest.

[3] In the presence of S9, cells were incubated with *p*-nitrotoluene or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 18 to 19 hours. Colcemid was added for the last 2 to 3 hours of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

[4] Significance of percent cells with aberrations tested by linear regression trend test vs. log of the dose.

[5] Precipitate of *p*-nitrotoluene formed at this concentration.

* Positive (≥20% increase over solvent control).

APPENDIX E

Unscheduled DNA Synthesis Assays of *o*-, *m*-, and *p*-Nitrotoluenes in F344/N Rats and B6C3F$_1$ Mice

Introduction

Studies of the ability of the 3 nitrotoluene isomers to induce s-phase and unscheduled DNA synthesis in hepatocytes were performed at Hazelton Laboratories of America, Rockville, MD, using an *in vivo/in vitro* unscheduled DNA synthesis assay (UDS) with male F344 rats, according to the methods of Mirsalis *et al.* (1985). Similar studies with female rats, and male and female B6C3F$_1$ mice were performed with *o*-nitrotoluene.

Materials and Methods

Procurement and Characterization of o-, m-, and p-Nitrotoluenes

o- and *m*-Nitrotoluenes and 2,6-dinitrotoluene (positive control) were obtained from Aldrich Chemical Co. (Milwaukee, WI), and *p*-nitrotoluene from Eastman Kodak Co. (Rochester, NY). Cumulative analytical data of each isomer and the control article indicated a purity of greater than 96%. The test material and positive control were formulated in corn oil for dosing.

Study Design

F344 rats and B6C3F$_1$ mice were obtained from Taconic Farms, Inc. (Germantown, NY) and held for 7 to 8 weeks to allow them to become acclimated to laboratory conditions. Rats were group housed (3 per cage), and mice were individually housed. Animals used in these studies were assigned to treatment groups by weight class using a computer-generated randomization procedure. After the acclimation period, animals were given a single oral gavage dose of 0, 100, 200, or 500 mg/kg for male rats, and 0, 200, 500, or 750 mg/kg for female rats and male and female mice. The animals were approximately 11 to 12 weeks of age at dosing. Dose volume was 5 ml/kg for rats and 10 ml/kg for mice. At specified timepoints, 3 rats or 6 mice were selected from each group for the collection of hepatocytes for UDS determination. Animals were weighed prior to dosing and at termination, and were observed for toxic effects.

At the time points specified, animals were anesthetized with sodium pentobarbitol, and the livers were perfused with Hanks' balanced salts containing ethylene glycol-bis(b-aminoethylether)-N,N-tetra-acetic acid (EGTA) and HEPES buffer at pH 7.2 for 2 - 4 minutes and with a collagenase solution for 4-10 minutes (Williams, 1980; Mirsalis *et al.*, 1982; 1985). The

liver was removed and hepatocytes were obtained by mechanical dispersion of the excised liver tissue in Williams' Medium C with collagenase.

Cells were cultured on plastic coverslips in Williams Medium E for 1.5 to 2 hours at 35°C. Unattached cells were then removed and cultures refed with 2.5 ml Williams' Medium E (without fetal bovine serum) containing ^{3}H-thymidine. Attachment efficiency was determined for each culture using trypan blue dye exclusion *in situ*; all cultures were found to contain >80% viable cells.

After a labeling period of approximately 4 hours, the cultures were refed with Williams' Medium E (without fetal bovine serum) containing unlabeled thymidine, and returned to the incubator for 14 to 19 hours. The incubations then were terminated by washing them with Williams' Medium E without serum. The nuclei were swollen by the addition of 1% sodium citrate to the dishes containing the coverslips, for 8-12 minutes, after which the cells were fixed in glacial acetic acid:ethanol (1:3), washed with deionized water, and dried for at least 24 hours. The coverslips were mounted on glass slides, dipped in Kodak NTB2 photographic emulsion, and dried. Coated slides were stored for 7 days at 4°C in light-tight boxes containing Drierite. The emulsions were developed in D19, fixed, and stained with Williams' modified hematoxylin and eosin procedure.

Evaluation of Unscheduled DNA Synthesis

The cells were examined microscopically at approximately 1500X magnification under oil immersion. UDS was measured by counting nuclear grains and subtracting the largest number of grains from 3 nuclear-sized areas adjacent to each nucleus (background count). This value is referred to as the net nuclear grain count (NNG) and can be a negative number if the number of grains in any background area exceeds the number of grains in the nucleus. The NNG was determined for 30 randomly selected cells on each coverslip. The mean NNG was determined from triplicate coverslips, if available (90 total nuclei), for each treated animal (2 or 3 animals per dose level).

The test was considered positive if an increase in the mean net nuclear grain count was observed to at least 5 grains per nucleus in excess of the concurrent vehicle control, or the percentage of nuclei with 5 or more net grains was increased above 10% of the examined population, in excess of the concurrent vehicle control.

The test is considered negative if the mean net grain count for all groups is less than 1 above the concurrent control value, and/or the percent of nuclei with 5 or more net grain counts does not increase more than 2% above the concurrent vehicle control.

The percentage of cells in S-phase was calculated as those cells exhibiting nuclei blackened by grains too numerous to count. Approximately 2000 cells were counted from randomly selected areas of each slide. For each dose, 3 slides were scored for each of 3 animals (6000 total cells). Significance of the response was determined using the Student's t-test modified for unpaired observations with unequal variances.

Results

Selected results from the UDS studies with *o*-nitrotoluene in male and female rats and mice are shown in Table 1. The average net nuclear grains in the vehicle control cultures was -2.57 for male rats. For the treatment to be considered positive, a net nuclear grain count 5 in excess of this, or 2.43, was required. Thus, the 200 and 500 mg/kg dose level responses were considered indicative of UDS. A positive response for UDS was also seen in female rats and female mice at the top dose (750 mg/kg).

A comparison of UDS activity of the 3 nitrotoluene isomers was performed using similar doses given to male F344 rats (Table 2). From these data, it is apparent that the *o*-nitrotoluene was the only isomer which was positive for induction of UDS.

TABLE 1 Unscheduled DNA Synthesis 12 Hours after Single Oral Gavage Dose of *o*-Nitrotoluene[a]

Dose (mg/kg)	Male Rats	Female Rats	Male Mice	Female Mice
0	-2.57 ± 0.18	-5.96 ± 0.59	-3.66 ± 0.60	-5.06 ± 0.68
100	-0.05 ± 0.47*	NT	NT	NT
200	5.64 ± 0.57**	-2.24 ± 1.00*	-2.67 ± 0.20	-3.15 ± 0.13*
500	13.11 ± 1.14**	0.94 ± 0.93**	-3.60 ± 0.35	-3.05 ± 0.23*
750	NT	1.45 ± 0.93*	-0.60 ± 0.80*	-1.41 ± 0.79**

a Figures indicate mean nuclear grain count ± standard deviation.
* Significantly different from control group (P=0.05).
** Significantly different from control group (P=0.01).
NT No measurements taken.

TABLE 2 Unscheduled DNA Synthesis in Male Rats 12 Hours after Single Oral Gavage Dose of *o*-, *m*-, and *p*-Nitrotoluene[a]

Dose (mg/kg)	*o*-Nitrotoluene	*m*-Nitrotoluene	*p*-Nitrotoluene
0	-2.57 ± 0.18	-3.77 ± 0.35	-2.11 ± 0.41
100	-0.05 ± 0.47*	-3.84 ± 0.23	-2.35 ± 0.24
200	5.64 ± 0.57**	-2.66 ± 0.73	-2.39 ± 0.74
500	13.11 ± 1.14**	-3.44 ± 0.60	-2.49 ± 0.61

a Figures indicate mean nuclear grain count ± standard deviation.
* Significantly different from control group (P=0.05).
** Significantly different from control group (P=0.01).
NT No measurements taken .

o-Nitrotoluene was also found to increase the number of hepatocytes in S-phase, cultured from both male and female rats (Table 3). Mice did not show this response to *o*-nitrotoluene, and neither rats nor mice of either sex showed an increase in S-phase hepatocytes following dosing with *m*- or *p*-nitrotoluene in this assay (data not shown).

TABLE 3 Percent Liver Cells in S-Phase 24 Hours after Single Oral Gavage Dose of *o*-Nitrotoluene[a]

Dose (mg/kg)	Male Rats	Female Rats	Male Mice	Female Mice
0	0.66 ± 0.18	0.58 ± 0.27	0.18 ± 0.05	0.21 ± 0.03
100	0.86 ± 0.42	NT	NT	NT
200	3.61 ± 0.94	2.40 ± 0.70*	0.28 ± 0.04	0.56 ± 0.30
500	3.20 ± 0.47*	7.17 ± 0.70**	0.46 ± 0.16	0.25 ± 0.06
750	NT	12.98 ± 3.90**	0.17 ± 0.10	0.18 ± 0.03

[a] Figures indicate mean nuclear grain count ± standard deviation.
* Significantly different from control group (P=0.05).
** Significantly different from control group (P=0.01).
NT No measurements taken .