National Toxicology Program
Toxicity Report Series
Number 43

NTP Technical Report
on Comparative Toxicity Studies of

o-, *m*-, and *p*-Chloroaniline

(CAS Nos. 95-51-2, 108-42-9, and 106-47-8)

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

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

Note to the Reader

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- 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.

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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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PEER REVIEW

The draft report on the toxicity studies of *o*-chloroaniline, *m*-chloroaniline, and *p*-chloroaniline was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

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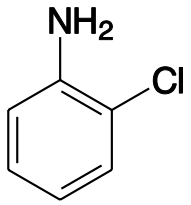
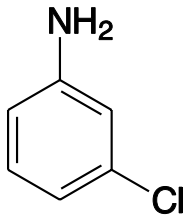
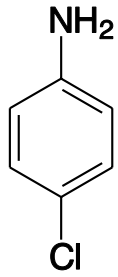
CONTENTS

ABSTRACT

	***o*-Chloroaniline**	***m*-Chloroaniline**	***p*-Chloroaniline**
			
CAS Number	95-51-2	108-42-9	106-47-8
Synonyms	*o*-aminochlorobenzene	*m*-aminochlorobenzene	*p*-aminochlorobenzene
	2-chlorobenzenamine	3-chlorobenzenamine	4-chlorobenzenamine
	2-chloroaniline	3-chloroaniline	4-chloroaniline
	2-chlorophenylamine	3-chlorophenylamine	4-chlorophenylamine

Molecular Formula C_6H_6ClN
Molecular Weight 127.57

Chlorinated anilines are used as intermediates in the manufacture of dyes, drugs, and agricultural agents. In comparative 13-week studies conducted to determine the structure-toxicity relationships of *o*-, *m*-, and *p*-chloroaniline, groups of 10 male and 10 female F344/N rats and B6C3F$_1$ mice were administered 0, 10, 20, 40, 80, or 160 mg *o*- or *m*-chloroaniline per kilogram body weight in dilute hydrochloric acid by gavage. Animals were evaluated for hematology, clinical chemistry, histopathology, and reproductive system effects. Genetic toxicity studies of *o*- and *m*-chloroaniline *in vivo* and *in vitro* were also conducted. The results of the *o*- and *m*-chloroaniline studies were compared to results from the *p*-chloroaniline studies performed previously under similar experimental conditions by the same laboratory; doses in the *p*-chloroaniline studies were 0, 5, 10, 20, 40, and 80 mg/kg for rats and 0, 7.5, 15, 30, 60, and 120 mg/kg for mice.

The hematopoietic system was the target of *o*-, *m*-, and *p*-chloroaniline in rats and mice. Neither the *o*- nor the *p*- isomer had an adverse effect on survival; the death of one female rat in the 160 mg/kg *m*-chloroaniline group during week 12 was possibly secondary to methemoglobinemia. The final mean body weights and weight gains of male rats in the highest dose group in each study and female mice in the 160 mg/kg group in the *o*-chloroaniline study were significantly less than those of the respective controls. Clinical findings of toxicity included a transient bluish discoloration of the genital and footpad regions in rats administered *o*- or *m*-chloroaniline and tremors in rats and mice administered *o*-chloroaniline and in mice administered

m-chloroaniline; these effects occurred primarily in the 80 and 160 mg/kg groups. Methemoglobin concentrations were increased in dosed rats and mice in all studies and resulted in a secondary anemia; the severity of the anemia increased with increasing dose. Microscopic lesions considered related to chemical administration in rats and mice included hemosiderin pigmentation in the bone marrow, kidney, liver, and spleen; hematopoiesis in the liver and spleen; and erythroid cell hyperplasia in the bone marrow. These lesions reflected the response to hemolytic anemia and methemoglobinemia induced by the chloroanilines. A comparative analysis of the results suggests that *p*-chloroaniline is the most potent of the chloroaniline isomers in the induction of methemoglobin formation in rats and mice, followed by *m*-chloroaniline and then by *o*-chloroaniline. This order of potency was also observed for changes in other hematology parameters and in spleen weights, gross and microscopic lesions, and the severity of hemosiderin deposition.

Although the *o*-, *m*-, and *p*- isomers of chloroaniline all exhibit genetic toxicity, the profiles of activity among the three isomers are not identical. *p*-Chloroaniline was mutagenic in all assays in which it was tested, including the *Salmonella* assay, the mouse lymphoma assay, *in vitro* Chinese hamster ovary cell cytogenetics assays, and the *in vivo* mouse bone marrow micronucleus assay; in contrast, *o*- and *m*-chloroaniline gave mixed results among the various assays in which each was tested.

In conclusion, chloroaniline isomers are hematotoxic and have the same pattern of toxicity in rats and mice. Hematotoxicity occurred at all doses in these studies. *p*-Chloroaniline induces the most severe hematotoxic effect, followed by *m*-chloroaniline, then *o*-chloroaniline. Each of the three isomers is more toxic to rats than to mice. *p*-Chloroaniline is clearly genotoxic in various test systems, while the results for the *o*- and *m*- isomers are inconsistent and indicate weak or no genotoxic effects.

INTRODUCTION

PHYSICAL PROPERTIES, PRODUCTION, USE, AND EXPOSURE

o-Chloroaniline and *m*-chloroaniline are synthesized by the reduction of 1-chloro-2-nitrobenzene and 1-chloro-3-nitrobenzene, respectively (*Merck Index*, 1983). Some of the physical properties of these aromatic amines are given in Table 1.

TABLE 1 Physical Properties of *o*-Chloroaniline and *m*-Chloroaniline[a]

Parameter	*o*-Chloroaniline	*m*-Chloroaniline
Boiling point	208.84° C	230.5° C
Density at 22° C	1.12114	1.2150
Vapor pressure	0.17 mm Hg at 20° C	<0.1 mm Hg at 30° C
Solubility	Practically insoluble in water; soluble in acids and organic solvents	Practically insoluble in water; soluble in organic solvents

[a] HSDB, 1995

o-Chloroaniline is used as a dye intermediate and as an intermediate in the manufacture of petroleum solvents, rubber, and fungicides (IARC, 1982; *Hawley's Condensed Chemical Dictionary*, 1987). The Toxic Substances Control Act Chemical Substances Inventory reported in 1977 that between 100,000 and 1,000,000 pounds were produced in the United States and approximately 200,000 pounds were imported (USEPA, 1983).

m-Chloroaniline is used as an intermediate in the production of azo dyes and pigments, pharmaceuticals, insecticides, and herbicides (*Hawley's Condensed Chemical Dictionary*, 1987). Its major use is in the manufacture of the herbicide chlorpropham; in 1978, 4,500,000 grams of *m*-chloroaniline were produced (HSDB, 1995).

Chloroanilines may be released into the environment from process/waste emissions involved in their production or use and may also enter the environment as degradation products of various pesticides (Hargesheimer *et al.*, 1981) or from residues in food (HSDB, 1995). Chloroanilines bond with humic materials in water and soil (Lyman *et al.*, 1982). Detectable levels of *m*-chloroaniline in the Rhine River and its tributaries have been attributed to wastewater effluents from chemical and other industrial plants (Greve and Wegman, 1975). Following the application of the herbicide propanil to irrigated fields, *m*-chloroaniline was identified as a propanil-derived metabolite in fish and algae of irrigation reservoirs (Barabanova and Motoenkov, 1973).

o-Chloroaniline and *m*-chloroaniline have also been designated as hazardous wastes by the U.S. Environmental Protection Agency (40 CFR, Part 61).

Occupational exposure to *o*- and *m*-chloroaniline occurs primarily by dermal contact or inhalation. NIOSH surveys showed that approximately 18,138 workers were exposed to *o*-chloroaniline in the U.S. between 1972 and 1974 (NIOSH, 1990a) and approximately 851 workers were exposed to *m*-chloroaniline between 1981 and 1983 (NIOSH, 1990b).

DISPOSITION AND METABOLISM

Urinary metabolites in an unspecified strain of rats were analyzed 24 hours after the intragastric administration in oil of 150 mg *m*-chloroaniline per kilogram body weight (Böhme and Grunow, 1969). Aromatic hydroxylation was the primary route of metabolism for *m*-chloroaniline-derived material in the urine. *m*-Chloroaniline was preferentially hydroxylated at the carbon opposite to the amino group, forming 2-chloro-4-aminophenol; 38.6% of the administered dose was excreted in this form. A smaller percentage of the administered dose (16.4%) was excreted as *ortho*-hydroxy congeners. Of the two potential forms of *o*-hydroxy metabolites, 2-amino-4-chlorophenol was detected in the greatest amount. The other *o*-hydroxy compound, 2-amino-6-chlorophenol, was found in trace quantities as the glucuronide or sulfate, and 0.8% of the administered dose was excreted unchanged. The phenolic metabolites of *m*-chloroaniline were eliminated solely in the conjugated form; only trace amounts of free phenol were found. *N*-Acetyl derivatives of aminochlorophenols were also detected. Bray *et al.* (1956) reported that in female rabbits administered 0.1 g/kg *o*-chloroaniline (route not specified), *o*-chloroaniline is metabolized to 2-amino-3-chlorophenol and 4-amino-3-chlorophenol.

TOXICITY

The major toxic effect of aniline and its aromatic amine and chlorinated derivatives is methemoglobin formation in erythrocytes, resulting from the oxidation of heme iron from the ferrous to the ferric state (McLean *et al.*, 1969; de Bruin, 1976; Chhabra *et al.*, 1990). Aniline derivatives are only effective as methemoglobin-inducing agents *in vivo*, and it has therefore been speculated that the ultimate hematotoxic species are aminophenol or N-hydroxylamine metabolites (Smith, 1996). Because methemoglobin is physiologically inactive and cannot bind reversibly with oxygen, the cooperativity between heme groups that is required for the loading and unloading of oxygen is compromised, and a smaller proportion of blood oxygen is available for release into tissues. Circulating levels of methemoglobin have been shown to produce a greater impairment in peripheral oxygen transport than an equivalent true anemia produced by a reduction in erythrocyte count (Darling and

Roughton, 1942). Methemoglobinemia is usually a transient effect due to intraerythrocytic mechanisms that facilitate the conversion of methemoglobin to hemoglobin, but it may be sustained under repeated chemical exposure. The resulting generalized hypoxia may lead to secondary central nervous system and cardiac disorders.

Toxic Effects in Humans

Exposure to chloroanilines has been associated with methemoglobinemia, with potential liver and kidney damage, in humans (Benya and Cornish, 1994).

Toxic Effects in Animals

Median lethal inhalation concentrations (LC_{50}) or oral or dermal lethal doses (LD_{50}) of *o*-, *m*-, and *p*-chloroaniline are given in Table 2.

The lowest lethal concentration (LC_{Lo}) of *m*-chloroaniline is 900 ppm in rats exposed by inhalation for 6 hours (RTECS, 1995). LD_{Lo}s of *o*-, *m*-, and *p*-chloroaniline in cats following subcutaneous injection are 310, 125, and 125 mg/kg, respectively (Lehmann, 1933); following intravenous administration, LD_{Lo}s in dogs are 50 mg/kg for *m*-chloroaniline and 100 mg/kg for *p*-chloroaniline (Kiese, 1963).

The formation of methemoglobin was followed over 5 hours in a group of five cats administered 0.25 mmol *o*- or *m*-chloroaniline orally (McLean *et al.*, 1969). For *o*-chloroaniline, the percentage of methemoglobin in the blood was greatest (62%) 2 hours after treatment and was decreased to 41% after 5 hours. Five hours after dosing with *m*-chloroaniline, the methemoglobin concentration was 58%. The methemoglobin-generating potential of *m*-chloroaniline was roughly equivalent to that of aniline and *o*-chloroaniline but was substantially less than that of *p*-chloroaniline. Watanabe *et al.* (1976) reported a 12% increase in methemoglobin 5 hours after rats received a single intraperitoneal injection of *o*-chloroaniline. These studies showed that chloroanilines tend to produce a long-lasting methemoglobin response, which has been associated with the formation of Heinz bodies in erythrocytes (Rentsch, 1968). A similar rank order of methemoglobin-inducing activity for the chloroaniline isomers has been reported in dogs (Kiese, 1963).

TABLE 2 Summary of Selected Animal Toxicity Data for *o*-Chloroaniline, *m*-Chloroaniline, and *p*-Chloroaniline

Species	Route of Exposure	LC_{50} or LD_{50}	Reference
***o*-Chloroaniline**			
Mouse	Inhalation	2.00 mmol/kg	RTECS, 1995 #
Mouse	Dermal	256 mg/kg	RTECS, 1995 #
Cat	Inhalation	1.74 mmol/kg	RTECS, 1995 #
Cat	Dermal	222 mg/kg	RTECS, 1995 #
***m*-Chloroaniline**			
Rat	Oral	256 mg/kg	Malkova, 1966
Rat	Dermal	250 mg/kg	Izmerov *et al.*, 1982
Rat	Intraperitoneal	200 mg/kg	RTECS, 1995
Mouse	Inhalation	550 mg/m^3/4H	Izmerov *et al.*, 1982
Mouse	Inhalation	2.62 mmol/kg	RTECS, 1995
Mouse	Dermal	334 mg/kg	RTECS, 1995
Mouse	Intraperitoneal	200 mg/kg	RTECS, 1995
Guinea pig	Oral	250 mg/kg	Malkova, 1966
Guinea pig	Intraperitoneal	100 mg/kg	RTECS, 1995
Cat	Inhalation	1.75 mmol/kg	RTECS, 1995
Cat	Dermal	223 mg/kg	RTECS, 1995
***p*-Chloroaniline**			
Rat	Oral	300 mg/kg	Khamuev, 1967
Rat	Dermal	3,200 mg/kg	Sziza and Podhragyai, 1957
Rat	Intraperitoneal	420 mg/kg	Sziza and Podhragyai, 1957
Mouse	Oral	100 mg/kg	RTECS, 1995
Mouse	Inhalation	1.79 mmol/kg	RTECS, 1995
Mouse	Dermal	228 mg/kg	RTECS, 1995
Mouse	Intraperitoneal	200 mg/kg	RTECS, 1995
Rabbit	Dermal	360 mg/kg	Smyth *et al.*, 1962
Guinea pig	Oral	350 mg/kg	Izmerov *et al.*, 1982
Cat	Inhalation	1.88 mmol/kg	RTECS, 1995
Cat	Dermal	239 mg/kg	RTECS, 1995

The nephrotoxic potential of aniline and of *o*-, *m*-, and *p*-chloroaniline was assessed in Fischer 344 rats after single intraperitoneal injections ranging from 0.4 to 1.5 mmol/kg (Rankin *et al.*, 1986). *In vitro* tests were also performed in which renal cortical slices were incubated with aniline or a derivative and *p*-aminohippurate (PAH) or tetraethylammonium. Intraperitoneal injection of *o*-chloroaniline (1.0 mmol/kg) caused decreased urine volume, elevated urea nitrogen concentration, and decreased basal and lactate-stimulated PAH accumulation. Eight of 12 rats administered 1.5 mmol/kg *m*-chloroaniline died. Renal effects including hematuria, proteinuria, decreased urine volume, and increased urea nitrogen were observed in animals receiving 1.5 mmol/kg *m*- or *p*-chloroaniline. In rats and mice administered *p*-chloroaniline for 13 weeks, methemoglobin formation, hemolytic anemia, splenomegaly, and extramedullary hematopoiesis were observed (Chhabra *et al.*, 1990).

Reproductive Effects

No sperm abnormalities were observed in male (CBA × BALB/c)F_1 mice administered an intraperitoneal dose of 25 to 500 mg/kg *o*-chloroaniline daily for 5 days (Topham, 1980).

Carcinogenicity

A literature review revealed no references that pertained to prechronic toxicity or chronic carcinogenicity studies of *o*- or *m*-chloroaniline in rodents. In addition, no epidemiologic studies or case reports examining the relationship between exposure to *o*- or *m*-chloroaniline and human cancer were found in the literature (HSDB, 1995).

Aniline hydrochloride has been shown to be carcinogenic in rats (Gralla *et al.*, 1979). Azobenzene, D & C Red No. 9, dapsone, and *o*-toluidine hydrochloride, all structurally related to aniline, are carcinogenic in rats (NCI, 1977, 1979a,b; NTP, 1982). In 2-year studies, groups of 50 male and 50 female F344/N rats were administered 2, 6, or 18 mg *p*-chloroaniline per kilogram body weight in water by gavage; groups of 50 male and 50 female B6C3F_1 mice were administered 3, 10, or 30 mg/kg *p*-chloroaniline (NTP, 1989). There was clear evidence of carcinogenicity in the male rat and equivocal evidence in the female rat, as indicated by an increased number of rare sarcomas of the spleen. Pheochromocytomas also appeared to be associated with *p*-chloroaniline treatment. Compound-related splenic fibrosis was observed in male and female rats. There was some evidence of carcinogenicity in the male mouse, as indicated by increased incidences of hepatocellular neoplasms and hemangiosarcomas of the spleen and liver. There was no evidence of carcinogenic activity in female B6C3F_1 mice that were treated with *p*-chloroaniline for 2 years.

Genetic Toxicity

Positive results were obtained with *o*-chloroaniline in tests for induction of differential growth inhibition in a DNA repair-deficient strain of *Escherichia coli* (Rosenkranz and Poirier, 1979; Thompson *et al.*, 1983); these findings indicate DNA damage. Positive results were also obtained for induction of mutations in L5178Y mouse lymphoma cells (McGregor *et al.*, 1991). These positive responses contrast with the negative results reported for all assays in which *m*-chloroaniline was tested. Other assays in which *o*-chloroaniline was tested yielded negative results. *o*-Chloroaniline did not induce mutations in *E. coli* (Thompson *et al.*, 1983) or *Salmonella typhimurium* (Garner and Nutman, 1977; Rosenkranz and Poirier, 1979; Simmon, 1979; Zimmer *et al.*, 1980; Miyata *et al.*, 1981; Thompson *et al.*, 1983; Zeiger *et al.*, 1987), with or without S9; unscheduled DNA synthesis in Fischer 344 rat hepatocytes exposed *in vitro* (Thompson *et al.*, 1983); or sperm head abnormalities in (CBA × BALB/c)F_1 mice treated with up to 500 mg/kg for 5 days (Topham, 1980). Additional data are needed for a complete assessment of the genotoxicity of *o*-chloroaniline.

The short-term genotoxicity test data available for *m*-chloroaniline, although limited in type and amount, indicate that *m*-chloroaniline is not mutagenic. No induction of gene mutations was observed with *m*-chloroaniline, with or without S9, in *E. coli* (Thompson *et al.*, 1983) or *S. typhimurium* (Garner and Nutman, 1977; Miyata *et al.*, 1981; Thompson *et al.*, 1983; Lyons *et al.*, 1985; Zeiger *et al.*, 1987). Likewise, results of mammalian cell genotoxicity tests with *m*-chloroaniline show no induction of unscheduled DNA synthesis in Fischer 344 rat hepatocytes (Thompson *et al.*, 1983) or sperm head abnormalities in (CBA × BALB/c)F_1 mice treated with up to 400 mg/kg per day for 5 days (Topham, 1980).

STUDY RATIONALE AND DESIGN

o-Chloroaniline and *m*-chloroaniline were selected for study to evaluate the structure-activity relationship for toxicity among the three isomers of chloroaniline. The third isomer, *p*-chloroaniline, has been evaluated previously by the NTP (1989). Gavage was chosen as the route of exposure for *o*-chloroaniline and *m*-chloroaniline because *p*-chloroaniline was studied by that route due to its instability in feed. Endpoints evaluated during these 13-week studies included histopathology and clinical pathology in F344/N rats and B6C3F_1 mice. The reproductive toxicity of *o*-chloroaniline and *m*-chloroaniline was assessed by the evaluation of testicular and spermatozoal parameters and by determination of the length of the estrous cycle. In addition, the genetic toxicity of these chemicals was assessed in studies in *S. typhimurium* and cultured Chinese hamster ovary cells and by determinations of the induction of trifluorothymidine resistance in L5178Y mouse lymphoma cells and of micronuclei in bone marrow cells of rats and mice from the 13-week studies.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF *O*-CHLOROANILINE AND *M*-CHLOROANILINE

Single lots of *o*-chloroaniline (Lot 04921TX) and *m*-chloroaniline (Lot 04214PV) were obtained from Aldrich Chemical Company (Milwaukee, WI). Information on identity and purity was provided by the supplier.

o-Chloroaniline, a colorless liquid, and *m*-chloroaniline, a pale yellow liquid, were identified by infrared spectroscopy; each spectrum was consistent with a literature reference (*Aldrich Library of FT-IR Spectra*, 1985) and with that expected for the chemical structure. Gas chromatography indicated a purity greater than 99% for each chemical.

Throughout the 13-week studies, *o*-chloroaniline and *m*-chloroaniline were stored in amber glass bottles sealed with Teflon®-lined lids at room temperature; periodic reanalyses performed by the study laboratory using high-performance liquid chromatography (HPLC) and infrared spectroscopy indicated no decomposition.

DOSE FORMULATIONS

Gavage solutions were prepared by mixing *o*-chloroaniline or *m*-chloroaniline with 1 M hydrochloric acid, sonicating for 20 minutes, and diluting to volume with deionized water. The pH was measured and adjusted with hydrochloric acid (pH 1.5 to 2.0). The dose levels are expressed as base *o*- or *m*-chloroaniline.

Stability studies of the gavage solutions were performed by the study laboratory using HPLC. The results indicated that 1 mg/mL solutions of *o*-chloroaniline and *m*-chloroaniline were stable for 35 days when stored sealed, in the dark, at room temperature or at 5° C; the solutions were stable for 3 hours under simulated dosing conditions.

During the 13-week studies, the gavage solutions were stored at 5° C in containers resistant to ultraviolet/visible light. The study laboratory periodically analyzed the gavage solutions and animal room samples by HPLC. All dose formulations administered to rats and mice were within 10% of the theoretical concentrations. All animal room samples for rats in the *o*-chloroaniline study and rats and mice in the *m*-chloroaniline studies were within 10% of the theoretical concentrations; four of five animal room samples for mice in the *o*-chloroaniline study were within 10% of the theoretical concentrations.

TOXICITY STUDY DESIGNS

Core Studies

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories (Raleigh, NC) and were 36 days old at receipt. Rats and mice were quarantined 11 to 14 days and were approximately 7 weeks old when the studies began. Blood samples were collected from five male and five female rats and mice at the beginning of the *o*-chloroaniline and *m*-chloroaniline 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 3.

The doses for the 13-week studies were selected based on literature values. In the core studies, groups of 10 male and 10 female rats and mice were administered 0, 10, 20, 40, 80, or 160 mg *o*-chloroaniline or *m*-chloroaniline per kilogram body weight in dilute hydrochloric acid by gavage 5 days a week for 13 weeks. Rats were housed five per cage by sex and mice were housed individually. NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) and water (Columbus Municipal Supply) were available *ad libitum*. Animal rooms were maintained at 69° to 75° F and 35% to 65% relative humidity, with 12 hours of fluorescent light per day and at least 10 room air changes per hour.

Complete necropsies were performed on all core study animals. 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 vehicle control animals, all animals in the highest dose groups with 60% survival, and all animals that died early. Gross lesions and selected organs of rats and mice in lower dose groups were examined until a no-observed-effect level was determined. Organs weighed and tissues examined microscopically are listed in Table 3.

Upon completion of the laboratory pathologist's histopathologic 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). During the quality assessment and PWG review of *m*-chloroaniline, slides from the studies of *p*-chloroaniline (NTP, 1989) were reviewed; for comparison purposes, data were reported with terminology and severity grading consistent with those used in the *o*- and *m*-chloroaniline studies.

Supplemental Evaluations

Clinical Pathology

Clinical pathology studies were performed on rats designated for clinical pathology testing and on all core study rats and mice at the end of the 13-week studies. Ten animals per sex and dose level were evaluated. Blood for hematology and clinical chemistry evaluations was collected from clinical pathology study rats on days 3 and 23; blood was collected from core study rats and mice at the end of the studies. The animals were anesthetized with a CO_2:O_2 mixture, and blood samples were drawn from the retroorbital sinus. Samples for hematology analysis were placed in micro-collection tubes (Sarstedt, Inc., Nümbrecht, Germany) coated with potassium EDTA; samples for clinical chemistry evaluations were placed in similar tubes devoid of anticoagulant. The latter samples were allowed to clot; the samples were then centrifuged and serum was removed.

Hematologic determinations were made on a Serono-Baker Diagnostics System 9000 Hematology Analyzer (Serono-Baker Diagnostics, Allentown, PA) with reagents obtained from the manufacturer. The parameters that were evaluated are listed in Table 3. Differential leukocyte counts and morphologic evaluation of blood cells were determined by light microscopy from blood smears stained with modified Wright-Giemsa. Smears made from blood samples stained with new methylene blue or brilliant cresyl blue were examined microscopically for quantitative determination of reticulocytes and Heinz bodies. Neutralized cyanide was added to a buffered hemolysate to convert methemoglobin to cyanmethemoglobin, which was then measured spectrophotometrically.

Clinical chemistry variables were measured with a Hitachi 704® chemistry analyzer (Boehringer Mannheim, Indianapolis, IN). The parameters that were evaluated are listed in Table 3. Reagent for assays of sorbitol dehydrogenase was obtained from Sigma Chemical Company (St. Louis, MO); other reagents were obtained from the equipment manufacturer.

Sperm Motility and Vaginal Cytology Evaluations

Vaginal cytology and sperm motility evaluations were performed on core study rats and mice at the end of the studies. Ten male and 10 female rats from the vehicle control, 40, 80, and 160 mg/kg groups were evaluated. The parameters that were evaluated are listed in Table 3. Methods were those described in the NTP Statement of Work (NTP, 1991). Briefly, for the 12 days prior to sacrifice, the vaginal vaults of 10 female rats and mice per dose group were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left epididymis was isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus

epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

TABLE 3 Experimental Design and Materials and Methods in the 13-Week Gavage Studies of *o*-Chloroaniline and *m*-Chloroaniline

	o-Chloroaniline Studies	*m*-Chloroaniline Studies
EXPERIMENTAL DESIGN		
Study Laboratory	Battelle Columbus Laboratories (Columbus, OH)	Same as *o*-chloroaniline studies
Strain and Species	F344/N rats B6C3F$_1$ mice	Same as *o*-chloroaniline studies
Animal Source	Charles River Breeding Laboratories (Raleigh, NC)	Same as *o*-chloroaniline studies
Size of Study Groups	10 males and 10 females	Same as *o*-chloroaniline studies
Doses	0, 10, 20, 40, 80, or 160 mg/kg body weight in dilute hydrochloric acid by gavage 5 days a week for 13 weeks plus 2 days	Same as *o*-chloroaniline studies
Date of First Dose	Rats: 27 January 1992 (males), 28 January 1992 (females) Mice: 29 January 1992 (males), 30 January 1992 (females)	Rats: 16 March 1992 (males), 17 March 1992 (females) Mice: 18 March 1992 (males), 19 March 1992 (females)
Date of Last Dose and Necropsy	Rats: 28 April 1992 (males), 29 April 1992 (females) Mice: 30 April 1992 (males), 1 May 1992 (females)	Rats: 16 June 1992 (males), 17 June 1992 (females) Mice: 18 June 1992 (males), 19 June 1992 (females)
Type and Frequency of Observation	Animals were observed twice daily and were weighed at the start of the study, weekly thereafter, and at necropsy. Clinical observations were recorded weekly.	Same as *o*-chloroaniline studies
Necropsy	Complete necropsies were performed on all animals in the core studies. The following organs were weighed: heart, right kidney, liver, lungs, spleen, right testis, and thymus.	Same as *o*-chloroaniline studies
Histopathologic Examination	Histopathologic evaluations were performed on all animals in the vehicle control and 160 mg/kg groups. 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), liver (two sections), lungs, lymph nodes (mandibular and mesenteric), mammary gland, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pituitary gland, preputial glands, prostate gland, salivary gland, seminal vesicle, small intestine (duodenum, jejunum, ileum), spinal cord/sciatic nerve (if neurological 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 and tissue masses of rats and mice in all lower dose groups were examined. Tissues examined in lower dose groups included the bone marrow, kidneys, liver, and spleen in rats and the spleen in mice.	Histopathologic evaluations were performed on all animals in the vehicle control and 160 mg/kg groups. Tissues routinely examined were the same as those examined in the *o*-chloroaniline studies. Tissues examined in lower dose groups included the bone marrow, kidneys, liver, and spleen in rats and mice.

TABLE 3 Experimental Design and Materials and Methods in the 13-Week Gavage Studies of *o*-Chloroaniline and *m*-Chloroaniline (continued)

	o-Chloroaniline Studies	*m*-Chloroaniline Studies
Supplemental Evaluations		
Clinical Pathology	Blood for hematology and clinical chemistry evaluations was collected on days 3 and 23 from rats in the clinical pathology study groups. Core study rats and mice were evaluated at the end of the studies. Hematology parameters included hematocrit, hemoglobin concentration, erythrocyte count, reticulocyte count, nucleated erythrocyte count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelet count, leukocyte count and differential, methemoglobin concentration, and Heinz body count. Clinical chemistry parameters included urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile salts.	Same as *o*-chloroaniline studies
Sperm Motility and Vaginal Cytology Evaluations	Sperm motility and vaginal cytology evaluations were performed on core study animals at the end of the studies. Rats and mice in the 0, 40, 80, and 160 mg/kg groups were evaluated. Male rats and mice were evaluated for necropsy body and reproductive tissue weights, epididymal spermatozoal data, and spermatogenesis. Females were evaluated for necropsy body weight, estrous cycle length, and the percentage of cycle spent in the various stages.	Same as *o*-chloroaniline studies
ANIMAL MAINTENANCE		
Time Held Before Study	Rats: 11 days (males), 12 days (females) Mice: 13 days (males), 14 days (females)	Same as *o*-chloroaniline studies
Age When Study Began	Approximately 7 weeks	Same as *o*-chloroaniline studies
Age When Killed	20 weeks	Same as *o*-chloroaniline studies
Method of Animal Distribution	Animals were distributed randomly into groups of approximately equal initial mean body weight.	Same as *o*-chloroaniline studies
Diet	NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in pellet form and water (Columbus Municipal Supply) were available *ad libitum*.	Same as *o*-chloroaniline studies
Animal Room Environment	Rats were housed five per cage by sex and mice were housed individually. The temperature was maintained at 69° to 75° F and relative humidity at 35% to 65%, with at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day.	Same as *o*-chloroaniline studies

STATISTICAL METHODS

Calculation and Analysis of Lesion Incidences

The incidences of lesions as presented in Appendixes A and B are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. The Fisher exact test, a procedure based on the overall proportion of affected animals, was used to determine significance (Gart *et al.*, 1979).

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, hematology, spermatid, and epididymal spermatozoal 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 a 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. Extreme values identified by the statistical test were reviewed by NTP personnel before being eliminated from the analysis.

Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), 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.

QUALITY ASSURANCE

The animal studies of *o*-chloroaniline and *m*-chloroaniline were performed in compliance with United States Food and Drug Administration Good Laboratory Practices regulations (21 CFR, Part 58). The Quality

Assurance Unit of EG&G Mason Research Institute performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

GENETIC TOXICITY STUDIES

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger *et al.* (1987). *o*-Chloroaniline and *m*-chloroaniline were sent to the testing laboratories as coded aliquots and were incubated with the *S. typhimurium* tester strains TA97, 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 *o*- or *m*-chloroaniline. The high dose was limited by toxicity.

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.

Mouse Lymphoma Mutagenicity Test Protocol

The experimental protocol is presented in detail by McGregor *et al.* (1991). *o*-Chloroaniline and *m*-chloroaniline were supplied as coded aliquots. The high dose was limited by toxicity. L5178Y mouse lymphoma cells were maintained at 37° C as suspension cultures in supplemented Fischer's medium; normal cell cycling time was approximately 10 hours. To reduce the number of spontaneously occurring trifluorothymidine-resistant cells, subcultures were exposed once to medium containing THMG (thymidine, hypoxanthine, methotrexate, and glycine) for 1 day, to medium containing THG for 1 day, and to normal medium for 3 to 5 days. For cloning, the horse serum content was increased and Noble agar was added.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 mL of medium. This volume included the S9 fraction in those

experiments performed with metabolic activation. Incubation with *o*- or *m*-chloroaniline continued for 4 hours, at which time the medium plus chemical was removed and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, cells were plated in medium and soft agar supplemented with trifluorothymidine (TFT) for selection of TFT-resistant cells and plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% CO_2 for 10 to 12 days. The assays were initially performed without S9 and were repeated using freshly prepared S9 from the livers of Aroclor 1254-induced male F344/N rats.

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

Chinese Hamster Ovary Cell Cytogenetics Protocols

Testing was performed as reported by Galloway *et al.* (1987). *m*-Chloroaniline was supplied as a coded aliquot. The aliquot was 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 *m*-chloroaniline; the high dose was limited by toxicity. A single flask per dose was used.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 32.8 hours with *m*-chloroaniline in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 32.8 hours, the medium containing *m*-chloroaniline 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. The incubation time of 32.8 hours was extended from the normal time of 26 hours to offset *m*-chloroaniline-induced cell cycle delay and to ensure a sufficient number of scorable (second-division metaphase) cells. In the SCE test with S9, cells were incubated with *m*-chloroaniline, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no *m*-chloroaniline, and incubation proceeded for an additional 25.5 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 per cell from each dose level.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (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.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with *m*-chloroaniline for 18.8 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 *m*-chloroaniline and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 8.5 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 based on the cell cycle information obtained in the SCE test; because cell cycle delay was anticipated in the trial without S9, the incubation period was extended.

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. One hundred 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).

Chromosomal aberration data are presented as the percentages 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.

Rat and Mouse Bone Marrow Micronucleus Test Protocol

Preliminary range-finding studies were performed; factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by chemical exposure. In the bone marrow micronucleus evaluations, male F344/N rats received three intraperitoneal injections of *o*- or *m*-chloroaniline dissolved in corn oil at 24-hour intervals. Male B6C3F$_1$ mice received one intraperitoneal injection of *o*- or *m*-chloroaniline or three injections of *m*-chloroaniline at 24-hour intervals. The total dosing volume was 0.4 mL. Solvent control rats and mice were injected with corn oil only; the positive control animals received injections of 25 mg/kg cyclophosphamide. Twenty-four hours after the final injection, the animals were killed and smears of the bone marrow cells obtained from the femurs were prepared. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for frequency of micronucleated cells in each of five animals per dose group. In addition, the percentage of PCEs among the total erythrocyte population in the bone marrow was scored for each dose group as a measure of toxicity; no bone marrow toxicity was observed.

The results were tabulated as the mean of the pooled results from all animals within a treatment group, plus or minus the standard error of the mean. The frequency of micronucleated cells among normochromatic erythrocytes was analyzed by a statistical software package that tested for increasing trend over dose groups using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (Margolin *et al.*, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial was considered positive if the trend test P value was greater than or equal to 0.025 or if the P value for any single dose group was greater than or equal to 0.025 divided by the number of dose groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 13-week studies were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Mouse Peripheral Blood Micronucleus Protocol

A detailed discussion of this assay is presented in MacGregor *et al.* (1990). At the end of the 13-week toxicity study, blood was obtained from male and female mice and smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with a chromatin-specific fluorescent dye (acridine orange) and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes in each of five animals per dose group. Statistical analyses were performed as described for the rat and mouse bone marrow micronucleus analyses.

RESULTS

13-WEEK GAVAGE STUDIES IN F344/N RATS

In the *o*-chloroaniline study, one male in the 40 mg/kg group and one female in the 20 mg/kg group died of undetermined causes before the end of the study (Table 4); one female rat in the 160 mg/kg group in the *m*-chloroaniline study died during week 12 of suspected erythrotoxicity (Table 5). The final mean body weights and mean body weight gains of male rats in the 160 mg/kg groups in both studies were significantly less than those of the respective vehicle controls; in both studies, the final mean body weights and mean body weight gains of dosed females were similar to those of the vehicle controls (Tables 4 and 5 and Figures 1 and 2).

In both studies, clinical findings of toxicity were generally observed beginning 30 minutes after dosing. In the *o*-chloroaniline study, rats in the 80 and 160 mg/kg groups frequently had tremors, and females in the 160 mg/kg group appeared thin during the second week of the study. In the *o*-chloroaniline study, male and female rats administered 40 mg/kg or greater developed a bluish discoloration of the genital and footpad regions. In male and female rats in the 80 and 160 mg/kg groups in the *m*-chloroaniline study, a similar discoloration of the ear, nasal, genital, and footpad regions was observed.

TABLE 4 Survival and Body Weights of F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
MALE					
0	10/10	135 ± 2	326 ± 3	191 ± 2	
10	10/10	134 ± 2	321 ± 6	187 ± 5	99
20	10/10	136 ± 2	330 ± 6	194 ± 5	101
40	9/10[c]	132 ± 2	324 ± 4	190 ± 3	99
80	10/10[d]	135 ± 2	317 ± 5	184 ± 5	97
160	10/10	133 ± 3	304 ± 4**	170 ± 3**	93
FEMALE					
0	10/10	112 ± 1	185 ± 2	73 ± 2	
10	10/10	113 ± 2	186 ± 3	73 ± 3	101
20	9/10[e]	113 ± 1	189 ± 3	77 ± 3	102
40	10/10	110 ± 2	178 ± 3	69 ± 3	97
80	10/10	111 ± 2	180 ± 2	69 ± 3	98
160	10/10	111 ± 1	178 ± 2	66 ± 2	96

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Number surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Week of death: 2
[d] The initial and final body weights of one rat are not included in the means.
[e] Week of death: 6

TABLE 5 Survival and Body Weights of F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
MALE					
0	10/10	156 ± 2	323 ± 6	166 ± 7	
10	10/10	154 ± 1	321 ± 9	167 ± 8	100
20	10/10	155 ± 2	311 ± 7	156 ± 6	96
40	10/10	156 ± 1	320 ± 5	165 ± 5	99
80	10/10	156 ± 2	313 ± 6	157 ± 6	97
160	10/10	157 ± 1	289 ± 6**	132 ± 7**	90
FEMALE					
0	10/10	124 ± 1	183 ± 3	59 ± 3	
10	10/10	124 ± 1	186 ± 3	62 ± 3	102
20	10/10	123 ± 1	185 ± 3	62 ± 3	101
40	10/10	123 ± 1	186 ± 3	62 ± 2	101
80	10/10	124 ± 1	178 ± 3	54 ± 2	97
160	9/10[c]	126 ± 1	184 ± 3	58 ± 2	100

** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Number surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Week of death: 12

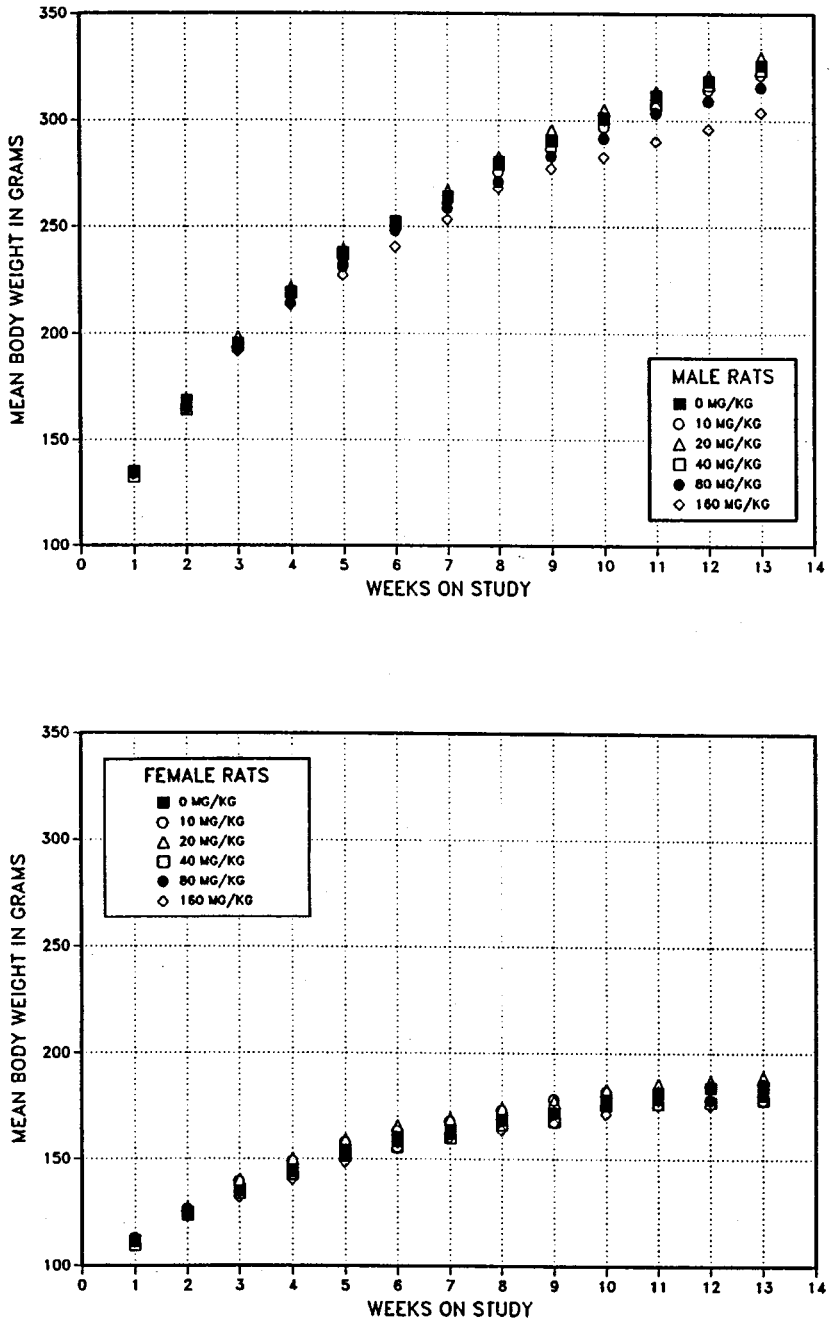


FIGURE 1 Body Weights of F344/N Rats Administered *o*-Chloroaniline by Gavage for 13 Weeks

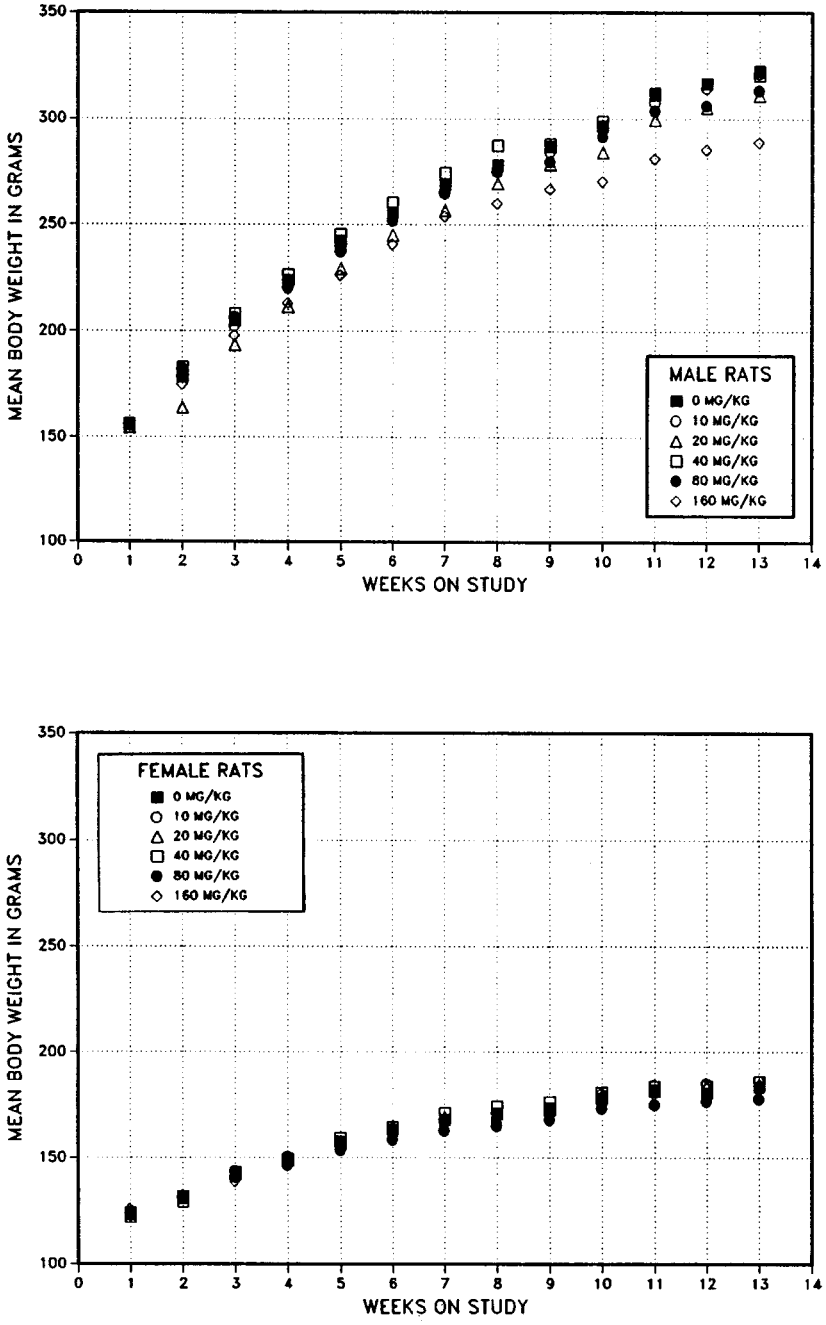


FIGURE 2 Body Weights of F344/N Rats Administered *m*-Chloroaniline by Gavage for 13 Weeks

o-Chloroaniline: Hematology and clinical chemistry data are listed in Tables 6 and D1. Administration of *o*-chloroaniline caused a minimal to marked, dose- and time-dependent methemoglobinemia, evidenced by increased methemoglobin concentrations, in all groups of dosed male and female rats at week 13. Increased methemoglobin concentrations also occurred in males in the 80 and 160 mg/kg groups and in females administered 40 mg/kg or greater on day 3; by day 23, methemoglobin concentrations were increased in males administered 40 mg/kg or greater and females administered 20 mg/kg or greater. In all dosed groups, the increases in methemoglobin concentration became more severe with time. Female rats appeared to be more susceptible to the methemoglobin-inducing effects of *o*-chloroaniline, based on the occurrence of methemoglobinemia in groups receiving lower doses or at earlier time points than in males.

A minimal to mild, dose- and time-related anemia, evidenced by decreases in hematocrit (Hct) values, hemoglobin (Hgb) concentrations, and erythrocyte (RBC) counts, occurred in males in the 160 mg/kg group and females in the 80 and 160 mg/kg groups on day 23. By week 13, the anemia occurred in males administered 80 or 160 mg/kg and females administered 20 mg/kg or greater. The anemia was characterized as macrocytic, normochromic, and responsive. Evidence of macrocytosis included increases in the mean cell volume (MCV) in female rats in the 160 mg/kg group on day 23 and in males administered 80 or 160 mg/kg and females administered 40 mg/kg or greater at week 13. The macrocytosis was attributed to the increased numbers of larger reticulocytes in the bloodstream and would be consistent with an erythropoietic response to anemia. Normochromic RBCs were evidenced by the lack of change in mean cell hemoglobin concentration (MCHC). Evidence of an erythropoietic response was demonstrated by increases in reticulocyte and nucleated erythrocyte (NRBC) counts. Reticulocyte counts were increased in males in the 160 mg/kg group and females in the 80 and 160 mg/kg group on day 23 and in males administered 40 mg/kg or greater and females administered 20 mg/kg or greater at week 13. Increases in NRBC counts accompanied the increases in reticulocyte counts. Reticulocytosis can cause slightly increased mean cell hemoglobin (MCH) values; thus, the minimal increases in MCH on day 23 and at week 13 in males in the 160 mg/kg group and females in the 80 and 160 mg/kg groups would be consistent with the increased reticulocyte counts in these groups. Dosed female rats appeared to be more susceptible to the development of anemia than males; this finding is consistent with the sex difference observed for the methemoglobin results.

TABLE 6 Selected Hematology Data for F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline[a]

	Dose (mg/kg)					
	Vehicle Control	10	20	40	80	160
MALE						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	9	10	10	10
Week 13	10	10	10	9	9	10
Methemoglobin (g/dL)						
Day 3	0.28 ± 0.03	0.24 ± 0.02	0.34 ± 0.03	0.31 ± 0.04	0.53 ± 0.02**	1.24 ± 0.08**
Day 23	0.15 ± 0.02	0.14 ± 0.02	0.20 ± 0.03[b]	0.39 ± 0.05**	0.81 ± 0.07**	1.70 ± 0.10**
Week 13	0.30 ± 0.02	0.40 ± 0.02**	0.59 ± 0.04**	0.84 ± 0.04**	1.59 ± 0.06**	2.79 ± 0.09**
Hematocrit (%)						
Day 3	39.8 ± 0.5	40.2 ± 0.7	40.3 ± 0.4	40.7 ± 0.4	40.2 ± 0.5	41.1 ± 0.4
Day 23	45.3 ± 0.3	45.9 ± 0.6	45.4 ± 0.5	45.6 ± 0.5	45.4 ± 0.4	42.9 ± 0.3**
Week 13	45.4 ± 0.4	45.7 ± 0.3	45.7 ± 0.3	44.6 ± 0.4	43.2 ± 0.6*	43.7 ± 0.3**
Hemoglobin (g/dL)						
Day 3	14.2 ± 0.1	14.3 ± 0.1	14.1 ± 0.1	14.1 ± 0.1	14.3 ± 0.1	14.3 ± 0.1
Day 23	16.2 ± 0.1	16.0 ± 0.1	16.1 ± 0.1	16.2 ± 0.1	16.1 ± 0.2	15.1 ± 0.1**
Week 13	16.1 ± 0.1	16.2 ± 0.1	16.1 ± 0.2	15.6 ± 0.1*	15.2 ± 0.2**	15.4 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 3	6.63 ± 0.08	6.65 ± 0.12	6.70 ± 0.08	6.75 ± 0.06	6.71 ± 0.09	6.83 ± 0.06
Day 23	7.86 ± 0.06	8.02 ± 0.11	7.98 ± 0.10	7.97 ± 0.10	8.00 ± 0.10	7.41 ± 0.06**
Week 13	8.55 ± 0.07	8.63 ± 0.06	8.71 ± 0.07	8.48 ± 0.10	7.91 ± 0.13**	7.52 ± 0.07**
Reticulocytes ($10^6/\mu L$)						
Day 3	0.15 ± 0.02	0.13 ± 0.03	0.13 ± 0.02	0.12 ± 0.02	0.19 ± 0.02	0.15 ± 0.03
Day 23	0.16 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.29 ± 0.04**
Week 13	0.16 ± 0.01	0.15 ± 0.02	0.19 ± 0.01	0.21 ± 0.02*	0.32 ± 0.04**	0.46 ± 0.02**
Nucleated erythrocytes ($10^3/\mu L$)						
Day 3	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.03 ± 0.01	0.01 ± 0.01
Day 23	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.05 ± 0.02
Week 13	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01*	0.13 ± 0.03**
Mean cell volume (fL)						
Day 3	60.1 ± 0.2	60.4 ± 0.2	60.1 ± 0.2	60.4 ± 0.3	59.9 ± 0.2	60.1 ± 0.1
Day 23	57.6 ± 0.2	57.2 ± 0.2	56.9 ± 0.2	57.2 ± 0.1	56.7 ± 0.4**	58.0 ± 0.1
Week 13	53.1 ± 0.2	53.0 ± 0.2	52.4 ± 0.2	52.6 ± 0.3	54.6 ± 0.4*	58.1 ± 0.5**
Mean cell hemoglobin (pg)						
Day 3	21.4 ± 0.1	21.6 ± 0.3	21.0 ± 0.2*	20.9 ± 0.1*	21.3 ± 0.3	21.0 ± 0.1
Day 23	20.6 ± 0.1	20.0 ± 0.2	20.2 ± 0.2	20.3 ± 0.2	20.1 ± 0.2*	20.4 ± 0.1
Week 13	18.9 ± 0.1	18.8 ± 0.1	18.5 ± 0.1	18.4 ± 0.2	19.2 ± 0.2	20.5 ± 0.2**
Heinz bodies (%)						
Week 13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.2 ± 0.6**

TABLE 6 Selected Hematology Data for F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline (continued)

	Dose (mg/kg)					
	Vehicle Control	**10**	**20**	**40**	**80**	**160**
FEMALE						
n						
Day 3	10	10	10	10	10	10
Day 23	10	9	10	10	10	10
Week 13	10	10	9	10	10	10
Methemoglobin (g/dL)						
Day 3	0.19 ± 0.03	0.24 ± 0.02	0.23 ± 0.03	0.46 ± 0.02**	1.11 ± 0.09**	1.92 ± 0.09**
Day 23	0.21 ± 0.03	0.26 ± 0.03[b]	0.47 ± 0.02**	0.94 ± 0.09**	1.69 ± 0.09**	2.46 ± 0.10**
Week 13	0.37 ± 0.04	0.54 ± 0.03**	0.83 ± 0.02**	1.53 ± 0.07**	2.35 ± 0.06**	2.80 ± 0.07**
Hematocrit (%)						
Day 3	41.9 ± 0.6	41.8 ± 0.4	42.8 ± 0.6	42.1 ± 0.5	43.2 ± 0.4	43.5 ± 0.5
Day 23	46.6 ± 0.6	45.9 ± 0.4	46.4 ± 0.3	45.7 ± 0.6	43.9 ± 0.5**	41.8 ± 0.3**
Week 13	45.2 ± 0.4	45.1 ± 0.4	44.4 ± 0.4	44.2 ± 0.4	42.5 ± 0.4**	41.9 ± 0.3**
Hemoglobin (g/dL)						
Day 3	14.5 ± 0.2	14.6 ± 0.1	14.7 ± 0.2	14.6 ± 0.2	14.9 ± 0.1	14.9 ± 0.1
Day 23	16.3 ± 0.2	16.2 ± 0.1	16.4 ± 0.2	16.0 ± 0.2	15.0 ± 0.1**	14.6 ± 0.1**
Week 13	15.7 ± 0.1	15.6 ± 0.1	15.2 ± 0.1*	15.0 ± 0.2**	14.3 ± 0.1**	14.6 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 3	6.87 ± 0.11	6.86 ± 0.05	6.99 ± 0.10	6.87 ± 0.10	7.13 ± 0.10	7.23 ± 0.09*
Day 23	7.74 ± 0.11	7.72 ± 0.05	7.79 ± 0.07	7.66 ± 0.10	7.27 ± 0.08**	6.57 ± 0.05**
Week 13	7.78 ± 0.08	7.74 ± 0.05	7.53 ± 0.06**	7.34 ± 0.08**	6.82 ± 0.04**	6.41 ± 0.07**
Reticulocytes ($10^6/\mu L$)						
Day 3	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Day 23	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.18 ± 0.02	0.27 ± 0.02**	0.48 ± 0.04**
Week 13	0.13 ± 0.01	0.13 ± 0.01	0.18 ± 0.01**	0.19 ± 0.02**	0.34 ± 0.01**	0.47 ± 0.02**
Nucleated erythrocytes ($10^3/\mu L$)						
Day 3	0.02 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Day 23	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.08 ± 0.02**	0.21 ± 0.07**
Week 13	0.03 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.06 ± 0.02	0.12 ± 0.02**	0.34 ± 0.10**
Mean cell volume (fL)						
Day 3	61.1 ± 0.3	60.9 ± 0.3	61.2 ± 0.4	61.3 ± 0.4	60.6 ± 0.3	60.2 ± 0.3
Day 23	60.3 ± 0.3	59.5 ± 0.3	59.6 ± 0.4	59.7 ± 0.4	60.3 ± 0.2	63.7 ± 0.3**
Week 13	58.1 ± 0.2	58.3 ± 0.3	59.0 ± 0.4	60.2 ± 0.2**	62.2 ± 0.3**	65.5 ± 0.2**
Mean cell hemoglobin (pg)						
Day 3	21.1 ± 0.1	21.4 ± 0.1	21.0 ± 0.1	21.3 ± 0.1	20.9 ± 0.2	20.6 ± 0.1*
Day 23	21.1 ± 0.2	20.9 ± 0.2	21.0 ± 0.1	20.9 ± 0.1	20.7 ± 0.1	22.2 ± 0.1**
Week 13	20.2 ± 0.1	20.2 ± 0.1	20.1 ± 0.1	20.5 ± 0.2	21.0 ± 0.1**	22.8 ± 0.2**
Heinz bodies (%)						
Week 13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.7 ± 0.7**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=10

Morphologic review of RBCs revealed Heinz bodies, schistocytes, keratocytes, spherocytes, and acanthocytes, occurring occasionally or at minimally increased numbers, in males and females in the 160 mg/kg groups at all time points. The Heinz bodies were 1- to 3-μm spherical structures seen as nipples protruding from the RBC surface. Increased numbers of polychromatophilic red cells occurred in males and females in the 160 mg/kg groups on day 23 and at week 13. The presence of treatment-related altered red cell morphology (e.g. Heinz bodies, schistocytes, keratocytes, spherocytes, and acanthocytes) is consistent with red cell injury and suggests that the anemia was of hemolytic origin. The presence of Heinz bodies indicates that hemoglobin was a target of the oxidative injury and is consistent with the methemoglobinemia. The increase in polychromasia in dosed rats would be consistent with a bone marrow response to the anemia.

An apparent minimal to mild leukocytosis, evidenced by increased total leukocyte (WBC) counts, occurred in females in the 80 and 160 mg/kg groups on day 23 and in males administered 40 mg/kg or greater and females administered 20 mg/kg or greater at week 13 (Table D1). Estimates of WBC counts from blood smears, however, did not support the quantitative results, suggesting that the automated WBC counts were erroneously elevated because of the presence of nonlysed reticulocytes or RBC fragments, Heinz bodies, or large platelets, which were counted as WBCs by the automated cell counter. Platelet counts were minimally increased in males in the 160 mg/kg group and females in the 80 and 160 mg/kg groups on day 23; at week 13, platelet counts were increased in males administered 80 or 160 mg/kg and females administered 20 mg/kg or greater (Table D1). The increased platelet counts could have been related to a general increase in hematopoietic activity or could have been erroneously elevated due to the presence of free Heinz bodies and/or RBC fragments in the circulation.

Evidence of increased hepatocellular leakage and/or altered function was demonstrated by increased sorbitol dehydrogenase (SDH) activities and bile salt concentrations in dosed males and females at week 13 (Table D1). The increases in these parameters were minimal to mild and were most pronounced in the 160 mg/kg groups.

Absolute and relative spleen weights of male and female rats generally increased with increasing dose (Tables 7 and C1). The absolute spleen weights of male rats administered 80 or 160 mg/kg and females administered 20 mg/kg or greater were significantly greater than those of the vehicle controls; relative spleen weights of males and females in the 40, 80, and 160 mg/kg groups were also significantly greater than those of the vehicle controls. Differences in the relative heart, right kidney, liver, and right testis weights of dosed males and relative heart and liver weights of females in the 160 mg/kg group (Table C1) generally reflected differences in necropsy body weights.

TABLE 7 Spleen Weights and Spleen-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline[a]

	Dose (mg/kg)					
	Vehicle Control	10	20	40	80	160
MALE						
n	10	10	10	9	9	10
Necropsy body wt	334 ± 3	328 ± 6	334 ± 7	329 ± 4	319 ± 5	304 ± 5**
Spleen						
Absolute	0.684 ± 0.014	0.663 ± 0.012	0.681 ± 0.018	0.750 ± 0.013	0.861 ± 0.043**	1.104 ± 0.024**
Relative	2.05 ± 0.04	2.02 ± 0.02	2.05 ± 0.05	2.28 ± 0.03*	2.70 ± 0.13**	3.63 ± 0.06**
FEMALE						
n	10	10	9	10	10	10
Necropsy body wt	183 ± 3	189 ± 3	190 ± 3	176 ± 3	178 ± 2	174 ± 2*
Spleen						
Absolute	0.419 ± 0.009	0.432 ± 0.007	0.459 ± 0.010*	0.460 ± 0.010*	0.594 ± 0.013**	0.907 ± 0.018**
Relative	2.28 ± 0.03	2.29 ± 0.02	2.42 ± 0.04	2.61 ± 0.05**	3.33 ± 0.06**	5.23 ± 0.09**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Spleen weights (absolute weights) and body weights are given in grams; spleen-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

The spleens of all rats in the 80 and 160 mg/kg groups were enlarged and dark red. There were no other treatment-related gross findings.

Minimal to mild hemosiderin pigmentation was observed microscopically in the spleen, liver, and renal cortex of male and female rats in the 160 mg/kg groups and in the spleen of females in the 80 mg/kg group (Tables 8, A1, and A2). Hematopoietic cell proliferation and capsule fibrosis of minimal to mild severity occurred in the spleens of males and females in the 80 and 160 mg/kg groups. All males and females administered 80 or 160 mg/kg *o*-chloroaniline and one male administered 40 mg/kg had minimal to mild hyperplasia of the bone marrow erythroid cells.

There were no significant differences in sperm motility or vaginal cytology parameters between dosed and vehicle control males or females (Tables E1 and E2).

TABLE 8 Incidence of Selected Lesions in F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline

	Dose (mg/kg)					
	Vehicle Control	10	20	40	80	160
MALE						
Bone marrow[a]	10	10	10	10	10	10
Erythroid cell, hyperplasia[b]	0	0	0	1 (2.0)[c]	10** (1.6)	10** (2.0)
Kidney	10	10	10	10	10	10
Cortex, pigmentation, hemosiderin	0	0	0	0	0	10** (1.4)
Liver	10	—[d]	1	1	—	10
Kupffer cell, pigmentation, hemosiderin	0		0	0		10** (1.0)
Spleen	10	10	10	10	10	10
Hematopoietic cell proliferation	0	0	0	0	10** (1.8)	10** (2.0)
Pigmentation, hemosiderin	0	0	0	0	0	9** (1.4)
Capsule, fibrosis	0	0	0	0	10** (1.0)	9** (2.0)
FEMALE						
Bone marrow	10	10	10	10	10	10
Erythroid cell, hyperplasia	0	0	0	0	10** (1.8)	10** (2.0)
Kidney	10	10	10	10	10	10
Cortex, pigmentation, hemosiderin	0	0	0	0	0	10** (1.1)
Liver	10	—	1	—	—	10
Kupffer cell, pigmentation, hemosiderin	0		0			10** (1.0)
Spleen	10	10	10	10	10	10
Hematopoietic cell proliferation	0	0	0	0	10** (2.0)	10** (1.9)
Pigmentation, hemosiderin	0	0	0	0	9** (1.8)	9** (1.9)
Capsule, fibrosis	0	0	0	0	10** (1.0)	10** (2.0)

** Significantly different ($P \leq 0.01$) from the vehicle control group by the Fisher exact test
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, and 4=marked
[d] Not examined

m-Chloroaniline: Hematology and clinical chemistry data are listed in Tables 9 and D2. In general, hematological and serum chemistry alterations similar to those occurring in the *o*-chloroaniline study occurred in the *m*-chloroaniline study; however, the changes caused by *m*-chloroaniline were more severe. Administration of *m*-chloroaniline caused a dose-dependent methemoglobinemia, evidenced by increased methemoglobin concentrations in all dosed groups at all time points, which was similar to, but more severe than, that occurring in the *o*-chloroaniline study. In males and females, the increases in methemoglobin concentration in the 10, 20, and 40 mg/kg groups became more severe with time; the methemoglobinemia in the 80 and 160 mg/kg groups was most severe at day 3 and appeared to ameliorate slightly on day 23 and at week 13. As in the *o*-chloroaniline study, female rats appeared to be more susceptible to the methemoglobin-inducing effects of *m*-chloroaniline than males, as evidenced by the greater severity of methemoglobinemia in all dosed groups of females at all time points.

As with *o*-chloroaniline administration, a dose-related, macrocytic, normochromic, responsive anemia of minimal to moderate severity occurred in males administered 20 mg/kg or greater and in all dosed groups of females (Table 9). The anemia was evidenced by decreased Hct values, Hgb concentrations, and RBC counts. The macrocytosis was evidenced by increased MCV values. Elevations in MCH values accompanied and reflected the increases in MCV. The increases in reticulocyte and NRBC counts would be consistent with an erythropoietic response to the anemia. There were no consistent changes in MCHC in dosed males or females.

Microscopic evaluation of blood smears revealed minimally to moderately increased numbers of Heinz bodies, schistocytes, keratocytes, spherocytes, acanthocytes, stomatocytes, and eccentrocytes in males and females in the 160 mg/kg groups at all time points but were most prominent on day 23 and at week 13. Altered RBC morphology also occurred in the lower dosed groups. The presence of Heinz bodies, schistocytes, keratocytes, spherocytes, and eccentrocytes is consistent with oxidative injury to mature RBCs and suggests that the anemia was of hemolytic origin. A marked increase in the numbers of polychromatophilic red cells occurred in the 160 mg/kg groups on day 23 and at week 13; increased polychromasia also occurred in the 80 mg/kg groups. The increase in polychromasia in dosed rats would be consistent with a bone marrow response to the anemia. On day 23, basophilic stippling occurred in rats in the 160 mg/kg groups; this would also be consistent with a bone marrow response.

TABLE 9 Selected Hematology Data for F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline[a]

	Dose (mg/kg)					
	Vehicle Control	10	20	40	80	160
MALE						
n	10	10	10	10	10	10
Methemoglobin (g/dL)						
Day 3	0.39 ± 0.04	0.56 ± 0.05**	1.64 ± 0.15**	2.54 ± 0.17**	4.57 ± 0.26**	6.13 ± 0.09**
Day 23	0.21 ± 0.03	0.80 ± 0.07**	1.70 ± 0.10**	3.12 ± 0.12**	4.79 ± 0.15**	5.11 ± 0.25**
Week 13	0.31 ± 0.02	1.11 ± 0.06**	2.13 ± 0.06**	3.65 ± 0.19**	4.89 ± 0.20**	5.83 ± 0.18**
Hematocrit (%)						
Day 3	42.5 ± 0.6	43.5 ± 0.7	44.6 ± 0.4	42.8 ± 0.4	42.5 ± 0.4	41.8 ± 0.5
Day 23	49.8 ± 1.4	46.0 ± 0.3	43.4 ± 0.2**	43.6 ± 0.3**	42.7 ± 0.5**	42.4 ± 0.4**
Week 13	46.7 ± 0.8	46.0 ± 0.6	44.8 ± 0.4*	44.9 ± 0.9**	43.1 ± 0.5**	47.4 ± 1.0*
Hemoglobin (g/dL)						
Day 3	15.0 ± 0.2	15.1 ± 0.2	15.5 ± 0.2	14.7 ± 0.1	14.7 ± 0.1	14.6 ± 0.1
Day 23	17.6 ± 0.4	16.3 ± 0.1*	15.5 ± 0.1**	15.2 ± 0.1**	15.2 ± 0.2**	15.0 ± 0.1**
Week 13	16.0 ± 0.3	15.8 ± 0.2	15.3 ± 0.1**	15.6 ± 0.3**	15.4 ± 0.1**	15.5 ± 0.3*
Erythrocytes ($10^6/\mu L$)						
Day 3	7.16 ± 0.11	7.39 ± 0.14	7.54 ± 0.07	7.24 ± 0.07	7.15 ± 0.08	6.94 ± 0.10
Day 23	8.81 ± 0.23	8.36 ± 0.07	7.84 ± 0.04**	7.53 ± 0.06**	6.73 ± 0.08**	5.52 ± 0.06**
Week 13	8.89 ± 0.13	8.75 ± 0.11	8.33 ± 0.08**	7.79 ± 0.16**	6.62 ± 0.07**	5.83 ± 0.08**
Reticulocytes ($10^6/\mu L$)						
Day 3	0.29 ± 0.01	0.29 ± 0.02	0.30 ± 0.03	0.31 ± 0.03	0.37 ± 0.02*	0.40 ± 0.03*
Day 23	0.16 ± 0.01	0.20 ± 0.01*	0.25 ± 0.02**	0.41 ± 0.03**	0.48 ± 0.03**	0.82 ± 0.06**
Week 13	0.12 ± 0.01	0.18 ± 0.01**	0.21 ± 0.01**	0.36 ± 0.03**	0.57 ± 0.03**	1.85 ± 0.11**
Nucleated erythrocytes ($10^3/\mu L$)						
Day 3	0.03 ± 0.01	0.01 ± 0.01	0.07 ± 0.04	0.04 ± 0.02	0.08 ± 0.03	0.26 ± 0.08**
Day 23	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.07 ± 0.03*	0.49 ± 0.15**	4.16 ± 0.32**[b]
Week 13	0.01 ± 0.01	0.01 ± 0.01	0.06 ± 0.02*	0.10 ± 0.03**	0.15 ± 0.04**	0.54 ± 0.07**
Mean cell volume (fL)						
Day 3	59.4 ± 0.3	58.8 ± 0.2	59.2 ± 0.2	59.1 ± 0.2	59.4 ± 0.2	60.3 ± 0.3
Day 23	56.5 ± 0.3	55.1 ± 0.1	55.4 ± 0.2	57.8 ± 0.1	63.4 ± 0.3**	76.9 ± 0.6**
Week 13	52.5 ± 0.2	52.6 ± 0.2	53.8 ± 0.2**	57.6 ± 0.2**	65.2 ± 0.3**	81.3 ± 1.1**
Mean cell hemoglobin (pg)						
Day 3	20.9 ± 0.2	20.5 ± 0.2	20.5 ± 0.1	20.3 ± 0.2*	20.6 ± 0.1	21.0 ± 0.2
Day 23	19.9 ± 0.1	19.6 ± 0.1	19.8 ± 0.1	20.2 ± 0.2	22.7 ± 0.1**	27.1 ± 0.2**
Week 13	18.0 ± 0.2	18.1 ± 0.1	18.4 ± 0.1	20.1 ± 0.1**	23.3 ± 0.2**	26.7 ± 0.3**
Mean cell hemoglobin concentration (g/dL)						
Day 3	35.3 ± 0.2	34.8 ± 0.3	34.7 ± 0.1*	34.3 ± 0.3**	34.6 ± 0.1**	34.8 ± 0.1*
Day 23	35.3 ± 0.3	35.5 ± 0.2	35.8 ± 0.2	34.9 ± 0.2	35.7 ± 0.2	35.3 ± 0.2
Week 13	34.4 ± 0.2	34.3 ± 0.1	34.2 ± 0.2	34.9 ± 0.2	35.8 ± 0.2*	32.8 ± 0.3*
Heinz bodies (%)						
Week 13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.7 ± 0.9**	16.4 ± 1.1**	1.7 ± 0.6**

TABLE 9 Selected Hematology Data for F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline (continued)

	Dose (mg/kg)					
	Vehicle Control	10	20	40	80	160
FEMALE						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	9	10	10	10
Week 13	10	10	10	10	10	9
Methemoglobin (g/dL)						
Day 3	0.20 ± 0.04	0.62 ± 0.06**	1.94 ± 0.12**	3.95 ± 0.13**	6.09 ± 0.30**	7.32 ± 0.26**
Day 23	0.30 ± 0.03	1.21 ± 0.09**	2.60 ± 0.11**	4.24 ± 0.13**	5.36 ± 0.13**	6.89 ± 0.16**
Week 13	0.26 ± 0.02	1.54 ± 0.06**	2.95 ± 0.11**	4.72 ± 0.21**	5.68 ± 0.23**	6.56 ± 0.22**
Hematocrit (%)						
Day 3	44.8 ± 0.4	44.9 ± 0.6	44.7 ± 0.4	45.6 ± 0.4	43.6 ± 0.5	43.3 ± 0.5
Day 23	47.4 ± 0.4	46.3 ± 0.4	42.9 ± 0.5**	43.6 ± 0.5**	40.3 ± 0.5**	43.6 ± 0.5**
Week 13	47.3 ± 0.7	45.3 ± 0.4*	45.0 ± 0.8*	43.5 ± 0.4**	42.0 ± 0.6**	44.6 ± 0.4**
Hemoglobin (g/dL)						
Day 3	15.6 ± 0.1	15.6 ± 0.2	15.4 ± 0.2	15.7 ± 0.2	14.9 ± 0.1**	15.4 ± 0.2*
Day 23	16.3 ± 0.1	15.6 ± 0.1**	14.5 ± 0.1**	15.1 ± 0.1**	14.4 ± 0.1**	15.2 ± 0.1**
Week 13	15.8 ± 0.2	15.3 ± 0.1	15.0 ± 0.3**	14.6 ± 0.2**	14.6 ± 0.2**	15.2 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 3	7.27 ± 0.07	7.30 ± 0.08	7.30 ± 0.08	7.48 ± 0.09	7.06 ± 0.06	6.82 ± 0.07**
Day 23	7.85 ± 0.07	7.69 ± 0.05	6.92 ± 0.07**	6.69 ± 0.07**	5.66 ± 0.05**	5.39 ± 0.09**
Week 13	8.14 ± 0.11	7.63 ± 0.07**	7.40 ± 0.13**	6.71 ± 0.07**	5.85 ± 0.08**	5.40 ± 0.09**
Reticulocytes ($10^6/\mu L$)						
Day 3	0.19 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.02	0.26 ± 0.02**	0.30 ± 0.02**
Day 23	0.12 ± 0.01	0.15 ± 0.01*	0.34 ± 0.01**	0.38 ± 0.03**	0.68 ± 0.04**	1.12 ± 0.05**
Week 13	0.10 ± 0.01	0.16 ± 0.01**	0.21 ± 0.02**	0.33 ± 0.03**	0.59 ± 0.04**	0.98 ± 0.08**
Nucleated erythrocytes ($10^3/\mu L$)						
Day 3	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.06 ± 0.02	0.61 ± 0.20**
Day 23	0.02 ± 0.01	0.01 ± 0.01	0.09 ± 0.03	0.20 ± 0.05**	1.57 ± 0.36**	1.35 ± 0.39**
Week 13	0.01 ± 0.01	0.06 ± 0.03[c]	0.08 ± 0.03*	0.14 ± 0.04**	0.42 ± 0.10**	0.45 ± 0.19**
Mean cell volume (fL)						
Day 3	61.7 ± 0.3	61.6 ± 0.3	61.2 ± 0.3	61.0 ± 0.3	61.8 ± 0.3	63.5 ± 0.3**
Day 23	60.4 ± 0.2	60.2 ± 0.3	62.0 ± 0.3**	65.2 ± 0.3**	71.3 ± 0.7**	81.1 ± 0.6**
Week 13	58.2 ± 0.1	59.4 ± 0.1**	60.8 ± 0.2**	64.9 ± 0.2**	71.7 ± 0.3**	82.8 ± 0.8**
Mean cell hemoglobin (pg)						
Day 3	21.5 ± 0.1	21.4 ± 0.2	21.1 ± 0.1	21.0 ± 0.1*	21.1 ± 0.1	22.6 ± 0.1
Day 23	20.8 ± 0.1	20.3 ± 0.1	20.9 ± 0.1	22.6 ± 0.1**	25.4 ± 0.2**	28.3 ± 0.3**
Week 13	19.4 ± 0.1	20.1 ± 0.2**	20.3 ± 0.2**	21.8 ± 0.1**	25.0 ± 0.2**	28.2 ± 0.3**
Mean cell hemoglobin concentration (g/dL)						
Day 3	34.9 ± 0.2	34.8 ± 0.3	34.5 ± 0.1	34.4 ± 0.2	34.1 ± 0.2	35.6 ± 0.2
Day 23	34.4 ± 0.2	33.7 ± 0.2	33.7 ± 0.2	34.6 ± 0.2	35.7 ± 0.5*	34.9 ± 0.2
Week 13	33.4 ± 0.2	33.9 ± 0.3	33.4 ± 0.2	33.6 ± 0.2	34.9 ± 0.2**	34.1 ± 0.2**
Heinz bodies (%)						
Week 13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 0.4**	10.0 ± 1.0**	13.5 ± 2.3**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Shirley's test
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9 #
[c] n=8 #

An apparent mild to marked leukocytosis, evidenced by increased WBC counts, occurred in female rats in the 80 and 160 mg/kg groups on day 23 and in males administered 40 mg/kg or greater and females administered 20 mg/kg or greater at week 13. Estimates of WBC counts from blood smears, however, did not support the quantitative results, suggesting that the automated WBC counts were erroneously elevated because of the presence of nonlysed reticulocytes or RBC fragments, Heinz bodies, or large platelets, which were counted as WBCs by the automated cell counter. A thrombocytosis, evidenced by mild increases in platelet counts, occurred in males and females in the 80 and 160 mg/kg groups on day 3 and in various dosed groups on day 23. At week 13, however, a thrombocytopenia, evidenced by mildly to moderately decreased platelet counts, occurred in males in the 80 and 160 mg/kg groups and females in the 160 mg/kg group.

Biochemical evidence of increased hepatocellular leakage and/or altered function was demonstrated by increased SDH activities and bile salt concentrations in dosed males and females at week 13 (Table D2). The increases in these parameters were minimal to mild and were most pronounced in the 160 mg/kg groups. In contrast, alanine aminotransferase activity, another biomarker of hepatocellular leakage, was decreased in males administered 20 mg/kg or greater and in females in the 160 mg/kg group. Serum creatine kinase activity, a marker of muscle injury, was minimally increased in the 160 mg/kg groups. Alkaline phosphatase activity was minimally decreased in the 160 mg/kg groups; this decrease would be consistent with decreased feed consumption (feed consumption data not available). Minimally increased creatinine concentrations in all dosed groups of males and females could suggest a minimally decreased glomerular filtration rate. However, the creatinine concentrations of dosed rats were within physiological ranges and did not demonstrate a dose relationship, and serum urea nitrogen concentrations, another marker of the glomerular filtration rate, were not increased. This suggests that the elevated creatinine concentrations were not toxicologically relevant and may reflect a nonphysiological mechanism such as compound-related interference with the analytical method.

The absolute and relative spleen weights of male and female rats increased with increasing dose, and the spleen weights of rats administered 20 mg/kg or greater were significantly greater than those of the vehicle controls (Tables 10 and C2). The absolute and relative heart, right kidney, liver, and thymus weights of female rats in the 160 mg/kg group were significantly greater than those of the vehicle controls; females in the 80 mg/kg group also had greater absolute and relative liver weights than those of the vehicle controls (Table C2). The absolute and relative lung weights of females administered 20 mg/kg or greater were significantly less than those of the vehicle controls; however, these differences were not dose related.

TABLE 10 Spleen Weights and Spleen-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline[a]

	Dose (mg/kg)					
	Vehicle Control	10	20	40	80	160
MALE						
n	10	10	10	10	10	10
Necropsy body wt	326 ± 6	327 ± 8	317 ± 7	325 ± 6	319 ± 6	293 ± 6**
Spleen						
Absolute	0.701 ± 0.030	0.788 ± 0.015	0.859 ± 0.011*	1.275 ± 0.024**	2.483 ± 0.068**	3.373 ± 0.098**
Relative	2.15 ± 0.10	2.42 ± 0.04	2.72 ± 0.05**	3.93 ± 0.07**	7.78 ± 0.11**	11.52 ± 0.18**
FEMALE						
n	10	10	10	10	10	9
Necropsy body wt	183 ± 3	185 ± 3	186 ± 3	183 ± 2	179 ± 3	185 ± 3
Spleen						
Absolute	0.451 ± 0.017	0.504 ± 0.008	0.603 ± 0.015**	0.885 ± 0.015**	1.614 ± 0.050**	2.349 ± 0.046**
Relative	2.47 ± 0.07	2.72 ± 0.04	3.25 ± 0.06**	4.83 ± 0.09**	9.04 ± 0.23**	12.75 ± 0.28**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Spleen weights (absolute weights) and body weights are given in grams; spleen-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

At necropsy, male and female rats administered 40 mg/kg or greater had enlarged, darkened spleens. Microscopic examination revealed hematopoietic cell proliferation and hemosiderin pigmentation in the spleens of rats in all dosed groups (Tables 11, A3, and A4). The increases in the incidences of these lesions were significant in all dosed groups, and the severity generally increased with dose. Additionally, congestion occurred in most males and females administered 20 mg/kg or greater and in one male in the 10 mg/kg group; severity ranged from minimal in the 20 mg/kg group to moderate in the 160 mg/kg group. The incidences and severity of capsule fibrosis and cellular infiltration were also significantly greater in males and females administered 40 mg/kg or greater than in the vehicle controls.

The incidences of hyperplasia of the bone marrow erythroid cells in male and female rats administered 40 mg/kg or greater and hemosiderin pigmentation in the bone marrow of males and females in the 80 and 160 mg/kg groups were significantly greater than those in the vehicle controls; the severity of these lesions was dose related (Tables 11, A3, and A4).

TABLE 11 Incidence of Selected Lesions in F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline

	Dose (mg/kg)					
	Vehicle Control	10	20	40	80	160
MALE						
Bone marrow[a]	10	10	10	10	10	10
Pigmentation, hemosiderin[b]	0	0	0	0	10** (1.5)[c]	10** (1.9)
Erythroid cell, hyperplasia	0	0	0	10** (1.0)	10** (1.6)	10** (2.9)
Kidney	10	10	10	10	10	10
Cortex, pigmentation, hemosiderin	0	0	0	10** (1.2)	10** (1.7)	10** (3.0)
Liver	10	10	10	10	10	10
Hematopoietic cell proliferation	0	1 (1.0)	0	0	9** (1.0)	9** (1.0)
Kupffer cell, pigmentation, hemosiderin	0	0	0	9** (1.0)	10** (1.0)	10** (1.2)
Spleen	10	10	10	10	10	10
Congestion	0	1 (1.0)	9** (1.1)	10** (2.3)	10** (2.9)	10** (3.0)
Hematopoietic cell proliferation	0	7** (1.0)	10** (1.0)	10** (1.5)	10** (1.2)	10** (1.8)
Pigmentation, hemosiderin	0	6** (1.0)	10** (1.0)	10** (1.8)	10** (1.5)	10** (2.4)
Capsule, fibrosis	0	2 (1.0)	2 (1.0)	8** (1.3)	10** (2.3)	10** (2.0)
Capsule, cellular infiltration	1 (1.0)	2 (1.0)	2 (1.0)	10** (2.0)	10** (2.4)	10** (2.0)
FEMALE						
Bone marrow	10	10	10	10	10	10
Cellular infiltration, histiocyte	0	1 (1.0)	2 (1.0)	3 (1.0)	0	1 (1.0)
Myelofibrosis	3 (1.3)	1 (1.0)	0	1 (1.0)	1 (2.0)	1 (1.0)
Pigmentation, hemosiderin	0	0	0	3 (1.0)	10** (1.3)	9** (2.0)
Erythroid cell, hyperplasia	0	0	0	6** (1.0)	10** (1.6)	10** (3.0)
Kidney	10	10	10	10	10	10
Cortex, pigmentation, hemosiderin	0	0	1 (1.0)	10** (1.2)	10** (2.0)	10** (2.9)
Liver	10	10	10	10	10	10
Hematopoietic cell proliferation	2 (1.0)	0	1 (1.0)	10** (1.0)	10** (1.0)	10** (1.0)
Kupffer cell, pigmentation, hemosiderin	0	0	0	10** (1.0)	10** (1.0)	10** (1.8)
Spleen	10	10	10	10	10	10
Congestion	0	0	8** (1.0)	9** (1.3)	10** (2.2)	10** (2.8)
Hematopoietic cell proliferation	0	5* (1.0)	8** (1.0)	10** (1.3)	10** (2.5)	9** (2.0)
Pigmentation, hemosiderin	0	10** (1.0)	10** (1.0)	9** (2.3)	10** (1.9)	10** (2.8)
Capsule, fibrosis	0	0	0	6** (1.3)	10** (2.1)	9** (1.6)
Capsule, cellular infiltration	1 (1.0)	1 (1.0)	0	9** (1.4)	10** (2.0)	8** (1.9)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, and 4=marked

Hemosiderin pigmentation also occurred with dose-related increases in severity in the Kupffer cells of the liver and in the renal cortex in male and female rats administered 40 mg/kg or greater (Tables 11, A3, and A4). Additionally, females in these groups and males in the 80 and 160 mg/kg groups had significantly greater incidences of minimal hematopoietic cell proliferation in the liver than did the vehicle controls.

The sperm motility of males in the 160 mg/kg group was significantly less than that of the vehicle controls (Table E3). The decrease in sperm motility in this group could have been due to a significant reduction in body weight. In the absence of a dose-related effect in rats or any similar effect in mice, this observation was not considered to be biologically significant. The percentage of time spent in the various estrous stages and the estrous cycle length of dosed and vehicle control females were similar (Table E4).

p-Chloroaniline: The results of the 16-day, 13-week, and 2-year *p*-chloroaniline studies have been published (NTP, 1989). There were no deaths or clinical findings related to *p*-chloroaniline administration in the 13-week rat study. The final mean body weight and mean body weight gain of males in the 80 mg/kg group were less than those of the vehicle controls.

Blood samples for hematology analyses were collected 72 hours after the final dose was administered for males and 24 hours after the final dose for females. All dosed groups of males and females had significantly lower Hct values, Hgb concentrations, and RBC counts and significantly greater methemoglobin concentrations than did the vehicle controls (Table 12). Males administered 10 mg/kg or greater had significantly higher NRBC counts, MCV, MCH, and MCHC than the vehicle controls, and males in the 40 mg/kg group had significantly higher WBC, lymphocyte, and segmented neutrophil counts than the vehicle controls. Females in all dosed groups had significantly higher WBC and lymphocyte counts and MCV than the vehicle controls. The segmented neutrophil counts and MCH of females administered 10 mg/kg or greater were significantly higher than those of the vehicle controls. Females administered 20 mg/kg or greater had significantly higher NRBC counts and MCHC than the vehicle controls.

The absolute spleen weights of male and female rats administered *p*-chloroaniline increased with increasing dose. The differences were significant for males administered 20 mg/kg or greater (versus the 5 mg/kg group; spleen weights of vehicle control males were not recorded) and for all groups of dosed females.

The histopathologic slides of lesions from the *p*-chloroaniline study were reviewed in parallel with those from the *o*- and *m*-chloroaniline studies to ensure that diagnostic terminology and severity grading criteria were consistent between the studies. The spectrum of lesions caused by *p*-chloroaniline (Table 13) and the sites of toxicity were similar to those for the *o*- and *m*- isomers.

Males and females in all dosed groups had significantly greater incidences of splenic congestion and hematopoietic cell proliferation than the vehicle controls; males in all dosed groups except the 40 mg/kg group also had significantly increased incidences of hemosiderin pigmentation in the spleen (Table 13). Males in the 40 mg/kg group and females administered 20 mg/kg or greater had significantly increased incidences of capsule fibrosis in the spleen. Males in all dosed groups and females administered 10 mg/kg or greater had significantly greater incidences of bone marrow erythroid cell hyperplasia and renal cortex hemosiderin pigmentation than did the vehicle controls. Males and females administered 10 mg/kg or greater had significantly increased incidences of hematopoietic cell proliferation in the liver; males administered 20 mg/kg or greater and females administered 10 mg/kg or greater had significantly increased incidences of Kupffer cell hemosiderin pigmentation.

TABLE 12 Selected Hematology Data for F344/N Rats in the 13-Week Gavage Study of *p*-Chloroaniline[a]

	Dose (mg/kg)					
	Vehicle Control	5	10	20	40	80
MALE						
n	10	10	9	10	10	10
Methemoglobin (% hemoglobin)	0.08 ± 0.04	0.59 ± 0.10*	0.70 ± 0.24*	0.68 ± 0.20*	0.68 ± 0.19*	0.86 ± 0.16**
Hematocrit (%)	45.5 ± 0.4	42.8 ± 0.5**	42.4 ± 0.2**	42.7 ± 0.4**	39.4 ± 0.3**	36.5 ± 0.4**
Hemoglobin (g/dL)	15.5 ± 0.1	14.7 ± 0.2**	14.6 ± 0.1**	15.1 ± 0.1**	14.2 ± 0.1**	13.4 ± 0.2**
Erythrocytes ($10^6/\mu L$)	9.14 ± 0.06	8.68 ± 0.10**	8.18 ± 0.04**	7.48 ± 0.06**	6.07 ± 0.06**	4.90 ± 0.07**
Nucleated erythrocytes ($10^3/\mu L$)	0.00 ± 0.00	1.70 ± 0.50	3.44 ± 0.60*	2.90 ± 0.46*	8.70 ± 1.36**	23.80 ± 1.59**
Mean cell volume (fL)	50.1 ± 0.2	49.4 ± 0.2	51.8 ± 0.3**	56.9 ± 0.3**	65.1 ± 0.4**	74.7 ± 0.4**
Mean cell hemoglobin (pg)	16.9 ± 0.1	17.0 ± 0.1	17.9 ± 0.1**	20.1 ± 0.1**	23.5 ± 0.2**	27.3 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	34.0 ± 0.1	34.3 ± 0.2	34.5 ± 0.1**	35.3 ± 0.1**	35.9 ± 0.2**	36.6 ± 0.1**
Leukocytes ($10^3/\mu L$)	5.49 ± 0.22	6.28 ± 0.23	6.60 ± 0.20	6.55 ± 0.11	12.55 ± 0.82**	3.47 ± 0.23**
Segmented neutrophils ($10^3/\mu L$)	0.74 ± 0.10	0.86 ± 0.07	1.19 ± 0.14	1.00 ± 0.08	2.07 ± 0.33**	0.66 ± 0.09
Lymphocytes ($10^3/\mu L$)	4.64 ± 0.27	5.36 ± 0.22	5.38 ± 0.13	5.50 ± 0.12	10.34 ± 0.64**	2.76 ± 0.17**
FEMALE						
n	10	10	10	10	10	9
Methemoglobin (% hemoglobin)	0.46 ± 0.13	1.35 ± 0.15**	1.85 ± 0.18**	1.73 ± 0.21**	2.40 ± 0.15**	3.68 ± 0.45**
Hematocrit (%)	45.7 ± 0.3	43.8 ± 0.3**	43.3 ± 0.4**	42.5 ± 0.3**	39.8 ± 0.6**	36.3 ± 0.5**
Hemoglobin (g/dL)	15.1 ± 0.1	14.4 ± 0.1**	14.3 ± 0.1**	14.8 ± 0.2**	13.7 ± 0.2**	13.0 ± 0.1**
Erythrocytes ($10^6/\mu L$)	8.33 ± 0.05	7.77 ± 0.05**	7.27 ± 0.08**	6.49 ± 0.06**	5.69 ± 0.09**	5.06 ± 0.06**
Nucleated erythrocytes ($10^3/\mu L$)	1.40 ± 0.54	3.10 ± 0.61	4.40 ± 0.62	7.90 ± 1.05*	22.60 ± 3.62**	24.44 ± 1.68**
Mean cell volume (fL)	55.0 ± 0.0	56.3 ± 0.2**	59.3 ± 0.2**	65.1 ± 0.2**	69.9 ± 0.3**	72.2 ± 0.2**
Mean cell hemoglobin (pg)	18.1 ± 0.1	18.5 ± 0.1	19.6 ± 0.1**	22.8 ± 0.2**	24.0 ± 0.2**	25.8 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	33.1 ± 0.1	32.8 ± 0.1	33.2 ± 0.3	35.0 ± 0.2**	34.3 ± 0.2**	35.7 ± 0.3**
Leukocytes ($10^3/\mu L$)	4.57 ± 0.32	6.04 ± 0.22*	8.09 ± 0.28**	9.70 ± 0.55**	10.26 ± 0.78**	6.49 ± 0.39**
Segmented neutrophils ($10^3/\mu L$)	0.68 ± 0.08	0.86 ± 0.08	1.17 ± 0.14*	1.64 ± 0.24**	2.26 ± 0.24**	1.33 ± 0.15**
Lymphocytes ($10^3/\mu L$)	3.84 ± 0.27	5.14 ± 0.23*	6.81 ± 0.23**	7.99 ± 0.48**	7.93 ± 0.65**	5.13 ± 0.33**

* Significantly different ($P \le 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \le 0.01$
[a] NTP, 1989. Data are given as mean ± standard error. Statistical tests were performed on unrounded data.

TABLE 13 Incidence of Selected Lesions in F344/N Rats in the 13-Week Gavage Study of *p*-Chloroaniline[a]

	Dose (mg/kg)					
	Vehicle Control	5	10	20	40	80
MALE						
Bone marrow[b]	10	10	10	10	10	10
Erythroid cell, hyperplasia[c]	0	6** (1.2)[d]	9** (1.0)	10** (1.5)	10** (2.3)	10** (2.8)
Kidney	10	10	10	10	10	10
Cortex, pigmentation, hemosiderin	0	10** (1.0)	9** (1.0)	10** (1.1)	10** (2.1)	10** (3.0)
Liver	10	10	10	10	10	10
Hematopoietic cell proliferation	0	2 (1.0)	8** (1.0)	5* (1.0)	7** (1.0)	10** (1.0)
Kupffer cell, pigmentation, hemosiderin	0	0	0	7** (1.0)	10** (1.1)	10** (1.9)
Spleen	10	10	10	10	10	10
Congestion	1 (2.0)	10** (1.0)	10** (1.7)	10** (2.3)	10** (3.0)	10** (2.9)
Hematopoietic cell proliferation	0	5* (1.4)	10** (1.5)	10** (1.5)	10** (1.5)	10** (2.1)
Pigmentation, hemosiderin	1 (2.0)	9** (1.4)	10** (1.6)	9** (1.9)	3 (1.0)	9** (1.6)
Capsule, fibrosis	0	0	0	2 (1.0)	10** (1.3)	2 (1.0)
FEMALE						
Bone marrow	10	10	10	10	10	10
Erythroid cell, hyperplasia	0	2 (1.0)	9** (1.0)	10** (2.0)	10** (3.0)	10** (3.1)
Kidney	10	10	10	10	10	10
Cortex, pigmentation, hemosiderin	0	2 (1.0)	9** (1.0)	10** (1.0)	10** (3.3)	10** (3.5)
Liver	10	10	10	10	10	10
Hematopoietic cell proliferation	2 (1.0)	5 (1.0)	7* (1.0)	8* (1.0)	9** (1.0)	9** (1.0)
Kupffer cell, pigmentation, hemosiderin	0	1 (1.0)	10** (1.0)	10** (1.2)	10** (1.3)	10** (1.9)
Spleen	10	10	10	10	10	10
Congestion	0	10** (1.3)	10** (1.9)	10** (2.9)	10** (2.7)	10** (3.1)
Hematopoietic cell proliferation	4 (1.5)	10** (1.5)	10** (1.9)	10** (1.4)	10** (2.4)	10** (1.6)
Pigmentation, hemosiderin	9 (1.8)	10 (1.6)	10 (1.9)	9 (2.2)	10 (1.3)	9 (2.0)
Capsule, fibrosis	0	3 (1.0)	3 (1.0)	10** (1.0)	7** (1.0)	5* (1.0)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] NTP, 1989 (Slides were reviewed by a quality assessment pathologist and are presented in this report with terminology and severity grading consistent with those used for the *o*- and *m*-chloroaniline data.)
[b] Number of animals with tissue examined microscopically
[c] Number of animals with lesion
[d] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, and 4=marked

13-WEEK GAVAGE STUDIES IN B6C3F$_1$ MICE

Three mice in the *o*-chloroaniline study and nine mice in the *m*-chloroaniline study died due to gavage accidents (Tables 14 and 15); no deaths were attributed to chemical effects. In the *o*-chloroaniline study, the final mean body weight and mean body weight gain of females in the 160 mg/kg group were significantly less than those of the vehicle controls (Table 14 and Figure 3). In the *m*-chloroaniline study, the final mean body weights and mean body weight gains of dosed male and female mice were similar to those of the vehicle controls (Table 15 and Figure 4).

Males and females in the 160 mg/kg groups in both studies had tremors that developed immediately after dosing and persisted for 10 to 15 minutes; mice in the 160 mg/kg groups in the *m*-chloroaniline study also had ataxia.

TABLE 14 Survival and Body Weights of B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g) #			Final Weight Relative to Controls (%)
		Initial	Final	Change	
MALE					
0	9/10[c]	25.1 ± 0.3	35.4 ± 1.5	10.3 ± 1.4	
10	9/10[d]	25.2 ± 0.3	35.2 ± 0.9	9.9 ± 0.7	99
20	10/10	25.4 ± 0.3	35.5 ± 1.0	10.1 ± 0.9	100
40	9/10[c]	25.6 ± 0.4	35.3 ± 0.4	9.6 ± 0.3	100
80	10/10	25.1 ± 0.2	35.0 ± 0.5	9.9 ± 0.4	99
160	9/10[c]	24.8 ± 0.3	33.5 ± 0.9	8.7 ± 0.9	95
FEMALE					
0	10/10	20.6 ± 0.3	30.0 ± 0.8	9.3 ± 0.7	
10	10/10	20.4 ± 0.3	31.4 ± 1.0	11.0 ± 0.8	105
20	9/10[e]	20.6 ± 0.3	31.1 ± 0.7	10.5 ± 0.6	104
40	10/10	20.6 ± 0.4	29.4 ± 0.7	8.8 ± 0.5	98
80	10/10	20.5 ± 0.2	30.5 ± 0.7	10.0 ± 0.7	102
160	9/10[e]	20.4 ± 0.2	27.6 ± 0.5*	7.2 ± 0.4*	92

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

[a] Number surviving at 13 weeks/number initially in group.

[b] Weights and weight changes are given as mean ± standard error.

[c] One mouse in this group died during blood collection at the end of the study, after the final weighing; body weight data for this animal are included in the means.

[d] Week of death: 2 (gavage accident)

[e] One mouse in this group died due to a gavage accident during the final week of the study; body weight data for this animal are included in the means.

TABLE 15 Survival and Body Weights of B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
MALE					
0	9/10	25.5 ± 0.3	35.0 ± 1.1	9.6 ± 1.1	
10	10/10	25.6 ± 0.3	35.3 ± 1.4	9.7 ± 1.1	101
20	7/10	25.3 ± 0.4	33.6 ± 0.6	8.4 ± 0.4	96
40	10/10	25.1 ± 0.4	33.9 ± 0.4	8.9 ± 0.3	97
80	10/10	25.7 ± 0.3	35.3 ± 0.4	9.5 ± 0.4	101
160	10/10	25.1 ± 0.3	35.3 ± 0.8	10.2 ± 0.9	101
FEMALE					
0	9/10	21.1 ± 0.2	28.2 ± 0.7	7.2 ± 0.7	
10	9/10	21.1 ± 0.3	28.4 ± 0.7	7.4 ± 0.6	101
20	10/10	20.9 ± 0.2	27.8 ± 0.7	6.9 ± 0.7	99
40	8/10	21.3 ± 0.2	29.6 ± 1.2	8.3 ± 1.1	105
80	10/10	20.9 ± 0.3	27.9 ± 0.4	6.9 ± 0.5	99
160	9/10	21.4 ± 0.2	28.3 ± 0.5	6.9 ± 0.5	100

[a] Number surviving at 13 weeks/number initially in group. All early deaths were due to gavage accidents and occurred during the first 2 weeks of the study.

[b] Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.

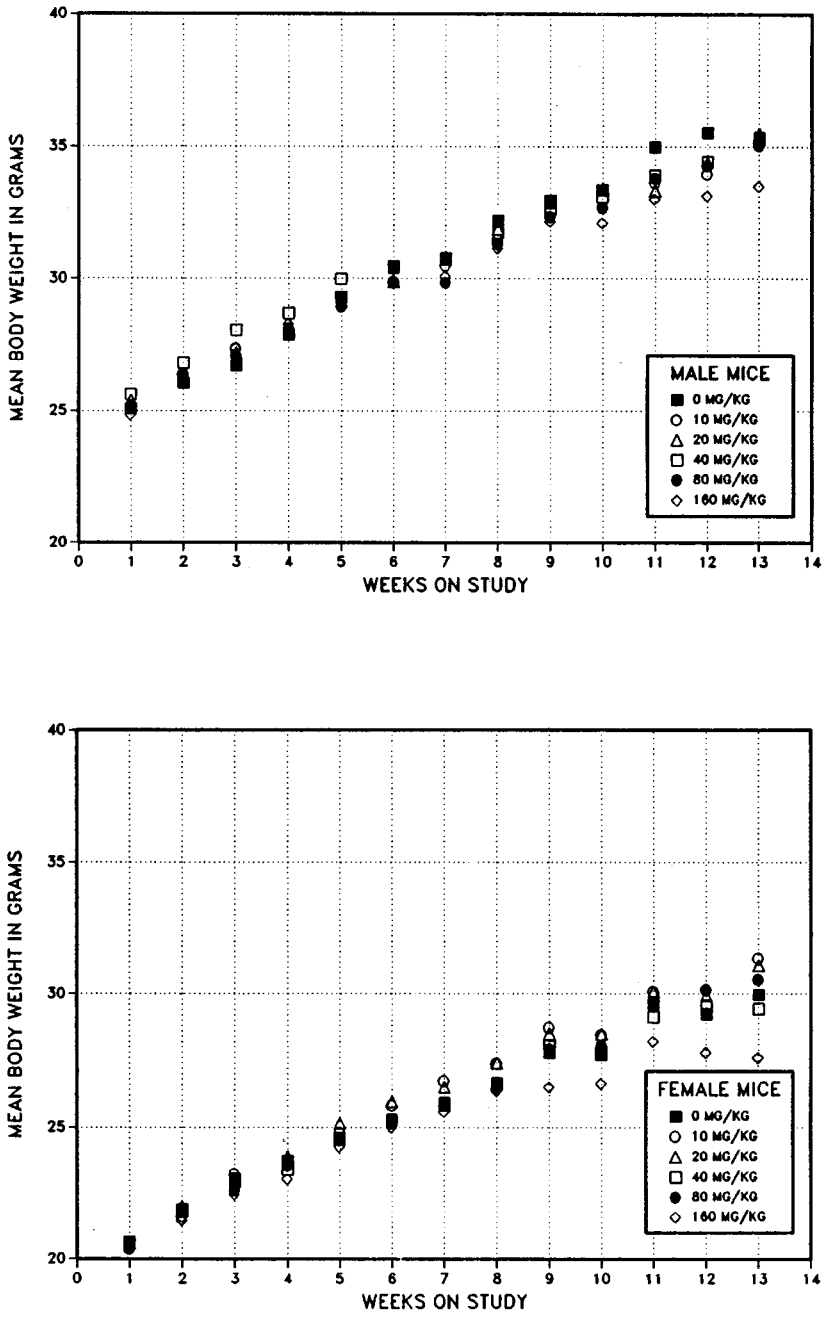


FIGURE 3 Body Weights of B6C3F$_1$ Mice Administered *o*-Chloroaniline by Gavage for 13 Weeks

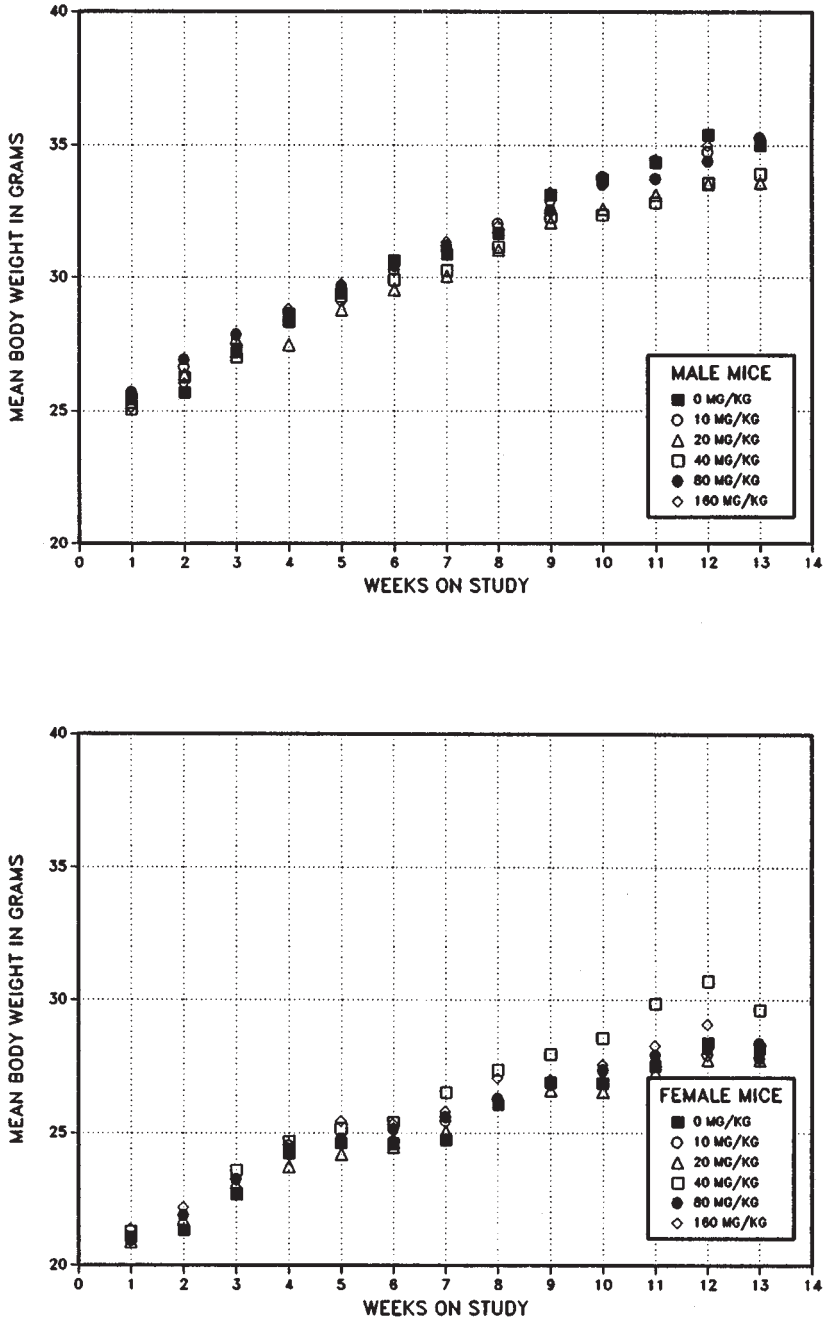


FIGURE 4 Body Weights of B6C3F$_1$ Mice Administered *m*-Chloroaniline by Gavage for 13 Weeks

o-Chloroaniline: Hematology and clinical chemistry data are listed in Tables 16 and D3. Administration of *o*-chloroaniline caused a dose-dependent methemoglobinemia evidenced by minimal to marked increases in methemoglobin concentration in all dosed groups of males and females. Evidence of a minimal anemia was demonstrated by treatment-related decreases in Hct values and RBC counts in the 80 and 160 mg/kg groups of males and females. In the 160 mg/kg groups, Hgb concentrations were inappropriately increased in males and less severely affected in females compared to the changes in Hct and RBC; these inappropriate or disproportionate responses would be consistent with erroneously elevated Hgb concentrations due to the presence of large numbers of Heinz bodies. MCV was not affected, indicating that the anemia was normocytic. MCHC elevations in males in the 80 and 160 mg/kg groups and females in the 160 mg/kg group indicate that the anemia was hyperchromic and are consistent with either a hemolytic process or the presence of large numbers of Heinz bodies causing erroneously elevated Hgb concentrations; increased MCH values accompanied the MCHC increases and would reflect similar effects. Reticulocyte counts were increased in males administered 80 or 160 mg/kg and females administered 160 mg/kg; these increases would be consistent with an erythropoietic response to the anemia.

Microscopic evaluation of blood smears revealed minimally to moderately increased numbers of Heinz bodies and schistocytes in males and females in the 160 mg/kg groups at 13 weeks. The Heinz bodies were 1- to 3-μm spherical structures that were freely scattered between red cells or that occurred as nipples protruding from the RBC surface. Altered RBC morphology also occurred in the 80 mg/kg groups. The presence of Heinz bodies and schistocytes is consistent with oxidative red cell injury and suggests that the anemia was of hemolytic origin. Increased numbers of polychromatophilic red cells also occurred in the 160 mg/kg groups; increased polychromasia would be consistent with the increased reticulocyte counts and indicates a bone marrow response to the anemia. A mild leukocytosis, evidenced by an increased WBC count, occurred in females in the 160 mg/kg group; this increase was apparently related to an elevation in neutrophil count. However, estimates of WBC counts from blood smears did not support the quantitative results, suggesting that the automated leukocyte counts were erroneously elevated due to the presence of nonlysed reticulocytes or RBC fragments or of Heinz bodies.

No biologically significant differences in clinical chemistry parameters were observed in dosed mice (Table D3).

TABLE 16 Selected Hematology Data for B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline[a]

	Dose (mg/kg)					
	Vehicle Control	10	20	40	80	160
MALE						
n	10	9	10	10	10	10
Methemoglobin (g/dL)	0.15 ± 0.02	0.24 ± 0.03*	0.27 ± 0.03**	0.44 ± 0.03**	0.79 ± 0.04**[b]	2.12 ± 0.32**
Hematocrit (%)	50.2 ± 0.5	51.1 ± 1.0	50.2 ± 0.7	48.2 ± 0.8	47.0 ± 0.7**	45.5 ± 0.7**
Hemoglobin (g/dL)	16.6 ± 0.1	16.7 ± 0.3	16.6 ± 0.1	16.2 ± 0.2	16.1 ± 0.1	17.3 ± 0.3
Erythrocytes ($10^6/\mu$L)	10.10 ± 0.10	10.34 ± 0.21	10.16 ± 0.15	9.77 ± 0.16	9.37 ± 0.13**	9.10 ± 0.14**
Reticulocytes ($10^6/\mu$L)	0.24 ± 0.02	0.22 ± 0.02	0.28 ± 0.03	0.22 ± 0.02	0.38 ± 0.03**	0.65 ± 0.04**
Mean cell volume (fL)	49.7 ± 0.1	49.4 ± 0.1	49.4 ± 0.3	49.3 ± 0.2	50.2 ± 0.2	50.0 ± 0.2
Mean cell hemoglobin (pg)	16.5 ± 0.2	16.2 ± 0.2	16.3 ± 0.2	16.6 ± 0.1	17.2 ± 0.2*	19.0 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	33.1 ± 0.4	32.8 ± 0.3	33.1 ± 0.3	33.7 ± 0.3	34.3 ± 0.3*	37.9 ± 0.4**
Heinz bodies (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	6.4 ± 0.8**
FEMALE						
n	10	10	9	10	10	9
Methemoglobin (g/dL)	0.14 ± 0.02	0.22 ± 0.02*	0.24 ± 0.02**	0.48 ± 0.03**	1.08 ± 0.13**	2.39 ± 0.16**
Hematocrit (%)	47.7 ± 0.9	46.7 ± 0.6	46.5 ± 0.7	46.8 ± 0.5	45.4 ± 0.3*	44.4 ± 0.6**
Hemoglobin (g/dL)	16.7 ± 0.3	16.2 ± 0.1	15.9 ± 0.1*	16.1 ± 0.1*	15.6 ± 0.1**	16.3 ± 0.2*
Erythrocytes ($10^6/\mu$L)	9.59 ± 0.21	9.46 ± 0.13	9.36 ± 0.11	9.45 ± 0.11	9.19 ± 0.07	8.80 ± 0.11**
Reticulocytes ($10^6/\mu$L)	0.29 ± 0.02	0.30 ± 0.03	0.31 ± 0.03	0.34 ± 0.02	0.38 ± 0.03	0.71 ± 0.06**
Mean cell volume (fL)	49.7 ± 0.2	49.5 ± 0.2	49.7 ± 0.2	49.5 ± 0.2	49.4 ± 0.1	50.5 ± 0.3
Mean cell hemoglobin (pg)	17.4 ± 0.2	17.2 ± 0.1	17.0 ± 0.1	17.1 ± 0.1	17.0 ± 0.1	18.6 ± 0.1*
Mean cell hemoglobin concentration (g/dL)	35.0 ± 0.2	34.7 ± 0.2	34.3 ± 0.3	34.5 ± 0.2	34.4 ± 0.1	36.8 ± 0.2
Heinz bodies (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.4 ± 0.5**

* Significantly different ($P \le 0.05$) from the vehicle control group by Dunn's or Shirley's test
** Significantly different ($P \le 0.01$) from the vehicle control group by Shirley's test
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9

Absolute and relative spleen weights of male and female mice generally increased with increasing dose, and the spleen weights of males and females in the 80 and 160 mg/kg groups were significantly greater than those of the vehicle controls (Tables 17 and C3). The relative heart and right testis weights of males and the absolute and relative heart weights of females administered 160 mg/kg were also greater than those of the vehicle controls.

TABLE 17 Spleen Weights and Spleen-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline[a]

	Dose (mg/kg)					
	Vehicle Control	**10**	**20**	**40**	**80**	**160**
MALE						
n	9	9	10	9	10	9
Necropsy body wt	36.1 ± 1.3	34.7 ± 1.0	35.6 ± 1.0	35.0 ± 0.4	35.4 ± 0.6	33.4 ± 1.1
Spleen						
Absolute	0.074 ± 0.003	0.077 ± 0.003	0.077 ± 0.001	0.084 ± 0.003	0.109 ± 0.005**	0.155 ± 0.005**
Relative	2.06 ± 0.09	2.22 ± 0.09	2.18 ± 0.07	2.39 ± 0.07	3.09 ± 0.17**	4.67 ± 0.15**
FEMALE						
n	10	10	9	10	10	9
Necropsy body wt	28.7 ± 0.7	30.8 ± 1.2	30.7 ± 0.6	28.5 ± 0.7	30.2 ± 0.6	27.8 ± 0.6
Spleen						
Absolute	0.093 ± 0.004	0.102 ± 0.004	0.111 ± 0.007	0.106 ± 0.004	0.131 ± 0.006**	0.205 ± 0.009**
Relative	3.27 ± 0.16	3.32 ± 0.11	3.66 ± 0.29	3.74 ± 0.14	4.33 ± 0.18**	7.36 ± 0.24**

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

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

At necropsy, male and female mice in the 160 mg/kg groups had enlarged, darkened spleens. Microscopically, the spleens of one male in the 80 mg/kg group, all males in the 160 mg/kg group, and most females administered 40 mg/kg or greater had hemosiderin pigmentation (Tables 18, B1, and B2). Hematopoietic cell proliferation was observed in the spleens of males administered 40 mg/kg or greater and females in the 80 and 160 mg/kg groups. The severity of these lesions increased with increasing dose.

TABLE 18 Incidence of Lesions of the Spleen in B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline

	Dose (mg/kg)					
	Vehicle Control	**10**	**20**	**40**	**80**	**160**
MALE						
Spleen[a]	10	10	10	10	10	10
Hematopoietic cell proliferation[b]	0	0	0	6** (1.0)[c]	10** (1.5)	10** (1.6)
Pigmentation, hemosiderin	0	0	0	0	1 (1.0)	10** (1.6)
FEMALE						
Spleen	10	10	10	10	10	10
Hematopoietic cell proliferation	0	0	0	0	9** (1.8)	10** (2.0)
Pigmentation, hemosiderin	0	0	0	10** (1.0)	9** (1.3)	10** (1.8)

** Significantly different ($P \leq 0.01$) from the vehicle control group by the Fisher exact test
[a] Number of animals with spleen examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesion in affected animals: 1=minimal, 2=mild, 3=moderate, and 4=marked

There were no biologically significant differences in sperm motility or vaginal cytology parameters between male or female dosed and vehicle control mice (Tables E5 and E6).

m-Chloroaniline: Hematology and clinical chemistry data are listed in Tables 19 and D4. As in the *o*-chloroaniline study, administration of *m*-chloroaniline caused a dose-dependent methemoglobinemia, evidenced by minimal to marked increases in methemoglobin concentration in all dosed groups of males and females at week 13. The methemoglobinemia was more severe in the *m*-chloroaniline study. Evidence of a mild to moderate anemia was demonstrated by treatment-related decreases in Hct values and RBC counts in males administered 20 mg/kg or greater and females administered 40 mg/kg or greater. The Hgb concentrations in the 80 and 160 mg/kg groups were inappropriately unchanged or increased in relation to the changes in Hct values and RBC counts. This inappropriate response would be consistent with the erroneous elevation of Hgb concentrations due to the presence of large numbers of Heinz bodies. An erythropoietic response to the anemia was evidenced by increased reticulocyte counts. Similar to that occurring in the *o*-chloroaniline study, the anemia was normocytic and hyperchromic, as evidenced by the lack of change in MCV and the minimal to moderate increases in MCHC. Increased MCH values accompanied the changes in MCHC; the increases in these parameters would be consistent with either a hemolytic process or the presence of large numbers of Heinz bodies causing erroneously elevated Hgb concentrations.

Microscopic evaluation of blood smears revealed moderately increased numbers of Heinz bodies and schistocytes in males and females in the 160 mg/kg groups at 13 weeks; occasional keratocytes and acanthocytes were also observed. Increased numbers of Heinz bodies and schistocytes also occurred in the 40 and 80 mg/kg groups. The presence of Heinz bodies and schistocytes is consistent with oxidative red cell injury and suggests that the anemia was of hemolytic origin. Increased numbers of polychromatophilic red cells occurred in the 80 and 160 mg/kg groups; increased polychromasia would be consistent with a bone marrow response to the anemia. While similar RBC alterations occurred in the *o*-chloroaniline study, the incidences, types, and severities of the alterations caused by *m*-chloroaniline administration were more pronounced. A minimal leukocytosis, evidenced by an increased WBC count, occurred in females in the 160 mg/kg group; this increase was related to an increase in lymphocyte count. Because the differences between females in the vehicle control and 160 mg/kg groups were minor, substantiating the leukocytosis by estimating WBC numbers from blood smears was not feasible; however, based on the findings in the *o*-chloroaniline study, the increased automated leukocyte counts in the *m*-chloroaniline study may have been erroneously elevated due to the presence of Heinz bodies, RBC fragments, and/or reticulocytes in the bloodstream.

No biologically significant differences in clinical chemistry parameters were observed in dosed mice (Table D4).

TABLE 19 Selected Hematology Data for B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline[a]

	Dose (mg/kg)					
	Vehicle Control	10	20	40	80	160
MALE						
n	9	10	7	10	10	10
Methemoglobin (g/dL)	0.19 ± 0.03	0.28 ± 0.04	0.50 ± 0.08**	1.72 ± 0.27**	3.22 ± 0.39**	3.77 ± 0.09**
Hematocrit (%)	49.9 ± 0.6	49.2 ± 0.6	46.9 ± 0.3**	45.2 ± 0.3**	44.4 ± 0.7**	42.1 ± 0.5**
Hemoglobin (g/dL)	16.8 ± 0.1	16.6 ± 0.1	15.8 ± 0.1*	15.8 ± 0.3**	16.3 ± 0.1	17.3 ± 0.2
Erythrocytes ($10^6/\mu L$)	10.24 ± 0.13	10.15 ± 0.12	9.57 ± 0.09**	9.18 ± 0.07**	9.08 ± 0.15**	8.52 ± 0.12**
Reticulocytes ($10^6/\mu L$)	0.20 ± 0.01	0.24 ± 0.02	0.20 ± 0.02	0.33 ± 0.04**	0.38 ± 0.03**	0.95 ± 0.08**
Mean cell volume (fL)	48.7 ± 0.2	48.5 ± 0.2	49.0 ± 0.2	49.2 ± 0.2	48.9 ± 0.1	49.4 ± 0.4*
Mean cell hemoglobin (pg)	16.4 ± 0.1	16.4 ± 0.1	16.5 ± 0.1	17.2 ± 0.4*	18.0 ± 0.2**	20.3 ± 0.4**
Mean cell hemoglobin concentration (g/dL)	33.7 ± 0.2	33.7 ± 0.2	33.8 ± 0.2	34.9 ± 0.7*	36.7 ± 0.4**	41.1 ± 0.7**
Heinz bodies (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 1.4	2.4 ± 0.7**	13.8 ± 3.5**
FEMALE						
n	9	9	10	8	10	9
Methemoglobin (g/dL)	0.18 ± 0.03	0.30 ± 0.03*	0.60 ± 0.04**	1.86 ± 0.29**	3.62 ± 0.21**	4.60 ± 0.19**
Hematocrit (%)	47.4 ± 0.4	48.3 ± 0.5	47.6 ± 0.4	45.9 ± 0.4*	43.9 ± 0.3**	44.0 ± 0.6**
Hemoglobin (g/dL)	16.3 ± 0.1	16.4 ± 0.1	16.2 ± 0.1	15.7 ± 0.1	15.8 ± 0.2	16.8 ± 0.2
Erythrocytes ($10^6/\mu L$)	9.62 ± 0.10	9.89 ± 0.11	9.76 ± 0.12	9.38 ± 0.08	9.00 ± 0.06**	8.95 ± 0.13**
Reticulocytes ($10^6/\mu L$)	0.19 ± 0.02	0.24 ± 0.01	0.26 ± 0.01*	0.30 ± 0.02**	0.57 ± 0.03**	0.99 ± 0.11**
Mean cell volume (fL)	49.2 ± 0.2	48.9 ± 0.1	48.8 ± 0.3	48.9 ± 0.2	48.7 ± 0.2	49.1 ± 0.4
Mean cell hemoglobin (pg)	16.9 ± 0.1	16.6 ± 0.1	16.6 ± 0.2	16.8 ± 0.1	17.6 ± 0.2*	18.8 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	34.3 ± 0.2	33.9 ± 0.1	34.0 ± 0.2	34.3 ± 0.1	36.0 ± 0.3**	38.3 ± 0.4**
Heinz bodies (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 0.6**	5.4 ± 1.4**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.

The absolute and relative spleen weights of male and female mice increased with increasing dose, and the spleen weights of males administered 40 mg/kg or greater and females administered 20 mg/kg or greater were significantly greater than those of the vehicle controls (Tables 20 and C4).

TABLE 20 Spleen Weights and Spleen-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline[a]

	Dose (mg/kg)					
	Vehicle Control	**10**	**20**	**40**	**80**	**160**
MALE						
n	9	10	7	10	10	10
Necropsy body wt	36.2 ± 0.9	35.4 ± 1.3	33.8 ± 0.6	34.4 ± 0.4	35.1 ± 0.3	35.6 ± 1.0
Spleen						
Absolute	0.081 ± 0.002	0.081 ± 0.002	0.088 ± 0.005	0.111 ± 0.003**	0.163 ± 0.003**	0.295 ± 0.012**
Relative	2.27 ± 0.09	2.32 ± 0.09	2.60 ± 0.15	3.25 ± 0.11**	4.65 ± 0.07**	8.32 ± 0.39**
FEMALE						
n	9	9	10	8	10	9
Necropsy body wt	28.4 ± 0.7	28.8 ± 0.7	28.9 ± 0.8	29.9 ± 1.2	28.1 ± 0.5	28.0 ± 0.7
Spleen						
Absolute	0.094 ± 0.003	0.104 ± 0.005	0.116 ± 0.004*	0.138 ± 0.004**	0.193 ± 0.009**	0.282 ± 0.009**
Relative	3.33 ± 0.15	3.63 ± 0.13	4.05 ± 0.14*	4.67 ± 0.21**	6.87 ± 0.29**	10.11 ± 0.22**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[a] Spleen weights (absolute weights) and body weights are given in grams; spleen-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

At necropsy, the spleens of males and females in the 80 and 160 mg/kg groups were enlarged. Hemosiderin pigmentation was observed microscopically in the spleens of mice in all dosed groups, and the incidences were significant in males administered 20 mg/kg or greater and in all dosed groups of females (Tables 21, B3, and B4). Males administered 40 mg/kg or greater and females administered 20 mg/kg or greater also had significantly greater incidences of hematopoietic cell proliferation than those in the vehicle controls; three males in the 10 mg/kg group and two females in the 10 mg/kg group also had this lesion. The severity of the spleen lesions increased with increasing dose.

Hemosiderin pigmentation was observed in the bone marrow of male mice administered 40 mg/kg or greater and females in all dosed groups; the severity of this lesion generally increased with increasing dose (Tables 21, B3, and B4). Four males and two females in the 160 mg/kg groups had bone marrow erythroid cell hyperplasia; this lesion was of minimal to mild severity in males and minimal severity in females. Additionally, one female in the 40 mg/kg group had moderate myeloid cell hyperplasia.

The livers of three male and nine female mice in the 80 mg/kg groups and nine males and nine females in the 160 mg/kg group had Kupffer cell pigmentation consistent with hemosiderin; this lesion was of minimal severity in males and females in the 80 mg/kg groups and minimal to mild in males and females in the 160 mg/kg groups (Tables 21, B3, and B4). Eight males and seven females administered 160 mg/kg and one female administered 80 mg/kg had mild hematopoietic cell proliferation in the liver. Six females in the 160 mg/kg group also had mild pigmentation in the kidney.

No significant differences in sperm motility or vaginal cytology parameters occurred in male or female mice dosed with *m*-chloroaniline (Tables E7 and E8).

TABLE 21 Incidence of Selected Lesions in B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline

	Dose (mg/kg)					
	Vehicle Control	10	20	40	80	160
MALE						
Bone marrow[a]	10	10	10	10	10	10
Pigmentation, hemosiderin[b]	0	0	0	10** (1.1)[c]	10** (2.1)	10** (3.6)
Erythroid cell, hyperplasia	0	0	0	0	0	4* (1.3)
Liver	10	—[d]	3	—	3	10
Hematopoietic cell proliferation	0		0		0	8** (1.0)
Kupffer cell, pigmentation, hemosiderin	0		0		3 (1.0)	9** (1.4)
Spleen	10	10	10	10	10	10
Hematopoietic cell proliferation	0	0	3 (1.0)	10** (1.9)	10** (2.8)	10** (3.8)
Pigmentation, hemosiderin	0	1 (1.0)	4* (1.5)	10** (1.9)	10** (3.0)	10** (4.0)
FEMALE						
Bone marrow	10	10	10	10	10	10
Pigmentation, hemosiderin	0	4* (1.8)	8** (1.1)	7** (1.6)	10** (2.0)	9** (2.8)
Erythroid cell, hyperplasia	0	0	0	0	0	2 (1.0)
Kidney	10	1	—	2	—	10
Cortex, pigmentation, hemosiderin	0	0		0		6** (1.0)
Liver	10	1	—	2	9	10
Hematopoietic cell proliferation	0	0		0	1 (1.0)	7** (1.0)
Kupffer cell, pigmentation, hemosiderin	0	0		0	9** (1.0)	9** (2.0)
Spleen	10	10	10	10	10	10
Hematopoietic cell proliferation	0	2 (1.0)	7** (1.4)	7** (2.0)	10** (2.7)	9** (3.6)
Pigmentation, hemosiderin	0	9** (1.3)	10** (1.8)	8** (2.4)	10** (3.3)	9** (3.9)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, and 4=marked
[d] Not examined

p-Chloroaniline: The results of the 16-day, 13-week, and 2-year *p*-chloroaniline studies have been published (NTP, 1989). There were no deaths or clinical findings related to *p*-chloroaniline administration in the 13-week mouse study. The final mean body weights and mean body weight gains of dosed mice were similar to those of the vehicle controls.

The Hct values and RBC counts of male mice administered 15 mg/kg or greater were significantly less than those of the vehicle controls (Table 22). Males in all dosed groups had significantly greater methemoglobin concentrations than the vehicle controls. Females in all dosed groups had significantly lower Hct values than the vehicle controls; for females administered 15 mg/kg or greater, RBC counts were significantly lower and methemoglobin concentrations were significantly greater than those of the vehicle controls. Males and females in the 60 and 120 mg/kg groups had significantly greater Hgb concentrations than the vehicle controls. NRBC counts and MCV of males and females in the 120 mg/kg groups and MCH and MCHC of males and females administered 30 mg/kg or greater were significantly higher than those of the vehicle controls; males in the 15 mg/kg group also had significantly higher MCH than the vehicle controls. Females in the 60 and 120 mg/kg groups had significantly higher segmented neutrophil counts than the vehicle controls. Male and female mice in the 30 and 120 mg/kg groups had moderately to markedly increased numbers of Heinz bodies compared to the vehicle controls. Moderate to marked polychromasia and poikilocytosis were also observed in dosed mice.

Absolute spleen weights increased with increasing dose. The differences were significant for all groups of dosed males and for females administered 30 mg/kg or greater.

The histopathologic slides of lesions from the *p*-chloroaniline study were reviewed in parallel with those from the *o*- and *m*-chloroaniline studies to ensure that diagnostic terminology and severity grading criteria were consistent between the studies. The spectrum of lesions caused by *p*-chloroaniline (Table 23) and the sites of toxicity were similar to those for the *o*- and *m*- isomers.

All dosed groups of male and female mice had significantly increased incidences of hematopoietic cell proliferation and hemosiderin pigmentation in the spleen (Table 23). Males and females administered 120 mg/kg had significantly increased incidences of bone marrow erythroid cell hyperplasia. Incidences of renal cortex hemosiderin pigmentation were increased in males and females in the 120 mg/kg groups and females in the 60 mg/kg group. Males and females in the 60 and 120 mg/kg groups had Kupffer cell hemosiderin pigmentation. The severity of these lesions generally increased with increasing dose.

TABLE 22 Selected Hematology Data for B6C3F$_1$ Mice in the 13-Week Gavage Study of *p*-Chloroaniline[a]

	Dose (mg/kg)					
	Vehicle Control	**7.5**	**15**	**30**	**60**	**120**
MALE						
n	10	10	10	10	10	8
Methemoglobin (% hemoglobin)	0.63 ± 0.08	1.72 ± 0.19**	1.77 ± 0.14**	2.36 ± 0.17**	2.84 ± 0.39**	3.80 ± 0.20**
Hematocrit (%)	48.7 ± 0.4	46.9 ± 0.5	45.5 ± 1.3**	43.8 ± 0.5**	40.4 ± 0.4**	32.6 ± 0.8**
Hemoglobin (g/dL)	16.7 ± 0.1	15.9 ± 0.2	16.1 ± 0.6	16.3 ± 0.3	18.4 ± 0.2*	17.2 ± 0.3*
Erythrocytes ($10^6/\mu$L)	10.66 ± 0.07	10.24 ± 0.13	9.79 ± 0.35**	9.26 ± 0.10**	8.78 ± 0.09**	6.86 ± 0.16**
Nucleated erythrocytes ($10^3/\mu$L)	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.20	0.30 ± 0.15	0.50 ± 0.31	3.13 ± 0.83**
Mean cell volume (fL)	45.7 ± 0.2	45.9 ± 0.7	46.5 ± 0.5	47.2 ± 0.4	46.3 ± 0.3	47.9 ± 0.4**
Mean cell hemoglobin (pg)	15.7 ± 0.0	15.5 ± 0.2	16.5 ± 0.3*	17.6 ± 0.3**	20.9 ± 0.3**	25.1 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	34.2 ± 0.1	34.0 ± 0.2	35.4 ± 0.5	37.3 ± 0.4**	45.3 ± 0.7**	52.6 ± 0.7**
FEMALE						
n	9	10	10	9	7	10
Methemoglobin (% hemoglobin)	0.29 ± 0.07	0.30 ± 0.11	1.65 ± 0.19**	2.88 ± 0.36**	3.22 ± 0.15**	3.32 ± 0.26**
Hematocrit (%)	49.8 ± 0.7	47.3 ± 0.8*	47.2 ± 0.5*	45.7 ± 0.5**	41.4 ± 1.5**	35.4 ± 0.7**
Hemoglobin (g/dL)	16.8 ± 0.2	16.1 ± 0.2	16.6 ± 0.2	17.1 ± 0.2	19.6 ± 0.6**	18.1 ± 0.2**
Erythrocytes ($10^6/\mu$L)	10.69 ± 0.15	10.30 ± 0.16	10.18 ± 0.15*	9.63 ± 0.10**	8.93 ± 0.33**	7.26 ± 0.15**
Nucleated erythrocytes ($10^3/\mu$L)	0.22 ± 0.15	0.00 ± 0.00	0.10 ± 0.10	0.00 ± 0.00	0.86 ± 0.40	5.80 ± 1.22**
Mean cell volume (fL)	46.3 ± 0.3	46.0 ± 0.3	46.4 ± 0.4	47.6 ± 0.2	46.9 ± 0.6	48.8 ± 0.4**
Mean cell hemoglobin (pg)	15.7 ± 0.1	15.7 ± 0.1	16.3 ± 0.1	17.8 ± 0.2**	22.0 ± 0.6**	25.0 ± 0.4**
Mean cell hemoglobin concentration (g/dL)	33.9 ± 0.2	34.1 ± 0.1	35.1 ± 0.2	37.5 ± 0.4**	47.2 ± 1.1**	51.3 ± 0.8**
Segmented neutrophils ($10^3/\mu$L)	1.13 ± 0.19	0.83 ± 0.15	1.30 ± 0.18	1.60 ± 0.21	1.89 ± 0.24*	1.75 ± 0.15*

* Significantly different ($P \le 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \le 0.01$
[a] NTP, 1989. Data are given as mean ± standard error. Statistical tests were performed on unrounded data.

TABLE 23 Incidence of Selected Lesions in B6C3F$_1$ Mice in the 13-Week Gavage Study of *p*-Chloroaniline[a]

	Dose (mg/kg)					
	Vehicle Control	7.5	15	30	60	120
MALE						
Bone marrow[b]	10	10	10	10	10	10
Erythroid cell, hyperplasia[c]	0	0	0	0	0	6** (1.7)[d]
Kidney	10	10	10	10	10	10
Cortex, pigmentation, hemosiderin	0	0	0	0	0	9** (2.1)
Liver	10	10	10	10	10	10
Hematopoietic cell proliferation	1 (1.0)	0	2 (1.0)	0	0	1 (1.0)
Kupffer cell, pigmentation, hemosiderin	0	0	0	2 (1.0)	10** (1.4)	9** (2.8)
Spleen	10	10	10	10	10	10
Hematopoietic cell proliferation	0	8** (1.0)	10** (1.6)	10** (2.8)	10** (1.9)	8** (2.6)
Pigmentation, hemosiderin	0	9** (1.0)	9** (1.2)	9** (1.3)	10** (1.5)	7** (2.7)
FEMALE						
Bone marrow	10	10	10	10	10	10
Erythroid cell, hyperplasia	0	0	0	0	0	4* (1.3)
Kidney	10	10	10	10	10	10
Cortex, pigmentation, hemosiderin	0	0	0	0	4* (1.0)	10** (2.5)
Liver	10	10	10	10	10	10
Hematopoietic cell proliferation	2 (1.0)	0	1 (1.0)	2 (1.0)	0	6 (1.5)
Kupffer cell, pigmentation, hemosiderin	0	0	0	0	7** (1.0)	10** (2.8)
Spleen	10	10	10	10	10	10
Hematopoietic cell proliferation	1 (1.0)	9** (1.2)	10** (1.3)	9** (2.4)	8** (3.3)	10** (3.1)
Pigmentation, hemosiderin	0	10** (1.0)	10** (1.1)	9** (1.4)	7** (2.0)	10** (2.9)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test

** $P \leq 0.01$

[a] NTP, 1989 (Slides were reviewed by a quality assessment pathologist and are presented in this report with terminology and severity grading consistent with those used for the *o*- and *m*-chloroaniline data.)

[b] Number of animals with tissue examined microscopically

[c] Number of animals with lesion

[d] Average severity of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, and 4=marked

GENETIC TOXICITY

o-Chloroaniline (1 to 3,333 μg/plate) and *m*-chloroaniline (1 to 2,000 μg/plate) were each tested with a preincubation protocol at two laboratories for mutagenicity in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537, with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9; no mutagenic activity by either chemical was observed (Tables F1 and F2; Zeiger *et al.*, 1987).

In contrast to the lack of mutagenic activity seen in *S. typhimurium*, *o*-chloroaniline induced significant increases in trifluorothymidine mutations in L5178Y mouse lymphoma cells treated with S9 activation enzymes (Table F3; McGregor *et al.*, 1991), and *m*-chloroaniline induced significant increases in trifluorothymidine mutations in mouse lymphoma cells treated with and without S9 activation enzymes (Table F4). The lowest effective dose of *o*-chloroaniline that produced a mutagenic response was 300 μg/mL. *o*-Chloroaniline produced a significant increase in mutant colonies only at the highest concentration tested (400 μg/mL) in the second trial performed without S9; results of the first trial without S9 were considered to be inconclusive, because although no increase in mutant colonies was observed, the relative total growth (33%) observed at the highest dose indicated that higher concentrations of *o*-chloroaniline could have been tested. The lowest effective doses of *m*-chloroaniline producing a mutagenic response were 300 μg/mL without S9 and 240 μg/mL with S9.

m-Chloroaniline also induced sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells with and without S9 (Tables F5 and F6). In the sister chromatid exchange test without S9, a delayed harvest protocol was used to offset cell cycle delay induced by *m*-chloroaniline and maximize the number of second-division metaphase cells available for scoring. Positive responses were obtained at concentrations of 80 μg/mL without S9 and 600 μg/mL with S9. In the chromosomal aberrations test without S9, a delayed harvest protocol was used, and the lowest effective dose was 300.2 μg/mL. With S9, only the highest nonlethal dose tested (598.9 μg/mL) induced a significant increase in chromosomal aberrations.

Results from an *in vivo* rat bone marrow micronucleus test with *o*-chloroaniline were positive (Table F7). Both trials showed a dose-related increase ($P < 0.01$) in micronucleated erythrocytes following a series of three injections of *o*-chloroaniline administered at 24-hour intervals. However, results of a mouse bone marrow micronucleus test were concluded to be negative (Table F9). In the first trial, a positive response was obtained at two of the four doses tested in mice; a second trial showed no significant increase in the frequency of micronucleated erythrocytes at these or at higher doses of *o*-chloroaniline. Neither *o*- nor *m*-chloroaniline exposure led to an increase in the frequencies of micronucleated normochromatic erythrocytes in peripheral blood samples obtained from male and female mice at the end of the 13-week studies.

Despite the positive responses recorded in *in vitro* mammalian cell mutagenicity tests, results from *in vivo* rat and mouse bone marrow micronucleus tests with *m*-chloroaniline were negative (Tables F8 and F10). In the single trial performed with male rats, only two dose groups survived, but these were sufficient for a valid test; no significant increase in the number of micronucleated erythrocytes was observed in rats (Table F8). In the mouse micronucleus tests, results of the first trial shown in Table F10 were positive ($P < 0.001$), but the results of a second trial performed with the same experimental protocol (single intraperitoneal injection) were negative ($P = 0.946$). Subsequently, a third trial was conducted with a different protocol for chemical administration (three injections versus a single injection, to increase the total amount of chemical administered), and the results from this third trial were again negative. Thus, *m*-chloroaniline was concluded to be negative in the mouse bone marrow micronucleus test.

In conclusion, although neither *o*-chloroaniline nor *m*-chloroaniline induced mutations in *S. typhimurium*, both chemicals were genotoxic to mammalian cells *in vitro*. *In vivo*, *o*-chloroaniline induced micronuclei in the bone marrow erythrocytes of rats, but not mice. *m*-Chloroaniline did not induce micronuclei in either rats or mice. The data from genetic toxicity tests conducted with *p*-chloroaniline indicated a broader range of activity for this isomer, with positive results noted in all assays in which it was tested, including the *S. typhimurium* assay, the mouse lymphoma assay, *in vitro* Chinese hamster ovary cell cytogenetics assays, and the *in vivo* mouse bone marrow micronucleus assay.

DISCUSSION

As reported for aniline (Kiese, 1966) and its chlorinated derivatives (McLean *et al.*, 1969; Chhabra *et al.*, 1990), the hematopoietic system was the major target for toxicity in rats and mice exposed to *o*- or *m*-chloroaniline. The pattern of toxic effects caused by *o*- and *m*-chloroaniline in the current studies was similar in rats and mice. Methemoglobin formation and the accompanying hemolytic anemia, extramedullary hematopoiesis, and Heinz body formation were indicative of erythrocyte toxicity induced by chloroanilines. In the current studies, dose-related increases in spleen weights occurred in rats and mice. Similar enlargements of the spleen were reported in rats administered aniline (Gralla *et al.*, 1979) and in rats and mice administered *p*-chloroaniline (Chhabra *et al.*, 1990). Splenomegaly in dosed animals was attributed to the sequestration of damaged erythrocytes in splenic sinusoids and to a compensatory increase in hematopoiesis. These spleen effects appeared to be secondary to erythrocyte toxicity, as there were no direct or primary microscopic abnormalities in the spleen.

The lowest doses of chloroaniline isomers affecting various toxicity parameters are given in Table 22. At equivalent doses, *m*-chloroaniline was more potent than *o*-chloroaniline in producing erythrocyte toxicity. Additionally, toxicity was induced at lower doses by *m*-chloroaniline than by *o*-chloroaniline. The magnitude of methemoglobin response was also much greater in the *m*-chloroaniline studies; a high concentration of methemoglobin was found at each evaluation period in the 160 mg/kg groups, suggesting that a sustained maximal response had been achieved. In contrast, a more gradual increase in methemoglobin concentration occurred in the *o*-chloroaniline studies. These results are consistent with studies of methemoglobin formation in cats (McLean *et al.*, 1969), in which *m*- and *p*-halo aniline derivatives appeared to be the most potent methemoglobin inducers. In previous studies with *p*-chloroaniline, significant increases in methemoglobin concentration occurred at gavage doses that were lower than the doses used in the present studies (NTP, 1989; Chhabra *et al.*, 1990). These findings suggest that *p*-chloroaniline is the most potent of the chloroaniline isomers in the induction of methemoglobin formation in rats and mice, followed by *m*-chloroaniline, then *o*-chloroaniline. A similar order of potency occurred with changes in other hematology parameters, spleen weights, gross and microscopic abnormalities, and the severity of hemosiderin deposition. The varying potency among chloroaniline isomers and quantitative species differences in toxicity could be related to possible dissimilarity in the ratios of bioactivation or inactivation of reactive intermediates of the isomers (Rankin *et al.*, 1993).

TABLE 22 Lowest Doses Causing Adverse Effects in F344/N Rats and B6C3F$_1$ Mice in the 13-Week Gavage Studies of *o*-, *m*-, and *p*-Chloroaniline[a]

	Isomer		
	ortho	*meta*	*para*
RATS			
Male			
Hematotoxicity	≤10	≤10	≤5
Increased spleen weight	40	20	≤5
Histopathologic lesions			
Bone marrow	80	40	≤5
Kidney	160	40	≤5
Liver	80	40	≤10
Spleen	80	≤10	≤5
Female			
Hematotoxicity	≤10	≤10	≤5
Increased spleen weight	40	20	≤5
Histopathologic lesions			
Bone marrow	80	40	≤10
Kidney	160	40	≤10
Liver	80	40	≤10
Spleen	80	≤10	≤5
MICE			
Male			
Hematotoxicity	≤10	20	≤7.5
Increased spleen weight	80	40	≤7.5
Histopathologic lesions			
Bone marrow	—[b]	40	120
Kidney	—	—	120
Liver	—	160	60
Spleen	40	20	≤7.5
Female			
Hematotoxicity	≤10	≤10	≤7.5
Increased spleen weight	40	20	15
Histopathologic lesions			
Bone marrow	—	10	120
Kidney	—	160	60
Liver	—	80	60
Spleen	40	≤10	≤7.5

[a] Doses are given in milligrams per kilogram body weight. *o*-Chloroaniline and *m*-chloroaniline doses were 0, 10, 20, 40, 80, and 160 mg/kg; *p*-chloroaniline doses were 0, 5, 10, 20, 40, and 80 mg/kg for rats and 0, 7.5, 15, 30, 60, and 120 mg/kg for mice (NTP, 1989).

[b] No adverse effects observed

The methemoglobin response to each isomer in all groups was consistently greater in rats than in male or female mice. Except for cats (Thompson *et al.*, 1989), there does not appear to be a significant difference in the susceptibility of hemoglobin from different species to be oxidized to methemoglobin (Smith, 1996). Short-term exposure of humans to chloroanilines produces cyanosis, a manifestation of methemoglobin formation. Long-term exposure may result in reversible anemia (Linch, 1974). The methemoglobin can be reduced to hemoglobin in mammalian species by NADH-dependent methemoglobin reductase located in the erythrocytes. A 10-fold difference in the activity of this enzyme exists among various species. Enzymic activity is five times higher in rat erythrocytes and 10 times higher in mouse erythrocytes than in human erythrocytes (Smith, 1996), suggesting that humans are more susceptible to this particular toxic effect of aniline and its isomers. The difference in enzyme activity could explain the more severe methemoglobinemia in rats than in mice.

Female rats appeared to be more susceptible to the formation of methemoglobin and the development of anemia than male rats in the *o*- and *m*-chloroaniline studies. The mechanism for this sex difference is unknown. Generally, female rats have slightly fewer but larger erythrocytes than male rats (Jain, 1986); this may predispose the erythrocytes of female rats to be more sensitive to oxidative injury. Additionally, male animals may be better able to respond to an anemia due to the stimulatory effect of androgens on erythropoiesis (Wintrobe, 1981; Jain, 1986).

Methemoglobin concentrations were increased in rats administered 40 (females), 80, or 100 mg/kg *o*-chloroaniline or any dose of *m*-chloroaniline at the earliest sampling time (day 3), indicating that marked oxidative red cell injury developed rapidly after administration. Methemoglobin concentrations have been shown to increase in cats and Wistar rats within hours after oral or intraperitoneal administration of *o*- or *m*-chloroaniline (McLean *et al.*, 1969; Watanabe *et al.*, 1976). For rats in the *m*-chloroaniline study, the greatest methemoglobin concentrations occurred in the 160 mg/kg groups on day 3; methemoglobin concentrations in these groups declined somewhat by day 23, remaining stable at week 13. This observation suggests that increased activity of the enzyme systems involved in methemoglobin reduction occurred and could be explained by an absolute increase in enzyme-rich reticulocytes that appeared in response to the anemia. Similar time-dependent methemoglobin concentration findings have been observed for rats exposed to other methemoglobin-forming compounds (Travlos *et al.*, 1996; NTP, 1998). In contrast, the severity of the methemoglobinemia increased with time in the lower dose groups and for male and female rats in the *o*-chloroaniline study, suggesting that the red cell injury may not have been as severe, and thus red cells with oxidized hemoglobin were allowed to remain in the bloodstream longer.

The increased platelet counts that occurred in the *o*- and *m*-chloroaniline studies could have been related to a general increase in hematopoietic activity or to a physiologic response resulting in splenic contraction, or the counts could have been erroneously elevated due to the presence of free Heinz bodies or erythrocyte fragments

in the bloodstream. The decreased platelet counts that occurred in dosed male and female rats at the end of the *m*-chloroaniline study would be consistent with the greatly increased spleen weights. In animals, the spleen will sequester approximately a third of the circulating platelet mass (Jain, 1986). In cases of splenomegaly, there is abnormal pooling of platelets within the spleen, resulting in thrombocytopenia; the magnitude of thrombocytopenia is related to spleen size.

The biochemical evidence of increased hepatocellular leakage and/or altered liver function suggests that the liver was a target of toxic effects of *o*- and *m*-chloroaniline in rats. However, effects on the liver could have been secondary to the decreased erythrocyte oxygen-carrying capacity, related to the methemoglobinemia, which resulted in mild hepatocellular hypoxia and increased cell membrane permeability.

Based on the methemoglobin concentrations, a no-observed-adverse-effect level for hematotoxicity was not determined for any of the isomers in the current studies. However, the data can be used to establish reference dose concentrations or minimum risk levels for chlorinated anilines.

Although the *o*-, *m*-, and *p*- isomers of chloroaniline all exhibit genetic toxicity, the profiles of activity are not consistent. *p*-Chloroaniline (NTP, 1989) was mutagenic in all assays in which it was tested, including the *Salmonella typhimurium* assay (Mortelmans *et al.*, 1986), the mouse lymphoma assay, *in vitro* Chinese hamster ovary cell cytogenetics assays, and the *in vivo* mouse bone marrow micronucleus test (NTP, unpublished data). In contrast, neither *o*- nor *m*-chloroaniline demonstrated consistent responses among the various assays in which the compounds were tested. Neither chemical was mutagenic in *S. typhimurium*, but both compounds induced gene mutations in mouse lymphoma cells. Only *m*-chloroaniline was tested for induction of chromosomal damage in cultured Chinese hamster ovary cells, and the results were positive. However, *m*-chloroaniline did not induce micronuclei (an indication of chromosomal damage) in bone marrow erythrocytes of rats or mice following intraperitoneal injection or gavage administration. In contrast, *o*-chloroaniline was positive for induction of micronuclei in rat, but not mouse, bone marrow cells. Thus, neither *o*- nor *m*-chloroaniline exhibits the spectrum of mutagenic activity seen with *p*-chloroaniline.

The available epidemiological data are insufficient to allow a conclusion as to the carcinogenicity of anilines to humans (IARC, 1982). Aniline produced splenic sarcomas in male F344 rats (Goodman *et al.*, 1984). *p*-Chloroaniline produced splenic sarcomas in male rats and hepatocellular neoplasms and hemangiosarcomas in the liver and spleen of male mice (Ward *et al.*, 1980; Chhabra, 1990). *o*-Toluidine hydrochloride and azobenzene, compounds structurally related to aniline, have also been shown to be carcinogens in male and female rats (NCI, 1979a,b); two other structurally related compounds, 4,4′-sulfonyldianiline (dapsone) and D & C Red No. 9, have been shown to be carcinogens in male rats (NCI, 1977; NTP, 1982). Goodman *et al.* (1984) studied splenic lesions from male rats in these NCI and NTP studies and proposed that fibrosis of the

splenic parenchyma was a potential preneoplastic lesion; the authors postulated that methemoglobin bound with aniline compounds or their reactive intermediates was broken down in the red pulp of the spleen. Reactive metabolites were released to bind with mesenchymal tissue, resulting in fibrosis that progressed to neoplasm formation. There is a strong possibility for the nongenetic mechanism in the formation of splenic neoplasms (Bus and Popp, 1987). However, aniline has been shown to induce DNA damage *in vivo* in the liver and kidney of rats, suggesting a direct genotoxic mechanism as well (Parodi *et al.*, 1982a,b; McCarthy *et al.*, 1985). *o*-Chloroaniline and *m*-chloroaniline have not been evaluated for potential carcinogenicity in laboratory animals. The toxicity data for *o*- and *m*-chloroaniline suggest that if progression of erythrocyte toxicity leads to the formation of splenic lesions in rats, then *o*- and *m*-chloroaniline would likely be positive in the rodent carcinogen bioassay. However, if carcinogenicity is dependent on genotoxicity, then the *o*- and *m*- isomers, which display weaker genotoxic activity, may not be carcinogenic in a rodent bioassay. It is therefore difficult to predict carcinogenic activity of *o*- and *m*-chloroaniline based on the available information.

In conclusion, chloroaniline isomers are hematotoxic and have the same pattern of toxicity in rats and mice. No-observed-adverse-effect levels for hematotoxicity were not attained in these studies. The structure-toxicity relationship establishes *p*-chloroaniline as the most toxic isomer, followed by *m*-chloroaniline, then *o*-chloroaniline. The magnitude of toxicity induced is greater in rats than in mice for each of the three isomers. *p*-Chloroaniline is clearly genotoxic in various test systems, while results for *o*- and *m*-chloroaniline are inconsistent and indicate that genotoxic effects, if any, are weak.

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APPENDIX A
SUMMARY OF NONNEOPLASTIC LESIONS IN RATS

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early death						
Natural death				1		
Survivors						
Terminal sacrifice	10	10	10	9	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)		(1)	(1)		(10)
Hepatodiaphragmatic nodule	1 (10%)					
Kupffer cell, pigmentation, hemosiderin						10 (100%)
Cardiovascular System						
Heart	(10)			(1)		(10)
Myocardium, degeneration, chronic, focal	2 (20%)					2 (20%)
Endocrine System						
None						
General Body System						
None						
Genital System						
Preputial gland	(10)			(1)		(10)
Inflammation, chronic active	6 (60%)					6 (60%)
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Erythroid cell, hyperplasia				1 (10%)	10 (100%)	10 (100%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation					10 (100%)	10 (100%)
Pigmentation, hemosiderin						9 (90%)
Capsule, fibrosis					10 (100%)	9 (90%)
Integumentary System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Mineralization, diffuse	9 (90%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	9 (90%)
Mineralization, focal	1 (10%)					
Cortex, pigmentation, hemosiderin						10 (100%)

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early death						
Natural death			1			
Survivors						
Terminal sacrifice	10	10	9	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)		(1)			(10)
Kupffer cell, pigmentation, hemosiderin						10 (100%)
Cardiovascular System						
Heart	(10)		(1)			(10)
Myocardium, degeneration, chronic, focal						1 (10%)
Endocrine System						
None						
General Body System						
None						
Genital System						
Clitoral gland	(10)		(1)			(10)
Inflammation, chronic active	3 (30%)					1 (10%)
Ovary	(10)		(1)			(10)
Periovarian tissue, cyst	1 (10%)					
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Erythroid cell, hyperplasia					10 (100%)	10 (100%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation					10 (100%)	10 (100%)
Pigmentation, hemosiderin					9 (90%)	9 (90%)
Capsule, fibrosis					10 (100%)	10 (100%)
Integumentary System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Mineralization, diffuse	10 (100%)	10 (100%)	9 (90%)	10 (100%)	10 (100%)	9 (90%)
Cortex, pigmentation, hemosiderin						10 (100%)

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation		1 (10%)			9 (90%)	9 (90%)
Hemorrhage	1 (10%)					
Inflammation, acute		1 (10%)				
Inflammation, chronic active	1 (10%)					
Necrosis, focal		1 (10%)				
Necrosis, multifocal		1 (10%)				
Vacuolization cytoplasmic				1 (10%)		
Kupffer cell, pigmentation, hemosiderin				9 (90%)	10 (100%)	10 (100%)
Pancreas	(10)					(10)
Acinus, atrophy						1 (10%)
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	10 (100%)					5 (50%)
Endocrine System						
Thyroid gland	(10)					(10)
Ultimobranchial cyst	1 (10%)					1 (10%)
General Body System						
None						
Genital System						
Preputial gland	(10)					(10)
Inflammation, chronic	1 (10%)					4 (40%)
Inflammation, chronic, focal	1 (10%)					
Inflammation, chronic, multifocal	4 (40%)					
Inflammation, chronic, suppurative	1 (10%)					1 (10%)
Inflammation, granulomatous						1 (10%)
Testes	(10)					(10)
Atrophy						1 (10%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Pigmentation, hemosiderin					10 (100%)	10 (100%)
Erythroid cell, hyperplasia				10 (100%)	10 (100%)	10 (100%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Congestion		1 (10%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Fibrosis			1 (10%)			
Hematopoietic cell proliferation		7 (70%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Pigmentation, hemosiderin		6 (60%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Capsule, fibrosis		2 (20%)	2 (20%)	8 (80%)	10 (100%)	10 (100%)
Capsule, infiltration cellular	1 (10%)	2 (20%)	2 (20%)	10 (100%)	10 (100%)	10 (100%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)					(10)
Hemorrhage, focal	1 (10%)					1 (10%)
Alveolar epithelium, hyperplasia	1 (10%)					
Artery, mineralization	6 (60%)					5 (50%)
Interstitium, inflammation, chronic, focal	1 (10%)					
Interstitium, inflammation, chronic, multifocal						1 (10%)
Vein, mineralization						1 (10%)
Nose	(10)					(10)
Lateral wall, inflammation, granulomatous	1 (10%)					
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, chronic, multifocal		1 (10%)				
Mineralization	10 (100%)	10 (100%)	9 (90%)	10 (100%)	10 (100%)	10 (100%)
Cortex, pigmentation, hemosiderin				10 (100%)	10 (100%)	10 (100%)
Renal tubule, regeneration, focal	2 (20%)			1 (10%)	2 (20%)	
Renal tubule, regeneration, multifocal	7 (70%)	8 (80%)	7 (70%)	2 (20%)		

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early death						
Natural death						1
Survivors						
Terminal sacrifice	10	10	10	10	10	9
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(9)					(10)
Muscularis, inflammation, chronic, focal						1 (10%)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	2 (20%)		1 (10%)	10 (100%)	10 (100%)	10 (100%)
Hepatodiaphragmatic nodule	1 (10%)					
Inflammation, chronic, multifocal	1 (10%)					
Pigmentation	3 (30%)					
Bile duct, inflammation, chronic, focal						1 (10%)
Kupffer cell, pigmentation, hemosiderin				10 (100%)	10 (100%)	10 (100%)
Pancreas	(10)					(10)
Acinus, atrophy	1 (10%)					
Cardiovascular System						
Heart	(10)					(10)
Cardiomyopathy	4 (40%)					5 (50%)
Endocrine System						
Thyroid gland	(10)		(1)			(10)
Cyst			1 (100%)			
Ultimobranchial cyst	1 (10%)					
General Body System						
None						
Genital System						
Clitoral gland	(10)					(10)
Inflammation, chronic	1 (10%)					
Inflammation, chronic, focal	1 (10%)					1 (10%)
Inflammation, chronic, multifocal	3 (30%)					6 (60%)
Inflammation, chronic, multifocal, suppurative	1 (10%)					

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, multifocal, histiocyte						1 (10%)
Infiltration cellular, histiocyte		1 (10%)	2 (20%)	3 (30%)		
Myelofibrosis	3 (30%)	1 (10%)			1 (10%)	1 (10%)
Pigmentation, hemosiderin				3 (30%)	10 (100%)	9 (90%)
Erythroid cell, hyperplasia				6 (60%)	10 (100%)	10 (100%)
Erythroid cell, myelofibrosis				1 (10%)		
Lymph node			(1)			
Lumbar, pigmentation			1 (100%)			
Lymph node, mesenteric	(10)					(10)
Hyperplasia, histiocytic	1 (10%)					
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Congestion			8 (80%)	9 (90%)	10 (100%)	10 (100%)
Hematopoietic cell proliferation		5 (50%)	8 (80%)	10 (100%)	10 (100%)	9 (90%)
Pigmentation, hemosiderin		10 (100%)	10 (100%)	9 (90%)	10 (100%)	10 (100%)
Capsule, fibrosis				6 (60%)	10 (100%)	9 (90%)
Capsule, infiltration cellular	1 (10%)	1 (10%)		9 (90%)	10 (100%)	8 (80%)
Lymphoid follicle, atrophy						1 (10%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)					(10)
Congestion						1 (10%)
Inflammation, chronic active, focal						1 (10%)
Inflammation, chronic active, multifocal	1 (10%)					
Arteriole, inflammation, granulomatous						1 (10%)
Artery, mineralization	3 (30%)					5 (50%)
Vein, mineralization						1 (10%)
Nose	(10)					(10)
Respiratory epithelium, inflammation, chronic						1 (10%)

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Mineralization	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	9 (90%)
Cortex, inflammation, chronic, focal		1 (10%)				
Cortex, pigmentation, hemosiderin			1 (10%)	10 (100%)	10 (100%)	10 (100%)
Renal tubule, regeneration, focal	2 (20%)		1 (10%)			

APPENDIX B
SUMMARY OF NONNEOPLASTIC LESIONS IN MICE

TABLE B1 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early death						
Accidental death		1				
Survivors						
Died last week of study	1			1		1
Terminal sacrifice	9	9	10	9	10	9
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(1)		(1)		(10)
Inflammation, suppurative		1 (100%)				
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation				6 (60%)	10 (100%)	10 (100%)
Pigmentation, hemosiderin					1 (10%)	10 (100%)
Thymus	(10)	(1)		(1)		(10)
Inflammation, suppurative		1 (100%)				
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B1 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Respiratory System						
Pleura		(1)				
Inflammation, suppurative		1 (100%)				
Special Senses System						
None						
Urinary System						
Kidney	(10)	(1)		(1)		(10)
Nephropathy	1 (10%)					

TABLE B2 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Died last week of study			1			1
Terminal sacrifice	10	10	9	10	10	9
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
None						
Cardiovascular System						
None						
Endocrine System						
Parathyroid gland	(7)		(1)			(9)
Cyst						1 (11%)
Thyroid gland	(10)		(1)			(10)
Cyst						1 (10%)
Inflammation, granulomatous			1 (100%)			
General Body System						
None						
Genital System						
Ovary	(10)		(1)	(1)		(10)
Cyst				1 (100%)		
Hematopoietic System						
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation					9 (90%)	10 (100%)
Pigmentation, hemosiderin				10 (100%)	9 (90%)	10 (100%)
Thymus	(10)		(1)			(10)
Hemorrhage						1 (10%)
Inflammation, granulomatous			1 (100%)			
Integumentary System						
None						

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B2 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)		(1)			(10)
Hemorrhage, focal						1 (10%)
Special Senses System						
None						
Urinary System						
None						

TABLE B3 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental deaths	1		3			
Survivors						
Terminal sacrifice	9	10	7	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)		(3)			(10)
Foreign body			1 (33%)			
Inflammation, suppurative	1 (10%)					
Perforation	1 (10%)					
Liver	(10)		(3)		(3)	(10)
Hematopoietic cell proliferation						8 (80%)
Kupffer cell, pigmentation, hemosiderin					3 (100%)	9 (90%)
Cardiovascular System						
Heart	(10)		(3)			(10)
Inflammation, suppurative			2 (67%)			
Myocardium, degeneration						1 (10%)
Pericardium, inflammation, suppurative	1 (10%)		1 (33%)			
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Pigmentation, hemosiderin				10 (100%)	10 (100%)	10 (100%)
Erythroid cell, hyperplasia						4 (40%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation			3 (30%)	10 (100%)	10 (100%)	10 (100%)
Pigmentation, hemosiderin		1 (10%)	4 (40%)	10 (100%)	10 (100%)	10 (100%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B3 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)		(3)			(10)
Foreign body	1 (10%)		1 (33%)			
Serosa, inflammation, suppurative	1 (10%)		3 (100%)			
Special Senses System						
None						
Urinary System						
None						

TABLE B4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidental deaths	1	1		2		1
Survivors						
Terminal sacrifice	9	9	10	8	10	9
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Esophagus	(10)	(1)		(2)		(10)
Hemorrhage	1 (10%)					
Ulcer				1 (50%)		
Liver	(10)	(1)		(2)	(9)	(10)
Hematopoietic cell proliferation					1 (11%)	7 (70%)
Necrosis, focal						1 (10%)
Kupffer cell, pigmentation, hemosiderin					9 (100%)	9 (90%)
Cardiovascular System						
Heart	(10)	(1)		(2)		(10)
Pericardium, inflammation, suppurative		1 (100%)		1 (50%)		
Endocrine System						
None						
General Body System						
None						
Genital System						
Ovary		(1)	(1)	(2)		
Cyst			1 (100%)			
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Pigmentation, hemosiderin		4 (40%)	8 (80%)	7 (70%)	10 (100%)	9 (90%)
Erythroid cell, hyperplasia						2 (20%)
Myeloid cell, hyperplasia, diffuse				1 (10%)		
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation		2 (20%)	7 (70%)	7 (70%)	10 (100%)	9 (90%)
Pigmentation, hemosiderin		9 (90%)	10 (100%)	8 (80%)	10 (100%)	9 (90%)
Thymus	(10)	(1)		(2)		(10)
Atrophy				1 (50%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B4 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(1)		(2)		(10)
Foreign body				2 (100%)		1 (10%)
Hemorrhage	1 (10%)					
Serosa, inflammation, suppurative		1 (100%)		2 (100%)		1 (10%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(1)		(2)		(10)
Hyperplasia, lymphoid	1 (10%)					
Infarct	1 (10%)					
Cortex, pigmentation, hemosiderin						6 (60%)

APPENDIX C
ORGAN WEIGHTS AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE C1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
MALE						
n	10	10	10	9	9	10
Necropsy body wt	334 ± 3	328 ± 6	334 ± 7	329 ± 4	319 ± 5	304 ± 5**
Heart						
Absolute	0.973 ± 0.017	0.972 ± 0.026	1.045 ± 0.027	1.009 ± 0.016	1.024 ± 0.028	0.950 ± 0.013
Relative	2.91 ± 0.04	2.96 ± 0.05	3.14 ± 0.07*	3.07 ± 0.05*	3.21 ± 0.07**	3.13 ± 0.06**
Right kidney						
Absolute	1.129 ± 0.024	1.110 ± 0.027	1.131 ± 0.029	1.148 ± 0.018	1.157 ± 0.024	1.115 ± 0.015
Relative	3.38 ± 0.06	3.38 ± 0.05	3.39 ± 0.04	3.49 ± 0.04	3.63 ± 0.05**	3.67 ± 0.04**
Liver						
Absolute	12.186 ± 0.363	12.169 ± 0.409	12.524 ± 0.428	12.655 ± 0.293	12.772 ± 0.335	11.478 ± 0.226
Relative	36.48 ± 0.95	37.02 ± 0.81	37.51 ± 0.84	38.44 ± 0.62	40.05 ± 0.88*	37.71 ± 0.41*
Lungs						
Absolute	1.362 ± 0.046[b]	1.556 ± 0.061	1.550 ± 0.059	1.461 ± 0.055	1.390 ± 0.063	1.376 ± 0.045
Relative	4.08 ± 0.13[b]	4.74 ± 0.17*	4.65 ± 0.17*	4.44 ± 0.14	4.35 ± 0.17	4.52 ± 0.14
Spleen						
Absolute	0.684 ± 0.014	0.663 ± 0.012	0.681 ± 0.018	0.750 ± 0.013	0.861 ± 0.043**	1.104 ± 0.024**
Relative	2.05 ± 0.04	2.02 ± 0.02	2.05 ± 0.05	2.28 ± 0.03*	2.70 ± 0.13**	3.63 ± 0.06**
Right testis						
Absolute	1.483 ± 0.013	1.468 ± 0.020	1.507 ± 0.027	1.514 ± 0.029	1.448 ± 0.038	1.461 ± 0.019
Relative	4.44 ± 0.03	4.49 ± 0.10	4.53 ± 0.09	4.61 ± 0.12	4.54 ± 0.10	4.81 ± 0.06**
Thymus						
Absolute	0.301 ± 0.015	0.318 ± 0.018	0.325 ± 0.012	0.319 ± 0.010	0.310 ± 0.011	0.300 ± 0.012
Relative	0.90 ± 0.04	0.97 ± 0.06	0.97 ± 0.03	0.97 ± 0.03	0.98 ± 0.04	0.99 ± 0.04
FEMALE						
n	10	10	9	10	10	10
Necropsy body wt	183 ± 3	189 ± 3	190 ± 3	176 ± 3	178 ± 2	174 ± 2*
Heart						
Absolute	0.608 ± 0.014	0.638 ± 0.015	0.654 ± 0.009	0.619 ± 0.011	0.627 ± 0.011	0.641 ± 0.018
Relative	3.32 ± 0.09	3.38 ± 0.09	3.45 ± 0.05	3.51 ± 0.05	3.52 ± 0.05	3.69 ± 0.08**
Right kidney						
Absolute	0.640 ± 0.011	0.650 ± 0.011	0.657 ± 0.009	0.615 ± 0.010	0.623 ± 0.007	0.621 ± 0.010
Relative	3.49 ± 0.05	3.44 ± 0.05	3.47 ± 0.04	3.50 ± 0.07	3.49 ± 0.03	3.58 ± 0.05
Liver						
Absolute	6.058 ± 0.123	6.341 ± 0.178	6.373 ± 0.139	6.000 ± 0.141	6.111 ± 0.087	6.082 ± 0.111
Relative	33.06 ± 0.66	33.55 ± 0.77	33.57 ± 0.44	34.03 ± 0.50	34.29 ± 0.47	35.05 ± 0.42*
Lungs						
Absolute	1.024 ± 0.029	1.092 ± 0.045	1.088 ± 0.066	1.006 ± 0.040	1.019 ± 0.036	1.021 ± 0.040
Relative	5.60 ± 0.19	5.77 ± 0.21	5.76 ± 0.41	5.69 ± 0.17	5.70 ± 0.16	5.89 ± 0.22
Spleen						
Absolute	0.419 ± 0.009	0.432 ± 0.007	0.459 ± 0.010*	0.460 ± 0.010*	0.594 ± 0.013**	0.907 ± 0.018**
Relative	2.28 ± 0.03	2.29 ± 0.02	2.42 ± 0.04	2.61 ± 0.05**	3.33 ± 0.06**	5.23 ± 0.09**
Thymus						
Absolute	0.235 ± 0.010	0.232 ± 0.009	0.237 ± 0.008	0.222 ± 0.007	0.218 ± 0.008	0.230 ± 0.007
Relative	1.28 ± 0.05	1.23 ± 0.05	1.25 ± 0.03	1.26 ± 0.03	1.22 ± 0.04	1.33 ± 0.05

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** Significantly different ($P \leq 0.01$) from the vehicle control group by Williams' test
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

TABLE C2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
MALE						
n	10	10	10	10	10	10
Necropsy body wt	326 ± 6	327 ± 8	317 ± 7	325 ± 6	319 ± 6	293 ± 6**
Heart						
Absolute	0.961 ± 0.025	1.010 ± 0.039	0.988 ± 0.039	0.989 ± 0.025	1.041 ± 0.031	1.034 ± 0.028
Relative	2.94 ± 0.06	3.08 ± 0.06	3.11 ± 0.08	3.04 ± 0.05	3.26 ± 0.07**	3.54 ± 0.09**
Right kidney						
Absolute	1.096 ± 0.031	1.114 ± 0.044	1.070 ± 0.031	1.115 ± 0.027	1.126 ± 0.031	1.076 ± 0.027
Relative	3.36 ± 0.07	3.40 ± 0.06	3.37 ± 0.04	3.43 ± 0.03	3.53 ± 0.06*	3.68 ± 0.05**
Liver						
Absolute	11.940 ± 0.359	11.790 ± 0.537	11.651 ± 0.405	11.973 ± 0.476	12.023 ± 0.338	10.785 ± 0.355
Relative	36.65 ± 1.13	35.90 ± 0.83	36.67 ± 0.62	36.72 ± 0.97	37.69 ± 0.62	36.80 ± 0.66
Lungs						
Absolute	1.573 ± 0.048	1.568 ± 0.062	1.447 ± 0.075	1.442 ± 0.059	1.480 ± 0.066	1.296 ± 0.068**
Relative	4.83 ± 0.15	4.81 ± 0.19	4.55 ± 0.18	4.45 ± 0.20	4.63 ± 0.16	4.43 ± 0.21
Spleen						
Absolute	0.701 ± 0.030	0.788 ± 0.015	0.859 ± 0.011*	1.275 ± 0.024**	2.483 ± 0.068**	3.373 ± 0.098**
Relative	2.15 ± 0.10	2.42 ± 0.04	2.72 ± 0.05**	3.93 ± 0.07**	7.78 ± 0.11**	11.52 ± 0.18**
Right testis						
Absolute	1.467 ± 0.031	1.455 ± 0.025	1.457 ± 0.030	1.435 ± 0.029	1.516 ± 0.018	1.363 ± 0.054
Relative	4.51 ± 0.11	4.46 ± 0.05	4.61 ± 0.07	4.41 ± 0.06	4.76 ± 0.06	4.68 ± 0.22
Thymus						
Absolute	0.282 ± 0.017	0.293 ± 0.013	0.260 ± 0.010	0.283 ± 0.011	0.321 ± 0.016	0.298 ± 0.019
Relative	0.86 ± 0.04	0.89 ± 0.03	0.82 ± 0.03	0.87 ± 0.03	1.01 ± 0.05*	1.02 ± 0.06**
FEMALE						
n	10	10	10	10	10	9
Necropsy body wt	183 ± 3	185 ± 3	186 ± 3	183 ± 2	179 ± 3	185 ± 3
Heart						
Absolute	0.631 ± 0.014	0.634 ± 0.011	0.637 ± 0.014	0.660 ± 0.010	0.648 ± 0.014	0.728 ± 0.016**
Relative	3.47 ± 0.09	3.42 ± 0.04	3.43 ± 0.08	3.60 ± 0.05	3.63 ± 0.07	3.95 ± 0.08**
Right kidney						
Absolute	0.629 ± 0.008	0.627 ± 0.010	0.642 ± 0.009	0.644 ± 0.009	0.645 ± 0.013	0.681 ± 0.012**
Relative	3.45 ± 0.06	3.39 ± 0.05	3.45 ± 0.04	3.52 ± 0.05	3.62 ± 0.06*	3.69 ± 0.04**
Liver						
Absolute	5.810 ± 0.153	6.171 ± 0.093	6.091 ± 0.122	6.099 ± 0.109	6.479 ± 0.319*	7.311 ± 0.147**
Relative	31.81 ± 0.50	33.31 ± 0.47	32.83 ± 0.77	33.28 ± 0.43	36.26 ± 1.58**	39.62 ± 0.60**
Lungs						
Absolute	1.207 ± 0.033	1.109 ± 0.044[b]	1.082 ± 0.034*	1.100 ± 0.036*	0.991 ± 0.043**	1.105 ± 0.041**
Relative	6.63 ± 0.23	6.00 ± 0.25[b]	5.84 ± 0.22*	6.01 ± 0.21*	5.56 ± 0.24**	5.98 ± 0.18**
Spleen						
Absolute	0.451 ± 0.017	0.504 ± 0.008	0.603 ± 0.015**	0.885 ± 0.015**	1.614 ± 0.050**	2.349 ± 0.046**
Relative	2.47 ± 0.07	2.72 ± 0.04	3.25 ± 0.06**	4.83 ± 0.09**	9.04 ± 0.23**	12.75 ± 0.28**
Thymus						
Absolute	0.208 ± 0.007	0.242 ± 0.010	0.227 ± 0.009	0.215 ± 0.008	0.235 ± 0.010	0.244 ± 0.012*
Relative	1.14 ± 0.04	1.31 ± 0.05	1.22 ± 0.04	1.17 ± 0.04	1.32 ± 0.05*	1.32 ± 0.07*

* Significantly different (P≤0.05) from the vehicle control group by Williams' test
** P≤0.01
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9

TABLE C3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
MALE						
n	9	9	10	9	10	9
Necropsy body wt	36.1 ± 1.3	34.7 ± 1.0	35.6 ± 1.0	35.0 ± 0.4	35.4 ± 0.6	33.4 ± 1.1
Heart						
Absolute	0.166 ± 0.004	0.172 ± 0.006	0.168 ± 0.005	0.171 ± 0.005	0.179 ± 0.005	0.173 ± 0.004
Relative	4.61 ± 0.10	4.99 ± 0.23	4.75 ± 0.13	4.88 ± 0.11	5.06 ± 0.21	5.22 ± 0.12*
Right kidney						
Absolute	0.309 ± 0.008	0.306 ± 0.008	0.293 ± 0.007	0.292 ± 0.008	0.300 ± 0.007	0.290 ± 0.007
Relative	8.60 ± 0.19	8.86 ± 0.29	8.28 ± 0.23	8.32 ± 0.19	8.49 ± 0.23	8.72 ± 0.22
Liver						
Absolute	1.777 ± 0.049	1.694 ± 0.061	1.769 ± 0.035	1.721 ± 0.037	1.782 ± 0.029	1.663 ± 0.042
Relative	49.33 ± 0.80	48.78 ± 1.08	49.83 ± 0.52	49.12 ± 0.89	50.33 ± 0.71	49.93 ± 0.80
Lungs						
Absolute	0.199 ± 0.005	0.212 ± 0.006	0.208 ± 0.007	0.206 ± 0.006	0.207 ± 0.007	0.200 ± 0.006
Relative	5.54 ± 0.20	6.16 ± 0.27	5.92 ± 0.30	5.89 ± 0.20	5.84 ± 0.22	6.05 ± 0.31
Spleen						
Absolute	0.074 ± 0.003	0.077 ± 0.003	0.077 ± 0.001	0.084 ± 0.003	0.109 ± 0.005**	0.155 ± 0.005**
Relative	2.06 ± 0.09	2.22 ± 0.09	2.18 ± 0.07	2.39 ± 0.07	3.09 ± 0.17**	4.67 ± 0.15**
Right testis						
Absolute	0.119 ± 0.002	0.119 ± 0.004	0.122 ± 0.003	0.122 ± 0.003	0.122 ± 0.002	0.125 ± 0.004
Relative	3.31 ± 0.11	3.45 ± 0.13	3.44 ± 0.09	3.48 ± 0.06	3.44 ± 0.08	3.76 ± 0.12**
Thymus						
Absolute	0.052 ± 0.004	0.051 ± 0.004	0.053 ± 0.002	0.044 ± 0.003	0.049 ± 0.002	0.050 ± 0.004
Relative	1.43 ± 0.10	1.46 ± 0.10	1.48 ± 0.06	1.26 ± 0.09	1.37 ± 0.06	1.51 ± 0.14
FEMALE						
n	10	10	9	10	10	9
Necropsy body wt	28.7 ± 0.7	30.8 ± 1.2	30.7 ± 0.6	28.5 ± 0.7	30.2 ± 0.6	27.8 ± 0.6
Heart						
Absolute	0.138 ± 0.003	0.152 ± 0.004	0.149 ± 0.005	0.142 ± 0.002	0.147 ± 0.003	0.154 ± 0.005**
Relative	4.81 ± 0.10	4.97 ± 0.17	4.86 ± 0.17	4.99 ± 0.09	4.89 ± 0.10	5.54 ± 0.14**
Right kidney						
Absolute	0.200 ± 0.005	0.201 ± 0.004	0.207 ± 0.003	0.193 ± 0.005	0.202 ± 0.002	0.203 ± 0.004
Relative	6.97 ± 0.13	6.57 ± 0.18	6.75 ± 0.14	6.79 ± 0.13	6.72 ± 0.15	7.32 ± 0.09
Liver						
Absolute	1.426 ± 0.053	1.581 ± 0.055	1.507 ± 0.032	1.406 ± 0.050	1.511 ± 0.040	1.457 ± 0.046
Relative	49.64 ± 1.27	51.51 ± 0.97	49.27 ± 1.35	49.21 ± 0.93	50.08 ± 1.17	52.42 ± 1.06
Lungs						
Absolute	0.198 ± 0.007	0.211 ± 0.010	0.211 ± 0.007	0.205 ± 0.009	0.208 ± 0.007	0.209 ± 0.008
Relative	6.91 ± 0.26	6.88 ± 0.26	6.93 ± 0.30	7.18 ± 0.22	6.91 ± 0.22	7.51 ± 0.23
Spleen						
Absolute	0.093 ± 0.004	0.102 ± 0.004	0.111 ± 0.007	0.106 ± 0.004	0.131 ± 0.006**	0.205 ± 0.009**
Relative	3.27 ± 0.16	3.32 ± 0.11	3.66 ± 0.29	3.74 ± 0.14	4.33 ± 0.18**	7.36 ± 0.24**
Thymus						
Absolute	0.052 ± 0.002	0.059 ± 0.004	0.054 ± 0.001	0.053 ± 0.002	0.055 ± 0.002	0.051 ± 0.002
Relative	1.84 ± 0.10	1.91 ± 0.07	1.76 ± 0.04	1.87 ± 0.06	1.82 ± 0.06	1.85 ± 0.07

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

** $P \leq 0.01$

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

TABLE C4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
MALE						
n	9	10	7	10	10	10
Necropsy body wt	36.2 ± 0.9	35.4 ± 1.3	33.8 ± 0.6	34.4 ± 0.4	35.1 ± 0.3	35.6 ± 1.0
Heart						
Absolute	0.187 ± 0.007	0.176 ± 0.008	0.181 ± 0.006	0.175 ± 0.003	0.189 ± 0.008	0.189 ± 0.006
Relative	5.20 ± 0.22	4.99 ± 0.24	5.34 ± 0.14	5.10 ± 0.09	5.40 ± 0.20	5.31 ± 0.14
Right kidney						
Absolute	0.301 ± 0.008	0.297 ± 0.007	0.292 ± 0.010	0.301 ± 0.006	0.299 ± 0.006	0.297 ± 0.008
Relative	8.33 ± 0.27	8.43 ± 0.17	8.63 ± 0.23	8.75 ± 0.17	8.52 ± 0.13	8.35 ± 0.20
Liver						
Absolute	1.834 ± 0.044	1.824 ± 0.056	1.755 ± 0.047	1.795 ± 0.028	1.782 ± 0.023	1.807 ± 0.052
Relative	50.81 ± 1.34	51.75 ± 1.53	51.87 ± 0.88	52.24 ± 0.98	50.82 ± 0.36	50.82 ± 0.83
Lungs						
Absolute	0.249 ± 0.014	0.234 ± 0.012	0.225 ± 0.011	0.217 ± 0.010	0.223 ± 0.010[b]	0.215 ± 0.007*
Relative	6.90 ± 0.42	6.68 ± 0.43	6.68 ± 0.36	6.33 ± 0.30	6.31 ± 0.25[b]	6.08 ± 0.25
Spleen						
Absolute	0.081 ± 0.002	0.081 ± 0.002	0.088 ± 0.005	0.111 ± 0.003**	0.163 ± 0.003**	0.295 ± 0.012**
Relative	2.27 ± 0.09	2.32 ± 0.09	2.60 ± 0.15	3.25 ± 0.11**	4.65 ± 0.07**	8.32 ± 0.39**
Right testis						
Absolute	0.120 ± 0.003	0.121 ± 0.004	0.123 ± 0.002	0.124 ± 0.003	0.122 ± 0.002	0.119 ± 0.003
Relative	3.34 ± 0.09	3.44 ± 0.14	3.64 ± 0.07	3.59 ± 0.06	3.48 ± 0.05	3.34 ± 0.08
Thymus						
Absolute	0.043 ± 0.002	0.042 ± 0.002	0.040 ± 0.003	0.048 ± 0.003	0.044 ± 0.002	0.044 ± 0.002
Relative	1.21 ± 0.06	1.18 ± 0.05	1.17 ± 0.07	1.40 ± 0.08	1.27 ± 0.06	1.24 ± 0.04
FEMALE						
n	9	9	10	8	10	9
Necropsy body wt	28.4 ± 0.7	28.8 ± 0.7	28.9 ± 0.8	29.9 ± 1.2	28.1 ± 0.5	28.0 ± 0.7
Heart						
Absolute	0.142 ± 0.004	0.154 ± 0.008	0.152 ± 0.005	0.151 ± 0.005	0.147 ± 0.003	0.155 ± 0.004
Relative	5.04 ± 0.20	5.37 ± 0.31	5.28 ± 0.19	5.09 ± 0.17	5.23 ± 0.13	5.56 ± 0.18
Right kidney						
Absolute	0.212 ± 0.006	0.211 ± 0.008	0.215 ± 0.003	0.211 ± 0.006	0.209 ± 0.003	0.221 ± 0.006
Relative	7.50 ± 0.26	7.34 ± 0.18	7.49 ± 0.18	7.11 ± 0.23	7.47 ± 0.12	7.95 ± 0.27
Liver						
Absolute	1.483 ± 0.031	1.530 ± 0.043	1.533 ± 0.051	1.515 ± 0.061	1.479 ± 0.035	1.523 ± 0.027
Relative	52.36 ± 1.36	53.21 ± 0.96	53.10 ± 1.10	50.84 ± 1.04	52.64 ± 0.78	54.67 ± 1.44
Lungs						
Absolute	0.218 ± 0.007	0.247 ± 0.012	0.232 ± 0.012	0.226 ± 0.014	0.222 ± 0.010	0.212 ± 0.008
Relative	7.69 ± 0.26	8.58 ± 0.35	8.07 ± 0.44	7.61 ± 0.49	7.91 ± 0.34	7.61 ± 0.32
Spleen						
Absolute	0.094 ± 0.003	0.104 ± 0.005	0.116 ± 0.004*	0.138 ± 0.004**	0.193 ± 0.009**	0.282 ± 0.009**
Relative	3.33 ± 0.15	3.63 ± 0.13	4.05 ± 0.14*	4.67 ± 0.21**	6.87 ± 0.29**	10.11 ± 0.22**
Thymus						
Absolute	0.048 ± 0.001	0.053 ± 0.004	0.051 ± 0.003	0.053 ± 0.003	0.047 ± 0.002	0.048 ± 0.001
Relative	1.69 ± 0.05	1.87 ± 0.14	1.76 ± 0.09	1.79 ± 0.10	1.69 ± 0.06	1.71 ± 0.04

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

** P≤0.01

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

[b] n=9

APPENDIX D
HEMATOLOGY AND CLINICAL CHEMISTRY RESULTS

TABLE D1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
MALE						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	9	10	10	10
Week 13	10	10	10	9	9	10
Hematocrit (%)						
Day 3	39.8 ± 0.5	40.2 ± 0.7	40.3 ± 0.4	40.7 ± 0.4	40.2 ± 0.5	41.1 ± 0.4
Day 23	45.3 ± 0.3	45.9 ± 0.6	45.4 ± 0.5	45.6 ± 0.5	45.4 ± 0.4	42.9 ± 0.3**
Week 13	45.4 ± 0.4	45.7 ± 0.3	45.7 ± 0.3	44.6 ± 0.4	43.2 ± 0.6*	43.7 ± 0.3**
Hemoglobin (g/dL)						
Day 3	14.2 ± 0.1	14.3 ± 0.1	14.1 ± 0.1	14.1 ± 0.1	14.3 ± 0.1	14.3 ± 0.1
Day 23	16.2 ± 0.1	16.0 ± 0.1	16.1 ± 0.1	16.2 ± 0.1	16.1 ± 0.2	15.1 ± 0.1**
Week 13	16.1 ± 0.1	16.2 ± 0.1	16.1 ± 0.2	15.6 ± 0.1*	15.2 ± 0.2**	15.4 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 3	6.63 ± 0.08	6.65 ± 0.12	6.70 ± 0.08	6.75 ± 0.06	6.71 ± 0.09	6.83 ± 0.06
Day 23	7.86 ± 0.06	8.02 ± 0.11	7.98 ± 0.10	7.97 ± 0.10	8.00 ± 0.10	7.41 ± 0.06**
Week 13	8.55 ± 0.07	8.63 ± 0.06	8.71 ± 0.07	8.48 ± 0.10	7.91 ± 0.13**	7.52 ± 0.07**
Reticulocytes ($10^6/\mu L$)						
Day 3	0.15 ± 0.02	0.13 ± 0.03	0.13 ± 0.02	0.12 ± 0.02	0.19 ± 0.02	0.15 ± 0.03
Day 23	0.16 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.29 ± 0.04**
Week 13	0.16 ± 0.01	0.15 ± 0.02	0.19 ± 0.01	0.21 ± 0.02*	0.32 ± 0.04**	0.46 ± 0.02**
Nucleated erythrocytes ($10^3/\mu L$)						
Day 3	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02	0.03 ± 0.01	0.01 ± 0.01
Day 23	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.05 ± 0.02
Week 13	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01*	0.13 ± 0.03**
Mean cell volume (fL)						
Day 3	60.1 ± 0.2	60.4 ± 0.2	60.1 ± 0.2	60.4 ± 0.3	59.9 ± 0.2	60.1 ± 0.1
Day 23	57.6 ± 0.2	57.2 ± 0.2	56.9 ± 0.2	57.2 ± 0.1	56.7 ± 0.4**	58.0 ± 0.1
Week 13	53.1 ± 0.2	53.0 ± 0.2	52.4 ± 0.2	52.6 ± 0.3	54.6 ± 0.4*	58.1 ± 0.5**
Mean cell hemoglobin (pg)						
Day 3	21.4 ± 0.1	21.6 ± 0.3	21.0 ± 0.2*	20.9 ± 0.1*	21.3 ± 0.3	21.0 ± 0.1
Day 23	20.6 ± 0.1	20.0 ± 0.2	20.2 ± 0.2	20.3 ± 0.2	20.1 ± 0.2*	20.4 ± 0.1
Week 13	18.9 ± 0.1	18.8 ± 0.1	18.5 ± 0.1	18.4 ± 0.2	19.2 ± 0.2	20.5 ± 0.2**
Mean cell hemoglobin concentration (g/dL)						
Day 3	35.6 ± 0.2	35.7 ± 0.4	34.9 ± 0.3	34.7 ± 0.2**	35.6 ± 0.3	34.9 ± 0.2
Day 23	35.7 ± 0.1	35.0 ± 0.2	35.6 ± 0.2	35.5 ± 0.2	35.4 ± 0.2	35.1 ± 0.2
Week 13	35.5 ± 0.2	35.6 ± 0.2	35.2 ± 0.2	34.9 ± 0.3	35.2 ± 0.1	35.3 ± 0.3
Platelets ($10^3/\mu L$)						
Day 3	983.8 ± 20.9	953.7 ± 11.8	956.6 ± 16.5	969.7 ± 14.1	987.2 ± 14.5	980.2 ± 13.9
Day 23	885.1 ± 16.1	865.9 ± 12.7	876.2 ± 19.3	869.5 ± 16.8	887.4 ± 16.8	938.5 ± 17.5*
Week 13	738.5 ± 7.2	745.6 ± 11.5	718.7 ± 13.5	778.6 ± 12.2	800.7 ± 15.2**	778.2 ± 9.9**
Leukocytes ($10^3/\mu L$)						
Day 3	6.35 ± 0.33	6.32 ± 0.21	6.56 ± 0.25	6.84 ± 0.40	6.69 ± 0.17	6.84 ± 0.32
Day 23	6.75 ± 0.19	5.91 ± 0.25	6.02 ± 0.24	6.03 ± 0.31	6.35 ± 0.39	7.18 ± 0.22
Week 13	5.50 ± 0.26	6.04 ± 0.19	5.94 ± 0.18	6.57 ± 0.22**	6.77 ± 0.30**	7.07 ± 0.27**

TABLE D1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
MALE (continued)						
Hematology (continued)						
Segmented neutrophils ($10^3/\mu L$)						
Day 3	1.97 ± 0.23	1.86 ± 0.19	2.01 ± 0.14	2.45 ± 0.23	2.36 ± 0.21	2.21 ± 0.20
Day 23	1.42 ± 0.11	1.12 ± 0.07	1.21 ± 0.11	1.18 ± 0.09	1.36 ± 0.10	1.43 ± 0.11
Week 13	1.09 ± 0.10	1.34 ± 0.12	1.32 ± 0.09	1.26 ± 0.12	1.59 ± 0.09**	1.44 ± 0.16*
Lymphocytes ($10^3/\mu L$)						
Day 3	4.27 ± 0.19	4.38 ± 0.24	4.45 ± 0.23	4.28 ± 0.21	4.19 ± 0.15	4.50 ± 0.12
Day 23	5.26 ± 0.16	4.69 ± 0.21	4.73 ± 0.17	4.79 ± 0.28	4.91 ± 0.31	5.66 ± 0.26
Week 13	4.25 ± 0.19	4.60 ± 0.17	4.49 ± 0.23	5.19 ± 0.23**	5.06 ± 0.22**	5.51 ± 0.27**
Monocytes ($10^3/\mu L$)						
Day 3	0.09 ± 0.02	0.07 ± 0.01	0.07 ± 0.02	0.07 ± 0.02	0.13 ± 0.02	0.11 ± 0.02
Day 23	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Week 13	0.06 ± 0.01	0.06 ± 0.02	0.05 ± 0.01	0.07 ± 0.02	0.07 ± 0.02	0.09 ± 0.02
Eosinophils ($10^3/\mu L$)						
Day 3	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.02 ± 0.01
Day 23	0.03 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.04 ± 0.01	0.06 ± 0.02	0.06 ± 0.02
Week 13	0.10 ± 0.03	0.03 ± 0.01	0.08 ± 0.02	0.05 ± 0.01	0.04 ± 0.02	0.04 ± 0.02
Methemoglobin (g/dL)						
Day 3	0.28 ± 0.03	0.24 ± 0.02	0.34 ± 0.03	0.31 ± 0.04	0.53 ± 0.02**	1.24 ± 0.08**
Day 23	0.15 ± 0.02	0.14 ± 0.02	0.20 ± 0.03[b]	0.39 ± 0.05**	0.81 ± 0.07**	1.70 ± 0.10**
Week 13	0.30 ± 0.02	0.40 ± 0.02**	0.59 ± 0.04**	0.84 ± 0.04**	1.59 ± 0.06**	2.79 ± 0.09**
Heinz bodies (%)						
Week 13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.2 ± 0.6**
Clinical Chemistry						
n	10	10	10	9	9	10
Urea nitrogen (mg/dL)	24.3 ± 0.5	25.0 ± 0.6	23.4 ± 0.4	24.2 ± 0.4	23.3 ± 0.4	23.2 ± 0.5
Creatinine (mg/dL)	0.79 ± 0.01	0.76 ± 0.02	0.78 ± 0.01	0.77 ± 0.02	0.80 ± 0.03	0.79 ± 0.01
Total protein (g/dL)	7.3 ± 0.1	7.2 ± 0.1	7.3 ± 0.1	7.2 ± 0.1	7.3 ± 0.1	7.2 ± 0.1
Albumin (g/dL)	4.7 ± 0.0	4.8 ± 0.0	4.8 ± 0.0	4.8 ± 0.1	4.9 ± 0.1	4.9 ± 0.0*
Alanine aminotransferase (IU/L)	53 ± 3	51 ± 1	49 ± 2	60 ± 6	51 ± 3	47 ± 1
Alkaline phosphatase (IU/L)	464 ± 13	479 ± 8	464 ± 10	465 ± 14	451 ± 13	444 ± 8
Creatine kinase (IU/L)	308 ± 23	278 ± 18	271 ± 36	310 ± 57	356 ± 51	387 ± 48
Sorbitol dehydrogenase (IU/L)	26 ± 1	22 ± 1	21 ± 1	28 ± 2	29 ± 3	29 ± 1
Bile salts (μmol/L)	18.1 ± 1.8	23.4 ± 2.7	19.9 ± 1.8	33.4 ± 3.7**	29.8 ± 4.7**	34.3 ± 3.2**

TABLE D1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
FEMALE						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 23	10	9	10	10	10	10
Week 13	10	10	9	10	10	10
Hematocrit (%)						
Day 3	41.9 ± 0.6	41.8 ± 0.4	42.8 ± 0.6	42.1 ± 0.5	43.2 ± 0.4	43.5 ± 0.5
Day 23	46.6 ± 0.6	45.9 ± 0.4	46.4 ± 0.3	45.7 ± 0.6	43.9 ± 0.5**	41.8 ± 0.3**
Week 13	45.2 ± 0.4	45.1 ± 0.4	44.4 ± 0.4	44.2 ± 0.4	42.5 ± 0.4**	41.9 ± 0.3**
Hemoglobin (g/dL)						
Day 3	14.5 ± 0.2	14.6 ± 0.1	14.7 ± 0.2	14.6 ± 0.2	14.9 ± 0.1	14.9 ± 0.1
Day 23	16.3 ± 0.2	16.2 ± 0.1	16.4 ± 0.2	16.0 ± 0.2	15.0 ± 0.1**	14.6 ± 0.1**
Week 13	15.7 ± 0.1	15.6 ± 0.1	15.2 ± 0.1*	15.0 ± 0.2**	14.3 ± 0.1**	14.6 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 3	6.87 ± 0.11	6.86 ± 0.05	6.99 ± 0.10	6.87 ± 0.10	7.13 ± 0.10	7.23 ± 0.09*
Day 23	7.74 ± 0.11	7.72 ± 0.05	7.79 ± 0.07	7.66 ± 0.10	7.27 ± 0.08**	6.57 ± 0.05**
Week 13	7.78 ± 0.08	7.74 ± 0.05	7.53 ± 0.06**	7.34 ± 0.08**	6.82 ± 0.04**	6.41 ± 0.07**
Reticulocytes ($10^6/\mu L$)						
Day 3	0.11 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Day 23	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.18 ± 0.02	0.27 ± 0.02**	0.48 ± 0.04**
Week 13	0.13 ± 0.01	0.13 ± 0.01	0.18 ± 0.01**	0.19 ± 0.02**	0.34 ± 0.01**	0.47 ± 0.02**
Nucleated erythrocytes ($10^3/\mu L$)						
Day 3	0.02 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.03 ± 0.01	0.00 ± 0.00	0.01 ± 0.01
Day 23	0.01 ± 0.01	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.08 ± 0.02**	0.21 ± 0.07**
Week 13	0.03 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.06 ± 0.02	0.12 ± 0.02**	0.34 ± 0.10**
Mean cell volume (fL)						
Day 3	61.1 ± 0.3	60.9 ± 0.3	61.2 ± 0.4	61.3 ± 0.4	60.6 ± 0.3	60.2 ± 0.3
Day 23	60.3 ± 0.3	59.5 ± 0.3	59.6 ± 0.4	59.7 ± 0.4	60.3 ± 0.2	63.7 ± 0.3**
Week 13	58.1 ± 0.2	58.3 ± 0.3	59.0 ± 0.4	60.2 ± 0.2**	62.2 ± 0.3**	65.5 ± 0.2**
Mean cell hemoglobin (pg)						
Day 3	21.1 ± 0.1	21.4 ± 0.1	21.0 ± 0.1	21.3 ± 0.1	20.9 ± 0.2	20.6 ± 0.1*
Day 23	21.1 ± 0.2	20.9 ± 0.2	21.0 ± 0.1	20.9 ± 0.1	20.7 ± 0.1	22.2 ± 0.1**
Week 13	20.2 ± 0.1	20.2 ± 0.1	20.1 ± 0.1	20.5 ± 0.2	21.0 ± 0.1**	22.8 ± 0.2**
Mean cell hemoglobin concentration (g/dL)						
Day 3	34.5 ± 0.2	35.1 ± 0.3	34.4 ± 0.2	34.7 ± 0.1	34.4 ± 0.2	34.2 ± 0.2
Day 23	34.9 ± 0.2	35.2 ± 0.3	35.3 ± 0.2	35.1 ± 0.3	34.3 ± 0.2	34.9 ± 0.2
Week 13	34.7 ± 0.2	34.6 ± 0.2	34.2 ± 0.3	34.1 ± 0.2	33.7 ± 0.2*	34.8 ± 0.2
Platelets ($10^3/\mu L$)						
Day 3	958.9 ± 16.8	941.5 ± 13.1	962.6 ± 14.9	938.2 ± 15.5	971.5 ± 19.7	991.7 ± 17.7
Day 23	887.3 ± 11.1	866.6 ± 16.4	847.0 ± 9.6	873.2 ± 13.2	957.8 ± 7.5**	930.7 ± 13.8*
Week 13	721.5 ± 12.4	734.2 ± 13.7	769.6 ± 22.1**	803.1 ± 12.9**	862.2 ± 15.6**	801.2 ± 20.9**
Leukocytes ($10^3/\mu L$)						
Day 3	6.75 ± 0.19	6.47 ± 0.28	6.88 ± 0.25	6.56 ± 0.38	6.59 ± 0.18	6.83 ± 0.29
Day 23	5.79 ± 0.24	5.39 ± 0.27	5.83 ± 0.16	5.75 ± 0.18	6.71 ± 0.22*	9.19 ± 0.63**
Week 13	4.90 ± 0.22	5.20 ± 0.28	5.68 ± 0.20*	5.59 ± 0.35*	6.52 ± 0.38**	11.40 ± 1.23**

TABLE D1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
FEMALE (continued)						
Hematology (continued)						
Segmented neutrophils ($10^3/\mu L$)						
Day 3	1.35 ± 0.12	1.38 ± 0.19	1.34 ± 0.14	1.52 ± 0.18	1.38 ± 0.11	1.45 ± 0.17
Day 23	1.30 ± 0.13	1.19 ± 0.14	1.17 ± 0.07	0.94 ± 0.09	1.45 ± 0.11	1.65 ± 0.11
Week 13	1.13 ± 0.10	1.10 ± 0.09	1.07 ± 0.09	1.11 ± 0.08	1.50 ± 0.09*	2.37 ± 0.32**
Lymphocytes ($10^3/\mu L$)						
Day 3	5.19 ± 0.16	4.98 ± 0.17	5.31 ± 0.15	4.93 ± 0.28	5.08 ± 0.21	5.24 ± 0.26
Day 23	4.29 ± 0.23	4.00 ± 0.23	4.43 ± 0.09	4.63 ± 0.18	5.02 ± 0.18*	7.29 ± 0.60**
Week 13	3.64 ± 0.20	3.99 ± 0.21	4.50 ± 0.20**	4.39 ± 0.30**	4.89 ± 0.38**	8.84 ± 1.02**
Monocytes ($10^3/\mu L$)						
Day 3	0.14 ± 0.02	0.06 ± 0.01	0.18 ± 0.03	0.09 ± 0.01	0.09 ± 0.02	0.12 ± 0.03
Day 23	0.16 ± 0.03	0.13 ± 0.03	0.16 ± 0.02	0.12 ± 0.02	0.16 ± 0.04	0.15 ± 0.03
Week 13	0.06 ± 0.02	0.04 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.12 ± 0.03
Eosinophils ($10^3/\mu L$)						
Day 3	0.07 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.03 ± 0.01*	0.03 ± 0.02*	0.01 ± 0.01**
Day 23	0.05 ± 0.01	0.07 ± 0.01	0.10 ± 0.03	0.06 ± 0.01	0.08 ± 0.02	0.10 ± 0.04
Week 13	0.06 ± 0.02	0.07 ± 0.01	0.05 ± 0.01	0.04 ± 0.02	0.08 ± 0.02	0.06 ± 0.03
Methemoglobin (g/dL)						
Day 3	0.19 ± 0.03	0.24 ± 0.02	0.23 ± 0.03	0.46 ± 0.02**	1.11 ± 0.09**	1.92 ± 0.09**
Day 23	0.21 ± 0.03	0.26 ± 0.03[b]	0.47 ± 0.02**	0.94 ± 0.09**	1.69 ± 0.09**	2.46 ± 0.10**
Week 13	0.37 ± 0.04	0.54 ± 0.03**	0.83 ± 0.02**	1.53 ± 0.07**	2.35 ± 0.06**	2.80 ± 0.07**
Heinz bodies (%)						
Week 13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	10.7 ± 0.7**
Clinical Chemistry						
n	10	10	9	10	10	10
Urea nitrogen (mg/dL)	22.6 ± 0.8	25.3 ± 0.4	24.7 ± 0.6	23.2 ± 0.7	22.0 ± 0.5	22.2 ± 0.8
Creatinine (mg/dL)	0.74 ± 0.02	0.77 ± 0.02	0.77 ± 0.02	0.78 ± 0.02	0.78 ± 0.02	0.77 ± 0.02
Total protein (g/dL)	7.1 ± 0.1	7.0 ± 0.1	7.3 ± 0.1	7.2 ± 0.1	7.1 ± 0.1	6.9 ± 0.1
Albumin (g/dL)	4.9 ± 0.1	4.9 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	5.0 ± 0.1	4.9 ± 0.1
Alanine aminotransferase (IU/L)	50 ± 3	53 ± 6	68 ± 10	60 ± 7	45 ± 2	42 ± 3
Alkaline phosphatase (IU/L)	440 ± 38	409 ± 10	427 ± 6	435 ± 45	389 ± 9	378 ± 6*
Creatine kinase (IU/L)	308 ± 35	243 ± 28	230 ± 17	321 ± 61	301 ± 33	274 ± 21
Sorbitol dehydrogenase (IU/L)	22 ± 2	23 ± 2	31 ± 3*	31 ± 2**	30 ± 1**	28 ± 1**
Bile salts (μmol/L)	35.5 ± 4.9	36.6 ± 3.3	38.1 ± 5.1	42.9 ± 5.7	51.4 ± 7.3	55.1 ± 5.9*

* Significantly different ($P \le 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \le 0.01$
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=10

TABLE D2 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
MALE						
Hematology						
n	10	10	10	10	10	10
Hematocrit (%)						
Day 3	42.5 ± 0.6	43.5 ± 0.7	44.6 ± 0.4	42.8 ± 0.4	42.5 ± 0.4	41.8 ± 0.5
Day 23	49.8 ± 1.4	46.0 ± 0.3	43.4 ± 0.2**	43.6 ± 0.3**	42.7 ± 0.5**	42.4 ± 0.4**
Week 13	46.7 ± 0.8	46.0 ± 0.6	44.8 ± 0.4*	44.9 ± 0.9**	43.1 ± 0.5**	47.4 ± 1.0*
Hemoglobin (g/dL)						
Day 3	15.0 ± 0.2	15.1 ± 0.2	15.5 ± 0.2	14.7 ± 0.1	14.7 ± 0.1	14.6 ± 0.1
Day 23	17.6 ± 0.4	16.3 ± 0.1*	15.5 ± 0.1**	15.2 ± 0.1**	15.2 ± 0.2**	15.0 ± 0.1**
Week 13	16.0 ± 0.3	15.8 ± 0.2	15.3 ± 0.1**	15.6 ± 0.3**	15.4 ± 0.1**	15.5 ± 0.3*
Erythrocytes ($10^6/\mu L$)						
Day 3	7.16 ± 0.11	7.39 ± 0.14	7.54 ± 0.07	7.24 ± 0.07	7.15 ± 0.08	6.94 ± 0.10
Day 23	8.81 ± 0.23	8.36 ± 0.07	7.84 ± 0.04**	7.53 ± 0.06**	6.73 ± 0.08**	5.52 ± 0.06**
Week 13	8.89 ± 0.13	8.75 ± 0.11	8.33 ± 0.08**	7.79 ± 0.16**	6.62 ± 0.07**	5.83 ± 0.08**
Reticulocytes ($10^6/\mu L$)						
Day 3	0.29 ± 0.01	0.29 ± 0.02	0.30 ± 0.03	0.31 ± 0.03	0.37 ± 0.02*	0.40 ± 0.03*
Day 23	0.16 ± 0.01	0.20 ± 0.01*	0.25 ± 0.02**	0.41 ± 0.03**	0.48 ± 0.03**	0.82 ± 0.06**
Week 13	0.12 ± 0.01	0.18 ± 0.01**	0.21 ± 0.01**	0.36 ± 0.03**	0.57 ± 0.03**	1.85 ± 0.11**
Nucleated erythrocytes ($10^3/\mu L$)						
Day 3	0.03 ± 0.01	0.01 ± 0.01	0.07 ± 0.04	0.04 ± 0.02	0.08 ± 0.03	0.26 ± 0.08**
Day 23	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.02	0.07 ± 0.03*	0.49 ± 0.15**	4.16 ± 0.32**[b]
Week 13	0.01 ± 0.01	0.01 ± 0.01	0.06 ± 0.02*	0.10 ± 0.03**	0.15 ± 0.04**	0.54 ± 0.07**
Mean cell volume (fL)						
Day 3	59.4 ± 0.3	58.8 ± 0.2	59.2 ± 0.2	59.1 ± 0.2	59.4 ± 0.2	60.3 ± 0.3
Day 23	56.5 ± 0.3	55.1 ± 0.1	55.4 ± 0.2	57.8 ± 0.1	63.4 ± 0.3**	76.9 ± 0.6**
Week 13	52.5 ± 0.2	52.6 ± 0.2	53.8 ± 0.2**	57.6 ± 0.2**	65.2 ± 0.3**	81.3 ± 1.1**
Mean cell hemoglobin (pg)						
Day 3	20.9 ± 0.2	20.5 ± 0.2	20.5 ± 0.1	20.3 ± 0.2*	20.6 ± 0.1	21.0 ± 0.2
Day 23	19.9 ± 0.1	19.6 ± 0.1	19.8 ± 0.1	20.2 ± 0.2	22.7 ± 0.1**	27.1 ± 0.2**
Week 13	18.0 ± 0.2	18.1 ± 0.1	18.4 ± 0.1	20.1 ± 0.1**	23.3 ± 0.2**	26.7 ± 0.3**
Mean cell hemoglobin concentration (g/dL)						
Day 3	35.3 ± 0.2	34.8 ± 0.3	34.7 ± 0.1*	34.3 ± 0.3**	34.6 ± 0.1**	34.8 ± 0.1*
Day 23	35.3 ± 0.3	35.5 ± 0.2	35.8 ± 0.2	34.9 ± 0.2	35.7 ± 0.2	35.3 ± 0.2
Week 13	34.4 ± 0.2	34.3 ± 0.1	34.2 ± 0.2	34.9 ± 0.2	35.8 ± 0.2*	32.8 ± 0.3*
Platelets ($10^3/\mu L$)						
Day 3	932.3 ± 7.5	967.0 ± 17.2	959.7 ± 13.5	962.4 ± 12.1	1,003.3 ± 14.2**	1,139.3 ± 18.0**
Day 23	817.0 ± 14.6	823.6 ± 14.4	871.2 ± 9.4	919.2 ± 15.3**	897.5 ± 17.0*	783.0 ± 12.1
Week 13	837.3 ± 80.7	833.9 ± 7.7	827.1 ± 13.5	784.1 ± 23.1	517.3 ± 8.5**	473.9 ± 10.5**
Leukocytes ($10^3/\mu L$)						
Day 3	6.61 ± 0.25	6.36 ± 0.32	8.08 ± 0.40*	6.88 ± 0.25	7.73 ± 0.33*	17.00 ± 0.91**
Day 23	6.08 ± 0.31	6.58 ± 0.24	7.13 ± 0.23*	7.77 ± 0.42**	13.27 ± 0.57**	42.93 ± 1.70**
Week 13	6.43 ± 0.99	6.95 ± 0.18	6.55 ± 0.29	6.28 ± 0.36	5.71 ± 0.42	5.94 ± 0.31

TABLE D2 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
MALE (continued)						
Hematology (continued)						
Segmented neutrophils ($10^3/\mu L$)						
Day 3	1.58 ± 0.17	1.32 ± 0.15	1.84 ± 0.23	1.44 ± 0.12	1.73 ± 0.17	3.36 ± 0.50**
Day 23	1.31 ± 0.11	1.31 ± 0.11	1.52 ± 0.13	1.69 ± 0.20	2.78 ± 0.10**	10.06 ± 0.84**
Week 13	2.33 ± 0.77	1.91 ± 0.14	1.25 ± 0.13	1.50 ± 0.12	1.48 ± 0.21	1.79 ± 0.21
Lymphocytes ($10^3/\mu L$)						
Day 3	4.98 ± 0.32	4.97 ± 0.25	6.14 ± 0.21**	5.34 ± 0.23	5.91 ± 0.33	13.34 ± 0.73**
Day 23	4.61 ± 0.28	5.10 ± 0.20	5.36 ± 0.23	5.89 ± 0.30**	10.23 ± 0.62**	32.41 ± 1.26**
Week 13	3.94 ± 0.28	4.94 ± 0.20**	5.21 ± 0.30**	4.63 ± 0.26	4.08 ± 0.25	4.08 ± 0.17
Monocytes ($10^3/\mu L$)						
Day 3	0.05 ± 0.01	0.04 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.14 ± 0.02*
Day 23	0.12 ± 0.03	0.13 ± 0.03	0.14 ± 0.03	0.16 ± 0.04	0.18 ± 0.05	0.33 ± 0.15
Week 13	0.07 ± 0.01	0.05 ± 0.02	0.06 ± 0.02	0.08 ± 0.02	0.10 ± 0.03	0.05 ± 0.02
Eosinophils ($10^3/\mu L$)						
Day 3	0.00 ± 0.00	0.03 ± 0.01*	0.05 ± 0.02*	0.04 ± 0.03	0.04 ± 0.02	0.14 ± 0.05**
Day 23	0.03 ± 0.01	0.05 ± 0.02	0.11 ± 0.03	0.03 ± 0.02	0.08 ± 0.03	0.13 ± 0.07
Week 13	0.08 ± 0.02	0.05 ± 0.01	0.03 ± 0.02	0.08 ± 0.03	0.06 ± 0.02	0.03 ± 0.01
Methemoglobin (g/dL)						
Day 3	0.39 ± 0.04	0.56 ± 0.05**	1.64 ± 0.15**	2.54 ± 0.17**	4.57 ± 0.26**	6.13 ± 0.09**
Day 23	0.21 ± 0.03	0.80 ± 0.07**	1.70 ± 0.10**	3.12 ± 0.12**	4.79 ± 0.15**	5.11 ± 0.25**
Week 13	0.31 ± 0.02	1.11 ± 0.06**	2.13 ± 0.06**	3.65 ± 0.19**	4.89 ± 0.20**	5.83 ± 0.18**
Heinz bodies (%)						
Week 13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.7 ± 0.9**	16.4 ± 1.1**	1.7 ± 0.6**
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	21.7 ± 0.7	21.9 ± 0.5	21.8 ± 0.5	21.1 ± 0.5	21.8 ± 0.4	21.7 ± 0.3
Creatinine (mg/dL)	0.69 ± 0.01	0.78 ± 0.02**	0.78 ± 0.01**	0.82 ± 0.01**	0.84 ± 0.02**	0.88 ± 0.01**
Total protein (g/dL)	6.5 ± 0.3	6.9 ± 0.1	6.9 ± 0.1	6.8 ± 0.1	6.6 ± 0.1*	6.6 ± 0.0*
Albumin (g/dL)	4.4 ± 0.2	4.8 ± 0.1	4.8 ± 0.0*	4.7 ± 0.0	4.7 ± 0.1	4.9 ± 0.0**
Alanine aminotransferase (IU/L)	52 ± 5	51 ± 3	45 ± 1*	46 ± 1*	45 ± 2*	40 ± 1**
Alkaline phosphatase (IU/L)	437 ± 30	442 ± 12	447 ± 13	416 ± 9*	438 ± 12	413 ± 13*
Creatine kinase (IU/L)	401 ± 50	356 ± 33	394 ± 41	521 ± 52	396 ± 52	538 ± 39*
Sorbitol dehydrogenase (IU/L)	22 ± 1	30 ± 2**	29 ± 1**	33 ± 2**	41 ± 1**	43 ± 2**
Bile salts (μmol/L)	11.1 ± 1.1	14.0 ± 1.8	13.4 ± 1.6	12.7 ± 1.0	16.1 ± 2.3*	28.1 ± 2.2**

TABLE D2 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
FEMALE						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 23	10	10	9	10	10	10
Week 13	10	10	10	10	10	9
Hematocrit (%)						
Day 3	44.8 ± 0.4	44.9 ± 0.6	44.7 ± 0.4	45.6 ± 0.4	43.6 ± 0.5	43.3 ± 0.5
Day 23	47.4 ± 0.4	46.3 ± 0.4	42.9 ± 0.5**	43.6 ± 0.5**	40.3 ± 0.5**	43.6 ± 0.5**
Week 13	47.3 ± 0.7	45.3 ± 0.4*	45.0 ± 0.8*	43.5 ± 0.4**	42.0 ± 0.6**	44.6 ± 0.4**
Hemoglobin (g/dL)						
Day 3	15.6 ± 0.1	15.6 ± 0.2	15.4 ± 0.2	15.7 ± 0.2	14.9 ± 0.1**	15.4 ± 0.2*
Day 23	16.3 ± 0.1	15.6 ± 0.1**	14.5 ± 0.1**	15.1 ± 0.1**	14.4 ± 0.1**	15.2 ± 0.1**
Week 13	15.8 ± 0.2	15.3 ± 0.1	15.0 ± 0.3**	14.6 ± 0.2**	14.6 ± 0.2**	15.2 ± 0.1**
Erythrocytes ($10^6/\mu L$)						
Day 3	7.27 ± 0.07	7.30 ± 0.08	7.30 ± 0.08	7.48 ± 0.09	7.06 ± 0.06	6.82 ± 0.07**
Day 23	7.85 ± 0.07	7.69 ± 0.05	6.92 ± 0.07**	6.69 ± 0.07**	5.66 ± 0.05**	5.39 ± 0.09**
Week 13	8.14 ± 0.11	7.63 ± 0.07**	7.40 ± 0.13**	6.71 ± 0.07**	5.85 ± 0.08**	5.40 ± 0.09**
Reticulocytes ($10^6/\mu L$)						
Day 3	0.19 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.02	0.26 ± 0.02**	0.30 ± 0.02**
Day 23	0.12 ± 0.01	0.15 ± 0.01*	0.34 ± 0.01**	0.38 ± 0.03**	0.68 ± 0.04**	1.12 ± 0.05**
Week 13	0.10 ± 0.01	0.16 ± 0.01**	0.21 ± 0.02**	0.33 ± 0.03**	0.59 ± 0.04**	0.98 ± 0.08**
Nucleated erythrocytes ($10^3/\mu L$)						
Day 3	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.06 ± 0.02	0.61 ± 0.20**
Day 23	0.02 ± 0.01	0.01 ± 0.01	0.09 ± 0.03	0.20 ± 0.05**	1.57 ± 0.36**	1.35 ± 0.39**
Week 13	0.01 ± 0.01	0.06 ± 0.03[c]	0.08 ± 0.03*	0.14 ± 0.04**	0.42 ± 0.10**	0.45 ± 0.19**
Mean cell volume (fL)						
Day 3	61.7 ± 0.3	61.6 ± 0.3	61.2 ± 0.3	61.0 ± 0.3	61.8 ± 0.3	63.5 ± 0.3**
Day 23	60.4 ± 0.2	60.2 ± 0.3	62.0 ± 0.3**	65.2 ± 0.3**	71.3 ± 0.7**	81.1 ± 0.6**
Week 13	58.2 ± 0.1	59.4 ± 0.1**	60.8 ± 0.2**	64.9 ± 0.2**	71.7 ± 0.3**	82.8 ± 0.8**
Mean cell hemoglobin (pg)						
Day 3	21.5 ± 0.1	21.4 ± 0.2	21.1 ± 0.1	21.0 ± 0.1*	21.1 ± 0.1	22.6 ± 0.1
Day 23	20.8 ± 0.1	20.3 ± 0.1	20.9 ± 0.1	22.6 ± 0.1**	25.4 ± 0.2**	28.3 ± 0.3**
Week 13	19.4 ± 0.1	20.1 ± 0.2**	20.3 ± 0.2**	21.8 ± 0.1**	25.0 ± 0.2**	28.2 ± 0.3**
Mean cell hemoglobin concentration (g/dL)						
Day 3	34.9 ± 0.2	34.8 ± 0.3	34.5 ± 0.1	34.4 ± 0.2	34.1 ± 0.2	35.6 ± 0.2
Day 23	34.4 ± 0.2	33.7 ± 0.2	33.7 ± 0.2	34.6 ± 0.2	35.7 ± 0.5*	34.9 ± 0.2
Week 13	33.4 ± 0.2	33.9 ± 0.3	33.4 ± 0.2	33.6 ± 0.2	34.9 ± 0.2**	34.1 ± 0.2**
Platelets ($10^3/\mu L$)						
Day 3	886.0 ± 13.7	876.8 ± 14.5	885.3 ± 19.9	928.4 ± 21.5	1,041.7 ± 20.3**	1,118.4 ± 14.0**
Day 23	819.9 ± 14.3	844.1 ± 8.6	922.6 ± 9.9**	911.7 ± 18.3*	917.0 ± 10.9**	734.4 ± 9.0
Week 13	721.6 ± 28.5	810.7 ± 20.3	849.4 ± 24.0	805.6 ± 14.7	732.4 ± 70.8	575.8 ± 15.4*
Leukocytes ($10^3/\mu L$)						
Day 3	7.56 ± 0.31	7.16 ± 0.23	7.05 ± 0.57	8.36 ± 0.41	11.88 ± 0.36**	34.22 ± 1.71**
Day 23	6.09 ± 0.26	6.57 ± 0.35	7.29 ± 0.34*	8.87 ± 0.21**	26.77 ± 1.88**[b]	39.18 ± 2.48**
Week 13	5.58 ± 0.18	5.79 ± 0.49[c]	6.15 ± 0.28	7.22 ± 0.40**	5.93 ± 0.42*[b]	7.94 ± 0.25**

TABLE D2 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
FEMALE (continued)						
Hematology (continued)						
Segmented neutrophils ($10^3/\mu L$)						
Day 3	1.64 ± 0.17	1.48 ± 0.23	1.58 ± 0.24	1.86 ± 0.23	2.93 ± 0.27**	8.44 ± 0.85**
Day 23	1.28 ± 0.10	1.45 ± 0.11	1.36 ± 0.14	1.53 ± 0.14	4.85 ± 0.47**[b]	6.29 ± 0.63**
Week 13	1.42 ± 0.16	1.68 ± 0.37[c]	1.33 ± 0.17	1.71 ± 0.19	1.44 ± 0.12[b]	1.41 ± 0.11
Lymphocytes ($10^3/\mu L$)						
Day 3	5.77 ± 0.22	5.57 ± 0.18	5.39 ± 0.39	6.40 ± 0.30	8.78 ± 0.34**	25.11 ± 1.24**
Day 23	4.70 ± 0.25	5.04 ± 0.30	5.77 ± 0.31*	7.20 ± 0.19**	21.49 ± 1.47**[b]	32.35 ± 1.96**
Week 13	4.02 ± 0.17	3.98 ± 0.30[c]	4.70 ± 0.15*	5.38 ± 0.34**	4.43 ± 0.35*[b]	6.46 ± 0.22**
Monocytes ($10^3/\mu L$)						
Day 3	0.12 ± 0.03	0.08 ± 0.03	0.05 ± 0.02	0.07 ± 0.03	0.06 ± 0.03	0.42 ± 0.14
Day 23	0.06 ± 0.01	0.03 ± 0.01	0.11 ± 0.03	0.08 ± 0.03	0.20 ± 0.08[b]	0.20 ± 0.09
Week 13	0.05 ± 0.01	0.07 ± 0.02[c]	0.06 ± 0.01	0.08 ± 0.02	0.03 ± 0.02[b]	0.05 ± 0.02
Eosinophils ($10^3/\mu L$)						
Day 3	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.02	0.11 ± 0.03*	0.17 ± 0.06
Day 23	0.04 ± 0.02	0.06 ± 0.02	0.05 ± 0.01	0.06 ± 0.02	0.19 ± 0.09[b]	0.34 ± 0.10*
Week 13	0.09 ± 0.02	0.06 ± 0.02[c]	0.05 ± 0.02	0.05 ± 0.02	0.03 ± 0.01*[b]	0.02 ± 0.01**
Methemoglobin (g/dL)						
Day 3	0.20 ± 0.04	0.62 ± 0.06**	1.94 ± 0.12**	3.95 ± 0.13**	6.09 ± 0.30**	7.32 ± 0.26**
Day 23	0.30 ± 0.03	1.21 ± 0.09**	2.60 ± 0.11**	4.24 ± 0.13**	5.36 ± 0.13**	6.89 ± 0.16**
Week 13	0.26 ± 0.02	1.54 ± 0.06**	2.95 ± 0.11**	4.72 ± 0.21**	5.68 ± 0.23**	6.56 ± 0.22**
Heinz bodies (%)						
Week 13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.3 ± 0.4**	10.0 ± 1.0**	13.5 ± 2.3**
Clinical Chemistry						
n	10	10	10	10	10	9
Urea nitrogen (mg/dL)	23.0 ± 0.8	23.6 ± 0.7	23.2 ± 0.7	24.5 ± 0.8	23.2 ± 0.5	24.1 ± 0.5
Creatinine (mg/dL)	0.72 ± 0.01	0.79 ± 0.01**	0.78 ± 0.02**	0.86 ± 0.03**	0.82 ± 0.01**	0.83 ± 0.02**
Total protein (g/dL)	7.1 ± 0.1	7.2 ± 0.1	6.8 ± 0.3	7.2 ± 0.1	6.8 ± 0.2	6.7 ± 0.1*
Albumin (g/dL)	5.0 ± 0.0	5.2 ± 0.1	4.9 ± 0.2	5.2 ± 0.1	5.0 ± 0.1	5.0 ± 0.1
Alanine aminotransferase (IU/L)	66 ± 7	53 ± 5	56 ± 10	52 ± 4	52 ± 5	39 ± 2**
Alkaline phosphatase (IU/L)	365 ± 13	374 ± 7	351 ± 25	351 ± 8	329 ± 13*	331 ± 13*
Creatine kinase (IU/L)	292 ± 30	268 ± 24	344 ± 31	312 ± 29	342 ± 22	392 ± 37*
Sorbitol dehydrogenase (IU/L)	25 ± 2	32 ± 3*	36 ± 3**	40 ± 2**	41 ± 3**	40 ± 1**
Bile salts (μmol/L)	25.8 ± 2.6	28.0 ± 3.0	27.6 ± 3.3	30.4 ± 3.6	38.5 ± 4.1*	47.5 ± 2.1**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9 #
[c] n=8 #

TABLE D3 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
MALE						
Hematology						
n	10	9	10	10	10	10
Hematocrit (%)	50.2 ± 0.5	51.1 ± 1.0	50.2 ± 0.7	48.2 ± 0.8	47.0 ± 0.7**	45.5 ± 0.7**
Hemoglobin (g/dL)	16.6 ± 0.1	16.7 ± 0.3	16.6 ± 0.1	16.2 ± 0.2	16.1 ± 0.1	17.3 ± 0.3
Erythrocytes ($10^6/\mu$L)	10.10 ± 0.10	10.34 ± 0.21	10.16 ± 0.15	9.77 ± 0.16	9.37 ± 0.13**	9.10 ± 0.14**
Reticulocytes ($10^6/\mu$L)	0.24 ± 0.02	0.22 ± 0.02	0.28 ± 0.03	0.22 ± 0.02	0.38 ± 0.03**	0.65 ± 0.04**
Nucleated erythrocytes ($10^3/\mu$L)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	49.7 ± 0.1	49.4 ± 0.1	49.4 ± 0.3	49.3 ± 0.2	50.2 ± 0.2	50.0 ± 0.2
Mean cell hemoglobin (pg)	16.5 ± 0.2	16.2 ± 0.2	16.3 ± 0.2	16.6 ± 0.1	17.2 ± 0.2*	19.0 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	33.1 ± 0.4	32.8 ± 0.3	33.1 ± 0.3	33.7 ± 0.3	34.3 ± 0.3*	37.9 ± 0.4**
Platelets ($10^3/\mu$L)	1,092.3 ± 43.1	1,050.1 ± 29.1	1,050.2 ± 11.0	1,025.7 ± 22.4	1,041.6 ± 10.8	989.6 ± 30.6
Leukocytes ($10^3/\mu$L)	5.42 ± 0.22	5.33 ± 0.24	5.45 ± 0.24	5.52 ± 0.37	6.09 ± 0.58	7.23 ± 0.94
Segmented neutrophils ($10^3/\mu$L)	1.46 ± 0.24	1.30 ± 0.11	1.61 ± 0.12	1.73 ± 0.20	1.80 ± 0.18[b]	2.17 ± 0.49
Lymphocytes ($10^3/\mu$L)	3.80 ± 0.13	3.96 ± 0.21	3.79 ± 0.17	3.65 ± 0.24	3.52 ± 0.28	4.90 ± 0.65
Monocytes ($10^3/\mu$L)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Eosinophils ($10^3/\mu$L)	0.16 ± 0.03	0.08 ± 0.03	0.04 ± 0.01*	0.13 ± 0.03	0.10 ± 0.02[b]	0.16 ± 0.04
Methemoglobin (g/dL)	0.15 ± 0.02	0.24 ± 0.03*	0.27 ± 0.03**	0.44 ± 0.03**	0.79 ± 0.04**[b]	2.12 ± 0.32**
Heinz bodies (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	6.4 ± 0.8**
Clinical Chemistry						
n	9	9	10	9	10	9
Urea nitrogen (mg/dL)	45.1 ± 3.2	41.8 ± 3.5	39.4 ± 1.9	42.4 ± 2.9	41.2 ± 2.9	44.0 ± 2.7
Creatinine (mg/dL)	0.47 ± 0.02	0.44 ± 0.02	0.43 ± 0.02	0.42 ± 0.02	0.41 ± 0.02	0.43 ± 0.02
Total protein (g/dL)	4.9 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	4.7 ± 0.1
Albumin (g/dL)	3.4 ± 0.1	3.3 ± 0.1	3.4 ± 0.0	3.3 ± 0.0	3.3 ± 0.1	3.2 ± 0.0*
Alanine aminotransferase (IU/L)	142 ± 13	167 ± 23	162 ± 18	163 ± 21	142 ± 15	136 ± 10
Alkaline phosphatase (IU/L)	138 ± 5	137 ± 4	133 ± 4	130 ± 4	133 ± 4	133 ± 4
Creatine kinase (IU/L)	1,950 ± 586[c]	1,125 ± 318[d]	1,068 ± 192	1,193 ± 294	2,486 ± 406	1,811 ± 351
Sorbitol dehydrogenase (IU/L)	85 ± 2	84 ± 5	80 ± 4	78 ± 3[c]	80 ± 4	79 ± 5
Bile salts (μmol/L)	8.7 ± 0.5	9.6 ± 1.3	7.6 ± 0.5	8.5 ± 0.3[c]	10.4 ± 1.3	8.9 ± 1.2

TABLE D3 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
FEMALE						
Hematology						
n	10	10	9	10	10	9
Hematocrit (%)	47.7 ± 0.9	46.7 ± 0.6	46.5 ± 0.7	46.8 ± 0.5	45.4 ± 0.3*	44.4 ± 0.6**
Hemoglobin (g/dL)	16.7 ± 0.3	16.2 ± 0.1	15.9 ± 0.1*	16.1 ± 0.1*	15.6 ± 0.1**	16.3 ± 0.2*
Erythrocytes ($10^6/\mu$L)	9.59 ± 0.21	9.46 ± 0.13	9.36 ± 0.11	9.45 ± 0.11	9.19 ± 0.07	8.80 ± 0.11**
Reticulocytes ($10^6/\mu$L)	0.29 ± 0.02	0.30 ± 0.03	0.31 ± 0.03	0.34 ± 0.02	0.38 ± 0.03	0.71 ± 0.06**
Nucleated erythrocytes ($10^3/\mu$L)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	49.7 ± 0.2	49.5 ± 0.2	49.7 ± 0.2	49.5 ± 0.2	49.4 ± 0.1	50.5 ± 0.3
Mean cell hemoglobin (pg)	17.4 ± 0.2	17.2 ± 0.1	17.0 ± 0.1	17.1 ± 0.1	17.0 ± 0.1	18.6 ± 0.1*
Mean cell hemoglobin concentration (g/dL)	35.0 ± 0.2	34.7 ± 0.2	34.3 ± 0.3	34.5 ± 0.2	34.4 ± 0.1	36.8 ± 0.2
Platelets ($10^3/\mu$L)	965.7 ± 32.2	899.1 ± 20.7	951.1 ± 34.0	899.0 ± 16.1	933.3 ± 18.4	974.7 ± 20.5
Leukocytes ($10^3/\mu$L)	4.76 ± 0.26	4.87 ± 0.23	4.50 ± 0.38	4.31 ± 0.24	4.95 ± 0.23	7.31 ± 0.77**
Segmented neutrophils ($10^3/\mu$L)	1.17 ± 0.13	1.34 ± 0.16	1.38 ± 0.26	1.16 ± 0.18	1.39 ± 0.17	3.29 ± 0.68**
Lymphocytes ($10^3/\mu$L)	3.45 ± 0.21	3.44 ± 0.17	2.97 ± 0.15	3.06 ± 0.18	3.48 ± 0.21	3.89 ± 0.32
Monocytes ($10^3/\mu$L)	0.04 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.02 ± 0.01
Eosinophils ($10^3/\mu$L)	0.11 ± 0.03	0.07 ± 0.02	0.14 ± 0.04	0.08 ± 0.01	0.06 ± 0.02	0.11 ± 0.02
Methemoglobin (g/dL)	0.14 ± 0.02	0.22 ± 0.02*	0.24 ± 0.02**	0.48 ± 0.03**	1.08 ± 0.13**	2.39 ± 0.16**
Heinz bodies (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.4 ± 0.5**
Clinical Chemistry						
n	10	10	8	10	10	9
Urea nitrogen (mg/dL)	33.9 ± 4.4[b]	29.2 ± 1.4	37.3 ± 3.3	37.2 ± 4.0	37.3 ± 2.1	33.1 ± 2.7
Creatinine (mg/dL)	0.44 ± 0.02[b]	0.41 ± 0.02	0.43 ± 0.02	0.44 ± 0.02	0.42 ± 0.01	0.42 ± 0.02
Total protein (g/dL)	5.1 ± 0.1	5.1 ± 0.1	4.9 ± 0.1	5.0 ± 0.1	4.8 ± 0.1*	4.8 ± 0.1*
Albumin (g/dL)	3.7 ± 0.1[b]	3.7 ± 0.1	3.4 ± 0.1	3.6 ± 0.0	3.5 ± 0.1	3.5 ± 0.1*
Alanine aminotransferase (IU/L)	100 ± 4[b]	111 ± 14	123 ± 26	139 ± 16	104 ± 15	99 ± 10
Alkaline phosphatase (IU/L)	206 ± 9	193 ± 7	191 ± 11[b]	203 ± 6	179 ± 6	188 ± 7
Creatine kinase (IU/L)	553 ± 162[c]	459 ± 133[b]	307 ± 38[e]	928 ± 250[c]	667 ± 88[b]	867 ± 187
Sorbitol dehydrogenase (IU/L)	57 ± 2	58 ± 1	57 ± 2[b]	66 ± 6	63 ± 2*	62 ± 3
Bile salts (μmol/L)	11.5 ± 0.3	11.1 ± 0.4[b]	11.8 ± 1.3[b]	11.9 ± 1.1	14.7 ± 1.3	11.3 ± 0.5

* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test
** Significantly different (P≤0.01) from the vehicle control group by Shirley's test
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9 #
[c] n=8 #
[d] n=6
[e] n=4

TABLE D4 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline[a]

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
MALE						
n	9	10	7	10	10	10
Hematology						
Hematocrit (%)	49.9 ± 0.6	49.2 ± 0.6	46.9 ± 0.3**	45.2 ± 0.3**	44.4 ± 0.7**	42.1 ± 0.5**
Hemoglobin (g/dL)	16.8 ± 0.1	16.6 ± 0.1	15.8 ± 0.1*	15.8 ± 0.3**	16.3 ± 0.1	17.3 ± 0.2
Erythrocytes ($10^6/\mu$L)	10.24 ± 0.13	10.15 ± 0.12	9.57 ± 0.09**	9.18 ± 0.07**	9.08 ± 0.15**	8.52 ± 0.12**
Reticulocytes ($10^6/\mu$L)	0.20 ± 0.01	0.24 ± 0.02	0.20 ± 0.02	0.33 ± 0.04**	0.38 ± 0.03**	0.95 ± 0.08**
Nucleated erythrocytes ($10^3/\mu$L)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	48.7 ± 0.2	48.5 ± 0.2	49.0 ± 0.2	49.2 ± 0.2	48.9 ± 0.1	49.4 ± 0.4*
Mean cell hemoglobin (pg)	16.4 ± 0.1	16.4 ± 0.1	16.5 ± 0.1	17.2 ± 0.4*	18.0 ± 0.2**	20.3 ± 0.4**
Mean cell hemoglobin concentration (g/dL)	33.7 ± 0.2	33.7 ± 0.2	33.8 ± 0.2	34.9 ± 0.7*	36.7 ± 0.4**	41.1 ± 0.7**
Platelets ($10^3/\mu$L)	1,126 ± 27	1,062 ± 14	1,021 ± 11**	1,072 ± 26	1,050 ± 17	1,112 ± 19
Leukocytes ($10^3/\mu$L)	6.46 ± 0.52	5.14 ± 0.36	5.40 ± 0.67	6.65 ± 0.54	5.89 ± 0.26	7.36 ± 0.60
Segmented neutrophils ($10^3/\mu$L)	1.56 ± 0.20	1.30 ± 0.19	1.34 ± 0.17	1.86 ± 0.38	1.11 ± 0.14	1.17 ± 0.20
Lymphocytes ($10^3/\mu$L)	4.73 ± 0.35	3.72 ± 0.30	3.95 ± 0.66	4.67 ± 0.29	4.67 ± 0.21	6.07 ± 0.48
Monocytes ($10^3/\mu$L)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00
Eosinophils ($10^3/\mu$L)	0.17 ± 0.04	0.11 ± 0.04	0.11 ± 0.03	0.11 ± 0.02	0.11 ± 0.03	0.12 ± 0.03
Methemoglobin (g/dL)	0.19 ± 0.03	0.28 ± 0.04	0.50 ± 0.08**	1.72 ± 0.27**	3.22 ± 0.39**	3.77 ± 0.09**
Heinz bodies (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 1.4	2.4 ± 0.7**	13.8 ± 3.5**
Clinical Chemistry						
Urea nitrogen (mg/dL)	31.0 ± 1.6	28.3 ± 1.4	36.1 ± 4.3	29.0 ± 1.6	32.1 ± 1.8	32.7 ± 2.6
Creatinine (mg/dL)	0.40 ± 0.00	0.40 ± 0.00	0.43 ± 0.02*	0.42 ± 0.01	0.41 ± 0.01	0.43 ± 0.02
Total protein (g/dL)	5.0 ± 0.1	5.1 ± 0.1	5.1 ± 0.1	5.1 ± 0.1	5.0 ± 0.1	4.8 ± 0.1
Albumin (g/dL)	3.5 ± 0.1	3.6 ± 0.1	3.5 ± 0.1	3.5 ± 0.1	3.5 ± 0.0	3.4 ± 0.0*
Alanine aminotransferase (IU/L)	173 ± 31	153 ± 25	195 ± 32	189 ± 40	175 ± 40	166 ± 22
Alkaline phosphatase (IU/L)	134 ± 3	128 ± 3	134 ± 7	129 ± 5	121 ± 3*	110 ± 2**
Creatine kinase (IU/L)	528 ± 123	387 ± 85	398 ± 124[b]	392 ± 77	309 ± 53[c]	478 ± 120
Sorbitol dehydrogenase (IU/L)	67 ± 3	63 ± 3	68 ± 5	63 ± 2[c]	74 ± 10[c]	65 ± 3[c]
Bile salts (μmol/L)	14.4 ± 1.1	14.9 ± 1.1	17.6 ± 2.3	14.0 ± 1.1	14.5 ± 1.4	14.1 ± 1.1

TABLE D4 Hematology and Clinical Chemistry Data for B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline (continued)

	Vehicle Control	10 mg/kg	20 mg/kg	40 mg/kg	80 mg/kg	160 mg/kg
FEMALE						
n	9	9	10	8	10	9
Hematology						
Hematocrit (%)	47.4 ± 0.4	48.3 ± 0.5	47.6 ± 0.4	45.9 ± 0.4*	43.9 ± 0.3**	44.0 ± 0.6**
Hemoglobin (g/dL)	16.3 ± 0.1	16.4 ± 0.1	16.2 ± 0.1	15.7 ± 0.1	15.8 ± 0.2	16.8 ± 0.2
Erythrocytes ($10^6/\mu L$)	9.62 ± 0.10	9.89 ± 0.11	9.76 ± 0.12	9.38 ± 0.08	9.00 ± 0.06**	8.95 ± 0.13**
Reticulocytes ($10^6/\mu L$)	0.19 ± 0.02	0.24 ± 0.01	0.26 ± 0.01*	0.30 ± 0.02**	0.57 ± 0.03**	0.99 ± 0.11**
Nucleated erythrocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	49.2 ± 0.2	48.9 ± 0.1	48.8 ± 0.3	48.9 ± 0.2	48.7 ± 0.2	49.1 ± 0.4
Mean cell hemoglobin (pg)	16.9 ± 0.1	16.6 ± 0.1	16.6 ± 0.2	16.8 ± 0.1	17.6 ± 0.2*	18.8 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	34.3 ± 0.2	33.9 ± 0.1	34.0 ± 0.2	34.3 ± 0.1	36.0 ± 0.3**	38.3 ± 0.4**
Platelets ($10^3/\mu L$)	979.3 ± 17.7	928.6 ± 16.0	991.7 ± 15.8	983.0 ± 20.3	984.9 ± 25.9	997.4 ± 31.9
Leukocytes ($10^3/\mu L$)	4.53 ± 0.40	4.79 ± 0.32	5.04 ± 0.43	4.80 ± 0.18	5.17 ± 0.33	6.38 ± 0.53**
Segmented neutrophils ($10^3/\mu L$)	0.95 ± 0.24	1.37 ± 0.24	1.32 ± 0.24	1.18 ± 0.20	1.00 ± 0.11	1.46 ± 0.28
Lymphocytes ($10^3/\mu L$)	3.52 ± 0.28	3.38 ± 0.23	3.65 ± 0.30	3.57 ± 0.18	4.10 ± 0.35*	4.79 ± 0.39**
Monocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.01*
Eosinophils ($10^3/\mu L$)	0.07 ± 0.02	0.04 ± 0.01	0.07 ± 0.02	0.06 ± 0.02	0.07 ± 0.02	0.11 ± 0.03
Methemoglobin (g/dL)	0.18 ± 0.03	0.30 ± 0.03*	0.60 ± 0.04**	1.86 ± 0.29**	3.62 ± 0.21**	4.60 ± 0.19**
Heinz bodies (%)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 0.6**	5.4 ± 1.4**
Clinical Chemistry						
Urea nitrogen (mg/dL)	25.8 ± 1.0	25.3 ± 2.1	27.7 ± 2.3	25.6 ± 1.7	27.8 ± 1.7	31.4 ± 1.2*
Creatinine (mg/dL)	0.41 ± 0.01	0.42 ± 0.02	0.43 ± 0.02	0.44 ± 0.02	0.45 ± 0.02	0.50 ± 0.02**
Total protein (g/dL)	5.0 ± 0.1	5.1 ± 0.1	5.0 ± 0.1	5.1 ± 0.1	4.9 ± 0.1	4.7 ± 0.1
Albumin (g/dL)	3.6 ± 0.1	3.7 ± 0.1	3.6 ± 0.1	3.8 ± 0.0	3.6 ± 0.1	3.5 ± 0.1
Alanine aminotransferase (IU/L)	118 ± 15[d]	125 ± 23	111 ± 14	128 ± 21	123 ± 15	99 ± 10[d]
Alkaline phosphatase (IU/L)	204 ± 8	206 ± 10	196 ± 9	203 ± 5	197 ± 7	177 ± 5*
Creatine kinase (IU/L)	545 ± 110	600 ± 98	732 ± 140	434 ± 89	772 ± 188	422 ± 53
Sorbitol dehydrogenase (IU/L)	57 ± 2[d]	69 ± 11	59 ± 5	58 ± 2	56 ± 2	65 ± 2[d]
Bile salts (μmol/L)	12.9 ± 1.1	12.6 ± 1.0	11.0 ± 0.8	10.9 ± 0.3	12.0 ± 1.0	14.2 ± 2.1

* Significantly different ($P \le 0.05$) from the vehicle control group by Dunn's or Shirley's test
** $P \le 0.01$
[a] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=6 #
[c] n=9 #
[d] n=8

APPENDIX E
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

TABLE E1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline[a]

Study Parameters	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
n	10	8	9	10
Weights (g)				
Necropsy body weight	334 ± 3	329 ± 4[b]	319 ± 5*	304 ± 5**
Left epididymis	0.436 ± 0.005	0.441 ± 0.006	0.427 ± 0.008	0.422 ± 0.005
Left cauda epididymis	0.148 ± 0.003	0.150 ± 0.005	0.143 ± 0.003	0.139 ± 0.004
Left testis	1.56 ± 0.02	1.57 ± 0.01	1.49 ± 0.03	1.50 ± 0.01
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.64 ± 0.26	10.14 ± 0.22	9.45 ± 0.29	10.09 ± 0.17
Spermatid heads (10^7/testis)	15.02 ± 0.46	15.97 ± 0.41	14.03 ± 0.27	15.15 ± 0.35
Spermatid count (mean/10^{-4} mL suspension)	75.10 ± 2.30	79.84 ± 2.03	70.17 ± 1.34	75.73 ± 1.74
Epididymal spermatozoal measurements				
Motility (%)	70.23 ± 2.79	72.31 ± 2.88[b]	71.71 ± 4.03	75.20 ± 1.99
Concentration (10^6/g cauda epididymal tissue)	965 ± 46	1,011 ± 32[b]	858 ± 38	945 ± 40

* Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's test
** $P \leq 0.01$
[a] Data are presented as mean ± standard error. Differences from the vehicle control group for left epididymal, cauda epididymal, and testis weights, spermatid measurements, and epididymal spermatozoal measurements are not significant by Dunn's or Shirley's test.
[b] n=9

TABLE E2 # Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Gavage Study of *o*-Chloroaniline[a]

Study Parameters	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
n	10	10	10	10
Necropsy body weight (g)	183 ± 3	176 ± 3	178 ± 2	174 ± 2**
Estrous cycle length (days)	4.95 ± 0.14	4.80 ± 0.11	4.95 ± 0.05	4.90 ± 0.07
Estrous stages (% of cycle)				
Diestrus	40.0	34.2	40.0	39.2
Proestrus	16.7	18.3	15.0	18.3
Estrus	23.3	25.0	26.7	20.8
Metestrus	20.0	22.5	18.3	21.7

** Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's test
[a] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, dosed groups do not differ significantly from the vehicle control group in the relative length of time spent in the estrous stages.

TABLE E3 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline[a]

Study Parameters	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
n	10	9	10	10
Weights (g)				
Necropsy body weight	326 ± 6	323 ± 5	319 ± 6	293 ± 6**
Left epididymis	0.423 ± 0.009	0.430 ± 0.007	0.437 ± 0.006	0.421 ± 0.004
Left cauda epididymis	0.141 ± 0.006	0.140 ± 0.003	0.151 ± 0.003	0.142 ± 0.003
Left testis	1.53 ± 0.03	1.50 ± 0.02	1.56 ± 0.02	1.50 ± 0.03
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.20 ± 0.20	9.53 ± 0.30	9.28 ± 0.14	9.66 ± 0.17
Spermatid heads (10^7/testis)	14.02 ± 0.22	14.28 ± 0.38	14.50 ± 0.20	14.42 ± 0.24
Spermatid count (mean/10^{-4} mL suspension)	70.10 ± 1.09	71.39 ± 1.91	72.48 ± 1.00	72.10 ± 1.22
Epididymal spermatozoal measurements				
Motility (%)	67.56 ± 1.08	66.36 ± 1.53	65.06 ± 2.62	57.52 ± 3.43**
Concentration (10^6/g cauda epididymal tissue)	980 ± 35	988 ± 66	1,018 ± 33	924 ± 30

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

[a] Data are presented as mean ± standard error. Differences from the vehicle control group for left epididymal, cauda epididymal, and testis weights, spermatid measurements, and epididymal spermatozoal concentration are not significant by Dunn's test.

TABLE E4 # Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Gavage Study of *m*-Chloroaniline[a]

Study Parameters	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
n	10	10	10	9
Necropsy body weight (g)	183 ± 3	183 ± 2	179 ± 3	185 ± 3
Estrous cycle length (days)	4.75 ± 0.11	4.95 ± 0.05	4.95 ± 0.05	4.89 ± 0.07
Estrous stages (% of cycle)				
Diestrus	30.8	35.8	35.0	35.2
Proestrus	12.5	12.5	10.0	11.1
Estrus	35.8	32.5	32.5	32.4
Metestrus	20.8	19.2	22.5	21.3

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

TABLE E5 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline[a]

Study Parameters	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
n	9	9	10	9
Weights (g)				
Necropsy body weight	36.1 ± 1.3	35.0 ± 0.4	35.4 ± 0.6	33.4 ± 1.1
Left epididymis	0.041 ± 0.001	0.042 ± 0.001	0.040 ± 0.001	0.041 ± 0.001
Left cauda epididymis	0.013 ± 0.000	0.014 ± 0.000	0.012 ± 0.000	0.012 ± 0.000
Left testis	0.112 ± 0.002	0.114 ± 0.002	0.114 ± 0.002	0.118 ± 0.001*
Spermatid measurements				
Spermatid heads (10^7/g testis)	21.58 ± 0.93	21.86 ± 0.69	20.69 ± 0.47	21.23 ± 0.38
Spermatid heads (10^7/testis)	2.42 ± 0.11	2.49 ± 0.07	2.36 ± 0.05	2.50 ± 0.05
Spermatid count (mean/10^{-4} mL suspension)	75.61 ± 3.41	77.69 ± 2.10	73.88 ± 1.58	78.25 ± 1.66
Epididymal spermatozoal measurements				
Motility (%)	62.26 ± 1.51	67.30 ± 1.16	60.04 ± 3.84	57.58 ± 2.56
Concentration (10^6/g cauda epididymal tissue)	1,371 ± 142	1,558 ± 127	1,557 ± 102	1,691 ± 187

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

[a] Data are presented as mean ± standard error. Differences from the vehicle control group for necropsy body weight, left epididymal and cauda epididymal weights, spermatid measurements, and epididymal spermatozoal concentration are not significant by Dunn's or Shirley's test.

TABLE E6 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Gavage Study of *o*-Chloroaniline[a]

Study Parameters	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
n	10	10	10	10
Necropsy body weight (g)	28.7 ± 0.7	28.5 ± 0.7	30.2 ± 0.6	27.4 ± 0.6
Estrous cycle length (days)	4.10 ± 0.10	4.05 ± 0.05	4.35 ± 0.15	4.10 ± 0.07
Estrous stages (% of cycle)				
Diestrus	32.5	27.5	28.3	31.0
Proestrus	23.3	25.0	23.3	23.9
Estrus	22.5	24.2	27.5	24.8
Metestrus	20.8	23.3	20.8	20.4
Uncertain diagnoses	0.8	0.0	0.0	0.0

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

TABLE E7 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline[a]

Study Parameters	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
n	9	10	10	10
Weights (g)				
Necropsy body weight	36.2 ± 0.9	34.4 ± 0.4	35.1 ± 0.3	35.6 ± 1.0
Left epididymis	0.043 ± 0.001	0.043 ± 0.001	0.043 ± 0.001	0.042 ± 0.001
Left cauda epididymis	0.014 ± 0.001	0.013 ± 0.001	0.014 ± 0.001	0.014 ± 0.000
Left testis	0.117 ± 0.002	0.117 ± 0.003	0.118 ± 0.002	0.116 ± 0.003
Spermatid measurements				
Spermatid heads (10^7/g testis)	21.26 ± 0.48	20.68 ± 0.48	20.29 ± 0.42	21.03 ± 0.65
Spermatid heads (10^7/testis)	2.49 ± 0.06	2.40 ± 0.06	2.40 ± 0.07	2.43 ± 0.06
Spermatid count (mean/10^{-4} mL suspension)	77.89 ± 2.01	75.15 ± 1.78	75.05 ± 2.12	75.78 ± 1.90
Epididymal spermatozoal measurements				
Motility (%)	68.28 ± 0.96	68.85 ± 1.37	68.13 ± 1.72	67.44 ± 3.15
Concentration (10^6/g cauda epididymal tissue)	2,175 ± 195	2,110 ± 90	2,066 ± 128	2,035 ± 154

[a] Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn's test.

TABLE E8 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Gavage Study of *m*-Chloroaniline[a]

Study Parameters	Vehicle Control	40 mg/kg	80 mg/kg	160 mg/kg
n	9	8	9	9
Necropsy body weight (g)	28.4 ± 0.7	29.9 ± 1.2	28.1 ± 0.5[b]	28.0 ± 0.7
Estrous cycle length (days)	4.28 ± 0.15	4.06 ± 0.06	4.22 ± 0.12[c]	4.17 ± 0.12
Estrous stages (% of cycle)				
Diestrus	25.0	27.1	34.2	30.6
Proestrus	18.5	22.9	20.0	21.3
Estrus	32.4	28.1	25.0	25.0
Metestrus	24.1	21.9	20.8	23.1

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

[b] n=10 #

[c] Estrous cycle longer than 12 days or unclear in 1 of 10 mice

APPENDIX F
GENETIC TOXICOLOGY

TABLE F1 Mutagenicity of *o*-Chloroaniline in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
STUDY PERFORMED AT SRI INTERNATIONAL						
TA100	0	124 ± 11.5	105 ± 4.0	146 ± 9.1	113 ± 2.4	149 ± 11.8
	10	132 ± 5.5		125 ± 6.3		146 ± 3.8
	33	128 ± 9.4	132 ± 4.7	127 ± 2.3	125 ± 2.4	165 ± 2.9
	100	134 ± 1.7	112 ± 8.8	148 ± 6.1	118 ± 4.1	162 ± 5.2
	333	127 ± 6.4	114 ± 8.3	144 ± 9.6	135 ± 8.0	159 ± 2.8
	1,000	101 ± 10.7	103 ± 6.5	130 ± 6.8	95 ± 5.5	148 ± 9.4
	3,333		0 ± 0.0[c]		0 ± 0.0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control[d]		253 ± 30.8	921 ± 60.5	1,796 ± 24.3	501 ± 22.4	1,007 ± 26.8
TA1535	0	19 ± 0.3	10 ± 2.0	8 ± 2.3	13 ± 2.9	36 ± 10.1
	10	18 ± 1.5		4 ± 1.2		12 ± 2.0
	33	15 ± 5.4	10 ± 1.3	11 ± 1.3	7 ± 0.3	10 ± 2.2
	100	22 ± 4.7	8 ± 1.9	5 ± 1.5	5 ± 0.7	11 ± 3.2
	333	26 ± 1.2	4 ± 1.9	4 ± 0.9	7 ± 2.7	7 ± 0.7
	1,000	25 ± 3.8	4 ± 0.9	6 ± 0.9	6 ± 1.8	8 ± 0.9
	3,333		0 ± 0.0[c]		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		236 ± 17.9	333 ± 47.0	511 ± 7.7	163 ± 26.2	289 ± 31.4
TA97	0	144 ± 14.6	108 ± 3.6	213 ± 2.6	121 ± 6.9	215 ± 19.8
	10	179 ± 15.0		190 ± 6.0		230 ± 8.7
	33	190 ± 11.9	127 ± 12.5	203 ± 11.5	129 ± 16.5	237 ± 5.8
	100	187 ± 5.2	120 ± 6.1	196 ± 11.7	117 ± 13.6	212 ± 10.5
	333	168 ± 4.7	118 ± 11.2	191 ± 6.1	132 ± 2.7	204 ± 6.1
	1,000	79 ± 6.4	111 ± 7.0	191 ± 8.3	119 ± 7.4	154 ± 3.7
	3,333		0 ± 0.0[c]		0 ± 0.0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		1,003 ± 26.4	923 ± 83.6	617 ± 45.0	414 ± 35.4	425 ± 5.7
TA98	0	15 ± 2.8	26 ± 3.6	23 ± 1.7	24 ± 2.3	33 ± 2.6
	10	10 ± 2.4		18 ± 0.9		29 ± 5.8
	33	15 ± 1.5	20 ± 2.5	25 ± 1.9	31 ± 5.0	34 ± 4.7
	100	10 ± 1.5	24 ± 2.5	29 ± 5.8	26 ± 8.2	33 ± 6.1
	333	9 ± 2.0	26 ± 5.0	21 ± 2.0	34 ± 2.2	36 ± 2.2
	1,000	11 ± 1.5	31 ± 3.2	28 ± 1.7	34 ± 3.8	30 ± 0.9
	3,333		Toxic		0 ± 0.0[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		752 ± 21.6	763 ± 74.9	1,562 ± 64.7	163 ± 26.2	846 ± 25.2

TABLE F1 Mutagenicity of *o*-Chloroaniline in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
STUDY PERFORMED AT MICROBIOLOGICAL ASSOCIATES, INC.						
TA100	0	92 ± 3.0	83 ± 2.0	116 ± 5.0	96 ± 2.8	102 ± 7.0
	10	77 ± 5.5	74 ± 5.8		94 ± 1.2	
	33	86 ± 0.6	81 ± 3.5	113 ± 2.7	82 ± 8.7	127 ± 1.2
	100	80 ± 3.8	79 ± 2.9	121 ± 6.7	82 ± 0.9	123 ± 4.7
	333	80 ± 8.7	75 ± 3.5	116 ± 3.6	99 ± 5.8	121 ± 7.0
	1,000	76 ± 5.5[c]	79 ± 8.4[c]	113 ± 7.3[c]	92 ± 4.3[c]	110 ± 6.1[c]
	2,000			14 ± 13.7[c]		0 ± 0.0[c]
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		343 ± 15.8	390 ± 5.8	537 ± 47.0	649 ± 11.9	498 ± 77.4
TA1535	0	18 ± 1.7	7 ± 1.5	8 ± 1.5	11 ± 0.7	12 ± 1.5
	10	17 ± 2.0	10 ± 0.9		8 ± 1.5	
	33	14 ± 2.0	7 ± 1.8	8 ± 1.2	11 ± 0.6	9 ± 1.0
	100	15 ± 1.9	9 ± 2.6	6 ± 1.2	10 ± 1.5	8 ± 0.9
	333	14 ± 1.3	10 ± 0.3	10 ± 1.7	6 ± 0.6	4 ± 0.6
	1,000	20 ± 1.5[c]	7 ± 0.9[c]	10 ± 0.9	11 ± 2.1[c]	8 ± 0.6
	2,000			10 ± 0.9[c]		Toxic
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		253 ± 7.5	61 ± 3.0	53 ± 4.6	140 ± 2.5	104 ± 4.8
TA97	0	88 ± 3.0	97 ± 6.1	103 ± 1.2	121 ± 8.3	131 ± 2.9
	10	80 ± 9.5	114 ± 7.4		115 ± 4.4	
	33	81 ± 5.3	103 ± 8.5	94 ± 4.4	105 ± 4.7	119 ± 1.2
	100	86 ± 6.5	104 ± 7.6	66[e]	111 ± 7.5	114 ± 15.9
	333	93 ± 3.8	94 ± 7.3	123 ± 12.3	120 ± 5.6	129 ± 8.3
	1,000	70 ± 2.1[c]	102 ± 6.4[c]	109 ± 4.1	96 ± 5.0[c]	115 ± 5.2[c]
	2,000			95 ± 4.4[c]		55 ± 19.8[c]
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		191 ± 4.4	459 ± 11.8	529 ± 40.3	1,139 ± 18.6	481 ± 5.2
TA98	0	12 ± 1.2	25 ± 3.5	59 ± 0.6	26 ± 2.1	50 ± 5.2
	10	15 ± 1.5	27 ± 2.3		32 ± 3.0	
	33	12 ± 0.0	25 ± 1.5	59 ± 3.3	27 ± 0.9	56 ± 4.3
	100	11 ± 2.8	25 ± 4.6	52 ± 5.0	28 ± 2.0	53 ± 3.8
	333	12 ± 0.3	25 ± 3.2	56 ± 3.3	27 ± 0.0	53 ± 8.5
	1,000	12 ± 0.3[c]	26 ± 1.7[c]	49 ± 6.3[c]	33 ± 1.9[c]	46 ± 3.9[c]
	2,000			3 ± 0.5[c]		12 ± 5.8[c]
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		167 ± 7.5	70 ± 5.0	140 ± 16.4	267 ± 4.9	133 ± 15.0

TABLE F1 Mutagenicity of *o*-Chloroaniline in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9 #	+10% hamster S9	+10% rat S9
STUDY PERFORMED AT MICROBIOLOGICAL ASSOCIATES (continued)				
TA1537	0	5 ± 0.9	8 ± 1.5 #	9 ± 0.6
	33	8 ± 0.7	9 ± 3.5	7 ± 1.5
	100	6 ± 1.0	6 ± 2.3	8 ± 0.7
	333	5 ± 1.5	8 ± 1.5	8 ± 2.3
	1,000	4 ± 1.7[c]	10 ± 2.3 #	7 ± 0.9
	2,000	5 ± 1.5[c]#	7 ± 0.7[c]	4 ± 2.0[c]
Trial summary		Negative	Negative	Negative
Positive control		71 ± 2.9	70 ± 6.7	51 ± 8.7

[a] The detailed protocol and the data from the SRI International study are presented in Zeiger *et al*. (1987); 0 μg/plate was the solvent control.
[b] Revertants are presented as the mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97 and TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for trials with metabolic activation with all strains was 2-aminoanthracene.
[e] Due to contamination, only one of three plates was scorable.

TABLE F2 Mutagenicity of *m*-Chloroaniline in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9	+10% hamster S9	+10% rat S9
STUDY PERFORMED AT CASE WESTERN RESERVE UNIVERSITY				
TA100	0	85 ± 4.9	143 ± 8.8	186 ± 2.2
	10	83 ± 1.5	183 ± 18.0	130 ± 5.2
	33	72 ± 1.2	214 ± 6.4	139 ± 4.2
	100	89 ± 4.5	190 ± 6.2	149 ± 7.5
	333	87 ± 2.1	201 ± 15.0	181 ± 13.1
	1,000	72 ± 2.8	156 ± 5.0	148 ± 5.5
Trial summary		Negative	Equivocal	Negative
Positive control[c]		1,137 ± 21.3	1,175 ± 177.2	2,784 ± 45.1
TA1535	0.0	5 ± 0.3	11 ± 1.2	18 ± 2.4
	3.3	10 ± 2.3	17 ± 3.5	19 ± 5.2
	10.0	5 ± 1.0	11 ± 2.6	10 ± 0.6
	33.0	8 ± 2.1	15 ± 2.6	12 ± 1.0
	100.0	5 ± 1.3	13 ± 2.3	19 ± 1.5
	333.0	5 ± 0.7	10 ± 3.1	16 ± 2.2
Trial summary		Negative	Negative	Negative
Positive control		770 ± 67.0	175 ± 33.6	129 ± 14.8
TA1537	0.0	4 ± 0.7	11 ± 1.2	15 ± 2.5
	3.3	3 ± 0.7	10 ± 0.7	6 ± 0.6
	10.0	2 ± 0.3	12 ± 0.5	8 ± 1.9
	33.0	2 ± 0.3	8 ± 1.2	6 ± 1.7
	100.0	2 ± 0.7	6 ± 0.6	5 ± 0.6
	333.0	1 ± 0.6	5 ± 1.2	7 ± 1.5
Trial summary		Negative	Negative	Negative
Positive control		303 ± 85.3	127 ± 7.2	261 ± 26.3
TA98	0.0	19 ± 0.9	27 ± 0.6	29 ± 1.7
	1.0			29 ± 2.3
	3.3			36 ± 4.2
	10.0	21 ± 1.5	28 ± 2.1	46 ± 3.8
	33.0	17 ± 1.9	33 ± 5.8	21 ± 3.5
	100.0	20 ± 5.7	43 ± 2.0	23 ± 6.8
	333.0	19 ± 2.3	32 ± 2.3	6 ± 2.2
	1,000.0	19 ± 3.8	27 ± 3.8	
Trial summary		Negative	Negative	Negative
Positive control		219 ± 8.6	764 ± 29.4	497 ± 51.3 #

TABLE F2 Mutagenicity of *m*-Chloroaniline in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9	+hamster S9 10%	+hamster S9 30%	+rat S9 10%	+rat S9 30%
STUDY PERFORMED AT MICROBIOLOGICAL ASSOCIATES, INC.						
TA100	0	98 ± 4.4	94 ± 4.2	121 ± 2.4	112 ± 2.2	120 ± 5.5
	33	94 ± 2.3	101 ± 3.8	110 ± 3.5	104 ± 7.2	139 ± 5.2
	100	95 ± 3.8	103 ± 9.4	120 ± 9.5	101 ± 2.3	139 ± 10.0
	333	98 ± 6.7	97 ± 7.1	130 ± 4.8	107 ± 2.3	127 ± 3.2
	1,000	61 ± 7.9[d]	89 ± 3.3[d]	122 ± 9.0	103 ± 3.8[d]	116 ± 7.2
	1,500	48 ± 21.2[d]	12 ± 10.0[d]		Toxic	
	2,000			100 ± 9.0[d]		74 ± 9.7[d]
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		310 ± 6.0	409 ± 10.7	728 ± 9.0	570 ± 13.7	560 ± 58.0
TA1535	0	20 ± 3.0	10 ± 0.0	13 ± 1.9	10 ± 1.0	13 ± 0.9
	33	15 ± 3.2	12 ± 1.5	11 ± 1.7	10 ± 1.8	9 ± 2.0
	100	16 ± 0.9	8 ± 2.0	9 ± 1.5	10 ± 1.8	13 ± 1.8
	333	14 ± 2.0	13 ± 2.3	9 ± 0.7	8 ± 0.7	8 ± 0.9
	1,000	12 ± 1.0[d]	10 ± 1.2	13 ± 0.9	8 ± 1.8[d]	7 ± 1.7
	1,500	15 ± 5.0[d]	4 ± 1.0[d]		Toxic	
	2,000			12 ± 2.5[d]		Toxic
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		220 ± 12.0	59 ± 2.8	228 ± 53.1	145 ± 9.8	143 ± 0.3
TA97	0	80 ± 2.3	87 ± 3.4	162 ± 6.6	118 ± 3.8	158 ± 7.7
	33	97 ± 5.0	114 ± 1.9	172 ± 12.1	118 ± 3.8	157 ± 9.5
	100	100 ± 8.6	99 ± 4.0	184 ± 2.3	107 ± 4.5	151 ± 3.6
	333	77 ± 6.4	107 ± 3.1	172 ± 12.7	111 ± 3.2	146 ± 5.3
	1,000	72 ± 3.7[d]	82 ± 1.2[d]	137 ± 7.8[d]	91 ± 3.5[d]	130 ± 6.8[d]
	1,500	Toxic	77 ± 9.0[d]		Toxic	
	2,000			Toxic		Toxic
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		223 ± 13.7	524 ± 10.0	553 ± 21.7	1,021 ± 5.2	471 ± 47.7
TA98	0	12 ± 1.2	21 ± 2.3	47 ± 0.7	25 ± 1.5	34 ± 3.8
	33	17 ± 1.9	26 ± 2.1	41 ± 5.1	24 ± 1.5	36 ± 1.2
	100	10 ± 1.3	27 ± 3.2	41 ± 2.9	24 ± 0.7	34 ± 2.9
	333	10 ± 1.5	29 ± 5.0	40 ± 2.9	25 ± 2.4	38 ± 3.8
	1,000	9 ± 1.7[d]	29 ± 1.8[d]	42 ± 3.7	19 ± 2.1[d]	32 ± 4.2
	1,500	Toxic	24 ± 8.2[d]		9 ± 3.2[d]	
	2,000			46 ± 1.5[d]		25 ± 12.5[d]
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		161 ± 2.2	83 ± 9.5	110 ± 8.4	245 ± 11.8	166 ± 11.3

TABLE F2 Mutagenicity of *m*-Chloroaniline in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -S9 #	+10% hamster S9	+10% rat S9
STUDY PERFORMED AT MICROBIOLOGICAL ASSOCIATES, INC. (continued)				
TA1537	0	8 ± 0.9	13 ± 1.0	10 ± 2.0
	33	11 ± 2.3	12 ± 0.9	6 ± 1.5
	100	10 ± 1.3	9 ± 1.9	8 ± 0.3
	333	7 ± 0.6	9 ± 1.5	8 ± 0.3
	1,000 #	7 ± 2.4	11 ± 1.5	9 ± 2.4
	2,000	3 ± 2.5[d]	10 ± 2.0[d,#]	Toxic
Trial summary		Negative	Negative	Negative
Positive control		74 ± 3.5	120 ± 11.9	62 ± 2.6

[a] The detailed protocol and the data from the Case Western Reserve University study are presented in Zeiger *et al*. (1987); 0 μg/plate was the solvent control.
[b] Revertants are presented as the mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97 and TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for trials with metabolic activation with all strains was 2-aminoanthracene.
[d] Slight toxicity

TABLE F3 Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by *o*-Chloroaniline[a]

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction[c]
-S9						
Trial 1						
Dimethylsulfoxide						
		119	126	191	53	
		107	86	160	50	
		103	99	163	53	
		111	89	179	54	52
Methyl methanesulfonate						
	15	39	19	378	322	
		42	19	382	306	314*
o-Chloroaniline						
	31.25	112	93	165	49	
	62.5	101	85	130	43	
		94	92	196	70	56
	125	104	96	180	58	
		117	97	249	71	65
	250	118	70	242	68	
	500	109	31	226	69	
		110	36	213	65	67
Trial 2						
Dimethylsulfoxide						
		97	100	74	25	
		80	97	61	25	
		83	119	77	31	
		60	84	55	31	28
Methyl methanesulfonate						
	15	25	34	167	223	
		30	27	174	196	209*
o-Chloroaniline						
	25	89	83	89	33	
		78	93	70	30	32
	50	77	76	65	28	
		94	95	104	37	32
	100	71	86	60	28	
		69	106	105	51	40
	200	71	70	92	43	
		78	71	79	34	38
	400	58	22	100	58	
		71	25	94	44	51*

TABLE F3 Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by *o*-Chloroaniline (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9						
Trial 1						
Dimethylsulfoxide						
		91	97	239	88	
		86	114	187	73	
		64	89	246	127	
		63	100	256	136	106
Methylcholanthrene						
	2.5	34	16	407	395	
		39	15	462	393	394*
o-Chloroaniline						
	200	51	47	217	143	
		44	41	182	139	141
	300	44	40	237	178	
		49	42	376	258	218*
	400	57	40	551	320	
		58	38	519	300	310*
	500	53	22	449	280	
		92	43	434	157	219*
	600	71	24	445	209	
		86	39	425	166	187*
	700	Lethal				
		Lethal				
Trial 2						
Dimethylsulfoxide						
		79	90	75	32	
		77	84	69	30	
		81	125	46	19	27
Methylcholanthrene						
	2.5	53	32	419	263	
		44	30	350	266	264*
o-Chloroaniline						
	300	70	43	146	69	
		74	37	132	59	64*
	400	72	36	278	128	
		82	33	370	150	139*
	500	75	23	599	268	
		67	30	421	210	239*
	600	71	16	818	382	
		71	19	616	291	336*
	700	69	8	482	233	
		76	8	531	233	233*

* Significant positive response ($P \leq 0.05$)

[a] Study was performed at Inveresk Research International. The experimental protocol and these data are presented in McGregor *et al.* (1991). All doses were tested in triplicate; the average of the three tests is presented in the table.

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

[c] Mean from three replicate plates of approximately 10^6 cells each

TABLE F4 Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by *m*-Chloroaniline[a]

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction[c]
-S9						
Trial 1						
Dimethylsulfoxide						
		72	98	172	79	
		99	101	239	81	
		94	106	216	77	
		83	94	255	103	85
Methyl methanesulfonate						
	15	30	24	242	272	
		24	19	288	400	336*
m-Chloroaniline						
	100	70	85	165	79	
		61	85	179	98	88
	200	76	60	213	94	
		77	70	213	92	93
	300	23	26	139	200	
		36	35	216	200	200*
	400	27	23	161	200	
		36	23	213	200	200*
	500	35	10	211	200	
		29	9	176	200	200*
Trial 2						
Dimethylsulfoxide						
		97	109	131	45	
		110	108	86	26	
		98	94	83	28	
		94	90	113	40	35
Methyl methanesulfonate						
	15	44	30	198	149	
		55	37	250	152	151*
m-Chloroaniline						
	160	113	76	142	42	
		83	62	72	29	35
	240	93	41	121	43	
		90	56	136	50	47
	320	84	25	145	57	
		73	31	122	55	56*
	400	102	26	187	61	
		79	24	156	66	64*
	480	86	10	226	87	
		84	10	222	88	88*

TABLE F4 Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by *m*-Chloroaniline (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9 (continued)						
Trial 3						
Dimethylsulfoxide						
		102	90	42	14	
		107	107	88	27	
		94	109	44	16	
		92	93	79	29	21
Methyl methanesulfonate						
	15	42	31	117	93	
		41	26	120	97	95*
m-Chloroaniline						
	440	73	14	71	33	
		70	12	58	28	30
	460	74	14	48	22	
		85	11	55	22	22
	480	110	10	80	24	
		78	9	77	33	29
	500	100	8	77	26	
		61	8	84	46	36
	520	63	6	84	45	
		57	6	87	51	48*
+S9						
Trial 1						
Dimethylsulfoxide						
		108	115	145	45	
		92	117	85	31	
		90	84	110	41	
		104	83	146	47	41
Methylcholanthrene						
	2.5	56	35	544	322	
		50	31	591	391	357*
m-Chloroaniline						
	240	74	52	185	84	
		68	40	191	93	89*
	320	56	35	123	73	
		56	28	150	90	82*
	400	66	16	146	73	
		62	24	132	71	72*
	480	56	16	149	89	
		102	11	269	88	88*
	560	39	6	134	115	
		46	5	163	119	117*

TABLE F4 Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by *m*-Chloroaniline (continued)

Compound	Concentration (μg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9 (continued)						
Trial 2						
Dimethylsulfoxide						
		61	124	56	31	
		67	108	65	32	
		67	88	62	31	
		62	79	57	31	31
Methylcholanthrene						
	2.5	40	30	413	341	
		47	33	350	248	295*
m-Chloroaniline						
	160	66	98	76	38	
		79	85	106	45	42
	240	66	74	60	30	
		51	47	71	46	38
	320	66	60	77	39	
		60	45	82	45	42
	400	71	40	122	57	
		79	38	153	65	61*
	480	90	30	149	55	
		63	28	91	48	52*
	560	66	21	131	67	
		73	20	145	67	67*

* Significant positive response ($P \leq 0.05$)

[a] Study was performed at Inveresk Research International. The experimental protocol is presented in McGregor *et al*. (1991). All doses were tested in triplicate; the average of the three tests is presented in the table.

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

[c] Mean from three replicate plates of approximately 10^6 cells each

TABLE F5 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by *m*-Chloroaniline[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Summary: Positive								
Dimethylsulfoxide		50	1,039	473	0.45	9.5	25.5	
Mitomycin-C	0.001	50	1,024	673	0.65	13.5	25.5	44.37
	0.010	5	99	236	2.38	47.2	25.5	423.64
m-Chloroaniline	80.0	50	1,017	580	0.57	11.6	32.8[c]	25.27*
	89.4	50	1,029	632	0.61	12.6	32.8[c]	34.91*
	100.0	50	1,031	651	0.63	13.0	32.8[c]	38.70*
					P < 0.001[d]			
+S9								
Trial 1								
Summary: Equivocal								
Dimethylsulfoxide		50	1,027	457	0.44	9.1	25.5	
Cyclophosphamide	0.3	50	1,034	631	0.61	12.6	25.5	37.14
	2.0	5	102	135	1.32	27.0	25.5	197.43
m-Chloroaniline	111	50	1,019	422	0.41	8.4	25.5	-6.93
	370	50	1,036	479	0.46	9.6	25.5	3.90
	1,100	50	1,026	540	0.52	10.8	25.5	18.28
					P=0.001			
Trial 2								
Summary: Weakly positive								
Dimethylsulfoxide		50	1,018	491	0.48	9.8	25.5	
Cyclophosphamide	0.3	50	1,029	616	0.59	12.3	25.5	24.12
	2.0	5	104	132	1.26	26.4	25.5	163.16
m-Chloroaniline	198	50	1,027	523	0.50	10.5	25.5	5.59
	402	50	1,023	554	0.54	11.1	25.5	12.28
	600	50	1,020	617	0.60	12.3	25.5	25.42*
					P < 0.001			

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

[a] Study was performed at Litton Bionetics, Inc. A detailed description of the protocol is presented in Galloway *et al.* (1987). SCE = sister chromatid exchange; BrdU = bromodeoxyuridine.

[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells

[c] Because *m*-chloroaniline induced a delay in the cell division cycle, harvest time was extended to maximize the proportion of second-division cells available for analysis.

[d] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose

TABLE F6 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by *m*-Chloroaniline[a]

-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 (%)
Harvest time: 20.8 hours[b]					Harvest time: 10.5 hours				
Summary: Positive					Summary: Weakly positive				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	0	0.00	0.0		100	3	0.03	2.0
Mitomycin-C					Cyclophosphamide				
0.0625	50	14	0.28	26.0	50	100	13	0.13	13.0
m-Chloroaniline					*m*-Chloroaniline				
252.1	100	5	0.05	4.0	397.7	100	3	0.03	3.0
300.2	100	13	0.13	12.0*	497.1	100	10	0.10	6.0
400.3	100	13	0.13	11.0*	598.9	100	32	0.32	19.0*
				$P < 0.001$[c]					$P < 0.001$

* # Positive ($P < 0.05$)

[a] Study was performed at Litton Bionetics, Inc. A detailed description of the protocol is presented in Galloway *et al.* (1987). Abs = aberrations.

[b] Because *m*-chloroaniline induced significant cell cycle delay, incubation time prior to addition of Colcemid was lengthened to provide sufficient metaphase cells at harvest.

[c] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose

TABLE F7 Induction of Micronuclei in Bone Marrow Cells of F344/N Rats by *o*-Chloroaniline[a]

	Dose (mg/kg)	Micronucleated PCEs/1,000 PCEs[b]
Trial 1		
Corn oil		1.50 ± 0.42
Cyclophosphamide	25	12.75 ± 1.30
o-Chloroaniline	200	1.60 ± 0.58
	400	2.50 ± 0.61
	550	4.00 ± 1.26
		P=0.007[c]
Trial 2		
Corn oil		0.60 ± 0.37
Cyclophosphamide	25	13.60 ± 0.81
o-Chloroaniline	200	1.50 ± 0.35
	400	2.38 ± 0.52
	550	7.40 ± 3.70*
		P<0.001

* Positive (P<0.008)
[a] PCEs = polychromatic erythrocytes. Data are presented as mean ± standard error for groups of five animals.
[b] Two thousand polychromatic erythrocytes were scored per animal.
[c] Significance of micronucleated PCEs/1,000 PCEs tested by a one-tailed trend test

TABLE F8 Induction of Micronuclei in Bone Marrow Cells of F344/N Rats by *m*-Chloroaniline[a]

	Dose (mg/kg)	Micronucleated PCEs/1,000 PCEs[b]
Corn oil		1.5 ± 0.4
Cyclophosphamide	25	12.8 ± 1.3
m-Chloroaniline	25	1.5 ± 0.4
	100	1.8 ± 0.6
		P=0.276[c]

[a] PCEs = polychromatic erythrocytes. Data are presented as mean ± standard error for groups of five animals.
[b] Two thousand polychromatic erythrocytes were scored per animal.
[c] Significance of micronucleated PCEs/1,000 PCEs tested by a one-tailed trend test

TABLE F9 Induction of Micronuclei in Bone Marrow Cells of B6C3F$_1$ Mice by *o*-Chloroaniline[a]

	Dose (mg/kg)	Micronucleated PCEs/1,000 PCEs[b]
Trial 1		
Corn oil		1.1 ± 0.3
Cyclophosphamide	25 #	13.0 ± 2.0
o-Chloroaniline	125	2.3 ± 0.9
	250	2.8 ± 0.6*
	500	3.4 ± 0.6*
	750	1.4 ± 0.4
	1,000 #	Lethal
		P=0.202[c]
Trial 2		
Corn oil		3.8 ± 1.9
Cyclophosphamide	25 #	12.6 ± 2.3
o-Chloroaniline	125	1.5 ± 0.4
	250	2.2 ± 0.5
	500	2.0 ± 0.7
	750	1.7 ± 0.3
	1,000 #	3.2 ± 1.5
		P=0.537

* Positive ($P < 0.005$)
[a] PCEs = polychromatic erythrocytes. Data are presented as mean ± standard error for groups of five animals.
[b] Two thousand polychromatic erythrocytes were scored per animal.
[c] Significance of micronucleated PCEs/1,000 PCEs tested by a one-tailed trend test

TABLE F10 Induction of Micronuclei in Bone Marrow Cells of B6C3F$_1$ Mice by *m*-Chloroaniline[a]

	Dose (mg/kg)	Micronucleated PCEs/1,000 PCEs[b]
Trial 1[c]		
Corn oil		1.1 ± 0.3
Cyclophosphamide	25	13.0 ± 2.0
m-Chloroaniline	100	1.4 ± 0.4
	200	1.0 ± 0.4
	400	3.1 ± 0.7*
		P < 0.001[d]
Trial 2[c]		
Corn oil		3.8 ± 1.9
Cyclophosphamide	25	12.6 ± 2.3
m-Chloroaniline	200	1.0 ± 0.4
	400	2.4 ± 0.9
	500	1.2 ± 0.3
		P=0.946
Trial 3[e]		
Corn oil		1.2 ± 0.2
Cyclophosphamide	25	16.9 ± 3.3
m-Chloroaniline	100	2.3 ± 0.9
	200	2.3 ± 0.3
		P=0.066

* Positive (P < 0.008)
[a] PCEs = polychromatic erythrocytes. Data are presented as mean ± standard error for groups of five animals.
[b] Two thousand polychromatic erythrocytes were scored per animal.
[c] Bone marrow was sampled 24 hours after mice received a single intraperitoneal injection.
[d] Significance of micronucleated PCEs/1,000 PCEs tested by a one-tailed trend test
[e] Mice received three intraperitoneal injections at 24-hour intervals; bone marrow was sampled 24 hours after the third injection.

TABLE F11 Frequency of Micronuclei in Peripheral Blood of B6C3F$_1$ Mice Treated with *o*-Chloroaniline for 13 Weeks[a]

	Dose (mg/kg)	Micronucleated NCEs/1,000 NCEs[b]
Male		
	0	1.0 ± 0.2
	10	1.1 ± 0.3
	20	1.3 ± 0.2
	40	1.6 ± 0.3
	80	1.3 ± 0.3
	160	0.7 ± 0.2
		P < 0.835[c]
Female		
	0	0.7 ± 0.3
	10	1.2 ± 0.3
	20	0.8 ± 0.1
	40	0.9 ± 0.2
	80	0.9 ± 0.2
	160	0.8 ± 0.3
		P=0.597

[a] NCEs = normochromatic erythrocytes. Data are presented as mean ± standard error for groups of five animals.
[b] Two thousand normochromatic erythrocytes were scored per animal. Differences from the control group were not significant at P < 0.005.
[c] Significance of micronucleated NCEs/1,000 NCEs tested by a one-tailed trend test, significant at P < 0.025.

TABLE F12 Frequency of Micronuclei in Peripheral Blood of B6C3F$_1$ Mice Treated with *m*-Chloroaniline for 13 Weeks[a]

	Dose (mg/kg)	Micronucleated NCEs/1,000 NCEs[b]
Male		
	0	0.9 ± 0.2
	10	1.1 ± 0.1
	20	1.4 ± 0.1
	40	1.1 ± 0.2
	80	1.0 ± 0.2
	160	1.2 ± 0.3
		P < 0.422[c]
Female		
	0	0.6 ± 0.2
	10	1.1 ± 0.3
	20	1.0 ± 0.0
	40	1.4 ± 0.2
	80	1.0 ± 0.2
	160	0.9 ± 0.2
		P=0.491

[a] NCEs = normochromatic erythrocytes. Data are presented as mean ± standard error for groups of five animals.
[b] Two thousand normochromatic erythrocytes were scored per animal. Differences from the control group were not significant at P < 0.005.
[c] Significance of micronucleated NCEs/1,000 NCEs tested by a one-tailed trend test, significant at P < 0.025.