National Toxicology Program
Toxicity Report Series
Number 33

NTP Technical Report
on Toxicity Studies of

2-Chloronitrobenzene and 4-Chloronitrobenzene

(CAS Nos. 88-73-3 and 100-00-5)

Administered by Inhalation
to F344/N Rats and B6C3F$_1$ Mice

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Post Office Box 12233
Research Triangle Park, NC 27709

NIH Publication 93-3382
July 1993

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

Foreword

The National Toxicology Program (NTP) is made up of four charter agencies of the United States Department of Health and Human Services (DHHS):

- the National Cancer Institute (NCI) of the National Institutes of Health;
- the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health;
- the National Center for Toxicological Research (NCTR) of the Food and Drug Administration; and
- the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control.

In July 1981, the Carcinogenesis Bioassay Testing Program was transferred from NCI to NIEHS. NTP coordinates the relevant Public Health Service programs, staff, and resources that are concerned with basic and applied research and with biological assay development and validation.

NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

To carry out its mission, NTP designs and conducts studies to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's toxic potential.

The studies described in this toxicity study report were performed under the direction of NIEHS and were conducted in compliance with NTP laboratory health and safety requirements. These studies met or exceeded all applicable federal, state, and local health and safety regulations. Animal care and use were in accord and compliance with the Public Health Service Policy on Humane Care and Use of Animals.

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CONTRIBUTORS

This NTP report on the toxicity studies of 2-chloronitrobenzene and 4-chloronitrobenzene is based primarily on 2-week and 13-week studies that took place in 1989.

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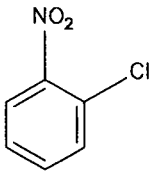
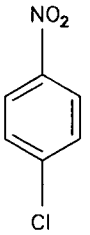
2-Chloronitrobenzene



CAS Number 88-73-3
Synonyms *o*-chloronitrobenzene
2-chloro-1-nitrobenzene
ONCB

4-Chloronitrobenzene



100-00-5
p-chloronitrobenzene
4-chloro-1-nitrobenzene
PNCB

Molecular Formula $C_6H_4ClNO_2$
Molecular Weight 157.56

2-Chloronitrobenzene and 4-chloronitrobenzene are oily yellow solids that are used primarily as chemical intermediates in the production of dyes, lumber preservatives, drugs, and photographic chemicals. Although these chemicals are solids at room temperature, the vapor pressures of these chemicals are sufficiently high to result in significant inhalation exposure. Toxicity studies of 2-chloronitrobenzene and 4-chloronitrobenzene were performed by exposing male and female F344/N rats and B6C3F$_1$ mice to the chemicals by whole-body inhalation 6 hours per day, 5 days per week, for 2 weeks or 13 weeks. Animals were evaluated for histopathology, clinical chemistry (rats), hematology (rats), and reproductive system effects. In separate studies, the dermal absorption of the chemicals was compared, and the absorption, distribution, metabolism, and excretion were partially characterized following oral administration to male F344/N rats. 2-Chloronitrobenzene and 4-chloronitrobenzene were also administered orally to CD-1 Swiss mice for evaluation of reproductive and developmental toxicity. Genetic effects were evaluated in *Salmonella typhimurium*, in Chinese hamster ovary cells, and in *Drosophila melanogaster*.

The highest exposure concentrations used in the 2-week and 13-week studies were limited by technical factors in vapor generation to 18 ppm (115.2 mg/m^3) for 2-chloronitrobenzene

and 24 ppm (153.6 mg/m^3) for 4-chloronitrobenzene. Other concentrations were 0, 1.1, 2.3, 4.5, and 9 ppm (0, 7, 14.7, 28.8, and 57.6 mg/m^3) for 2-chloronitrobenzene and 0, 1.5, 3, 6, and 12 ppm (0, 9.6, 19.2, 38.4, and 76.8 mg/m^3) for 4-chloronitrobenzene.

In 2-week studies with 2-chloronitrobenzene, all rats survived to the end of the study. One of five male mice exposed to 18 ppm died, but weight gains of exposed rats and mice were not affected. Exposed rats and mice had concentration-related increases in liver weights, and spleen weights were increased in rats and mice exposed to 18 ppm. Histopathologic findings in rats were limited to hemosiderin deposition in the liver and spleen at the highest exposure concentration. Exposed mice, primarily those in the 18 ppm groups, had coagulative necrosis, hepatocytomegaly, and granulomatous inflammation in the liver. Splenic changes including increased hematopoietic cell proliferation and hemosiderin deposition occurred at concentrations as low as 4.5 ppm.

In 13-week studies with 2-chloronitrobenzene, all rats survived to the end of the study; 2 of 10 male mice exposed to 18 ppm died. Body weight gains of exposed rats and mice were similar to or somewhat higher than those of the respective controls. Methemoglobinemia occurred in rats and resulted in a normocytic, normochromic anemia that became responsive by the end of the study. Exposed rats and mice had increased liver weights, but these increases were not as great as those seen in the 2-week studies. Spleen weights were increased in exposed rats. Histopathologic changes in rats included increased basophilia of centrilobular hepatocytes, pigmentation and regeneration of the proximal convoluted tubules of the kidney, and hyperplasia of the nasal cavity respiratory epithelium. In mice, hepatocellular necrosis, cytomegaly, mineralization, and chronic inflammation occurred in the liver, primarily in mice in the 18 ppm group, and hematopoietic activity in the spleen was increased.

In 2-week studies with 4-chloronitrobenzene, all rats and mice survived to the end of the studies. Body weight gains of exposed rats were similar to those of the controls; body weight gains of exposed mice were greater than those of the controls. Liver and spleen weights were increased in exposed rats and mice. In rats, histopatholologic changes in the liver were limited to an increase in hemosiderin pigment in Kupffer cells. The spleens of exposed rats were congested and had increased hematopoietic activity and hemosiderin deposition. Kidneys of exposed male rats had lesions consistent with hyaline droplet nephropathy. The proximal convoluted tubules of exposed female rats contained

hemosiderin. Microscopic changes in exposed mice primarily involved increased hematopoietic activity in the spleen and hemosiderin pigmentation in the spleen, liver, and proximal convoluted tubules in the kidney.

In 13-week studies with 4-chloronitrobenzene, there were no deaths that were clearly related to exposure to 4-chloronitrobenzene. Body weight gains of exposed rats and mice were either equal to or greater than those of the controls. A more severe methemoglobinemia developed in rats exposed to 4-chloronitrobenzene than occurred in rats exposed to 2-chloronitrobenzene, and this methemoglobinemia resulted in a responsive macrocytic, hyperchromic anemia. Spleen weights were markedly greater in exposed rats and mice than in controls. In exposed rats, lesions in the spleen, liver, and kidney were similar to those described for the 2-week study. Additionally, increased hematopoietic cell proliferation in bone marrow, histiocytic hyperplasia in mediastinal lymph nodes, testicular atrophy, and chronic inflammation of the harderian gland occurred in exposed rats. In exposed mice, microscopic changes in the spleen and liver were similar to those noted in the 2-week study. Additional lesions included increased hematopoiesis and hemosiderin deposition in the bone marrow of exposed males and females and squamous cell hyperplasia of the forestomach epithelium in female mice.

In reproductive system assessments, there was evidence of decreased spermatogenesis in rats exposed to either 2- or 4-chloronitrobenzene. In mice, effects were limited to a decrease in sperm motility in males exposed to 2-chloronitrobenzene and an increase in estrous cycle length in females exposed to 4-chloronitrobenzene. In continuous breeding studies, a progressive decrease in fertility was noted in CD-1 Swiss mice receiving 4-chloronitrobenzene by oral gavage; fertility was not affected in mice administered 2-chloronitrobenzene by oral gavage.

Percutaneous absorption of [^{14}C]-2-chloronitrobenzene and [^{14}C]-4-chloronitrobenzene was demonstrated in rats. For doses ranging from 0.65 to 65 mg/kg of either chemical, 33% to 40% of 2-chloronitrobenzene and 51% to 62% of 4-chloronitrobenzene were absorbed under nonocclusive conditions. Oral absorption was somewhat higher than dermal absorption for both chemicals, and metabolism was complicated, with over 20 unidentified metabolites isolated from urine of rats given either 2- or 4-chloronitrobenzene.

2-Chloronitrobenzene and 4-chloronitrobenzene were mutagenic in *Salmonella typhimurium* with S9 activation. In addition, both compounds induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells; requirements for S9 activation varied among testing laboratories. Neither compound induced sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* treated as adults or as larvae.

In summary, inhalation exposure of rats and mice to 2- or 4-chloronitrobenzene resulted in methemoglobin formation and oxidative damage to red blood cells, leading to a regenerative anemia and a recognized spectrum of tissue damage and changes secondary to erythrocyte injury. In addition, numerous other lesions that were considered primary toxic effects occurred following exposure. These included renal hyaline droplet accumulation and testicular atrophy in male rats exposed to 4-chloronitrobenzene and hyperplasia of the respiratory epithelium in rats exposed to 2-chloronitrobenzene. A no-observed-adverse-effect level (NOAEL) for rats was not achieved, as increases in methemoglobin and histopathologic changes occurred at exposure concentrations as low as 1.1 ppm for 2-chloronitrobenzene and 1.5 ppm for 4-chloronitrobenzene in the 13-week studies. The NOAEL for histopathologic injury in mice was 4.5 ppm for 2-chloronitrobenzene and 6 ppm for 4-chloronitrobenzene.

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated the draft report on the toxicity studies of 2-chloronitrobenzene and 4-chloronitrobenzene on December 2, 1992, 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 are appropriate and to ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

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SUMMARY OF PEER REVIEW COMMENTS

On December 2, 1992, the Technical Reports Review Subcommittee of the Board of Scientific Counselors for the National Toxicology Program met in Research Triangle Park, NC, to review the draft technical report on toxicity studies of 2-chloronitrobenzene and 4-chloronitrobenzene.

Dr. John R. Bucher, NIEHS, introduced the short-term toxicity studies of 2-chloronitrobenzene and 4-chloronitrobenzene by reviewing the rationale for the studies, experimental designs, and results.

Dr. van Zwieten, a principal reviewer, thought the document was well written and had no major criticisms. He asked that the term "eosinophilic microgranulomas" in the mediastinal lymph nodes of rats in the 13-week study of 4-chloronitrobenzene be clarified. He also wondered if the diagnosis "cytoplasmic basophilia" in the liver of mice in the 13-week 4-chloronitrobenzene study was the most appropriate terminology for the lesion.

Mr. Beliczky, a second principal reviewer, thought that more information about the experimental design should be added to the abstract. He indicated that several sections of the introduction could be updated, and wondered if more recent NIOSH estimates of occupational exposure were available. He asked for clarification of the factors that limited the maximum vapor concentrations in the studies, and suggested that the methods for handling chamber exhaust be added to the report. He also suggested that the report be divided into two reports, one for each chemical, to make the studies easier to follow.

Dr. Carlson, a member of the panel, wished to know the time interval between the end of the exposures and the collection of blood samples for methemoglobin analyses. He wanted to preserve the focus of the report on the comparison of the toxicities of the chemicals and did not want to see the report divided.

Dr. Bucher responded by agreeing to many of the suggested additions to the report. He stated that the diagnostic terms would be reexamined and changed if necessary. He indicated that the desire to prevent exposure of the animals to aerosols was the technical factor that limited the maximum concentrations used in the studies. He agreed that the time interval between exposure and methemoglobin assay was critical and promised to add

that information to the report. He also welcomed the comments of the panel concerning the inclusion of studies of more than one chemical in a report. He stated that chemical studies are reported together to facilitate comparisons and increase the efficiency of report preparation and review.

Following a short discussion of other studies that might be performed with the chloronitrobenzenes, Dr. Klaassen accepted the report on behalf of the peer review panel.

INTRODUCTION

Physical Properties, Production, Use, and Exposure

2-Chloronitrobenzene and 4-chloronitrobenzene are yellow, oily solids with a sweet odor. The two isomers of chloronitrobenzene are manufactured by reacting chlorobenzene with cold, fuming nitric acid. The products of this reaction are 4-chloronitrobenzene (70%), 2-chloronitrobenzene (29%), and 3-chloronitrobenzene (1%) (Roberts *et al.*, 1971). Some of the physical properties of 2-chloronitrobenzene and 4-chloronitrobenzene are given in Table 1.

Table 1 Physical Properties of 2-Chloronitrobenzene and 4-Chloronitrobenzene[1]

Parameter	2-Chloronitrobenzene	4-Chloronitrobenzene
Melting point	32°-33° C	82°-84° C
Boiling point	245°-246° C	242° C
Vapor pressure at 25° C	0.4 mm Hg	0.15 mm Hg
Solubility		
Water at 20° C	0.028% by wt	0.03% by wt
Organic solvents	Soluble in alcohol, benzene, and ether	Soluble in boiling alcohol, ether, and carbon disulfide
Log octanol/water partition coefficient	2.24	2.39

[1] Adapted from Nair *et al.* (1985).

In 1990, an estimated 140 million pounds (approximately 62 million kg) of mixed nitrochlorobenzenes were manufactured in the United States (SRI, 1992). Major production facilities include Monsanto at Sauget, Illinois, and DuPont at Deepwater, New Jersey. Several companies are known to import chloronitrobenzenes (isomer unspecified) (USEPA, 1985).

2-Chloronitrobenzene is used primarily in the manufacture of 2,4-dinitrochlorobenzene, 2-nitrophenol, and 2-nitroaniline, which are ingredients of dyes, lumber preservatives, and photographic chemicals. 4-Chloronitrobenzene is used as an intermediate in the production of 4-nitrophenol, nitroaniline, phenacetin, acetaminophen, and 4-aminophenol.

End products include parathion, sulfur and azo-fast dyes, pharmaceuticals, rubber chemicals, antioxidants, gasoline gum inhibitors, corrosion inhibitors, and photographic chemicals (NIOSH/OSHA, 1978).

According to the National Occupational Exposure Survey taken between 1980 and 1983, 2215 workers in 23 plants were potentially exposed to 2-chloronitrobenzene, and 2948 workers in 30 plants were potentially exposed to 4-chloronitrobenzene (NIOSH, 1984). Exposure levels of approximately 0.03 mg/m^3 2-chloronitrobenzene and 0.05 mg/m^3 4-chloronitrobenzene have been reported in the DuPont plant (Dastur, 1983); Monsanto reported chloronitrobenzene exposure levels in their facility ranging from 0.11 to 0.45 mg/m^3 (Keating, 1983). In the 1950's and 1960's, exposure to higher chloronitrobenzene concentrations was more common, and clinical cases of cyanosis were seen at the DuPont plant (Linch, 1974). Exposure occurs by inhalation and by dermal contact.

Eight-hour, time-weighted average workplace exposure limits for 4-chloronitrobenzene in air were set at 0.1 ppm (0.64 mg/m^3) by the American Conference of Governmental Industrial Hygienists (ACGIH, 1991-1992) and 1 mg/m^3 (0.15 ppm) by the Occupational Safety and Health Administration (Federal Register 39, 23540, 1974). The 4-chloronitrobenzene exposure limit carries a skin notation, indicating that dermal exposure could be harmful. No exposure limit has been recommended for 2-chloronitrobenzene. These standards were based on hematologic findings in exposed workers.

The general population is not expected to come in contact with either chloronitrobenzene isomer except through environmental contamination. 2-Chloronitrobenzene, an industrial pollutant in the lower Mississippi River (Rosen, 1981), has been found in the tissues of fish near a plant at Sauget, Illinois, at a concentration of 0.24 mg/kg; 60 miles south of St. Louis, Missouri, at a concentration of 0.12 mg/kg; and 150 miles from the Sauget plant, at concentrations ranging from 0.006 to 0.027 mg/kg (Yurawecz and Puma, 1983). 4-Chloronitrobenzene has been identified in waste water from a plant that manufactured nitrobenzene, nitrophenol, aniline, and oil additives (Shafer *et al.*, 1979).

Disposition and Metabolism

The metabolism of 2-chloronitrobenzene and 4-chloronitrobenzene in rabbits was described by Bray *et al.* (1956). Pathways for the metabolism of 2-chloronitrobenzene are outlined in Figure 1. The major products result from reduction of the nitro group to the amine and hydroxylation of the benzene ring. The phenols formed are excreted primarily in the urine, conjugated with glucuronic acid (about 20% to 40%), sulfate (about 20%), or mercapturic acid (less than 10%). About 10% of the doses administered to rabbits were recovered from the urine as chloroaniline.

Ridley *et al.* (1983) studied the metabolism of 4-chloronitrobenzene in male rats given a single 200 mg/kg oral dose of [^{14}C]-4-chloronitrobenzene. Seventy-two hours after dosing, 74.6% of the radiolabel was recovered in the urine and 20.5% was recovered in the feces. Most of the radiolabel remaining in the carcass was found in the spleen and blood. Two major early urinary metabolites were a nitrochlorophenol, excreted as a glucuronide or sulfate, and an *N*-acetyl cysteine conjugate of nitrobenzene. The latter presumably arises from the dechlorination of 4-chloronitrobenzene catalyzed by glutathione-S-transferase. Two other urinary metabolites were tentatively identified as aminochlorophenol and *N*-acetyl aminochlorophenol.

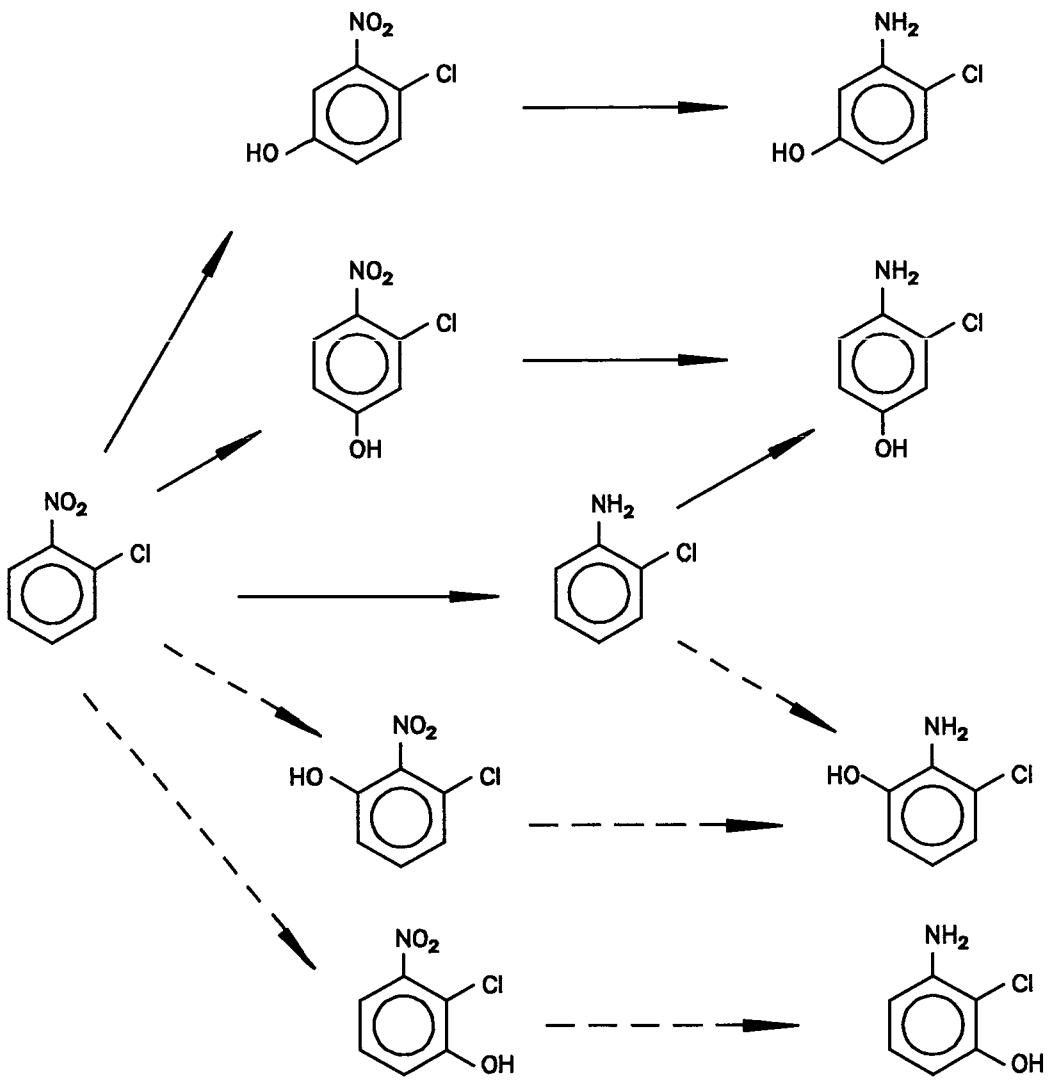

FIGURE 1 Phenolic Metabolites of 2-Chloronitrobenzene Excreted (Free or Conjugated) in Rabbit Urine (Adapted from Bray *et al.*, 1956)

Toxicity

TOXIC EFFECTS IN HUMANS

In 1923, an English chemical firm began manufacturing 2-chloronitrobenzene and 4-chloronitrobenzene. The nitration of chlorobenzene was carried out in a large tank, and the resulting hot oil was cooled in trays. 4-Chloronitrobenzene was crystallized, and the supernatant, 2-chloronitrobenzene, was run off into drums. 4-Chloronitrobenzene was shoveled into a centrifuge for drying and then placed in casks. Workers generally required hospitalization after about 3 days of exposure. Symptoms included a slate gray appearance, headache, and dyspnea on exertion. Blood serum was often a port wine color, and erythrocytes were large and occasionally deformed. Workers received treatments of atropine, ether, pituitary extract, and a coffee enema. Despite this, all recovered over a period of several weeks. The physicians recognized the similarity of the symptoms to those of aniline poisoning and proposed that the chloronitrobenzenes were converted to chloroanilines through the "reducing power of hemoglobin, or of the tissues themselves." At a second plant in Switzerland, similar cases were even more prevalent. The higher number of cases had been attributed to the continental workmen having a lower tolerance than the British laborers, but the physicians correctly suggested that the higher altitude in Switzerland may exaggerate the toxic effect on the blood (Renshaw and Ashcroft, 1926). Subsequent publications on occupational exposures to chloronitrobenzenes have noted the same general symptoms as the report by Renshaw and Ashcroft.

TOXIC EFFECTS IN ANIMALS

The acute oral LD_{50} for 2-chloronitrobenzene was reported to be 140 mg/kg in CF-1 mice and 270 mg/kg in Sprague-Dawley rats. For 4-chloronitrobenzene, the acute oral LD_{50} was reported to be 1410 mg/kg for CF-1 mice and 810 mg/kg for Sprague-Dawley rats (Vernot *et al.*, 1977). In rabbits, the dermal LD_{50} of 2-chloronitrobenzene was 400 mg/kg, and the dermal LD_{50} for 4-chloronitrobenzene was 3040 mg/kg (Monsanto Corporation, 1986). Studies conducted by Monsanto Corporation indicate that neither 2-chloronitrobenzene nor 4-chloronitrobenzene is irritating to rabbit skin, and each isomer causes only slight, transient corneal cloudiness when placed in the eye of rabbits (Dastur, 1983). The compounds are not considered corrosive.

The primary toxic response associated with the administration of chloronitrobenzenes to animals is methemoglobin formation. Ridley *et al.* (1983) measured methemoglobin levels

in male rats administered a single oral dose of 200 mg/kg 4-chloronitrobenzene. Six hours after dosing, treated rats had methemoglobin levels of 14.2%, compared to levels of 1.0% in the controls. Peak methemoglobin levels (not reported) were observed 24 hours after dosing, and decreased to 12.1% by 72 hours. Watanabe *et al.* (1976) gave male Wistar rats a single intraperitoneal dose of 100 µmol/kg 4-chloronitrobenzene in propylene glycol. Five hours after dosing, methemoglobin levels were 16.3%. Male Wistar rats given a single intraperitoneal dose of 100 µmol/kg 2-chloronitrobenzene had methemoglobin levels of 20.6% after 5 hours. Incubation of 2-chloronitrobenzene with hemoglobin *in vitro* did not cause increases in methemoglobin levels (Watanabe *et al.*, 1976). These findings are consistent with a requirement of *in vivo* reduction of the chloronitrobenzenes to chloronitroanilines for methemoglobin formation. Methemoglobin formation caused by nitrobenzene has been demonstrated to occur only after reduction of nitrobenzene to aniline by gut microflora. Germ-free or antibiotic-treated animals were unable to convert sufficient nitrobenzene to aniline to cause methemoglobin formation (Reddy *et al.*, 1976). Carr *et al.* (1979) demonstrated similar results with 4-chloronitrobenzene.

Hasegawa and Sato (1963) injected rabbits with a single 500 mg/kg dose of 4-chloronitrobenzene in sesame oil to study Heinz body formation. During the first 7 hours after dosing, methemoglobin levels increased rapidly, and within 24 hours all erythrocytes contained Heinz bodies. Nishida *et al.* (1982) reported increased numbers of Heinz bodies and increased osmotic fragility of erythrocytes in rabbits that received a single subcutaneous dose of 50 to 200 mg/kg 4-chloronitrobenzene.

Davydova (1967) gave oral doses of 0, 0.0025, 0.005, 0.025, or 5.0 mg/kg 2- or 4-chloronitrobenzene to rats (strain and sex unspecified) daily for 7 months. Effects were noted only at the highest dose of each chemical. Rats dosed with 4-chloronitrobenzene had slightly increased levels of methemoglobin (0.5% in treated rats versus 0.2% in control rats) after 2 months of dosing, while methemoglobin levels in rats treated with 2-chloronitrobenzene were increased (0.4% in treated rats versus 0.2% in controls) after 6 months of dosing. The changes in methemoglobin levels were accompanied by increases in reticulocyte counts and numbers of Heinz bodies. Blood alkaline phosphatase activity increased slightly in rats dosed with 4-chloronitrobenzene, but not in rats dosed with 2-chloronitrobenzene.

Nair *et al.* (1986a) exposed Sprague-Dawley rats to 0, 9.9, 30, or 59 mg/m^3 (0, 1.6, 5, or 10 ppm) 2-chloronitrobenzene vapor for 6 hours per day, 5 days per week, for 4 weeks. The only findings were in animals exposed to 30 or 59 mg/m^3 and included increased blood methemoglobin levels (up to 6%) compared to those of the controls (0.3%) and mild decreases in hemoglobin concentration and hematocrit. Spleen and liver weights were increased by about 30% at the highest exposure level. Microscopic examination revealed increased extramedullary hematopoiesis in the spleen of animals exposed to 30 or 59 mg/m^3 and hemosiderosis in the spleen of rats at all exposure levels.

In another 4-week inhalation study, rats were exposed to 0, 5, 15, or 45 mg/m^3 (0, 0.82, 2.5, or 7.5 ppm) 4-chloronitrobenzene in ethylene glycol monoethyl ether for 6 hours daily, 5 days per week (Nair *et al.*, 1986b). Cyanosis was diagnosed in all exposed groups. Increases in blood methemoglobin levels and decreases in hemoglobin, hematocrit, and erythrocyte counts were exposure concentration related. Leukocyte counts were elevated and spleen and liver weights were increased in rats exposed to 45 mg/m^3. Microscopic examination revealed congestion, increased extramedullary hematopoiesis, and hemosiderosis in the spleen at all exposure levels (Nair *et al.*, 1986b).

BIOCHEMICAL TOXICITY

Brain succinate dehydrogenase activity was decreased 24 hours after dosing in rats that received a subcutaneous dose of 4.69 mmol/kg 4-chloronitrobenzene (Goldstein, 1976). However, the respiration rate of brain slices was not affected. Oxygen consumption was inhibited 50% in Ehrlich ascites cells in 1 mM 4-chloronitrobenzene and in Chinese hamster V79 lung cells in 0.1 mM 4-chloronitrobenzene (Bigalow *et al.*, 1978). Kimes and Carr (1982) reported that 4-chloronitrobenzene inhibited the activity of type B monoamine oxidase obtained from rabbit liver mitochondria (K_i = 0.43 µM).

TERATOGENICITY AND REPRODUCTIVE EFFECTS

Nair *et al.* (1985) administered 4-chloronitrobenzene by gavage to Sprague-Dawley rats at doses of 0, 5, 15, or 45 mg/kg per day on gestation days 6 through 19 and to New Zealand rabbits at doses of 0, 5, 15, or 40 mg/kg per day on gestation days 7 through 19. In rats, no mortality of dams occurred, but high-dose dams had reduced weight gain and increased spleen weights. The number of resorptions per dam (5.6 ± 5.8 in high-dose dams versus 0.5 ± 0.7 in controls) and the incidence of skeletal malformations (29 in 10 litters from

high-dose dams versus two in two litters from controls) were significantly higher in the high-dose group than in the controls. No effects were seen at the lower doses. In rabbits, 44% of the high-dose dams died. No significant maternal or fetal toxicity or teratogenic effects were seen at the lower doses.

In a reproduction study of 4-chloronitrobenzene, doses of 0.1, 0.7, or 5.0 mg/kg per day were administered by gavage in corn oil to male and female Sprague-Dawley rats beginning 14 weeks prior to mating and continuing throughout gestation and lactation (Nair *et al.*, 1989). Part of the F_1 generation received the same doses beginning 18 weeks prior to mating and continuing throughout gestation and lactation. There were no dose-related effects on weight gain, mortality, or clinical signs. Slight decreases in the pregnancy rate and male fertility index were reported in the 5.0 mg/kg group in the F_0 generation.

CARCINOGENICITY

Weisburger *et al.* (1978) reported the results of 2-year dietary studies of 2-chloronitrobenzene and 4-chloronitrobenzene. The chemicals were administered in feed for 18 months to groups of 25 male CD rats and 25 male and 25 female CD-1 mice. The mice were held for 3 additional months and the rats for 6 months before sacrifice. The dietary levels of 2-chloronitrobenzene and 4-chloronitrobenzene were adjusted during the study when evidence of toxicity was found. The exposure levels used are listed in Table 2.

TABLE 2 Concentrations of 2-Chloronitrobenzene and 4-Chloronitrobenzene Administered to CD Rats and CD-1 Mice in Feed for 18 Months[1]

	2-Chloronitrobenzene		4-Chloronitrobenzene	
	Concentration (ppm)	Duration (months)	Concentration (ppm)	Duration (months)
Male rats	0, 1000, and 2000	6	0, 2000, and 4000	3
	0, 500, and 1000	12	0, 250, and 500	2
			0, 500, and 1000	13
Mice	0, 3000, and 6000	8	0, 3000, and 6000	18
	0, 1500, and 3000	10		

[1] Weisburger *et al.* (1978).

The incidences of liver tumors were increased in female mice exposed to 2-chloronitrobenzene. Male and female mice exposed to 4-chloronitrobenzene had increased incidences of malignant vascular tumors.

Nair *et al.* (1989) reported that 4-chloronitrobenzene, when administered by gavage in corn oil for 2 years at doses of 0, 0.1, 0.7, or 5.0 mg/kg per day to groups of 60 Sprague-Dawley rats, was not carcinogenic. Increased methemoglobin levels and slight anemia were noted in rats in the high-dose groups. In other 2-year studies, the administration of *p*-chloroaniline caused an increased incidence of fibrosarcomas in the spleen of male rats and increased incidences of hepatocellular tumors and hemangiosarcomas in male mice (Chhabra *et al.*, 1991).

GENETIC TOXICITY

Genotoxicity data for 2-chloronitrobenzene and 4-chloronitrobenzene are limited. Both chemicals were mutagenic in *Salmonella typhimurium* when tested in the presence of induced liver S9 (Haworth *et al.*, 1983), and mutagenic activity was also reported in one strain of *S. typhimurium* in the absence of S9 activation (Haworth *et al.*, 1983; Shimizu *et al.*, 1983). Neither compound induced sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* treated as adults (Zimmering *et al.*, 1985) or as larvae (Zimmering *et al.*, 1989). A positive response was reported with 4-chloronitrobenzene for induction of DNA repair synthesis in rat hepatocytes *in vitro*, as measured by alkaline elution (Cesarone *et al.*, 1984). Also, an increase in the occurrence of single-strand DNA breaks was observed in brain, kidney, and liver cells of Swiss mice treated with 4-chloronitrobenzene *in vivo* (Cesarone *et al.*, 1983). 4-Chloronitrobenzene induced sister chromatid exchanges in the presence of S9 and chromosomal aberrations with and without S9, although the positive responses for chromosomal aberrations occurred at doses that were severely toxic (Galloway *et al.*, 1987).

The results of studies of the structural analogue 3-chloronitrobenzene were negative with and without S9 activation in *S. typhimurium* mutagenicity assays (Simmon *et al.*, 1977; Haworth *et al.*, 1983; Shimizu *et al.*, 1983) and in Chinese hamster ovary cell tests for induction of chromosomal aberrations and sister chromatid exchanges (Galloway *et al.*, 1987).

Study Rationale and Design

2-Chloronitrobenzene and 4-chloronitrobenzene were nominated to the NTP for general and reproductive toxicity and metabolism studies by the U.S. Environmental Protection Agency based on the high production volumes and evidence of significant worker exposure and environmental contamination. Existing carcinogenicity studies were considered inadequate. Inhalation was chosen as the route of exposure for the toxicity evaluations because 2-chloronitrobenzene and 4-chloronitrobenzene are known to be sufficiently volatile to result in toxic concentrations in air and because inhalation is a major route of worker exposure. Endpoints evaluated in the inhalation studies included histopathology and clinical pathology in F344/N rats and B6C3F$_1$ mice. The disposition and metabolism of 2-chloronitrobenzene and 4-chloronitrobenzene in rats were studied following single or repeated administration by the oral and dermal routes. The effects of these chemicals on reproduction were assessed by evaluation of testicular and spermatozoal parameters and determination of the length of the estrous cycle in animals in the 13-week inhalation studies and by performance of continuous breeding studies in mice given 2- or 4-chloronitrobenzene by oral gavage in corn oil. In addition, the genetic toxicity of these chemicals was assessed in *in vitro* studies in *S. typhimurium* and Chinese hamster ovary cells and in *in vivo* studies of sex-linked recessive lethal mutations in *D. melanogaster*.

MATERIALS AND METHODS

Procurement and Characterization of 2-Chloronitrobenzene and 4-Chloronitrobenzene

Single lots of 2-chloronitrobenzene (Lot ET 00210KM) and 4-chloronitrobenzene (Lot ET 02513BT) were obtained from the Aldrich Chemical Company (Milwaukee, WI). These lots were used throughout the 2-week and 13-week studies.

2-Chloronitrobenzene: Chemical analyses performed by Midwest Research Institute (MRI; Kansas City, MO) identified the chemical, a pale yellow, flaky solid, as 2-chloronitrobenzene. The melting point of the 2-chloronitrobenzene samples agreed with literature references. Infrared, ultraviolet/visible, and nuclear magnetic resonance spectra were consistent with the structure of 2-chloronitrobenzene and with literature references (*Sadtler Standard Spectra*). The results of elemental analyses for carbon, hydrogen, nitrogen, and chlorine agreed with theoretical values for 2-chloronitrobenzene. Karl Fischer analysis indicated less than 0.3% water. Functional group titration by nitro reduction with titanium (III) chloride followed by back titration with standardized ferric ammonium sulfate indicated a purity of 97% ± 3%. Thin-layer chromatography (TLC) by two solvent systems indicated a major product spot only. Gas chromatography by two systems indicated no impurities with areas greater than 0.1% relative to the major peak area. The cumulative data indicated a purity greater than 99% for 2-chloronitrobenzene.

Stability studies performed by MRI with gas chromatography indicated that 2-chloronitrobenzene was stable as a bulk chemical for 2 weeks when stored under a nitrogen headspace, protected from light, at temperatures up to 60° C. At the study laboratory, the bulk chemical was stored in amber glass containers under a nitrogen headspace at approximately 20° C. The study laboratory monitored the stability of the bulk chemical with gas chromatography throughout the studies; no degeneration of 2-chloronitrobenzene was observed.

4-Chloronitrobenzene: Chemical analyses performed by MRI identified the chemical, a pale yellow, crystalline solid, as 4-chloronitrobenzene. Infrared, ultraviolet/visible, and nuclear magnetic resonance spectra were consistent with the structure of 4-chloronitrobenzene and with literature references (*Sadtler Standard Spectra*). The melting point of the

4-chloronitrobenzene samples was consistent with literature references, and the results of elemental analyses for carbon, hydrogen, nitrogen, and chlorine agreed with theoretical values for 4-chloronitrobenzene. Karl Fischer analysis indicated less than 0.3% water. Functional group titration by nitro reduction with titanium (III) chloride followed by back titration with standardized ferric ammonium sulfate indicated a purity of 97% ± 1%. Thin-layer chromatography (TLC) by two solvent systems indicated a major product spot only. Gas chromatography by two systems resolved a single impurity; the first system indicated an area of approximately 0.4% and the second an area of approximately 0.3% for the impurity relative to the major peak area. The cumulative data indicated a purity of approximately 99% for 4-chloronitrobenzene.

Stability studies performed by MRI with gas chromatography indicated that 4-chloronitrobenzene was stable as a bulk chemical for at least 2 weeks at temperatures up to 60° C when stored protected from light. At the study laboratory, the bulk chemical was stored in plastic bags inside metal cans at approximately 20° C. The study laboratory monitored the stability of the bulk chemical with gas chromatography throughout the studies; no degeneration of 4-chloronitrobenzene was observed.

Vapor Generation System

The 2-chloronitrobenzene and 4-chloronitrobenzene vapor exposures were conducted with an automated data acquisition and control system. A central computer (HP 9816; Hewlett-Packard, Palo Alto, CA) monitored and controlled the basic chamber functions (chemical concentration, airflow, vacuum, temperature, and relative humidity) in the exposure rooms with on-line data collected by HP-85B computers and other data collected by an Intelligent Interface System (Model 53A-IBX; Colorado Data Systems, Englewood, CO). Animals were exposed and maintained in inhalation exposure chambers developed at Battelle Pacific Northwest Laboratories and produced by Harford System Division of Lab Products, Incorporated (Aberdeen, MD). Each chamber had an active mixing volume of 1.7 m^3.

All vapor transport lines and all airflows except the individual chamber dilution air inlet flows were heated and/or insulated to prevent crystallization of 2-chloronitrobenzene and 4-chloronitrobenzene, due to the low volatility and low melting points of these compounds. Bulk 4-chloronitrobenzene was transferred into a flask and attached to a vapor generator with a rotary evaporation system (Büchi Rotavapor Model EL-131S; Büchi Laboratoriums Technik AG, Flavil, Switzerland). Bulk 2-chloronitrobenzene was melted by immersion of

the storage container in a warm-water bath prior to being transferred to the flask. The flask was immersed in a hot-oil bath and then rotated; a stream of heated nitrogen was metered into the flask. The resulting vapor was forced into a condenser with temperature maintained by a water bath in all 2-chloronitrobenzene studies and in the 2-week 4-chloronitrobenzene studies and by circulating oil in the 13-week 4-chloronitrobenzene studies. Condensate was returned to the rotating flask.

Generator output was set to provide vapor to meet target concentrations in the exposure chambers plus a surplus (20% 2-chloronitrobenzene or 30% 4-chloronitrobenzene) delivery line flow to offset losses to the surfaces of the delivery system and to allow for adjustments to the vapor delivery system's operating parameters. Unused delivery line flow was vented to waste.

The temperature of the saturated vapor leaving the condenser was well above room temperature. Vapor was transported to the exposure room via heated Teflon® transport line (E.I. DuPont deNemours and Co., Wilmington, DE) that was maintained at a temperature above the exit vapor temperature. An Air-Vac® (Air-Vac Engineering Co., Milford, CT) pump drew the vapor through the line and injected it into the heated, filtered dilution air stream of a vapor distribution manifold. Flow and concentration were automatically controlled. Temperature-controlled Teflon delivery lines carried the vapor from the distribution manifold to the exposure chambers. An Air-Vac pump at the junction of each delivery line withdrew the appropriate amount of vapor from the manifold. The vapor passed through a pneumatic three-way valve and was directed to the chamber or to the chamber exhaust system. Chambers were exhausted by a damped downstream vacuum; the exhaust was diluted with building air, passed through HEPA filters, and vented to the atmosphere.

Concentration Monitoring

2-Chloronitrobenzene and 4-chloronitrobenzene vapor concentrations were monitored with a gas chromatographic system (HP 5890) equipped with an electron capture detector and an HP 3393 integrator. This system was used to measure the 2- or 4-chloronitrobenzene concentration in the exposure and control chambers, the exposure room, an on-line standard, and nitrogen blank samples. Samples from multiple positions were taken via a 12-port stream select valve fed by sampling lines. Calibration of the on-line chamber monitor was based on quantitative analysis of grab samples taken from the exposure

chambers; these samples were analyzed with an off-line gas chromatographic system calibrated with gravimetrically prepared standards of 2- or 4-chloronitrobenzene.

The concentration of 2- or 4-chloronitrobenzene in the chamber was defined by the correlation between chamber concentrations determined by analysis of the grab samples and the on-line monitor peak area at the times of sample collection. Possible drift in the calibration of the on-line gas chromatograph was determined by monitoring an on-line standard. Neat 2- or 4-chloronitrobenzene in a nitrogen carrier gas was used as the standard.

In the 2-week and 13-week 4-chloronitrobenzene studies, on-line monitor drift occurred more frequently than expected. When drift was noted at the start of an exposure day, exposure was continued at the generator settings from the previous day or at slightly lower settings. Chamber grab samples were collected and analyzed, and the gas chromatograph was recalibrated. Replacement of the on-line monitor in the 13-week study with that used in the 2-chloronitrobenzene studies and changing the on-line carrier gas to tank nitrogen did not significantly decrease drift.

Mean chamber concentrations were calculated from daily monitoring data (Table 3). The mean concentrations in all chambers for the 2-week 2-chloronitrobenzene studies were between 96% and 105% of the target concentrations, with relative standard deviations ranging from 4% to 8%. At least 88% of all individual concentration measurements were within 10% of target concentrations except for measurements for the 18 ppm chamber, where 82% of readings for rats and 81% of readings for mice were within 10% of the target concentration. The mean concentrations in all chambers for the 2-week 4-chloronitrobenzene studies were between 96% and 103% of the target concentrations, with relative standard deviations ranging from 8% to 21%; for each chamber, 60% to 86% of the individual concentration measurements were within 10% of target concentrations. The mean concentrations in all chambers for the 13-week 2-chloronitrobenzene studies were between 98% and 101% of the target concentrations, with relative standard deviations ranging from 6% to 8%; at least 89% of all individual concentration measurements were within 10% of target concentrations. The mean concentrations in all chambers for the 13-week 4-chloronitrobenzene studies were between 99% and 100% of the target concentrations, with relative standard deviations ranging from 6% to 8%; at least 88% of all individual concentration measurements were within 10% of target

concentrations. The low volatility of 4-chloronitrobenzene caused prolonged buildup times and large variations in concentration resulting from small temperature changes, making the concentration difficult to control. The low volatility of both chloronitrobenzenes was the primary factor limiting the maximum exposure concentrations used in these studies. The generation of higher concentrations would have required the use of aerosols, but this method was not appropriate for these studies.

Chamber Characterization

CONCENTRATION UNIFORMITY

During the 2-week and 13-week studies, the uniformity of vapor concentration throughout each exposure chamber was measured prior to the start of the studies and once during the studies. The uniformity of all chambers was within the specified limits of ±5% for all studies.

CONCENTRATION BUILDUP AND DECAY

During the 2-week and 13-week studies, buildup and decay rates were measured prior to the start of the studies without animals and during the studies with animals to determine whether the presence of animals in the chambers would affect these rates. The time following the start of exposure for the 2- or 4-chloronitrobenzene concentration to reach 90% of the final stable concentration in the chamber (T_{90}) and the time following the termination of generation for the vapor concentration to decay to 10% of the stable concentration (T_{10}) were determined.

For the 2-week 2-chloronitrobenzene studies, T_{90} ranged from 13 to 20 minutes without animals in the chambers and from 16 to 25 minutes with animals present. T_{10} ranged from 8 to 18 minutes without animals and from 9 to 18 minutes with animals. A T_{90} of 20 minutes was chosen for the 2-chloronitrobenzene studies. For the 2-week 4-chloronitrobenzene studies, T_{90} ranged from 9 to 17 minutes without animals in the chamber and 8 to 23 minutes with animals present. T_{10} ranged from 14 to 18 minutes without animals and from 18 to 33 minutes with animals present. A T_{90} of 18 minutes was used for the 4-chloronitrobenzene studies.

TABLE 3 Mean Chamber Concentrations of 2-Chloronitrobenzene and 4-Chloronitrobenzene in the 2-Week and 13-Week Inhalation Studies in F344/N Rats and B6C3F$_1$ Mice

Target Concentration (ppm)	Mean ± SD	Target ± RSD[1]	Maximum	Minimum	Samples within Range[2] (%)
RATS					
2-Week 2-Chloronitrobenzene Study					
0	—[3]	—	0.03	0.00	100
1.1	1.11 ± 0.06	101 ± 6	1.22	0.76	96
2.3	2.31 ± 0.10	100 ± 4	2.58	1.98	96
4.5	4.38 ± 0.26	97 ± 6	4.84	3.51	88
9	9.03 ± 0.45	100 ± 5	10.0	7.52	92
18	18.9 ± 1.56	105 ± 8	24.1	15.7	82
2-Week 4-Chloronitrobenzene Study					
0	0.000 ± 0.002	—	0.03	0.00	99
1.5	1.44 ± 0.30	96 ± 21	2.34	0.12	60
3	2.93 ± 0.46	98 ± 16	4.95	1.80	72
6	6.05 ± 0.67	101 ± 11	9.25	4.11	81
12	12.0 ± 1.24	100 ± 10	17.8	8.22	79
24	24.5 ± 2.21	102 ± 9	29.0	16.8	77
13-Week 2-Chloronitrobenzene Study					
0	0.003 ± 0.004	—	0.02	0.00	99
1.1	1.11 ± 0.07	101 ± 6	1.67	0.79	93
2.3	2.28 ± 0.13	99 ± 6	2.57	1.42	93
4.5	4.45 ± 0.27	99 ± 6	5.33	2.06	93
9	8.84 ± 0.70	98 ± 8	17.2	4.08	89
18	17.8 ± 1.20	99 ± 7	20.4	5.88	94
13-Week 4-Chloronitrobenzene Study					
0	<MDL	—	0.071	<MDL	—
1.5	1.50 ± 0.10	100 ± 7	1.87	0.99	90
3	2.97 ± 0.21	99 ± 7	3.77	0.51	89
6	5.98 ± 0.35	100 ± 6	7.33	3.26	91
12	12.0 ± 0.96	100 ± 8	20.3	2.21	88
24	23.9 ± 1.71	100 ± 7	31.9	10.1	88
MICE					
2-Week 2-Chloronitrobenzene Study					
0	—	—	0.03	0.00	100
1.1	1.11 ± 0.06	101 ± 6	1.22	0.76	96
2.3	2.31 ± 0.10	101 ± 4	2.58	1.98	97
4.5	4.39 ± 0.26	98 ± 6	4.84	3.51	88
9	9.05 ± 0.45	101 ± 5	10.0	7.52	93
18	18.9 ± 1.58	105 ± 8	24.1	15.7	81
2-Week 4-Chloronitrobenzene Study					
0	0.000 ± 0.003	—	0.03	0.00	98
1.5	1.45 ± 0.30	97 ± 21	2.34	0.12	63
3	2.97 ± 0.40	99 ± 14	4.95	1.80	80
6	6.11 ± 0.61	102 ± 10	9.25	4.11	86
12	12.1 ± 1.09	101 ± 9	17.8	8.64	84
24	24.7 ± 1.95	103 ± 8	29.0	17.2	80

TABLE 3 Mean Chamber Concentrations of 2-Chloronitrobenzene and 4-Chloronitrobenzene in the 2-Week and 13-Week Inhalation Studies in F344/N Rats and B6C3F$_1$ Mice (continued)

Target Concentration (ppm)	Mean ± SD	Target ± RSD[1]	Maximum	Minimum	Samples within Range[2] (%)
MICE (continued)					
13-Week 2-Chloronitrobenzene Study					
0	0.003 ± 0.004	—	0.02	0.00	99
1.1	1.11 ± 0.07	101 ± 6	1.67	0.79	92
2.3	2.28 ± 0.13	99 ± 6	2.57	1.42	92
4.5	4.45 ± 0.27	99 ± 6	5.33	2.06	93
9	8.84 ± 0.69	98 ± 8	17.2	4.08	89
18	17.8 ± 1.20	99 ± 7	20.4	5.88	94
13-Week 4-Chloronitrobenzene Study					
0	<MDL	—	0.071	<MDL	—
1.5	1.49 ± 0.10	100 ± 7	1.87	0.99	90
3	2.97 ± 0.22	99 ± 7	3.77	0.51	89
6	5.97 ± 0.35	100 ± 6	7.33	3.26	90
12	12.0 ± 0.98	100 ± 8	20.3	2.21	88
24	23.9 ± 1.75	100 ± 7	31.9	10.1	88

[1] Mean concentration ± relative standard deviation as a percent of target concentration.
[2] Samples within 10% of the target concentration (or less than the MDL for target concentration = 0 ppm) were considered to be within range. MDL = minimum detectable limit. For 2-week studies, MDL = 0.04 ppm 2-chloronitrobenzene or 0.01 ppm 4-chloronitrobenzene. For 13-week studies, MDL = 0.01 ppm 2-chloronitrobenzene or 0.015 ppm 4-chloronitrobenzene.
[3] Readings less than 0 ppm occurred as a result of equipment variations.

For the 13-week 2-chloronitrobenzene studies, T_{90} ranged from 14 to 19 minutes without animals in the chambers and 17 to 26 minutes with animals present. A T_{90} of 20 minutes was chosen for the 2-chloronitrobenzene studies, but T_{90} was changed to 25 minutes after the third week of the studies to reflect the actual buildup of the chemical in the chambers with animals present. For the 13-week 4-chloronitrobenzene studies, T_{90} ranged from 10 to 12 minutes without animals in the chambers and from 13 to 18 minutes with animals present. T_{10} ranged from 12 to 14 minutes without animals and from 20 to 42 minutes with animals in the chambers. A T_{90} value of 15 minutes was chosen for the 4-chloronitrobenzene studies; T_{90} was changed to 18 minutes on Day 16 (mice) or 17 (rats) of exposure to reflect the actual chemical buildup in the chambers.

STABILITY STUDIES

The stability of 2-chloronitrobenzene and 4-chloronitrobenzene in the exposure chambers with and without animals present, in the generator reservoir, in the vapor transport line

(2-week studies only), and in the distribution manifold was confirmed by gas chromatography with flame ionization detection (all studies) and electron capture detection (2-week 2-chloronitrobenzene studies only). Samples for the 2-chloronitrobenzene studies were collected from the 1.1 and 18 ppm chambers, and samples for the 4-chloronitrobenzene studies were collected from the 1.5 and 24 ppm chambers. The samples of 2-chloronitrobenzene and 4-chloronitrobenzene were screened for 1,2-dichlorobenzene, azobenzene, and isomers of nitrophenol, nitroaniline, chloroaniline, and chloronitrobenzene. No degradation of 2- or 4-chloronitrobenzene was detected during the 13-week studies.

Toxicity Study Designs

BASE STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY) for the 2-week 2-chloronitrobenzene studies and from Simonsen Laboratories (Gilroy, CA) for the 2-week 4-chloronitrobenzene studies and all 13-week studies. Rats and mice used in the 2-week studies and the 13-week 2-chloronitrobenzene studies were approximately 4 weeks old at receipt; rats and mice used in the 13-week 4-chloronitrobenzene study were 3 weeks old at receipt. Rats and mice were quarantined for 11 to 13 days and were approximately 5 to 6 weeks old when the studies began. For all studies, blood samples were collected from rats and mice of each sex 3 weeks after receipt and at the end of the studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b); all results were negative. Additional details concerning the study design are provided in Table 4.

During the 2-week studies, groups of five rats and five mice of each sex were exposed to 0, 1.1, 2.3, 4.5, 9, or 18 ppm 2-chloronitrobenzene vapor or 0, 1.5, 3, 6, 12, or 24 ppm 4-chloronitrobenzene vapor through whole-body exposure for 6 hours plus T_{90} per day, 5 days per week, excluding weekends and holidays, for 12 exposure days. The maximum exposure concentrations for both the 2-week and 13-week studies were the same and were limited by the low vapor pressures of the chemicals. In the 13-week base studies, groups of 10 rats and 10 mice of each sex were exposed to 0, 1.1, 2.3, 4.5, 9, or 18 ppm 2-chloronitrobenzene vapor or 0, 1.5, 3, 6, 12, or 24 ppm 4-chloronitrobenzene vapor through whole-body exposure for 6 hours plus T_{90} per day, 5 days per week, excluding weekends and holidays, for 13 weeks.

For all studies, rats and mice were housed in individual cages within the exposure chambers. City water (Richland, WA) was available *ad libitum*, and NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form was available *ad libitum* except during the daily exposure periods. Animal rooms were maintained with 12 hours of fluorescent light per day.

Complete necropsies were performed on all base-study animals in the 2-week and 13-week studies. The heart, right kidney, liver, lungs, spleen, right testis, and thymus of each animal were weighed. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all animals in the control and the highest exposure groups and all animals that died before the end of the studies. Gross lesions and selected organs of rats and mice in lower exposure groups were examined until a no-observed-effect level was determined. Tissues examined microscopically are listed in Table 4.

Upon completion of the laboratory pathologist's histologic evaluation, 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. 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).

SUPPLEMENTAL EVALUATIONS

Summaries of the continuous breeding and the disposition and metabolism studies are given in Appendices D and F, respectively. These studies were performed under separate contracts and were independent from the inhalation studies that are the principal subject of this report.

Clinical Pathology

Clinical pathology evaluations were conducted on 10 rats per sex and exposure level during the 13-week studies. In the 2-chloronitrobenzene study, blood samples for hematology and clinical chemistry evaluations were collected immediately following exposure from rats designated for clinical pathology testing on Days 1 (methemoglobin only), 4, and 23 and from base-study rats at the end of the studies. Blood samples for hematology and clinical chemistry evaluations in the 4-chloronitrobenzene study were collected immediately following exposure from rats designated for clinical pathology testing on Days 3 and 23 and from base-study rats at the end of the study. At all time points, rats were anesthetized with a CO_2:room air gas mixture, and blood samples were drawn from the retroorbital sinus. Blood for hematology determinations was placed in tubes containing potassium EDTA as the anticoagulant. Blood for clinical chemistry evaluations was placed in tubes devoid of anticoagulant and allowed to clot at room temperature; the samples were then centrifuged and the serum was removed. All hematologic and biochemical analyses were performed on the day of sample collection.

Hematology determinations were performed with an Ortho ELT-8/ds hematology analyzer (Ortho Instruments, Westwood, MA). The parameters that were evaluated are listed in Table 4. Manual hematocrit determinations were performed by the microhematocrit method with a Damon/IEC MB microcentrifuge and Damon/IEC capillary reader (International Equipment Company, Needham Heights, MA) for comparison with automated hematocrit values. Differential leukocyte counts and morphologic evaluation of blood cells were determined microscopically from blood smears stained with Wright-Giemsa. Smears made from blood samples stained with new methylene blue were examined microscopically with a Miller disc for the quantitative determination of reticulocytes. Methemoglobin concentrations were measured within approximately 30 minutes of sample collection with an IL CO-Oximeter (Instrumentation Laboratory, Inc., Lexington, MA) calibrated for rat carboxyhemoglobin.

Clinical chemistry variables were measured with an Abbott VP (Abbott Laboratories, Abbott Park, IL) or a Roche Cobas Fara chemistry analyzer (Roche Diagnostic Systems, Inc, Montclair, NJ). The parameters that were evaluated are listed in Table 4. Reagents for assay of sorbitol dehydrogenase activity and bile acid concentration were obtained from Sigma Chemical Company (St. Louis, MO); reagents for the other endpoints were obtained from the manufacturer.

Sperm Morphology and Vaginal Cytology in Rats and Mice

At the end of the 13-week studies, vaginal cytology and sperm morphology evaluations were performed on base-study rats and mice (10 animals per sex) from the 0, 4.5, 9, and 18 ppm groups in the 2-chloronitrobenzene study and from the 0, 6, 12, and 24 ppm groups in the 4-chloronitrobenzene study. Methods were those outlined in the National Toxicology Program's Sperm Motility Vaginal Cytology Evaluation protocol (NTP, 1982). Briefly, for the 12 days prior to sacrifice, the vaginal vaults of 10 females of each species per exposure group were lavaged, and the aspirated lavage fluid and cells were stained with Toluidine Blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (*i.e.*, diestrus, proestrus, estrus, and metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body and weighed. Test yolk (rats) or Tyrode's buffer (mice) was applied to slides, and a small incision was made in the cauda. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five microscopic fields per slide.

Following completion of sperm motility estimates, each left cauda was placed in phosphate-buffered saline solution. Cauda were finely minced and the tissue was incubated and then heat fixed. Sperm density was then determined microscopically with the aid of a hemacytometer.

TABLE 4 Experimental Design and Materials and Methods in the 2-Week and 13-Week Inhalation Studies of 2-Chloronitrobenzene and 4-Chloronitrobenzene

EXPERIMENTAL DESIGN	
Study Laboratory	Battelle Pacific Northwest Laboratories (Richland, WA)
Size of Study Groups	2-Week Studies: five males and five females per species per exposure group 13-Week Studies: Base Studies: 10 males and 10 females per species per exposure group Clinical Pathology Studies: 10 male and 10 female rats per exposure group
Route of Administration	Inhalation
Exposure Concentration/Duration	2-Week Studies: *2-Chloronitrobenzene:* 0, 1.1, 2.3, 4.5, 9, or 18 ppm daily, 6 hours plus 20 minutes per day, 5 days per week, for 2 weeks (12 exposure days) *4-Chloronitrobenzene:* 0, 1.5, 3, 6, 12, or 24 ppm daily, 6 hours plus 18 minutes per day, 5 days per week, for 2 weeks (12 exposure days) 13-Week Studies: *2-Chloronitrobenzene:* 0, 1.1, 2.3, 4.5, 9, or 18 ppm daily, 6 hours plus 20 to 25 minutes per day, 5 days per week, for 13 weeks *4-Chloronitrobenzene:* 0, 1.5, 3, 6, 12, or 24 ppm daily, 6 hours plus 15 to 18 minutes per day, 5 days per week, for 13 weeks
Date of First Exposure	2-Week Studies: *2-Chloronitrobenzene:* Rats: 3 October 1988 Mice: 4 October 1988 *4-Chloronitrobenzene:* Rats: 16 January 1989 Mice: 17 January 1989 13-Week Studies: *2-Chloronitrobenzene:* Rats: 17 April 1989 (males), 18 April 1989 (females) Mice: 18 April 1989 (males), 19 April 1989 (females) *4-Chloronitrobenzene:* Rats: 24 July 1989 (males), 25 July 1989 (females) Mice: 25 July 1989
Date of Last Exposure	2-Week Studies: *2-Chloronitrobenzene:* Rats: 18 October 1988 Mice: 19 October 1988 *4-Chloronitrobenzene:* Rats: 31 January 1989 Mice: 1 February 1989 13-Week Studies: *2-Chloronitrobenzene:* Rats: 19 July 1989 (males), 20 July 1989 (females) Mice: 17 July 1989 (males), 18 July 1989 (females) *4-Chloronitrobenzene:* Rats: 25 October 1989 (males), 26 October 1989 (females) Mice: 23 October 1989 (males), 24 October 1989 (females)

TABLE 4 Experimental Design and Materials and Methods in the 2-Week and 13-Week Inhalation Studies of 2-Chloronitrobenzene and 4-Chloronitrobenzene (continued)

Necropsy Dates	2-Week Studies: *2-Chloronitrobenzene:* Rats: 19 October 1988 Mice: 20 October 1988 *4-Chloronitrobenzene:* Rats: 1 February 1989 Mice: 2 February 1989 13-Week Studies: *2-Chloronitrobenzene:* Rats: 20 July 1989 (males), 21 July 1989 (females) Mice: 18 July 1989 (males), 19 July 1989 (females) *4-Chloronitrobenzene:* Rats: 26 October 1989 (males), 27 October 1989 (females) Mice: 24 October 1989 (males), 25 October 1989 (females)
Type and Frequency of Observation	2-Week Studies: Animals were observed twice daily and were weighed on Days 1 and 8 and at necropsy. Clinical observations were recorded daily. 13-Week Studies: Animals were observed twice daily and were weighed at the start of the study, weekly thereafter, and at necropsy. Clinical observations were recorded weekly.
Necropsy and Histologic Examinations	2-Week Studies: Complete necropsies were performed on all animals. Histopathologic evaluations were performed on all animals in the control and highest exposure groups and all animals that died early. The following tissues were examined: gross lesions, kidneys, larynx, liver, lungs and attached tracheobronchial lymph nodes, nose, spleen, testes, and trachea. Gross lesions of rats and mice in all lower exposure groups were examined. Target organs identified and examined until a no-observed-effect level was determined included: liver and spleen of rats and mice in the 2-chloronitrobenzene studies, and kidneys, liver, and spleen of rats and mice in the 4-chloronitrobenzene studies. 13-Week Studies: Complete necropsies were performed on all animals from the base studies. Histopathologic evaluations were performed on all animals in the control and highest exposure groups and all animals that died early. The following tissues were examined: adrenal glands, brain (three sections), clitoral glands, esophagus, eyes (if grossly abnormal), femur and marrow, gallbladder (mice only), gross lesions and tissue masses, heart, kidneys, large intestine (cecum, colon, rectum), larynx, liver, lungs, lymph nodes (bronchial, mandibular, mediastinal, and mesenteric), mammary gland, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial glands, prostate gland, salivary gland, seminal vesicle, small intestine (duodenum, jejunum, ileum), spinal cord/sciatic nerve (if neurologic signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thigh muscle, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only). Gross lesions of rats and mice in all lower exposure groups were examined. Target organs identified and examined in all lower exposure groups included: kidneys, liver, nasal cavity, and spleen of rats in the 2-chloronitrobenzene study; liver and spleen of mice in the 2-chloronitrobenzene study; bone marrow, harderian gland, kidneys, liver, mediastinal lymph node, spleen, and testes of rats in the 4-chloronitrobenzene study; and bone marrow, forestomach, liver, and spleen of mice in the 4-chloronitrobenzene study.

TABLE 4 Experimental Design and Materials and Methods in the 2-Week and 13-Week Inhalation Studies of 2-Chloronitrobenzene and 4-Chloronitrobenzene (continued)

Supplemental Evaluations	Clinical Pathology Studies: Hematology and clinical chemistry evaluations were conducted for rats in the 13-week 2-chloronitrobenzene and 4-chloronitrobenzene studies. In the 2-chloronitrobenzene study, blood was collected on Days 1, 4, and 23 from rats in the clinical pathology special study group. Animals in the base study were evaluated at the end of the study. In the 4-chloronitrobenzene study, blood was collected on Days 3 and 23 from the clinical pathology special study rats and at the end of the study from rats in the base study. Hematology parameters evaluated included hematocrit (HCT), hemoglobin (HGB) concentration, erythrocyte (RBC) count, reticulocyte count, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count, leukocyte (WBC) count and differential, and methemoglobin. (Only methemoglobin was measured on Day 1 of the 2-chloronitrobenzene study.) Clinical chemistry parameters evaluated included urea nitrogen (UN), creatinine, total protein, albumin, globulin, alanine aminotransferase (ALT), alkaline phosphatase (AP), creatine kinase (CK), sorbitol dehydrogenase (SDH), and bile acids. Sperm Morphology and Vaginal Cytology Evaluations: Sperm morphology and vaginal cytology evaluations were performed on base-study animals at the end of the 13-week studies. Animals in the 0, 4.5, 9, and 18 ppm groups in the 2-chloronitrobenzene studies and the 0, 6, 12, and 24 ppm groups in the 4-chloronitrobenzene studies were evaluated. Male rats and mice were evaluated for necropsy body and reproductive tissue weights, spermatozoal data, and spermatogenesis. Females were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spent in the various stages.
ANIMALS AND ANIMAL MAINTENANCE	
Strain and Species	F344/N rats B6C3F$_1$ mice
Animal Source	2-Week Studies: *2-Chloronitrobenzene:* Taconic Farms (Germantown, NY) *4-Chloronitrobenzene:* Simonsen Laboratories (Gilroy, CA) 13-Week Studies: Simonsen Laboratories (Gilroy, CA)
Time Held Before Study	2-Week Studies: *2-Chloronitrobenzene:* rats, 11 days; mice, 12 days *4-Chloronitrobenzene:* rats, 12 days; mice, 13 days 13-Week Studies: *2-Chloronitrobenzene:* Rats: 11 days (males), 12 days (females) Mice: 12 days (males), 13 days (females) *4-Chloronitrobenzene:* Rats: 11 days (males), 12 days (females) Mice: 12 days
Age When Placed on Study	2-Week Studies: 6 weeks 13-Week Studies: *2-Chloronitrobenzene:* 6 weeks *4-Chloronitrobenzene:* 5 weeks
Age When Killed	2-Week Studies: 8 weeks 13-Week Studies: *2-Chloronitrobenzene:* 19 weeks *4-Chloronitrobenzene:* 18 weeks
Method of Animal Distribution	Animals were weighed and were randomized with a computer program.

TABLE 4 Experimental Design and Materials and Methods in the 2-Week and 13-Week Inhalation Studies of 2-Chloronitrobenzene and 4-Chloronitrobenzene (continued)

Diet	NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form, available *ad libitum* except during exposure periods, and deionized, softened water (City of Richland), available *ad libitum*
Animal Room Environment	Rats and mice were housed in individual cages in the exposure chambers for all studies. The temperature was maintained at 75° ± 3° F and relative humidity at 55% ± 15%, with 12-18 air changes per hour. Fluorescent light was provided for 12 hours per day.

Genetic Toxicity Studies

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Haworth *et al.* (1983). 2-Chloronitrobenzene and 4-chloronitrobenzene were sent to the testing laboratories as coded aliquots. The procedure for the standard *Salmonella typhimurium*/microsome plate test was described by Ames *et al.*, 1975. For the preincubation experiments, the chemicals were incubated with the *S. typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of 2- or 4-chloronitrobenzene. The high dose was limited by toxicity. Positive assays were repeated under the conditions that elicited the positive response. Because of the number of tests performed with 2-chloronitrobenzene, the results that were published in Haworth *et al.* (1983) are presented in abbreviated form, with repeat trials not included in this report.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1987). 2-Chloronitrobenzene and 4-chloronitrobenzene were supplied as coded aliquots. The aliquots were tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges

(SCEs) and chromosomal aberrations (Abs) both in the presence and the absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of 2- or 4-chloronitrobenzene; the high dose was limited by toxicity. A single flask per dose was used, and trials yielding equivocal or positive results were repeated.

In the SCE test without S9, CHO cells were incubated for 26 hours with 2- or 4-chloronitrobenzene in McCoy's 5A medium supplemented with fetal bovine serum, *l*-glutamine, and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing 2- or 4-chloronitrobenzene was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with 2- or 4-chloronitrobenzene, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no 2- or 4-chloronitrobenzene, and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with 2- or 4-chloronitrobenzene for 8 to 12 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with 2- or 4-chloronitrobenzene and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 8 to 12 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was extended as necessary to counter cell cycle delay induced by 2-chloronitrobenzene and 4-chloronitrobenzene.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. Up to 200 first-division metaphase cells were scored at

each dose level. Classes of aberrations recorded included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

DROSOPHILA MELANOGASTER TEST PROTOCOL

The assays for induction of sex-linked recessive lethal (SLRL) mutations were performed with adult flies as described by Zimmering *et al.* (1985) and with larvae as described by Zimmering *et al.* (1989). 2-Chloronitrobenzene and 4-chloronitrobenzene were supplied as coded aliquots, and were assayed in the SLRL test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no response was obtained, 2-chloronitrobenzene and 4-chloronitrobenzene were retested by injection into adult males.

To administer a chemical by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament and the tip was broken off to allow delivery of the test solution. Injection was performed either manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 µL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector, which automatically delivered a calibrated volume. Flies were anaesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of 2- or 4-chloronitrobenzene at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. Oral exposure was achieved by allowing Canton-S males to feed for 72 hours on a solution of 2- or 4-chloronitrobenzene in 5% sucrose. In the injection experiments, 24- to 72-hour-old Canton-S males were treated with a solution of 2- or 4-chloronitrobenzene dissolved in saline and allowed to recover for 24 hours. For the larval feeding experiment, Canton-S females and males were mated and eggs were exposed in vials containing standard cornmeal food with 2- or 4-chloronitrobenzene in solvent (5%) ethanol or solvent alone (Zimmering *et al.*, 1989). Adult emergent males were mated at an age of approximately 24 hours with two successive harems of three to five *Basc* females to establish two single-day broods. In the adult exposures, treated males were mated to three *Basc* females for 3 days and were given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case,

sample sperm from successive matings were treated at successively earlier postmeiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event, and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution). A cluster was identified and all data from the male in question were discarded. Presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

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 with the parametric multiple comparisons procedures of Williams (1971, 1972) or Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed with the nonparametric multiple comparisons methods of Shirley (1977) or 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. The extreme values chosen by the statistical test were subject to approval by NTP personnel. In addition, values indicated by the laboratory report as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF VAGINAL CYTOLOGY DATA

Because 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 simultaneous equality of measurements across dose levels.

ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

A positive response in the *S. typhimurium* assay is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that was not dose related, not reproducible, or not of sufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

ANALYSIS OF CHINESE HAMSTER OVARY CELL CYTOGENETICS DATA

For the SCE data, statistical analyses were conducted on the slopes of the dose-response curves (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses indicated that the trial was positive. A statistically significant trend ($P<0.05$), in the absence of any responses reaching 20% above background, led to a call of equivocal (Galloway *et al.*, 1987).

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose-response curve and individual dose points (Galloway *et al.*, 1987). For a single trial, a statistically significant ($P<0.05$) difference for one dose point and a significant trend ($P<0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend, in the absence of a statistically significant increase at any one dose point, led to a conclusion of equivocal activity.

Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

ANALYSIS OF *DROSOPHILA MELANOGASTER* DATA

Sex-linked recessive lethal data were analyzed by simultaneous comparison with the concurrent and historical controls using a normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered positive if the P-value was less than or equal to 0.01 and the mutation frequency in the tested group was greater than 0.10% or if the P-value was less than or equal to 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if the P-value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or if the P-value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P-value was greater than or equal to 0.10 or if the frequency in the treatment group was less than 0.10%.

Quality Assurance

The animal studies of 2-chloronitrobenzene and 4-chloronitrobenzene were performed in compliance with U.S. Food and Drug Administration Good Laboratory Practices regulations (21 CFR 58). The Quality Assurance Unit of Battelle Pacific Northwest Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

RESULTS

2-Week Inhalation Studies in F344/N Rats

All rats exposed to 2- or 4-chloronitrobenzene survived until the end of the studies (Tables 5 and 6). The final mean body weights of all exposed groups in both studies were similar to those of the controls (Tables 5 and 6). Clinical signs of toxicity were noted in rats in the 18 ppm groups in the 2-chloronitrobenzene study; these findings included apparent dehydration and nasal discharge in males and females and hypoactivity, ataxia, and pallor in males. Decreased urination and defecation were also noted in males and females. In the 4-chloronitrobenzene study, clinical signs related to exposure included hypoactivity and pale skin in males and females exposed to 24 ppm and hypoactivity in males and females exposed to 12 ppm.

TABLE 5 Survival and Weight Gain of F344/N Rats in the 2-Week Inhalation Study of 2-Chloronitrobenzene

Concentration (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
0	5/5	118	184	66	
1.1	5/5	120	188	68	102
2.3	5/5	117	187	70	102
4.5	5/5	119	189	70	103
9	5/5	118	189	71	103
18	5/5	119	179	60	97
FEMALE					
0	5/5	99	134	35	
1.1	5/5	100	136	36	102
2.3	5/5	101	139	38	104
4.5	5/5	100	139	39	104
9	5/5	100	137	37	103
18	5/5	102	138	36	103

[1] Number surviving at 2 weeks/number of animals per dose group.
[2] Mean weight change.
[3] (Dose group mean/control group mean) × 100.

TABLE 6 Survival and Weight Gain of F344/N Rats in the 2-Week Inhalation Study of 4-Chloronitrobenzene

Concentration (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
0	5/5	104	178	74	
1.5	5/5	109	184	75	103
3	5/5	107	179	72	100
6	5/5	107	188	81	105
12	5/5	106	185	79	104
24	5/5	110	182	72	102
FEMALE					
0	5/5	93	130	37	
1.5	5/5	94	131	37	101
3	5/5	94	131	37	101
6	5/5	94	134	40	103
12	5/5	92	131	39	101
24	5/5	94	127	33	98

[1] Number surviving at 2 weeks/number of animals per dose group.
[2] Mean weight change.
[3] (Dose group mean/control group mean) × 100.

2-Chloronitrobenzene: Male and female rats exposed to 2-chloronitrobenzene had exposure-related increases in absolute and relative liver weights; absolute and relative spleen weights were increased in males and females in the 18 ppm groups, and relative kidney weights were slightly increased in males exposed to 18 ppm.

No gross lesions were attributed to 2-chloronitrobenzene exposure. Microscopic findings related to exposure were observed in the liver and spleen. In the liver, minimal deposition of golden-brown pigment was present in portal areas and around central veins in all rats exposed to 18 ppm. The pigment was typically within macrophages and stained positive for iron, indicating that the pigment was hemosiderin. In the spleen, hemosiderin pigment deposition of mild severity was observed in all rats in the 18 ppm groups. There were no clear histopathologic changes that could account for the increases in liver weights or spleen weights.

4-Chloronitrobenzene: There were exposure-related increases in absolute and relative spleen and liver weights in exposed males and females; other changes possibly related to

exposure to 4-chloronitrobenzene included increased absolute and relative heart weights and relative kidney weights in males and females in the 24 ppm groups and decreased absolute and relative thymus weights in males.

Gross lesions related to exposure to 4-chloronitrobenzene included enlargement and dark discoloration of the spleen in all male and female rats exposed to 12 or 24 ppm and in three of five males and one of five females exposed to 6 ppm. The spleen, kidney, and liver were identified microscopically as target tissues following 2 weeks of exposure to 4-chloronitrobenzene.

Microscopic changes in the spleen included congestion, increased hematopoietic cell proliferation, and hemosiderin deposition. Congestion consisting of blood-filled sinusoids in the red pulp was present in all females exposed to 3 ppm or greater and in most males exposed to 6 ppm or greater. A minimal to mild increase in hematopoietic cell activity, primarily erythropoiesis, occurred in the spleens of most male and female rats exposed to 4-chloronitrobenzene at a concentration of 6 ppm or greater. The amount of iron-positive pigment (hemosiderin) in the spleen increased with increasing concentration in males and females; the no-effect level of this pigmentation was 1.5 ppm in males, whereas a no-effect level was not achieved in females.

Microscopic changes in the kidney were observed in the proximal convoluted tubules of male and female rats in the 12 and 24 ppm groups. The kidney change in male rats consisted primarily of accumulation of eosinophilic hyaline droplets within the cytoplasm of tubular epithelial cells (hyaline droplet nephropathy). In females, these cells were stippled with small brown pigment granules. In exposed male rats, staining for iron (hemosiderin) indicated a minimal increase in the number of small, iron-positive granules superimposed on the hyaline droplet change. Iron-positive granules were clearly increased in number and size in exposed females; a smaller number of pigment granules were iron-negative. A minimal amount of iron-positive pigment (hemosiderin) was seen in the sinusoidal Kupffer cells in the liver of all males in the 24 ppm group and most female rats exposed to 6 ppm or greater.

13-Week Inhalation Studies in F344/N Rats

All rats in the 13-week 2-chloronitrobenzene study survived until the end of the study (Table 7); in the 4-chloronitrobenzene study, one female rat exposed to 3 ppm was killed moribund due to malocclusion (Table 8). The mean body weight gains of exposed animals in each study were similar to those of the respective controls (Tables 7 and 8 and Figures 2 and 3). There were no clear clinical signs of toxicity in either study.

TABLE 7 Survival and Weight Gain of F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene

Concentration (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
0	10/10	98	324	226	
1.1	10/10	102	341	239	105
2.3	10/10	99	333	234	103
4.5	10/10	100	338	238	104
9	10/10	100	330	230	102
18	10/10	96	313	217	97
FEMALE					
0	10/10	87	186	99	
1.1	10/10	85	186	100	100
2.3	10/10	88	197	108	106
4.5	10/10	86	190	103	102
9	10/10	85	190	105	102
18	10/10	88	190	102	102

[1] Number surviving at 13 weeks/number of animals per dose group.
[2] Mean weight change.
[3] (Dose group mean/control group mean) × 100.

TABLE 8 Survival and Weight Gain of F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene

Concentration (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
0	10/10	100	333	233	
1.5	10/10	102	342	240	103
3	10/10	103	335	232	101
6	10/10	98	339	241	102
12	10/10	103	326	224	98
24	10/10	104	340	236	102
FEMALE					
0	10/10	87	186	99	
1.5	10/10	89	187	99	101
3	9/10	87	197	111	106
6	10/10	86	191	105	102
12	10/10	85	191	106	103
24	10/10	87	196	110	105

[1] Number surviving at 13 weeks/number of animals per dose group.
[2] Mean weight change.
[3] (Dose group mean/control group mean) × 100.

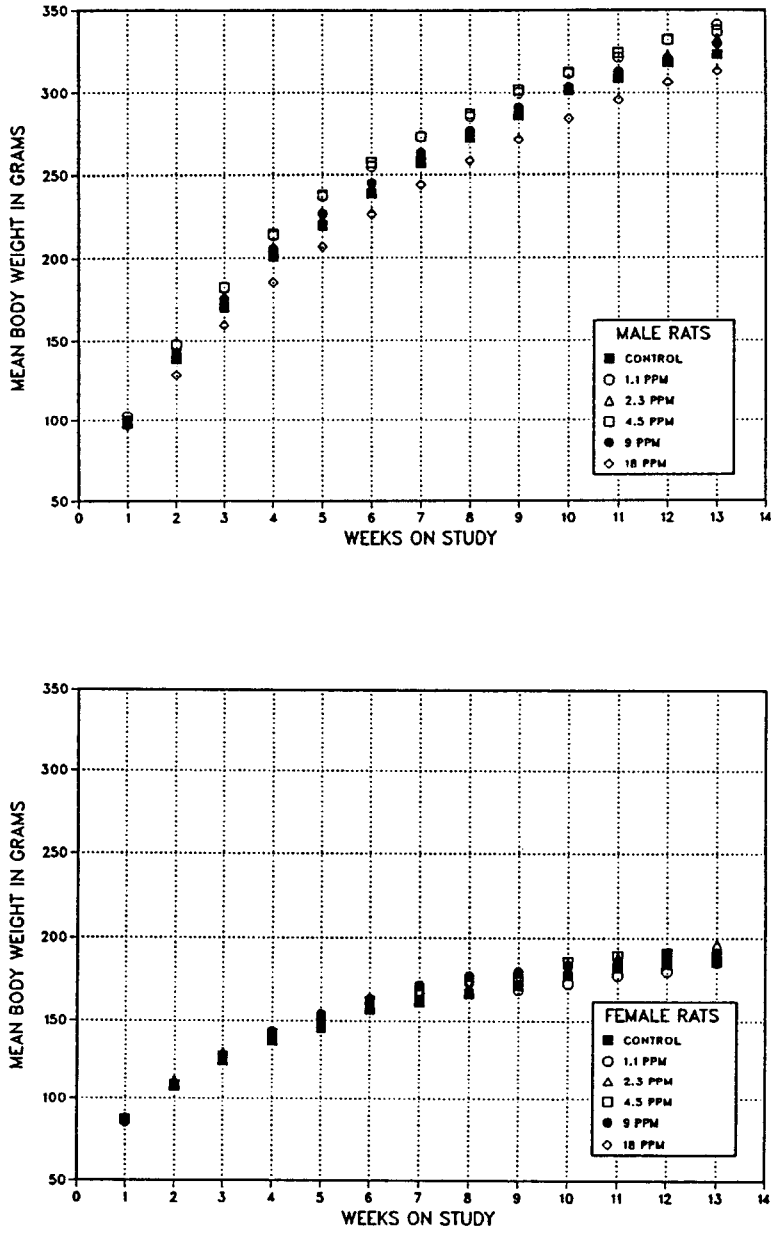

FIGURE 2 Body Weights of F344/N Rats Administered 2-Chloronitrobenzene by Inhalation for 13 Weeks

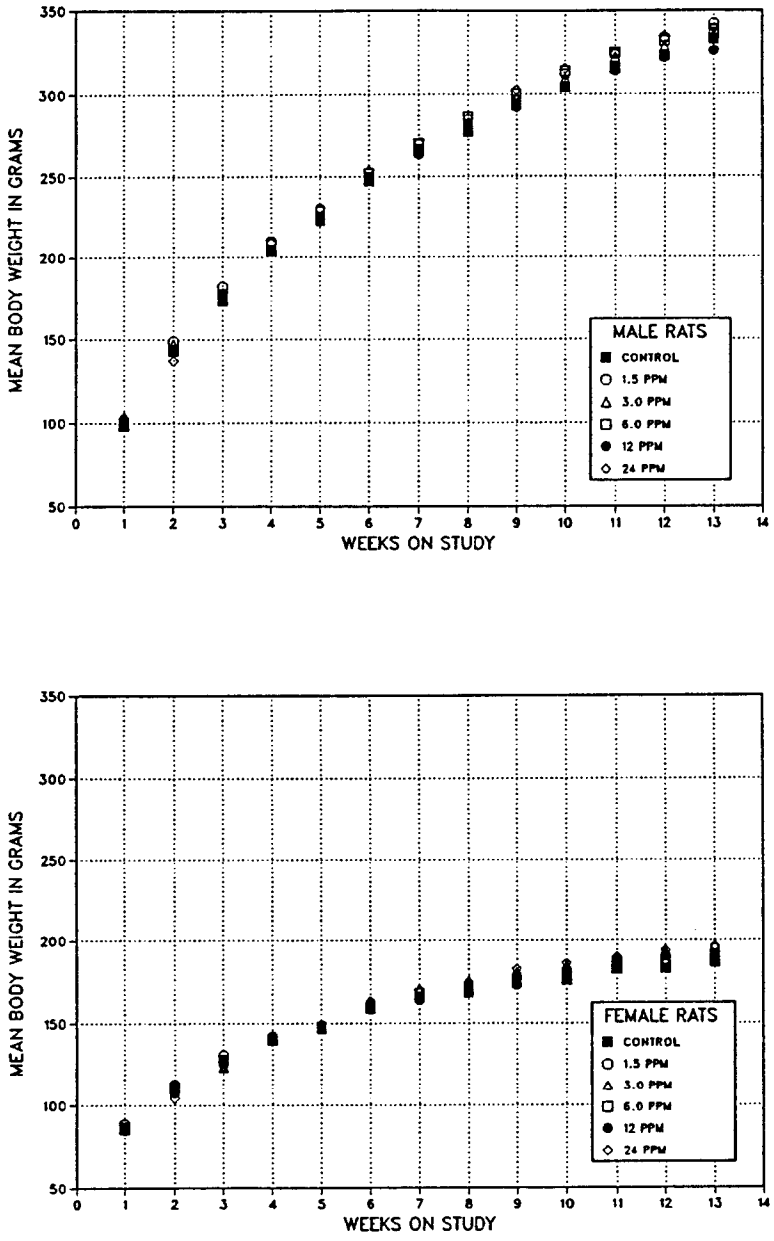

FIGURE 3 Body Weights of F344/N Rats Administered 4-Chloronitrobenzene by Inhalation for 13 Weeks

2-Chloronitrobenzene: Hematology findings were consistent with methemoglobinemia and a normocytic, normochromic anemia with evidence of a hematopoietic response by the end of the study. Methemoglobin concentrations were elevated in males and females in most exposure groups after the first exposure day (data not shown) and remained elevated at all time points in the 2-chloronitrobenzene study (Table 9 and Appendix B). By Day 23, all exposure groups of male rats had elevated methemoglobin concentrations, and by Week 13, methemoglobin concentrations were significantly increased in all exposure groups of males and females. In general, mild, treatment-related decreases in hematocrit (HCT), hemoglobin (HGB) concentrations, and erythrocyte (RBC) counts occurred in exposed male and female rats (Table 9). Male rats exposed to 18 ppm were affected at all time points; decreases in these parameters also occurred in male rats at lower exposure levels, but with less consistency and at fewer time points. Decreases in HCT and HGB concentrations occurred at Week 13 in males in all but the lowest (1.1 ppm) exposure group. Decreases in HCT, HGB concentrations, and RBC counts occurred in exposed female rats; however, the changes were less consistent than those in males for all exposure groups. As in male rats, female rats in the 18 ppm group had the most changes occurring at any time point, and most exposure groups had some change by Week 13 (Table 9). Minor decreases in the RBC indices, including mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC), occurred in exposed males and females (Table 9). In male rats, decreases in MCV and MCH occurred in the 18 ppm group at Day 4. In female rats, MCV, MCH and MCHC values were mildly decreased in the 9 and 18 ppm groups. Increases in reticulocyte counts occurred in males and females in the 9 and 18 ppm groups at Week 13 (Table 9). This change also occurred in female rats exposed to 18 ppm on Days 4 and 23 and in the 4.5 ppm group at Week 13. Increases in nucleated RBC numbers occurred in male and female rats in the 18 ppm groups at Week 13 (Table 9). Increased numbers of nucleated RBCs also occurred in males in the 9 ppm group at Week 13 and in males in the 18 ppm group on Day 4. Platelet counts were significantly decreased in male and female rats in the 9 and 18 ppm groups on Day 4. At Week 13, increases in leukocyte (WBC) counts occurred in male rats in all exposure groups (Table 9); these increases were accompanied by increased numbers of lymphocytes at all exposure levels, although the increase in the 2.3 ppm group was not significant. This change is consistent with a physiologic response to stress.

TABLE 9 Selected Hematology Data for F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

	Concentration (ppm)					
	0	1.1	2.3	4.5	9	18
MALE						
n						
Day 4	10	10	10	10	10	10
Day 23	8	10	9	10	10	6
Week 13	10	10	10	10	10	10
Hematocrit (automated) (%)						
Day 4	36.7 ± 0.3	36.8 ± 0.2	36.5 ± 0.3	36.8 ± 0.3	35.5 ± 0.2**	33.3 ± 0.5**
Day 23	45.1 ± 0.7	43.2 ± 0.5*	44.8 ± 0.5	43.6 ± 0.3	43.6 ± 0.4	41.2 ± 0.4**
Week 13	46.5 ± 0.4	46.2 ± 0.2	45.5 ± 0.2*	45.1 ± 0.2**	45.0 ± 0.3**	42.6 ± 0.3**
Hemoglobin (g/dL)						
Day 4	12.1 ± 0.1	12.1 ± 0.1	11.9 ± 0.1	12.1 ± 0.1	11.5 ± 0.1**	10.9 ± 0.2**
Day 23	15.3 ± 0.2	14.7 ± 0.2*	15.3 ± 0.2	14.8 ± 0.1	14.8 ± 0.1	14.1 ± 0.1**
Week 13	15.0 ± 0.1	14.9 ± 0.1	14.6 ± 0.1*	14.5 ± 0.1**	14.4 ± 0.1**	13.7 ± 0.1**
Erythrocytes (10^6/μL)						
Day 4	6.21 ± 0.06	6.16 ± 0.05	6.11 ± 0.08	6.18 ± 0.07	5.99 ± 0.04*	6.07 ± 0.12*
Day 23	8.05 ± 0.16	7.61 ± 0.09*	7.95 ± 0.11	7.74 ± 0.08	7.77 ± 0.07	7.31 ± 0.07**
Week 13	9.16 ± 0.08	9.11 ± 0.04	8.99 ± 0.05	8.88 ± 0.05**	8.86 ± 0.06**	8.43 ± 0.07**
Reticulocytes (10^6/μL)						
Day 4	0.81 ± 0.05	0.90 ± 0.05	0.86 ± 0.03	0.92 ± 0.05	0.91 ± 0.05[2]	0.77 ± 0.04
Day 23	0.23 ± 0.02	0.24 ± 0.02	0.24 ± 0.02[3]	0.27 ± 0.03	0.25 ± 0.02	0.24 ± 0.04
Week 13	0.248 ± 0.009	0.282 ± 0.013*	0.283 ± 0.017	0.279 ± 0.012	0.331 ± 0.018**	0.384 ± 0.023**
Nucleated erythrocytes (10^3/μL)						
Day 4	0.29 ± 0.07	0.41 ± 0.06	0.39 ± 0.06	0.37 ± 0.06	0.34 ± 0.05	0.57 ± 0.08**
Day 23	0.00 ± 0.00	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01
Week 13	0.05 ± 0.02	0.05 ± 0.03	0.07 ± 0.03	0.10 ± 0.03	0.15 ± 0.03*	0.16 ± 0.03**
Mean cell volume (fL)						
Day 4	59.0 ± 0.7	59.7 ± 0.3	59.7 ± 0.3	59.7 ± 0.4	59.3 ± 0.3	54.7 ± 0.2**
Day 23	56.0 ± 0.4	56.8 ± 0.2	56.4 ± 0.3	56.2 ± 0.3	56.1 ± 0.1	56.5 ± 0.4
Week 13	50.7 ± 0.2	50.8 ± 0.2	50.6 ± 0.2	50.8 ± 0.1	50.7 ± 0.2	50.5 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.5 ± 0.2	19.7 ± 0.1	19.5 ± 0.1	19.6 ± 0.1	19.3 ± 0.1	17.9 ± 0.2**
Day 23	19.1 ± 0.1	19.3 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.1 ± 0.1	19.2 ± 0.1
Week 13	16.4 ± 0.0	16.4 ± 0.1	16.3 ± 0.1	16.3 ± 0.1	16.3 ± 0.1	16.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.9 ± 0.2	33.0 ± 0.1	32.7 ± 0.1	32.9 ± 0.1	32.5 ± 0.2	32.7 ± 0.2
Day 23	34.0 ± 0.1	34.0 ± 0.1	34.1 ± 0.1	34.1 ± 0.1	33.9 ± 0.2	34.1 ± 0.2
Week 13	32.3 ± 0.1	32.3 ± 0.1	32.1 ± 0.1	32.2 ± 0.1	32.1 ± 0.1	32.1 ± 0.2
Platelets (10^3/μL)						
Day 4	782.6 ± 15.4	757.7 ± 21.9	791.3 ± 10.5	754.2 ± 11.7	730.8 ± 13.0*	484.2 ± 46.1**
Day 23	610.0 ± 16.9	619.1 ± 10.4	610.2 ± 16.5	635.0 ± 12.5	643.5 ± 20.5	699.3 ± 18.2**
Week 13	513.8 ± 9.4	534.7 ± 8.4	525.5 ± 5.9	522.6 ± 27.1	553.5 ± 7.2**	575.8 ± 9.1**
Leukocytes (10^3/μL)						
Day 4	8.40 ± 0.26	8.19 ± 0.41	9.23 ± 0.31	9.00 ± 0.34	8.23 ± 0.39	10.14 ± 0.57*
Day 23	6.65 ± 0.24	6.70 ± 0.32	6.95 ± 0.45	6.70 ± 0.35	7.40 ± 0.49	6.73 ± 0.60
Week 13	6.07 ± 0.20	7.13 ± 0.34*	6.80 ± 0.24*	6.93 ± 0.32*	7.58 ± 0.31**	7.43 ± 0.44**

TABLE 9 Selected Hematology Data for F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene (continued)

	Concentration (ppm)					
	0	1.1	2.3	4.5	9	18
MALE (continued)						
Segmented neutrophils (10^3/μL)						
Day 4	1.48 ± 0.11	1.14 ± 0.11	1.43 ± 0.14	1.42 ± 0.12	1.52 ± 0.14	1.85 ± 0.17
Day 23	0.99 ± 0.09	0.87 ± 0.06	0.80 ± 0.07	0.89 ± 0.08	0.75 ± 0.11	0.80 ± 0.10
Week 13	1.24 ± 0.11	1.28 ± 0.14	1.39 ± 0.13	1.27 ± 0.17	1.09 ± 0.14	0.95 ± 0.10
Lymphocytes (10^3/μL)						
Day 4	6.89 ± 0.18	6.95 ± 0.32	7.69 ± 0.26	7.50 ± 0.30	6.67 ± 0.35	8.19 ± 0.47
Day 23	5.63 ± 0.30	5.77 ± 0.32	6.05 ± 0.45	5.76 ± 0.36	6.60 ± 0.46	5.86 ± 0.57
Week 13	4.74 ± 0.23	5.74 ± 0.35*	5.32 ± 0.21	5.60 ± 0.24*	6.44 ± 0.30**	6.44 ± 0.47**
Methemoglobin (g/dL)						
Day 4	0.09 ± 0.01	0.11 ± 0.01	0.12 ± 0.01**	0.17 ± 0.01**	0.24 ± 0.01**	1.14 ± 0.09**
Day 23	0.14 ± 0.02	0.15 ± 0.01*	0.19 ± 0.01**	0.23 ± 0.02**	0.41 ± 0.02**	0.55 ± 0.01**
Week 13	0.15 ± 0.01	0.21 ± 0.01**	0.26 ± 0.01**	0.36 ± 0.01**	0.55 ± 0.01**	0.87 ± 0.02**
FEMALE						
n						
Day 4	10	10	10	10	10	10
Day 23	9	10	10	10	10	8
Week 13	10	10	10	10	10	10
Hematocrit (automated) (%)						
Day 4	39.5 ± 0.4	39.0 ± 0.4	38.7 ± 0.3	38.8 ± 0.5	38.3 ± 0.4	35.5 ± 0.4**
Day 23	45.8 ± 0.3	45.7 ± 0.3	45.7 ± 0.3	45.1 ± 0.2*	45.4 ± 0.2	43.5 ± 0.6**
Week 13	47.9 ± 0.4	46.8 ± 0.2	47.2 ± 0.4	45.9 ± 0.3**	45.0 ± 0.3**	42.6 ± 0.3**
Hemoglobin (g/dL)						
Day 4	13.1 ± 0.1	12.9 ± 0.1	12.8 ± 0.1	12.8 ± 0.2	12.5 ± 0.2**	11.5 ± 0.1**
Day 23	15.9 ± 0.1	15.8 ± 0.1	15.9 ± 0.1	15.6 ± 0.1*	15.7 ± 0.1	15.1 ± 0.2**
Week 13	15.5 ± 0.1	15.1 ± 0.1**	15.2 ± 0.1	14.7 ± 0.1**	14.3 ± 0.1**	13.4 ± 0.1**
Erythrocytes (10^6/μL)						
Day 4	6.77 ± 0.09	6.69 ± 0.08	6.67 ± 0.08	6.66 ± 0.12	6.63 ± 0.09	6.48 ± 0.09
Day 23	8.09 ± 0.04	8.07 ± 0.05	8.10 ± 0.06	8.02 ± 0.06	8.24 ± 0.06	7.99 ± 0.12
Week 13	8.80 ± 0.06	8.58 ± 0.06*	8.64 ± 0.06	8.41 ± 0.06**	8.24 ± 0.05**	7.84 ± 0.05**
Reticulocytes (10^6/μL)						
Day 4	0.84 ± 0.05[3]	0.84 ± 0.04	0.76 ± 0.05[2]	0.79 ± 0.05[2]	0.72 ± 0.05[2]	0.59 ± 0.05**[2]
Day 23	0.15 ± 0.02	0.19 ± 0.02	0.18 ± 0.03	0.12 ± 0.01[2]	0.19 ± 0.04[2]	0.31 ± 0.06**
Week 13	0.19 ± 0.01	0.22 ± 0.01	0.21 ± 0.02	0.25 ± 0.02**	0.25 ± 0.01**	0.37 ± 0.02**
Nucleated erythrocytes (10^3/μL)						
Day 4	0.25 ± 0.07	0.17 ± 0.03	0.32 ± 0.05	0.26 ± 0.06	0.15 ± 0.04	0.25 ± 0.08
Day 23	0.03 ± 0.02	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.02
Week 13	0.06 ± 0.03	0.09 ± 0.03	0.09 ± 0.03	0.10 ± 0.04	0.11 ± 0.02	0.19 ± 0.03**
Mean cell volume (fL)						
Day 4	58.7 ± 0.3	58.2 ± 0.3	58.2 ± 0.3	58.3 ± 0.3	57.9 ± 0.3	54.8 ± 0.3**
Day 23	56.6 ± 0.3	56.7 ± 0.2	56.4 ± 0.2	56.2 ± 0.3	55.0 ± 0.3**	54.5 ± 0.4**
Week 13	54.5 ± 0.2	54.8 ± 0.2	54.5 ± 0.2	54.7 ± 0.3	54.6 ± 0.3	54.4 ± 0.3

TABLE 9 Selected Hematology Data for F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene (continued)

	Concentration (ppm)					
	0	1.1	2.3	4.5	9	18
FEMALE (continued)						
Mean cell hemoglobin (pg)						
Day 4	19.4 ± 0.1	19.3 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	18.9 ± 0.1**	17.7 ± 0.1**
Day 23	19.6 ± 0.1	19.6 ± 0.1	19.6 ± 0.1	19.4 ± 0.1	19.0 ± 0.1**	18.8 ± 0.1**
Week 13	17.6 ± 0.1	17.6 ± 0.1	17.6 ± 0.1	17.5 ± 0.1	17.3 ± 0.0**	17.1 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.2 ± 0.1	33.1 ± 0.1	33.1 ± 0.1	33.0 ± 0.1	32.6 ± 0.1**	32.3 ± 0.1**
Day 23	34.6 ± 0.1	34.7 ± 0.1	34.7 ± 0.2	34.6 ± 0.2	34.5 ± 0.1	34.6 ± 0.2
Week 13	32.4 ± 0.1	32.2 ± 0.1	32.2 ± 0.2	32.0 ± 0.2	31.8 ± 0.2**	31.5 ± 0.1**
Platelets (10^3/μL)						
Day 4	771.0 ± 31.8	736.1 ± 22.1	733.3 ± 11.0	721.7 ± 28.2	686.5 ± 18.4*	462.5 ± 40.4**
Day 23	638.4 ± 12.6	631.5 ± 18.3	580.0 ± 19.9*	592.5 ± 14.5*	612.7 ± 15.5	605.3 ± 19.0
Week 13	646.1 ± 29.9	560.8 ± 17.3	572.3 ± 39.8	631.4 ± 24.0	610.0 ± 21.2	603.2 ± 18.4
Leukocytes (10^3/μL)						
Day 4	7.87 ± 0.57	8.58 ± 0.48	9.01 ± 0.53	8.36 ± 0.29	9.00 ± 0.41	9.48 ± 0.55
Day 23	7.44 ± 0.34	7.17 ± 0.24	7.72 ± 0.45	6.34 ± 0.31	7.36 ± 0.41	8.43 ± 0.59
Week 13	7.12 ± 0.49	6.19 ± 0.39	6.55 ± 0.50	6.62 ± 0.37	6.65 ± 0.27	6.51 ± 0.33
Segmented neutrophils (10^3/μL)						
Day 4	0.834 ± 0.093	0.978 ± 0.106	1.345 ± 0.127**	0.980 ± 0.108*	1.028 ± 0.116	1.327 ± 0.126**
Day 23	0.90 ± 0.13	0.87 ± 0.09	0.84 ± 0.15	0.84 ± 0.11	0.78 ± 0.08	0.83 ± 0.11
Week 13	1.25 ± 0.11	1.33 ± 0.13	1.28 ± 0.18	1.41 ± 0.23	1.12 ± 0.12	0.93 ± 0.14*
Lymphocytes (10^3/μL)						
Day 4	7.02 ± 0.52	7.56 ± 0.44	7.61 ± 0.48	7.35 ± 0.24	7.89 ± 0.31	8.01 ± 0.49
Day 23	6.45 ± 0.34	6.23 ± 0.25	6.81 ± 0.40	5.49 ± 0.27	6.49 ± 0.43	7.55 ± 0.62
Week 13	5.83 ± 0.46	4.82 ± 0.34	5.23 ± 0.51	5.18 ± 0.26	5.46 ± 0.30	5.56 ± 0.32
Methemoglobin (g/dL)						
Day 4	0.09 ± 0.01	0.11 ± 0.01	0.14 ± 0.01**	0.17 ± 0.01**	0.25 ± 0.01**	1.04 ± 0.08**
Day 23	0.16 ± 0.01	0.18 ± 0.01	0.22 ± 0.01**	0.30 ± 0.01**	0.47 ± 0.02**	0.71 ± 0.04**
Week 13	0.19 ± 0.01	0.22 ± 0.01**	0.28 ± 0.01**	0.35 ± 0.01**	0.51 ± 0.01**	0.79 ± 0.03**

[1] Data are given as mean ± standard error.
[2] n=9.
[3] n=8.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

Significant changes in the clinical chemistry endpoints for the 2-chloronitrobenzene study are shown in Table 10 and Appendix B. In general, there were mild, exposure-related increases in albumin and total protein concentrations in male and female rats in the 9 and 18 ppm groups on Day 23 and at Week 13, consistent with dehydration. Serum activities of alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) were mildly increased in different male and female exposure groups at various time points, suggesting hepatocellular injury. The most pronounced change occurred in males and females in the 18 ppm groups on Day 4, and in male rats, most exposure groups were affected on Day 4. Additionally, SDH activities were increased in males in the 9 and 18 ppm groups at most

time points and in female rats in these groups at all time points. Increased bile acid concentrations, indicative of cholestasis, occurred on Day 4 in male rats exposed to 2.3, 4.5, 9, or 18 ppm and in females exposed to 18 ppm. Decreases in serum activity of alkaline phosphatase (AP) occurred on Day 23 in males in the 9 ppm group and females in the 4.5, 9, and 18 ppm groups. At Week 13, decreases in AP activity occurred in males in the 4.5, 9, and 18 ppm groups and females in the 18 ppm group, likely due to a reduction in feed consumption.

TABLE 10 Selected Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

	Concentration (ppm)					
	0	1.1	2.3	4.5	9	18
MALE						
n						
Day 4	10	10	10	10	10	10
Day 23	8	10	9	10	10	6
Week 13	10	10	10	10	10	10
Total protein (g/dL)						
Day 4	5.5 ± 0.1	5.7 ± 0.1	5.8 ± 0.1	5.7 ± 0.0	5.7 ± 0.1	5.2 ± 0.1
Day 23	6.16 ± 0.07	6.12 ± 0.06	6.38 ± 0.05*	6.35 ± 0.06	6.64 ± 0.09**	6.65 ± 0.10**
Week 13	7.0 ± 0.1	7.2 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.3 ± 0.1**	7.5 ± 0.1**
Albumin (g/dL)						
Day 4	3.3 ± 0.0	3.4 ± 0.0	3.4 ± 0.1	3.4 ± 0.0	3.5 ± 0.1	3.3 ± 0.1
Day 23	3.6 ± 0.1	3.7 ± 0.0	3.8 ± 0.0*	3.8 ± 0.1*	3.9 ± 0.1**	3.9 ± 0.1**
Week 13	4.0 ± 0.0	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.3 ± 0.1**	4.3 ± 0.0**
Globulin (g/dL)						
Day 4	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.0	2.3 ± 0.1	2.0 ± 0.1
Day 23	2.5 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.7 ± 0.1
Week 13	3.0 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	3.2 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	44 ± 1	48 ± 1	50 ± 2*	53 ± 2**	57 ± 3**	212 ± 19**
Day 23	35 ± 1	33 ± 1	36 ± 1	33 ± 1	39 ± 2	47 ± 3**
Week 13	62 ± 5	57 ± 3	60 ± 4	54 ± 3	49 ± 1*	55 ± 2
Alkaline phosphatase (IU/L)						
Day 4	888 ± 26	897 ± 33	838 ± 21	815 ± 24	847 ± 24	1045 ± 38
Day 23	554 ± 16	592 ± 21	543 ± 25	512 ± 18	482 ± 18*	498 ± 14
Week 13	343 ± 8	338 ± 6	323 ± 12	298 ± 10**	294 ± 9**	298 ± 10**
Sorbitol dehydrogenase (IU/L)						
Day 4	10 ± 1	12 ± 0**	14 ± 1**	15 ± 1**	16 ± 1**	34 ± 4**
Day 23	9 ± 0	9 ± 0	10 ± 0	10 ± 0	14 ± 1**	16 ± 1**
Week 13	20 ± 2	20 ± 2	21 ± 3	21 ± 3	22 ± 1	28 ± 2**
Bile acids (μmol/L)						
Day 4	18.97 ± 0.85	24.14 ± 2.00	22.66 ± 1.49*	24.70 ± 0.93**	27.22 ± 1.30**	75.41 ± 5.82**[2]
Day 23	17.00 ± 1.26	18.88 ± 1.57	20.52 ± 1.06	18.68 ± 0.79	21.87 ± 2.56	13.92 ± 1.09[3]
Week 13	19.04 ± 0.53	21.95 ± 1.36	22.03 ± 0.69*[2]	22.76 ± 1.84	22.34 ± 0.80*	20.45 ± 0.81

TABLE 10 Selected Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene (continued)

	Concentration (ppm)					
	0	1.1	2.3	4.5	9	18
FEMALE						
n						
Day 4	10	10	10	10	10	10
Day 23	9	10	10	10	10	8
Week 13	10	10	10	10	10	10
Total protein (g/dL)						
Day 4	5.6 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	5.2 ± 0.1
Day 23	6.1 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.5 ± 0.1**	6.5 ± 0.1**
Week 13	7.4 ± 0.1	7.3 ± 0.1	7.4 ± 0.1	7.5 ± 0.1	7.7 ± 0.1	7.8 ± 0.1*
Albumin (g/dL)						
Day 4	3.6 ± 0.1	3.7 ± 0.0	3.8 ± 0.0	3.8 ± 0.1*	3.9 ± 0.1**	3.5 ± 0.1
Day 23	3.7 ± 0.0	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	4.0 ± 0.0**	4.0 ± 0.0**
Week 13	4.5 ± 0.0	4.5 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.9 ± 0.0**	4.8 ± 0.1**
Globulin (g/dL)						
Day 4	2.0 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	1.8 ± 0.1
Day 23	2.4 ± 0.1	2.5 ± 0.1	2.4 ± 0.0	2.4 ± 0.0	2.5 ± 0.1	2.5 ± 0.1
Week 13	2.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	41 ± 2	40 ± 1	38 ± 1	35 ± 1*	38 ± 1	137 ± 20
Day 23	33 ± 1	33 ± 1	36 ± 1	36 ± 4	35 ± 1	45 ± 3**
Week 13	58 ± 4	50 ± 2	58 ± 3	53 ± 2	47 ± 2*	41 ± 2**
Alkaline phosphatase (IU/L)						
Day 4	736 ± 25	731 ± 16	725 ± 17	700 ± 25	675 ± 30	838 ± 81
Day 23	459 ± 16	435 ± 18	444 ± 13	415 ± 20*	379 ± 11**	342 ± 15**[4]
Week 13	309 ± 11	310 ± 16	294 ± 12	274 ± 14	278 ± 16	242 ± 14**
Sorbitol dehydrogenase (IU/L)						
Day 4	9 ± 1	9 ± 0	9 ± 1	9 ± 0	12 ± 1**	20 ± 1**
Day 23	11 ± 1	12 ± 1	12 ± 1	11 ± 1[2]	14 ± 1**	23 ± 2**
Week 13	19 ± 1	18 ± 1	23 ± 1	22 ± 1	24 ± 1*	26 ± 2**
Bile acids (μmol/L)						
Day 4	19.25 ± 1.10	19.95 ± 1.15	17.74 ± 0.68	20.41 ± 1.06	21.92 ± 0.94	34.71 ± 3.39**
Day 23	16.44 ± 1.07	12.87 ± 0.57	18.38 ± 3.42	18.56 ± 2.83	16.34 ± 0.55	17.55 ± 0.41
Week 13	24.10 ± 2.64	20.65 ± 2.01	20.55 ± 1.82	19.20 ± 0.94	19.93 ± 1.68	20.50 ± 1.72

[1] Data are given as mean ± standard error.
[2] n=9.
[3] n=5.
[4] n=7.
* 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.

The absolute and relative liver weights of rats exposed to 2-chloronitrobenzene increased with exposure concentration, and these increases in liver weights were significant for males exposed to 2.3 ppm or greater and females exposed to 4.5 ppm or greater (Table 11). Absolute and relative spleen weights of males exposed to 18 ppm and females exposed to 4.5, 9, or 18 ppm were significantly increased. Relative right kidney weights of males in the 9 and 18 ppm groups and absolute and relative right kidney weights of females in the 18 ppm group were significantly increased (Appendix A). In males exposed to 18 ppm, absolute and relative lung weights were significantly decreased.

At necropsy, dark spleens in 2 of 10 males and 1 of 10 females in the 18 ppm group were the only gross findings attributed to exposure to 2-chloronitrobenzene. Microscopically, the liver, kidney, spleen, and nasal cavity were identified as target tissues for 2-chloronitrobenzene toxicity.

In the liver, cytoplasmic basophilia of centrilobular hepatocytes was observed in all male rats and most female rats in the 9 and 18 ppm groups (Table 12). The amount of cytoplasm of affected hepatocytes was slightly increased, and the cytoplasm was mottled by clumped basophilic material interspersed with finely granular "ground-glass" areas. This change was of minimal severity.

In the kidney, cytoplasmic pigment within proximal convoluted tubule cells was seen in male rats exposed to 4.5 ppm or greater and female rats exposed to 9 or 18 ppm (Table 12). All rats in the 18 ppm group were affected. The cytoplasmic pigment was granular and brown in H&E-stained sections; special stains of selected slides revealed it to be iron negative and PAS positive and, therefore, presumably a lipofuscin pigment. A concentration-dependent increase in the incidence and severity of tubule regeneration was also observed in exposed male rats (Table 12). No morphologic changes consistent with hyaline droplet nephropathy were observed in male rats; the absence of protein droplet accumulation was confirmed by Mallory-Heidenhain special stains.

TABLE 11 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

	Concentration (ppm)					
	0	1.1	2.3	4.5	9	18
MALE						
n	10	10	10	10	10	10
Necropsy body wt	334 ± 7	350 ± 7	343 ± 7	349 ± 8	340 ± 7	323 ± 6
Right kidney						
Absolute	1.124 ± 0.032	1.153 ± 0.063	1.184 ± 0.032	1.242 ± 0.032	1.218 ± 0.029	1.225 ± 0.029
Relative	3.36 ± 0.04	3.28 ± 0.15	3.45 ± 0.04	3.56 ± 0.04	3.59 ± 0.04*	3.79 ± 0.04**
Liver						
Absolute	11.820 ± 0.356	13.102 ± 0.401*	12.987 ± 0.356*	14.126 ± 0.576**	14.160 ± 0.398**	15.543 ± 0.346**
Relative	35.33 ± 0.58	37.48 ± 1.26	37.81 ± 0.48*	40.40 ± 0.84**	41.67 ± 0.61**	48.07 ± 0.68**
Spleen						
Absolute	0.631 ± 0.016	0.680 ± 0.014[2]	0.650 ± 0.020	0.659 ± 0.018	0.669 ± 0.012	0.753 ± 0.018**
Relative	1.89 ± 0.03	1.93 ± 0.04[2]	1.89 ± 0.03	1.89 ± 0.02	1.97 ± 0.02	2.33 ± 0.04**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	191 ± 3	188 ± 4	200 ± 4	193 ± 3	196 ± 5	193 ± 4
Right kidney						
Absolute	0.641 ± 0.009	0.641 ± 0.014	0.720 ± 0.054	0.666 ± 0.017	0.696 ± 0.016	0.739 ± 0.028*
Relative	3.36 ± 0.02	3.42 ± 0.08	3.57 ± 0.21	3.44 ± 0.07	3.55 ± 0.05	3.83 ± 0.09**
Liver						
Absolute	6.658 ± 0.191	6.751 ± 0.124	7.397 ± 0.203*	7.610 ± 0.221**	8.594 ± 0.273**	9.773 ± 0.362**
Relative	34.86 ± 0.75	36.00 ± 0.68	36.91 ± 0.61	39.29 ± 0.72**	43.73 ± 0.54**	50.67 ± 1.07**
Spleen						
Absolute	0.422 ± 0.006	0.420 ± 0.009	0.440 ± 0.012	0.463 ± 0.008*	0.468 ± 0.010**	0.538 ± 0.020**
Relative	2.21 ± 0.04	2.24 ± 0.04	2.20 ± 0.03	2.40 ± 0.05*	2.39 ± 0.05*	2.80 ± 0.09**

[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] n=9.
* Significantly different (P≤0.05) from the control group by Williams' test.
** Significantly different (P≤0.01) from the control group by Williams' test.

Congestion of the spleen, consisting of increased red blood cells within the red pulp parenchyma, was observed in control and exposed rats. Slightly increased severity in exposed males and slightly increased incidences in exposed females suggested a possible exposure-related effect (Table 12). No increase in spleen hemosiderin pigment was apparent in exposed rats.

TABLE 12 Incidence and Severity of Selected Lesions in F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

	Concentration (ppm)					
	0	1.1	2.3	4.5	9	18
MALE						
n	10	10	10	10	10	10
Kidney						
Tubule pigment	0	0	0	4 (1.0)	4 (1.0)	10 (1.0)
Tubule regeneration	1 (1.0)	4 (1.0)	6 (1.0)	9 (1.2)	8 (1.0)	10 (1.3)
Liver						
Cytoplasmic basophilia	0	0	0	0	10 (1.0)	10 (1.0)
Nasal cavity						
Respiratory epithelium hyperplasia	4 (1.0)	9 (1.0)	8 (1.0)	10 (1.2)	10 (1.4)	9 (1.1)
Spleen						
Congestion	8 (1.4)	9 (1.6)	10 (1.5)	10 (1.6)	10 (1.4)	10 (1.9)
FEMALE						
n	10	10	10	10	10	10
Kidney						
Tubule pigment	0	0	0	0	10 (1.0)	10 (3.0)
Tubule regeneration	0	0	0	0	0	0
Liver						
Cytoplasmic basophilia	0	0	0	0	6 (1.0)	8 (1.0)
Nasal cavity						
Respiratory epithelium hyperplasia	0	8 (1.0)	9 (1.0)	10 (1.1)	9 (1.1)	6 (1.2)
Spleen						
Congestion	4 (1.0)	4 (1.0)	7 (1.0)	3 (1.0)	9 (1.0)	10 (1.0)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

Hyperplasia/hypertrophy of the respiratory epithelium was a treatment-related effect observed in the nasal cavity (Table 12). This effect was restricted to the dorsal meatus and nasoturbinate of the most anterior nasal section (at the level of the incisor teeth). The change was characterized by multifocal infoldings of tall columnar epithelial cells in this area that produced a scalloped mucosal surface or small intraepithelial cysts. These invaginations were lined by flattened ciliated cells or goblet-like cells and sometimes contained wispy strands of mucoid material; occasionally, dilated glandular structures with luminal neutrophils were also present in the lamina propria in these areas. Although a few control male rats had small, widely scattered mucosal infoldings of a similar nature and concentration-related effects were not clear, a more extensive occurrence of this lesion in exposed male rats appeared to be related to 2-chloronitrobenzene exposure.

Sperm morphology and vaginal cytology evaluations were performed on rats exposed to 0, 4.5, 9, or 18 ppm 2-chloronitrobenzene (Appendix C). The left cauda epididymal weight, spermatid heads per testis, and spermatid count of males in the 18 ppm group were significantly lower than those of control males; there were no significant changes in females.

4-Chloronitrobenzene: The hematology findings were consistent with methemoglobinemia and a responsive macrocytic, hyperchromic anemia, typical for a hemolytic anemia. Methemoglobin concentrations were elevated in all groups of exposed male and female rats at all time points (Table 13 and Appendix B). Decreases in HCT, HGB concentrations, and RBC counts occurred in all groups of exposed male and female rats by Week 13. In male rats, these changes involved most exposure groups at Day 3 and all but the lowest (1.5 ppm) exposure group at Day 23. In female rats, decreases in HCT and RBC counts occurred only in the 24 ppm group on Day 3; HCT, HGB concentrations, and RBC counts were decreased in all but the 1.5 ppm group on Day 23. In contrast to the results of the 2-chloronitrobenzene study, RBC indices (MCV, MCH, and MCHC) were increased in exposed male and female rats (Table 13). All three parameters were consistently increased in males and females in the 12 and 24 ppm groups on Day 23 and at Week 13. MCHC was also elevated on Day 3 in males and females exposed to 24 ppm and on Day 3 and at Week 13 in females in other exposure groups. Elevations in MCV and MCH values also occurred in male rats in the 6 ppm group and female rats in all lower exposure groups at Week 13. The only exception to the increases in RBC indices occurred in female rats on Day 3. At this time point, females at all exposure concentrations except 24 ppm had decreased MCV, MCH, or MCV and MCH values. Reticulocyte counts were significantly increased in male and female rats in the 12 and 24 ppm groups at all time points except in males in the 12 ppm group on Day 3 (Table 13). On Day 23 and at Week 13, reticulocyte counts were also increased in males exposed to 6 ppm and females exposed to 3 or 6 ppm, and at Week 13, the reticulocyte count in males in the 3 ppm group was increased. The males exposed to 1.5 ppm had increased reticulocyte counts on Day 3 and at Week 13. Increased numbers of nucleated RBCs accompanied the increases in reticulocyte counts in exposed male and female rats at most time points (Table 13). Platelet counts were increased in male and female rats at various exposure levels at one or more time points (Table 13). In the 12 and 24 ppm groups, the increases occurred on Days 3 and 23, while in the lower exposure groups, significant increases occurred on Day 23 (males and females) and at Week 13 (males only). In general, WBC counts were

increased in male and female rats in various exposure groups on Day 23 and at Week 13 (Table 13). Increases occurred in male and female rats in the 12 and 24 ppm groups and in females in the 3 and 6 ppm groups. In rats exposed to 12 or 24 ppm, increased WBC counts were often accompanied by increases in both segmented neutrophil and lymphocyte numbers; these changes are consistent with an erroneously elevated WBC count caused by reticulocytes resistant to lysis being counted as leukocytes during the automated count. Increased lymphocyte numbers also occurred in females in the 1.5, 3, and 6 ppm groups on Day 23, at Week 13, or at both time points. At Day 3, however, the WBC count of male rats in the 24 ppm group was significantly decreased; this change was accompanied by an increased number of segmented neutrophils and a decreased lymphocyte count and is consistent with a stress (endogenous steroid release) response.

Clinical chemistry changes that occurred in the 4-chloronitrobenzene study are shown in Table 14 and Appendix B. In general, decreases in globulin and/or total protein concentrations occurred in male and female rats in the 6, 12, and 24 ppm groups at various time points. Most decreases occurred on Day 23 and at Week 13. As in the 2-chloronitrobenzene study, increases in SDH activities occurred in various exposure groups at different time points. The 24 ppm groups were most frequently affected. Elevations in bile acid concentrations occurred in males in the 3, 6, 12, and 24 ppm groups at almost every time point. Bile acid levels were also increased in female rats. However, significant increases did not occur at Week 13 as in the male rats, and bile acid levels were significantly increased on both Day 3 and Day 23 only for females in the 12 and 24 ppm groups. Decreases in serum AP activity occurred at all time points in males exposed to 12 or 24 ppm and females exposed to 6, 12, or 24 ppm. AP activities were also significantly decreased in other exposure groups of females on Day 23 and in males and females at Week 13.

TABLE 13 Selected Hematology Data for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

	Concentration (ppm)					
	0	1.5	3	6	12	24
MALE						
n	10	10	10	10	10	10
Hematocrit (automated) (%)						
Day 3	41.2 ± 0.5	41.4 ± 0.7	39.5 ± 0.4*	40.7 ± 0.5	39.2 ± 0.4**	38.2 ± 0.3**
Day 23	46.4 ± 0.4	45.2 ± 0.4*	44.2 ± 0.6**	42.7 ± 0.4**	41.0 ± 0.3**	39.1 ± 0.4**
Week 13	46.8 ± 0.3	44.9 ± 0.2**	43.8 ± 0.3**	41.9 ± 0.3**	39.9 ± 0.2**	36.1 ± 0.5**
Hemoglobin (g/dL)						
Day 3	13.3 ± 0.1	13.3 ± 0.2	12.7 ± 0.2**	13.1 ± 0.2	12.6 ± 0.2**	12.9 ± 0.1*
Day 23	15.0 ± 0.1	14.6 ± 0.1	14.4 ± 0.2**	13.9 ± 0.1**	13.7 ± 0.1**	13.2 ± 0.2**
Week 13	14.9 ± 0.1	14.2 ± 0.1**	13.9 ± 0.1**	13.3 ± 0.1**	13.4 ± 0.1**	12.6 ± 0.2**
Erythrocytes (10^6/μL)						
Day 3	7.00 ± 0.09	6.94 ± 0.12	6.62 ± 0.09**	6.96 ± 0.11	6.72 ± 0.13	6.53 ± 0.08**
Day 23	8.06 ± 0.09	7.93 ± 0.06	7.76 ± 0.12*	7.49 ± 0.08**	6.89 ± 0.06**	5.79 ± 0.10**
Week 13	9.00 ± 0.06	8.69 ± 0.04**	8.40 ± 0.04**	7.83 ± 0.04**	7.13 ± 0.06**	5.73 ± 0.07**
Reticulocytes (10^6/μL)						
Day 3	0.48 ± 0.03	0.68 ± 0.04**	0.56 ± 0.03	0.50 ± 0.04	0.53 ± 0.02	0.67 ± 0.04**
Day 23	0.31 ± 0.02	0.32 ± 0.02	0.36 ± 0.02	0.46 ± 0.02**	0.72 ± 0.03**	1.16 ± 0.04**
Week 13	0.17 ± 0.01	0.27 ± 0.02**	0.30 ± 0.02**	0.42 ± 0.02**	0.59 ± 0.03**	0.91 ± 0.03**
Nucleated erythrocytes (10^3/μL)						
Day 3	0.13 ± 0.03	0.15 ± 0.05	0.27 ± 0.05*	0.18 ± 0.04	0.23 ± 0.06	0.38 ± 0.08*
Day 23	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.25 ± 0.04**	0.63 ± 0.07**	3.39 ± 0.25**
Week 13	0.03 ± 0.01	0.07 ± 0.03	0.10 ± 0.03*	0.23 ± 0.04**	0.50 ± 0.08**	0.93 ± 0.11**
Mean cell volume (fL)						
Day 3	58.9 ± 0.2	59.8 ± 0.3	59.7 ± 0.3	58.8 ± 0.3	58.4 ± 0.5	58.6 ± 0.3
Day 23	57.5 ± 0.2	56.9 ± 0.2	57.1 ± 0.3	57.0 ± 0.2	59.5 ± 0.4**	67.7 ± 0.8**
Week 13	51.9 ± 0.1	51.7 ± 0.2	52.2 ± 0.1	53.6 ± 0.2**	55.7 ± 0.3**	62.9 ± 0.3**
Mean cell hemoglobin (pg)						
Day 3	19.0 ± 0.1	19.1 ± 0.1	19.1 ± 0.1	18.8 ± 0.1	18.7 ± 0.1	19.8 ± 0.3
Day 23	18.7 ± 0.1	18.4 ± 0.1	18.6 ± 0.1	18.6 ± 0.1	19.9 ± 0.1**	22.8 ± 0.2**
Week 13	16.5 ± 0.1	16.4 ± 0.1	16.6 ± 0.1	17.0 ± 0.1**	18.8 ± 0.2**	22.1 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 3	32.2 ± 0.1	32.0 ± 0.1	32.0 ± 0.1	32.1 ± 0.1	32.0 ± 0.1	33.8 ± 0.4*
Day 23	32.4 ± 0.1	32.4 ± 0.1	32.6 ± 0.1	32.5 ± 0.1	33.3 ± 0.2**	33.8 ± 0.3**
Week 13	31.8 ± 0.1	31.6 ± 0.2	31.8 ± 0.1	31.8 ± 0.1	33.7 ± 0.2**	35.0 ± 0.2**
Platelets (10^3/μL)						
Day 3	774.2 ± 16.0	774.7 ± 8.5	793.4 ± 41.7	728.8 ± 13.8	796.9 ± 10.8	899.6 ± 21.6**
Day 23	584.4 ± 31.7	638.9 ± 9.3	674.0 ± 17.0**	698.0 ± 11.8**	708.3 ± 10.7**	767.3 ± 19.0**
Week 13	507.5 ± 9.0	596.8 ± 15.4*	629.0 ± 20.2**	658.1 ± 23.4**	547.2 ± 19.1	465.6 ± 30.5
Leukocytes (10^3/μL)						
Day 3	8.54 ± 0.44	9.33 ± 0.25	8.62 ± 0.31	7.90 ± 0.28	7.55 ± 0.32	6.23 ± 0.26**
Day 23	7.33 ± 0.50	6.61 ± 0.48	7.71 ± 0.45	7.66 ± 0.51	9.08 ± 0.52*	12.33 ± 1.07**
Week 13	7.76 ± 0.57	8.24 ± 0.38	8.00 ± 0.35	8.54 ± 0.36	7.75 ± 0.35	9.56 ± 0.53**
Segmented neutrophils (10^3/μL)						
Day 3	1.06 ± 0.11	1.17 ± 0.13	1.24 ± 0.13	1.19 ± 0.10	1.22 ± 0.10	1.85 ± 0.13**
Day 23	0.84 ± 0.07	1.09 ± 0.17	1.02 ± 0.11	0.94 ± 0.13	1.17 ± 0.10*	1.66 ± 0.13**
Week 13	1.20 ± 0.07[2]	1.33 ± 0.14	1.38 ± 0.17	1.32 ± 0.10	1.31 ± 0.09	2.00 ± 0.30*

TABLE 13 Selected Hematology Data for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene (continued)

	Concentration (ppm)					
	0	1.5	3	6	12	24
MALE (continued)						
Lymphocytes (10^3/μL)						
Day 3	7.43 ± 0.41	8.13 ± 0.21	7.34 ± 0.28	6.69 ± 0.26	6.31 ± 0.30*	4.35 ± 0.20**
Day 23	6.45 ± 0.49	5.48 ± 0.36	6.64 ± 0.42	6.70 ± 0.50	7.86 ± 0.48*	10.67 ± 1.05**
Week 13	6.01 ± 0.39	6.62 ± 0.28	6.48 ± 0.34	7.02 ± 0.28	6.23 ± 0.34	7.30 ± 0.30*
Methemoglobin (g/dL)						
Day 3	0.08 ± 0.00	0.19 ± 0.01**	0.31 ± 0.02**	0.60 ± 0.04**	1.31 ± 0.09**	4.13 ± 0.26**
Day 23	0.13 ± 0.01	0.32 ± 0.02**	0.57 ± 0.02**	0.98 ± 0.03**	1.90 ± 0.07**	2.97 ± 0.23**
Week 13	0.16 ± 0.01	0.50 ± 0.01**	0.73 ± 0.01**	1.22 ± 0.04**	2.08 ± 0.06**	2.96 ± 0.05**
FEMALE						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	8
Week 13	10	10	9	10	10	10
Hematocrit (automated) (%)						
Day 3	43.0 ± 0.5	43.5 ± 0.6	42.7 ± 0.5	42.9 ± 0.5	41.9 ± 0.3	39.8 ± 0.5**
Day 23	48.0 ± 0.3	47.3 ± 0.6	45.5 ± 0.3**	42.9 ± 0.5**	39.7 ± 0.3**	40.0 ± 0.5**
Week 13	48.7 ± 0.3	44.2 ± 0.4**	42.6 ± 0.3**	41.6 ± 0.3**	39.8 ± 0.3**	34.5 ± 0.5**
Hemoglobin (g/dL)						
Day 3	13.5 ± 0.2	13.9 ± 0.2	13.6 ± 0.2	13.5 ± 0.1	13.0 ± 0.1	14.0 ± 0.2
Day 23	15.3 ± 0.1	15.2 ± 0.2	14.5 ± 0.1**	13.7 ± 0.2**	13.4 ± 0.1**	13.9 ± 0.2**
Week 13	15.4 ± 0.1	14.2 ± 0.1**	13.6 ± 0.1**	13.7 ± 0.1**	13.7 ± 0.1**	12.3 ± 0.2**
Erythrocytes (10^6/μL)						
Day 3	7.06 ± 0.10	7.38 ± 0.12	7.22 ± 0.12	7.32 ± 0.11	7.03 ± 0.10	6.58 ± 0.08**
Day 23	8.15 ± 0.06	8.14 ± 0.10	7.75 ± 0.04**	7.27 ± 0.10**	6.30 ± 0.08**	5.67 ± 0.08**
Week 13	8.68 ± 0.06	7.77 ± 0.10**	7.41 ± 0.05**	7.00 ± 0.06**	6.36 ± 0.08**	4.87 ± 0.09**
Reticulocytes (10^6/μL)						
Day 3	0.48 ± 0.02	0.45 ± 0.02	0.46 ± 0.02	0.49 ± 0.02	0.55 ± 0.02*	0.69 ± 0.03**
Day 23	0.18 ± 0.01	0.21 ± 0.01	0.31 ± 0.02**	0.48 ± 0.02**	0.72 ± 0.03**	1.20 ± 0.04**
Week 13	0.17 ± 0.02	0.21 ± 0.01[2]	0.38 ± 0.02**	0.54 ± 0.03**	0.81 ± 0.07**	1.51 ± 0.07**
Nucleated erythrocytes (10^3/μL)						
Day 3	0.18 ± 0.03	0.14 ± 0.04	0.16 ± 0.04	0.15 ± 0.02	0.18 ± 0.04	0.81 ± 0.18**
Day 23	0.06 ± 0.02	0.09 ± 0.04	0.12 ± 0.05	0.25 ± 0.05**	1.13 ± 0.22**	1.97 ± 0.27**
Week 13	0.06 ± 0.03	0.18 ± 0.04*	0.29 ± 0.07**	0.82 ± 0.13**	1.29 ± 0.11**	4.96 ± 0.74**
Mean cell volume (fL)						
Day 3	61.0 ± 0.3	58.9 ± 0.5*	59.2 ± 0.4*	58.5 ± 0.3**	59.7 ± 0.7	60.5 ± 0.2
Day 23	58.8 ± 0.3	58.2 ± 0.3	58.7 ± 0.4	58.9 ± 0.3	63.2 ± 0.6**	70.6 ± 0.3**
Week 13	55.9 ± 0.1	56.9 ± 0.3**	57.7 ± 0.2**	59.4 ± 0.2**	62.5 ± 0.4**	70.8 ± 0.4**
Mean cell hemoglobin (pg)						
Day 3	19.1 ± 0.1	18.8 ± 0.1	18.9 ± 0.1	18.5 ± 0.1*	18.5 ± 0.1*	21.3 ± 0.4
Day 23	18.8 ± 0.1	18.7 ± 0.1	18.7 ± 0.1	18.9 ± 0.1	21.2 ± 0.1**	24.4 ± 0.4**
Week 13	17.8 ± 0.1	18.2 ± 0.2*	18.3 ± 0.1**	19.5 ± 0.1**	21.5 ± 0.2**	25.3 ± 0.2**

TABLE 13 Selected Hematology Data for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene (continued)

	Concentration (ppm)					
	0	1.5	3	6	12	24
FEMALE (continued)						
Mean cell hemoglobin concentration (g/dL)						
Day 3	31.4 ± 0.1	31.9 ± 0.1**	31.9 ± 0.2**	31.6 ± 0.1*	31.0 ± 0.2	35.2 ± 0.7**
Day 23	31.9 ± 0.1	32.1 ± 0.1	31.9 ± 0.1	32.0 ± 0.2	33.6 ± 0.2**	34.7 ± 0.5**
Week 13	31.7 ± 0.1	32.0 ± 0.2	31.9 ± 0.1	32.9 ± 0.1**	34.4 ± 0.2**	35.8 ± 0.1**
Platelets (10^3/μL)						
Day 3	804.7 ± 47.4	722.8 ± 20.6	777.1 ± 27.2	754.4 ± 25.4	964.6 ± 48.7*	1166.9 ± 53.5**
Day 23	616.4 ± 7.1	606.3 ± 19.7	666.6 ± 12.3**	712.4 ± 24.5**	698.1 ± 13.8**	734.1 ± 30.5**
Week 13	536.5 ± 18.9	666.9 ± 30.8	783.7 ± 35.1	706.6 ± 32.5	563.6 ± 22.5	478.0 ± 24.3
Leukocytes (10^3/μL)						
Day 3	8.68 ± 0.67	8.31 ± 0.51	8.69 ± 0.39	8.64 ± 0.40	7.84 ± 0.22	9.61 ± 1.14
Day 23	7.26 ± 0.31	7.03 ± 0.38	8.68 ± 0.48*	9.14 ± 0.50**	11.72 ± 0.58**	12.15 ± 1.09**
Week 13	7.77 ± 0.40	8.73 ± 0.31	9.23 ± 0.51*	8.90 ± 0.59	9.66 ± 0.67*	19.63 ± 1.57**
Segmented neutrophils (10^3/μL)						
Day 3	0.88 ± 0.07	0.84 ± 0.12	0.84 ± 0.09	0.96 ± 0.12	0.90 ± 0.14	2.02 ± 0.32**
Day 23	0.86 ± 0.11	1.02 ± 0.17	1.28 ± 0.21	1.21 ± 0.16	1.32 ± 0.10*	1.26 ± 0.19*
Week 13	1.47 ± 0.16	1.51 ± 0.20	1.42 ± 0.11	1.23 ± 0.09	2.39 ± 0.28*	4.04 ± 0.28**
Lymphocytes (10^3/μL)						
Day 3	7.77 ± 0.64	7.43 ± 0.43	7.81 ± 0.37	7.65 ± 0.32	6.88 ± 0.18	7.57 ± 0.95
Day 23	6.34 ± 0.34	5.96 ± 0.29	7.38 ± 0.50	7.90 ± 0.40*	10.36 ± 0.57**	10.89 ± 1.04**
Week 13	6.19 ± 0.34	7.06 ± 0.23*	7.69 ± 0.52*	7.49 ± 0.56*	7.12 ± 0.55	15.19 ± 1.33**
Methemoglobin (g/dL)						
Day 3	0.07 ± 0.01	0.20 ± 0.01**	0.38 ± 0.02**	0.72 ± 0.04**	1.79 ± 0.09**	5.90 ± 0.43**
Day 23	0.14 ± 0.01	0.48 ± 0.02**	0.82 ± 0.02**	1.44 ± 0.06**	2.87 ± 0.17**	3.88 ± 0.25**
Week 13	0.16 ± 0.01	0.63 ± 0.03**	0.90 ± 0.03**	1.69 ± 0.04**	2.50 ± 0.09**	2.85 ± 0.07**

[1] Data are given as mean ± standard error.
[2] 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 14 Selected Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

	Concentration (ppm)					
	0	1.5	3	6	12	24
MALE						
n	10	10	10	10	10	10
Total protein (g/dL)						
Day 3	5.5 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.7 ± 0.1	5.4 ± 0.1	5.6 ± 0.1
Day 23	6.43 ± 0.04	6.44 ± 0.05	6.28 ± 0.06	6.24 ± 0.08*	6.19 ± 0.06**	6.15 ± 0.08**
Week 13	7.1 + 0.1	7.1 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	6.7 ± 0.1**	6.6 ± 0.1**
Albumin (g/dL)						
Day 3	3.4 ± 0.1	3.5 ± 0.1	3.5 ± 0.0	3.6 ± 0.1**	3.4 ± 0.1	3.4 ± 0.0
Day 23	3.7 ± 0.0	3.7 ± 0.0	3.7 ± 0.1	3.6 ± 0.0	3.6 ± 0.1	3.7 ± 0.0
Week 13	4.1 ± 0.0	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1
Globulin (g/dL)						
Day 3	2.1 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.2 ± 0.1
Day 23	2.8 ± 0.0	2.8 ± 0.0	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.0*	2.5 ± 0.1**
Week 13	3.1 ± 0.1	3.1 ± 0.0	3.1 ± 0.1	3.0 ± 0.1	2.8 ± 0.0**	2.6 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 3	44 ± 1	43 ± 1	43 ± 1	47 ± 2	46 ± 1	42 ± 1
Day 23	36.1 ± 0.6	33.4 ± 0.8*	32.9 ± 1.0*	32.6 ± 1.3**	31.7 ± 0.8**	30.6 ± 0.8**
Week 13	58 ± 5	63 ± 5	56 ± 3	54 ± 5	48 ± 1	36 ± 2**
Alkaline phosphatase (IU/L)						
Day 3	935 ± 17	905 ± 19	984 ± 73	962 ± 57	862 ± 24*[2]	792 ± 18**
Day 23	552 ± 14	541 ± 18	566 ± 32	483 ± 50	485 ± 15**	470 ± 13**
Week 13	411 ± 18	365 ± 12*	371 ± 11	373 ± 13	355 ± 9**	293 ± 17**
Sorbitol dehydrogenase (IU/L)						
Day 3	15 ± 0	16 ± 0	14 ± 1	17 ± 0**	20 ± 0**	21 ± 1**
Day 23	14.7 ± 0.7	14.0 ± 0.5	14.4 ± 0.4	15.7 ± 0.6	17.5 ± 1.2	17.6 ± 1.0*
Week 13	24 ± 3	26 ± 3	23 ± 1	25 ± 2	23 ± 1	22 ± 2
Bile acids (μmol/L)						
Day 3	17.70 ± 0.62	19.70 ± 1.16	22.40 ± 1.39*	26.44 ± 2.09**[2]	30.90 ± 1.25**	24.80 ± 0.90**
Day 23	20.30 ± 1.03	20.80 ± 0.73	21.11 ± 1.06[2]	23.80 ± 1.15*	33.70 ± 2.60**	43.50 ± 4.18**
Week 13	23.20 ± 3.09	26.30 ± 2.21	27.30 ± 2.15*	26.20 ± 1.61*	29.70 ± 1.87**	35.00 ± 3.90**
FEMALE						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	8
Week 13	10	10	9	10	10	10
Total protein (g/dL)						
Day 3	5.6 ± 0.1	6.0 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.7 ± 0.0
Day 23	6.3 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.0 ± 0.1*
Week 13	7.36 ± 0.09	7.18 ± 0.09	7.32 ± 0.12	7.11 ± 0.10	7.09 ± 0.07*	6.84 ± 0.12**
Albumin (g/dL)						
Day 3	3.5 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.8 ± 0.0**	3.6 ± 0.1	3.5 ± 0.1
Day 23	3.8 ± 0.0	3.9 ± 0.1	4.0 ± 0.0	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1
Week 13	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1

TABLE 14 Selected Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene (continued)

	Concentration (ppm)					
	0	1.5	3	6	12	24
FEMALE (continued)						
Globulin (g/dL)						
Day 3	2.2 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.0 ± 0.0**	2.0 ± 0.1*	2.1 ± 0.0
Day 23	2.5 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.1 ± 0.1*
Week 13	3.1 ± 0.1	2.9 ± 0.1	3.0 ± 0.1	2.8 ± 0.1**	2.8 ± 0.1**	2.6 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 3	39 ± 2	41 ± 1	40 ± 1	40 ± 2	38 ± 1	40 ± 2
Day 23	31 ± 1	34 ± 1	32 ± 1	31 ± 1	31 ± 1	33 ± 1
Week 13	46 ± 2	46 ± 2	49 ± 4	44 ± 1	39 ± 2*	36 ± 2**
Alkaline phosphatase (IU/L)						
Day 3	861 ± 67	820 ± 69	736 ± 20	688 ± 29**	675 ± 19**	642 ± 23**
Day 23	493 ± 51	473 ± 40	389 ± 13*	388 ± 11**	364 ± 12**	360 ± 12**
Week 13	404 ± 9	377 ± 11	358 ± 16*	342 ± 15**	335 ± 14**	265 ± 14**
Sorbitol dehydrogenase (IU/L)						
Day 3	15 ± 0	16 ± 1	16 ± 1	17 ± 0**	19 ± 0**	23 ± 1**
Day 23	20 ± 1	19 ± 1	19 ± 1	21 ± 1	23 ± 1**	26 ± 1**
Week 13	19 ± 1	20 ± 1	21 ± 1	21 ± 1	22 ± 1	23 ± 2
Bile acids (μmol/L)						
Day 3	15.80 ± 1.04	24.30 ± 3.16**	17.90 ± 1.09	22.30 ± 2.79*	23.40 ± 1.29**	24.11 ± 3.35**[2]
Day 23	19.90 ± 1.93	20.60 ± 2.44	18.80 ± 0.76	20.10 ± 1.03	23.90 ± 1.11*	27.75 ± 1.95**
Week 13	22.20 ± 3.84	25.90 ± 3.37	23.11 ± 2.90	34.20 ± 6.78	25.50 ± 3.22	29.60 ± 3.62

[1] Data are given as mean ± standard error.
[2] n=9.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test.

Absolute and relative spleen weights were markedly increased in males and females exposed to 4-chloronitrobenzene, and heart, liver, and thymus weights in females were mildly increased with increasing concentration (Table 15 and Appendix A). Spleen weights were significantly increased in males exposed to 3 ppm or greater. Spleen and liver weights were significantly increased in females exposed to 6 ppm or greater. Absolute and relative heart and thymus weights were slightly increased in females in the 12 and 24 ppm groups. In males in the 24 ppm group, the relative heart weight and absolute and relative kidney, liver, and thymus weights were significantly increased and absolute and relative right testis weights were significantly decreased (Appendix A). In females exposed to 24 ppm, absolute and relative right kidney weights were significantly increased.

TABLE 15 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

	Concentration (ppm)					
	0	1.5	3	6	12	24
MALE						
n	10	10	10	10	10	10
Necropsy body wt	342 ± 4	354 ± 9	347 ± 4	348 ± 6	337 ± 5	346 ± 7
Heart						
Absolute	0.998 ± 0.018	1.004 ± 0.019	0.987 ± 0.014	1.014 ± 0.025	0.999 ± 0.015	1.047 ± 0.019
Relative	2.92 ± 0.04	2.84 ± 0.03	2.85 ± 0.03	2.91 ± 0.04	2.97 ± 0.03	3.03 ± 0.03*
Liver						
Absolute	12.055 ± 0.373	12.680 ± 0.445	12.206 ± 0.284	12.849 ± 0.357	12.425 ± 0.230	14.323 ± 0.367**
Relative	35.19 ± 0.86	35.79 ± 0.58	35.18 ± 0.52	36.89 ± 0.68	36.91 ± 0.40	41.35 ± 0.45**
Spleen						
Absolute	0.637 ± 0.017	0.716 ± 0.018	0.789 ± 0.039*	0.983 ± 0.024**	1.659 ± 0.062**[2]	3.280 ± 0.079**
Relative	1.86 ± 0.04	2.02 ± 0.02	2.28 ± 0.11**	2.82 ± 0.05**	4.92 ± 0.15**[2]	9.48 ± 0.17**
Right testis						
Absolute	1.369 ± 0.053	1.410 ± 0.031	1.369 ± 0.026	1.404 ± 0.031	1.351 ± 0.029	1.057 ± 0.066**
Relative	4.01 ± 0.16	3.99 ± 0.04	3.95 ± 0.07	4.03 ± 0.05	4.01 ± 0.05	3.07 ± 0.21**
Thymus						
Absolute	0.374 ± 0.019	0.370 ± 0.011	0.386 ± 0.014	0.351 ± 0.012	0.360 ± 0.021	0.434 ± 0.014*
Relative	1.09 ± 0.05	1.05 ± 0.03	1.11 ± 0.03	1.01 ± 0.04	1.08 ± 0.07	1.25 ± 0.03*
FEMALE						
n	10	9	9	10	10	10
Necropsy body wt	192 ± 5	195 ± 5	202 ± 6	196 ± 4	197 ± 6	200 ± 2
Heart						
Absolute	0.632 ± 0.014	0.621 ± 0.010	0.657 ± 0.019	0.657 ± 0.012	0.690 ± 0.021*	0.738 ± 0.013**
Relative	3.30 ± 0.05	3.20 ± 0.05	3.25 ± 0.04	3.36 ± 0.04	3.51 ± 0.05**	3.70 ± 0.04**
Liver						
Absolute	5.922 ± 0.243	6.212 ± 0.198	6.712 ± 0.284*	6.786 ± 0.242**	6.844 ± 0.230**	7.704 ± 0.134**
Relative	30.89 ± 0.87	31.96 ± 0.95	33.19 ± 0.86	34.65 ± 0.72**	34.80 ± 0.82**	38.63 ± 0.74**
Spleen						
Absolute	0.394 ± 0.010	0.449 ± 0.011	0.563 ± 0.018*	0.844 ± 0.032**	1.490 ± 0.056**	3.097 ± 0.091**
Relative	2.06 ± 0.05	2.31 ± 0.07	2.80 ± 0.10	4.32 ± 0.14**	7.61 ± 0.34**	15.55 ± 0.53**
Thymus						
Absolute	0.267 ± 0.014	0.279 ± 0.014	0.289 ± 0.018	0.292 ± 0.013	0.359 ± 0.034**	0.391 ± 0.029**
Relative	1.39 ± 0.05	1.43 ± 0.06	1.43 ± 0.07	1.50 ± 0.07	1.83 ± 0.18**	1.95 ± 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] 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' test.

At necropsy, the incidence of enlarged or enlarged and darkened spleens in male and female rats increased with increasing concentration. The kidneys of most females exposed to 12 or 24 ppm were also darkened. Mediastinal lymph nodes were enlarged in many male and female rats in the 12 and 24 ppm groups.

Treatment-related effects were observed microscopically in the spleen, kidney, liver, bone marrow, lymph nodes, testis, and harderian gland. Multiple effects in the spleen were attributed to exposure to 4-chloronitrobenzene (Table 16). Congestion of the red pulp was present in all exposed rats of each sex, and the severity of the change increased with increasing exposure concentration. All treated male and female rats exhibited a minimal increase in hemosiderin pigment, and the incidence of hematopoietic cell proliferation was increased in most animals exposed to 3.0 ppm or greater. Increased hematopoietic activity was generally only minimal, with a slight exposure effect evident only in females in the 24 ppm group. Capsular fibrosis of the spleen occurred with concentration-dependent increases in incidence and severity; this lesion was characterized by focal or multifocal fibrous thickenings of the capsule accompanied by mononuclear inflammatory cell infiltrates (Table 16).

Effects of 4-chloronitrobenzene exposure in the kidney were observed in proximal tubule epithelial cells (Table 16). In H&E-stained sections, the nature of the change was different between males and females. In male rats, there was a concentration-dependent increase in the amount of eosinophilic hyaline droplets within the cytoplasm of tubule epithelial cells (hyaline droplet nephropathy). Additionally, a few small brown pigment granules were detected in the tubule epithelial cells of most males in the 12 and 24 ppm groups. The primary tubule lesion in female rats was accumulation of brown pigment granules; the accumulation occurred to a much greater degree than in males. This pigment was present in all females exposed to 6 ppm or greater and increased in severity with increasing exposure concentration (Table 16). Special stains were performed on selected kidney sections to further characterize the tubule pigment. In males, Mallory-Heidenhain stains confirmed the presence of protein-positive hyaline droplets; iron stains were negative, and there was an equivocal increase in PAS-positive granules. In contrast, most of the brown tubule pigment granules seen in H&E-stained sections of exposed female rats stained PAS positive, although the number of iron-positive granules in exposed females was also increased above that in the controls.

The only chemical-related effect in the liver was increased pigment in the Kupffer cells; this golden-brown pigment was interpreted to be hemosiderin. This pigment was observed as a minimal change in males in the 12 and 24 ppm groups, while in females, a clear exposure effect on incidence and severity was observed in all exposure groups except the lowest (1.5 ppm) exposure group (Table 16).

Increased hematopoietic cell proliferation was an exposure-related effect in the bone marrow of male and female rats. The increased hematopoiesis primarily involved erythroid cells, occurred at all exposure levels except 1.5 ppm, and increased in incidence and severity with increasing concentration (Table 16).

Enlarged mediastinal lymph nodes noted at necropsy corresponded microscopically to histiocytic hyperplasia (Table 16). This change, which occurred in rats in the 12 and 24 ppm groups, was of mild to moderate severity and consisted of increased numbers of histiocytes forming microgranulomas.

All male rats in the 24 ppm group had minimal to moderate testicular atrophy characterized by decreased cellularity of seminiferous tubules (Table 16).

In the harderian gland, the incidences of infiltrates of chronic inflammatory cells in male and female rats exposed to 24 ppm and in females exposed to 12 ppm were notably higher than the incidence in the controls (Table 16), suggesting an exposure-related effect. Small, focal interstitial aggregates of lymphocytes were observed in some control rats; however, lymphocytic infiltrates were multifocal and more extensive in many rats exposed to 4-chloronitrobenzene.

Sperm morphology and vaginal cytology evaluations were performed on rats exposed to 0, 6, 12, or 24 ppm 4-chloronitrobenzene (Appendix C). In males exposed to 24 ppm, the left epididymal, cauda epididymal, and testis weights; number of spermatid heads per testis; spermatid count; and spermatozoal concentration were significantly lower than those of control males. Estrous cycle length was decreased in all groups of females exposed to 4-chloronitrobenzene.

TABLE 16 Incidence and Severity of Selected Lesions in F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

	Concentration (ppm)					
	0	1.5	3	6	12	24
MALE						
n	10	10	10	10	10	10
Bone marrow						
Hematopoietic cell proliferation	0	0	3 (1.0)	10 (1.8)	10 (2.0)	10 (2.8)
Harderian gland						
Chronic inflammation	2 (1.0)	1 (1.0)	1 (1.0)	3 (1.0)	1 (1.0)	8 (2.2)
Kidney						
Hyaline droplet nephropathy	0	8 (1.0)	9 (1.0)	10 (1.0)	10 (1.2)	10 (3.0)
Tubule pigment	0	0	0	0	8 (1.0)	10 (1.0)
Liver						
Hemosiderin	0	0	0	0	9 (1.0)	10 (1.0)
Mediastinal lymph node						
Histiocytic hyperplasia	0	0	0	0	4 (1.5)	9 (2.3)
Spleen						
Congestion	0	10 (1.0)	10 (1.4)	10 (1.9)	10 (2.8)	10 (3.0)
Hemosiderin	0	10 (1.0)	10 (1.1)	10 (1.0)	10 (1.0)	10 (1.0)
Hematopoietic cell proliferation	0	0	10 (1.0)	9 (1.0)	10 (1.0)	10 (1.0)
Capsular fibrosis	0	0	4 (1.0)	8 (1.0)	10 (1.8)	10 (2.1)
Testis						
Atrophy	1 (4.0)	2 (1.5)	1 (1.0)	0	1 (1.0)	10 (1.6)
FEMALE						
n	10	10	10	10	10	10
Bone marrow						
Hematopoietic cell proliferation	0	0	9 (1.2)	10 (2.2)	10 (3.0)	10 (3.8)
Harderian gland						
Chronic inflammation	1 (1.0)	2 (1.0)	4 (1.7)	5 (1.6)	8 (2.2)	10 (3.0)
Kidney						
Hyaline droplet nephropathy	0	0	0	0	0	0
Tubule pigment	0	0	0	10 (1.0)	10 (2.0)	10 (3.0)
Liver						
Hemosiderin	0	0	7 (1.0)	10 (1.1)	10 (1.8)	10 (2.4)
Mediastinal lymph node						
Histiocytic hyperplasia	0	0	0	0	6 (1.5)	10 (2.7)
Spleen						
Congestion	0	10 (1.0)	10 (1.4)	10 (1.8)	10 (2.0)	10 (3.0)
Hemosiderin	3 (1.0)	10 (1.0)	9 (1.0)	10 (1.0)	10 (1.0)	10 (1.0)
Hematopoietic cell proliferation	0	0	9 (1.0)	10 (1.0)	9 (1.0)	10 (2.0)
Capsular fibrosis	0	0	2 (1.0)	10 (1.2)	10 (2.3)	10 (2.3)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

2-Week Inhalation Studies in B6C3F$_1$ Mice

One male mouse in the 18 ppm group died prior to exposure on Day 2 of the 2-chloronitrobenzene study (Table 17); there were no deaths in the 4-chloronitrobenzene study (Table 18). In the 2-chloronitrobenzene study, body weight gains of exposed mice were variable, and differences between body weight gains of exposed and control mice did not appear to be related to exposure to 2-chloronitrobenzene. In the 4-chloronitrobenzene study, the body weight gains of males and females in the 24 ppm groups were notably increased compared to those of the controls (Table 18).

Clinical signs of toxicity in the 2-chloronitrobenzene study were limited to hypoactivity, abnormal posture, and dyspnea in mice in the 18 ppm groups, especially males. In the 4-chloronitrobenzene study, clinical signs of toxicity included hypoactivity and dyspnea in males and females in the 24 ppm groups and hypoactivity in male mice in the 12 ppm group.

TABLE 17 Survival and Weight Gain of B6C3F$_1$ Mice in the 2-Week Inhalation Study of 2-Chloronitrobenzene

Concentration (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
0	5/5	24.4	27.3	2.9	
1.1	5/5	24.6	27.9	3.3	102
2.3	5/5	24.7	28.6	3.9	105
4.5	5/5	25.0	27.2	2.2	100
9	5/5	25.1	28.4	3.3	104
18	4/5[4]	25.2	27.5	2.3	101
FEMALE					
0	5/5	20.1	23.5	3.4	
1.1	5/5	19.7	23.3	3.6	99
2.3	5/5	19.7	23.0	3.3	98
4.5	5/5	19.8	23.0	3.2	98
9	5/5	19.8	23.5	3.7	100
18	5/5	19.9	23.9	4.0	102

[1] Number surviving at 2 weeks/number of animals per dose group.
[2] Mean weight change.
[3] (Dose group mean/control group mean) × 100.
[4] Day of death: 2.

TABLE 18 Survival and Weight Gain of B6C3F$_1$ Mice in the 2-Week Inhalation Study of 4-Chloronitrobenzene

Concentration (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
0	5/5	24.9	27.1	2.2	
1.5	5/5	24.9	28.3	3.4	104
3	5/5	24.9	28.6	3.7	106
6	5/5	25.1	28.1	3.0	104
12	5/5	25.0	28.3	3.3	104
24	5/5	25.3	30.0	4.7	111
FEMALE					
0	5/5	20.2	23.1	2.9	
1.5	5/5	19.7	23.4	3.7	101
3	5/5	19.4	23.2	3.8	100
6	5/5	20.3	23.4	3.1	101
12	5/5	20.2	23.8	3.6	103
24	5/5	20.0	25.0	5.0	108

[1] Number surviving at 2 weeks/number of animals per dose group.
[2] Mean weight change.
[3] (Dose group mean/control group mean) × 100.

2-Chloronitrobenzene: Absolute and relative liver weights increased markedly with increasing concentration in male and female mice. Absolute and relative kidney and spleen weights in males and females exposed to 18 ppm were significantly greater than those of the controls.

Gross lesions were observed in the livers of two of five male mice and one of five female mice in the 18 ppm groups at necropsy. The livers were pale and had rough, granular surfaces. The liver and spleen were identified microscopically as target organs. The most severe exposure-related lesions occurred in the liver, where necrosis of centrilobular hepatocytes was the primary effect. Necrosis was coagulative in nature or was evidenced by mineral deposits in centrilobular areas. A granulomatous inflammatory response accompanied the necrotic/mineralized foci and consisted of macrophages with yellow-brown cytoplasmic pigment (iron negative and presumably lipofuscin), multinucleated giant cells, and variable numbers of lymphocytes and fibroblasts. Necrosis with associated inflammation was observed only in mice in the 18 ppm groups. Additionally, most mice in the 9 and 18 ppm groups had enlargement of viable centrilobular hepatocytes. This

change, diagnosed as hepatocytomegaly, was characterized by centrilobular cells with increased amounts of finely granular, basophilic-staining cytoplasm.

Splenic changes consisted of hematopoietic cell proliferation and pigmentation. Increased hematopoietic activity was observed in all males exposed to 18 ppm that survived to the end of the study and also occurred with increasing incidence and severity in females in the 4.5, 9, and 18 ppm groups. The splenic pigment, which was golden brown in H&E-stained sections and which was located in the red pulp, was positive for iron and was thus identified as hemosiderin. Hemosiderin accumulation in the spleen was mild in severity and occurred in four of five males and all females exposed to 18 ppm. One male mouse in the 18 ppm group died early and had a diffusely dark, discolored liver that corresponded histologically to severe centrilobular congestion and necrosis.

4-Chloronitrobenzene: Absolute and relative liver and spleen weights increased with increasing concentration. At necropsy, gross findings attributed to 4-chloronitrobenzene exposure were limited to enlarged and dark spleens and were observed in three of five male mice and four of five female mice exposed to 24 ppm.

The spleen was identified microscopically as the primary target tissue of 4-chloronitrobenzene toxicity. Splenic effects consisted of increased hematopoietic cell proliferation and pigmentation. Minimal to mild increases in primarily erythropoietic activity occurred in all mice in the 12 and 24 ppm groups and in some mice exposed to 6 ppm. A similar exposure concentration response pattern was observed in an associated minimal to mild increase of iron-positive pigment (hemosiderin). Although no lesions were detected in H&E-stained sections of liver and kidney, iron stains revealed subtle increases in the amount of hemosiderin pigment in the cortical tubule epithelial cells and Kupffer cells of these tissues.

13-Week Inhalation Studies in B6C3F$_1$ Mice

Two male mice in the 18 ppm group in the 2-chloronitrobenzene study died during Week 12 (Table 19) and one male in the 6 ppm group in the 4-chloronitrobenzene study died during Week 8 (Table 20). The mean body weight gains of most groups of males and females exposed to 2-chloronitrobenzene or 4-chloronitrobenzene were equal to or greater than the mean body weight gains of the controls (Tables 19 and 20 and Figures 4 and 5). There were no clinical signs of toxicity related to 2- or 4-chloronitrobenzene exposure.

TABLE 19 Survival and Weight Gain of B6C3F$_1$ Mice in the 13-Week Inhalation Study of 2-Chloronitrobenzene

Concentration (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
0	10/10	23.0	35.5	12.5	
1.1	10/10	23.2	36.4	13.2	103
2.3	10/10	23.1	35.3	12.2	99
4.5	10/10	22.9	34.0	11.2	96
9	10/10	23.0	36.1	13.1	102
18	8/10[4]	23.1	35.2	12.2	99
FEMALE					
0	10/10	19.5	29.8	10.2	
1.1	10/10	19.6	31.7	12.0	106
2.3	10/10	20.0	33.1	13.0	111
4.5	10/10	19.7	32.0	12.3	108
9	10/10	19.8	33.7	13.9	113
18	10/10	19.6	33.5	13.9	112

[1] Number surviving at 13 weeks/number of animals per dose group.
[2] Mean weight change.
[3] (Dose group mean/control group mean) × 100.
[4] Week of death: 12.

TABLE 20 Survival and Weight Gain of B6C3F$_1$ Mice in the 13-Week Inhalation Study of 4-Chloronitrobenzene

Concentration (ppm)	Survival[1]	Mean Body Weight (grams)			Final Weight Relative to Controls (%)[3]
		Initial	Final	Change[2]	
MALE					
0	10/10	24.1	34.2	10.1	
1.5	10/10	24.1	35.5	11.5	104
3	10/10	24.2	35.7	11.4	104
6	9/10[4]	23.9	34.0	10.1	100
12	10/10	24.1	34.8	10.7	102
24	10/10	23.7	35.6	12.0	104
FEMALE					
0	10/10	20.0	31.3	11.3	
1.5	10/10	19.9	31.9	12.0	102
3	10/10	19.8	31.9	12.1	102
6	10/10	20.1	29.5	9.4	94
12	10/10	19.4	31.6	12.2	101
24	10/10	19.2	32.2	13.0	103

[1] Number surviving at 13 weeks/number of animals per dose group.
[2] Mean weight change.
[3] (Dose group mean/control group mean) x 100.
[4] Week of death: 8.

2-Chloronitrobenzene: Absolute and relative right kidney weights and relative liver weights increased with increasing exposure concentration, and were significantly increased in males exposed to 2.3 ppm or greater (Table 21 and Appendix A). Absolute liver weights of males exposed to 9 or 18 ppm were also significantly increased. Absolute liver weights in all groups of exposed females, relative liver weights in females exposed to 9 or 18 ppm, and absolute right kidney weights in females exposed to 2.3 ppm or greater were significantly increased (Table 21).

Pale discoloration of the liver was noted grossly at necropsy in 6 of 10 male mice and 1 of 10 female mice in the 18 ppm groups. The spleen was grossly enlarged in three females in the 9 ppm group and four females in the 18 ppm group.

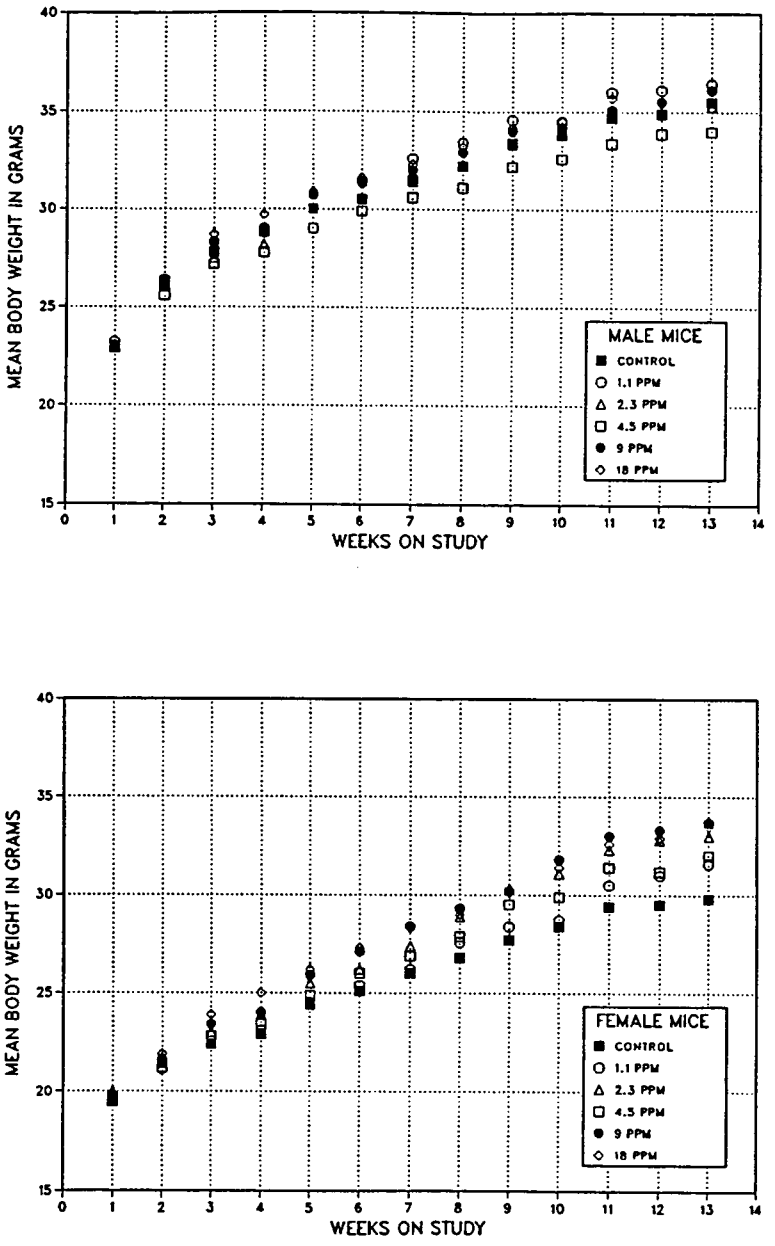

FIGURE 4 Body Weights of B6C3F$_1$ Mice Administered 2-Chloronitrobenzene by Inhalation for 13 Weeks

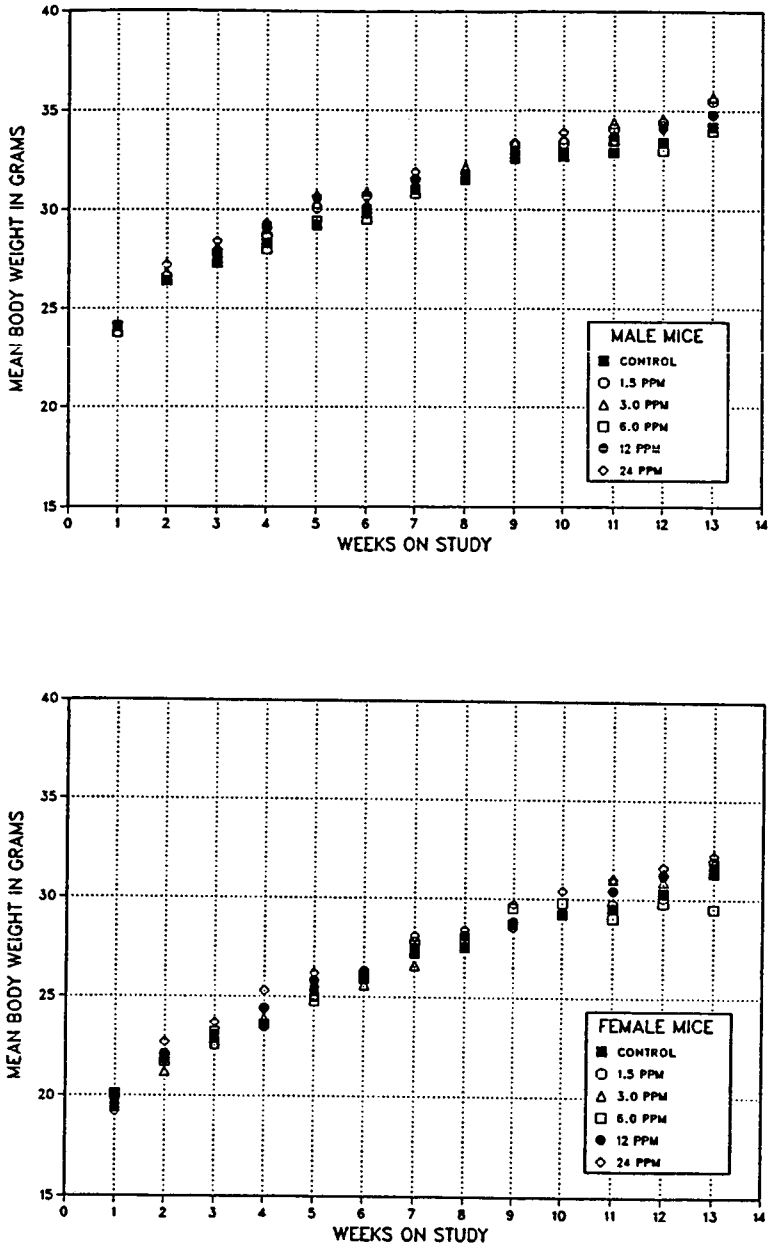

FIGURE 5 Body Weights of B6C3F$_1$ Mice Administered 4-Chloronitrobenzene by Inhalation for 13 Weeks

TABLE 21 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

	Concentration (ppm)					
	0	1.1	2.3	4.5	9	18
MALE						
n	10	10	10	10	10	8
Necropsy body wt	36.7 ± 0.7	37.2 ± 0.3	36.2 ± 0.9	34.9 ± 0.8	36.9 ± 1.0	35.8 ± 1.0
Right kidney						
Absolute	0.318 ± 0.010	0.335 ± 0.007	0.344 ± 0.007*	0.348 ± 0.012*	0.353 ± 0.005**	0.354 ± 0.009**
Relative	8.69 ± 0.27	9.01 ± 0.16	9.54 ± 0.21*	10.00 ± 0.34**	9.62 ± 0.21**	9.91 ± 0.22**
Liver						
Absolute	1.713 ± 0.039	1.835 ± 0.057	1.816 ± 0.059	1.794 ± 0.044	2.025 ± 0.066**	2.279 ± 0.103**
Relative	46.75 ± 0.76	49.30 ± 1.33	50.24 ± 1.22*	51.46 ± 0.82**	54.92 ± 0.83**	63.51 ± 1.46**
Spleen						
Absolute	0.072 ± 0.003	0.073 ± 0.003	0.071 ± 0.002	0.065 ± 0.002	0.071 ± 0.002	0.075 ± 0.003
Relative	1.97 ± 0.09	1.96 ± 0.07	1.97 ± 0.05	1.86 ± 0.05	1.93 ± 0.05	2.10 ± 0.05
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	30.1 ± 0.8	32.6 ± 0.8	34.5 ± 1.1*	33.0 ± 1.0*	34.7 ± 1.2**	33.9 ± 1.3**
Right kidney						
Absolute	0.205 ± 0.008	0.223 ± 0.005	0.239 ± 0.003**	0.231 ± 0.004**	0.244 ± 0.012**	0.237 ± 0.003**
Relative	6.81 ± 0.24	6.87 ± 0.18	6.97 ± 0.20	7.04 ± 0.21	7.09 ± 0.38	7.07 ± 0.24
Liver						
Absolute	1.472 ± 0.040	1.625 ± 0.042*	1.768 ± 0.050**	1.723 ± 0.052**	1.933 ± 0.048**	2.234 ± 0.065**
Relative	49.00 ± 1.25	49.93 ± 0.97	51.32 ± 0.86	52.31 ± 1.24	56.00 ± 0.97**	66.37 ± 2.15**
Spleen						
Absolute	0.092 ± 0.003	0.090 ± 0.002	0.093 ± 0.003	0.094 ± 0.004	0.098 ± 0.003	0.101 ± 0.004[2]
Relative	3.06 ± 0.09	2.77 ± 0.08	2.71 ± 0.12	2.85 ± 0.10	2.85 ± 0.12	3.04 ± 0.13[2]

[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] n=9.
* Significantly different (P≤0.05) from the control group by Williams' test.
** Significantly different (P≤0.01) from the control group by Williams' test.

As in the 2-week study, the liver and spleen were histologically identified as target organs following 13 weeks of exposure to 2-chloronitrobenzene. Liver lesions were diagnosed as hepatocellular necrosis and mineralization, chronic inflammation, and hepatocytomegaly (Table 22). Necrosis, mineralization, and inflammation were observed primarily in mice exposed to 18 ppm. In most instances, there was little or no acute hepatocellular necrosis; liver changes were evidenced instead by mineralized cells associated with chronic inflammatory changes of fibrosis and accumulations of mononuclear inflammatory cells,

including macrophages containing yellow-brown pigment. Foci of mineralization and inflammation were typically in subcapsular locations. Enlargement of hepatocytes (cytomegaly) occurred in all male and female mice in the 18 ppm groups and, to a minimal degree, in all male mice exposed to 9 ppm. Affected hepatocytes had enlarged nuclei and increased amounts of cytoplasm; the cytoplasm was mottled and had perinuclear basophilia and finely granular, "ground-glass" eosinophilia peripherally.

Increased hematopoietic activity, primarily erythropoiesis, in the red pulp of the spleen was a minimal treatment effect in both sexes of mice, particularly in females in the 18 ppm group, but also, to a lesser degree, in females in the 9 ppm group (Table 22). The splenic hemosiderosis seen in the 2-week study was not observed in the 13-week study.

In the two males in the 18 ppm group that died before the scheduled sacrifice, livers were darkly discolored. This finding was attributed microscopically to diffuse, severe sinusoidal congestion with hepatocellular degeneration and necrosis.

Sperm morphology and vaginal cytology evaluations were performed on mice in the 0, 4.5, 9, and 18 ppm groups (Appendix C). Sperm motility was significantly decreased in all groups of males exposed to 2-chloronitrobenzene. No significant changes occurred in exposed females.

4-Chloronitrobenzene: Spleen weights of males and females exposed to 12 or 24 ppm 4-chloronitrobenzene were markedly increased compared to those of the controls (Table 23 and Appendix A). Relative liver weights of males and females showed a mild increase with increasing exposure concentration. Absolute right kidney weights of all groups of exposed males and of females exposed to 3 ppm or greater were significantly increased.

TABLE 22 Incidence and Severity of Selected Lesions in B6C3F$_1$ Mice in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

	Concentration (ppm)					
	0	1.1	2.3	4.5	9	18
MALE						
n	10	10	10	10	10	10
Liver						
Cytomegaly	0	0	0	0	10 (1.0)	10 (1.7)
Necrosis/mineralization	0	0	0	0	0	8 (1.9)
Sinusoidal congestion	0	0	0	0	0	2[2] (4.0)
Chronic inflammation	0	0	0	0	0	5 (2.0)
Spleen						
Hematopoietic cell proliferation	0	0	0	0	0	4 (1.0)
FEMALE						
n	10	10	10	10	10	10
Liver						
Cytomegaly	0	0	0	0	0	10 (2.0)
Necrosis/mineralization	0	0	0	0	1 (1.0)	4 (1.2)
Sinusoidal congestion	0	0	0	0	0	0
Chronic inflammation	0	0	0	0	0	1 (1.0)
Spleen						
Hematopoietic cell proliferation	3 (1.0)	0	0	0	10 (1.0)	8 (1.2)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.
[2] Both incidences were animals that died before the end of the study.

At necropsy, treatment-related gross lesions consisted of enlarged and dark spleens in male and female mice exposed to 24 ppm and female mice exposed to 12 ppm. Microscopically, the spleen, bone marrow, liver, and forestomach were identified as target tissues of 4-chloronitrobenzene toxicity. A spectrum of splenic lesions was observed, including minimal to mild congestion, increased hematopoietic cell proliferation, and pigmentation that was interpreted to be hemosiderin (Table 24). Congestion was observed in all mice in the 24 ppm groups, while increased hematopoietic activity and hemosiderin deposition occurred in all mice exposed to 24 ppm and most mice exposed to 12 ppm. Lower incidences of increased hematopoiesis were observed in females at all lower exposure levels.

Increased hematopoiesis and hemosiderin deposition were also treatment effects in the bone marrow, primarily at the 24 ppm exposure level, and were of minimal severity (Table 24). Also present in the bone marrow were small, round to oval bodies interpreted to be red blood cell fragments; this finding was observed in all males and 9 of 10 females exposed to 24 ppm.

TABLE 23 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

	Concentration (ppm)					
	0	1.5	3	6	12	24
MALE						
n	10	10	10	9	10	10
Necropsy body wt	34.9 ± 0.8	36.7 ± 1.0	36.7 ± 0.9	35.3 ± 1.4	35.8 ± 0.5	36.8 ± 0.7
Right kidney						
Absolute	0.300 ± 0.006	0.330 ± 0.011*	0.336 ± 0.012*	0.324 ± 0.010*	0.327 ± 0.005*	0.337 ± 0.008**
Relative	8.63 ± 0.20	8.99 ± 0.21	9.15 ± 0.23	9.24 ± 0.30	9.15 ± 0.17	9.18 ± 0.27
Liver						
Absolute	1.596 ± 0.051	1.678 ± 0.045	1.733 ± 0.041	1.704 ± 0.059	1.759 ± 0.045*	1.868 ± 0.050**
Relative	45.81 ± 1.24	45.77 ± 1.05	47.32 ± 1.09	48.44 ± 1.40	49.21 ± 1.26	50.82 ± 1.27**
Spleen						
Absolute	0.065 ± 0.002	0.068 ± 0.002	0.067 ± 0.002	0.064 ± 0.002	0.079 ± 0.002**	0.160 ± 0.006**
Relative	1.87 ± 0.07	1.85 ± 0.05	1.83 ± 0.06	1.83 ± 0.07	2.21 ± 0.06*	4.36 ± 0.19**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	31.5 ± 0.9	32.3 ± 1.1	33.5 ± 1.3	31.1 ± 0.8	33.1 ± 0.8	33.0 ± 0.4
Right kidney						
Absolute	0.205 ± 0.007	0.215 ± 0.003	0.220 ± 0.005*	0.220 ± 0.004*	0.222 ± 0.004*	0.240 ± 0.002**
Relative	6.53 ± 0.21	6.73 ± 0.22	6.64 ± 0.28	7.11 ± 0.14	6.73 ± 0.19	7.27 ± 0.07*
Liver						
Absolute	1.468 ± 0.027	1.535 ± 0.049	1.618 ± 0.050	1.552 ± 0.048	1.760 ± 0.055**	1.889 ± 0.024**
Relative	46.78 ± 0.89	47.72 ± 1.00	48.49 ± 1.02	50.07 ± 1.34*	53.10 ± 0.97**	57.25 ± 0.82**
Spleen						
Absolute	0.093 ± 0.004	0.095 ± 0.004	0.097 ± 0.003	0.099 ± 0.003	0.130 ± 0.005**	0.253 ± 0.007**
Relative	2.98 ± 0.16	2.95 ± 0.10	2.93 ± 0.15	3.20 ± 0.09	3.94 ± 0.16**	7.68 ± 0.27**

[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).
* Significantly different (P≤0.05) from the control group by Williams' test.
** Significantly different (P≤0.01) from the control group by Williams' test.

TABLE 24 Incidence and Severity of Selected Lesions in B6C3F$_1$ Mice in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

	Concentration (ppm)					
	0	1.5	3	6	12	24
MALE						
n	10	10	10	10	10	10
Bone marrow						
Hyperplasia	0	0	0	0	3 (1.0)	9 (1.2)
Hemosiderin	0	0	0	0	0	10 (1.1)
Red blood cell fragments	0	0	0	0	0	10 (1.0)
Forestomach						
Hyperplasia	0	0	0	0	0	1 (1.0)
Liver						
Hemosiderin	0	0	0	0	0	10 (1.2)
Necrosis	0	0	0	0	1 (1.0)	5 (1.0)
Cytoplasmic basophilia	0	0	0	0	0	4 (1.0)
Spleen						
Congestion	0	0	0	0	1 (1.0)	10 (1.9)
Hemosiderin	0	0	0	0	10 (1.1)	10 (2.0)
Hematopoietic cell proliferation	0	0	0	0	7 (1.0)	10 (2.0)
FEMALE						
n	10	10	10	10	10	10
Bone marrow						
Hyperplasia	0	0	0	0	0	10 (1.0)
Hemosiderin	0	0	0	0	0	8 (1.0)
Red blood cell fragments	0	0	0	0	0	9 (1.0)
Forestomach						
Hyperplasia	0	0	0	0	0	7 (2.0)
Liver						
Hemosiderin	0	0	0	0	0	10 (1.0)
Necrosis	0	0	0	0	0	0
Cytoplasmic basophilia	0	0	0	0	0	0
Spleen						
Congestion	0	0	0	0	0	10 (1.9)
Hemosiderin	0	0	0	0	10 (1.1)	10 (2.0)
Hematopoietic cell proliferation	0	1 (1.0)	1 (1.0)	2 (1.0)	9 (1.2)	10 (2.0)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.

Hemosiderin deposition in Kupffer cells, the most common finding in the liver, was limited to male and female mice in the 24 ppm groups (Table 24). Less frequent and minimal changes attributed to exposure to 4-chloronitrobenzene included single cell necroses in 5 of 10 males in the 24 ppm group and one male in the 12 ppm group and centrilobular cytoplasmic basophilia of hepatocytes in 4 of 10 males in the 24 ppm group.

Squamous cell hyperplasia of the forestomach epithelium was found in 7 of 10 female mice in the 24 ppm group (Table 24). This change was minimal to moderate in severity and consisted of focal thickening of the epithelium due to increased cell layers and associated hyperkeratosis. Focal hyperplasia typically involved the mucosa along the greater curvature of the forestomach between the limiting ridges. No similar effect was clearly evident in males exposed to 24 ppm.

Sperm morphology and vaginal cytology evaluations were performed on mice in the 0, 6, 12, and 24 ppm groups (Appendix C). The estrous cycle length of females exposed to 24 ppm was significantly increased. No significant changes occurred in males exposed to 4-chloronitrobenzene.

Continuous Breeding Studies in CD-1 Swiss Mice

2-Chloronitrobenzene and 4-chloronitrobenzene were each administered in corn oil by gavage to CD-1 Swiss mice for 7 days prior to cohousing and for 98 days of continuous breeding. Toxic and reproductive effects on breeding animals and effects on survival and body weights of pups were evaluated. The last litters of control and high-dose breeding pairs were reared and assessed for fertility effects. These studies are summarized in Appendix D. Although mice dosed with 2-chloronitrobenzene had significant changes in organ weights and methemoglobin levels compared to control mice and pup weights of the F_1 generation were notably lower than those of the controls by Day 21, no effects on fertility were noted in F_0 or F_1 mice. This is in general agreement with the rather minor effects noted with this isomer in the sperm motility assays in the inhalation study. In contrast, the fertility of mice dosed with 4-chloronitrobenzene decreased progressively with the duration of dosing and with increasing dose. Spleen and liver weights of F_1 mice dosed with 4-chloronitrobenzene were significantly greater than those of the controls, and the estrous cycle length of dosed F_1 females was significantly increased, perhaps influencing the observed fertility of the mating pairs. Survival and body weights of pups were significantly decreased.

Genetic Toxicity Studies

Both 2-chloronitrobenzene and 4-chloronitrobenzene are genotoxic *in vitro*. 2-Chloronitrobenzene was tested in four separate studies conducted by two different laboratories for mutagenicity in *Salmonella typhimurium*. The results of the first two assays are published in Haworth *et al.* (1983) and a summary of those data is given in Table E1. Complete data from two unpublished studies are also presented in Table E1. The results of these studies show that 2-chloronitrobenzene was mutagenic in *S. typhimurium* strain TA100 in the presence of induced hamster or rat liver S9. A positive response was also obtained in strain TA98 in trials conducted with 30% hamster S9.

4-Chloronitrobenzene was tested for mutagenicity in *S. typhimurium* by two different laboratories (Table E2; Haworth *et al.*, 1983). Positive responses were obtained with strains TA100 and TA1535 in the presence of induced S9. A positive response was also obtained with TA1535 in the absence of S9.

Both 2-chloronitrobenzene and 4-chloronitrobenzene induced sister chromatid exchanges (Tables E3 and E4) and chromosomal aberrations (Tables E5 and E6) in Chinese hamster ovary cells. 2-Chloronitrobenzene was tested by two laboratories for induction of sister chromatid exchanges and chromosomal aberrations. Results of the first study with 2-chloronitrobenzene showed an increase in sister chromatid exchanges only in the absence of S9; in the second study, sister chromatid exchanges were significantly increased only in the presence of S9 at the highest dose tested in each of two trials. The results of the chromosomal aberrations tests with 2-chloronitrobenzene also differed between the two laboratories; in the first study, there was a small increase in chromosomal aberrations in the absence of S9, and this response was judged to be equivocal. In the second study, there was a significant increase in chromosomal aberrations at the highest dose tested in each of two trials performed with S9.

4-Chloronitrobenzene induced sister chromatid exchanges in Chinese hamster ovary cells in the presence of S9 at all doses tested (Table E4; Galloway *et al.*, 1987). The control value of sister chromatid exchanges in the first trial with S9 was exceptionally low, but the positive response repeated in the second trial, where a higher control frequency of sister chromatid exchanges occurred. 4-Chloronitrobenzene induced chromosomal aberrations with and without S9 under conditions which produced severe toxicity, as evidenced by cell cycle delay and the small number of scorable cells (Table E6; Galloway *et al.*, 1987). The

low solubility of 4-chloronitrobenzene at concentrations of 500 µg/mL and above may have been a factor in the variable response among trials.

Neither 2-chloronitrobenzene nor 4-chloronitrobenzene induced sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when administered to adults either by feeding or by injection (Zimmering *et al.*, 1985) or to larvae by feeding (Zimmering *et al.*, 1989) (Tables E7 and E8).

Disposition and Metabolism Studies

The disposition and metabolism of 2-chloronitrobenzene and 4-chloronitrobenzene were assessed in male F344 rats. Disposition and metabolism were studied following a single oral dose in young adult rats and following repeated oral dosing in young adult and geriatric rats, and absorption of each isomer was determined following topical administration to young adult rats. These studies are presented in Appendix F.

In summary, studies of 2-chloronitrobenzene and 4-chloronitrobenzene established that these compounds are readily absorbed from the gastrointestinal tract and skin, rapidly metabolized to a large number of metabolites, and rapidly excreted, primarily in urine. The disposition and metabolite profile of these compounds were quite similar and were apparently unaffected by age at doses up to 65 mg/kg. The only parameter that varied with compound was the induction of methemoglobin, which was three- to fourfold greater following administration of 4-chloronitrobenzene.

DISCUSSION

The results of the inhalation studies with 2-chloronitrobenzene and 4-chloronitrobenzene were in general agreement with expected findings based on previous studies in the literature (Renshaw and Ashcroft, 1926; Hasegawa and Sato, 1963; Nair *et al.*, 1986a,b). Clearly, the formation of methemoglobin in the studies with each isomer, but particularly with 4-chloronitrobenzene, is a major toxic effect, and many of the lesions described in these studies are recognized as secondary to methemoglobin-associated decreases in erythrocyte (RBC) life span and anemia. These lesions include effects on the spleen (hemosiderin accumulation, capsular fibrosis, and increased hematopoietic cell proliferation), liver (Kupffer cell hemosiderin accumulation), bone marrow (increased hemosiderin and hematopoietic cell proliferation), and kidney (tubule hemosiderin). However, other findings, including hyaline droplet nephropathy and degeneration of the testis in male rats, inflammation of the harderian gland in rats, and hyperplasia of the forestomach epithelium in mice exposed to 4-chloronitrobenzene, as well as hyperplasia of the nasal cavity epithelium in rats exposed to 2-chloronitrobenzene, have not previously been described. The studies of Nair *et al.* (1989) demonstrated a marginal effect, a decrease in fertility of Sprague-Dawley rats, caused by oral administration of 4-chloronitrobenzene, and this finding was confirmed in the continuous breeding studies in mice outlined in the studies reported here.

The highest methemoglobin concentrations in rats, approximately 4 to 6 g/dL, occurred after administration of 24 ppm 4-chloronitrobenzene on Day 3. The methemoglobin concentrations were highest on Day 4 of the 2-chloronitrobenzene study, but the levels in rats exposed to the highest (18 ppm) concentration (approximately 1 to 1.2 g/dL methemoglobin) were lower than those in the 4-chloronitrobenzene study. In both studies, the methemoglobin concentrations declined somewhat by Day 23 and remained stable at Week 13, suggesting increased activity of the enzyme systems involved in methemoglobin reduction or an absolute increase in enzyme-rich reticulocytes that appeared in response to the anemia.

Both 2-chloronitrobenzene and 4-chloronitrobenzene caused responsive anemias, as evidenced by increases in reticulocyte counts and nucleated erythrocytes and decreases in hematocrit (HCT), hemoglobin (HGB) concentrations, and RBC counts. In the

4-chloronitrobenzene study, the anemia was macrocytic, as indicated by increased mean cell volume (MCV), and hyperchromic, as indicated by increased mean cell hemoglobin concentration (MCHC), and was consistent with a hemolytic anemia. The macrocytosis was related to the increased numbers of reticulocytes and the hyperchromia indicated increased release of HGB into the plasma, suggesting increased RBC fragility. In the 2-chloronitrobenzene study, the anemia was responsive but was not typical for a hemolytic process. In general, the anemia was normocytic and normochromic. (In the female rats, there was a tendency for smaller RBC size and decreased MCHC.) Normocytic, normochromic anemias often occur when there is a depression of erythrogenesis, and usually these anemias are nonresponsive. However, there was evidence of an erythropoietic response (indicated by increased numbers of reticulocytes) on Day 23 in female rats and in both sexes at Week 13, suggesting that the erythropoietic system was not suppressed. The anemia that occurred was mild and therefore would not be expected to produce a strong stimulus for an erythrogenic response. Also, the methemoglobinemia was mild and may not have resulted in oxidative damage to the RBCs as severe as that occurring in the rats exposed to 4-chloronitrobenzene. Any Heinz bodies that may have resulted from the methemoglobinemia in rats would have been removed by the efficient pitting function of the spleen, resulting in smaller RBCs remaining in the circulation.

Although the highest exposure concentration used was only somewhat higher for 4-chloronitrobenzene (24 ppm) than for 2-chloronitrobenzene (18 ppm), the degree of RBC and tissue injury was markedly greater with the 4-isomer at similar exposure concentrations. This was evidenced by the more extensive tissue deposition of hemosiderin with 4-chloronitrobenzene than with 2-chloronitrobenzene and by the persistence of hemosiderin in tissues (observed at 2 weeks and 13 weeks) with 4-chloronitrobenzene, but not with 2-chloronitrobenzene. No compensatory hematopoietic cell proliferation was present in rats at 13 weeks in the 2-chloronitrobenzene study, in contrast to the splenic and bone marrow response with 4-chloronitrobenzene.

In addition to hemosiderin deposition and increased hematopoietic cell proliferation, additional splenic effects that were seen and attributed to RBC toxicity included congestion and capsular fibrosis; the full spectrum of splenic effects (hemosiderin, increased hematopoietic cell proliferation, congestion, and capsular fibrosis) was manifested in rats exposed to 4-chloronitrobenzene. This spectrum is consistent with that seen in studies of aniline and other nitroaromatic compounds known to produce methemoglobin, in

particular *p*-chloroaniline (Chhabra *et al.*, 1991), which would be the presumed metabolite resulting from 4-chloronitrobenzene reduction.

The actual mechanism by which nitroaromatic compounds cause the partial oxidation of heme iron from ferrous to ferric is not fully known. This effect does not occur in *in vitro* incubations, and it is thought that bioactivation of nitro compounds to aminophenols or hydroxylamines is required (Smith, 1991). This has been shown to involve gut microflora (Carr *et al.*, 1979). Thus the kinetics of methemoglobin formation may not directly parallel blood concentrations of the chloronitrobenzenes. Although there does not appear to be a great difference in the susceptibility of hemoglobin from different species to be oxidized to methemoglobin, there is a marked difference in the rate at which methemoglobin can be reduced to hemoglobin within the RBC, with the rates in rodents being higher than that observed in RBCs from humans (Smith, 1991). Based on this difference, humans may be more susceptible than rats or mice to toxic effects associated with the methemoglobin-producing action of the chloronitrobenzenes.

The liver was a primary target organ for toxic effects of 2-chloronitrobenzene and 4-chloronitrobenzene in rats and mice. Liver weights were increased in both species following 2 weeks of exposure to either chemical and remained increased to a lesser degree at 13 weeks. In both chloronitrobenzene studies, serum activities of alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), or both were increased in exposed rats, suggesting enzyme leakage from hepatocytes. The observed increases in bile acid levels would also support a hepatotoxic effect and would be consistent with cholestasis. Although necrosis was suggested by the clinical pathology results, the most consistent microscopic change was an increase in hemosiderin deposition in Kupffer cells, and after 13 weeks there was evidence of cytomegaly in mice and cytoplasmic basophilia in rats, suggesting induction of metabolizing enzymes. The results of the repeated-dose disposition and metabolism studies did not, however, suggest a significant enhancement of the rate of metabolism of the chemicals. Clear evidence of hepatocellular necrosis and granulomatous inflammation was observed only in mice. This was rather specific to the 2-chloronitrobenzene isomer, as only slight evidence of focal necrosis in the liver was observed in mice exposed to 4-chloronitrobenzene.

A clear difference in the toxic effects of the chloronitrobenzenes was the induction of hyaline droplet accumulation in the kidney of rats exposed to the 4- but not the 2-isomer.

This lesion was a mild form of hyaline droplet nephropathy, with little evidence of the typical secondary sequelae of tubule cell necrosis and degeneration and accumulation of protein casts in the tubule lumen. A similar isomer-specific induction of this lesion was noted with 1,2-dichlorobenzene and 1,4-dichlorobenzene (NTP, 1985, 1987), with the 1,4-isomer being an inducer while the 1,2-isomer was not.

Several pigments accumulated in renal tubule cells in the studies reported here. Pigment granules were observed in the renal tubule cells of rats exposed to either 2- or 4-chloronitrobenzene. At 2 weeks, only rats exposed to 4-chloronitrobenzene exhibited granules; the pigment at this time was iron-positive hemosiderin and likely reflected the greater acute erythrotoxicity of 4-chloronitrobenzene relative to 2-chloronitrobenzene. However, by Week 13, granules were evident in rats exposed to either isomer, and at this time the pigment was considered to be a predominantly lipofuscin type, based on mostly iron-negative and PAS-positive staining results. The source of this lipofuscin-like pigment is uncertain. A similar PAS-positive pigment interpreted to be lipofuscin was observed in the nitrotoluene studies in rats; it was associated with both the *o*- and *p*-isomers (NTP, 1992a).

An inflammatory lesion in the harderian gland in rats was noted in the 13-week 4-chloronitrobenzene study. This is not a common target organ in short-term toxicity studies, and there is no explanation for this finding. Sialodacryoadenitis (SDAV) infection may cause similar lesions, but viral titers to SDAV were negative, and other lesions associated with this infection were not present.

Increases in platelet counts occurred in rats at various time points and exposure levels in the 4-chloronitrobenzene study. Platelet counts can increase for several reasons, including a physiologic thrombocytosis with increased platelet mobilization from the splenic and nonsplenic (pulmonary) pools. This is believed to be a response to either epinephrine release or increased exercise and does not involve increased production. Reactive thrombocytosis occurs with several conditions and involves increased production. The increase in reticulocytes could indicate a reactive hematopoietic process involving the megakaryocytic series, and the platelet increase would be consistent with a reactive thrombocytosis. An exception to the thrombocytosis occurred in the 2-chloronitrobenzene study; male and female rats in the 9 and 18 ppm groups had decreased platelet counts at

Day 4. Thrombocytopenia can occur in a variety of situations, resulting from decreased production, increased utilization or loss, or abnormal distribution or sequestration.

Atrophy of seminiferous tubules was noted in the 13-week studies in rats at the highest exposure concentration of 4-chloronitrobenzene. Similar testicular degeneration in rats occurred in 13-week studies of *o*-, *m*-, and *p*-nitrotoluenes (NTP, 1992b) and nitrobenzoic acids (NTP, unpublished). The results of sperm morphology and vaginal cytology evaluations showed mild decreases in spermatid counts in rats exposed to either 2- or 4-chloronitrobenzene, and sperm motility was lower in rats exposed to the highest concentration of 4-chloronitrobenzene and in all exposure groups of mice in the 2-chloronitrobenzene study. The estrous cycle was slightly lengthened in mice exposed to 24 ppm 4-chloronitrobenzene. Continuous breeding studies in CD-1 Swiss mice were performed primarily to determine if the relatively mild changes seen in reproductive parameters in the screening assays would translate into significant deficits in fertility in more exhaustive studies. The continuous breeding study with 4-chloronitrobenzene did demonstrate a decrease in fertility and other adverse effects; thus these findings corresponded with the results of the screening studies in rats.

In comparing the effects of 2-chloronitrobenzene and 4-chloronitrobenzene described in these studies, it should be noted that the same concentrations were used in the 2-week and the 13-week studies, with the highest exposure concentrations limited to 18 ppm for 2-chloronitrobenzene and 24 ppm for 4-chloronitrobenzene by technical factors in the generation of the vapors. Because of the relatively low vapor pressures of these chemicals, to achieve concentrations higher than those used would have required the generation of aerosols. It is unlikely that the toxic effects seen in these studies were influenced greatly by the use of inhalation as the route of exposure. The hyperplasia observed in the nasal cavity of rats exposed to 2-chloronitrobenzene may represent an irritant effect that is route specific, but the extent of dermal and oral absorption (approximately 50% to 80%) demonstrated with each of the chemicals in male rats suggests that significant toxicity could be expected following any of the possible routes of exposure. The observation of hyperplasia of the forestomach epithelium in mice exposed to 4-chloronitrobenzene suggests that significant oral exposure could have occurred in these inhalation studies, possibly through grooming activities. Results of absorption, disposition, and metabolism studies established that both chloronitrobenzenes are readily absorbed from the

gastrointestinal tract and skin, are metabolized to a large number of metabolites, and are relatively rapidly excreted, primarily in urine.

In summary, inhalation exposure of rats and mice to 2- or 4-chloronitrobenzene resulted in methemoglobin formation and oxidative damage to red blood cells, leading to a regenerative anemia and a recognized spectrum of tissue damage and changes secondary to erythrocyte injury. In addition, numerous other lesions that were considered primary toxic effects occurred following exposure. These included renal hyaline droplet accumulation and testicular atrophy in male rats exposed to 4-chloronitrobenzene and hyperplasia of the respiratory epithelium in rats exposed to 2-chloronitrobenzene. A no-observed-adverse-effect level (NOAEL) for rats was not achieved, as increases in methemoglobin and histopathologic changes occurred at exposure concentrations as low as 1.1 ppm for 2-chloronitrobenzene and 1.5 ppm for 4-chloronitrobenzene in the 13-week studies. The NOAEL for histopathologic injury in mice was 4.5 ppm for 2-chloronitrobenzene and 6 ppm for 4-chloronitrobenzene.

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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 F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

	0 ppm	1.1 ppm	2.3 ppm	4.5 ppm	9 ppm	18 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	334 ± 7	350 ± 7	343 ± 7	349 ± 8	340 ± 7	323 ± 6
Heart						
Absolute	0.970 ± 0.030	0.977 ± 0.051	0.995 ± 0.020	0.983 ± 0.019	0.980 ± 0.027	0.943 ± 0.016
Relative	2.90 ± 0.04	2.78 ± 0.12	2.90 ± 0.02	2.82 ± 0.03	2.88 ± 0.04	2.92 ± 0.04
Right kidney						
Absolute	1.124 ± 0.032	1.153 ± 0.063	1.184 ± 0.032	1.242 ± 0.032	1.218 ± 0.029	1.225 ± 0.029
Relative	3.36 ± 0.04	3.28 ± 0.15	3.45 ± 0.04	3.56 ± 0.04	3.59 ± 0.04*	3.79 ± 0.04**
Liver						
Absolute	11.820 ± 0.356	13.102 ± 0.401*	12.987 ± 0.356*	14.126 ± 0.576**	14.160 ± 0.398**	15.543 ± 0.346**
Relative	35.33 ± 0.58	37.48 ± 1.26	37.81 ± 0.48*	40.40 ± 0.84**	41.67 ± 0.61**	48.07 ± 0.68**
Lungs						
Absolute	1.606 ± 0.057	1.615 ± 0.097	1.602 ± 0.028	1.596 ± 0.056	1.582 ± 0.050	1.398 ± 0.028*
Relative	4.80 ± 0.11	4.59 ± 0.23	4.68 ± 0.11	4.57 ± 0.08	4.66 ± 0.12	4.33 ± 0.07*
Spleen						
Absolute	0.631 ± 0.016	0.680 ± 0.014[2]	0.650 ± 0.020	0.659 ± 0.018	0.669 ± 0.012	0.753 ± 0.018**
Relative	1.89 ± 0.03	1.93 ± 0.04[2]	1.89 ± 0.03	1.89 ± 0.02	1.97 ± 0.02	2.33 ± 0.04**
Right testis						
Absolute	1.358 ± 0.014	1.273 ± 0.074	1.344 ± 0.028	1.401 ± 0.025	1.386 ± 0.033	1.322 ± 0.020
Relative	4.07 ± 0.06	3.63 ± 0.19	3.92 ± 0.04	4.03 ± 0.06	4.09 ± 0.07	4.09 ± 0.04
Thymus						
Absolute	0.348 ± 0.011	0.371 ± 0.011[2]	0.342 ± 0.015	0.372 ± 0.012	0.336 ± 0.006	0.349 ± 0.012
Relative	1.04 ± 0.04	1.05 ± 0.03[2]	0.99 ± 0.03	1.07 ± 0.03	0.99 ± 0.02	1.08 ± 0.03

TABLE A1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene (continued)

	0 ppm	1.1 ppm	2.3 ppm	4.5 ppm	9 ppm	18 ppm
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	191 ± 3	188 ± 4	200 ± 4	193 ± 3	196 ± 5	193 ± 4
Heart						
Absolute	0.641 ± 0.009	0.625 ± 0.014	0.653 ± 0.016	0.626 ± 0.015	0.645 ± 0.020	0.663 ± 0.013
Relative	3.36 ± 0.02	3.33 ± 0.06	3.26 ± 0.05	3.24 ± 0.08	3.28 ± 0.05	3.45 ± 0.05
Right kidney						
Absolute	0.641 ± 0.009	0.641 ± 0.014	0.720 ± 0.054	0.666 ± 0.017	0.696 ± 0.016	0.739 ± 0.028*
Relative	3.36 ± 0.02	3.42 ± 0.08	3.57 ± 0.21	3.44 ± 0.07	3.55 ± 0.05	3.83 ± 0.09**
Liver						
Absolute	6.658 ± 0.191	6.751 ± 0.124	7.397 ± 0.203*	7.610 ± 0.221**	8.594 ± 0.273**	9.773 ± 0.362**
Relative	34.86 ± 0.75	36.00 ± 0.68	36.91 ± 0.61	39.29 ± 0.72**	43.73 ± 0.54**	50.67 ± 1.07**
Lungs						
Absolute	1.045 ± 0.022	1.023 ± 0.031	1.060 ± 0.035[2]	1.042 ± 0.031	1.026 ± 0.023	1.034 ± 0.026
Relative	5.48 ± 0.12	5.44 ± 0.12	5.34 ± 0.14[2]	5.39 ± 0.14	5.24 ± 0.08	5.38 ± 0.11
Spleen						
Absolute	0.422 ± 0.006	0.420 ± 0.009	0.440 ± 0.012	0.463 ± 0.008*	0.468 ± 0.010**	0.538 ± 0.020**
Relative	2.21 ± 0.04	2.24 ± 0.04	2.20 ± 0.03	2.40 ± 0.05*	2.39 ± 0.05*	2.80 ± 0.09**
Thymus						
Absolute	0.259 ± 0.006	0.252 ± 0.009	0.280 ± 0.016	0.258 ± 0.005	0.276 ± 0.014	0.268 ± 0.007
Relative	1.36 ± 0.04	1.34 ± 0.04	1.39 ± 0.06	1.34 ± 0.03	1.41 ± 0.06	1.40 ± 0.04

[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] n=9.
* Significantly different (P≤0.05) from the control group by Williams' test.
** Significantly different (P≤0.01) from the control group by Williams' test.

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

	0 ppm	1.5 ppm	3 ppm	6 ppm	12 ppm	24 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	342 ± 4	354 ± 9	347 ± 4	348 ± 6	337 ± 5	346 ± 7
Heart						
Absolute	0.998 ± 0.018	1.004 ± 0.019	0.987 ± 0.014	1.014 ± 0.025	0.999 ± 0.015	1.047 ± 0.019
Relative	2.92 ± 0.04	2.84 ± 0.03	2.85 ± 0.03	2.91 ± 0.04	2.97 ± 0.03	3.03 ± 0.03*
Right kidney						
Absolute	1.144 ± 0.024	1.176 ± 0.034	1.235 ± 0.065	1.188 ± 0.016	1.174 ± 0.019	1.252 ± 0.033*
Relative	3.34 ± 0.06	3.32 ± 0.04	3.56 ± 0.18	3.42 ± 0.05	3.49 ± 0.03	3.62 ± 0.05*
Liver						
Absolute	12.055 ± 0.373	12.680 ± 0.445	12.206 ± 0.284	12.849 ± 0.357	12.425 ± 0.230	14.323 ± 0.367**
Relative	35.19 ± 0.86	35.79 ± 0.58	35.18 ± 0.52	36.89 ± 0.68	36.91 ± 0.40	41.35 ± 0.45**
Lungs						
Absolute	1.652 ± 0.038	1.644 ± 0.068	1.561 ± 0.103	1.636 ± 0.062	1.511 ± 0.049	1.616 ± 0.073
Relative	4.83 ± 0.09	4.65 ± 0.15	4.49 ± 0.28	4.69 ± 0.14	4.48 ± 0.10	4.68 ± 0.20
Spleen						
Absolute	0.637 ± 0.017	0.716 ± 0.018	0.789 ± 0.039*	0.983 ± 0.024**	1.659 ± 0.062**[2]	3.280 ± 0.079**
Relative	1.86 ± 0.04	2.02 ± 0.02	2.28 ± 0.11**	2.82 ± 0.05**	4.92 ± 0.15**[2]	9.48 ± 0.17**
Right testis						
Absolute	1.369 ± 0.053	1.410 ± 0.031	1.369 ± 0.026	1.404 ± 0.031	1.351 ± 0.029	1.057 ± 0.066**
Relative	4.01 ± 0.16	3.99 ± 0.04	3.95 ± 0.07	4.03 ± 0.05	4.01 ± 0.05	3.07 ± 0.21**
Thymus						
Absolute	0.374 ± 0.019	0.370 ± 0.011	0.386 ± 0.014	0.351 ± 0.012	0.360 ± 0.021	0.434 ± 0.014*
Relative	1.09 ± 0.05	1.05 ± 0.03	1.11 ± 0.03	1.01 ± 0.04	1.08 ± 0.07	1.25 ± 0.03*

TABLE A2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene (continued)

	0 ppm	1.5 ppm	3 ppm	6 ppm	12 ppm	24 ppm
FEMALE						
n	10	9	9	10	10	10
Necropsy body wt	192 ± 5	195 ± 5	202 ± 6	196 ± 4	197 ± 6	200 ± 2
Heart						
Absolute	0.632 ± 0.014	0.621 ± 0.010	0.657 ± 0.019	0.657 ± 0.012	0.690 ± 0.021*	0.738 ± 0.013**
Relative	3.30 ± 0.05	3.20 ± 0.05	3.25 ± 0.04	3.36 ± 0.04	3.51 ± 0.05**	3.70 ± 0.04**
Right kidney						
Absolute	0.662 ± 0.017	0.671 ± 0.016	0.697 ± 0.024	0.692 ± 0.020	0.691 ± 0.016	0.758 ± 0.010**
Relative	3.46 ± 0.09	3.45 ± 0.06	3.46 ± 0.09	3.54 ± 0.09	3.52 ± 0.07	3.80 ± 0.06**
Liver						
Absolute	5.922 ± 0.243	6.212 ± 0.198	6.712 ± 0.284*	6.786 ± 0.242**	6.844 ± 0.230**	7.704 ± 0.134**
Relative	30.89 ± 0.87	31.96 ± 0.95	33.19 ± 0.86	34.65 ± 0.72**	34.80 ± 0.82**	38.63 ± 0.74**
Lungs						
Absolute	1.109 ± 0.040	1.059 ± 0.035	1.076 ± 0.046	1.050 ± 0.023	1.064 ± 0.025	1.138 ± 0.025
Relative	5.80 ± 0.19	5.44 ± 0.11	5.32 ± 0.15	5.38 ± 0.13	5.42 ± 0.11	5.71 ± 0.14
Spleen						
Absolute	0.394 ± 0.010	0.449 ± 0.011	0.563 ± 0.018*	0.844 ± 0.032**	1.490 ± 0.056**	3.097 ± 0.091**
Relative	2.06 ± 0.05	2.31 ± 0.07	2.80 ± 0.10	4.32 ± 0.14**	7.61 ± 0.34**	15.55 ± 0.53**
Thymus						
Absolute	0.267 ± 0.014	0.279 ± 0.014	0.289 ± 0.018	0.292 ± 0.013	0.359 ± 0.034**	0.391 ± 0.029**
Relative	1.39 ± 0.05	1.43 ± 0.06	1.43 ± 0.07	1.50 ± 0.07	1.83 ± 0.18**	1.95 ± 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] 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' test.

TABLE A3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

	0 ppm	1.1 ppm	2.3 ppm	4.5 ppm	9 ppm	18 ppm
MALE						
n	10	10	10	10	10	8
Necropsy body wt	36.7 ± 0.7	37.2 ± 0.3	36.2 ± 0.9	34.9 ± 0.8	36.9 ± 1.0	35.8 ± 1.0
Heart						
Absolute	0.179 ± 0.008	0.179 ± 0.007	0.185 ± 0.008	0.166 ± 0.005	0.178 ± 0.007	0.171 ± 0.008
Relative	4.88 ± 0.17	4.81 ± 0.16	5.14 ± 0.26	4.77 ± 0.16	4.82 ± 0.08	4.79 ± 0.17
Right kidney						
Absolute	0.318 ± 0.010	0.335 ± 0.007	0.344 ± 0.007*	0.348 ± 0.012*	0.353 ± 0.005**	0.354 ± 0.009**
Relative	8.69 ± 0.27	9.01 ± 0.16	9.54 ± 0.21*	10.00 ± 0.34**	9.62 ± 0.21**	9.91 ± 0.22**
Liver						
Absolute	1.713 ± 0.039	1.835 ± 0.057	1.816 ± 0.059	1.794 ± 0.044	2.025 ± 0.066**	2.279 ± 0.103**
Relative	46.75 ± 0.76	49.30 ± 1.33	50.24 ± 1.22*	51.46 ± 0.82**	54.92 ± 0.83**	63.51 ± 1.46**
Lungs						
Absolute	0.247 ± 0.011	0.266 ± 0.017	0.272 ± 0.013	0.231 ± 0.007	0.251 ± 0.015	0.269 ± 0.036
Relative	6.74 ± 0.27	7.15 ± 0.45	7.56 ± 0.43	6.61 ± 0.10	6.81 ± 0.36	7.52 ± 1.03
Spleen						
Absolute	0.072 ± 0.003	0.073 ± 0.003	0.071 ± 0.002	0.065 ± 0.002	0.071 ± 0.002	0.075 ± 0.003
Relative	1.97 ± 0.09	1.96 ± 0.07	1.97 ± 0.05	1.86 ± 0.05	1.93 ± 0.05	2.10 ± 0.05
Right testis						
Absolute	0.120 ± 0.005	0.123 ± 0.003	0.124 ± 0.003	0.127 ± 0.002	0.125 ± 0.001	0.121 ± 0.003
Relative	3.27 ± 0.12	3.30 ± 0.08	3.43 ± 0.10	3.65 ± 0.07*	3.40 ± 0.08	3.40 ± 0.12
Thymus						
Absolute	0.052 ± 0.002[2]	0.054 ± 0.002	0.050 ± 0.003	0.046 ± 0.003	0.049 ± 0.003	0.048 ± 0.004
Relative	1.43 ± 0.06[2]	1.45 ± 0.06	1.37 ± 0.06	1.32 ± 0.07	1.33 ± 0.07	1.34 ± 0.11

TABLE A3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of 2-Chloronitrobenzene (continued)

	0 ppm	1.1 ppm	2.3 ppm	4.5 ppm	9 ppm	18 ppm
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	30.1 ± 0.8	32.6 ± 0.8	34.5 ± 1.1*	33.0 ± 1.0*	34.7 ± 1.2**	33.9 ± 1.3**
Heart						
Absolute	0.145 ± 0.004	0.144 ± 0.004	0.146 ± 0.003	0.144 ± 0.003	0.150 ± 0.004	0.144 ± 0.002
Relative	4.82 ± 0.11	4.43 ± 0.10*	4.26 ± 0.12*	4.39 ± 0.12*	4.37 ± 0.18*	4.30 ± 0.17*
Right kidney						
Absolute	0.205 ± 0.008	0.223 ± 0.005	0.239 ± 0.003**	0.231 ± 0.004**	0.244 ± 0.012**	0.237 ± 0.003**
Relative	6.81 ± 0.24	6.87 ± 0.18	6.97 ± 0.20	7.04 ± 0.21	7.09 ± 0.38	7.07 ± 0.24
Liver						
Absolute	1.472 ± 0.040	1.625 ± 0.042*	1.768 ± 0.050**	1.723 ± 0.052**	1.933 ± 0.048**	2.234 ± 0.065**
Relative	49.00 ± 1.25	49.93 ± 0.97	51.32 ± 0.86	52.31 ± 1.24	56.00 ± 0.97**	66.37 ± 2.15**
Lungs						
Absolute	0.220 ± 0.006	0.215 ± 0.004	0.226 ± 0.004	0.226 ± 0.011	0.225 ± 0.006	0.210 ± 0.006
Relative	7.33 ± 0.22	6.62 ± 0.16	6.59 ± 0.18	6.89 ± 0.39	6.57 ± 0.31	6.26 ± 0.26**
Spleen						
Absolute	0.092 ± 0.003	0.090 ± 0.002	0.093 ± 0.003	0.094 ± 0.004	0.098 ± 0.003	0.101 ± 0.004[2]
Relative	3.06 ± 0.09	2.77 ± 0.08	2.71 ± 0.12	2.85 ± 0.10	2.85 ± 0.12	3.04 ± 0.13[2]
Thymus						
Absolute	0.058 ± 0.003	0.066 ± 0.003	0.067 ± 0.003	0.067 ± 0.003	0.068 ± 0.003*	0.069 ± 0.003*
Relative	1.93 ± 0.10	2.01 ± 0.07	1.96 ± 0.08	2.02 ± 0.06	1.95 ± 0.07	2.03 ± 0.08

[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] n=).

* 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' test.

TABLE A4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

	0 ppm	1.5 ppm	3 ppm	6 ppm	12 ppm	24 ppm
MALE						
n	10	10	10	9	10	10
Necropsy body wt	34.9 ± 0.8	36.7 ± 1.0	36.7 ± 0.9	35.3 ± 1.4	35.8 ± 0.5	36.8 ± 0.7
Heart						
Absolute	0.159 ± 0.005	0.165 ± 0.005	0.169 ± 0.006	0.157 ± 0.006	0.159 ± 0.003	0.164 ± 0.005
Relative	4.56 ± 0.11	4.49 ± 0.10	4.60 ± 0.11	4.45 ± 0.14	4.45 ± 0.11	4.46 ± 0.14
Right kidney						
Absolute	0.300 ± 0.006	0.330 ± 0.011*	0.336 ± 0.012*	0.324 ± 0.010*	0.327 ± 0.005*	0.337 ± 0.008**
Relative	8.63 ± 0.20	8.99 ± 0.21	9.15 ± 0.23	9.24 ± 0.30	9.15 ± 0.17	9.18 ± 0.27
Liver						
Absolute	1.596 ± 0.051	1.678 ± 0.045	1.733 ± 0.041	1.704 ± 0.059	1.759 ± 0.045*	1.868 ± 0.050**
Relative	45.81 ± 1.24	45.77 ± 1.05	47.32 ± 1.09	48.44 ± 1.40	49.21 ± 1.26	50.82 ± 1.27**
Lungs						
Absolute	0.208 ± 0.005	0.219 ± 0.005	0.211 ± 0.006	0.227 ± 0.009	0.215 ± 0.006	0.212 ± 0.005
Relative	5.98 ± 0.14	5.99 ± 0.17	5.75 ± 0.11	6.42 ± 0.14	6.01 ± 0.13	5.77 ± 0.11
Spleen						
Absolute	0.065 ± 0.002	0.068 ± 0.002	0.067 ± 0.002	0.064 ± 0.002	0.079 ± 0.002**	0.160 ± 0.006**
Relative	1.87 ± 0.07	1.85 ± 0.05	1.83 ± 0.06	1.83 ± 0.07	2.21 ± 0.06*	4.36 ± 0.19**
Right testis						
Absolute	0.116 ± 0.003	0.124 ± 0.003	0.120 ± 0.004	0.121 ± 0.003	0.116 ± 0.003	0.120 ± 0.002
Relative	3.34 ± 0.09	3.38 ± 0.05	3.28 ± 0.11	3.43 ± 0.09	3.25 ± 0.06	3.28 ± 0.08
Thymus						
Absolute	0.046 ± 0.003	0.042 ± 0.003	0.045 ± 0.003	0.044 ± 0.004	0.041 ± 0.002	0.049 ± 0.003
Relative	1.31 ± 0.07	1.14 ± 0.07	1.23 ± 0.08	1.26 ± 0.09	1.15 ± 0.05	1.31 ± 0.07

TABLE A4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Inhalation Study of 4-Chloronitrobenzene (continued)

	0 ppm	1.5 ppm	3 ppm	6 ppm	12 ppm	24 ppm
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	31.5 ± 0.9	32.3 ± 1.1	33.5 ± 1.3	31.1 ± 0.8	33.1 ± 0.8	33.0 ± 0.4
Heart						
Absolute	0.144 ± 0.003	0.138 ± 0.003	0.136 ± 0.002	0.141 ± 0.003	0.140 ± 0.002	0.140 ± 0.004
Relative	4.60 ± 0.15	4.33 ± 0.18	4.11 ± 0.15*	4.55 ± 0.10	4.25 ± 0.11	4.24 ± 0.09
Right kidney						
Absolute	0.205 ± 0.007	0.215 ± 0.003	0.220 ± 0.005*	0.220 ± 0.004*	0.222 ± 0.004*	0.240 ± 0.002**
Relative	6.53 ± 0.21	6.73 ± 0.22	6.64 ± 0.28	7.11 ± 0.14	6.73 ± 0.19	7.27 ± 0.07*
Liver						
Absolute	1.468 ± 0.027	1.535 ± 0.049	1.618 ± 0.050	1.552 ± 0.048	1.760 ± 0.055**	1.889 ± 0.024**
Relative	46.78 ± 0.89	47.72 ± 1.00	48.49 ± 1.02	50.07 ± 1.34*	53.10 ± 0.97**	57.25 ± 0.82**
Lungs						
Absolute	0.210 ± 0.007	0.222 ± 0.007	0.214 ± 0.004	0.216 ± 0.005	0.207 ± 0.004	0.215 ± 0.006
Relative	6.68 ± 0.17	6.91 ± 0.21	6.45 ± 0.20	6.97 ± 0.16	6.28 ± 0.21	6.50 ± 0.12
Spleen						
Absolute	0.093 ± 0.004	0.095 ± 0.004	0.097 ± 0.003	0.099 ± 0.003	0.130 ± 0.005**	0.253 ± 0.007**
Relative	2.98 ± 0.16	2.95 ± 0.10	2.93 ± 0.15	3.20 ± 0.09	3.94 ± 0.16**	7.68 ± 0.27**
Thymus						
Absolute	0.054 ± 0.002	0.061 ± 0.002	0.061 ± 0.002	0.054 ± 0.003	0.063 ± 0.004	0.061 ± 0.002
Relative	1.71 ± 0.06	1.91 ± 0.08	1.83 ± 0.07	1.73 ± 0.07	1.87 ± 0.09	1.85 ± 0.05

[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).

* 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' test.

APPENDIX B

Hematology and Clinical Chemistry Results

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

	0 ppm	1.1 ppm	2.3 ppm	4.5 ppm	9 ppm	18 ppm
MALE						
n						
Day 4	10	10	10	10	10	10
Day 23	8	10	9	10	10	6
Week 13	10	10	10	10	10	10
Hematocrit (automated) (%)						
Day 4	36.7 ± 0.3	36.8 ± 0.2	36.5 ± 0.3	36.8 ± 0.3	35.5 ± 0.2**	33.3 ± 0.5**
Day 23	45.1 ± 0.7	43.2 ± 0.5[2]	44.8 ± 0.5	43.6 ± 0.3	43.6 ± 0.4	41.2 ± 0.4**
Week 13	46.5 ± 0.4	46.2 ± 0.2	45.5 ± 0.2*	45.1 ± 0.2**	45.0 ± 0.3**	42.6 ± 0.3**
Hematocrit (manual) (%)						
Day 4	38.4 ± 0.4	38.8 ± 0.3	38.7 ± 0.4	38.7 ± 0.3	37.7 ± 0.2	35.8 ± 0.7**[2]
Day 23	47.3 ± 0.7	45.1 ± 0.5*	46.5 ± 0.5	45.6 ± 0.3	45.5 ± 0.4*	43.8 ± 0.6**
Week 13	46.4 ± 0.5	46.1 ± 0.3	45.9 ± 0.2	45.3 ± 0.3	45.7 ± 0.3	43.5 ± 0.3**
Hemoglobin (g/dL)						
Day 4	12.1 ± 0.1	12.1 ± 0.1	11.9 ± 0.1	12.1 ± 0.1	11.5 ± 0.1**	10.9 ± 0.2**
Day 23	15.3 ± 0.2	14.7 ± 0.2*	15.3 ± 0.2	14.8 ± 0.1	14.8 ± 0.1	14.1 ± 0.1**
Week 13	15.0 ± 0.1	14.9 ± 0.1	14.6 ± 0.1*	14.5 ± 0.1**	14.4 ± 0.1**	13.7 ± 0.1**
Erythrocytes (10^6/μL)						
Day 4	6.21 ± 0.06	6.16 ± 0.05	6.11 ± 0.08	6.18 ± 0.07	5.99 ± 0.04*	6.07 ± 0.12*
Day 23	8.05 ± 0.16	7.61 ± 0.09*	7.95 ± 0.11	7.74 ± 0.08	7.77 ± 0.07	7.31 ± 0.07**
Week 13	9.16 ± 0.08	9.11 ± 0.04	8.99 ± 0.05	8.88 ± 0.05**	8.86 ± 0.06**	8.43 ± 0.07**
Reticulocytes (10^6/μL)						
Day 4	0.81 ± 0.05	0.90 ± 0.05	0.86 ± 0.03	0.92 ± 0.05	0.91 ± 0.05[2]	0.77 ± 0.04
Day 23	0.23 ± 0.02	0.24 ± 0.02	0.24 ± 0.02[3]	0.27 ± 0.03	0.25 ± 0.02	0.24 ± 0.04
Week 13	0.248 ± 0.009	0.282 ± 0.013*	0.283 ± 0.017	0.279 ± 0.012	0.331 ± 0.018**	0.384 ± 0.023**
Nucleated erythrocytes (10^3/μL)						
Day 4	0.29 ± 0.07	0.41 ± 0.06	0.39 ± 0.06	0.37 ± 0.06	0.34 ± 0.05	0.57 ± 0.08**
Day 23	0.00 ± 0.00	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.02	0.02 ± 0.01
Week 13	0.05 ± 0.02	0.05 ± 0.03	0.07 ± 0.03	0.10 ± 0.03	0.15 ± 0.03*	0.16 ± 0.03**
Mean cell volume (fL)						
Day 4	59.0 ± 0.7	59.7 ± 0.3	59.7 ± 0.3	59.7 ± 0.4	59.3 ± 0.3	54.7 ± 0.2**
Day 23	56.0 ± 0.4	56.8 ± 0.2	56.4 ± 0.3	56.2 ± 0.3	56.1 ± 0.1	56.5 ± 0.4
Week 13	50.7 ± 0.2	50.8 ± 0.2	50.6 ± 0.2	50.8 ± 0.1	50.7 ± 0.2	50.5 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.5 ± 0.2	19.7 ± 0.1	19.5 ± 0.1	19.6 ± 0.1	19.3 ± 0.1	17.9 ± 0.2**
Day 23	19.1 ± 0.1	19.3 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	19.1 ± 0.1	19.2 ± 0.1
Week 13	16.4 ± 0.0	16.4 ± 0.1	16.3 ± 0.1	16.3 ± 0.1	16.3 ± 0.1	16.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.9 ± 0.2	33.0 ± 0.1	32.7 ± 0.1	32.9 ± 0.1	32.5 ± 0.2	32.7 ± 0.2
Day 23	34.0 ± 0.1	34.0 ± 0.1	34.1 ± 0.1	34.1 ± 0.1	33.9 ± 0.2	34.1 ± 0.2
Week 13	32.3 ± 0.1	32.3 ± 0.1	32.1 ± 0.1	32.2 ± 0.1	32.1 ± 0.1	32.1 ± 0.2
Platelets (10^3/μL)						
Day 4	782.6 ± 15.4	757.7 ± 21.9	791.3 ± 10.5	754.2 ± 11.7	730.8 ± 13.0*	484.2 ± 46.1**
Day 23	610.0 ± 16.9	619.1 ± 10.4	610.2 ± 16.5	635.0 ± 12.5	643.5 ± 20.5	699.3 ± 18.2**
Week 13	513.8 ± 9.4	534.7 ± 8.4	525.5 ± 5.9	522.6 ± 27.1	553.5 ± 7.2**	575.8 ± 9.1**
Leukocytes (10^3/μL)						
Day 4	8.40 ± 0.26	8.19 ± 0.41	9.23 ± 0.31	9.00 ± 0.34	8.23 ± 0.39	10.14 ± 0.57*
Day 23	6.65 ± 0.24	6.70 ± 0.32	6.95 ± 0.45	6.70 ± 0.35	7.40 ± 0.49	6.73 ± 0.60
Week 13	6.07 ± 0.20	7.13 ± 0.34*	6.80 ± 0.24*	6.93 ± 0.32*	7.58 ± 0.31**	7.43 ± 0.44**

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene (continued)

	0 ppm	1.1 ppm	2.3 ppm	4.5 ppm	9 ppm	18 ppm
MALE (continued)						
Segmented neutrophils ($10^3/\mu L$)						
Day 4	1.48 ± 0.11	1.14 ± 0.11	1.43 ± 0.14	1.42 ± 0.12	1.52 ± 0.14	1.85 ± 0.17
Day 23	0.99 ± 0.09	0.87 ± 0.06	0.80 ± 0.07	0.89 ± 0.08	0.75 ± 0.11	0.80 ± 0.10
Week 13	1.24 ± 0.11	1.28 ± 0.14	1.39 ± 0.13	1.27 ± 0.17	1.09 ± 0.14	0.95 ± 0.10
Lymphocytes ($10^3/\mu L$)						
Day 4	6.89 ± 0.18	6.95 ± 0.32	7.69 ± 0.26	7.50 ± 0.30	6.67 ± 0.35	8.19 ± 0.47
Day 23	5.63 ± 0.30	5.77 ± 0.32	6.05 ± 0.45	5.76 ± 0.36	6.60 ± 0.46	5.86 ± 0.57
Week 13	4.74 ± 0.23	5.74 ± 0.35*	5.32 ± 0.21	5.60 ± 0.24*	6.44 ± 0.30**	6.44 ± 0.47**
Monocytes ($10^3/\mu L$)						
Day 4	0.03 ± 0.01	0.06 ± 0.02	0.07 ± 0.02	0.06 ± 0.03	0.02 ± 0.02	0.00 ± 0.00
Day 23	0.01 ± 0.01	0.02 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.04 ± 0.02	0.01 ± 0.01
Week 13	0.05 ± 0.03	0.06 ± 0.04	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Eosinophils ($10^3/\mu L$)						
Day 4	0.01 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.01 ± 0.01	0.06 ± 0.03
Day 23	0.02 ± 0.01	0.03 ± 0.01	0.07 ± 0.02	0.03 ± 0.01	0.01 ± 0.01	0.07 ± 0.01
Week 13	0.05 ± 0.01	0.04 ± 0.02	0.07 ± 0.03	0.05 ± 0.02	0.04 ± 0.02	0.03 ± 0.02
Methemoglobin (g/dL)						
Day 4	0.09 ± 0.01	0.11 ± 0.01	0.12 ± 0.01**	0.17 ± 0.01**	0.24 ± 0.01**	1.14 ± 0.09**
Day 23	0.14 ± 0.02	0.15 ± 0.01*	0.19 ± 0.01**	0.23 ± 0.02**	0.41 ± 0.02**	0.55 ± 0.01**
Week 13	0.15 ± 0.01	0.21 ± 0.01**	0.26 ± 0.01**	0.36 ± 0.01**	0.55 ± 0.01**	0.87 ± 0.02**
FEMALE						
n						
Day 4	10	10	10	10	10	10
Day 23	9	10	10	10	10	8
Week 13	10	10	10	10	10	10
Hematocrit (automated) (%)						
Day 4	39.5 ± 0.4	39.0 ± 0.4	38.7 ± 0.3	38.8 ± 0.5	38.3 ± 0.4	35.5 ± 0.4**
Day 23	45.8 ± 0.3	45.7 ± 0.3	45.7 ± 0.3	45.1 ± 0.2*	45.4 ± 0.2	43.5 ± 0.6**
Week 13	47.9 ± 0.4	46.8 ± 0.2	47.2 ± 0.4	45.9 ± 0.3**	45.0 ± 0.3**	42.6 ± 0.3**
Hematocrit (manual) (%)						
Day 4	41.0 ± 0.4	40.9 ± 0.5	40.3 ± 0.4	40.6 ± 0.5	39.5 ± 0.6	37.0 ± 0.7**
Day 23	46.5 ± 0.5	47.2 ± 0.3	47.2 ± 0.4	46.2 ± 0.3	46.8 ± 0.3	45.1 ± 0.6
Week 13	46.7 ± 0.4	46.1 ± 0.3	46.6 ± 0.5	45.5 ± 0.4	44.3 ± 0.3**	42.6 ± 0.4**
Hemoglobin (g/dL)						
Day 4	13.1 ± 0.1	12.9 ± 0.1	12.8 ± 0.1	12.8 ± 0.2	12.5 ± 0.2**	11.5 ± 0.1**
Day 23	15.9 ± 0.1	15.8 ± 0.1	15.9 ± 0.1	15.6 ± 0.1*	15.7 ± 0.1	15.1 ± 0.2**
Week 13	15.5 ± 0.1	15.1 ± 0.1**	15.2 ± 0.1	14.7 ± 0.1**	14.3 ± 0.1**	13.4 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 4	6.77 ± 0.09	6.69 ± 0.08	6.67 ± 0.08	6.66 ± 0.12	6.63 ± 0.09	6.48 ± 0.09
Day 23	8.09 ± 0.04	8.07 ± 0.05	8.10 ± 0.06	8.02 ± 0.06	8.24 ± 0.06	7.99 ± 0.12
Week 13	8.80 ± 0.06	8.58 ± 0.06*	8.64 ± 0.06	8.41 ± 0.06**	8.24 ± 0.05**	7.84 ± 0.05**
Reticulocytes ($10^6/\mu L$)						
Day 4	0.84 ± 0.05[3]	0.84 ± 0.04	0.76 ± 0.05[2]	0.79 ± 0.05[2]	0.72 ± 0.05[2]	0.59 ± 0.05**[2]
Day 23	0.15 ± 0.02	0.19 ± 0.02	0.18 ± 0.03	0.12 ± 0.01[2]	0.19 ± 0.04[2]	0.31 ± 0.06**
Week 13	0.19 ± 0.01	0.22 ± 0.01	0.21 ± 0.02	0.25 ± 0.02**	0.25 ± 0.01**	0.37 ± 0.02**

TABLE B1 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene (continued)

	0 ppm	1.1 ppm	2.3 ppm	4.5 ppm	9 ppm	18 ppm
FEMALE (continued)						
Nucleated erythrocytes (10^3/μL)						
Day 4	0.25 ± 0.07	0.17 ± 0.03	0.32 ± 0.05	0.26 ± 0.06	0.15 ± 0.04	0.25 ± 0.08
Day 23	0.03 ± 0.02	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.02
Week 13	0.06 ± 0.03	0.09 ± 0.03	0.09 ± 0.03	0.10 ± 0.04	0.11 ± 0.02	0.19 ± 0.03**
Mean cell volume (fL)						
Day 4	58.7 ± 0.3	58.2 ± 0.3	58.2 ± 0.3	58.3 ± 0.3	57.9 ± 0.3	54.8 ± 0.3**
Day 23	56.6 ± 0.3	56.7 ± 0.2	56.4 ± 0.2	56.2 ± 0.3	55.0 ± 0.3**	54.5 ± 0.4**
Week 13	54.5 ± 0.2	54.8 ± 0.2	54.5 ± 0.2	54.7 ± 0.3	54.6 ± 0.3	54.4 ± 0.3
Mean cell hemoglobin (pg)						
Day 4	19.4 ± 0.1	19.3 ± 0.1	19.2 ± 0.1	19.2 ± 0.1	18.9 ± 0.1**	17.7 ± 0.1**
Day 23	19.6 ± 0.1	19.6 ± 0.1	19.6 ± 0.1	19.4 ± 0.1	19.0 ± 0.1**	18.8 ± 0.1**
Week 13	17.6 ± 0.1	17.6 ± 0.1	17.6 ± 0.1	17.5 ± 0.1	17.3 ± 0.0**	17.1 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.2 ± 0.1	33.1 ± 0.1	33.1 ± 0.1	33.0 ± 0.1	32.6 ± 0.1**	32.3 ± 0.1**
Day 23	34.6 ± 0.1	34.7 ± 0.1	34.7 ± 0.2	34.6 ± 0.2	34.5 ± 0.1	34.6 ± 0.2
Week 13	32.4 ± 0.1	32.2 ± 0.1	32.2 ± 0.2	32.0 ± 0.2	31.8 ± 0.2**	31.5 ± 0.1**
Platelets (10^3/μL)						
Day 4	771.0 ± 31.8	736.1 ± 22.1	733.3 ± 11.0	721.7 ± 28.2	686.5 ± 18.4*	462.5 ± 40.4**
Day 23	638.4 ± 12.6	631.5 ± 18.3	580.0 ± 19.9*	592.5 ± 14.5*	612.7 ± 15.5	605.3 ± 19.0
Week 13	646.1 ± 29.9	560.8 ± 17.3	572.3 ± 39.8	631.4 ± 24.0	610.0 ± 21.2	603.2 ± 18.4
Leukocytes (10^3/μL)						
Day 4	7.87 ± 0.57	8.58 ± 0.48	9.01 ± 0.53	8.36 ± 0.29	9.00 ± 0.41	9.48 ± 0.55
Day 23	7.44 ± 0.34	7.17 ± 0.24	7.72 ± 0.45	6.34 ± 0.31	7.36 ± 0.41	8.43 ± 0.59
Week 13	7.12 ± 0.49	6.19 ± 0.39	6.55 ± 0.50	6.62 ± 0.37	6.65 ± 0.27	6.51 ± 0.33
Segmented neutrophils (10^3/μL)						
Day 4	0.834 ± 0.093	0.978 ± 0.106	1.345 ± 0.127**	0.980 ± 0.108*	1.028 ± 0.116	1.327 ± 0.126**
Day 23	0.90 ± 0.13	0.87 ± 0.09	0.84 ± 0.15	0.84 ± 0.11	0.78 ± 0.08	0.83 ± 0.11
Week 13	1.25 ± 0.11	1.33 ± 0.13	1.28 ± 0.18	1.41 ± 0.23	1.12 ± 0.12	0.93 ± 0.14*
Lymphocytes (10^3/μL)						
Day 4	7.02 ± 0.52	7.56 ± 0.44	7.61 ± 0.48	7.35 ± 0.24	7.89 ± 0.31	8.01 ± 0.49
Day 23	6.45 ± 0.34	6.23 ± 0.25	6.81 ± 0.40	5.49 ± 0.27	6.49 ± 0.43	7.55 ± 0.62
Week 13	5.83 ± 0.46	4.82 ± 0.34	5.23 ± 0.51	5.18 ± 0.26	5.46 ± 0.30	5.56 ± 0.32
Monocytes (10^3/μL)						
Day 4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02*
Day 23	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Week 13	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
Eosinophils (10^3/μL)						
Day 4	0.01 ± 0.01	0.03 ± 0.01	0.05 ± 0.03	0.02 ± 0.01	0.05 ± 0.02	0.11 ± 0.03**
Day 23	0.07 ± 0.02	0.06 ± 0.03	0.06 ± 0.03	0.01 ± 0.01	0.08 ± 0.02	0.04 ± 0.02
Week 13	0.01 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.06 ± 0.02	0.02 ± 0.01
Methemoglobin (g/dL)						
Day 4	0.09 ± 0.01	0.11 ± 0.01	0.14 ± 0.01**	0.17 ± 0.01**	0.25 ± 0.01**	1.04 ± 0.08**
Day 23	0.16 ± 0.01	0.18 ± 0.01	0.22 ± 0.01**	0.30 ± 0.01**	0.47 ± 0.02**	0.71 ± 0.04**
Week 13	0.19 ± 0.01	0.22 ± 0.01**	0.28 ± 0.01**	0.35 ± 0.01**	0.51 ± 0.01**	0.79 ± 0.03**

[1] Data are given as mean ± standard error.
[2] n=9.
[3] n=8.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE B2 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

	0 ppm	1.5 ppm	3 ppm	6 ppm	12 ppm	24 ppm
MALE						
n	10	10	10	10	10	10
Hematocrit (automated) (%)						
Day 3	41.2 ± 0.5	41.4 ± 0.7	39.5 ± 0.4*	40.7 ± 0.5	39.2 ± 0.4**	38.2 ± 0.3**
Day 23	46.4 ± 0.4	45.2 ± 0.4*	44.2 ± 0.6**	42.7 ± 0.4**	41.0 ± 0.3**	39.1 ± 0.4**
Week 13	46.8 ± 0.3	44.9 ± 0.2**	43.8 ± 0.3**	41.9 ± 0.3**	39.9 ± 0.2**	36.1 ± 0.5**
Hematocrit (manual) (%)						
Day 3	42.1 ± 0.4	42.1 ± 0.7	40.4 ± 0.4*	41.9 ± 0.5	40.2 ± 0.4**	38.9 ± 0.3**
Day 23	46.3 ± 0.4	45.3 ± 0.3	44.6 ± 0.5**	43.4 ± 0.5**	42.8 ± 0.4**	43.0 ± 0.6**
Week 13	46.2 ± 0.2	44.6 ± 0.3**	44.2 ± 0.4**	42.8 ± 0.3**	41.0 ± 0.2**	38.8 ± 0.6**
Hemoglobin (g/dL)						
Day 3	13.3 ± 0.1	13.3 ± 0.2	12.7 ± 0.2**	13.1 ± 0.2	12.6 ± 0.2**	12.9 ± 0.1*
Day 23	15.0 ± 0.1	14.6 ± 0.1	14.4 ± 0.2**	13.9 ± 0.1**	13.7 ± 0.1**	13.2 ± 0.2**
Week 13	14.9 ± 0.1	14.2 ± 0.1**	13.9 ± 0.1**	13.3 ± 0.1**	13.4 ± 0.1**	12.6 ± 0.2**
Erythrocytes (10^6/μL)						
Day 3	7.00 ± 0.09	6.94 ± 0.12	6.62 ± 0.09**	6.96 ± 0.11	6.72 ± 0.13	6.53 ± 0.08**
Day 23	8.06 ± 0.09	7.93 ± 0.06	7.76 ± 0.12*	7.49 ± 0.08**	6.89 ± 0.06**	5.79 ± 0.10**
Week 13	9.00 ± 0.06	8.69 ± 0.04**	8.40 ± 0.04**	7.83 ± 0.04**	7.13 ± 0.06**	5.73 ± 0.07**
Reticulocytes (10^6/μL)						
Day 3	0.48 ± 0.03	0.68 ± 0.04**	0.56 ± 0.03	0.50 ± 0.04	0.53 ± 0.02	0.67 ± 0.04**
Day 23	0.31 ± 0.02	0.32 ± 0.02	0.36 ± 0.02	0.46 ± 0.02**	0.72 ± 0.03**	1.16 ± 0.04**
Week 13	0.17 ± 0.01	0.27 ± 0.02**	0.30 ± 0.02**	0.42 ± 0.02**	0.59 ± 0.03**	0.91 ± 0.03**
Nucleated erythrocytes (10^3/μL)						
Day 3	0.13 ± 0.03	0.15 ± 0.05	0.27 ± 0.05*	0.18 ± 0.04	0.23 ± 0.06	0.38 ± 0.08*
Day 23	0.04 ± 0.02	0.04 ± 0.01	0.04 ± 0.01	0.25 ± 0.04**	0.63 ± 0.07**	3.39 ± 0.25**
Week 13	0.03 ± 0.01	0.07 ± 0.03	0.10 ± 0.03*	0.23 ± 0.04**	0.50 ± 0.08**	0.93 ± 0.11**
Mean cell volume (fL)						
Day 3	58.9 ± 0.2	59.8 ± 0.3	59.7 ± 0.3	58.8 ± 0.3	58.4 ± 0.5	58.6 ± 0.3
Day 23	57.5 ± 0.2	56.9 ± 0.2	57.1 ± 0.3	57.0 ± 0.2	59.5 ± 0.4**	67.7 ± 0.8**
Week 13	51.9 ± 0.1	51.7 ± 0.2	52.2 ± 0.1	53.6 ± 0.2**	55.7 ± 0.3**	62.9 ± 0.3**
Mean cell hemoglobin (pg)						
Day 3	19.0 ± 0.1	19.1 ± 0.1	19.1 ± 0.1	18.8 ± 0.1	18.7 ± 0.1	19.8 ± 0.3
Day 23	18.7 ± 0.1	18.4 ± 0.1	18.6 ± 0.1	18.6 ± 0.1	19.9 ± 0.1**	22.8 ± 0.2**
Week 13	16.5 ± 0.1	16.4 ± 0.1	16.6 ± 0.1	17.0 ± 0.1**	18.8 ± 0.2**	22.1 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 3	32.2 ± 0.1	32.0 ± 0.1	32.0 ± 0.1	32.1 ± 0.1	32.0 ± 0.1	33.8 ± 0.4*
Day 23	32.4 ± 0.1	32.4 ± 0.1	32.6 ± 0.1	32.5 ± 0.1	33.3 ± 0.2**	33.8 ± 0.3**
Week 13	31.8 ± 0.1	31.6 ± 0.2	31.8 ± 0.1	31.8 ± 0.1	33.7 ± 0.2**	35.0 ± 0.2**
Platelets (10^3/μL)						
Day 3	774.2 ± 16.0	774.7 ± 8.5	793.4 ± 41.7	728.8 ± 13.8	796.9 ± 10.8	899.6 ± 21.6**
Day 23	584.4 ± 31.7	638.9 ± 9.3	674.0 ± 17.0**	698.0 ± 11.8**	708.3 ± 10.7**	767.3 ± 19.0**
Week 13	507.5 ± 9.0	596.8 ± 15.4*	629.0 ± 20.2**	658.1 ± 23.4**	547.2 ± 19.1	465.6 ± 30.5
Leukocytes (10^3/μL)						
Day 3	8.54 ± 0.44	9.33 ± 0.25	8.62 ± 0.31	7.90 ± 0.28	7.55 ± 0.32	6.23 ± 0.26**
Day 23	7.33 ± 0.50	6.61 ± 0.48	7.71 ± 0.45	7.66 ± 0.51	9.08 ± 0.52*	12.33 ± 1.07**
Week 13	7.76 ± 0.57	8.24 ± 0.38	8.00 ± 0.35	8.54 ± 0.36	7.75 ± 0.35	9.56 ± 0.53**
Segmented neutrophils (10^3/μL)						
Day 3	1.06 ± 0.11	1.17 ± 0.13	1.24 ± 0.13	1.19 ± 0.10	1.22 ± 0.10	1.85 ± 0.13**
Day 23	0.84 ± 0.07	1.09 ± 0.17	1.02 ± 0.11	0.94 ± 0.13	1.17 ± 0.10*	1.66 ± 0.13**
Week 13	1.20 ± 0.07[2]	1.33 ± 0.14	1.38 ± 0.17	1.32 ± 0.10	1.31 ± 0.09	2.00 ± 0.30*

TABLE B2 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene (continued)

	0 ppm	1.5 ppm	3 ppm	6 ppm	12 ppm	24 ppm
MALE (continued)						
Lymphocytes (10^3/μL)						
Day 3	7.43 ± 0.41	8.13 ± 0.21	7.34 ± 0.28	6.69 ± 0.26	6.31 ± 0.30*	4.35 ± 0.20**
Day 23	6.45 ± 0.49	5.48 ± 0.36	6.64 ± 0.42	6.70 ± 0.50	7.86 ± 0.48*	10.67 ± 1.05**
Week 13	6.01 ± 0.39	6.62 ± 0.28	6.48 ± 0.34	7.02 ± 0.28	6.23 ± 0.34	7.30 ± 0.30*
Monocytes (10^3/μL)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01
Day 23	0.03 ± 0.02	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01	0.00 ± 0.00
Week 13	0.23 ± 0.06	0.24 ± 0.05	0.11 ± 0.04	0.16 ± 0.05	0.19 ± 0.06	0.24 ± 0.06
Eosinophils (10^3/μL)						
Day 3	0.06 ± 0.02	0.03 ± 0.01	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
Day 23	0.01 ± 0.01	0.03 ± 0.01	0.05 ± 0.02	0.02 ± 0.01	0.03 ± 0.01	0.00 ± 0.00
Week 13	0.08 ± 0.03	0.05 ± 0.04	0.03 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.01 ± 0.01*
Methemoglobin (g/dL)						
Day 3	0.08 ± 0.00	0.19 ± 0.01**	0.31 ± 0.02**	0.60 ± 0.04**	1.31 ± 0.09**	4.13 ± 0.26**
Day 23	0.13 ± 0.01	0.32 ± 0.02**	0.57 ± 0.02**	0.98 ± 0.03**	1.90 ± 0.07**	2.97 ± 0.23**
Week 13	0.16 ± 0.01	0.50 ± 0.01**	0.73 ± 0.01**	1.22 ± 0.04**	2.08 ± 0.06**	2.96 ± 0.05**
FEMALE						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	8
Week 13	10	10	9	10	10	10
Hematocrit (automated) (%)						
Day 3	43.0 ± 0.5	43.5 ± 0.6	42.7 ± 0.5	42.9 ± 0.5	41.9 ± 0.3	39.8 ± 0.5**
Day 23	48.0 ± 0.3	47.3 ± 0.6	45.5 ± 0.3**	42.9 ± 0.5**	39.7 ± 0.3**	40.0 ± 0.5**
Week 13	48.7 ± 0.3	44.2 ± 0.4**	42.6 ± 0.3**	41.6 ± 0.3**	39.8 ± 0.3**	34.5 ± 0.5**
Hematocrit (manual) (%)						
Day 3	43.1 ± 0.6	43.8 ± 0.6	42.9 ± 0.5	42.8 ± 0.5	42.4 ± 0.3	41.0 ± 0.4**
Day 23	47.0 ± 0.3	46.7 ± 0.6	45.6 ± 0.4*	43.3 ± 0.5**	41.6 ± 0.3**	45.3 ± 0.6**
Week 13	47.3 ± 0.4	44.2 ± 0.3**	43.1 ± 0.4**	42.0 ± 0.3**	41.0 ± 0.3**	39.5 ± 0.5**
Hemoglobin (g/dL)						
Day 3	13.5 ± 0.2	13.9 ± 0.2	13.6 ± 0.2	13.5 ± 0.1	13.0 ± 0.1	14.0 ± 0.2
Day 23	15.3 ± 0.1	15.2 ± 0.2	14.5 ± 0.1**	13.7 ± 0.2**	13.4 ± 0.1**	13.9 ± 0.2**
Week 13	15.4 ± 0.1	14.2 ± 0.1**	13.6 ± 0.1**	13.7 ± 0.1**	13.7 ± 0.1**	12.3 ± 0.2**
Erythrocytes (10^6/μL)						
Day 3	7.06 ± 0.10	7.38 ± 0.12	7.22 ± 0.12	7.32 ± 0.11	7.03 ± 0.10	6.58 ± 0.08**
Day 23	8.15 ± 0.06	8.14 ± 0.10	7.75 ± 0.04**	7.27 ± 0.10**	6.30 ± 0.08**	5.67 ± 0.08**
Week 13	8.68 ± 0.06	7.77 ± 0.10**	7.41 ± 0.05**	7.00 ± 0.06**	6.36 ± 0.08**	4.87 ± 0.09**
Reticulocytes (10^6/μL)						
Day 3	0.48 ± 0.02	0.45 ± 0.02	0.46 ± 0.02	0.49 ± 0.02	0.55 ± 0.02*	0.69 ± 0.03**
Day 23	0.18 ± 0.01	0.21 ± 0.01	0.31 ± 0.02**	0.48 ± 0.02**	0.72 ± 0.03**	1.20 ± 0.04**
Week 13	0.17 ± 0.02	0.21 ± 0.01[2]	0.38 ± 0.02**	0.54 ± 0.03**	0.81 ± 0.07**	1.51 ± 0.07**
Nucleated erythrocytes (10^3/μL)						
Day 3	0.18 ± 0.03	0.14 ± 0.04	0.16 ± 0.04	0.15 ± 0.02	0.18 ± 0.04	0.81 ± 0.18**
Day 23	0.06 ± 0.02	0.09 ± 0.04	0.12 ± 0.05	0.25 ± 0.05**	1.13 ± 0.22**	1.97 ± 0.27**
Week 13	0.06 ± 0.03	0.18 ± 0.04*	0.29 ± 0.07**	0.82 ± 0.13**	1.29 ± 0.11**	4.96 ± 0.74**

TABLE B2 Hematology Data for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene (continued)

	0 ppm	1.5 ppm	3 ppm	6 ppm	12 ppm	24 ppm
FEMALE (continued)						
Mean cell volume (fL)						
Day 3	61.0 ± 0.3	58.9 ± 0.5*	59.2 ± 0.4*	58.5 ± 0.3**	59.7 ± 0.7	60.5 ± 0.2
Day 23	58.8 ± 0.3	58.2 ± 0.3	58.7 ± 0.4	58.9 ± 0.3	63.2 ± 0.6**	70.6 ± 0.3**
Week 13	55.9 ± 0.1	56.9 ± 0.3**	57.7 ± 0.2**	59.4 ± 0.2**	62.5 ± 0.4**	70.8 ± 0.4**
Mean cell hemoglobin (pg)						
Day 3	19.1 ± 0.1	18.8 ± 0.1	18.9 ± 0.1	18.5 ± 0.1*	18.5 ± 0.1*	21.3 ± 0.4
Day 23	18.8 ± 0.1	18.7 ± 0.1	18.7 ± 0.1	18.9 ± 0.1	21.2 ± 0.1**	24.4 ± 0.4**
Week 13	17.8 ± 0.1	18.2 ± 0.2*	18.3 ± 0.1**	19.5 ± 0.1**	21.5 ± 0.2**	25.3 ± 0.2**
Mean cell hemoglobin concentration (g/dL)						
Day 3	31.4 ± 0.1	31.9 ± 0.1**	31.9 ± 0.2**	31.6 ± 0.1*	31.0 ± 0.2	35.2 ± 0.7**
Day 23	31.9 ± 0.1	32.1 ± 0.1	31.9 ± 0.1	32.0 ± 0.2	33.6 ± 0.2**	34.7 ± 0.5**
Week 13	31.7 ± 0.1	32.0 ± 0.2	31.9 ± 0.1	32.9 ± 0.1**	34.4 ± 0.2**	35.8 ± 0.1**
Platelets (10^3/μL)						
Day 3	804.7 ± 47.4	722.8 ± 20.6	777.1 ± 27.2	754.4 ± 25.4	964.6 ± 48.7*	1166.9 ± 53.5**
Day 23	616.4 ± 7.1	606.3 ± 19.7	666.6 ± 12.3**	712.4 ± 24.5**	698.1 ± 13.8**	734.1 ± 30.5**
Week 13	536.5 ± 18.9	666.9 ± 30.8	783.7 ± 35.1	706.6 ± 32.5	563.6 ± 22.5	478.0 ± 24.3
Leukocytes (10^3/μL)						
Day 3	8.68 ± 0.67	8.31 ± 0.51	8.69 ± 0.39	8.64 ± 0.40	7.84 ± 0.22	9.61 ± 1.14
Day 23	7.26 ± 0.31	7.03 ± 0.38	8.68 ± 0.48*	9.14 ± 0.50**	11.72 ± 0.58**	12.15 ± 1.09**
Week 13	7.77 ± 0.40	8.73 ± 0.31	9.23 ± 0.51*	8.90 ± 0.59	9.66 ± 0.67*	19.63 ± 1.57**
Segmented neutrophils (10^3/μL)						
Day 3	0.88 ± 0.07	0.84 ± 0.12	0.84 ± 0.09	0.96 ± 0.12	0.90 ± 0.14	2.02 ± 0.32**
Day 23	0.86 ± 0.11	1.02 ± 0.17	1.28 ± 0.21	1.21 ± 0.16	1.32 ± 0.10*	1.26 ± 0.19*
Week 13	1.47 ± 0.16	1.51 ± 0.20	1.42 ± 0.11	1.23 ± 0.09	2.39 ± 0.28*	4.04 ± 0.28**
Lymphocytes (10^3/μL)						
Day 3	7.77 ± 0.64	7.43 ± 0.43	7.81 ± 0.37	7.65 ± 0.32	6.88 ± 0.18	7.57 ± 0.95
Day 23	6.34 ± 0.34	5.96 ± 0.29	7.38 ± 0.50	7.90 ± 0.40*	10.36 ± 0.57**	10.89 ± 1.04**
Week 13	6.19 ± 0.34	7.06 ± 0.23*	7.69 ± 0.52*	7.49 ± 0.56*	7.12 ± 0.55	15.19 ± 1.33**
Monocytes (10^3/μL)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 13	0.04 ± 0.02	0.07 ± 0.03	0.08 ± 0.04	0.11 ± 0.04	0.12 ± 0.04	0.30 ± 0.11*
Eosinophils (10^3/μL)						
Day 3	0.03 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.02 ± 0.01
Day 23	0.06 ± 0.03	0.05 ± 0.02	0.03 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.00 ± 0.00*
Week 13	0.07 ± 0.03	0.09 ± 0.03	0.04 ± 0.03	0.07 ± 0.03	0.03 ± 0.01	0.10 ± 0.04
Methemoglobin (g/dL)						
Day 3	0.07 ± 0.01	0.20 ± 0.01**	0.38 ± 0.02**	0.72 ± 0.04**	1.79 ± 0.09**	5.90 ± 0.43**
Day 23	0.14 ± 0.01	0.48 ± 0.02**	0.82 ± 0.02**	1.44 ± 0.06**	2.87 ± 0.17**	3.88 ± 0.25**
Week 13	0.16 ± 0.01	0.63 ± 0.03**	0.90 ± 0.03**	1.69 ± 0.04**	2.50 ± 0.09**	2.85 ± 0.07**

[1] Data are given as mean ± standard error.
[2] 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 B3 Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

	0 ppm	1.1 ppm	2.3 ppm	4.5 ppm	9 ppm	18 ppm
MALE						
n						
Day 4	10	10	10	10	10	10
Day 23	8	10	9	10	10	6
Week 13	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	14.6 ± 0.9	14.4 ± 0.4	14.4 ± 0.3	15.4 ± 0.5	16.4 ± 0.5	13.8 ± 0.8
Day 23	14.8 ± 0.6	13.7 ± 0.5	14.6 ± 0.8	13.6 ± 0.3	15.1 ± 0.4	16.5 ± 0.6
Week 13	19.6 ± 0.8	20.4 ± 0.6	19.9 ± 0.7	19.3 ± 0.6	19.1 ± 0.6	19.8 ± 0.8
Creatinine (mg/dL)						
Day 4	0.63 ± 0.02	0.56 ± 0.02	0.55 ± 0.02	0.56 ± 0.02	0.61 ± 0.02	0.68 ± 0.01
Day 23	0.54 ± 0.02	0.54 ± 0.03	0.57 ± 0.02	0.53 ± 0.02	0.59 ± 0.03	0.57 ± 0.03
Week 13	0.62 ± 0.01	0.62 ± 0.01	0.64 ± 0.02	0.65 ± 0.02	0.64 ± 0.02	0.64 ± 0.02
Total protein (g/dL)						
Day 4	5.5 ± 0.1	5.7 ± 0.1	5.8 ± 0.1	5.7 ± 0.0	5.7 ± 0.1	5.2 ± 0.1
Day 23	6.16 ± 0.07	6.12 ± 0.06	6.38 ± 0.05*	6.35 ± 0.06	6.64 ± 0.09**	6.65 ± 0.10**
Week 13	7.0 ± 0.1	7.2 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.3 ± 0.1**	7.5 ± 0.1**
Albumin (g/dL)						
Day 4	3.3 ± 0.0	3.4 ± 0.0	3.4 ± 0.1	3.4 ± 0.0	3.5 ± 0.1	3.3 ± 0.1
Day 23	3.6 ± 0.1	3.7 ± 0.0	3.8 ± 0.0*	3.8 ± 0.1*	3.9 ± 0.1**	3.9 ± 0.1**
Week 13	4.0 ± 0.0	4.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.3 ± 0.1**	4.3 ± 0.0**
Globulin (g/dL)						
Day 4	2.2 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.0	2.3 ± 0.1	2.0 ± 0.1
Day 23	2.5 ± 0.1	2.4 ± 0.1	2.6 ± 0.1	2.6 ± 0.1	2.7 ± 0.1	2.7 ± 0.1
Week 13	3.0 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	3.1 ± 0.1	3.0 ± 0.1	3.2 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	44 ± 1	48 ± 1	50 ± 2*	53 ± 2**	57 ± 3**	212 ± 19**
Day 23	35 ± 1	33 ± 1	36 ± 1	33 ± 1	39 ± 2	47 ± 3**
Week 13	62 ± 5	57 ± 3	60 ± 4	54 ± 3	49 ± 1*	55 ± 2
Alkaline phosphatase (IU/L)						
Day 4	888 ± 26	897 ± 33	838 ± 21	815 ± 24	847 ± 24	1045 ± 38
Day 23	554 ± 16	592 ± 21	543 ± 25	512 ± 18	482 ± 18*	498 ± 14
Week 13	343 ± 8	338 ± 6	323 ± 12	298 ± 10**	294 ± 9**	298 ± 10**
Creatine kinase (IU/L)						
Day 4	456 ± 35	384 ± 24[2]	322 ± 18**	419 ± 18	380 ± 40*	327 ± 21**
Day 23	295 ± 22	227 ± 19	329 ± 52	318 ± 38	303 ± 33[2]	308 ± 40
Week 13	124 ± 16	128 ± 12	99 ± 15	168 ± 38	106 ± 18	117 ± 15[2]
Sorbitol dehydrogenase (IU/L)						
Day 4	10 ± 1	12 ± 0**	14 ± 1**	15 ± 1**	16 ± 1**	34 ± 4**
Day 23	9 ± 0	9 ± 0	10 ± 0	10 ± 0	14 ± 1**	16 ± 1**
Week 13	20 ± 2	20 ± 2	21 ± 3	21 ± 3	22 ± 1	28 ± 2**
Bile acids (μmol/L)						
Day 4	18.97 ± 0.85	24.14 ± 2.00	22.66 ± 1.49*	24.70 ± 0.93**	27.22 ± 1.30**	75.41 ± 5.82**[2]
Day 23	17.00 ± 1.26	18.88 ± 1.57	20.52 ± 1.06	18.68 ± 0.79	21.87 ± 2.56	13.92 ± 1.09[3]
Week 13	19.04 ± 0.53	21.95 ± 1.36	22.03 ± 0.69*[2]	22.76 ± 1.84	22.34 ± 0.80*	20.45 ± 0.81

TABLE B3 Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene (continued)

	0 ppm	1.1 ppm	2.3 ppm	4.5 ppm	9 ppm	18 ppm
FEMALE						
n						
Day 4	10	10	10	10	10	10
Day 23	9	10	10	10	10	8
Week 13	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	18.0 ± 0.6	16.9 ± 0.5	16.7 ± 0.6	16.8 ± 0.5	17.9 ± 0.5	14.8 ± 1.0
Day 23	15.8 ± 0.7	16.0 ± 0.6	16.5 ± 0.8	16.8 ± 0.6	17.1 ± 0.4	17.6 ± 0.5*
Week 13	21.3 ± 0.9	20.2 ± 0.5	19.8 ± 0.6	20.4 ± 0.7	19.8 ± 0.7	19.4 ± 0.7
Creatinine (mg/dL)						
Day 4	0.58 ± 0.01	0.57 ± 0.02	0.58 ± 0.01	0.59 ± 0.02	0.59 ± 0.01	0.58 ± 0.03
Day 23	0.50 ± 0.02	0.50 ± 0.02	0.51 ± 0.03	0.49 ± 0.02	0.55 ± 0.02	0.59 ± 0.02*
Week 13	0.63 ± 0.02	0.61 ± 0.02	0.62 ± 0.01	0.60 ± 0.02	0.58 ± 0.01	0.61 ± 0.03
Total protein (g/dL)						
Day 4	5.6 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	5.2 ± 0.1
Day 23	6.1 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.5 ± 0.1**	6.5 ± 0.1**
Week 13	7.4 ± 0.1	7.3 ± 0.1	7.4 ± 0.1	7.5 ± 0.1	7.7 ± 0.1	7.8 ± 0.1*
Albumin (g/dL)						
Day 4	3.6 ± 0.1	3.7 ± 0.0	3.8 ± 0.0	3.8 ± 0.1*	3.9 ± 0.1**	3.5 ± 0.1
Day 23	3.7 ± 0.0	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	4.0 ± 0.0**	4.0 ± 0.0**
Week 13	4.5 ± 0.0	4.5 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.9 ± 0.0**	4.8 ± 0.1**
Globulin (g/dL)						
Day 4	2.0 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.0 ± 0.1	1.8 ± 0.1
Day 23	2.4 ± 0.1	2.5 ± 0.1	2.4 ± 0.0	2.4 ± 0.0	2.5 ± 0.1	2.5 ± 0.1
Week 13	2.9 ± 0.1	2.8 ± 0.1	2.8 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	2.9 ± 0.1
Alanine aminotransferase (IU/L)						
Day 4	41 ± 2	40 ± 1	38 ± 1	35 ± 1*	38 ± 1	137 ± 20
Day 23	33 ± 1	33 ± 1	36 ± 1	36 ± 4	35 ± 1	45 ± 3**
Week 13	58 ± 4	50 ± 2	58 ± 3	53 ± 2	47 ± 2*	41 ± 2**
Alkaline phosphatase (IU/L)						
Day 4	736 ± 25	731 ± 16	725 ± 17	700 ± 25	675 ± 30	838 ± 81
Day 23	459 ± 16	435 ± 18	444 ± 13	415 ± 20*	379 ± 11**	342 ± 15**[4]
Week 13	309 ± 11	310 ± 16	294 ± 12	274 ± 14	278 ± 16	242 ± 14**
Creatine kinase (IU/L)						
Day 4	298 ± 38[2]	282 ± 27	313 ± 30	287 ± 20	282 ± 30	224 ± 25
Day 23	320 ± 47	271 ± 44	288 ± 35	243 ± 30	240 ± 31	280 ± 34
Week 13	103 ± 11	112 ± 18[2]	120 ± 16	114 ± 13[2]	98 ± 7	127 ± 23
Sorbitol dehydrogenase (IU/L)						
Day 4	9 ± 1	9 ± 0	9 ± 1	9 ± 0	12 ± 1**	20 ± 1**
Day 23	11 ± 1	12 ± 1	12 ± 1	11 ± 1[2]	14 ± 1**	23 ± 2**
Week 13	19 ± 1	18 ± 1	23 ± 1	22 ± 1	24 ± 1*	26 ± 2**
Bile acids (μmol/L)						
Day 4	19.25 ± 1.10	19.95 ± 1.15	17.74 ± 0.68	20.41 ± 1.06	21.92 ± 0.94	34.71 ± 3.39**
Day 23	16.44 ± 1.07	12.87 ± 0.57	18.38 ± 3.42	18.56 ± 2.83	16.34 ± 0.55	17.55 ± 0.41
Week 13	24.10 ± 2.64	20.65 ± 2.01	20.55 ± 1.82	19.20 ± 0.94	19.93 ± 1.68	20.50 ± 1.72

[1] Data are given as mean ± standard error.
[2] n=9.
[3] n=5.
[4] n=7.
* 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 B4 Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

	0 ppm	1.5 ppm	3 ppm	6 ppm	12 ppm	24 ppm
MALE						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	15.0 ± 0.6	12.5 ± 0.3*	12.5 ± 1.0**	13.9 ± 0.5	14.2 ± 0.4	14.7 ± 0.5
Day 23	14.4 ± 0.3	12.9 ± 0.5	13.9 ± 0.5	13.0 ± 0.9	13.3 ± 0.6	13.9 ± 0.7
Week 13	17.0 ± 1.5	19.2 ± 1.2	19.9 ± 0.8	19.2 ± 1.2	20.2 ± 0.7	20.1 ± 0.7
Creatinine (mg/dL)						
Day 3	0.50 ± 0.02	0.58 ± 0.02	0.52 ± 0.02	0.58 ± 0.03	0.53 ± 0.02	0.55 ± 0.03
Day 23	0.55 ± 0.02	0.53 ± 0.02	0.59 ± 0.02	0.60 ± 0.03	0.61 ± 0.02	0.68 ± 0.03**
Week 13	0.66 ± 0.02	0.67 ± 0.02	0.68 ± 0.01	0.70 ± 0.02	0.69 ± 0.02	0.74 ± 0.02**
Total protein (g/dL)						
Day 3	5.5 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.7 ± 0.1	5.4 ± 0.1	5.6 ± 0.1
Day 23	6.43 ± 0.04	6.44 ± 0.05	6.28 ± 0.06	6.24 ± 0.08*	6.19 ± 0.06**	6.15 ± 0.08**
Week 13	7.1 ± 0.1	7.1 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	6.7 ± 0.1**	6.6 ± 0.1**
Albumin (g/dL)						
Day 3	3.4 ± 0.1	3.5 ± 0.1	3.5 ± 0.0	3.6 ± 0.1**	3.4 ± 0.1	3.4 ± 0.0
Day 23	3.7 ± 0.0	3.7 ± 0.0	3.7 ± 0.1	3.6 ± 0.0	3.6 ± 0.1	3.7 ± 0.0
Week 13	4.1 ± 0.0	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.1
Globulin (g/dL)						
Day 3	2.1 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.1 ± 0.1	2.0 ± 0.1	2.2 ± 0.1
Day 23	2.8 ± 0.0	2.8 ± 0.0	2.6 ± 0.1	2.6 ± 0.1	2.6 ± 0.0*	2.5 ± 0.1**
Week 13	3.1 ± 0.1	3.1 ± 0.0	3.1 ± 0.1	3.0 ± 0.1	2.8 ± 0.0**	2.6 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 3	44 ± 1	43 ± 1	43 ± 1	47 ± 2	46 ± 1	42 ± 1
Day 23	36.1 ± 0.6	33.4 ± 0.8*	32.9 ± 1.0*	32.6 ± 1.3**	31.7 ± 0.8**	30.6 ± 0.8**
Week 13	58 ± 5	63 ± 5	56 ± 3	54 ± 5	48 ± 1	36 ± 2**
Alkaline phosphatase (IU/L)						
Day 3	935 ± 17	905 ± 19	984 ± 73	962 ± 57	862 ± 24*[2]	792 ± 18**
Day 23	552 ± 14	541 ± 18	566 ± 32	483 ± 50	485 ± 15**	470 ± 13**
Week 13	411 ± 18	365 ± 12*	371 ± 11	373 ± 13	355 ± 9**	293 ± 17**
Creatine kinase (IU/L)						
Day 3	274 ± 14	291 ± 13	270 ± 22[2]	369 ± 52[2]	297 ± 15	216 ± 20
Day 23	229 ± 26	220 ± 14	247 ± 17	279 ± 26	228 ± 18	217 ± 33
Week 13	95 ± 14	124 ± 14	137 ± 19	103 ± 12	117 ± 13	95 ± 11
Sorbitol dehydrogenase (IU/L)						
Day 3	15 ± 0	16 ± 0	14 ± 1	17 ± 0**	20 ± 0**	21 ± 1**
Day 23	14.7 ± 0.7	14.0 ± 0.5	14.4 ± 0.4	15.7 ± 0.6	17.5 ± 1.2	17.6 ± 1.0*
Week 13	24 ± 3	26 ± 3	23 ± 1	25 ± 2	23 ± 1	22 ± 2
Bile acids (µmol/L)						
Day 3	17.70 ± 0.62	19.70 ± 1.16	22.40 ± 1.39*	26.44 ± 2.09**[2]	30.90 ± 1.25**	24.80 ± 0.90**
Day 23	20.30 ± 1.03	20.80 ± 0.73	21.11 ± 1.06[2]	23.80 ± 1.15*	33.70 ± 2.60**	43.50 ± 4.18**
Week 13	23.20 ± 3.09	26.30 ± 2.21	27.30 ± 2.15*	26.20 ± 1.61*	29.70 ± 1.87**	35.00 ± 3.90**

TABLE B4 Clinical Chemistry Data for F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene (continued)

	0 ppm	1.5 ppm	3 ppm	6 ppm	12 ppm	24 ppm
FEMALE						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	10	10	10	8
Week 13	10	10	9	10	10	10
Urea nitrogen (mg/dL)						
Day 3	16.1 ± 1.0	13.5 ± 0.7	14.1 ± 0.5	14.0 ± 0.7	14.8 ± 0.5	13.6 ± 0.4
Day 23	15.1 ± 0.5	14.2 ± 0.6	15.8 ± 0.7	14.2 ± 0.8	14.1 ± 0.5	13.0 ± 0.5*
Week 13	19.9 ± 0.8	20.5 ± 0.8	20.4 ± 0.8	19.6 ± 0.6	19.2 ± 0.8	16.9 ± 0.7**
Creatinine (mg/dL)						
Day 3	0.51 ± 0.02	0.54 ± 0.02	0.50 ± 0.02	0.53 ± 0.02	0.55 ± 0.02	0.60 ± 0.03*
Day 23	0.58 ± 0.02	0.52 ± 0.01	0.56 ± 0.03	0.59 ± 0.01	0.64 ± 0.02	0.68 ± 0.03*
Week 13	0.68 ± 0.03	0.69 ± 0.02	0.71 ± 0.02	0.67 ± 0.03	0.73 ± 0.03	0.73 ± 0.03
Total protein (g/dL)						
Day 3	5.6 ± 0.1	6.0 ± 0.1	5.8 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.7 ± 0.0
Day 23	6.3 ± 0.1	6.2 ± 0.1	6.3 ± 0.1	6.3 ± 0.1	6.2 ± 0.1	6.0 ± 0.1*
Week 13	7.36 ± 0.09	7.18 ± 0.09	7.32 ± 0.12	7.11 ± 0.10	7.09 ± 0.07*	6.84 ± 0.12**
Albumin (g/dL)						
Day 3	3.5 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.8 ± 0.0**	3.6 ± 0.1	3.5 ± 0.1
Day 23	3.8 ± 0.0	3.9 ± 0.1	4.0 ± 0.0	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1
Week 13	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1
Globulin (g/dL)						
Day 3	2.2 ± 0.1	2.3 ± 0.1	2.1 ± 0.1	2.0 ± 0.0**	2.0 ± 0.1*	2.1 ± 0.0
Day 23	2.5 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.1 ± 0.1*
Week 13	3.1 ± 0.1	2.9 ± 0.1	3.0 ± 0.1	2.8 ± 0.1**	2.8 ± 0.1**	2.6 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 3	39 ± 2	41 ± 1	40 ± 1	40 ± 2	38 ± 1	40 ± 2
Day 23	31 ± 1	34 ± 1	32 ± 1	31 ± 1	31 ± 1	33 ± 1
Week 13	46 ± 2	46 ± 2	49 ± 4	44 ± 1	39 ± 2*	36 ± 2**
Alkaline phosphatase (IU/L)						
Day 3	861 ± 67	820 ± 69	736 ± 20	688 ± 29**	675 ± 19**	642 ± 23**
Day 23	493 ± 51	473 ± 40	389 ± 13*	388 ± 11**	364 ± 12**	360 ± 12**
Week 13	404 ± 9	377 ± 11	358 ± 16*	342 ± 15**	335 ± 14**	265 ± 14**
Creatine kinase (IU/L)						
Day 3	279 ± 21	339 ± 49	349 ± 40	284 ± 47	247 ± 15	252 ± 47
Day 23	224 ± 23	225 ± 29	207 ± 14	174 ± 21	242 ± 21	228 ± 32
Week 13	148 ± 23	132 ± 15	132 ± 26	191 ± 56	136 ± 22	164 ± 32
Sorbitol dehydrogenase (IU/L)						
Day 3	15 ± 0	16 ± 1	16 ± 1	17 ± 0**	19 ± 0**	23 ± 1**
Day 23	20 ± 1	19 ± 1	19 ± 1	21 ± 1	23 ± 1**	26 ± 1**
Week 13	19 ± 1	20 ± 1	21 ± 1	21 ± 1	22 ± 1	23 ± 2
Bile acids (μmol/L)						
Day 3	15.80 ± 1.04	24.30 ± 3.16**	17.90 ± 1.09	22.30 ± 2.79*	23.40 ± 1.29**	24.11 ± 3.35**[2]
Day 23	19.90 ± 1.93	20.60 ± 2.44	18.80 ± 0.76	20.10 ± 1.03	23.90 ± 1.11*	27.75 ± 1.95**
Week 13	22.20 ± 3.84	25.90 ± 3.37	23.11 ± 2.90	34.20 ± 6.78	25.50 ± 3.22	29.60 ± 3.62

[1] Data are given as mean ± standard error.
[2] 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.

APPENDIX C

Reproductive Tissue Evaluations and Estrous Cycle Characterization

TABLE C1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

Study Parameters	0 ppm	4.5 ppm	9 ppm	18 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	334 ± 7	349 ± 8	340 ± 7	323 ± 6
Left epididymis	0.257 ± 0.008[2]	0.254 ± 0.007	0.258 ± 0.003	0.245 ± 0.006
Left cauda epididymis	0.167 ± 0.003[2]	0.168 ± 0.006	0.162 ± 0.004	0.150 ± 0.003**
Left testis	1.46 ± 0.06	1.44 ± 0.02	1.45 ± 0.03	1.36 ± 0.02
Spermatid measurements				
Spermatid heads (10^7/g testis)	12.39 ± 0.70	11.89 ± 0.39	12.10 ± 0.45	11.34 ± 0.47
Spermatid heads (10^7/testis)	17.79 ± 0.73	17.09 ± 0.56	17.47 ± 0.36	15.44 ± 0.71*
Spermatid count (mean/10^{-4} mL suspension)	88.93 ± 3.66	85.43 ± 2.79	87.33 ± 1.82	77.20 ± 3.57*
Spermatozoal measurements				
Motility (%)	92.53 ± 1.28	92.59 ± 1.93	93.86 ± 0.62	94.20 ± 0.66
Concentration (10^6/g cauda epididymal tissue)	620 ± 33[2]	646 ± 28	595 ± 14	623 ± 44

[1] Data are presented as mean ± standard error. Differences from the control group for necropsy body weight are not significant by Dunnett's test. Differences from the control group for epididymal and testis weights, spermatid heads/g testis, and spermatozoal measurements are not significant by Dunn's or Shirley's test.
[2] n=9.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE C2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

Study Parameters	0 ppm	4.5 ppm	9 ppm	18 ppm
n	10	10	10	10
Necropsy body weight	191 ± 3	193 ± 3	196 ± 5	193 ± 4
Estrous cycle length (days)	5.00 ± 0.07	4.95 ± 0.09	4.90 ± 0.07	4.75 ± 0.13
Estrous stages (% of cycle)				
Diestrus	43.3	40.8	40.0	41.7
Proestrus	15.8	15.0	15.8	16.7
Estrus	22.5	24.2	26.7	23.3
Metestrus	18.3	20.0	17.5	18.3

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for necropsy body weight are not significant by Dunnett's test. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.

TABLE C3 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

Study Parameters	0 ppm	6 ppm	12 ppm	24 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	342 ± 4	348 ± 6	337 ± 5	346 ± 7
Left epididymis	0.263 ± 0.003	0.274 ± 0.006	0.259 ± 0.006	0.211 ± 0.011**
Left cauda epididymis	0.164 ± 0.005	0.166 ± 0.006	0.161 ± 0.005	0.126 ± 0.006**
Left testis	1.44 ± 0.02	1.42 ± 0.03	1.38 ± 0.03	1.07 ± 0.07**
Spermatid measurements				
Spermatid heads (10^7/g testis)	10.22 ± 0.34	11.36 ± 0.39	11.33 ± 0.68	9.16 ± 0.80
Spermatid heads (10^7/testis)	14.78 ± 0.61	16.13 ± 0.55	15.65 ± 1.01	10.03 ± 1.27*
Spermatid count (mean/10^{-4} mL suspension)	73.90 ± 3.05	80.65 ± 2.73	78.23 ± 5.06	50.15 ± 6.36*
Spermatozoal measurements				
Motility (%)	97.45 ± 0.90	98.23 ± 0.36	98.48 ± 0.34	57.99 ± 15.75
Concentration (10^6/g cauda epididymal tissue)	933 ± 67	830 ± 58	754 ± 58	517 ± 51**

[1] Data are presented as mean ± standard error. Differences from the control group for necropsy body weight are not significant by Dunnett's test. Differences from the control group for spermatid heads/g testis and spermatozoal motility are not significant by Dunn's test.

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

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

TABLE C4 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

Study Parameters	0 ppm	6 ppm	12 ppm	24 ppm[2]
n	10	10	10	10
Necropsy body weight	192 ± 5	196 ± 4	197 ± 6	200 ± 2
Estrous cycle length (days)	4.95 ± 0.05	4.45 ± 0.16*	4.50 ± 0.31*	4.05 ± 0.05**
Estrous stages (% of cycle)				
Diestrus	43.3	41.7	36.7	33.3
Proestrus	15.8	13.3	17.5	15.8
Estrus	20.8	23.3	24.2	25.8
Metestrus	20.0	21.7	21.7	25.0

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for necropsy body weight are not significant by Dunnett's test.

[2] Evidence suggests that females in the 24 ppm group differ significantly (P<0.01, Wilks' Criterion) from the control females in the relative length of time spent in the estrous stages. Females in this exposure group spent more time in estrus and metestrus and less time in diestrus than control females.

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

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

TABLE C5 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

Study Parameters	0 ppm	4.5 ppm	9 ppm	18 ppm
n	10	10	10	8
Weights (g)				
Necropsy body weight	36.7 ± 0.7	34.9 ± 0.8	36.9 ± 1.0	35.8 ± 1.0
Left epididymis	0.024 ± 0.001	0.024 ± 0.001	0.026 ± 0.000	0.024 ± 0.001
Left cauda epididymis	0.014 ± 0.001	0.015 ± 0.001	0.015 ± 0.001	0.013 ± 0.001
Left testis	0.117 ± 0.003	0.120 ± 0.002	0.124 ± 0.002	0.118 ± 0.002
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.34 ± 0.83	19.02 ± 0.97	19.16 ± 1.01	20.19 ± 1.03
Spermatid heads (10^7/testis)	2.26 ± 0.10	2.26 ± 0.10	2.36 ± 0.12	2.39 ± 0.13
Spermatid count (mean/10^{-4} mL suspension)	70.48 ± 3.12	70.63 ± 2.99	73.75 ± 3.74	74.69 ± 4.05
Spermatozoal measurements				
Motility (%)	90.42 ± 0.73	87.84 ± 1.13*	81.07 ± 2.71**	81.99 ± 2.78**
Concentration (10^6/g cauda epididymal tissue)	1511 ± 142	1366 ± 93	1211 ± 131	1436 ± 118

[1] Data are presented as mean ± standard error. Differences from the control group for necropsy body weight are not significant by Dunnett's test. Differences from the control group for epididymal, cauda epididymal, and testis weights, spermatid measurements, and spermatozoal concentration are not significant by Dunn's test.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE C6 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Inhalation Study of 2-Chloronitrobenzene[1]

Study Parameters	0 ppm	4.5 ppm	9 ppm	18 ppm
n	9	10	10	10
Necropsy body weight	30.1 ± 0.8[2]	33.0 ± 1.0*	34.7 ± 1.2**	33.9 ± 1.3**
Estrous cycle length (days)	4.22 ± 0.17	4.45 ± 0.17	4.55 ± 0.16	5.00 ± 0.57
Estrous stages (% of cycle)				
Diestrus	44.2	35.8	30.0	36.7
Proestrus	15.0	20.8	19.2	18.3
Estrus	27.5	28.3	31.7	30.8
Metestrus	13.3	15.0	19.2	14.2

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
[2] n=10.
* Significantly different (P≤0.05) from the control group by Williams' test.
** Significantly different (P≤0.01) from the control group by Williams' test.

TABLE C7 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

Study Parameters	0 ppm	6 ppm	12 ppm	24 ppm
n	10	9	10	10
Weights (g)				
Necropsy body weight	34.9 ± 0.8	35.3 ± 1.4	35.8 ± 0.5	36.8 ± 0.7
Left epididymis	0.028 ± 0.001	0.028 ± 0.001	0.030 ± 0.002	0.028 ± 0.001
Left cauda epididymis	0.017 ± 0.001	0.017 ± 0.001	0.017 ± 0.001	0.017 ± 0.001
Left testis	0.114 ± 0.003	0.117 ± 0.002	0.115 ± 0.002	0.114 ± 0.002
Spermatid measurements				
Spermatid heads (10^7/g testis)	20.61 ± 1.74	17.10 ± 1.20	17.61 ± 1.11	18.92 ± 1.21
Spermatid heads (10^7/testis)	2.31 ± 0.15	2.01 ± 0.16	2.02 ± 0.13	2.15 ± 0.14
Spermatid count (mean/10^{-4} mL suspension)	72.10 ± 4.79	62.86 ± 5.10	63.05 ± 4.22	67.10 ± 4.33
Spermatozoal measurements				
Motility (%)	93.25 ± 0.80	85.91 ± 5.81	92.20 ± 0.95	89.83 ± 1.55
Concentration (10^6/g cauda epididymal tissue)	1471 ± 162	1480 ± 185	1639 ± 150	1445 ± 116

[1] Data are presented as mean ± standard error. Differences from the control group for necropsy body weight are not significant by Dunnett's test. Differences from the control group for epididymal, cauda epididymal, and testis weights and spermatid and spermatozoal measurements are not significant by Dunn's test.

TABLE C8 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Inhalation Study of 4-Chloronitrobenzene[1]

Study Parameters	0 ppm	6 ppm[2]	12 ppm	24 ppm
n	10	9	10	10
Necropsy body weight	31.5 ± 0.9	31.1 ± 0.8[3]	33.1 ± 0.8	33.0 ± 0.4
Estrous cycle length (days)	4.15 ± 0.11	4.22 ± 0.15	4.25 ± 0.13	4.65 ± 0.15*
Estrous stages (% of cycle)				
Diestrus	36.7	32.5	30.0	30.0
Proestrus	20.0	21.7	23.3	19.2
Estrus	27.5	25.8	25.8	32.5
Metestrus	15.8	20.0	20.8	18.3

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for necropsy body weight are not significant by Dunnett's test. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
[2] Estrous cycle longer than 12 days or unclear in 1 of 10 mice.
[3] n=10.
* Significantly different (P≤0.05) from the control group by Shirley's test.

APPENDIX D

Continuous Breeding Studies

Final Report on the Reproductive Toxicity of 2-Chloronitrobenzene (CAS No. 88-73-3) in CD-1 Swiss Mice II
(Summary)

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Final Report on the Reproductive Toxicity of a 4-Chloronitrobenzene (CAS No. 100-00-5) in CD-1-Swiss Mice
(Summary)

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CONTINUOUS BREEDING STUDIES

Introduction

Because of the observed effects of exposure to chloronitrobenzenes on spermatid counts, sperm motility, and estrous cycle length in the 13-week inhalation studies, the effects of 2-chloronitrobenzene and 4-chloronitrobenzene on reproduction were assessed by the performance of continuous breeding studies in CD-1 Swiss mice given 2- or 4-chloronitrobenzene in corn oil by gavage. Reproductive assessment consists of four phases: dose finding, continuous breeding, identification of the affected sex, and offspring assessment.

The 2-week dose-finding phase is conducted to determine doses for the continuous breeding phase. During the continuous breeding phase, the effects of the maximum tolerated dose estimated in the 2-week studies and the two lower doses on fertility and reproduction are determined. If fertility is significantly affected during the continuous breeding phase, crossover mating trials are performed to determine if males, females, or both sexes are affected. Offspring assessment includes evaluation of reproductive performance of second-generation (F_1) mice from the final litters of the continuous breeding phase. Offspring assessment of mice from all dose groups is conducted when the fertility of first-generation (F_0) mice is significantly affected; otherwise, only offspring from control and high-dose breeding pairs are evaluated.

In the 2-chloronitrobenzene and 4-chloronitrobenzene studies, no significant effects on fertility were observed; therefore, no crossover mating trials were performed, and only pups from control and high-dose breeding pairs were maintained for offspring assessment. Complete results of these studies are available (NTP, 1991, 1992a).

Materials and Methods

CONTINUOUS BREEDING STUDIES

2-Chloronitrobenzene (Lot ET 00210KM) and 4-chloronitrobenzene (Lot ET 02513BT) were obtained from Aldrich Chemical Company (Milwaukee, WI). Gas chromatography indicated a purity greater than 99% for bulk 2-chloronitrobenzene and a purity of approximately 99% for 4-chloronitrobenzene. Stability studies indicated that 2-chloronitrobenzene in corn oil (2.5 mg/mL) is stable for up to 4 weeks and 4-chloronitrobenzene in corn oil (0.03 mg/mL) is stable for 3 weeks when stored in the dark at temperatures up to 60° C. The corn oil from each study contained less than 10 meq/kg peroxides.

Male and female VAF Crl:CD-1 (ICR)BR outbred albino mice used in the 2-chloronitrobenzene and 4-chloronitrobenzene continuous breeding studies were obtained from Charles River Breeding Laboratories (Kingston, NY, for 2-week study mice and Portage, MI, for continuous breeding study mice). Mice used in the 2-chloronitrobenzene studies and the 4-chloronitrobenzene continuous breeding study were 9 weeks old at receipt; mice used in the 2-week 4-chloronitrobenzene study were 6 weeks old at receipt. All mice were quarantined for 2 weeks before the start of the studies. Blood samples were collected periodically from sentinel mice and were analyzed for antibody titers to rodent viruses; all results from 2-chloronitrobenzene study mice were negative. All sera from mice in the 4-chloronitrobenzene study showed an antibody response to Minute Virus of Mice; however, no clinical signs of disease were detected in the study mice.

For the 2-week dose-setting studies, groups of eight mice per sex received 0, 20, 40, 80, 160, or 320 mg/kg 2-chloronitrobenzene or 0, 40, 80, 160, 320, or 640 mg/kg 4-chloronitrobenzene in corn oil by gavage. The doses for the continuous breeding phase were based on clinical signs, body weights, and water consumption data for mice in the 2-week studies. For the continuous breeding studies, groups of 20 breeding pairs received 40, 80, or 160 mg/kg 2-chloronitrobenzene or 62.5, 125, or 250 mg/kg 4-chloronitrobenzene in corn oil by gavage. For each of the 2-chloronitrobenzene and 4-chloronitrobenzene continuous breeding studies, 40 breeding pairs received the corn oil vehicle only. After the beginning of dosing, the mice were housed separately for 7 days and then were housed in breeding pairs for 98 days while being dosed; deionized water and NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form were available *ad libitum*. Body weights, water consumption, fertility, number of litters per pair, number of live pups per litter, proportion of pups born alive, sex ratio of live pups, and pup body weights were recorded. Spleen weights were recorded and blood samples were taken for determination of methemoglobin for 23 control and 21 high-dose F_0 mice from the 2-chloronitrobenzene study.

Following the continuous breeding period of the F_0 mice, the final litter of pups born to each control or high-dose breeding pair in the 5-week holding period was reared. Siblings were housed two per cage by sex and received the same doses as the F_0 mice. After weaning, 20 nonsibling F_1 mice of each sex were cohabited for 7 days and then housed singly through delivery of pups. Body weights, water consumption, fertility, number of litters per pair, number of live pups per litter, proportion of pups born alive, sex ratio of live pups, and pup body weights were recorded and mice were examined for the presence of a copulatory plug. At the end of the study, F_1 mice were necropsied; organ weights (liver, kidneys, testes, epididymides, prostate, seminal vesicles, and ovaries) and body weights were determined, sperm morphology and vaginal cytology evaluations were made for 12 days prior to necropsy, and blood samples were collected for methemoglobin analysis. Selected organs were fixed in 10% neutral buffered formalin. Ovaries were fixed in Bouin's fixative and testes and epididymides were fixed in paraformaldehyde. Testes and epididymides from five control and five high-dose males were embedded in glycol methacrylate, and sections were stained with PAS and hematoxylin.

STATISTICAL METHODS

For data expressed as proportions (fertility, mating, and pregnancy indices), the Cochran-Armitage test (Armitage, 1971) was used to test for dose-related trends. Each dose group was compared to the control group with a chi-square test (Conover, 1971). The number of litters and the number of live pups per litter were determined per fertile pair and then treatment group means were determined. The proportion of live pups was defined as the number of pups born alive divided by the total number of pups produced by each pair. The sex ratio was expressed as the number of male pups born alive divided by the total number of live pups born to each fertile pair. Dose group means for data with skewed distributions were analyzed using the nonparametric multiple comparisons methods of Shirley (1977) or 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 (Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunn). For offspring assessment data, Wilcoxon's test (Conover, 1971) was used, because a control group and only one dose group were tested. For vaginal cytology data, 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 simultaneous equality of measurements across dose levels.

Results

2-CHLORONITROBENZENE

2-Week Dose-Finding Study

All mice in the 320 mg/kg (high-dose) group died or were sacrificed moribund during the first 2 days of the study. Two control mice and one mouse in each of the 20, 40, and 80 mg/kg groups also died; these deaths were attributed to gavage trauma. Final mean body weights of all groups of mice surviving to the end of the study were similar to those of the controls. Water consumption by females in the 20 and 160 mg/kg groups during Week 1 and by males and females in the 40 mg/kg groups during Week 2 was significantly increased. Mice in the 160 mg/kg groups appeared weak and inactive following dosing during the first week of the study and were slightly cyanotic but active following dosing during the second week.

Continuous Breeding Study

One male in the 80 mg/kg group and three high-dose males died before the cohabitation period. During the continuous breeding period, two control females died (one due to uterine hemorrhage during delivery and one with cause of death undetermined). One male and one female in the 40 mg/kg group and one female in the 80 mg/kg group also died during the continuous breeding period. The final mean body weights of high-dose males and of females in the 80 mg/kg group were notably greater than those of the controls at 14 weeks (the end of the cohabitation period). Water consumption of dosed animals was generally similar to that of the controls. High-dose mice were inactive immediately following dosing during the first 10 days of the study; there were no other clinical signs of toxicity.

All dosed pairs were fertile, compared to 36 of 38 fertile control pairs (Table D1). The average number of litters per pair and cumulative days to litter for dosed pairs were similar to those of the controls. The mean body weights of dams at delivery were generally similar to the mean body weight of the controls for each litter; the mean body weight of high-dose dams was slightly greater than that of the controls during lactation, and the difference was significant at postnatal Days 0 and 21 (Table D1). The average number of live pups per litter and the average live pups per breeding pair were greater for the high-dose breeding pairs than for the controls (Table D2). The sex ratios and the pup weights for dosed breeding pairs were generally similar to those of the controls.

For the final litter of F_1 pups, the weights of male and female pups from breeding pairs in the 80 and 160 mg/kg groups and female pups from breeding pairs in the 40 mg/kg group were significantly less than those of control pups (Table D3). The survival of pups from high-dose breeding pairs was slightly

higher than that of the controls, and the difference was significant at Day 7. There were no clinical signs of toxicity.

At mating, the mean body weight of dosed F_1 mice was significantly greater than that of the control group; however, the mean body weight of dosed dams was similar to that of control dams at delivery (Table D4). Water consumption of dosed and control mice was similar. Mating, pregnancy, and fertility indices and average days to litter for dosed breeding pairs were similar to those of the controls (Table D4). There were no significant differences in survival, sex ratios, or pup weights between pups of dosed dams and control pups (Table D4), and there were no clinical signs of toxicity in F_1 mice or F_2 pups.

The absolute and relative spleen weights of F_0 mice receiving 160 mg/kg 2-chloronitrobenzene were increased by as much as 50% over those of the controls. Absolute and relative liver and spleen weights of dosed female F_1 mice were significantly greater than those of the controls. For dosed F_1 males, relative liver and spleen weights were significantly greater than those of the controls, and the relative seminal vesicle weight was significantly less than that of the controls. Methemoglobin levels ranged from 8% to 12% in F_0 and F_1 mice receiving 160 mg/kg. Sperm morphology and vaginal cytology evaluations of F_1 mice showed no effects of treatment with 2-chloronitrobenzene.

4-CHLORONITROBENZENE

2-Week Dose-Finding Study

All mice in the 640 mg/kg group died or were sacrificed moribund. Four control mice and five dosed mice also died, and most of these deaths were attributed to gavage trauma. The final mean body weight of females in the 320 mg/kg group was notably greater than that of the control group; the mean body weights of other dosed groups were similar to those of the controls. Water consumption was decreased for males and females in the 640 mg/kg groups, for females in the 320 mg/kg group during Week 1, and for females in the 40 mg/kg group during Week 2. Males and females in the 160 and 320 mg/kg groups became cyanotic.

Continuous Breeding Study

A number of deaths occurred during the study; five control mice, four mice receiving 62.5 mg/kg, three mice receiving 125 mg/kg, and six mice receiving 250 mg/kg died during the precohabitation period or during the continuous breeding period. Two deaths in each of the control and 125 mg/kg groups and one death in the high-dose group were due to gavage trauma; two deaths in each of the control and 62.5 mg/kg groups were due to fight wounds. The final mean body weights of dosed mice, especially for

the 125 and 250 mg/kg groups, were notably greater than those of the controls. The water consumption of high-dose mice was significantly lower than that of control mice.

All dosed and control pairs were fertile; however, the number of high-dose breeding pairs delivering litters declined by the second litter, and the difference was significant for the third and fourth litters (Table D5). The average number of litters per pair decreased slightly with increasing dose. Cumulative days to litter for dosed pairs were similar to those of the controls. The mean body weights of dosed dams at delivery were generally similar to the mean body weight of the controls for each litter; the mean body weights of dosed dams were generally greater than the mean body weight of control dams at delivery and during lactation of litter 5 (Table D5). The average number of live pups per litter, the average live pups per breeding pair, and the sex ratios of pups from dosed breeding pairs were similar to those of the controls (Table D6). The male and female pup weights from breeding pairs in the 125 and 250 mg/kg groups were significantly less than those of the controls, and the weights of male pups in the second and fourth litters of breeding pairs in the 62.5 mg/kg group were also significantly less than those of the controls.

For the final litter of F_1 pups, the weights of male and female pups from breeding pairs in the 125 and 250 mg/kg groups and female pups from breeding pairs in the 40 mg/kg group were significantly less than those of control pups at all time points (Table D7). The survival of female pups and the total survival of pups from high-dose breeding pairs were significantly less than the survival of the controls at all time points. The total survival of pups from breeding pairs in the 125 mg/kg group was slightly but significantly greater than that of the control pups by Day 7, but was slightly less than that of the control pups at Day 21; male pup survival was also slightly less than that of the controls at Day 21 (Table D7). There were no clinical signs of toxicity.

At mating, the mean body weights of dosed F_1 mice were similar to those of the controls. Water consumption by dosed and control mice was similar. Most high-dose mice were cyanotic. Mating, pregnancy, and fertility indices, average days to litter, and mean dam weight at delivery for dosed breeding pairs were similar to those of the controls (Table D8). There were no significant differences in survival or sex ratios for F_2 pups; however, the proportion of pups born alive and male and female pup weights for pups of dosed breeding pairs were significantly less than those of control pups (Table D8). There were no clinical signs of toxicity in F_2 pups.

For dosed male and female F_1 mice, absolute and relative liver weights were significantly greater than those of the controls. Spleens were not weighed, but were observed to be enlarged and dark in dosed mice. Methemoglobin concentrations were not determined. Results of sperm morphology evaluations of

F_1 mice showed no effects of treatment with 4-chloronitrobenzene; however, average estrous cycle length in dosed females was significantly increased (not shown).

To summarize, 2-chloronitrobenzene and 4-chloronitrobenzene were studied in a continuous breeding protocol in which CD-1 Swiss mice were administered the chemicals individually by gavage in corn oil, using doses up to approximately one-third to one-half those that caused mortality in 2-week range-finding studies. Results of the 2-chloronitrobenzene study were largely negative, and this was generally in agreement with the rather minor effects noted with this isomer in the sperm motility assays in the inhalation study. However, 4-chloronitrobenzene produced significant and progressive deficits in fertility in the F_0 generation, and weight gains of F_1 and F_2 pups were lower than those of the control pups. The same effect that lengthened estrous cycle in the inhalation study may have promoted the fertility effect observed in the continuous breeding study. The changes in pup weight could not be predicted based on the sperm morphology/vaginal cytology data and represent an additional effect; this effect may be secondary to methemoglobin-related hypoxia, which is generally more severe in animals treated with 4-chloronitrobenzene than in animals treated with 2-chloronitrobenzene.

TABLE D1 Fertility Data, Mean Body Weights, and Time to Delivery for F_0 CD-1 Swiss Mice in the Continuous Breeding Study of 2-Chloronitrobenzene[1]

Study Parameters	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
Pregnancy Index (pregnant females/cohabiting pairs)				
Litter 1	36/38 (95%)	18/18 (100%)	18/18 (100%)	17/17 (100%)
Litter 2	36/38 (95%)	18/18 (100%)	18/18 (100%)	17/17 (100%)
Litter 3	36/38 (95%)	18/18 (100%)	18/18 (100%)	17/17 (100%)
Litter 4	36/38 (95%)	18/18 (100%)	18/18 (100%)	17/17 (100%)
Litter 5	34/38 (89%)	17/18 (94%)	16/18 (89%)	15/17 (88%)
Average Litters per Pair	4.9 ± 0.0	4.9 ± 0.1	4.9 ± 0.1	4.9 ± 0.1
Cumulative Days to Litter				
Litter 1	22.3 ± 0.7	20.7 ± 0.3	22.1 ± 1.0	22.4 ± 1.3
Litter 2	42.1 ± 0.7	40.8 ± 0.7	42.2 ± 1.1	42.2 ± 1.4
Litter 3	63.1 ± 0.9	60.9 ± 0.8	62.6 ± 1.2	63.1 ± 2.0
Litter 4	83.5 ± 1.0	82.4 ± 1.3	83.2 ± 1.4	83.8 ± 2.1
Litter 5	102.9 ± 0.8	102.2 ± 1.3	102.3 ± 1.0	101.0 ± 0.8
Dam Weight at Delivery (g)				
n	36	18	18	17
Litter 1	32.1 ± 0.4	32.9 ± 0.7	33.4 ± 0.6	33.0 ± 0.6
Litter 2	35.3 ± 0.5	36.3 ± 0.7	36.4 ± 0.6	36.5 ± 0.6
Litter 3	37.5 ± 0.6[2]	38.2 ± 0.7	38.9 ± 0.6	38.1 ± 0.7
Litter 4	38.5 ± 0.6	39.7 ± 0.8	40.6 ± 0.9	40.9 ± 0.8*
Litter 5	39.4 ± 0.6[3]	40.3 ± 0.8[4]	41.8 ± 1.0[5]	41.4 ± 0.8[6]
Dam Weight During Lactation of Litter 5 (g)				
n	36	17	17	17
Lactation day 0	39.2 ± 0.6[2]	40.3 ± 0.8	41.3 ± 1.0	41.5 ± 0.7*
Lactation day 4	40.8 ± 0.6[2]	41.4 ± 1.1[5]	41.0 ± 0.8	42.9 ± 0.7
Lactation day 7	43.1 ± 0.7	43.5 ± 1.3	43.2 ± 1.0	44.2 ± 0.6
Lactation day 14	46.4 ± 0.8	46.6 ± 1.5	46.7 ± 1.2	47.3 ± 0.7
Lactation day 21	40.7 ± 0.8	42.6 ± 1.5	43.4 ± 1.3	44.9 ± 1.1*

[1] Data for litters per pair, cumulative days to litter, and dam weights are given as mean ± standard error. Differences from the control group for cumulative days to litter are not significant by Dunn's test.
[2] n=35.
[3] n=33.
[4] n=17.
[5] n=16.
[6] n=15.
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

TABLE D2 Mean Body Weights of F_1 CD-1 Swiss Mouse Pups in the Continuous Breeding Study of 2-Chloronitrobenzene[1]

Study Parameters	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
Litter 1				
Number of litters	36	18	18	17
Live pups/litter	9.6 ± 0.6	10.9 ± 0.7	9.1 ± 1.0	10.7 ± 0.8
Live pups/breeding pair (%)	91 ± 5	98 ± 2	88 ± 8	100 ± 0
Sex ratio[2] (%)	52 ± 2	52 ± 4	58 ± 5	44 ± 4
Male pup weight	1.56 ± 0.02	1.54 ± 0.03	1.58 ± 0.03	1.59 ± 0.05
Female pup weight	1.50 ± 0.02	1.48 ± 0.02	1.52 ± 0.03	1.54 ± 0.05
Litter 2				
Number of litters	36	18	18	17
Live pups/litter	11.3 ± 0.7	11.9 ± 0.6	12.4 ± 0.7	13.4 ± 0.4*
Live pups/breeding pair (%)	89 ± 5	98 ± 1	99 ± 1	100 ± 0
Sex ratio (%)	50 ± 2	50 ± 4	53 ± 4	44 ± 3
Male pup weight	1.57 ± 0.03	1.56 ± 0.03	1.57 ± 0.03	1.56 ± 0.03
Female pup weight	1.51 ± 0.02	1.52 ± 0.03	1.53 ± 0.03	1.49 ± 0.03
Litter 3				
Number of litters	36	18	18	17
Live pups/litter	11.4 ± 0.8	12.4 ± 0.9	13.9 ± 0.5*	14.1 ± 0.4*
Live pups/breeding pair (%)	88 ± 5	95 ± 5	100 ± 0	100 ± 0*
Sex ratio (%)	50 ± 2	51 ± 4	42 ± 3*	50 ± 2
Male pup weight	1.58 ± 0.02	1.56 ± 0.02	1.56 ± 0.03	1.52 ± 0.02
Female pup weight	1.54 ± 0.02	1.50 ± 0.03	1.52 ± 0.04	1.51 ± 0.02
Litter 4				
Number of litters	36	18	17	17
Live pups/litter	11.8 ± 0.7	12.6 ± 0.8	12.5 ± 1.1	13.0 ± 0.9
Live pups/breeding pair (%)	91 ± 4	97 ± 2	93 ± 6	100 ± 0*
Sex ratio (%)	51 ± 3	41 ± 4	49 ± 4	49 ± 4
Male pup weight	1.60 ± 0.02	1.57 ± 0.04	1.58 ± 0.03	1.62 ± 0.03
Female pup weight	1.55 ± 0.02	1.54 ± 0.04	1.55 ± 0.04	1.58 ± 0.04
Litter 5				
Number of litters	34	17	16	15
Live pups/litter	12.2 ± 0.7	12.7 ± 1.1	13.0 ± 1.0	13.3 ± 0.8
Live pups/breeding pair (%)	93 ± 4	94 ± 6	94 ± 6	99 ± 1
Sex ratio (%)	50 ± 3	49 ± 3	51 ± 3	48 ± 3
Male pup weight	1.57 ± 0.03	1.60 ± 0.03	1.52 ± 0.02	1.59 ± 0.03
Female pup weight	1.52 ± 0.02	1.52 ± 0.03	1.48 ± 0.02	1.54 ± 0.03
Litters 1 through 5				
Average live pups/litter	11.2 ± 0.5	12.1 ± 0.6	11.9 ± 0.7	12.9 ± 0.5*
Average live pups/breeding pair (%)	91 ± 3	96 ± 2	95 ± 3	100 ± 0*
Average sex ratio (%)	51 ± 1	48 ± 1	49 ± 1	48 ± 1
Average male pup weight	1.56 ± 0.02	1.56 ± 0.02	1.56 ± 0.02	1.56 ± 0.02
Average female pup weight	1.51 ± 0.02	1.51 ± 0.02	1.51 ± 0.02	1.52 ± 0.02

[1] Data for live pups/litter, pups/breeding pair, sex ratios, and pup weights are given as mean ± standard error. Pup weights are given in grams. Differences from the control group for pup weights are not significant by Dunn's test.
[2] Number of live male pups/number of live pups.
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

TABLE D3 Survival and Mean Body Weights of F_1 CD-1 Swiss Mouse Pups (Final Litter) in the Continuous Breeding Study of 2-Chloronitrobenzene[1]

Study Parameters	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
Day 0				
Number of litters	34	17	16	15
Live pups/breeding pair (%)	93 ± 4	94 ± 6	94 ± 6	99 ± 1
Male pup weight (g)	1.57 ± 0.03	1.60 ± 0.03	1.52 ± 0.02	1.59 ± 0.03
Female pup weight (g)	1.52 ± 0.02	1.52 ± 0.03	1.48 ± 0.02	1.54 ± 0.03
Day 4				
Male survival (%)	92 ± 3	90 ± 6	95 ± 3	98 ± 2
Female survival (%)	97 ± 2	95 ± 7	97 ± 3	99 ± 1
Total survival (%)	93 ± 3	92 ± 6	96 ± 3	99 ± 1
Male pup weight (g)	2.88 ± 0.07	2.77 ± 0.14	2.71 ± 0.08	2.77 ± 0.10
Female pup weight (g)	2.79 ± 0.07	2.68 ± 0.13	2.62 ± 0.08	2.66 ± 0.10
Day 7				
Male survival (%)	92 ± 3	90 ± 6	94 ± 3	98 ± 2
Female survival (%)	96 ± 2	95 ± 7	96 ± 3	99 ± 1
Total survival (%)	92 ± 3	92 ± 6	95 ± 3	99 ± 1*
Male pup weight (g)	4.34 ± 0.11	4.13 ± 0.21	4.04 ± 0.13	4.06 ± 0.15
Female pup weight (g)	4.21 ± 0.11	4.01 ± 0.21	3.88 ± 0.14	3.88 ± 0.16
Day 14				
Male survival (%)	92 ± 4	89 ± 6	93 ± 4	98 ± 2
Female survival (%)	95 ± 2	94 ± 7	96 ± 3	97 ± 1
Total survival (%)	92 ± 3	91 ± 6	94 ± 3	97 ± 1
Male weight (g)	6.92 ± 0.19	6.48 ± 0.31	6.35 ± 0.20	6.29 ± 0.23*
Female pup weight (g)	6.82 ± 0.17	6.36 ± 0.31	6.13 ± 0.23*	6.13 ± 0.25*
Day 21				
Male survival (%)	91 ± 4	89 ± 6	92 ± 3	98 ± 2
Female survival (%)	94 ± 2	93 ± 7	94 ± 4	96 ± 2
Total survival (%)	91 ± 3	91 ± 6	93 ± 3	97 ± 1
Male pup weight (g)	11.02 ± 0.32	9.91 ± 0.53	9.64 ± 0.39*	9.68 ± 0.48*
Female pup weight (g)	10.62 ± 0.28	9.58 ± 0.48*	9.17 ± 0.35*	9.36 ± 0.45*

[1] Survival and pup weight data are given as mean ± standard error.
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

TABLE D4 Reproductive, Survival, and Mean Body Weight Data for F_1 and F_2 CD-1 Swiss Mice in the Continuous Breeding Study of 2-Chloronitrobenzene[1]

Study Parameters	Vehicle Control	160 mg/kg
F_1 Adult Data		
Mating index[2]	20/20 (100%)	19/20 (95%)
Pregnancy index[3]	19/20 (95%)	19/20 (95%)
Fertility index[4]	19/20 (95%)	19/19 (100%)
Dam weight at delivery (g)	34 ± 1	34 ± 1
Days to litter	18.9 ± 0.1	18.9 ± 0.3
F_2 Pup Data		
Live male pups/litter	6.0 ± 0.4	6.2 ± 0.4
Live female pups/litter	5.6 ± 0.4	5.2 ± 0.4
Total live pups/litter	11.6 ± 0.4	11.4 ± 0.4
Live pups/breeding pair (%)	98 ± 1	100 ± 0
Sex ratio[5] (%)	52 ± 3	54 ± 3
Male pup weight	1.57 ± 0.03	1.55 ± 0.04
Female pup weight	1.51 ± 0.03	1.48 ± 0.03

[1] Data for dam weights, days to litter, pup survival, and pup weights are given as mean ± standard error. Differences from the control group for mating, pregnancy, and fertility indexes are not significant by the chi-square test. Differences from the control group for dam weights, days to litter, pup survival, sex ratio, and pup weights are not significant by Wilcoxon's test.

[2] Number of females with copulatory plug/number of cohabiting pairs.

[3] Number of fertile pairs/number of cohabiting pairs.

[4] Number of fertile pairs/number of females with copulatory plug.

[5] Number of live male pups/number of live pups.

TABLE D5 Fertility Data, Mean Body Weights, and Time to Delivery for F_0 CD-1 Swiss Mice in the Continuous Breeding Study of 4-Chloronitrobenzene[1]

Study Parameters	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Fertility Index (fertile pairs/cohabiting pairs)				
Litter 1	35/35 (100%)	16/16 (100%)	19/19 (100%)	14/14 (100%)
Litter 2	35/35 (100%)	16/16 (100%)	19/19 (100%)	13/14 (93%)
Litter 3	35/35 (100%)	16/16 (100%)	19/19 (100%)	12/14 (86%)*
Litter 4	35/35 (100%)	16/16 (100%)	18/19 (95%)	11/14 (79%)*
Litter 5	28/35 (80%)	11/16 (69%)	12/19 (63%)	8/14 (57%)
Average Litters per Pair	4.8 ± 0.1	4.7 ± 0.1	4.6 ± 0.1	4.1 ± 0.3
Cumulative Days to Litter				
Litter 1	22.3 ± 0.6	21.5 ± 0.8	23.1 ± 1.3	22.1 ± 1.3
Litter 2	42.7 ± 0.8	41.8 ± 0.9	43.7 ± 1.7	42.0 ± 1.6
Litter 3	62.7 ± 0.8	61.8 ± 0.9	63.6 ± 1.7	61.9 ± 1.7
Litter 4	83.9 ± 1.1	83.1 ± 1.1	85.6 ± 2.1	84.8 ± 2.2
Litter 5	102.5 ± 0.8	103.1 ± 1.4	101.9 ± 1.2	103.4 ± 1.4
Dam Weight at Delivery (g)				
n	35	16	19	14
Litter 1	39.0 ± 0.5	38.6 ± 0.5	40.3 ± 0.5	40.3 ± 0.7
Litter 2	43.1 ± 0.4	43.2 ± 0.6	44.9 ± 0.9*	44.8 ± 0.7[2]
Litter 3	45.5 ± 0.6	46.2 ± 0.8	47.9 ± 0.8*	46.8 ± 1.1[3]
Litter 4	47.5 ± 0.7	49.3 ± 0.8*	49.3 ± 1.2[4]	49.1 ± 1.5[5]
Litter 5	45.7 ± 0.5[6]	47.7 ± 1.2[5]	51.4 ± 1.9*[3]	50.0 ± 2.2*[7]
Dam Weight During Lactation of Litter 5 (g)				
n	30	11	13	8
Lactation day 0	45.7 ± 0.5	47.3 ± 1.2	50.7 ± 1.9*	50.4 ± 2.1*
Lactation day 4	48.6 ± 0.7	48.9 ± 1.0	49.9 ± 1.1	48.9 ± 1.5
Lactation day 7	50.2 ± 0.7	50.0 ± 1.1	52.2 ± 1.1	49.2 ± 1.7
Lactation day 14	52.2 ± 1.0	52.7 ± 0.8	54.7 ± 1.3	51.8 ± 1.8[8]
Lactation day 21	42.4 ± 0.6	43.7 ± 1.2	47.3 ± 1.3*	50.1 ± 1.6*

[1] Data for litters per pair, cumulative days to litter, and dam weights are given as mean ± standard error. Differences from the control group for litters per pair and cumulative days to litter are not significant by Dunn's test.
[2] n=13.
[3] n=12.
[4] n=18.
[5] n=11.
[6] n=28.
[7] n=8.
[8] n=7.
* Significantly different ($P \leq 0.05$) from the control group by the chi-square test (fertility index) or Shirley's test (body weights).

TABLE D6 Mean Body Weights of F_1 CD-1 Swiss Mouse Pups in the Continuous Breeding Study of 4-Chloronitrobenzene[1]

Study Parameters	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Litter 1				
Number of litters	35	16	19	14
Live pups/litter	11.5 ± 0.5	9.9 ± 1.1	11.9 ± 0.7	11.1 ± 0.6
Live pups/breeding pair (%)	100 ± 0	88 ± 9	100 ± 0	97 ± 2
Sex ratio[2] (%)	48 ± 2	49 ± 2	45 ± 4	45 ± 2
Male pup weight	1.71 ± 0.02	1.68 ± 0.03	1.59 ± 0.04*	1.39 ± 0.04*
Female pup weight	1.62 ± 0.02	1.63 ± 0.04	1.51 ± 0.04*	1.32 ± 0.04*
Litter 2				
Number of litters	35	16	19	13
Live pups/litter	12.4 ± 0.6	13.5 ± 0.5	14.5 ± 0.6*	12.0 ± 0.3
Live pups/breeding pair (%)	95 ± 3	100 ± 0	100 ± 0	99 ± 1
Sex ratio	49 ± 3	51 ± 3	52 ± 3	42 ± 5
Male pup weight	1.72 ± 0.02	1.62 ± 0.03*	1.50 ± 0.03*	1.41 ± 0.04*
Female pup weight	1.62 ± 0.02	1.57 ± 0.03	1.44 ± 0.02*	1.32 ± 0.04*
Litter 3				
Number of litters	35	16	19	12
Live pups/litter	13.3 ± 0.5	13.7 ± 0.5	13.4 ± 0.9	10.9 ± 0.9
Live pups/breeding pair (%)	98 ± 2	100 ± 0	99 ± 1	95 ± 5
Sex ratio	50 ± 3	48 ± 4	53 ± 2	53 ± 6
Male pup weight	1.68 ± 0.03	1.61 ± 0.02	1.51 ± 0.03*	1.42 ± 0.06*
Female pup weight	1.61 ± 0.02	1.57 ± 0.03	1.45 ± 0.03*	1.35 ± 0.06*
Litter 4				
Number of litters	35	16	18	11
Live pups/litter	11.4 ± 0.8	14.0 ± 0.4	13.4 ± 0.6	10.2 ± 1.1
Live pups/breeding pair (%)	90 ± 5	100 ± 0	99 ± 1	93 ± 4
Sex ratio	53 ± 3	53 ± 4	44 ± 3	48 ± 2
Male pup weight	1.71 ± 0.03	1.61 ± 0.03*	1.52 ± 0.03*	1.45 ± 0.04*
Female pup weight	1.63 ± 0.03	1.55 ± 0.04	1.47 ± 0.04*	1.38 ± 0.04*
Litter 5				
Number of litters	28	11	12	8
Live pups/litter	11.8 ± 0.6	11.3 ± 0.8	13.3 ± 0.8	11.3 ± 1.9
Live pups/breeding pair (%)	98 ± 2	100 ± 0	96 ± 2	86 ± 12
Sex ratio	51 ± 3	49 ± 3	57 ± 5	50 ± 3
Male pup weight	1.75 ± 0.03	1.69 ± 0.06	1.48 ± 0.03*	1.44 ± 0.06*
Female pup weight	1.68 ± 0.03	1.62 ± 0.05	1.40 ± 0.03*	1.35 ± 0.05*
Litters 1 through 5				
Average live pups/litter	12.1 ± 0.4	12.6 ± 0.3	13.2 ± 0.4	10.9 ± 0.5
Average live pups/breeding pair (%)	97 ± 1	98 ± 1	99 ± 0	95 ± 2
Average sex ratio	50 ± 1	50 ± 2	50 ± 2	47 ± 1
Average male pup weight	1.70 ± 0.02	1.62 ± 0.02*	1.51 ± 0.02*	1.40 ± 0.03*
Average female pup weight	1.62 ± 0.02	1.58 ± 0.02	1.46 ± 0.02*	1.31 ± 0.03*

[1] Data for live pups/litter, pups/breeding pair, sex ratios, and pup weights are given as mean ± standard error. Pup weights are given in grams. Differences from the control group for percent live pups and sex ratio are not significant by Dunn's test.

[2] Number of live male pups/number of live pups.

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

TABLE D7 Survival and Mean Body Weights of F_1 CD-1 Swiss Mouse Pups (Final Litter) in the Continuous Breeding Study of 4-Chloronitrobenzene[1]

Study Parameters	Vehicle Control	62.5 mg/kg	125 mg/kg	250 mg/kg
Day 0				
Number of litters	28	11	12	8
Live pups/breeding pair (%)	98 ± 2	100 ± 0	96 ± 2	86 ± 12
Male pup weight (g)	1.75 ± 0.03	1.69 ± 0.06	1.48 ± 0.03*	1.44 ± 0.06*
Female pup weight (g)	1.68 ± 0.03	1.62 ± 0.05	1.40 ± 0.03*	1.35 ± 0.05*
Day 4				
Male survival (%)	93 ± 5	100 ± 0	95 ± 3	80 ± 15
Female survival (%)	93 ± 5	98 ± 2	104 ± 8	79 ± 14*
Total survival (%)	93 ± 5	99 ± 1	95 ± 3	79 ± 14*
Male pup weight (g)	3.46 ± 0.09	3.35 ± 0.16	2.68 ± 0.15*	2.45 ± 0.17*
Female pup weight (g)	3.34 ± 0.09	3.27 ± 0.15	2.62 ± 0.16*	2.17 ± 0.15*
Day 7				
Male survival (%)	93 ± 5	100 ± 0	94 ± 3	80 ± 15
Female survival (%)	93 ± 5	98 ± 2	104 ± 8	79 ± 14*
Total survival (%)	93 ± 5	99 ± 1	95 ± 3*	79 ± 14*
Male pup weight (g)	5.35 ± 0.15	5.10 ± 0.23	4.22 ± 0.22*	3.93 ± 0.24*
Female pup weight (g)	5.14 ± 0.15	4.96 ± 0.23	4.01 ± 0.22*	3.48 ± 0.21*
Day 14				
Male survival (%)	93 ± 5	100 ± 0	93 ± 3	80 ± 15
Female survival (%)	93 ± 5	98 ± 2	103 ± 8	77 ± 14*
Total survival (%)	93 ± 5	99 ± 1	94 ± 3*	78 ± 14*
Male pup weight (g)	8.66 ± 0.30	8.28 ± 0.36	7.14 ± 0.35*	6.73 ± 0.39*
Female pup weight (g)	8.34 ± 0.29	8.25 ± 0.36	7.02 ± 0.38*	6.14 ± 0.30*
Day 21				
Male survival (%)	93 ± 5	100 ± 0	91 ± 4*	80 ± 15
Female survival (%)	92 ± 5	98 ± 2	103 ± 8	77 ± 14*
Total survival (%)	93 ± 5	99 ± 1	93 ± 3*	78 ± 14*
Male pup weight (g)	14.86 ± 0.44	14.33 ± 0.56	12.07 ± 0.63*	11.30 ± 0.62*
Female pup weight (g)	13.85 ± 0.42	13.65 ± 0.49	11.24 ± 0.61*	10.19 ± 0.52*

[1] Survival and pup weight data are given as mean ± standard error.
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

TABLE D8 Reproductive, Survival, and Mean Body Weight Data for F_1 and F_2 CD-1 Swiss Mice in the Continuous Breeding Study of 4-Chloronitrobenzene[1]

Study Parameters	Vehicle Control	250 mg/kg
F_1 Adult Data		
Mating index[2]	19/20 (95%)	20/20 (100%)
Pregnancy index[3]	19/20 (95%)	18/20 (90%)
Fertility index[4]	19/19 (100%)	18/20 (90%)
Days to litter	19.1 ± 0.4	18.8 ± 0.2
Dam weight at delivery (g)	36.6 ± 0.8	34.8 ± 0.7
F_2 Pup Data		
Live male pups/litter	5.8 ± 0.5	5.4 ± 0.6
Live female pups/litter	5.7 ± 0.6	5.0 ± 0.6
Total live pups/litter	11.6 ± 0.8	10.4 ± 1.1
Live pups/breeding pair (%)	95 ± 5	85 ± 7*
Sex ratio[5] (%)	51 ± 4	52 ± 3
Male pup weight (g)	1.56 ± 0.03	1.35 ± 0.02*
Female pup weight (g)	1.49 ± 0.02	1.28 ± 0.02*

[1] Data for dam weights, days to litter, pup survival, and pup weights are given as mean ± standard error. Differences from the control group for mating, pregnancy, and fertility indexes are not significant by the chi-square test. Differences from the control group for dam weights, days to litter, number of live pups/litter, and sex ratio are not significant by Wilcoxon's test.

[2] Number of females with copulatory plug/number of cohabiting pairs.

[3] Number of fertile pairs/number of cohabiting pairs.

[4] Number of fertile pairs/number of females with copulatory plug.

[5] Number of live male pups/number of live pups.

* Significantly different ($P \leq 0.05$) by Wilcoxon's test.

APPENDIX E

Genetic Toxicology

TABLE E1 Mutagenicity of 2-Chloronitrobenzene in *Salmonella typhimurium*[1]

Strain	Dose (μg/plate)	Revertants/plate[2] -S9	+10% hamster S9	+10% rat S9
STUDY PERFORMED AT SRI, INTERNATIONAL (Complete data published in Haworth *et al.*, 1983)				
TA100	0.0	118 ± 2.6	99 ± 5.8	88 ± 1.2
	10.0	118 ± 3.5	112 ± 9.6	106 ± 9.8
	33.3	116 ± 9.6	103 ± 1.5	121 ± 3.5
	100.0	131 ± 3.2	224 ± 14.6	138 ± 2.6
	333.3	124 ± 6.9	360 ± 18.7	149 ± 6.4
	1000.0	Toxic	Toxic	133 ± 68.1
Trial summary		Negative	Positive	Positive
Positive control[3]		359 ± 12.7	1603 ± 50.7	925 ± 55.2
TA98	0.0	30 ± 4.0	39 ± 5.4	47 ± 4.0
	10.0	36 ± 5.2	48 ± 6.4	46 ± 1.8
	33.3	35 ± 2.0	49 ± 5.7	43 ± 2.9
	100.0	31 ± 3.0	43 ± 5.9	44 ± 4.3
	333.3	29 ± 1.7	48 ± 6.7	40 ± 7.1
	1000.0	2 ± 2.3[4]	18 ± 11.4[4]	16 ± 2.7[4]
Trial summary		Negative	Negative	Negative
Positive control		594 ± 16.2	1970 ± 40.8	671 ± 26.8
STUDY PERFORMED AT EG&G MASON RESEARCH INSTITUTE				
Standard plate protocol				
TA100	0.0	101 ± 16.2	110 ± 8.0	87 ± 5.5
	62.5	100 ± 3.6	144 ± 11.9	109 ± 4.7
	125.0	126 ± 3.8	248 ± 12.2	136 ± 10.1
	250.0	122 ± 5.0	470 ± 21.2	199 ± 11.5
	500.0	124 ± 7.3	429 ± 74.6[4]	279 ± 5.5
	1000.0	133 ± 12.4	103 ± 36.4[4]	386 ± 3.2
Trial summary		Equivocal	Positive	Positive
Positive control		951 ± 27.5	3405 ± 60.8	3405 ± 60.8

TABLE E1 Mutagenicity of 2-Chloronitrobenzene in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9	Revertants/plate +10% hamster S9	Revertants/plate +10% rat S9
STUDY PERFORMED AT EG&G MASON RESEARCH INSTITUTE				
Preincubation protocol (Complete data published in Haworth *et al.*, 1983)				
TA100	0.0	109 ± 10.3	92 ± 11.2	92 ± 7.3
	62.5	115 ± 6.4	144 ± 11.9	115 ± 11.8
	125.0	109 ± 11.6	203 ± 7.7	118 ± 11.3
	250.0	121 ± 0.9	339 ± 21.9	130 ± 1.2
	500.0	92 ± 5.2[4]	44 ± 7.9[4]	91 ± 1.5[4]
	1000.0	Toxic	Toxic	Toxic
Trial summary		Negative	Positive	Equivocal
Positive control		2419 ± 19.1	3019 ± 91.8	2188 ± 32.9
TA98	0	19 ± 0.9	19 ± 1.9	20 ± 1.2
	6	16 ± 1.9	23 ± 3.3	21 ± 2.0
	20	16 ± 1.9	20 ± 1.5	20 ± 2.3
	60	18 ± 2.3	24 ± 3.2	24 ± 1.0
	200	28 ± 1.3	35 ± 3.2	25 ± 0.7
	600	Toxic	Toxic	18 ± 2.5[4]
Trial summary		Negative	Equivocal	Negative
Positive control		1630 ± 14.5	2797 ± 95.7	1353 ± 62.3

Strain	Dose (μg/plate)	Revertants/plate -S9	Revertants/plate +30% hamster S9	Revertants/plate +30% rat S9
STUDY PERFORMED AT SRI, INTERNATIONAL				
TA100	0	159 ± 9.0	157 ± 6.9	127 ± 2.1
	10	143 ± 3.2		
	33	156 ± 10.1	167 ± 6.0	
	100	142 ± 9.9	272 ± 10.9	165 ± 7.3
	133		374 ± 11.3	
	166		434 ± 9.1	
	250		567 ± 25.0	
	333	129 ± 13.9		195 ± 2.6
	666	Toxic		260 ± 7.5
	1000			236 ± 9.0
	1666			209 ± 10.3
Trial summary		Negative	Positive	Positive
Positive control		450 ± 6.3	697 ± 75.4	578 ± 41.9

TABLE E1 Mutagenicity of 2-Chloronitrobenzene in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9	+hamster S9 5%	+hamster S9 10%	+hamster S9 30%	+30% rat S9
STUDY PERFORMED AT SRI, INTERNATIONAL						
TA98	0	20 ± 0.0	33 ± 2.9	28 ± 3.2	27 ± 2.3	41 ± 5.0
	10	17 ± 1.7				
	33	16 ± 3.0	30 ± 4.6	26 ± 2.9	25 ± 2.9	35 ± 4.2
	66		35 ± 3.0	38 ± 3.8	37 ± 4.7	
	100	21 ± 0.6	47 ± 5.5	41 ± 3.5	46 ± 4.2	37 ± 4.4
	133		45 ± 5.9	43 ± 2.3	62 ± 6.7	
	166		53 ± 2.3	56 ± 1.9	58 ± 4.4	
	333	21 ± 3.3				45 ± 2.4
	666	Toxic				
	1000					30 ± 3.2
	1666					36 ± 6.0
Trial summary		Negative	Equivocal	Weakly Positive	Positive	Negative
Positive control		543 ± 21.3	1369 ±184.7	835 ± 79.5	491 ± 13.8	147 ± 18.8
TA100	0	89 ± 6.8			113 ± 7.8	152 ± 3.7
	3	127 ± 4.7				
	10	123 ± 4.4			108 ± 7.5	144 ± 2.3
	33	106 ± 8.1			146 ± 8.7	144 ± 8.1
	66				180 ± 3.7	
	100	129 ± 6.0			255 ± 17.9	167 ± 13.4
	166				380 ± 9.2	
	333	121 ± 4.3				196 ± 4.4
	666					234 ± 15.1
Trial summary		Equivocal			Positive	Equivocal
Positive control		906 ± 6.0			595 ± 47.8	405 ± 1.5
TA98	0	20 ± 4.1			14 ± 3.5	34 ± 7.2
	3	30 ± 2.3			13 ± 2.1	
	10	25 ± 3.8			13 ± 1.2	23 ± 2.8
	33	30 ± 1.5			21 ± 2.9	28 ± 2.2
	66				19 ± 1.5	
	100	28 ± 2.2			19 ± 1.8	23 ± 1.5
	166				40 ± 0.3	
	333	25 ± 1.0			37 ± 5.7	27 ± 3.1
	666					28 ± 0.9
Trial summary:		Negative			Weakly Positive	Negative
Positive control		575 ± 17.3			508 ± 45.4	152 ± 12.3

[1] 0 μg/plate is the solvent control.
[2] Revertants are presented as the mean ± standard error from three plates.
[3] The positive controls in the absence of metabolic activation were sodium azide (TA100) and 4-nitro-*o*-phenylenediamine (TA98). The positive control for trials with metabolic activation with both strains was 2-aminoanthracene.
[4] Slight toxicity.

TABLE E2 Mutagenicity of 4-Chloronitrobenzene in *Salmonella typhimurium*[1]

Strain	Dose (μg/plate)	Revertants/plate[2] -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3
STUDY PERFORMED AT EG&G MASON RESEARCH INSTITUTE							
Preincubation protocol							
TA100	0.0	98 ± 3.3	128 ± 3.3	117 ± 13.3	117 ± 7.2	105 ± 6.7	112 ± 3.7
	30.0	93 ± 3.8	150 ± 5.0		137 ± 12.3	131 ± 5.8	
	62.5			99 ± 3.5			132 ± 7.1
	100.0	93 ± 7.8	136 ± 5.8		152 ± 7.8	136 ± 4.4	
	250.0			99 ± 2.6			158 ± 13.7
	300.0	82 ± 5.5	116 ± 4.4		152 ± 17.6	147 ± 6.5	
	500.0			81 ± 6.0[3]			200 ± 11.0[3]
	1000.0	81 ± 4.1	114 ± 4.7	94 ± 4.3[3]	255 ± 28.0	178 ± 4.5[3]	235 ± 20.3[3]
	1500.0			69 ± 4.4[3]			189 ± 11.6[3]
	2000.0			61 ± 4.4[3]			148 ± 15.2[3]
	3000.0	28 ± 9.0	25 ± 13.9[3]		25 ± 17.4[3]	98 ± 14.2[3]	
Trial summary		Negative	Negative	Negative	Weakly Positive	Weakly Positive	Positive
Positive control[4]		1270 ± 4.5	1987 ± 29.7	2321 ± 123.2	3212 ± 76.1	2032 ± 30.8	3005 ± 74.6

TA100 (continued)

Dose (μg/plate)	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3
0.0	98 ± 9.2	113 ± 10.2	118 ± 10.3
30.0	94 ± 2.6	138 ± 0.3	
62.5			137 ± 6.6
100.0	108 ± 3.5	143 ± 5.4	
250.0			155 ± 6.1
300.0	129 ± 6.7	172 ± 12.6	
500.0			176 ± 5.9[3]
1000.0	192 ± 3.3	201 ± 21.1	183 ± 6.1[3]
1500.0			168 ± 6.3[3]
2000.0			174 ± 0.9[3]
3000.0	57 ± 17.8	37 ± 22.9[3]	
Trial summary	Weakly Positive	Weakly Positive	Weakly Positive
Positive control	2356 ± 38.1	1264 ± 52.9	2232 ± 45.4

TABLE E2 Mutagenicity of 4-Chloronitrobenzene in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9	Revertants/plate +10% hamster S9	Revertants/plate +10% rat S9
Standard plate protocol				
TA100	0.0	101 ± 3.5	104 ± 9.3	126 ± 13.2
	62.5	118 ± 10.7	135 ± 4.6	128 ± 3.7
	250.0	107 ± 12.0	171 ± 5.6	156 ± 8.4
	500.0	132 ± 4.1	301 ± 14.6	185 ± 6.6
	1000.0	114 ± 4.8	400 ± 16.5	215 ± 11.7
	1500.0	117 ± 10.5	344 ± 16.0[3]	203 ± 12.5
	2000.0	94 ± 10.2	68 ± 38.3[3]	194 ± 0.7
Trial summary		Negative	Positive	Weakly Positive
Positive control		1217 ± 103.7	3663 ± 158.3	2080 ± 16.3
Preincubation protocol				
TA1535	0	12 ± 2.1	9 ± 2.3	9 ± 1.2
	30	11 ± 2.3	8 ± 1.2	11 ± 0.3
	100	9 ± 1.2	12 ± 0.6	8 ± 1.2
	300	13 ± 0.6	15 ± 1.8	6 ± 0.7
	1000	13 ± 1.5	14 ± 1.5	15 ± 1.8
	3000	8 ± 1.5	3 ± 2.0[3]	10 ± 1.9
Trial summary		Negative	Negative	Negative
Positive control		929 ± 43.8	189 ± 31.8	87 ± 19.2
TA1537	0	6 ± 1.5	6 ± 0.3	9 ± 1.2
	30	8 ± 0.9	10 ± 0.6	10 ± 1.5
	100	6 ± 1.5	8 ± 2.3	5 ± 1.5
	300	5 ± 1.2	8 ± 1.7	7 ± 0.3
	1000	5 ± 0.9	7 ± 1.0	7 ± 1.8
	3000	2 ± 1.2	5 ± 1.8[3]	3 ± 2.2
Trial summary		Negative	Negative	Negative
Positive control		320 ± 29.3	298 ± 11.7	158 ± 6.1
TA98	0	17 ± 1.8	29 ± 3.3	26 ± 1.5
	30	17 ± 2.0	25 ± 3.2	17 ± 2.8
	100	22 ± 4.3	25 ± 3.2	22 ± 5.3
	300	21 ± 1.5	25 ± 3.2	25 ± 0.7
	1000	18 ± 2.0	45 ± 6.1	15 ± 2.7
	3000	10 ± 0.3	24 ± 4.9	9 ± 2.0
Trial summary		Negative	Negative	Negative
Positive control		1444 ± 58.4	2739 ± 69.7	2270 ± 61.5

TABLE E2 Mutagenicity of 4-Chloronitrobenzene in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate							
		-S9		+10% hamster S9			+10% rat S9		
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
STUDY PERFORMED AT SRI, INTERNATIONAL									
TA100	0.0	113 ± 6.7					85 ± 3.2		
	100.0	117 ± 8.4					113 ± 7.5		
	333.3	122 ± 6.6					163 ± 5.5		
	666.7	136 ± 3.8					313 ± 14.1		
	1000.0	141 ± 6.1					321 ± 19.9		
	3333.3	73 ± 11.8[3]					8 ± 8.0[3]		
Trial summary		Negative					Positive		
Positive control		461 ± 12.2					620 ± 9.5		
TA1535	0.0	12 ± 3.2		11 ± 1.7			12 ± 1.7		
	100.0	14 ± 3.5		12 ± 1.3			8 ± 2.0		
	333.3	21 ± 4.0		8 ± 2.3			12 ± 1.2		
	666.7	33 ± 1.5		49 ± 7.2			43 ± 0.7		
	1000.0	37 ± 4.4		70 ± 5.2			56 ± 4.8		
	3333.3	33 ± 8.7		6 ± 3.8[3]			9 ± 1.5[3]		
Trial summary		Positive		Positive			Positive		
Positive control		409 ± 42.7		303 ± 14.4			198 ± 12.2		
TA98	0.0			40 ± 3.4			31 ± 3.7		
	100.0			38 ± 4.0			33 ± 3.5		
	333.3			32 ± 3.5			32 ± 10.3		
	666.7			50 ± 15.4			48 ± 6.8		
	1000.0			70 ± 10.7			53 ± 4.6		
	3333.3			19 ± 12.6[3]			29 ± 12.5[3]		
Trial summary				Equivocal			Negative		
Positive control				1186 ± 38.4			350 ± 6.8		
TA100	0.0	124 ± 9.9	108 ± 1.8	131 ± 7.3	108 ± 11.7	109 ± 11.0	138 ± 7.0	176 ± 1.2	121 ± 7.5
	3.3	135 ± 2.6		145 ± 4.7			127 ± 19.6		
	33.3	129 ± 17.0		147 ± 5.6			131 ± 8.9		
	100.0	146 ± 5.8	109 ± 4.7	150 ± 0.9	117 ± 2.4	130 ± 4.8	170 ± 9.2	173 ± 6.8	140 ± 6.2
	333.3	126 ± 5.3	112 ± 9.4	150 ± 8.4	135 ± 7.4	189 ± 6.9	209 ± 9.8	182 ± 7.9	169 ± 8.2
	666.7		114 ± 7.4[3]		135 ± 5.8	322 ± 38.3		200 ± 6.7	215 ± 4.2[3]
	1000.0	155 ± 6.6	137 ± 3.2[3]	159 ± 9.2	214 ± 17.0	360 ± 29.4[3]	258 ± 13.3	259 ± 10.7	266 ± 17.0[3]
	3333.3		30 ± 6.7[3]		371 ± 26.1	231 ± 12.8[3]		325 ± 24.2	47 ± 15.4[3]
Trial summary		Equivocal	Negative	Negative	Positive	Positive	Positive	Weakly Positive	Positive
Positive control		550 ± 8.1	574 ± 21.1	556 ± 9.5	442 ± 16.7	1540 ± 28.6	596 ± 29.5	621 ± 5.1	761 ± 40.8

TABLE E2 Mutagenicity of 4-Chloronitrobenzene in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3
TA1535	0.0	25 ± 6.0	15 ± 2.4	24 ± 3.7	14 ± 0.9	15 ± 1.7	12 ± 1.2
	3.3	23 ± 2.0			18 ± 1.8		
	33.3	25 ± 2.2			15 ± 1.2		
	100.0	26 ± 3.8	19 ± 2.9	24 ± 2.2	24 ± 2.8	28 ± 5.0	11 ± 2.3
	333.3	31 ± 3.3	23 ± 2.7	23 ± 4.7	53 ± 0.9	44 ± 4.4	13 ± 2.3
	666.7		23 ± 3.3[3]	45 ± 1.9[3]		58 ± 8.9	46 ± 5.0
	1000.0	73 ± 3.1[3]	27 ± 5.2[3]	47 ± 6.1[3]	71 ± 2.7	56 ± 6.7	65 ± 2.6[3]
	3333.3		20 ± 0.6[3]	28 ± 4.2[3]		36 ± 3.3[3]	36 ± 12.3[3]
Trial summary		Equivocal	Negative	Weakly Positive	Positive	Positive	Positive
Positive control		438 ± 5.3	515 ± 4.9	406 ± 25.2	367 ± 6.2	303 ± 4.4	253 ± 25.1

TA1535 (continued)

Strain	Dose (μg/plate)	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3
	0.0	12 ± 2.9	18 ± 1.3	11 ± 0.9
	3.3	12 ± 2.9		
	33.3	15 ± 2.5		
	100.0	18 ± 3.0	21 ± 4.3	9 ± 2.3
	333.3	30 ± 2.8	28 ± 4.9	11 ± 2.5
	666.7		55 ± 5.2	45 ± 5.6[3]
	1000.0	60 ± 8.4	62 ± 2.3	78 ± 10.9[3]
	3333.3	40 ± 2.7	37 ± 3.4[3]	
Trial summary		Positive	Positive	Positive
Positive control		334 ± 51.3	197 ± 2.8	186 ± 17.7

Strain	Dose (μg/plate)	Revertants/plate -S9	+10% hamster S9	+10% rat S9
TA1537	0.0	11 ± 2.7	26 ± 3.5	20 ± 0.3
	3.3	14 ± 2.5	23 ± 1.5	15 ± 4.5
	33.3	16 ± 1.7	23 ± 2.5	22 ± 2.0
	100.0	11 ± 4.5	23 ± 4.4	26 ± 3.5
	333.3	12 ± 4.0	21 ± 4.7	22 ± 1.2
	1000.0	7 ± 0.6	15 ± 0.9	18 ± 2.3
Trial summary		Negative	Negative	Negative
Positive control		163 ± 24.0	467 ± 13.0	287 ± 3.6

TABLE E2 Mutagenicity of 4-Chloronitrobenzene in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% hamster S9 Trial 3	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA98	0.0	25 ± 2.4	35 ± 3.4	42 ± 4.9	38 ± 7.6	22 ± 5.0	35 ± 2.7
	3.3	21 ± 1.9	22 ± 1.0			26 ± 3.8	
	33.3	18 ± 3.0	31 ± 4.2			28 ± 3.7	
	100.0	17 ± 1.0	35 ± 1.9	42 ± 5.2	44 ± 8.0	26 ± 2.9	36 ± 4.7
	333.3	19 ± 3.1	50 ± 5.5	48 ± 8.0	44 ± 5.8	29 ± 0.3	41 ± 4.8
	666.7			60 ± 2.2	57 ± 12.8		32 ± 3.8
	1000.0	22 ± 3.3	86 ± 1.5	68 ± 4.0	65 ± 5.9	41 ± 4.4	32 ± 1.0[3]
	3333.3			39 ± 4.7[3]	33 ± 4.8[3]		17 ± 5.8[3]
Trial summary		Negative	Equivocal	Equivocal	Equivocal	Equivocal	Negative
Positive control		474 ± 16.3	934 ± 19.9	1058 ± 69.7	1271 ± 233.3	388 ± 25.1	434 ± 5.7

[1] The detailed protocol is presented in Haworth *et al.* (1983). 0 μg/plate is the solvent control.
[2] Revertants are presented as mean ± standard error from three plates.
[3] Slight toxicity.
[4] The positive controls in the absence of metabolic activation were sodium azide (TA1535 and TA100), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 2-Chloronitrobenzene[1]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)[2]
Study performed at Columbia University								
-S9								
Trial 1								
Summary: Weak positive								
Dimethylsulfoxide		50	1043	426	0.40	8.5	26.0	
Mitomycin-C	0.005	25	523	732	1.39	29.3	26.0	242.68
2-Chloronitrobenzene	5	50	1045	440	0.42	8.8	26.0	3.09
	16	50	1044	450	0.43	9.0	26.0	5.53
	50	50	1047	528	0.50	10.6	26.0	23.47*
								P=0.001[3]
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1049	428	0.40	8.6	26.0	
Mitomycin-C	0.005	25	525	619	1.17	24.8	26.0	188.98
2-Chloronitrobenzene	30	50	1047	454	0.43	9.1	26.0	6.28
	40	50	1047	427	0.40	8.5	26.0	-0.04
	50	50	1048	547	0.52	10.9	26.0	27.93*
	60	50	1044	473	0.45	9.5	26.0	11.04
	75	15	310	163	0.52	10.9	26.0	28.87*
								P<0.001
+S9								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1050	451	0.42	9.0	26.0	
Cyclophosphamide	1	50	1048	1117	1.06	22.3	26.0	148.14
2-Chloronitrobenzene	50	50	1051	480	0.45	9.6	26.0	6.33
	160	50	1047	500	0.47	10.0	26.0	11.18
	500	50	1049	511	0.48	10.2	26.0	13.41
								P=0.019

TABLE E3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 2-Chloronitrobenzene (continued)

Compound	Dose (μg/mL)	Total Cells	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)
Study performed at Sitek Laboratories								
-S9								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1049	386	0.36	7.7	26.0	
Mitomycin-C	0.001	50	1050	618	0.58	12.4	26.0	59.95
	0.004	10	210	265	1.26	26.5	26.0	242.94
2-Chloronitrobenzene	5	50	1052	450	0.42	9.0	26.0	16.25
	17	50	1049	443	0.42	8.9	26.0	14.77
	50	50	1047	453	0.43	9.1	26.0	17.58
								P=0.016
+S9								
Trial 1								
Summary: Weakly positive								
Dimethylsulfoxide		50	1052	412	0.39	8.2	26.0	
Cyclophosphamide	0.125	50	1047	647	0.61	12.9	26.0	57.79
	0.500	10	209	221	1.05	22.1	26.0	170.00
2-Chloronitrobenzene	50	50	1050	418	0.39	8.4	26.0	1.65
	167	50	1051	439	0.41	8.8	26.0	6.65
	500	50	1041	568	0.54	11.4	26.0	39.32*
								P<0.001

TABLE E3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 2-Chloronitrobenzene (continued)

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)
+S9 (continued)								
Trial 2								
Summary: Weakly positive								
Dimethylsulfoxide		50	1050	409	0.38	8.2	26.0	
Cyclophosphamide	0.125	50	1052	502	0.47	10.0	26.0	22.50
	0.500	10	210	216	1.02	21.6	26.0	164.06
2-Chloronitrobenzene	63	50	1048	453	0.43	9.1	26.0	10.97
	125	50	1049	432	0.41	8.6	26.0	5.72
	250	50	1051	503	0.47	10.1	26.0	22.87*
								P=0.003

[1] SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the protocol is presented by Galloway *et al.* (1987).
[2] Percentage increase in SCEs/chromosome of culture exposed to 2-chloronitrobenzene relative to those of culture exposed to solvent.
[3] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose.
* Positive (>20% increase over solvent control).

TABLE E4 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 4-Chloronitrobenzene[1]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Increase over Solvent (%)[2]
-S9								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1042	412	0.39	8.2	25.7	
Mitomycin-C	0.01	50	1037	1689	1.62	33.8	25.7	311.93
4-Chloronitrobenzene	100	50	1034	394	0.38	7.9	25.7	-3.63
	150	50	1034	481	0.46	9.6	30.4[3]	17.65
								P=0.007[4]
+S9								
Trial 1								
Summary: Positive								
Dimethylsulfoxide		50	1042	235	0.22	4.7	25.5	
Cyclophosphamide	2	50	1044	1349	1.29	27.0	25.5	472.96
4-Chloronitrobenzene	250	50	1029	367	0.35	7.3	25.5	58.15*
	400	50	1030	359	0.34	7.2	25.5	54.55*
	500	50	1027	427	0.41	8.5	25.5	84.36*
								P<0.001
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1040	318	0.30	6.4	25.5	
Cyclophosphamide	1.5	50	1041	1342	1.28	26.8	25.5	321.62
4-Chloronitrobenzene	300	50	1038	421	0.40	8.4	25.5	32.65*
	400	50	1040	409	0.39	8.2	25.5	28.62*
	500	50	1027	547	0.53	10.9	28.5	74.19*
								P<0.001

TABLE E4 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by 4-Chloronitrobenzene (continued)

[1] Study performed at Litton Bionetics, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the protocol is presented by Galloway *et al.* (1987).
[2] Percentage increase in SCEs/chromosome of culture exposed to 4-chloronitrobenzene relative to those of culture exposed to solvent.
[3] Because 4-chloronitrobenzene induced a delay in the cell division cycle, harvest time was extended to maximize the proportion of second division cells available for analysis.
[4] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose.
* Positive (>20% increase over solvent control).

TABLE E5 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 2-Chloronitrobenzene[1]

-S9					+S9				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
Study performed at Columbia University									
Trial 1 — Harvest time: 14.0 hours Summary: Equivocal					**Trial 1** — Harvest time: 14.0 hours Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	2	0.02	2.0		100	4	0.04	4.0
Mitomycin-C					Cyclophosphamide				
0.15	50	21	0.42	34.0	15	50	14	0.28	26.0
2-Chloronitrobenzene					2-Chloronitrobenzene				
16	100	8	0.08	7.0	50	100	8	0.08	8.0
50	100	8	0.08	8.0	160	100	6	0.06	6.0
160	100	11	0.11	9.0*	500	100	6	0.06	6.0
				P=0.023[2]					P=0.353
Study performed at Sitek Research Laboratories									
Trial 1 — Harvest time: 18.5 hours[3] Summary: Negative					**Trial 1** — Harvest time: 13.6 hours Summary: Weak positive				
Dimethylsulfoxide					Dimethylsulfoxide				
	200	3	0.02	1.5		200	3	0.02	1.5
Mitomycin-C					Cyclophosphamide				
0.4	25	66	2.64	88.0	20	25	13	0.52	40.0
2-Chloronitrobenzene					2-Chloronitrobenzene				
47	200	2	0.01	1.0	101	200	2	0.01	1.0
101	200	0	0.00	0.0	216	200	0	0.00	0.0
216	200	2	0.01	1.0	465	200	22	0.11	9.0*
				P=0.802					P<0.001

TABLE E5 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 2-Chloronitrobenzene (continued)

-S9					+S9				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
					Trial 2 — Harvest time: 13.6 hours				
					Summary: Weakly positive				
					Dimethylsulfoxide				
						200	3	0.02	1.5
					Cyclophosphamide				
					20	25	29	1.16	56.0
					2-Chloronitrobenzene				
					125	200	2	0.01	1.0
					250	200	3	0.02	1.5
					500	100	23	0.23	20.0*
									P<0.001

[1] Abs = aberrations. A detailed presentation of the protocol is found in Galloway *et al.* (1985, 1987).
[2] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose.
[3] Because 2-chloronitrobenzene induced significant cell cycle delay, incubation time prior to addition of Colcemid was lengthened to provide sufficient metaphases at harvest.
* Positive (P<0.05).

TABLE E6 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 4-Chloronitrobenzene[1]

-S9					+S9				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
Trial 1 — Harvest time: 10.5 hours Summary: Negative					**Trial 1** — Harvest time: 10.5 hours Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	2	0.02	2.0		100	4	0.04	4.0
	100	1	0.01	1.0					
					Cyclophosphamide				
Mitomycin-C					25	100	24	0.24	20.0
1	100	22	0.22	19.0					
					4-Chloronitrobenzene				
4-Chloronitrobenzene					50	100	4	0.04	4.0
50	100	1	0.01	1.0	167	100	5	0.05	5.0
167	100	2	0.02	2.0	500	100	1	0.01	1.0
500	100	0	0.00	0.0	5000	100	2	0.02	2.0
				P=0.665[2]					P=0.881
Trial 2 — Harvest time: 10.6 hours Summary: Weakly positive					**Trial 2** — Harvest time: 19.0 hours[3] Summary: Positive				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	2	0.02	2.0		100	0	0.00	0.0
	100	4	0.04	3.0					
					Cyclophosphamide				
Mitomycin-C					50	50	236	4.72	88.0
1	100	16	0.16	16.0					
					4-Chloronitrobenzene				
4-Chloronitrobenzene					600	35	6	0.17	17.0*
700	100	1	0.01	1.0	800	100	38	0.38	31.0*
800	84	5	0.06	6.0	900	100	4	0.04	3.0
900	33	8	0.24	24.0*					
				P<0.001					P=0.002

TABLE E6 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by 4-Chloronitrobenzene (continued)

-S9					+S9				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
Trial 3 — Harvest time: 19.0 hours[3]									
Summary: Weakly positive									
Dimethylsulfoxide									
	100	1	0.01	1.0					
	100	0	0.00	0.0					
Mitomycin-C									
1	100	26	0.26	21.0					
4-Chloronitrobenzene									
500	100	0	0.00	0.0					
600	50	0	0.00	0.0					
700	22	1	0.05	5.0*					
				P=0.013					

[1] Study performed at Litton Bionetics, Inc. Abs = aberrations. A detailed presentation of the protocol is found in Galloway *et al.* (1987).
[2] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose.
[3] Because 4-chloronitrobenzene induced significant cell cycle delay, incubation time prior to addition of Colcemid was lengthened to provide sufficient metaphases at harvest.
* Positive ($P<0.05$).

TABLE E7 Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by 2-Chloronitrobenzene[1]

Route of Exposure	Dose (ppm)	Incidence Deaths (%)	Incidence Sterility (%)	No. of Lethals/No. of X Chromosomes Tested Mating 1	Mating 2	Mating 3	Total[2]
Study performed at Brown University; data published in Zimmering *et al.* (1989)							
Larva feeding	59	1	0	1/1349	1/1344		2/2693 (0.07%)
	0			2/1341	0/1332		2/2673 (0.07%)
Larva feeding	60	10	0	3/1309	1/1326		4/2635 (0.15%)
	0			1/1281	0/1234		1/2515 (0.04%)
Study performed at Bowling Green State University; data published in Zimmering *et al.* (1985)							
Feeding	125	8	9	4/3481	1/1759	0/1847	5/7087 (0.07%)
	0			1/2282	0/785	1/520	2/3587 (0.06%)
Injection	10,000	15	9	0/3065	0/2635	1/2450	1/8150 (0.01%)
	0			1/1455	1/1345	4/1160	6/3960 (0.15%)

[1] Results were not significant at the 5% level (Margolin *et al.*, 1983).
[2] Total number of lethal mutations/total number of X chromosomes tested for up to three mating trials.

TABLE E8 Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by 4-Chloronitrobenzene[1]

Route of Exposure	Dose (ppm)	Incidence of Deaths (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested Mating 1	Mating 2	Mating 3	Total[2]
Study reported in Zimmering *et al.* (1989)							
Larva feeding	77	34	1	3/1307	1/1363		4/2670 (0.15%)
	0			1/1333	0/1320		1/2653 (0.04%)
Larva feeding	79	36	0	0/1311	0/1360		0/2671 (0.00%)
	0			0/1349	0/1327		0/2676 (0.00%)
Study reported in Zimmering *et al.* (1985)							
Feeding	100	23	8	4/4708	0/4736	6/4648	10/14,092 (0.07%)
	0			9/4681	3/4682	1/4717	13/14,080 (0.09%)
Injection	100	16	0	3/2374	3/2326	0/2377	6/7077 (0.08%)
	0			3/2308	2/2268	1/2111	6/6687 (0.09%)

[1] Studies performed at Brown University. Results were not significant at the 5% level (Margolin *et al.*, 1983).
[2] Total number of lethal mutations/total number of X chromosomes tested for up to three mating trials.

APPENDIX F

Disposition and Metabolism Studies

Studies of Chemical Disposition in Mammals

(Summary)

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DISPOSITION AND METABOLISM STUDIES

Introduction

A series of studies was performed in male F344 rats to assess the disposition and metabolism of 2-chloronitrobenzene and 4-chloronitrobenzene. Each isomer was tested independently, using the appropriate [^{14}C]-labeled compound as a tracer. The studies conducted included assessments of the disposition and metabolism of 2-chloronitrobenzene and 4-chloronitrobenzene following a single oral dose in young adult rats and following repeated oral dosing in young adult and geriatric rats, and determination of the absorption of each isomer following topical administration to young adult rats.

Single-dose studies were conducted to provide comparative data on the disposition and metabolism of [^{14}C]-labeled 2-chloronitrobenzene and 4-chloronitrobenzene following oral administration. The studies were designed to determine the effect of dose on absorption, extent of metabolism, relative amounts of parent compound and metabolites excreted, and rate of excretion. The dose levels selected for these studies were 2, 20, and 200 mg/kg. The high dose was selected based on the results of previous studies of 4-chloronitrobenzene, in which the administration of 200 mg/kg 4-chloronitrobenzene orally to rats resulted in methemoglobin production (Ridley *et al.*, 1983); the lower doses correspond to 0.1 and 0.01 fractions of the high dose. Additionally, tissue residues of 2-chloronitrobenzene and 4-chloronitrobenzene were determined at 24 hours, the time at which peak levels of methemoglobin (41%) had been observed in rats following 4-chloronitrobenzene administration, and at 72 hours, the time at which methemoglobin levels had decreased to 12% (Ridley *et al.*, 1983).

Repeated-dose studies were conducted to investigate the effect of the administration of multiple oral doses on the disposition and metabolism of 2-chloronitrobenzene and 4-chloronitrobenzene. In these studies, rats received 65 mg/kg 2- or 4-chloronitrobenzene daily by gavage for 11 days, with administration of [^{14}C]-labeled compound on Days 1, 5, and 9. The dose selected for these studies, 65 mg/kg, was based on the results of the single-dose studies and was chosen to be intermediate between the mid and high doses used in the single-dose studies. An interval of 4 days between the administration of each [^{14}C]-labeled dose was chosen based on the results of the single-dose studies, which showed that each radiolabeled dose should be cleared within this time frame.

One objective of the repeated-dose studies was to compare the disposition and metabolism profiles of [^{14}C]-labeled 2-chloronitrobenzene and 4-chloronitrobenzene administered at a dose level of 65 mg/kg on Day 1 to those observed in the single-dose studies at doses of 2, 20, and 200 mg/kg. In addition, the

effect of 4 or 8 days of pretreatment with 65 mg/kg of each compound (unlabeled) on the disposition and metabolism of labeled compound administered on Days 5 and 9 was also evaluated.

Similar repeated-dose studies, using a dose level of 65 mg/kg, were performed in geriatric rats to assess the effect of age on the disposition and metabolism of 2-chloronitrobenzene and 4-chloronitrobenzene.

Dermal absorption studies were performed to investigate the effect of dose on the absorption of 2-chloronitrobenzene and 4-chloronitrobenzene following topical application to male rats. The dose levels chosen were 0.65, 6.5, and 65 mg/kg. The 65 mg/kg dose was selected as the high dose because it was used extensively in the repeated oral dose studies; the lower doses correspond to 0.1 and 0.01 fractions of the high dose. The dermal route was selected because it is a major route of human exposure.

Materials and Methods

[^{14}C]-2-Chloronitrobenzene (ring labeled, Lot 2405-111) and [^{14}C]-4-chloronitrobenzene (ring labeled, Lot 2405-112) were obtained from New England Nuclear Research Products (Boston, MA). Each radiochemical was supplied in solution in methylene chloride, was 97% to 99% radiochemically pure, and had an activity of 51.17 mCi/mmol. Unlabeled 2-chloronitrobenzene (Lot ET 00210KM) and 4-chloronitrobenzene (Lot ET 02513BT) were obtained from Aldrich Chemicals (Milwaukee, WI).

In the oral dose studies, separate solutions of [^{14}C]-labeled 2-chloronitrobenzene and [^{14}C]-labeled 4-chloronitrobenzene were prepared in corn oil at the concentrations appropriate for each study. Similar solutions of unlabeled 2-chloronitrobenzene and 4-chloronitrobenzene were also prepared. Solutions of [^{14}C]-2-chloronitrobenzene and [^{14}C]-4-chloronitrobenzene were diluted with the corresponding solutions of unlabeled compound to obtain dose formulations with specific activities appropriate for achieving the desired sensitivities for detection of the compounds in tissue samples. Dose formulations for the dermal absorption studies were prepared in a similar manner, except that acetone was used as the vehicle.

Dose formulations were administered by gavage in the oral dose studies and by topical application to clipped dorsal skin in the dermal absorption studies. Following compound administration, urine and feces were collected at various intervals prior to sacrifice; urine was expressed from the bladder of each rat at the time of sacrifice and was combined with the final urine sample. The cages were then thoroughly washed with water. At sacrifice, blood was removed by cardiac puncture and transferred into heparinized Vacutainer® tubes (Becton-Dickinson, Rutherford, NJ); plasma and blood cells were separated by centrifugation and frozen on dry ice. Liver, kidney, heart, lung, brain, spleen, thymus, testes, and representative samples of adipose tissue and skeletal muscle were removed, weighed, and immediately

frozen on dry ice. All carcasses and urine, feces, cage wash, plasma, and tissue samples were stored at -10° C or below.

All radioactivity determinations were performed with a Tracor Analytic Mark III liquid scintillation system. Radioactivity in duplicate 0.1 mL samples of urine, plasma, and cage wash was quantified in ACS®. Duplicate samples of fecal paste and tissue preparations were combusted with a Packard Tricarb Oxidizer Model 306, and the [^{14}C]-radioactivity was trapped in Carbosorb® II (Packard Instrument Company, Inc., Downers Grove, IL) and was quantified in ACS® (single oral dose and repeated oral dose studies) or Permafluor® (oral dose studies in geriatric rats and dermal absorption studies). In the oral administration studies, urine samples were also analyzed for metabolites of 2-chloronitrobenzene and 4-chloronitrobenzene by high-performance liquid chromatography (HPLC). Additional details concerning study design and conduct are provided below.

SINGLE-DOSE STUDIES

In the single-dose studies, the concentrations of the [^{14}C]-labeled 2-chloronitrobenzene test solutions were 0.47, 4.02, and 40.0 mg/mL; concentrations of the [^{14}C]-labeled 4-chloronitrobenzene solutions were 0.47, 4.5, and 45.1 mg/mL. The dosing solutions were administered at 5 mL/kg to achieve doses of approximately 2, 20, and 200 mg/kg.

Groups of eight male F344 rats were administered 2, 20, or 200 mg/kg [^{14}C]-2-chloronitrobenzene or [^{14}C]-4-chloronitrobenzene by gavage. Urine and feces were collected from four rats per group for the entire 24 hour period between compound administration and sacrifice; for the remaining four rats per group, samples were collected at 0 through 4, 4 through 8, 8 through 24, 24 through 48, and 48 through 72 hours after compound administration, and the rats were sacrificed after 72 hours. Urine samples from three rats per dose group were analyzed for metabolites by HPLC. In addition to the tissues mentioned previously, bone marrow samples were also obtained at sacrifice.

REPEATED-DOSE STUDIES IN YOUNG ADULT AND GERIATRIC RATS

In the repeated-dose studies, the concentration of all [^{14}C]-labeled and unlabeled test solutions of 2-chloronitrobenzene and 4-chloronitrobenzene was 20 mg/mL of corn oil, administered at 3.25 mL/kg to achieve a dose of 65 mg/kg.

Groups of four young adult (10- to 12-week-old) or geriatric (19- to 20-month-old) male F344 rats received 65 mg/kg [^{14}C]-2-chloronitrobenzene or [^{14}C]-4-chloronitrobenzene by gavage on Days 1, 5, and 9.

Unlabeled 2- or 4-chloronitrobenzene in corn oil was administered at a dose level of 65 mg/kg on Days 2, 3, 4, 6, 7, 8, 10, and 11.

Urine and feces were collected at 0 through 4, 4 through 8, 8 through 24, 24 through 48, 48 through 72, and 72 through 96 hours after the administration of the first and second doses of the radiolabeled compound and at 0 through 4, 4 through 8, 8 through 24, 24 through 48, and 48 through 72 hours after the third dose of the radiolabeled compound. Following the determination of radioactivity in the urine samples from individual rats, the samples for each collection interval were pooled and were analyzed for metabolites by HPLC. The rats were transferred to clean Nalgene metabolism cages after administration of the radiolabeled dose on Days 5 and 9; cages were thorougly washed following transfer and again at the time of sacrifice. Blood samples (0.5 to 1.0 mL) for the determination of methemoglobin levels were obtained from the retroorbital sinus on the day before dosing, on Days 4 and 8, and at sacrifice on Day 12; methemoglobin levels were determined using a model 282 CO-Oximeter® system (Instrumentation Laboratory, Inc., Lexington, MA). The time of sacrifice on Day 12 corresponded to 72 hours after administration of the third dose of the radiolabeled compound and 24 hours after the last dose of unlabeled compound.

DERMAL ABSORPTION STUDIES

In the dermal absorption studies, the concentrations of [^{14}C]-labeled test solutions of 2-chloronitrobenzene and 4-chloronitrobenzene were 4, 40, and 400 mg/mL in acetone.

Groups of three male F344 rats received a single topical application of 0.65, 6.5, or 65 mg/kg [^{14}C]-2-chloronitrobenzene or [^{14}C]-4-chloronitrobenzene; the dose volume was approximately 163 µL/kg body weight. Each dose was applied evenly to a 4 cm^2 clipped area of the dorsal skin using an electronic digital pipette (Rainin Instrument Company, Woburn, MA). The treated area was covered with a nonocclusive protective device to restrict contact to the area of application and to prevent ingestion during grooming.

Urine and feces were collected at 0 through 4, 4 through 8, 8 through 24, 24 through 48, and 48 through 72 hours after application. Although urinary excretion of radiolabel was measured, urinary metabolite determinations were not performed. Volatiles were collected in ethanol traps at 0 through 4, 4 through 8, 8 through 24, 24 through 32, 32 through 48, 48 through 56, and 56 through 72 hours after compound application. Rats were sacrificed 72 hours after compound application. At sacrifice, the protective device covering the application site was carefully removed and saved for analysis. The application site was swabbed with gauze pads soaked in acetone; the swabs were saved for analysis. The area of clipped skin,

including the application site and the surrounding area, was excised and frozen on dry ice. All samples were stored at -10° C or below. Although samples of additional organs and tissues were collected at sacrifice, the only tissue analyzed was the skin at the application site.

ANALYSIS OF DISPOSITION STUDY DATA

Compound equivalents in biological samples were determined by dividing the dpm in the sample by the specific activity of the compound in dpm/nmol. Radioactivity in tissues was expressed as a percentage of the radioactivity administered to each rat both as a percentage per gram of tissue and as a total percentage per organ or tissue. Radioactivity in urine, feces, and cage washes was expressed as a percentage of the radioactivity administered to each rat, for each time interval and as a cumulative percentage. In the dermal absorption studies, radioactivity in ethanol traps, exposed skin, the protective device, and application site swabs was also expressed as a percentage of radioactivity administered to each rat. For repeated dose studies, radioactivity in samples was expressed relative to the last dose of radioactive compound administered prior to sample collection. Urinary metabolites were expressed as a percentage of the total radioactivity excreted during each time interval and as a percentage of the radioactivity administered to each rat, for each time interval and as a cumulative percentage. For all data, the means and standard deviations for each treatment group were calculated. For the sake of clarity, standard deviations are not presented in the data tables.

Results

ORAL ADMINISTRATION STUDIES

The results of the oral dose studies are presented in Tables F1 through F7.

Single-Dose Studies

In the oral dose studies, an estimate of the minimum extent of absorption was determined from the sum of radioactivity, expressed as percent of dose administered, collected in urine and that remaining in tissues at 72 hours. Following oral administration of 2, 20, or 200 mg/kg, at least 61% to 77% of the 2-chloronitrobenzene dose and at least 73% to 78% of the 4-chloronitrobenzene dose was absorbed (Tables F3 and F4). For 2-chloronitrobenzene, the extent of absorption was similar at the lower doses and increased somewhat at the high dose, while for 4-chloronitrobenzene, the extent of absorption was similar at all dose levels.

At all dose levels, the radioactivity was excreted primarily via the urinary route for both 2-chloronitrobenzene and 4-chloronitrobenzene. For 2-chloronitrobenzene, a greater percentage of

radioactivity was excreted in the urine and less was excreted in the feces at the high dose than at the lower doses; the decrease in fecal excretion at the highest dose suggests that the radioactivity defecated had entered the intestinal tract by biliary secretion. In addition, for both compounds, radioactivity was excreted in the urine and feces more slowly following administration of 200 mg/kg of 2- or 4-chloronitrobenzene than after administration of the lower doses; slow fecal excretion was also apparent at the lower doses of 4-chloronitrobenzene, suggesting the involvement of biliary secretion.

The concentration of [^{14}C]-2-chloronitrobenzene or [^{14}C]-4-chloronitrobenzene equivalents in tissues was measured 24 and 72 hours after dosing. The results, expressed in terms of percentage of dose administered, are presented in Tables F1 and F2. At the lower doses, about 6% and 3% of the administered 2-chloronitrobenzene dose and about 23% and 5% of the administered 4-chloronitrobenzene dose were found in the tissues at 24 and 72 hours, respectively. At the highest dose, which was more slowly excreted, the fractions increased to about 20% and 4% for 2-chloronitrobenzene at 24 and 72 hours, respectively, and to about 35% and 7% for 4-chloronitrobenzene at 24 and 72 hours, respectively. Although the absolute concentration of radioactivity increased with dose in all tissues examined for both compounds, the relative distribution of the administered dose and the clearance rate from various tissues varied with dose and with the isomer administered.

For 2-chloronitrobenzene, the concentration of radioactivity in all tissues (not shown) was proportional to dose at the two lower dose levels, and most tissues contained less than 0.1% of the dose administered (Table F1). However, at the high dose of 200 mg/kg [^{14}C]-2-chloronitrobenzene, a disproportionately greater percentage of administered radioactivity was found in all tissues except the liver, which contained a lower percentage of the administered dose than at the lower dose levels. In all tissues and at all dose levels, the concentration of 2-chloronitrobenzene equivalents declined between 24 and 72 hours. At 24 hours after administration of 2 or 20 mg/kg [^{14}C]-2-chloronitrobenzene, most of the radioactivity in the tissues was in the liver (4%), followed by fat, muscle, and kidney; the radioactivity was most concentrated in the liver and kidney. At the high dose of 200 mg/kg, the greatest percentage of radioactivity was in the fat (13%), followed by muscle, liver, and kidney; the radioactivity was most concentrated in fat, followed by kidney and liver. Seventy-two hours after dosing, the highest concentration of radiolabel and the greatest percentage of administered dose occurred in the liver for the lower dose groups; at the high dose at 72 hours, the greatest percentage of the administered dose of [^{14}C]-2-chloronitrobenzene also occurred in the liver, although the radiolabel was more concentrated in the kidney than in the liver and fat.

For 4-chloronitrobenzene, in all tissues with the exception of fat, the concentration of 4-chloronitrobenzene equivalents was proportional to dose (not shown); at the more slowly excreted highest dose, the concentrations in fat were disproportionately higher than at the lower doses at both time intervals (Table F2). In most tissues, the concentration of 4-chloronitrobenzene equivalents declined between 24 and 72 hours; however, at all dose levels, the concentration of 4-chloronitrobenzene equivalents in blood cells and spleen were essentially the same at both time points, indicating the retention of equivalents in these tissues. For all dose levels, at 24 hours the highest concentrations of equivalents were found in the fat, followed by the blood cells, kidney, liver, and spleen; at 72 hours, the highest concentrations were found in the blood cells due to the retention of equivalents in this tissue, followed by fat and spleen. At 24 hours, the greatest percentage of the radioactivity was in the fat (15% to 28%), followed by blood cells (2% to 3%) for all dose levels. At 72 hours, the greatest percentage of the radioactivity was in the blood cells (3%) for the lower dose levels and in fat (4%) and blood cells (2%) for the highest dose level.

Radioactivity in urine samples from all dose groups was resolved by HPLC analysis into up to 23 metabolites for 2-chloronitrobenzene (assigned the numbers I through XXIII) and up to 25 metabolites for 4-chloronitrobenzene (assigned letters A through Y).

For 2-chloronitrobenzene, the relative proportion of the metabolites excreted in urine was similar for the 2 and 20 mg/kg dose groups. There was one major metabolite, XXI, which accounted for 27% of the administered radioactivity, and a less major metabolite, XIX, which accounted for 8% of the administered dose. The other 21 metabolites were minor, each representing less than 5% of the dose. At the highest dose, which was more slowly excreted, the fractions excreted as metabolite XXI (23%) and XIX (6%) were relatively unchanged as compared with the lower dose groups. However, metabolite XI increased from 3% of the dose at the lower dose levels to 21% at the highest dose, and metabolite XV increased from 1% to 6% at the highest dose. The increase in metabolite XI was largely responsible for the increase in the fraction of the dose excreted in the urine at the highest dose and may reflect such changes as saturation of metabolic pathways and/or biliary secretion of metabolites at the highest dose level.

For 4-chloronitrobenzene, as with 2-chloronitrobenzene, the relative proportions of the metabolites excreted in urine were similar for the 2 and 20 mg/kg dose groups. There was one major metabolite, M, which accounted for 19% of the administered radioactivity, and four less major metabolites, F, O, Q, and W, each of which accounted for at least 5% of the administered dose. The other 20 metabolites were minor, each representing less than 5% of the dose. There was evidence of some disproportionate changes at the more slowly excreted highest dose (Table F4); the most notable change occurred for metabolite M,

which increased from about 13% of the dose at the lower dose levels to 19% of the dose at the highest dose level. In addition, the percentage of the administered radioactivity excreted as metabolite Q increased and that excreted as metabolite O decreased at the highest dose.

Repeated-Dose Studies in Young Adult Rats

2-Chloronitrobenzene: The disposition and metabolic characteristics of [^{14}C]-2-chloronitrobenzene following oral administration of a single 65 mg/kg dose were compared with those obtained in the single-dose study, following administration of 2, 20, or 200 mg/kg [^{14}C]-2-chloronitrobenzene (Table F3). The characteristics at 65 mg/kg were intermediate between those observed for the lower doses and those observed for the 200 mg/kg dose. At the 65 mg/kg dose level, a greater fraction of the administered dose was excreted in the urine and a lower fraction in the feces than at the lower doses; the fraction excreted in the urine was similar to that observed at the 200 mg/kg dose, but the fraction excreted in feces was greater. The rates of urinary excretion were similar at 2, 20, or 65 mg/kg and were faster than observed at 200 mg/kg. The rate of fecal excretion was slower at 65 mg/kg than at the lower doses but faster than at the 200 mg/kg dose level. The minimum extent absorption of 65 mg/kg 2-chloronitrobenzene was 71%, greater than the absorption observed at the lower doses (61%) but less than that observed at 200 mg/kg (77%). The pattern of urinary metabolite excretion after 65 mg/kg was also intermediate between that observed after the lower doses and that observed after 200 mg/kg. At all four dose levels, metabolites XXI and XIX were the major metabolites excreted; the percentages of radioactivity excreted as these two metabolites after 65 mg/kg 2-chloronitrobenzene were similar to those observed at the 200 mg/kg dose and were slightly lower than the percentages observed at the two lower doses. At 65 mg/kg, fractions excreted as some of the more minor metabolites, such as X and XV, were similar to those observed at the lower doses and differed significantly from the percentages observed at the highest dose. The percentage of the dose excreted as metabolite XI was greater than at the lower doses, but was less than that observed at the highest dose.

After 0, 4, or 8 days of pretreatment with 2-chloronitrobenzene, [^{14}C]-2-chloronitrobenzene-derived radioactivity was excreted primarily in the urine and primarily during the first 24 hours (Table F5). A similar fraction of the dose (71% to 74% for urine; 20% to 27% for feces) was excreted at all three treatment intervals. Radioactivity was excreted more rapidly in both urine and feces following pretreatment. In 0 to 8 hours, about 47% to 51% of the administered radioactivity was excreted in the urine after 4 or 8 days of pretreatment as compared with 26% without pretreatment; in the first 24 hours after administration, only 5% of the administered radioactive dose was excreted in the feces without pretreatment, whereas 18% to 21% was excreted in feces after 4 or 8 days of pretreatment. The more

rapid fecal excretion following pretreatment is consistent with enhanced biliary secretion of 2-chloronitrobenzene.

HPLC analysis of urine samples revealed the presence of up to 24 metabolites, including metabolites I through XXIII as identified in the single-dose studies and an additional metabolite designated as XIIA. At all treatment intervals, metabolite XXI was the major metabolite, accounting for about 20% of the administered radioactivity, and metabolites XI (8% to 13%) and XIX (4% to 6%) were less major metabolites. The fractions of the dose excreted as metabolites XXI and XIX were unaffected by pretreatment with 2-chloronitrobenzene, whereas metabolite XI decreased. The other 21 metabolites were minor without pretreatment, each representing less than 5% of the administered dose. The fraction of the dose excreted as several of these metabolites, including metabolites I, XIII, XVI, XVIII, increased with pretreatment, while the fraction of the dose excreted as metabolite II decreased with pretreatment.

Approximately 5% of the administered radioactivity was found in the tissues 72 hours after the Day 9 dose (Tables F1 and F5). Most of the radioactivity was in the liver (Table F1). The highest concentrations of radioactivity were in the liver and kidney.

Methemoglobin levels increased from about 1% in control blood obtained prior to treatment to about 6% on Days 4 and 8 and decreased slightly to 5% at sacrifice on Day 12 (Table F7). The increases were about one-third those observed in the repeated-dose study of 4-chloronitrobenzene.

4-Chloronitrobenzene: The disposition and metabolism of [^{14}C]-4-chloronitrobenzene following oral administration of a single 65 mg/kg dose were compared with those obtained in the single-dose study following administration of 2, 20, or 200 mg/kg [^{14}C]-4-chloronitrobenzene (Table F4). The extent of absorption of 65 mg/kg 4-chloronitrobenzene (74%) and the extent of urinary excretion (71%) were similar to those observed for all dose levels in the single-dose study. The rate of urinary and fecal excretion following administration of 65 mg/kg were similar to those observed for the two lower doses in the single-dose study, however; radioactivity was excreted more slowly at the 200 mg/kg dose than at the 2, 20, or 65 mg/kg dose levels. The same 25 metabolites of 4-chloronitrobenzene were identified in the urine in the single-dose and repeated-dose studies. The urinary metabolite profile obtained following the administration of a single dose of 65 mg/kg 4-chloronitrobenzene shared characteristics with the profiles observed for the lower dose levels and with the profile observed for the 200 mg/kg dose level. The percentage of the dose excreted as metabolites M and Q increased for both the 65 and 200 mg/kg dose groups, but the proportion of metabolite O in the urine after the 65 mg/kg dose remained similar to the

percentage seen for 2 and 20 mg/kg and did not decrease as was observed at the highest dose of 200 mg/kg.

After 0, 4, or 8 days of pretreatment with 65 mg/kg 4-chloronitrobenzene, [^{14}C]-4-chloronitrobenzene-derived radioactivity was excreted primarily via the urinary route (74% to 81% of the administered dose) (Table F6). Radioactivity was excreted more rapidly and slightly more extensively in the urine following pretreatment than after the initial dose. The 24-hour urinary excretion increased from 43% after the initial dose to 53% and 61%, respectively, following 4 and 8 days of pretreatment. The extent of urinary excretion increased from 74% after the initial dose to about 80% after 4 and 8 days of pretreatment. Pretreatment also resulted in more rapid fecal excretion, but only after 8 days of pretreatment. Minimum extent absorption increased from 74% without pretreatment to 81% to 82% after pretreatment.

Pretreatment with 65 mg/kg 4-chloronitrobenzene did not significantly alter the urinary metabolite profile for 4 (F, O, Q, and W) of the five major metabolites. However, the percentage of administered radioactivity excreted as metabolite M increased from 18% with no pretreatment to 25% following 4 or 8 days of pretreatment. In addition, the rate of excretion of metabolite M increased with pretreatment; the 0-to-24-hour excretion of metabolite M increased from 10% without pretreatment to 18% to 19% following pretreatment.

Approximately 2% of the administered radioactivity was found in the tissues 72 hours after the Day 9 dose (Tables F2 and F6). Most of the radioactivity was in blood cells and fat (Table F2). The highest concentrations of radioactivity were found in blood cells and spleen.

Methemoglobin levels increased from about 1% in control blood taken prior to treatment with 4-chloronitrobenzene to about 20% on Days 4 and 8 and decreased to about 14% at sacrifice on Day 12 (Table F7). The administration of 65 mg/kg 4-chloronitrobenzene resulted in methemoglobin levels approximately three times those observed following the administration of a similar dose of 2-chloronitrobenzene.

Oral Administration Studies in Geriatric Rats

2-Chloronitrobenzene: Disposition characteristics in geriatric rats given a single 65 mg/kg dose of 2-chloronitrobenzene paralleled those seen in young adult rats given a similar dose (Table F3). Geriatric rats exhibited slightly greater absorption, extent of urinary excretion, and extent of fecal excretion than young adults given 65 mg/kg; however, these observations may be a reflection of the much greater total recovery of radioactivity achieved for geriatric rats at this time point.

For all dose levels and for both young and geriatric rats, metabolite XXI was the major metabolite observed in the urine (Table F3); the percentage of the dose excreted as metabolite XXI was slightly greater in geriatric rats than in young adults given a single dose of 65 mg/kg 2-chloronitrobenzene and was similar to the fraction excreted by young rats given 2 or 20 mg/kg. A similar pattern was noted for metabolite XIX. The percentage of the dose excreted as metabolite XI was greater in geriatric rats than in young adults given 2 or 20 mg/kg 2-chloronitrobenzene but was less than observed in young adults given 65 mg/kg. Several metabolites that were considered to be of minor importance in young adults given 2, 20, or 65 mg/kg 2-chloronitrobenzene represented a greater fraction of excreted radioactivity in geriatric rats; these included metabolites I, X, and XV.

Minor changes occurred in the disposition and metabolic characteristics of 2-chloronitrobenzene in geriatric rats with pretreatment (Table F5). After repeated administration of 65 mg/kg 2-chloronitrobenzene, the fraction of the dose excreted in the urine was slightly lower, while the fraction excreted in feces remained unchanged. As in young adult rats given multiple doses of 2-chloronitrobenzene, the rate of fecal excretion by geriatric rats increased with pretreatment, but not to the same extent as observed in younger animals. The rate of urinary excretion (0 to 24 hours) decreased slightly after 8 days of pretreatment for geriatric rats, in contrast to findings in young adults at 0 to 8 hours and at 0 to 24 hours. In the geriatric rats, the fractions of the dose excreted as urinary metabolites XIX, XXI, and X decreased with pretreatment, while metabolite XI increased. In young adult rats, metabolites XIX and XXI were relatively unchanged with pretreatment and metabolite XI appeared to slightly decrease.

A slightly greater fraction (8%) of the administered radioactivity was found in the tissues of geriatric rats than in those of young adult rats (5%) 72 hours after the Day 9 dose (Tables F1 and F5). As in young adults, most of the radioactivity was in the liver (6%) (Table F1) and the highest concentrations of radioactivity were in the liver and kidney.

The administration of 2-chloronitrobenzene to geriatric rats resulted in an increase in methemoglobin levels similar to that seen in young adult rats, although a decrease after 8 days of pretreatment was not apparent in geriatric animals (Table F7).

4-Chloronitrobenzene: Individual variation in the values of distribution parameters among the geriatric rats was notable; nevertheless, trends were evident. The disposition and metabolic characteristics of 4-chloronitrobenzene in geriatric rats following administration of a single 65 mg/kg dose were more similar to those observed in young adult rats given 200 mg/kg than to those observed in young adults

given 2, 20, or 65 mg/kg (Table F4). The extent of fecal excretion was notably lower in geriatric rats than in young adults regardless of the dose administered. However, the extent of urinary excretion (0 to 96 hours) and therefore the minimum extent absorption in geriatric rats were similar to those in young adults. The urinary metabolite profile for metabolites M, O, and Q in geriatric rats given 65 mg/kg 4-chloronitrobenzene was similar to that observed in young adults given 200 mg/kg. However, geriatric rats had lower levels of metabolites F and W and higher levels of metabolite P than young adults.

Pretreatment of geriatric rats with 65 mg/kg 4-chloronitrobenzene did not affect the rate or extent of urinary excretion, but resulted in an increase in the extent of fecal excretion (Table F6). This is in contrast to the results for young adults, in which the rate and extent of urinary excretion increased with pretreatment while the extent of fecal excretion remained relatively constant with pretreatment. In both young and geriatric rats, the extent of absorption increased with pretreatment, although the increase was not apparent until after 8 days of pretreatment in the older animals.

As observed in young adult rats, the rate of excretion of the major metabolite, M, increased with pretreatment in geriatric rats, but the effect was less marked. Excretion of metabolites O, Q, and W also decreased with pretreatment in geriatric rats.

The percentage of [^{14}C]-4-chloronitrobenzene-derived radioactivity retained in the tissues of the geriatric rats (17%) was much greater than that retained in young adults (2%) 72 hours after the Day 9 dose (Tables F2 and F6). Most of the radioactivity was retained in the fat (11%), although 4% was retained in skeletal muscle and 2% was retained in the blood cells. The highest concentrations of radioactivity occurred in the fat (30% of dose/100 g tissue), followed by blood cells, testes, and spleen (11%, 9%, and 8% per 100 g tissue, respectively).

The administration of 4-chloronitrobenzene to geriatric rats resulted in an increase in methemoglobin levels slightly greater than that observed in young adults given a similar dose (Table F7). As observed in young adults, the methemoglobin levels exhibited an initial increase and then decreased slightly on Day 12.

DERMAL ABSORPTION STUDIES

The results of the dermal absorption studies are presented in Tables F8 and F9. The extent of dermal absorption was determined directly from the sum of radioactivity, expressed as percent of dose administered, collected in urine, feces, and cage washings. At the three doses tested, 0.65, 6.5, and 65 mg/kg, at least 33% to 40% of the 2-chloronitrobenzene dose and at least 51% to 62% of the

4-chloronitrobenzene dose was absorbed from the skin of rats within 72 hours under the nonocclusive conditions of these studies. The majority of the unabsorbed dose was recovered in the ethanol traps and in the protective device covering the application site; radioactivity in the ethanol traps was identified by HPLC as parent compound and represented 2- or 4-chloronitrobenzene that had evaporated from the application site. A greater percentage of the applied radioactivity was recovered in the ethanol traps for 2-chloronitrobenzene (27% to 32%) than for 4-chloronitrobenzene (13% to 15%), which suggests that the lower dermal absorption of 2-chloronitrobenzene may be due to the more rapid evaporation of the compound from the application site.

For 2-chloronitrobenzene, the extent of absorption increased with an increase in dose from 0.65 to 6.5 mg/kg, but increased only negligibly when the dose was increased to 65 mg/kg. For 4-chloronitrobenzene, only a negligible increase in absorption was noted when the dose increased from 0.65 to 6.5 mg/kg, but greater absorption occurred at the high dose of 65 mg/kg. These changes were not considered to be significant, however. (The extent of dermal absorption of 2-chloronitrobenzene and 4-chloronitrobenzene was not significantly affected by dose.)

For both 2-chloronitrobenzene and 4-chloronitrobenzene, the radioactivity was excreted primarily in the urine and, to a lesser extent, in the feces. Urinary excretion of radiolabel was greater for [^{14}C]-4-chloronitrobenzene (43% to 45% of the administered dose) than for [^{14}C]-2-chloronitrobenzene (21% to 28%), although this may be a reflection of the greater absorption of 4-chloronitrobenzene. The extent of urinary excretion of radioactivity was not significantly affected by dose over the range studied for either compound; however, the extent of fecal excretion of 4-chloronitrobenzene increased with dose. The initial rate of urinary excretion was also unaffected by dose for 2-chloronitrobenzene (16% to 21% in 24 hours), and was similar over the 0.65 to 6.5 mg/kg dose range for 4-chloronitrobenzene (26% to 28% in 24 hours); however, the rate of urinary excretion of 4-chloronitrobenzene was much lower at the high dose (12% in 24 hours). For both compounds, the initial rate of fecal excretion increased with dose over the 0.65 to 6.5 mg/kg range, but decreased notably at the high dose.

Discussion

The absorption and disposition of 2-chloronitrobenzene and 4-chloronitrobenzene were studied in adult and geriatric rats following oral administration and in adult rats following dermal administration. The oral and dermal routes were selected to support inhalation studies because these are considered to be likely additional routes of human exposure, because dermatitis has been associated with dermal exposure, and because it is thought that metabolism following exposure by either of these routes would be similar to that following inhalation. The fates of these compounds were studied in adult and geriatric

animals in an effort to detect possible age-related variations in absorption and clearance which might be relevant to predictions of toxicity to individuals of varying ages exposed in the work place. Metabolites of the chlorinated nitrobenzenes were isolated and quantified by HPLC. The metabolites were not identified because earlier metabolism studies by Bray *et al.* (1956) and Ridley *et al.* (1983) determined that chloronitrobenzenes are metabolized to various combinations of the respective hydroxylated chloronitrobenzenes, chlorinated anilines, and hydroxylated chlorinated anilines, each of which could undergo additional metabolism or one or more conjugation reactions.

Results of studies of the disposition of these chlorinated nitrobenzenes, administered orally at doses of 2, 20, 65, and 200 mg/kg, established that each of these compounds was readily absorbed from the gastrointestinal tract and rapidly metabolized and excreted. Absorption was linear with dose, and at the two lower doses accounted for at least 60% of the dose; absorption may have increased somewhat at the higher doses. The 200 mg/kg dose was more slowly excreted, but the slower excretion may have been due to the greater time necessary for absorption and metabolism or may reflect greater biliary excretion and reabsorption. Radioactivity derived from [^{14}C]-2-chloronitrobenzene was excreted in one major metabolite accounting for 27% of the total dose and in 22 to 23 lesser metabolites, evidencing extensive metabolism. Radioactivity derived from [^{14}C]-4-chloronitrobenzene was excreted in one major metabolite accounting for 19% of the total dose and in 24 minor metabolites. Metabolism was altered somewhat by dose in that, with 2-chloronitrobenzene and 4-chloronitrobenzene, some of the minor metabolites became more significant as the dose was increased.

The absorption, metabolism, and excretion of these compounds were not greatly affected by the age of the animals dosed. Geriatric animals, 19- to 20-month-old male rats, absorbed, metabolized, and cleared these compounds as readily as young adults at doses up to 65 mg/kg, the highest dose studied in geriatric animals, and the same metabolic patterns were seen in young and geriatric animals. When groups of adult or geriatric animals were dosed daily for up to 9 days with 65 mg/kg 2- or 4-chloronitrobenzene, variations in the rates of excretion in urine and feces were also minor. The relative amounts of major metabolites excreted varied little throughout the period. Greater variations were observed in the relative amounts of some of the minor metabolites excreted in urine, but none became major metabolites or ceased to be observed. It appears that absorption does not change with age and that the enzymes responsible for metabolism are not diminished to the point of being challenged by this dose in aged animals.

Studies of dermal absorption indicated that 2-chloronitrobenzene and 4-chloronitrobenzene are each relatively easily absorbed from the skin. Results of these studies indicated dermal absorption of

approximately 40% of each dose of 2-chloronitrobenzene and approximately 60% of each dose of 4-chloronitrobenzene. The absorbed material was readily metabolized and excreted as observed when administered orally. Absorption of 4-chloronitrobenzene is speculated to have been greater than that of 2-chloronitrobenzene, because the lower vapor pressure of 4-chloronitrobenzene resulted in lower losses due to volatilization from the skin. Another parameter, methemoglobin formation, showed marked variation with the compound administered, in agreement with other results of these studies. Administration of 1 to 11 daily doses of 65 mg/kg 2-chloronitrobenzene resulted in the formation of approximately 6% methemoglobin in blood, and this amount was sustained through the course of dosing. On the other hand, administration of similar daily doses of 4-chloronitrobenzene resulted in the formation of 20% methemoglobin after one dose, and this amount decreased to approximately 15% by Day 12. As with other parameters, formation of methemoglobin did not appear to be age dependent.

In summary, studies of 2-chloronitrobenzene and 4-chloronitrobenzene established that these compounds are readily absorbed from the gastrointestinal tract and skin, rapidly metabolized to a large number of metabolites, and rapidly excreted, primarily in urine. The disposition and metabolite profile of these compounds were quite similar and were apparently unaffected by age at doses up to 65 mg/kg. The only parameter that varied with compound was the induction of methemoglobin, which was three- to four-fold greater following administration of 4-chloronitrobenzene.

TABLE F1 Percentage of Dose in Tissues of Male F344 Rats at 24 and 72 Hours After Administration of the Final Dose of [^{14}C]-2-Chloronitrobenzene[1]

Tissue	Time (hours)	Single-Dose Study (mg/kg)			Repeated-Dose Studies[2]	
		2	20	200	Young	Geriatric
Plasma	24	0.05	0.07	0.39		
	72	0.01	0.01	0.07	0.02	0.06
Blood Cells	24	0.07	0.10	0.31		
	72	0.05	0.07	0.14	0.10	0.17
Liver	24	4.20	4.36	2.82		
	72	2.25	2.34	1.57	3.42	6.04
Kidney	24	0.20	0.25	0.65		
	72	0.12	0.14	0.50	0.52	0.80
Heart	24	<0.01	<0.01	0.02		
	72	<0.01	<0.01	0.00	0.01	0.01
Lung	24	0.01	0.01	0.05		
	72	0.01	0.01	0.02	0.01	0.01
Brain	24	<0.01	0.01	0.04		
	72	<0.01	<0.01	0.01	<0.01	0.00
Fat	24	0.61	0.92	12.47		
	72	0.09	0.10	1.18	0.29	0.52
Skeletal Muscle	24	0.28	0.36	4.03		
	72	0.10	0.09	0.38	0.28	0.56
Spleen	24	<0.01	<0.01	0.02		
	72	0.01	<0.01	0.02	0.02	0.02
Thymus	24	<0.01	<0.01	0.01		
	72	<0.01	<0.0	<0.01	<0.01	<0.01
Testes	24	0.01	0.01	0.05		
	72	<0.01	<0.01	0.01	0.02	0.02
Bone Marrow[3]	24	<0.01	0.00	<0.01		
	72	0.00	—	<0.01	—	—
Total	24	5.42	6.08	20.85		
	72	2.64	2.75	3.90	4.67	8.21

[1] Percentages were calculated from the organ weights and were based on the assumptions that plasma = 3.75%, blood cells = 3.75%, fat = 9.5%, and skeletal muscle = 47.5% of body weight.

[2] In the repeated-dose studies of young or geriatric rats administered 65 mg/kg 2-chloronitrobenzene, tissue samples were collected on Day 12, 72 hours after the administration of the final radiolabeled dose of 2-chloronitrobenzene and 24 hours after administration of the final dose of unlabeled 2-chloronitrobenzene.

[3] Mean percentage for 20 mg/kg dose at 72 hours was not calculated because radioactivity in all samples was less than twice background for the system. Bone marrow was not collected in the repeated-dose studies.

TABLE F2 Percentage of Dose in Tissues of Male F344 Rats at 24 and 72 Hours After Administration of the Final Dose of [^{14}C]-4-Chloronitrobenzene[1]

Tissue	Time (hours)	Single-Dose Study (mg/kg)			Repeated-Dose Studies[2]	
		2	20	200	Young	Geriatric
Plasma	24	0.30	0.33	0.42		
	72	0.03	0.04	0.08	0.03	0.14
Blood Cells	24	2.60	3.12	2.22		
	72	2.82	3.07	2.33	0.95	1.50
Liver	24	0.76	0.84	0.90		
	72	0.22	0.25	0.30	0.18	0.46
Kidney	24	0.26	0.30	0.34		
	72	0.05	0.06	0.09	0.05	0.13
Heart	24	0.03	0.04	0.04		
	72	0.02	0.02	0.02	0.01	0.02
Lung	24	0.04	0.05	0.06		
	72	0.03	0.03	0.03	0.02	0.07
Brain	24	0.03	0.04	0.05		
	72	0.01	0.01	0.01	0.00	0.02
Fat	24	15.05	17.56	28.14		
	72	0.80	1.40	3.68	0.59	10.66
Skeletal Muscle	24	1.68	2.09	2.80		
	72	0.30	0.32	0.45	0.19	3.75
Spleen	24	0.04	0.04	0.06		
	72	0.03	0.04	0.09	0.15	0.19
Thymus	24	0.01	0.01	0.02		
	72	<0.01	<0.01	<0.01	<0.01	0.03
Testes	24	0.05	0.05	0.07		
	72	0.01	0.01	0.02	0.01	0.07
Bone Marrow[3]	24	<0.01	<0.01	<0.01		
	72	0.00	0.00	0.00	—	—
Total	24	20.84	24.45	35.10		
	72	4.31	5.23	7.09	2.17	17.03

[1] Percentages were calculated from the organ weights and were based on the assumptions that plasma = 3.75%, blood cells = 3.75%, fat = 9.5%, and skeletal muscle = 47.5% of body weight.

[2] In the repeated-dose studies of young or geriatric rats administered 65 mg/kg 4-chloronitrobenzene, tissue samples were collected on Day 12, 72 hours after the administration of the final radiolabeled dose of 4-chloronitrobenzene and 24 hours after administration of the final dose of unlabeled 4-chloronitrobenzene.

[3] Bone marrow was not collected for the repeated-dose studies.

TABLE F3 Comparison of the Disposition and Metabolism of [^{14}C]-2-Chloronitrobenzene after a Single Oral Dose in Geriatric Male F344 Rats Administered 65 mg/kg with Those in Young Adult Male Rats Administered Doses of 2, 20, 65, or 200 mg/kg[1]

Parameter	Collection Period (hours)	Dose (mg/kg)				
		2	20	65		200
				adult	geriatric	
Minimum Extent Absorption[2]		62	61	71	85	77
Excreted Urine	0 - 24	56.4	53.0	60.6	63.5	39.2
	0 - 72	59.6	57.7	70.1	83.5	73.5
	0 - 96	—	—	70.9	85.1	—
Excreted Feces	0 - 24	21.9	19.8	5.4	1.4	0.0
	0 - 72	28.2	26.3	16.8	21.4	6.9
	0 - 96	—	—	19.9	22.4	—
Tissues	24	5.4	6.1	—	—	20.9
	72	2.6	2.8	—	—	3.9
Total Recovery[3]		91.2	88.0	93.1	107.5	85.9
Urinary Metabolites[4]						
I		0.6	0.7	1.8	6.4	0.1
X		2.9	2.4	3.2	6.3	0.2
XI		2.9	3.2	12.7	5.6	21.1
XV		0.4	1.0	0.3	4.5	5.9
XIX		8.2	8.4	6.4	7.4	5.9
XXI		27.3	26.4	21.4	25.2	23.2
Other Metabolites[5]		15.9	16.9	26.1	29.8	17.1
Total Metabolites		58.2	59.0	71.9	85.2	73.5

[1] Mean fractions of dose (%) for data from groups of three or four rats or duplicate analyses of pooled urine samples from three or four rats.

[2] Equal to the percent of the dose excreted in urine in 0-72 hours or 0-96 hours with the percent of dose in tissues at 72 hours. Extent of absorption was probably greater as there was evidence of biliary secretion.

[3] Recovery period of 0-96 hours for 65 mg/kg; 0-72 hours for all other doses.

[4] Recovery period of 0-48 hours for 2 and 20 mg/kg; 0-72 hours for 200 mg/kg; 0-96 hours for 65 mg/kg.

[5] Total of 16-18 other metabolites, each of which accounted for less than 5% of the dose.

TABLE F4 Comparison of the Disposition and Metabolism of [^{14}C]-4-Chloronitrobenzene after a Single Oral Dose in Geriatric Male F344 Rats Administered 65 mg/kg with Those in Young Adult Male Rats Administered Doses of 2, 20, 65, or 200 mg/kg[1]

Parameter	Collection Period (hours)	Dose (mg/kg)				
		2	20	65		200
				adult	geriatric	
Minimum Extent Absorption[2]		78	73	74	72	75
Excreted Urine	0 - 24	52.9	41.2	42.6	32.7	25.5
	0 - 72	73.9	68.2	70.9	63.1	68.0
	0 - 96	—	—	73.9	71.6	—
Excreted Feces	0 - 24	6.6	2.1	4.1	0.7	0.2
	0 - 72	11.8	10.3	12.9	4.7	12.3
	0 - 96	—	—	13.5	6.9	—
Tissues	24	20.8	24.5	—	—	35.1
	72	4.3	5.2	—	—	7.1
Total Recovery[3]		93.2	86.3	91.1	78.5	90.2
Urinary Metabolites[3]						
F		8.2	5.9	4.2	1.7	3.6
M		11.5	13.9	18.1	20.1	19.1
O		12.7	12.0	12.3	9.2	9.1
P		3.9	3.4	2.3	5.1	1.9
Q		8.2	8.7	16.1	12.3	11.1
W		13.7	10.2	11.1	9.4	12.8
Other Metabolites[4]		15.3	12.7	11.4	13.8	7.6
Total Metabolites		73.5	66.8	75.5	71.6	65.2

[1] Mean fractions of dose (%) for data from groups of three or four rats, or duplicate analyses of pooled urine samples from three or four rats.

[2] Equal to the percent of the dose excreted in urine in 0-72 hours or 0-96 hours, with the percent of dose in tissues at 72 hours. Extent of absorption was probably greater, as there was evidence of biliary secretion.

[3] Recovery period of 0-96 hours for 65 mg/kg; 0-72 hours for all other doses.

[4] Total of 19 other metabolites, each of which represented less than 5% of the dose.

TABLE F5 Effect of Pretreatment with 2-Chloronitrobenzene on the Disposition and Metabolism of [^{14}C]-2-Chloronitrobenzene in Young and Geriatric Male F344 Rats at Intervals During 11 Days of Treatment[1]

Parameter	Collection Period (hours)	Duration of Pretreatment with 65 mg/kg 2-chloronitrobenzene (days)					
		0		4		8	
		young	geriatric	young	geriatric	young	geriatric
Minimum Extent Absorption[2]		71	85	74	75	78	79
Excreted Urine	0 - 8	26.2	21.1	47.4	16.0	51.1	26.6
	0 - 24	60.6	63.5	70.8	61.3	69.6	52.2
	0 - 72	70.1	83.5	73.7	73.5	73.5	70.8
	0 - 96	70.9	85.1	74.4	75.1	—	—
Excreted Feces	0 - 24	5.4	1.4	18.1	7.0	21.4	7.5
	0 - 72	16.8	21.4	22.5	21.0	26.6	18.9
	0 - 96	19.9	22.4	23.0	21.7	—	—
Tissues	72	—	—	—	—	4.7	8.2
Total Recovery[3]		93.1	107.5	98.2	96.9	105.3	100.4
Urinary Metabolites[3]							
I		1.8	6.4	5.8	5.9	5.7	5.2
II		4.7	0.6	1.2	0.5	1.1	0.9
VI		0.8	3.4	0.7	5.9	1.0	5.2
VII		1.7	2.9	5.6	6.0	1.4	4.9
IX		0.3	3.6	0.5	4.7	0.4	4.2
X		3.2	6.3	4.8	1.1	1.7	—[4]
XI		12.7	5.6	10.7	8.7	8.0	10.1
XIII		0.7	4.4	0.3	4.6	4.2	4.3
XVI		0.8	0.6	0.9	1.3	5.7	1.2
XVIII		0.1	0.2	—[4]	0.9	3.2	1.1
XIX		6.4	7.4	4.2	3.6	4.7	2.6
XXI		21.4	25.2	18.3	13.1	20.1	10.6
Other Metabolites[5]		17.3	18.6	27.3[6]	18.8	16.3	20.5
Total Metabolites		71.9	85.2	74.5	75.1	73.5	70.8

[1] Data are given as a mean fraction of dose (percentage of radioactivity administered on Day 1, 5, or 9) for groups of three or four young or 4 geriatric rats, or duplicate analyses of pooled urine samples from three or four rats. Animals received 65 mg/kg [^{14}C]-2-chloronitrobenzene by gavage on Days 1, 5, and 9. Unlabeled 2-chloronitrobenzene was administered at a dose level of 65 mg/kg on Days 2, 3, 4, 6, 7, 8, 10, and 11.

[2] Equal to the percent of the dose excreted in urine in 0-72 hours or 0-96 hours with the dose in tissues at 72 hours. Extent of absorption was probably greater as there was evidence of biliary secretion.

[3] Recovery period of 0-96 hours for 0 and 4 days of pretreatment; 0-72 hours for 8 days of pretreatment.

[4] Not detectable.

[5] Total of 12 (young rats) or 10 (geriatric rats) other metabolites, each of which represented less than 5% of the dose and constituted a similar fraction of the Day 1 and Day 9 radiolabeled doses.

[6] Increase in the fraction as other metabolites due to increase in metabolite VIII from 1.4% of the Day 1 dose to 6.4% of the Day 5 dose.

TABLE F6 Effect of Pretreatment with 4-Chloronitrobenzene on the Disposition and Metabolism of [^{14}C]-4-Chloronitrobenzene in Young and Geriatric Male F344 Rats at Intervals During 11 Days of Treatment[1]

Parameter	Collection Period (hours)	Duration of Pretreatment with 65 mg/kg 2-chloronitrobenzene (days)					
		0		4		8	
		young	geriatric	young	geriatric	young	geriatric
Minimum Extent Absorption[2]		74	72	81	74	82	82
Excreted Urine	0 - 24	42.6	32.7	52.8	33.5	61.4	34.1
	0 - 72	70.9	63.1	78.8	66.1	80.3	65.2
	0 - 96	73.9	71.6	80.7	73.7	—	—
Excreted Feces	0 - 24	4.1	0.7	4.5	2.9	7.1	1.9
	0 - 72	12.9	4.7	13.8	8.7	14.9	10.6
	0 - 96	13.5	6.9	14.2	12.2	—	—
Tissues	72	—	—	—	—	2.2	17.0
Total Recovery[3]		91.1	78.5	97.1	85.9	99.5	98.2
Urinary Metabolites[3]							
F		4.2	1.7	4.1	1.6	5.0	2.0
M	0 - 24	(10.4)	(8.0)	(17.5)	(10.7)	(19.0)	(11.8)
	0-72 or 0-96	18.1	20.1	25.0	24.8	24.6	23.4
O		12.3	9.2	11.9	6.3	10.9	5.4
P		2.3	5.1	3.0	5.2	1.7	4.6
Q		16.1	12.3	17.4	11.0	16.2	8.5
W		11.1	9.4	10.8	8.3	9.6	6.7
Other Metabolites[4]		11.4	13.8	12.8	16.7	12.3	14.5
Total Metabolites		75.5	71.6	85.0	73.9	80.3	65.1

[1] Data are given as mean fraction of dose (percentage of radioactivity administered on Day 1, 5, or 9) for groups of three or four young rats or 4 geriatric rats, or duplicate analyses of pooled urine samples from three or four rats. Animals received 65 mg/kg [^{14}C]-4-chloronitrobenzene by gavage on Days 1, 5, and 9. Unlabeled 4-chloronitrobenzene was administered at a dose level of 65 mg/kg on Days 2, 3, 4, 6, 7, 8, 10, and 11.

[2] Equal to the percent of the dose excreted in urine in 0-72 hours or 0-96 hours with the percent of dose in tissues at 72 hours. Extent of absorption was probably greater, as there was evidence of biliary secretion.

[3] Recovery period of 0-96 hours for 0 and 4 days of pretreatment; 0-72 hours for 8 days of pretreatment.

[4] Total of 19 other metabolites, each of which represented less than 5% of the dose and constituted a similar fraction of the Day 1, Day 5, and Day 9 radiolabeled doses.

TABLE F7 Methemoglobin Levels in the Blood of Male F344 Rats at Intervals During Treatment with 2-Chloronitrobenzene or 4-Chloronitrobenzene at 65 mg/kg Daily for 11 Days[1]

Day	2-Chloronitrobenzene		4-Chloronitrobenzene	
	young	geriatric	young	geriatric
-1	0.9	0.7	1.0	0.8
4	6.4	—[2]	20.6	—[2]
8	5.8	6.1	18.8	25.8
12	4.6	6.2	14.4	17.4

[1] Mean of the data from 4 rats, expressed as percent.
[2] Value not obtained due to instrument malfunction.

TABLE F8 Recovery of [^{14}C] Radioactivity 72 Hours after Dermal Application of [^{14}C]-2-Chloronitrobenzene to Male F344 Rats[1]

Sample	Dose Level (mg/kg)		
	0.65	6.5	65
Exposed skin	0.4	0.7	0.4
Protective device	18.1	4.9	28.5
Gauze	1.1	0.3	0.8
Ethanol trap	32.3	26.5	32.3
Urine	20.5	24.4	27.5
Feces	11.0	14.6	11.7
Cagewash	1.0	1.0	1.2
Total unabsorbed[2]	51.9	32.4	62.0
Total absorbed[3]	32.6	40.0	40.3
Total recovered	84.5	72.4	102.3

[1] Data are given as mean dose recovered (%).
[2] Total unabsorbed represents radioactivity recovered in exposed skin, protective device, gauze, and ethanol traps.
[3] Total absorbed represents radioactivity recovered in urine, feces, and cage washings.

TABLE F9 Recovery of [^{14}C] Radioactivity 72 Hours after Dermal Application of [^{14}C]-4-Chloronitrobenzene to Male F344 Rats[1]

Sample	Dose Level (mg/kg)		
	0.65	6.5	65
Exposed skin	0.8	1.3	1.3
Protective device	15.4	7.7	8.8
Gauze	0.3	0.3	0.3
Ethanol trap	13.4	15.1	14.9
Urine	43.4	43.0	45.4
Feces	5.4	8.3	12.2
Cagewash	2.6	1.9	4.6
Total unabsorbed[2]	29.9	24.4	25.3
Total absorbed[3]	51.3	53.2	62.2
Total recovered	81.2	77.6	87.5

[1] Data are given as mean dose recovered (%).
[2] Total unabsorbed represents radioactivity recovered in exposed skin, protective device, gauze, and ethanol traps.
[3] Total absorbed represents radioactivity recovered in urine, feces, and cagewash.

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Toxicity Report Number	Chemical	Route of Exposure	Publication Number
1	Hexachloro-1,3-butadiene	Dosed Feed	91-3120
2	*n*-Hexane	Inhalation	91-3121
3	Acetone	Drinking Water	91-3122
4	1,2-Dichloroethane	Drinking Water, Gavage	91-3123
5	Cobalt Sulfate Heptahydrate	Inhalation	91-3124
6	Pentachlorobenzene	Dosed Feed	91-3125
7	1,2,4,5-Tetrachlorobenzene	Dosed Feed	91-3126
8	D & C Yellow No. 11	Dosed Feed	91-3127
9	*o*-Cresol *m*-Cresol *p*-Cresol	Dosed Feed	92-3128
10	Ethylbenzene	Inhalation	92-3129
11	Antimony Potassium Tartrate	Drinking Water, I.P. Inject.	92-3130
12	Castor Oil	Dosed Feed	92-3131
13	Trinitrofluorenone	Dermal, Dosed Feed	92-3132
14	*p*-Chloro-α,α,α-Trifluorotoluene	Gavage (corn oil, a-CD)	92-3133
15	*t*-Butyl Perbenzoate	Gavage	92-3134
16	Glyphosate	Dosed Feed	92-3135
17	Black Newsprint Ink	Dermal	92-3340
18	Methyl Ethyl Ketone Peroxide	Dermal	92-3341
19	Formic Acid	Inhalation	92-3342
20	Diethanolamine	Drinking Water, Dermal	92-3343
21	2-Hydroxy-4-Methoxybenzophenone	Dosed Feed, Drinking Water	92-3344
22	N, N-Dimethylformamide	Inhalation	93-3345
23	*o*-Nitrotoluene *m*-Nitrotoluene *p*-Nitrotoluene	Dosed Feed	92-3346
24	1,6-Hexanediamine	Inhalation	93-3347
25	Glutaraldehyde	Inhalation	93-3348
28	Tetrachlorophthalic Anhydride	Gavage	93-3351