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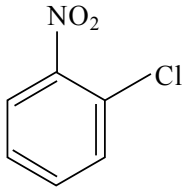
Provisional Peer-Reviewed Toxicity Values for

o-Chloronitrobenzene
(2-Chloronitrobenzene)
(CASRN 88-73-3)



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Commonly Used Abbreviations

BMD	Benchmark Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional inhalation reference concentration
p-RfD	provisional oral reference dose
RfC	inhalation reference concentration
RfD	oral reference dose
UF	uncertainty factor

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR *o*-CHLORONITROBENZENE (2-CHLORONITROBENZENE) (CASRN 88-73-3)

Background

On December 5, 2003, the U.S. Environmental Protection Agency's (U.S. EPA) Office of Superfund Remediation and Technology Innovation (OSRTI) revised its hierarchy of human health toxicity values for Superfund risk assessments, establishing the following three tiers as the new hierarchy:

1. U.S. EPA's Integrated Risk Information System (IRIS).
2. Provisional Peer-Reviewed Toxicity Values (PPRTVs) used in U.S. EPA's Superfund Program.
3. Other (peer-reviewed) toxicity values, including:
   - Minimal Risk Levels produced by the Agency for Toxic Substances and Disease Registry (ATSDR),
   - California Environmental Protection Agency (CalEPA) values, and
   - EPA Health Effects Assessment Summary Table (HEAST) values.

A PPRTV is defined as a toxicity value derived for use in the Superfund Program when such a value is not available in U.S. EPA's IRIS. PPRTVs are developed according to a Standard Operating Procedure (SOP) and are derived after a review of the relevant scientific literature using the same methods, sources of data, and Agency guidance for value derivation generally used by the U.S. EPA IRIS Program. All provisional toxicity values receive internal review by two U.S. EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all U.S. EPA programs, while PPRTVs are developed specifically for the Superfund Program.

Because new information becomes available and scientific methods improve over time, PPRTVs are reviewed on a 5-year basis and updated into the active database. Once an IRIS value for a specific chemical becomes available for Agency review, the analogous PPRTV for that same chemical is retired. It should also be noted that some PPRTV documents conclude that a PPRTV cannot be derived based on inadequate data.

Disclaimers

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the U.S. EPA

Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other U.S. EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300), or OSRTI.

INTRODUCTION

o-Chloronitrobenzene (2-chloronitrobenzene or 1-chloro-2-nitrobenzene) is an intermediate in the production of dyes, lumber preservatives, drugs and photographic chemicals (IARC, 1996). The empirical formula for *o*-chloronitrobenzene is $C_6H_4ClNO_2$ (see Figure 1).

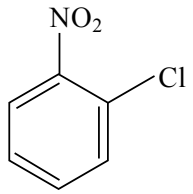

Figure 1. *o*-Chloronitrobenzene Structure

The U.S. Environmental Protection Agency (U.S. EPA, 2008) Integrated Risk Information System (IRIS) does not list a chronic oral reference dose (RfD), a chronic inhalation reference concentration (RfC), or a cancer assessment for *o*-chloronitrobenzene. Neither the Health Effects Assessment Summary Tables (HEAST; U.S. EPA, 1997) nor the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) included subchronic or chronic RfDs or RfCs for *o*-chloronitrobenzene. However, following guidelines for carcinogen risk assessment available at that time (i.e., U.S. EPA, 1984), the HEAST (U.S. EPA, 1997) listed *o*-chloronitrobenzene as a possible human carcinogen based on no evidence in humans, positive evidence in animals, and positive results in a few bacterial and all mammalian genotoxicity assays. U.S. EPA (1985) calculated an oral slope factor (OSF) of 2.5×10^{-2} per (mg/kg-day) for *o*-chloronitrobenzene in the Health and Environmental Effects Profile (HEEP) based on the incidence of hepatocellular carcinoma in female mice exposed to 2167 or 4333 ppm (time-weighted average; TWA) of *o*-chloronitrobenzene via diet for 18 months (Weisburger et al., 1978). The International Agency for Research on Cancer (IARC, 1996) concluded that *o*-chloronitrobenzene was not classifiable as to its carcinogenicity to humans (Group 3) based on an absence of data in humans and inadequate data in animals; IARC (1996) stated that the Weisburger et al. (1978) dietary study in rats and mice was inadequate for an evaluation.

o-Chloronitrobenzene was not included in the National Toxicology Program (NTP) 11th Report on Carcinogens (NTP, 2005). The Chemical Assessments and Related Activities (CARA) lists (U.S. EPA, 1991, 1994a) reported no documents relevant to the toxicity of *o*-chloronitrobenzene, other than the HEEP (U.S. EPA, 1985).

The U.S. Agency for Toxic Substances and Disease Registry (ATSDR, 2008) and the World Health Organization (WHO, 2008) had not reviewed the toxicity of *o*-chloronitrobenzene. The American Conference of Governmental Industrial Hygienists (ACGIH, 2007), the U.S. National Institute for Occupational Safety and Health (NIOSH, 2005), and the U.S. Occupational Safety and Health Administration (OSHA, 2008) had not established occupational exposure limits for *o*-chloronitrobenzene. However, OSHA (2008) listed a PEL-TWA of 1 mg/m^3 and ACGIH (2001a, 2007) listed a TLV-TWA of 0.1 ppm (0.64 mg/m^3) for *p*-chloronitrobenzene (listed as *p*-nitrochlorobenzene). Both occupational exposure limits for *p*-chloronitrobenzene included "skin" notations, indicating the likelihood that *p*-chloronitrobenzene could be absorbed through intact skin. In addition, ACGIH (2001a, 2007) classified *p*-chloronitrobenzene as a "confirmed animal carcinogen with unknown relevance to humans" and published a Biological Exposure Index (BEI) for methemoglobin inducers (ACGIH, 2001b), including the chloronitrobenzenes, of 1.5% methemoglobin, a form of hemoglobin that does not bind oxygen. Weisburger and Hudson (2001) and Woo and Lai (2001) published toxicity reviews on aromatic nitro, amino, and nitro-amino compounds, and their halogenated derivatives.

Literature searches were conducted for data relevant to the derivation of provisional toxicity values for *o*-chloronitrobenzene (CASRN 88-73-3) in May 2007 and a secondary check in June 2008 in MEDLINE, TOXLINE special. In addition, DART/ETIC; BIOSIS; TSCATS/TSCATS2, RTECS, CCRIS, HSDB and GENETOX (not date limited) and the Current Contents were reviewed.

REVIEW OF PERTINENT LITERATURE

Human Studies

Oral Exposure

No studies investigating the effects of subchronic or chronic oral exposure to *o*-chloronitrobenzene in humans could be identified.

Inhalation Exposure

Renshaw and Ashcroft (1926) described four workers' occupational exposures to *o*- and *p*-chloronitrobenzene, probably involving both inhalation and dermal exposure, which resulted in methemoglobinemia, slate gray appearance, headache, dyspnea on exertion, darkened blood serum, and large and occasionally deformed erythrocytes. However, the study authors provided no information on the total number of workers exposed or the air concentrations to which workers were exposed.

Jones et al. (2006) conducted a clinical study evaluating workers exposed to *o*- and *p*-chloronitrobenzene at a chemical manufacturing plant in Tainjing, China. Exposed (n = 39) and unexposed workers (n = 15) were given clinical examinations that included health complaints (e.g., fatigue, headache, dizziness, insomnia, eye and skin irritation, dyspnea),

physical examination, urinalysis, hematology, clinical chemistry, and analysis of blood for hemoglobin adducts with *o*- and *p*-chloronitrobenzene metabolites. Jones et al. (2006) measured air concentrations of *o*- and *p*-chloronitrobenzene using personal air monitors in a subset of exposed workers ($n = 19$). Median time weighted average (8-hour) air concentrations of *o*- and *p*-chloronitrobenzene were 0.37 and 0.87 mg/m^3, respectively; mean TWA exposures were 0.49 and 1.17 mg/m^3. The study authors reported no information regarding durations of exposure. Although the prevalence of complaints (fatigue, headache, dizziness) and abnormalities (splenomegaly, hepatomegaly) among exposed workers tended to be higher than in the control group, the differences were not statistically significant ($p > 0.05$). The study authors observed no differences between exposed and unexposed workers for urinalysis clinical chemistry or hematology including methemoglobin concentrations, hemoglobin concentration, red blood cell (RBC) counts, or leukocyte counts. The correlations between hemoglobin adducts and outcomes were not statistically significant (i.e., $p > 0.05$; correlation coefficients were not reported).

Animal Studies

Oral Exposure

Subchronic Exposure—The Screening Information Data Set (SIDS) on 1-chloro-2-nitrobenzene prepared by the Organization for Economic Cooperation Development (OECD) identified an unpublished subchronic oral toxicity study conducted by the Bayer (1991, 1993) AG Corporation in Germany. OECD/SIDS (2001) provided a brief summary of this study; however, it was not possible to obtain the complete report. According to OECD/SIDS (2001), this study followed OECD Guideline 407 and was conducted to Good Laboratory Practice (GLP) standards. Bayer (1991, 1993) administered diets containing 0, 50, 500, or 5000 ppm *o*-chloronitrobenzene to groups of male and female B6C3F1 mice (12/gender/group) for 5 weeks; an additional 6 mice/gender/group were included for an interim sacrifice after 1 week. Daily doses of *o*-chloronitrobenzene were reported as 0, 16, 167, or 1120 mg/kg-body weight in males and 0, 24, 220, or 1310 mg/kg-body weight in females. Based on findings reported by OECD/SIDS (2001), Bayer (1991, 1993) examined endpoints including mortality, clinical signs, food consumption, body weight, hematology, clinical chemistry, gross pathology, and histopathology. Unless indicated below, the OCED/SIDS (2001) summary did not report group means, incidence of effect, magnitude of effect, or statistical significance for any endpoint.

During treatment, one male in the 50-ppm group died (cause of death not reported), but Bayer (1991, 1993) observed no mortalities in the control, 500- or 5000-ppm groups. Bayer (1991, 1993) observed reduced food intake in males at 5000 ppm and females at $\geq$500 ppm, and decreased body weight gain in males and females in the 5000-ppm groups. Clinical signs of toxicity, including corneal opacity and narrowed palpebral fissure, were observed in males in the 5000-ppm group. No information on hematological effects was reported at the 1-week interim sacrifice. Effects on hematological parameters in males and females fed diets containing 5000 ppm *o*-chloronitrobenzene for 5 weeks were consistent with treatment-induced methemoglobinemia and subsequent anemia. These effects included reduced erythrocyte count, altered red blood cell morphology (anisocytosis [unequal sized red blood cells], poikilocytosis [abnormally shaped RBCs] and polychromatophilia), reduced hematocrit and hemoglobin; and increased methemoglobin (1.7% in males, 2.8% in females; control values not reported), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

After 1 week of treatment, males and females fed diets containing ≥500 ppm had increased serum "cholesterin" (cholesterol) content and significant changes (direction of change not specified) in the activities of cytochrome 450-dependent 7-ethoxycoumarin deethylase (EOD), epoxide hydroxylase (EH), aldrin epoxidase (ALD), and Phase II enzymes, including glutathion-S-transferase (GSH-T) and UDP-glucuronyltransferase (GLU-T) from unspecified tissues. Bayer (1991 and 1993) also reported decreased gluconeogenesis and glycogen in unspecified tissues. The study authors noted increased activities of EOD, EOR (not defined), GLU-T, ALD, GSH-T, and EH in males; and normal ALD activity and increased EOR, EH, GLU-T, EOD, and GSH-T in females. After 5 weeks of treatment with 5000 ppm, both the males and females exhibited increased bilirubin, AST, ALT, activated pentose phosphate cycle, and glycolysis. The males also exhibited increased alkaline phosphatase activity. Bayer (1991 and 1993) reported increases in spleen and liver weights (not specified whether absolute or relative weights) among males and females in the 5000-ppm group including liver weight increases of up to 89% in females. Males in the 5000-ppm group exhibited reduced testes weight. Histopathological examination revealed centrilobular hepatocytomegaly in males and females in the 500- and 5000-ppm groups and hemosiderin deposition in the spleen in males and females in the 5000-ppm group, but no histopathological changes in the kidneys or testes. Bayer (1991 and 1993) identified the hematopoietic system and the liver as targets of *o*-chloronitrobenzene in this study. Although anemia and other effects observed in this study appeared to have occurred primarily in the 5000-ppm group (1120 mg/kg-day in males and 1310 mg/kg-day in females), Bayer (1991 and 1993) noted some effects in the 500-ppm group. Although NOAEL and LOAEL values cannot conclusively be identified without review and analysis of the original study reports, it can be asserted that 500 ppm (167 mg/kg-day in males; 220 mg/kg-day in females) appears to be a 5-week LOAEL for effects on the hematopoietic system and the liver in mice, while 50 ppm (16 mg/kg-day in males; 22 mg/kg-day in females) appears to have been a NOAEL.

Matsumoto et al. (2006a) investigated the subchronic toxicity of oral *o*-chloronitrobenzene in two 13-week studies: one in F344 rats and one in BDF_1 mice. Rats (10/gender/group) were fed diets containing 0, 63, 250, 1000, 2000, or 4000 ppm and mice (10/gender/group) were fed diets containing 0, 78, 313, 1250, 2500, or 5000 ppm *o*-chloronitrobenzene (>99% purity). Matsumoto et al. (2006a) calculated the daily doses of *o*-chloronitrobenzene based on daily food consumption and mean body weights as follows:

- 0, 3.5, 13.8, 57.5, 119.5, or 234.1 mg/kg-day for male rats
- 0, 4.0, 15.5, 63.9, 133.3, or 249.3 mg/kg-day for female rats
- 0, 10.4, 43.6, 170.4, 345.1, or 684.1 mg/kg-day in male mice
- 0, 12.2, 49.5, 196.5, 400.3, or 762.5 mg/kg-day in female mice.

Matsumoto et al. (2006a) observed the animals daily for mortality and clinical signs, and they recorded food consumption and body weight. They collected blood samples at the end of the 13-week treatment period and analyzed the samples for hematology (RBC count, hemoglobin [Hgb], hematocrit [Hct] and mean corpuscular hemoglobin [MCH]), and clinical chemistry (total bilirubin, alanine aminotransferase [ALT] and aspartate aminotransferase [AST]), but did not measure methemoglobin (MetHgb). The study authors conducted necropsy on all animals, recorded body weights, and performed histopathological examination on comprehensive tissues at the end of treatment.

No mortalities occurred in rats exposed to dietary *o*-chloronitrobenzene at any concentration up to 4000 ppm for 13 weeks. Matsumoto et al. (2006a) observed no clinical signs of toxicity in the male or female rats, but noted that terminal body weights were significantly decreased (by ≥10%; other details not reported) compared to controls in male and female rats fed diets containing 4000 ppm *o*-chloronitrobenzene. The dose-dependent changes in hematology parameters were consistent with treatment-induced anemia (see Table 1). Matsumoto et al. (2006a) observed significant decreases in Hgb (≥250 ppm), RBC counts (≥1000 ppm), and Hct (≥1000 ppm), and increases in MCV (≥1000 ppm) among male rats. In female rats, the authors reported significantly decreased RBC counts and Hgb in all treated groups; Hct was decreased and MCV was increased at dietary concentrations ≥1000 ppm. Increases in clinical chemistry parameters, including total bilirubin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) indicate that exposure to dietary *o*-chloronitrobenzene is hepatotoxic (See Table 1). In male rats, the study authors observed dose-dependent increases in total bilirubin and ALT at concentrations ≥1000 ppm and AST at ≥2000 ppm. In female rats, they observed increased total bilirubin and ALT at dietary concentrations ≥2000 ppm; AST was increased at the highest dietary concentration (4000 ppm).

Table 1. Selected Hematology and Clinical Chemistry Parameters in F344 Rats Exposed to Dietary *o*-Chloronitrobenzene for 13 Weeks[a]

	Dietary Concentration (ppm)					
Parameter	0	63	250	1000	2000	4000
Males mg/kg-day	**0**	**3.5**	**13.8**	**57.5**	**119.5**	**234.1**
RBC count (10^6/µL)	9.49 ± 0.19[b]	9.44 ± 0.17	9.35 ± 0.20	8.78 ± 0.11[d]	8.42 ± 0.20[d]	7.83 ± 0.33[d]
Hgb (g/dL)	16.0 ± 0.4	15.8 ± 0.3	15.5 ± 0.4[d]	14.4 ± 0.2[d]	13.9 ± 0.3[d]	14.0 ± 0.5[d]
Hct (%)	46.1 ± 0.8	45.8 ± 0.9	45.1 ± 0.8	43.4 ± 0.5[d]	41.9 ± 0.8[d]	42.6 ± 1.1[d]
MCV (fL)	48.6 ± 0.3	48.5 ± 0.3	48.3 ± 0.4	49.4 ± 0.5[d]	49.8 ± 0.5[d]	54.5 ± 1.3[d]
Total bilirubin (mg/dL)	0.11 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.16 ± 0.01[c]	0.30 ± 0.08[d]	0.69 ± 0.13[d]
AST (IU/L)	60 ± 5	73 ± 24	74 ± 23	67 ± 13	139 ± 53[d]	223 ± 32[d]
ALT (IU/L)	40 ± 4	45 ± 9	45 ± 9	59 ± 12[c]	205 ± 77[d]	448 ± 71[d]
Females mg/kg-day	**0**	**4.0**	**15.5**	**63.9**	**133.3**	**249.3**
RBC count (10^6/µL)	8.84 ± 0.22	8.54 ± 0.16[d]	8.48 ± 0.14[d]	8.03 ± 0.21[d]	7.67 ± 0.23[d]	7.20 ± 0.18[d]
Hgb (g/dL)	16.1 ± 0.4	15.5 ± 0.3[d]	15.3 ± 0.3[d]	14.3 ± 0.4[d]	13.7 ± 0.4[d]	13.4 ± 0.4[d]
Hct (%)	44.6 ± 1.3	43.4 ± 0.8	43.7 ± 0.8	41.8 ± 1.2[d]	40.4 ± 1.0[d]	40.2 ± 1.1[d]
MCV (fL)	50.4 ±0.5	50.9 ± 0.5	51.5 ± 0.4	52.0 ± 0.4[d]	52.7 ± 1.0[d]	55.8 ± 0.4[d]
Total bilirubin (mg/dL)	0.15 ± 0.02	0.14 ± 0.02	0.14 ± 0.01	0.17 ± 0.01	0.21 ± 0.03[c]	0.41 ± 0.04[d]
AST (IU/L)	69 ± 14	65 ± 8	66 ± 12	68 ± 6	77 ± 10	132 ± 34[d]
ALT (IU/L)	36 ± 9	33 ± 4	37 ± 9	40 ± 7	60 ± 16[d]	144 ± 37[d]

[a]Matsumoto et al., 2006a
[b]Means ± standard deviation (SD); n = 10/gender/group (9/gender/group for 4000-ppm males)
[c]Significantly different from control ($p < 0.05$) by Dunnett test
[d]Significantly different from control ($p < 0.01$) by Dunnett test

The relative spleen and liver weights increased linearly with dose in male and female rats (Matsumoto et al., 2006a). However, the data were presented graphically and no information on statistical significance is included in the study report. Based on visual inspection of graphs, it is estimated that the increases in relative spleen and liver weights are approximately 3- and 2.5-fold, respectively, greater than control in males and females in the 4000-ppm group. The results of the histopathological examination showed that dietary exposure to *o*-chloronitrobenzene produced adverse effects to the bone marrow, spleen, and liver (see Table 2). The findings are consistent with accelerated RBC destruction (hemolytic anemia) and compensatory erythropoiesis to maintain erythrocyte mass and with hepatotoxicity. The incidence of erythropoiesis in bone marrow is significantly increased in male and female rats exposed to ≥2000 ppm. In the spleen, the incidences of congestion and hemosiderin deposition were significantly increased in males and females fed dietary concentrations ≥250 ppm and extramedullary hematopoiesis was increased at ≥1000 ppm. The incidence of capsule hyperplasia of the spleen, characterized by focal or multi-focal fibrous thickening, outward expansion of the capsule and incorporation of hematopoietic cells, was significantly increased in males and females exposed to ≥2000 ppm. In the liver, the incidence of hemosiderin deposition in Kupffer's cells was significantly increased in males and females fed ≥1000 ppm, although extramedullary hematopoiesis was not increased (details not shown). The incidence of centrilobular hepatocyte hypertrophy was increased in males fed ≥2000 ppm and females fed 4000 ppm, the study authors observed single-cell necrosis in males and females fed ≥1000 ppm, and they observed the hydropic degeneration of hepatocytes, characterized by ballooning hepatocytes with "watery materials," in males exposed to ≥1000 ppm and females exposed to ≥2000 ppm.

Based on significant decreases in RBC counts and Hgb in all *o*-chloronitrobenzene groups in female rats, Matsumoto et al. (2006a) identified a LOAEL of 63 ppm (4.0 mg/kg-day) for dietary exposure of rats to *o*-chloronitrobenzene for 13 weeks; a NOAEL was not identified.

In the mouse study, Matsumoto et al. (2006a) reported one death in a 1250-ppm group. However, the report is unclear as to whether the death occurred in a male or female mouse, nor did the authors report the time or cause of death. The study authors observed no clinical signs of toxicity in the male or female mice. Terminal body weights of males or females are not affected by treatment with *o*-chloronitrobenzene. The dose-dependent changes in the hematology parameters were consistent with treatment-induced anemia (see Table 3). In male mice, decreases were observed in RBC count, Hgb, and Hct at concentrations of ≥1250 ppm *o*-chloronitrobenzene; MCV was increased only in male mice fed 1250 ppm. In female mice, RBC count and Hgb were decreased at concentrations of ≥1250 ppm and Hct at concentrations ≥2500 ppm. MCV was increased in females exposed to 313 and 1250 ppm. Changes in serum ALT activity indicate that exposure to dietary *o*-chloronitrobenzene is hepatotoxic (see Table 3). In male mice, a dose-dependent increase in ALT was observed at concentrations ≥2500 ppm; however, AST was not increased in any *o*-chloronitrobenzene group. No changes in total bilirubin were observed in male mice. In female mice, dose-dependent increases were observed in ALT at concentrations ≥1250 and total bilirubin was increased at 5000 ppm; no changes in AST were observed.

Table 2. Incidence of Bone Marrow, Spleen and Liver Lesions in Groups of 10 F344 Rats Exposed to Dietary *o*-Chloronitrobenzene for 13 Weeks[a]

Organ	Lesion	Dietary Concentration (ppm)					
		0	63	250	1000	2000	4000
Males	**mg/kg-day**	**0**	**3.5**	**13.8**	**57.5**	**119.5**	**234.1**
Bone marrow	Increased erythropoiesis	0[b]	0	0	0	10[d]	10[d]
Spleen	Congestion	0	0	10[d]	10[d]	10[d]	10[d]
	Hemosiderin deposition	0	0	10[d]	10[d]	10[d]	10[d]
	Extramedullary hematopoiesis	0	0	0	10[d]	10[d]	10[d]
	Capsule hyperplasia	0	0	0	0	10[d]	10[d]
Liver	Hemosiderin deposition	0	0	0	5[c]	10[d]	10[d]
	Centrilobular hydropic degeneration	0	0	0	7[d]	10[d]	10[d]
	Single cell necrosis	0	0	0	8[d]	10[d]	10[d]
	Centrilobular hypertrophy	0	0	0	0	10[d]	10[d]
Females	**mg/kg-day**	**0**	**4**	**15.5**	**63.9**	**133.3**	**249.3**
Bone marrow	Increased erythropoiesis	0	0	0	0	10[d]	10[d]
Spleen	Congestion	0	0	9[d]	10[d]	10[d]	10[d]
	Hemosiderin deposition	0	0	10[d]	10[d]	10[d]	10[d]
	Extramedullary hematopoiesis	0	0	0	10[d]	10[d]	10[d]
	Capsule hyperplasia	0	0	0	0	6[d]	10[d]
Liver	Hemosiderin deposition	0	0	0	10[d]	10[d]	10[d]
	Centrilobular hydropic degeneration	0	0	0	1	10[d]	10[d]
	Single cell necrosis	0	0	0	8[d]	9[d]	10[d]
	Centrilobular hypertrophy	0	0	0	0	2	10[d]

[a]Matsumoto et al., 2006a
[b]Number of rats with lesions; $n = 10$/gender/group
[c]Significantly different from control ($p < 0.05$) by Fisher Exact test
[d]Significantly different from control ($p < 0.01$) by Fisher Exact test

Table 3. Selected Hematology and Clinical Chemistry Parameters in BDF_1 Mice Exposed to Dietary *o*-Chloronitrobenzene for 13 Weeks[a]

Parameter	Dietary Concentration (ppm)					
	0	78	313	1250	2500	5000
Males mg/kg-day	**0**	**10.4**	**43.6**	**170.4**	**345.1**	**684.1**
RBC count (10^6/μL)	10.82 ± 0.27[b]	10.77 ± 0.32	10.58 ± 0.26	10.20 ± 0.11[e]	9.95 ± 0.26[e]	9.57 ± 0.20[e]
Hgb (g/dL)	15.5 ± 0.3	15.5 ± 0.5	15.2 ± 0.3	14.9 ± 0.2[e]	14.6 ± 0.3[e]	–[c]
Hct (%)	48.7 ± 1.1	48.7 ± 1.4	48.3 ± 0.8	47.0 ± 0.7[e]	45.2 ± 1.3[e]	42.5 ± 1.1[e]
MCV (fL)	45.0 ± 0.6	45.2 ± 0.7	45.6 ± 0.7	46.0 ± 0.5[e]	45.5 ± 0.5	44.4 ± 0.9
Total bilirubin (mg/dL)	0.18 ± 0.03	0.19 ± 0.07	0.18 ± 0.05	0.17 ± 0.01	0.18 ± 0.02	0.20 ± 0.02
AST (IU/L)	46 ± 5	44 ± 7	44 ± 6	37 ± 6[d]	38 ± 5	52 ± 12
ALT (IU/L)	19 ± 5	19 ± 3	25 ± 6	26 ± 4	39 ± 8[e]	65 ± 14[e]
Females mg/kg-day	**0**	**12.2**	**49.5**	**196.5**	**400.3**	**762.5**
RBC count (10^6/μL)	10.60 ± 0.25	10.81 ± 0.35	10.59 ± 0.27	10.17 ± 0.18[e]	9.90 ± 0.25[e]	9.71 ± 0.20[e]
Hgb (g/dL)	15.6 ± 0.4	15.8 ± 0.4	15.7 ± 0.5	15.0 ± 0.3[d]	14.7 ± 0.4[e]	–[c]
Hct (%)	47.7 ± 1.0	49.1 ± 1.6	48.4 ± 1.1	47.0 ± 0.6	45.0 ± 1.0[e]	44.1 ± 0.9[e]
MCV (fL)	45.0 ± 0.5	45.4 ± 0.7	45.8 ± 0.5[d]	46.2 ± 0.7[e]	45.5 ± 0.6	45.5 ± 0.7
Total bilirubin (mg/dL)	0.18 ± 0.02	0.17 ± 0.02	0.17 ± 0.01	0.17 ± 0.03	0.20 ± 0.05	0.25 ± 0.07[e]
AST (IU/L)	56 ± 8	54 ± 14	54 ± 8	51 ± 13	54 ± 9	71 ± 32
ALT (IU/L)	20 ± 2	21 ± 4	22 ± 2	32 ± 7[d]	51 ± 12[e]	65 ± 39[e]

[a]Matsumoto et al., 2006a
[b]Means ± SD, n = 10 mice/gender/group in all groups, except males fed 313 or 1250 ppm (9 mice/group)
[c]Hgb data not available due to error in sample processing
[d]Significantly different from control ($p \leq 0.05$) by Dunnett test
[e]Significantly different from control ($p \leq 0.01$) by Dunnett test

Relative spleen and liver weights increased linearly with dose in male and female mice (Matsumoto et al., 2006a). The study authors presented the data graphically and no information on statistical significance is included in the study report. Based on visual inspection of graphs, it is estimated that the increase in relative spleen weights in males and females in the 5000-ppm group are approximately 2.5- and 3.5-fold greater than the control, respectively; it is estimated that the increase in relative liver weights in males and females in the 5000-ppm group is approximately 3-fold greater than the control. Results of the histopathological examination show that dietary exposure to *o*-chloronitrobenzene produced effects to the spleen and liver (see Table 4). The findings are consistent with accelerated RBC destruction (hemolytic anemia) and compensatory erythropoiesis to maintain erythrocyte mass and with hepatotoxicity. The study authors did not observe a treatment-related increase in the incidence of erythropoiesis in bone marrow in either the male or female mice (data not reported). In the spleens of male and female mice, the study authors observed a deposition of hemosiderin at concentrations ≥313 ppm and congestion and increased extramedullary hematopoiesis at concentrations ≥1250 ppm. In the livers, (Matsumoto et al., 2006a) observed the hemosiderin deposition in males and females fed diets containing ≥1250 ppm and centrilobular hypertrophy in males and females at concentrations ≥313 and 1250 ppm, respectively. The study authors observed centrilobular

nuclear enlargement with atypia (cell enlargement, varying nuclear size and shape, and coarse chromatin in the nucleus) in males treated with ≥313 ppm and females treated with 1250 ppm.

Table 4. Incidence of Spleen and Liver Lesions in Groups of 10 BDF_1 Mice Exposed to Dietary *o*-Chloronitrobenzene for 13 Weeks[a]

Organ	Lesion	Dietary Concentration (ppm)					
		0	78	313	1250	2500	5000
Males	**mg/kg-day**	**0**	**10.4**	**43.6**	**170**	**345**	**684**
Spleen	Congestion	0[b]	0	0	7[d]	10[d]	10[d]
	Hemosiderin deposition	0	0	5[c]	10[d]	10[d]	10[d]
	Extramedullary hematopoiesis	0	0	0	5[c]	10[d]	10[d]
Liver	Hemosiderin deposition	0	0	0	9[d]	10[d]	10[d]
	Centrilobular nuclear enlargement with atypia	0	0	10[d]	9[d]	10[d]	10[d]
	Centrilobular hypertrophy	0	0	6[d]	10[d]	10[d]	10[d]
Females	**mg/kg-day**	**0**	**12.2**	**49.5**	**196.5**	**400.3**	**762.5**
Spleen	Congestion	0	0	0	10[d]	10[d]	10[d]
	Hemosiderin deposition	0	0	5[c]	10[d]	10[d]	10[d]
	Extramedullary hematopoiesis	0	0	0	6[d]	10[d]	10[d]
Liver	Hemosiderin deposition	0	0	0	10[d]	10[d]	10[d]
	Centrilobular nuclear enlargement with atypia	0	0	1	10[d]	10[d]	10[d]
	Centrilobular hypertrophy	0	0	0	10[d]	10[d]	10[d]

[a]Matsumoto et al., 2006a
[b]Number of mice with lesions; $n = 10$/gender/group
[c]Significantly different from control ($p \leq 0.05$) by Fisher Exact test
[d]Significantly different from control ($p \leq 0.01$) by Fisher Exact test

Based on significant increases in the incidence of hemosiderin deposition in the spleens of male and female mice and of hepatic centrilobular nuclear enlargement and hypertrophy in male mice, Matsumoto et al. (2006a) identified subchronic NOAEL and LOAEL values of 78 and 313 ppm (43.6 and 170.4 mg/kg-day, respectively, in males and 49.5 and 196.5 mg/kg-day, respectively, in females) for dietary exposure of mice to *o*-chloronitrobenzene for 13 weeks.

Chronic Exposure—Matsumoto et al. (2006b) investigated the chronic toxicity and carcinogenicity of *o*-chloronitrobenzene in 2-year feeding studies in F344 rats and BDF_1 mice. The study authors fed the rats (50/gender/group) diets containing 0, 80, 400 or 2000 ppm *o*-chloronitrobenzene and the mice (50/gender/group) diets containing 0, 100, 500 or 2500 ppm *o*-chloronitrobenzene (>99% purity). Based on weekly (from week 0 to week 14) and monthly (from week 14 to study completion) records of food consumption and mean body weights, the study authors calculated the daily doses of *o*-chloronitrobenzene to be 0, 4, 19, or 99 mg/kg-day and 0, 4, 22, or 117 mg/kg-day for male and female rats, respectively, and 0, 11, 54, or 329 mg/kg-day and 0, 14, 69, or 396 mg/kg-day in male and female mice (Matsumoto et al.,

2006b). The study authors observed the animals daily for mortality and clinical signs. They recorded food consumption and body weights weekly from the beginning of treatment to week 14 and monthly thereafter. Blood samples were collected from all animals surviving to the end of treatment and analyzed for hematology (RBC count, Hgb, Hct, MCV, MCH, platelet count, reticulocyte count, and MetHgb) and clinical chemistry (total bilirubin, AST, ALT, lactate dehydrogenase [LDH], γ-glutamyl transpeptidase [γ-GTP], creatinine, and blood urea nitrogen [BUN]). Due to early mortality, the study authors did not obtain blood samples from the male rats fed 2000 ppm *o*-chloronitrobenzene. Matsumoto et al. (2006b) conducted necropsies on all animals; they also recorded organ weights and performed histopathological examinations on comprehensive tissues.

All male rats treated with diets containing 2000 ppm died before the end of the 2-year treatment period, with survival significantly decreased after ≥76 weeks of treatment (Most deaths occurred between treatment weeks 70 and 100 (data reported graphically). Matsumoto et al. (2006b) reported chronic progressive nephropathy as the cause of death in 47/50 males in the 2000-ppm group; causes of death were not reported for the 3 remaining male rats. Survival rates of males fed diets containing 80 or 400 ppm were 80 and 78%, respectively, and they were similar to controls (80%). Survival rates in female rats treated with 80, 400 and 2000 ppm *o*-chloronitrobenzene were 84, 90, and 78%, respectively, and they were similar to controls (82%). No information on clinical signs was reported for male or female rats. Terminal body weights were significantly decreased in males in the 400-ppm group (10% decrease; $p \leq 0.01$) and in females in the 2000-ppm group (18% decrease; $p \leq 0.01$); no treatment-related effects on body weight were observed in males treated with 80 ppm or females treated with 80 or 400 ppm. Food consumption is not different from the controls in any *o*-chloronitrobenzene group, except for males in the 2000-ppm group (data not reported).

Changes in hematology parameters in male and female rats (see Table 5) generally were consistent with treatment-induced anemia associated with elevated methemoglobin (Matsumoto et al., 2006b). Decreases in MCV and MCH were observed in males in the 80- and 400-ppm *o*-chloronitrobenzene groups. Although RBC counts were not decreased in any *o*-chloronitrobenzene group, Hgb and Hct were decreased in the 400-ppm group. Methemoglobin and platelet counts were significantly increased in males in the 400-ppm group. In female rats, changes in hematological parameters were observed at dietary concentrations ≥400 ppm. Decreases were observed in Hgb and MCH in the 400- and 2000-ppm groups and in RBC count and Hct in the 2000-ppm group. Increases in platelet count and methemoglobin were observed in the 400- and 2000-ppm groups and in reticulocyte count in the 2000-ppm group. Increases in clinical chemistry parameters indicated that exposure to dietary *o*-chloronitrobenzene produced hepatotoxicity and nephrotoxicity (see Table 5). In certain groups of male rats, significant increases in γ-GTP (≥80 ppm), creatinine (400 ppm) and BUN (400 ppm) were observed. However, LDH, a serum enzyme marker for tissue damage (including hemolytic anemia), was significantly decreased in males fed 400 ppm. No treatment-related effects were observed for total bilirubin, AST, or ALT in male rats. In female rats, significant increases were observed in total bilirubin (2000 ppm), ALT (2000 ppm), γ-GTP (≥400 ppm), creatinine (2000 ppm), and BUN (≥400 ppm); significant decreases were observed in LDH (≥400 ppm).

Table 5. Selected Hematology and Clinical Chemistry Parameters in F344 Rats Exposed to Dietary *o*-Chloronitrobenzene for 2 Years[a]

Parameter	Dietary Concentration (ppm)			
	0	80	400	2000
Males mg/kg-day	**0**	**4**	**19**	**99**
Number examined	***39***	***40***	***39***	***0***
RBC count (10^6/µL)	7.44 ± 1.94[b]	8.30 ± 1.24[d]	7.42 ± 1.18	–[c]
Hgb (g/dL)	12.9 ± 3.5	14.0 ± 2.1	12.3 ± 1.8[e]	–[c]
Hct (%)	37.1 ± 8.6	40.4 ± 4.8	35.5 ± 4.8[e]	–[c]
MCV (fL)	51.8 ± 10.0	49.2 ± 5.5[d]	48.1 ± 2.5[e]	–[c]
MCH (pg)	17.6 ± 2.8	16.9 ± 1.4[d]	16.6 ± 1.0[e]	–[c]
Platelet count (10^3/µL)	786 ± 274	833 ± 277	949 ± 154[e]	–[c]
Reticulocyte count (%)	5.7 ± 6.8	3.6 ± 2.8	3.4 ± 1.6	–[c]
MetHgb (%)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2[e]	–[c]
LDH (IU/L)	254 ± 296	176 ± 96	166 ± 90[d]	–[c]
γ-GTP (IU/L)	6 ± 3	13 ± 6[e]	35 ± 23[e]	–[c]
Creatinine (mg/dL)	0.6 ± 0.1	0.6 ± 0.1	0.9 ± 0.3[e]	–[c]
BUN (mg/dL)	18.0 ± 4.1	19.4 ± 3.2	43.9 ± 31.7[e]	–[c]
Females mg/kg-day	**0**	**4**	**22**	**117**
Number examined	***41***	***42***	***45***	***38***
RBC count (10^6/µL)	7.82 ± 0.86	7.61 ± 1.48	7.80 ± 0.55	6.71 ± 0.57[e]
Hgb (g/dL)	14.7 ± 1.6	14.2 ± 2.8	14.1 ± 1.2[e]	12.2 ± 0.9[e]
Hct (%)	40.7 ± 3.6	39.7 ± 6.1	39.8 ± 2.9	35.4 ± 2.4[e]
MCV (fL)	52.3 ± 2.9	53.8 ± 9.4	51.0 ± 1.7[e]	52.9 ± 2.3
MCH (pg)	18.9 ± 1.0	18.8 ± 1.3	18.1 ± 1.0[e]	18.2 ± 0.7[e]
Platelet count (10^3/µL)	612 ± 142	644 ± 154	725 ± 123[e]	730 ± 115[e]
Reticulocyte count (%)	3.3 ± 2.9	4.6 ± 8.1	3.1 ± 1.4	5.7 ± 1.4[d]
Methemoglobin (%)	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.2[d]	1.3 ± 0.4[d]
Total bilirubin (mg/dL)	0.15 ± 0.05	0.29 ± 0.96	0.14 ± 0.02	0.21 ± 0.03[e]
ALT (IU/L)	67 ± 70	73 ± 89	67 ± 28	134 ± 117[e]
LDH (IU/L)	268 ± 93	285 ± 229	200 ± 70[e]	189 ± 73[e]
γ-GTP (IU/L)	2 ± 2	3 ± 3	6 ± 3[e]	77 ± 25[e]
Creatinine (mg/dL)	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.2[d]
BUN (mg/dL)	16.1 ± 2.7	17.7 ± 4.8	18.0 ± 4.2[d]	28.8 ± 12.7[e]

[a]Matsumoto et al., 2006b
[b]Means ± SD
[c]Data could not be obtained due to early death of all animals
[d]Significantly different from control ($p \leq 0.05$) by Dunnett test
[e]Significantly different from control ($p \leq 0.01$) by Dunnett test

In male rats fed diets containing *o*-chloronitrobenzene, Matsumoto et al. (2006b) reported increases in relative kidney weights at 400 ppm and in relative liver weights at 80 and 400 ppm; no change in relative spleen weights were reported (see Table 6A). In female rats, relative liver and kidney weights were increased at dietary concentrations ≥400 ppm and relative spleen weights were increased at 2000 ppm (see Table 6B). Nonneoplastic lesions in the liver, kidney and spleen were observed in male and female rats; see Tables 6A and 6B summarize the details on lesion type and incidence data. The hepatic lesions were primarily pre-neoplastic in nature. In male rats, the incidences of these lesions (acidophilic and basophilic cell foci and spongiosis hepatis) were significantly increased in the 400-ppm group; in females, the incidences of acidophilic foci were increased in the 400- and 2000-ppm groups and the incidences of other liver lesions (clear cell foci, single cell necrosis, centrilobular hydropic degeneration, and brown pigment deposition) were increased in the 2000-ppm group; the incidences of basophilic foci were decreased in all treatment groups, compared to control. The incidences of nonneoplastic lesions of the kidney (chronic progressive nephropathy, urothelial hyperplasia, and brown pigment deposition) were significantly increased in males (400-ppm group); the severity of chronic progressive nephropathy increased from "slight" in the controls to "severe" in the 2000-ppm group.

In female rats, renal lesions included chronic progressive nephropathy (≥80 ppm), brown pigment deposition (≥400 ppm), and urothelial hyperplasia (2000 ppm); the severity of chronic progressive nephropathy increased from "slight" in controls to "moderate"-to-"marked" in the 2000-ppm group. In female rat spleens, the incidences of extramedullary hematopoiesis (80 ppm), erythrocyte engorgement (400 ppm), and hemosiderin deposition (400 ppm) were increased in male rats; in females, lesions of the spleen included hemosiderin deposition (≥400 ppm), angiectasis (2000 ppm), erythrocyte engorgement (≥400 ppm), and extramedullary hematopoiesis (2000 ppm). Based on significant, dose-related increases in the incidence and (2006b) indentified a LOAEL of 80 ppm (4 mg/kg-day) for chronic dietary exposure to *o*-chloronitrobenzene; a NOAEL was not established.

Matsumoto et al. (2006b) reported neoplastic lesions in the livers and kidneys of male and female rats fed diets containing *o*-chloronitrobenzene (see Table 7). The study authors did not include data from the 2000-ppm group in the statistical analyses because this dietary concentration exceeded the maximum tolerated dose (MTD). They reported dose-dependent increases in the incidences of hepatocellular adenomas and carcinomas in male and female rats, as indicated by results of the Peto's trend test. Although the incidences of hepatocellular adenomas and carcinomas in male rats fed 400 ppm were not significantly increased compared to control in pairwise tests, incidence data for both tumor types exceeded the maximum tumor incidence in Japanese Bioassay Research Center (JBRC) historical controls. The incidences of renal adenomas and carcinomas in males were not increased relative to control and did not exhibit positive dose-response trends, although the incidence of renal carcinomas in males fed 2000 ppm exceeded the maximum tumor incidence in the historical controls. In female rats, hepatocellular carcinomas and adenomas exhibited a positive dose-response trend and the incidence of both tumor types exceeded the maximum incidence in JBRC historical controls. In females fed 2000 ppm, the incidence of hepatocellular adenomas was significantly increased compared to control. The incidences of renal adenomas and carcinomas in females were not increased relative to controls and did not exhibit a positive dose-response trend, although the incidence of renal adenomas in females fed 2000 ppm exceeded the maximum tumor incidence in historical controls. The study authors concluded that exposure of male and female rats to dietary *o*-chloronitrobenzene produced dose-dependent increases in hepatocellular adenomas and

carcinomas. They also concluded that, although the marginally increased incidences of renal cell tumors appeared to be related to treatment, a relationship to chronic progressive nephropathy could not be ruled out.

Table 6A. Relative Organ Weights and Incidence of Nonneoplastic Lesions of the Liver, Kidney and Spleen in Male F344 Rats Exposed to Dietary *o*-Chloronitrobenzene for 2 Years[a]

Parameter	Dietary Concentration (ppm)			
	0	80	400	2000
Males mg/kg-day	**0**	**4**	**19**	**99**
Number examined for organ weight	***40***	***40***	***39***	***0***
Relative liver weight (%)	2.990 ± 0.672[b]	3.184 ± 0.408[e]	4.424 ± 0.471[f]	–[c]
Relative kidney weight (%)	0.782 ± 0.273	0.769 ± 0.102	0.961 ± 0.180[f]	–[c]
Relative spleen weight (%)	0.551 ± 0.684	0.393 ± 0.605	0.306 ± 0.085	–[c]
Number examined for histopathology	***50***	***50***	***50***	***50***
Clear cell foci (liver)	9[d]	7	6	4[e]
Acidophilic cell foci (liver)	2	3	24[f]	7[g]
Basophilic cell foci (liver)	6	7	20[f]	1[g]
Spongiosis hepatis	4	8	35[f]	0[g]
Single cell necrosis (liver)	0	0	0	18[g]
Fatty change (liver)	0	1	0	16[g]
Centrilobular hydropic degeneration (liver)	0	0	0	48[g]
Deposit of brown pigment (liver)	0	0	0	50[g]
Atypical tubule hyperplasia (kidney)	0	1	1	6[g]
Chronic progressive nephropathy	43 (1.6)	48 (2.0)	49[e] (3.2)	50[g] (4.0)
Mineralization of cortex (kidney)	0	0	2	44[g]
Urothelial hyperplasia (kidney)	0	1	32[f]	48[g]
Deposit of brown pigment (kidney)	0	0	42[f]	49[g]
Capsule hyperplasia (spleen)	0	0	0	49[g]
Angiectasis (spleen)	0	0	0	16[g]
Engorgement of erythrocytes (spleen)	0	3	11[f]	3
Extramedullary hematopoiesis (spleen)	2	8[e]	2	2[g]
Hemosiderin deposition, more than moderate (spleen)	1	2	7[e]	28[g]

[a]Matsumoto et al., 2006b
[b]Means ± SD
[c]Measurement was not obtained due to early death of all animals
[d]Number of animals with lesion; ()=average grade (1 = slight, 2 = moderate, 3 = marked, 4 = severe)
[e]Significantly different from control ($p \leq 0.05$)
[f]Significantly different from control ($p \leq 0.01$)
[g]Data from male rats in the 2000-ppm group were not included in statistical analysis

Table 6B. Relative Organ Weights and Incidences of Nonneoplastic Lesions of the Liver, Kidney and Spleen in Female F344 Rats Exposed to Dietary *o*-Chloronitrobenzene for 2 Years[a]

Parameter	Dietary Concentration (ppm)			
	0	80	400	2000
Females mg/kg-day	**0**	**4**	**22**	**117**
Number examined for organ weight	***41***	***42***	***45***	***39***
Relative liver weight (%)	2.641 ± 0.416[b]	2.972 ± 0.664	3.571 ± 0.376[e]	7.314 ± 0.845[e]
Relative kidney weight (%)	0.705 ± 0.071	0.717 ± 0.116	0.785 ± 0.088[e]	1.257 ± 0.187[e]
Relative spleen weight (%)	0.324 ± 0.350	0.445 ± 0.925	0.255 ± 0.079	0.522 ± 0.159[e]
Number examined for histopathology	***50***	***50***	***50***	***50***
Clear cell foci (liver)	2[c]	1	2	11[e]
Acidophilic cell foci (liver)	0	0	8[e]	36[e]
Basophilic cell foci (liver)	29	22	9[e]	5[e]
Spongiosis hepatis	0	0	0	1
Single cell necrosis (liver)	0	0	1	6[d]
Fatty change (liver)	0	0	0	0
Centrilobular hydropic degeneration (liver)	0	0	0	41[e]
Deposit of brown pigment (liver)	0	0	0	44[e]
Atypical tubule hyperplasia (kidney)	0	0	0	5[d]
Chronic progressive nephropathy	20 (1.3)	33[e] (1.3)	45[e] (1.4)	49[e] (2.7)
Mineralization of cortex (kidney)	0	0	0	0
Urothelial hyperplasia (kidney)	0	0	0	7[e]
Deposit of brown pigment (kidney)	0	0	48[e]	49[e]
Capsule hyperplasia (spleen)	0	0	0	46[e]
Angiectasis (spleen)	0	0	0	5[d]
Engorgement of erythrocytes (spleen)	0	1	5[d]	27[e]
Extramedullary hematopoiesis (spleen)	15	9	16	28[e]
Hemosiderin deposition , more than moderate (spleen)	9	14	23[e]	12

[a]Matsumoto et al., 2006b
[b]Means ± SD
[c]Number of animals with lesion; ()=average grade (1 = slight, 2 = moderate, 3 = marked, 4 = severe)
[d]Significantly different from control ($p \leq 0.05$)
[e]Significantly different from control ($p \leq 0.01$)

Table 7. Neoplastic Lesions in F344 Rats Exposed to Dietary *o*-Chloronitrobenzene for 2 Years[a]

Organ	Lesion Type	Dietary Concentration (ppm)			
		0	80	400	2000
Males	**mg/kg-day**	**0**	**4**	**19**	**99**
Liver	Hepatocellular adenoma	2[b,d]	3	7[f]	1[g]
	Hepatocellular carcinoma	0[e]	0	3[f]	1[g]
Kidney	Renal cell adenoma	0	1	0	1[g]
	Renal cell carcinoma	0	0	0	4[f,g]
Females	**mg/kg-day**	**0**	**4**	**22**	**117**
Liver	Hepatocellular adenoma	0[e]	0	2	20[c,f]
	Hepatocellular carcinoma	0[e]	0	0	4[f]
Kidney	Renal cell adenoma	0	0	0	2[f]
	Renal cell carcinoma	0	0	0	0

[a]Matsumoto et al., 2006b
[b]Number of animals with lesions, $n = 50$ rats/gender/group
[c]Significantly different from control ($p \leq 0.01$), Fisher's exact test
[d]Significant trend ($p \leq 0.05$), Peto's test
[e]Significant trend ($p \leq 0.01$), Peto's test
[f]Exceeds maximum tumor incidence in JBRC historical controls (historical data not reported)
[g]Data from male rats in the 2000-ppm group were not included in statistical analysis

Survival rates were significantly reduced in male and female mice exposed to dietary *o*-chloronitrobenzene (Matsumoto et al., 2006b). Survival rates of male mice were significantly decreased in the 500-ppm (34% survival at study termination) and 2500-ppm (16% survival at study termination) groups from treatment weeks 73 and 92, respectively, through the end of treatment; the terminal survival rate of male mice in the 100-ppm group (70% survival) was similar to controls (70% survival). Most deaths in the 500- and 2500-ppm groups occurred between treatment weeks 70 to 100 (data presented graphically) and were considered "causally related" to malignant liver tumors. The survival rate of female mice was significantly decreased in the 2500-ppm group from treatment week 71 through the end of treatment (10% survival at study termination). Most deaths occurred between treatment weeks 70 and 100 (data presented graphically) and were attributed to malignant liver tumors. Survival rates for females in the 100- and 500-ppm groups were 68 and 52% respectively, and they were not significantly different from the controls (58% survival). No additional information regarding causes of death or clinical signs in males and females in any treatment group was reported. Terminal body weights were significantly decreased by 22 and 40% in male mice fed 500 and 2500 ppm, respectively; terminal body weights in 100-ppm males were similar to controls. In female mice, terminal body weights were significantly decreased by 12 and 29% in the 500 and 2500-ppm groups, respectively, but were similar to control in the 100-ppm group. Food consumption was not different from control in any *o*-chloronitrobenzene group.

Reticulocytes were significantly increased in male and female mice fed diets with *o*-chloronitrobenzene concentrations ≥500 ppm (see Table 8), but no treatment-related changes were observed in RBC counts, platelet counts, Hgb, Hct, MCV, or MCH (Matsumoto et al.,

2006b). No data on methemoglobin were reported. Increases in clinical chemistry parameters indicated that exposure to dietary *o*-chloronitrobenzene produced hepatotoxicity and nephrotoxicity (see Table 8). Dose-dependent increases were observed in total bilirubin, AST, ALT, LDH, and γ-GTP in male and female mice in the 500- and 2500-ppm groups; BUN was significantly increased in the 2500-ppm females.

Table 8. Selected Hematology and Clinical Chemistry Parameters in BCF_1 Mice Exposed to Dietary *o*-Chloronitrobenzene for 2 Years[a]

Parameter	Dietary Concentration (ppm)			
	0	100	500	2500
Males mg/kg-day	**0**	**11**	**54**	**329**
Number examined for hematology	***33***	***33***	***14***	***8***
Reticulocyte count (%)	2.4 ± 1.6[b]	2.8 ± 2.3	9.4 ± 12.7[d]	8.0 ± 5.4[d]
Number examined for clinical chemistry	***34***	***34***	***14***	***8***
Total bilirubin (mg/dL)	0.15 ± 0.07	0.14 ± 0.03	0.29 ± 0.26[c]	0.38 ± 0.20[d]
AST (IU/L)	306 ± 787	156 ± 209	549 ± 590[d]	3136 ± 3412[d]
ALT (IU/L)	234 ± 579	120 ± 173	610 ± 759	2400 ± 2502[d]
LDH (IU/L)	929 ± 2145	495 ± 693	7530 ± 10481[d]	10515 ± 10479[d]
γ-GTP (IU/L)	2 ± 1	1 ± 1	3 ± 2[c]	74 ± 29[d]
BUN (mg/dL)	20.6 ± 3.0	21.3 ± 3.8	27.5 ± 22.7	21.0 ± 4.3
Females mg/kg-day	**0**	**14**	**69**	**396**
Number examined for hematology	***29***	***34***	***25***	***4***
Reticulocyte count (%)	4.7 ± 8.0[b]	3.1 ± 2.7	5.2 ± 3.9[d]	5.3 ± 1.0[c]
Number examined for clinical chemistry	***29***	***34***	***26***	***4***
Total bilirubin (mg/dL)	0.14 ± 0.03	0.16 ± 0.07	0.24 ± 0.16[d]	0.58 ± 0.12[d]
AST (IU/L)	94 ± 45	105 ± 122	449 ± 824[d]	1432 ± 796[d]
ALT (IU/L)	36 ± 27	51 ± 62	480 ± 816[d]	2115 ± 779[d]
LDH (IU/L)	409 ± 395	393 ± 528	2078 ± 4212[d]	6228 ± 2802[d]
γ-GTP (IU/L)	1 ± 1	1 ± 1	5 ± 8[d]	250 ± 30[d]
BUN (mg/dL)	17.5 ± 5.2	15.2 ± 3.0	21.3 ± 9.8	35.5 ± 15.1[c]

[a]Matsumoto et al., 2006b
[b]Means ± SD
[c]Significantly different from control ($p \leq 0.05$) by Dunnett test
[d]Significantly different from control ($p \leq 0.01$) by Dunnett test

Dose-dependent increases in the relative weights of liver, kidney, and spleen were observed in male mice fed diets containing 500 and 2500 ppm *o*-chloronitrobenzene (see Table 9) (Matsumoto et al., 2006b). In female mice, dose-dependent increases were observed in relative weights of liver and kidney in the 500- and 2500-ppm groups and in relative weights of spleens in the 500-ppm group. No increases were observed for relative organ weights in males and females in the 100-ppm group compared to control. Incidence data for nonneoplastic lesions and details on lesion types are summarized in Table 9. Lesions of the liver, centrilobular

hypertrophy, and nuclear enlargement were pre-neoplastic in nature; hemosiderin deposition in kidneys and spleens, and hematopoiesis in spleens were consistent with methemoglobin-induced hemolytic anemia and compensatory hematopoiesis. In male mice, dose-dependent increases in hepatocellular centrilobular hypertrophy (≥100 ppm) and hepatocellular centrilobular nuclear enlargement (≥500 ppm) were observed; hemosiderin deposition in kidneys was increased (≥500 ppm); and hemosiderin deposition (≥100 ppm) and extramedullary hematopoiesis (≥500 ppm) of the spleen were increased. In female mice, lesions included centrilobular hypertrophy of the liver (≥500 ppm), hemosiderin deposition of the kidneys (2500 ppm), and hemosiderin deposition and extramedullary hematopoiesis of the spleen (≥500 ppm). Based on significant increases in the incidences in male mice of hemosiderin deposition in the spleen and centrilobular hypertrophy of the liver, a LOAEL of 100 ppm (11 mg/kg-day) was identified for chronic dietary exposure to *o*-chloronitrobenzene; a NOAEL was not established.

Dose-dependent increases in the incidences of hepatocellular adenomas, hepatocellular carcinomas, and hepatoblastomas were observed in male and female mice fed diets containing *o*-chloronitrobenzene (see Table 10) (Matsumoto et al., 2006b). In males, the incidences of hepatocellular adenoma (≥100 ppm), hepatocellular carcinoma (2500 ppm), and hepatoblastoma (≥500 ppm) were increased compared to concurrent and historical controls. The three tumor types exhibited dose-dependent increases, as indicated by positive Peto's trend tests. In females, the incidences of hepatocellular adenoma (≥100 ppm), hepatocellular carcinoma (≥500 ppm), and hepatoblastoma (≥500 ppm) were increased compared to concurrent and historical controls. The three tumor types exhibited dose-dependent increases, as indicated by positive Peto's trend tests. In male and female mice fed diets containing 2500 ppm *o*-chloronitrobenzene, 41 and 69% of total malignant liver tumors metastasized, predominantly to lung, followed by bone marrow, peritoneum, and pancreas.

Table 9. Relative Organ Weights and Nonneoplastic Lesions of the Liver, Kidney and Spleen in BCF_1 Mice Exposed to Dietary *o*-Chloronitrobenzene for 2 Years[a]

Parameter	Dietary Concentration (ppm)			
	0	100	500	2500
Males mg/kg-day	**0**	**11**	**54**	**329**
Number examined for organ weight	***35***	***35***	***17***	***8***
Relative liver weight (%)	4.682 ± 3.366	5.101 ± 2.348	12.890 ± 6.586[e]	28.286 ± 4.120[e]
Relative kidney weight (%)	1.281 ± 0.222	1.510 ± 0.841	1.650 ± 0.180[e]	1.936 ± 0.160[e]
Relative spleen weight (%)	0.247 ± 0.205	0.308 ± 0.299	0.737 ± 0.888[e]	0.393 ± 0.158[d]
Number examined for nonneoplastic lesions	***50***	***50***	***50***	***50***
Centrilobular hypertrophy (liver)	0[c]	32[e]	42[e]	42[e]
Centrilobular nuclear enlargement (liver)	0	0	18[e]	6[d]
Hemosiderin deposition (kidney)	1	3	26[e]	32[e]
Extramedullary hematopoiesis (spleen)	18	14	37[e]	39[e]
Hemosiderin deposition , more than moderate (spleen)	9	20[d]	21[e]	40[e]
Females mg/kg-day	**0**	**14**	**69**	**396**
Number examined for organ weight	***29***	***34***	***26***	***5***
Relative liver weight (%)	4.147 ± 1.235	4.614 ± 2.557	12.174 ± 7.567[e]	33.269 ± 3.223[e]
Relative kidney weight (%)	1.220 ± 0.279	1.221 ± 0.291	1.569 ± 0.533[e]	1.721 ± 0.142[e]
Relative spleen weight (%)	0.477 ± 0.386	0.616 ± 0.883	0.874 ± 0.901[d]	0.550 ± 0.226
Number examined for nonneoplastic lesions	***50***	***50***	***50***	***50***
Centrilobular hypertrophy (liver)	0	0	29[e]	37[e]
Centrilobular nuclear enlargement (liver)	0	0	0	0
Hemosiderin deposition (kidney)	0	0	4	17[e]
Extramedullary hematopoiesis (spleen)	23	13	34[d]	43[e]
Hemosiderin deposition , more than moderate (spleen)	17	23	27[d]	45[e]

[a]Matsumoto et al., 2006b
[b]Means ± SD
[c]Number of animals with lesion
[d]Significantly different from control ($p \leq 0.05$)
[e]Significantly different from control ($p \leq 0.01$)

Table 10. Incidence of Neoplastic Lesions in Groups of 50 BDF_1 Mice Exposed to Dietary *o*-Chloronitrobenzene for 2 Years[a]

Organ	Lesion Type	Dietary Concentration (ppm)			
		0	100	500	2500
Males	**mg/kg-day**	**0**	**11**	**54**	**329**
Liver	Hepatocellular adenoma	19[b,e]	29[c,f]	30[c,f]	34[d,f]
	Hepatocellular carcinoma	15[e]	14	20	35[d,f]
	Hepatoblastoma	1[e]	6[f]	35[d,f]	44[d,f]
Females	**mg/kg-day**	**0**	**14**	**69**	**396**
Liver	Hepatocellular adenoma	8[e]	22[d,f]	48[d,f]	38[d,f]
	Hepatocellular carcinoma	0[e]	3	14[d,f]	48[d,f]
	Hepatoblastoma	0[e]	0	9[d,f]	28[d,f]

[a]Matsumoto et al., 2006b
[b]Number of animals with lesions, $n = 50$ rats/gender/group
[c]Significantly different from control ($p \leq 0.05$), Fisher's exact test
[d]Significantly different from control ($p \leq 0.05$), Fisher's exact test
[e]Significant trend ($p \leq 0.01$), Peto's trend test
[f]Exceeds maximum tumor incidence in JBRC historical controls (historical data not reported)

In a carcinogenicity study of 21 aromatic compounds, Weisburger et al. (1978) fed groups of male CD rats (25/group) diets containing 0, 1000, or 2000 ppm of *o*-chloronitrobenzene (97-99% purity) for 6 months, then diets containing 0, 500, or 1000 ppm for 12 months, followed by control diets for an observation period of 6 months. The time-weighted-average dietary concentrations over the 18-month exposure period were 0, 667, and 1333 ppm. Using reference values for male body weight and food consumption in U.S. EPA (1988), the doses for the 18-month exposures were calculated to be 0, 46, and 92 mg/kg-day. Rats that died during the first 6 month were discarded without necropsy. The remaining rats were given a complete gross necropsy; histopathological examination was performed on all gross lesions and tissue masses, and selected organs (lung, liver, spleen, kidney, adrenal glands, heart, urinary bladder, stomach, intestines, pituitaries, and reproductive organs). Information on survival, body weight gain, nonneoplastic lesions, numbers of tumors per animal, or the incidences of individual tumor types was not reported. The total number of animals bearing multiple tumors (1/22, 7/22, and 1/19 in the matched control, low- and high-dose groups, respectively) was significantly elevated in the low-dose animals compared to matched controls and "pooled" controls (14/111 male rats). "Pooled" controls included all 111 control male rats used during the period in which the 21 chemicals were tested. The tumor types usually included pituitary adenoma with adrenal tumor, thyroid adenocarcinoma, lymphosarcoma, cholangiocarcinoma of the liver or subcutaneous fibroma. Incidences of individual tumor types were not reported.

In this same study, Weisburger et al. (1978) fed groups of CD-1 mice (25/gender/group) diets containing 0, 3000, or 6000 ppm of *o*-chloronitrobenzene (97-99% purity) for 8 months, then diets containing 0, 1500, or 3000 ppm for 10 months, followed by the control diet for an observation period of 3 months. The time-weighted-average dietary concentrations during the 18-month exposure period were 0, 2167, and 4333 ppm. Using reference values for body weight and food consumption provided in U.S. EPA (1988), the average doses during the 18-month

exposure period were 0, 372, and 743 mg/kg-day for male mice and 0, 375, and 749 mg/kg-day for female mice. Mice that died during the first 6 months were discarded without necropsy. Remaining mice were given a complete gross necropsy; gross lesions, tissue masses, and selected organs (lung, liver, spleen, kidney, adrenal glands, heart, urinary bladder, stomach, intestines, and reproductive organs) were examined for histopathology. Information on survival, body weight gain, or nonneoplastic lesions was not reported. The incidences of hepatocellular carcinomas in male mice (3/18, 7/17, and 3/16 in matched control, low- and high-dose groups, respectively) were not significantly increased by treatment compared to matched controls; however, in the low-dose group, the incidence was significantly higher than pooled controls (7/99 male mice; see Table 11). The incidences of hepatocellular carcinoma in females (0/20, 5/22, and 5/19 for the matched control, low-, and high-dose groups, respectively) were significantly increased in both treatment groups compared to matched or pooled controls (1/102 female mice; see Table 11).

Table 11. Incidences of Liver Tumors in Male and Female CD-1 Mice Exposed to Dietary *o*-Chloronitrobenzene for 18 Months[a]

	Time-Weighted Average Dietary Doses (mg/kg-day)		
Gender	**0**	**372**	**743**
Males	3/18[b]	7/17[c]	3/16
Females	0/20	5/22[c,d]	5/19[c,d]

[a]Weisburger et al., 1978
[b]Number of animals with tumor/number of animals examined
[c]Significantly different from incidence in pooled controls (Males: 7/99; Females: 1/102), $p < 0.025$
[d]Significantly different from matched controls, $p < 0.05$

Developmental and Reproduction Studies—NTP (1993) evaluated the effects of *o*-chloronitrobenzene (>99% purity) on fertility and reproduction in Swiss CD-1 mice in a 2-week range-finding study and a 98-day continuous breeding study. In the 2-week study, groups of 8 mice/gender received 0, 20, 40, 80, 160, or 320 mg/kg-day of *o*-chloronitrobenzene in corn oil by gavage. Clinical signs, body weights, and water consumption data were recorded; frequency of observations was not reported. All mice in the 320-mg/kg-day group died or were sacrificed moribund during the first 2 days of dosing; other deaths in treatment and control groups were attributed to gavage trauma. Treatment had no effect on terminal body weights. Increased water consumption was observed during week 1 in females treated with 20 or 160 mg/kg-day and during week 2 in both genders treated with 40 mg/kg-day. Mice receiving 160 mg/kg-day appeared weak and inactive following dosing during week 1 and were slightly cyanotic but active following dosing during week 2. On the basis of these results, doses between 40 and 160 mg/kg-day were selected for the continuous breeding study.

In the continuous breeding study, NTP (1993) gavaged groups of 20 breeding pairs of Swiss CD-1 mice (F_0 generation) daily with 40, 80, or 160 mg/kg-day of *o*-chloronitrobenzene in corn oil for a 7-day pre-cohabitation period and a 98-day cohabitation period that produced 5 litters per pair. A control group of 40 breeding pairs was gavaged with corn oil only. The following reproductive endpoints were examined: number of litters/pair, number of live pups/litter, the proportion of pups born alive, the gender ratio of pups, and pup body weights. Endpoints assessed in adults included body weights recorded after each delivery and at termination, water consumption, and gross lesions at termination. Spleen weights were recorded

and blood samples for methemoglobin measurements were taken from 23 control and 21 high-dose F_0 mice. Following the continuous breeding of F_0 mice, the final litters (F_1) of the control and high-dose pairs were raised with the same treatment as the parents. After weaning of the F_1 mice, nonsiblings were housed for mating for 7 days and housed singly through delivery of F_2 pups; the same reproductive endpoints and adult body weights and water consumption were evaluated. In addition, sperm morphology and vaginal cytology evaluations were made for 12 days prior to necropsy of the F_1 mice. At necropsy of F_1 mice, weights were determined for livers, kidneys, testes, epididymides, prostates, seminal vesicles, and ovaries; blood samples were collected for methemoglobin analyses; and ovaries, testes, and epididymides were examined for histopathology.

No treatment-related deaths occurred among F_0 mice administered up to 160 mg/kg-day *o*-chloronitrobenzene by gavage (NTP, 1993). The only treatment-related clinical sign observed was inactivity of 160-mg/kg-day mice immediately following dosing, during the first 10 days of the study. F_0 mice administered 160 mg/kg-day had increased body weights (details not reported), increased absolute and relative spleen and liver weights (details not reported) and methemoglobin concentrations ranging from 8 to 12% (details for control mice not reported). No treatment-related effects on reproductive endpoints were observed in the F_0 mice. Male and female pups in the final F_1 mouse litters had significantly lower body weights at weaning. However, body weights at mating and terminal body weights of high-dose F_1 mice (the only treated F_1 animals that were raised) were significantly increased compared to control. However, terminal body weights were not reported. High-dose F_1 mice also had increased absolute and relative spleen and liver weights (details not reported) and methemoglobin concentrations exceeding 8% (details for control mice not reported); high-dose males had reduced relative seminal vesicle weights (details not reported). No reported fertility or reproductive parameters were affected by treatment in F_1 animals. In summary, the high dose of 160 mg/kg-day caused methemoglobinemia, increased spleen and liver weights, and reduced seminal vesicle weights in mice, but it caused no adverse effects on reproductive function. Since mid- and low-dose animals were not evaluated for methemoglobinemia or organ weights, it is not possible to determine the NOAEL or the lowest LOAEL for this study.

Monsanto Environmental Health Laboratory (MEHL) conducted a developmental toxicity study in Sprague-Dawley (Crl:CD® (SD)BR) rats (Monsanto, 1986). Groups of 25 mated females received 0, 25, 75, or 150 mg/kg-day of *o*-chloronitrobenzene by gavage in corn oil on gestational days (GD) 6–15. Rats were observed twice daily for mortality; each received a detailed physical examination for clinical signs on GD 0, 6–20, and just before scheduled sacrifice on GD 21. Body weights and food consumption were recorded on GD 0, 6, 10, 13, 16, and 21. At termination, all dams were subjected to a gross necropsy; the number and placement of live and dead fetuses, early and late resorptions, and the number of *corpora lutea* per ovary were recorded. Monsanto (1986) externally examined all live fetuses, determined genders and identified visceral or skeletal malformations and variations.

In rats treated with 150 mg/kg-day, severe toxicity and increased mortality (6/25 dead by GD 14) were observed; surviving females were sacrificed without necropsy or fetal examination (Monsanto, 1986). No cause of death was discovered in the post-mortem examination for the unscheduled deaths. One death occurred in the 75-mg/kg-day group on GD 9, but the cause of death was not determined. Body weight loss occurred during GD 6–10 in the 150-mg/kg-day group; a statistically nonsignificant reduction in body weight gain occurred in rats treated with 75 mg/kg-day during the same period. Changes in body weight were most likely related to

reduced food consumption in rats treated with 75 and 150 mg/kg during GD 6–10; body weight effects in 75-mg/kg-day rats were reversed later in gestation. Clinical signs in rats administered 150 mg/kg-day included urinary staining, cold and pale extremities, alopecia (baldness), piloerection (erection of hairs), and staining or encrustations on the face or forelimbs. An increase in alopecia and urinary staining was observed at 75 mg/kg-day, but no clinical signs were noted at 25 mg/kg-day. Treatment with 25 or 75 mg/kg-day had no effect on pregnancy rates, the mean number of live or dead fetuses, the number of late resorptions, total implantations, *corpora lutea*, or pre-implantation losses. A significant increase in early resorptions and corresponding post-implantation losses occurred in the 75-mg/kg-day group, but not in the 25-mg/kg-day group. Treatment with 25 or 75 mg/kg-day had no effect on fetal body weights or gender distributions. The total number of litters exhibiting external and skeletal malformations in the 25- and 75-mg/kg-day groups was similar to the control. An increase in the number of litters exhibiting the "cervical #7 rib" occurred in the 25- and 75-mg/kg-day groups, but it was statistically significant only at 75 mg/kg-day. NOAEL and LOAEL values of 25 and 75 mg/kg-day, respectively, were identified for maternal (clinical signs) and fetal (cervical #7 rib and early resorptions) toxicity in Sprague-Dawley (Crl:CD® (SD)BR) rats treated with *o*-chloronitrobenzene by gavage on GD 6–16.

Due to severe toxicity in rats treated with 150 mg/kg-day, a separate developmental toxicity experiment was conducted by the International Research and Development Corporation (IRDC) for Monsanto (1986). Groups of 25 mated Sprague Dawley (Crl:COBS®-CD®) female rats were gavaged with 0 or 100 mg/kg-day of *o*-chloronitrobenzene in corn oil on GD 6–15 using the same protocol described above for the MEHL study. On GD 20, one treated female died, but the cause of death was not determined. On GD 21, one control female delivered and was sacrificed and necropsied as scheduled; the pups received the same analyses as the fetal litters. Food consumption and body weights were reduced in the treated group during GD 6–10, but they were not significantly different from controls during the remaining period. This resulted in a slightly reduced mean body weight gains (~5% decrease) for the overall gestation period in treated animals compared to the controls. Treatment had no effect on clinical signs, gross necropsy findings, or uterine or fetal endpoints. In this study, 100 mg/kg-day could have been a freestanding LOAEL for maternal toxicity, however it was unclear whether the transiently reduced food intake and body weights in Sprague Dawley [Crl:COBS®-CD®] rats were meaningful adverse effects of treatment. However, this dose was a NOAEL for effects on fetal development.

Inhalation Exposure

Subchronic Exposure—The subchronic toxicity of airborne *o*-chloronitrobenzene has been examined in studies in rats (Haskell Laboratories, 1984; Nair et al., 1986; NTP, 1993; Travlos et al., 1996) and mice (NTP, 1993; Travlos et al., 1996). Haskell Laboratories (1984) conducted a 2-week study in Crl:CD (SD)BR rats. Groups of 16 male rats were exposed, head only, to 0.03, 0.16, and 0.53 mg/L of *o*-chloronitrobenzene (99.8% purity) "vapor and particulates," 6 hours/day, 5 days/week, for 2 weeks, followed by a 13-day recovery period; a control group was simultaneously exposed to air only. These exposure concentrations were equivalent to 30, 160, or 530 mg/m^3 or average daily exposures of 5.4, 29, or 95 mg/m^3. Animals were weighed and observed for mortality and clinical signs daily throughout the exposure and recovery periods. Urine and blood samples were obtained after 9 and 10 days of exposure, respectively, from 10 rats per group. Urine samples were analyzed for volume, color, sediment, osmolality, pH, occult blood, protein, sugar, bilirubin, acetone, and urobilinogen; blood samples were analyzed for hematology (RBC, leukocyte and platelet counts, differential

count of leukocytes, Hgb, MCV, Hct, MCH, MCHC, and MetHgb) and clinical chemistry (alkaline phosphatase [AP], ALT, AST, BUN, creatinine, total protein, and cholesterol). Necropsies and histopathological examinations of 23 tissues were performed on 5 rats per group after 10 days of exposure and 5 rats per group after the 13-day recovery period. Organs and tissues examined included the heart, kidneys, liver, lungs, spleen, testes, thymus, adrenal, brain cecum, colon, duodenum, epididymides, esophagus, eyes, ileum, jejunum, parathyroid, skin, sternum bone marrow, stomach, trachea, and thymic lymph nodes. The remaining 6 rats per group were designated for methemoglobin and hemoglobin measurements on alternate days during exposure and on day 13 of the recovery period.

In each of the mid- and high-exposure groups, one death occurred; the causes of death could not be determined (Haskell Laboratories, 1984). Body weights were significantly ($p \leq 0.05$) decreased by 7 % compared to controls in the low- and high-exposure groups at the end of the treatment period, but returned to control values within 2 days after the end of treatment; body weights in the mid-dose group were unaffected by treatment. Lung noise (rales) was observed in four rats during the first week of exposure at 160 mg/m^3, but in only one rat per group in the control and low exposure groups and in no rats in the high-exposure group. No other respiratory effects were reported. Cyanosis in six rats and hyperemia of the ears in one rat were observed in the high-exposure group. Significant hematological and clinical chemistry changes in rats exposed to 530 mg/m^3 were consistent with methemoglobinemia and possible hepatic damage (see Table 12). Methemoglobin was increased by almost 30-fold in rats exposed to 530 mg/m^3, with values returned to control concentrations at the end of the recovery period. RBC count, Hgb, and MCHC were significantly decreased and platelet count, MCV, and MCH were significantly increased in the 530-mg/m^3 group. No changes in hematological parameters were observed in rats exposed to 30 or 160 mg/m^3. In rats exposed to 530 mg/m^3, serum ALT, total protein, and BUN were slightly, but significantly, increased; cholesterol was increased in the 160- and 530-mg/m^{3}groups. No treatment-related effects on urinalysis parameters were observed.

Other than the transient increases in rales observed during the first week in mid-dose rats, Haskell Laboratories (1984) reported no other respiratory tract effects. Dose-related increases in liver and spleen weights and histopathological changes in the spleens of rats exposed to *o*-chloronitrobenzene were consistent with the development of methemoglobin-induced hemolytic anemia and mild hepatotoxicity (see Table 13). Mean absolute and relative spleen weights were significantly increased in high-exposure rats and mean absolute and relative liver weights were elevated in mid- and high-exposure rats. Histological changes occurred in the spleens (congestion in 1/10 rats and hemosiderosis in 4/10 rats) of rats exposed to 530 mg/m^3; no histopathological change in the spleens were observed in the control, 30-, or 160-mg/m^3 groups. Compound-related histological changes occurred in the liver (cellular lipid vacuolation and increased mitosis) in the 160- and 530-mg/m^3 groups. NOAEL and LOAEL values of 30 mg/m^3 (average daily concentration of 5.4 mg/m^3) and 160 mg/m^3 (average daily concentration of 29 mg/m^3), respectively, were identified for possible hepatotoxicity, based on increased absolute and relative liver weights and histological changes in male rats exposed to inhaled *o*-chloronitrobenzene for 2 weeks.

Table 12. Selected Hematology and Clinical Chemistry Parameters in Male CRL:CD(SD)BR Rats Exposed Head-Only to Airborne *o*-Chloronitrobenzene 6 hours/day, 5 days/week, for 2 Weeks[a]

Parameter	Exposure group (mg/m^3)			
	0	30	160	530
RBC count (10^6/µL)	6.93 ± 0.35[b]	7.02 ± 0.36	6.93 ± 0.39	5.89 ± 0.35[c]
Platelet count (10^3/µL)	885 ± 123	811 ± 103	959 ± 154	981 ± 70[c]
Hgb (g/dL)	15.8 ± 0.5	15.7 ± 0.5	15.5 ± 0.5	14.7 ± 0.4[c]
MCV (fL)	58 ± 2	57 ± 1	57 ± 2	67 ± 5[c]
MCH (pg)	23 ± 1	22 ± 1	22 ± 1	25 ± 1[c]
MCHC (g/dL)	39 ± 1	39 ± 2	39 ± 1	37 ± 1[c]
MetHgb (g %)	0.6	0.8	1.9	17.0[c]
ALT (IU)	34 ± 9	36 ± 3	35 ± 5	47 ± 13[c]
BUN (mg %)	17.6 ± 2.6	19.0 ± 3.7	19.0 ± 1.9	21.8 ± 2.1[c]
Total protein (g %)	5.7 ± 0.2	5.8 ± 0.1	5.9 ± 0.2	6.1 ± 0.3[c]
Cholesterol (mg %)	72 ± 5	67 ± 10	96 ± 18[b]	99 ± 13[c]

[a]Haskell Laboratories, 1984
[b]Means ± SD, or means (if SD not reported)
[c]Significantly different from control ($p \leq 0.05$)

Table 13. Absolute and Relative Organ Weights in Male Rats Exposed Head-Only to Airborne *o*-Chloronitrobenzene 6 hours/day, 5 days/week, for 2 Weeks[a]

Parameter	Exposure group (mg/m^3)			
	0	30	160	530
Absolute liver weight (g)[b]	10.690	10.80	12.428[c]	13.190[c]
Relative liver weight (%)[b]	3.778	3.833	4.443[c]	5.024[c]
Absolute spleen weight (g)[b]	0.544	0.448	0.566	0.868[c]
Relative spleen weight (%)[b]	0.192	0.171	0.202	0.329[c]

[a]Haskell Laboratories, 1984
[b]Means (SD or SE not reported)
[c]Significantly different from controls ($p \leq 0.05$)

Nair et al. (1986) exposed (whole body) groups of Sprague-Dawley rats (15/gender/group) to *o*-chloronitrobenzene vapor (99.71% purity) at measured concentrations of 0, 9.9, 30, and 59 mg/m^3 for 6 hours/day, 5 days/week, for 4 weeks. Rats were examined twice daily for signs of toxicity and mortality and given detailed physical examinations weekly; body weights were recorded weekly. All rats were given an ophthalmoscopic examination prior to initiation of exposure and just prior to termination of the study. After 2 weeks of exposure, methemoglobin concentrations were determined for 10 rats/gender/group. At the end of treatment, blood samples were obtained from 10 rats/gender/group and analyzed for hematology (RBC count, reticulocyte count, total and differential leukocyte count, RBC morphology, Hgb,

Hct, MetHgb, and clotting time) and clinical chemistry (ALT, AP, BUN, glucose, albumin, total protein, globulin, and electrolytes). At termination, all surviving animals were necropsied and organ weights were recorded for the brain, testes, kidneys, liver, lungs, and spleen. Microscopic examinations of gross lesions and sections from 37 tissues, including the thoracic cavity, trachea, and nasal turbinates, were conducted for 10 rats/gender from the control and high-exposure groups; spleens from the low- and mid-exposure rats also were examined for histopathology. No compound-related deaths, clinical signs, or effects on body weight, clinical chemistry, or ophthalmologic examination were observed (Nair et al., 1986). Effects on hematological parameters were consistent with treatment-induced methemoglobinemia and subsequent hemolytic anemia and compensatory hematopoiesis (see Table 14). Dose-dependent increases in methemoglobin were observed in males and females (statistically significant at $\geq$30 mg/m^3). Decreased RBC, Hgb, and Hct counts, and slightly increased reticulocyte counts were observed in males exposed to 59 mg/m^3 and in females exposed to $\geq$30 mg/m^3. Treatment-related increases in organ weights included the following: at $\geq$9.9 mg/m^3, relative liver weight in males; at $\geq$30 mg/m^3, absolute liver and spleen weights and relative kidney weights in both genders, absolute kidney weights in males and relative spleen weights in females; and at 59 mg/m^3, relative spleen weight in males (see Table 15). An exposure-related increase in the severity of hemosiderosis of the spleen (data presented graphically) was observed in both genders at $\geq$9.9 mg/m^3 and a slight increase in extramedullary hematopoiesis (details not reported) of the spleen was observed in both genders exposed to $\geq$30 mg/m^3. No treatment-related histopathological changes were observed in other tissues. Based on increased severity of hemosiderosis of the spleen in both genders of rats, a LOAEL of 9.9 mg/m^3 (average daily concentration of 1.8 mg/m^3) was identified; a NOAEL was not established.

Table 14. Selected Hematology Parameters in Sprague-Dawley Rats Exposed Whole-Body to Airborne *o*-Chloronitrobenzene 6 hours/day, 5 days/week, for 4 Weeks[a]

Parameter	Exposure group (mg/m^3)			
	0	9.9	30	59
Males				
RBC count (10^6/µL)	6.9 ± 0.5[b]	7.0 ± 0.3	7.1 ± 0.3	6.5 ± 0.5[c]
Reticulocyte count (% of RBC count)	2.3 ± 1.0	2.6 ± 0.7	2.2 ± 0.5	3.9 ± 1.6[d]
Hgb (g/dL)	15.6 ± 1.1	15.6 ± 0.9	15.5 ± 0.5	14.5 ± 0.8[d]
Hct (%)	43 ± 3	43 ± 3	42 ± 2	40 ± 3
MetHgb (g/dL)	0.3 ± 0.1	1.5 ± 0.4	3.2 ± 0.7[d]	5.7 ± 1.3[d]
Females				
RBC count (10^6/µL)	6.8 ± 0.3	6.7 ± 0.3	6.2 ± 0.2[d]	6.0 ± 0.3[d]
Reticulocyte count (% of RBC count)	2.2 ± 0.6	2.2 ± 0.7	3.0 ± 1.2	4.4 ± 1.4[d]
Hgb (g/dL)	15.3 ± 0.5	15.1 ± 0.5	14.1 ± 0.5[d]	13.7 ± 0.5[d]
Hct (%)	43 ± 2	43 ± 1	39 ± 2[d]	39 ± 2[d]
MetHgb (g/dL)	0.5 ± 0.2	1.5 ± 0.3	3.1 ± 0.3[d]	4.8 ± 0.6[d]

[a]Nair et al., 1986
[b]Means ± SD
[c]Significantly different from control ($p \leq 0.05$)
[d]Significantly different from control ($p \leq 0.01$)

Table 15. Selected Organ Weights in Sprague-Dawley Rats Exposed Whole-Body to Airborne *o*-Chloronitrobenzene 6 hours/day, 5 days/week, for 4 Weeks[a]

Parameter	Exposure group (mg/m^3)			
	0	9.9	30	59
Males				
Absolute liver weight (g)	12.9 ± 1.4[b]	14.2 ± 1.8	15.8 ± 1.6[d]	16.6 ± 2.2[d]
Relative liver weight (%)	3.6 ± 0.2	3.9 ± 0.4[c]	4.3 ± 0.4[d]	4.4 ± 0.3[d]
Absolute spleen weight (g)	0.68 ± 0.13	0.63 ± 0.08	0.78 ± 0.11[d]	0.89 ± 0.14[d]
Relative spleen weight (%)	0.19 ± 0.03	0.17 ± 0.02	0.21 ± 0.03	0.25 ± 0.04[d]
Absolute kidney weight (g)	2.4 ± 0.3	2.6 ± 0.3	2.7 ± 0.2[d]	2.7 ± 0.2[c]
Relative kidney weight (%)	6.7 ± 0.4	7.1 ± 0.6	7.5 ± 0.6[d]	7.4 ± 0.4[d]
Females				
Absolute liver weight (g)	8.4 ± 0.8	8.6 ± 0.8	9.4 ± 0.9[d]	10.2 ± 0.8[d]
Relative liver weight (%)	3.6 ± 0.2	3.7 ± 0.3	4.0 ± 0.2[d]	4.4 ± 0.3[c]
Absolute spleen weight (g)	0.49 ± 0.05	0.51 ± 0.08	0.64 ± 0.09[d]	0.59 ± 0.13[d]
Relative spleen weight (%)	0.21 ± 0.02	0.22 ± 0.04	0.28 ± 0.04[d]	0.26 ± 0.06[c]
Absolute kidney weight (g)	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.1	1.7 ± 0.2
Relative kidney weight (%)	6.8 ± 0.4	6.9 ± 0.4	7.3 ± 0.4[c]	7.3 ± 0.7[c]

[a]Nair et al., 1986
[b]Means ± SD
[c]Significantly different from control ($p \leq 0.05$)
[d]Significantly different from control ($p \leq 0.01$)

NTP (1993; Travlos et al., 1996) evaluated toxicity of airborne *o*-chloronitrobenzene in subchronic bioassays in rats and mice. Groups of F344/N rats (10/gender/group) were exposed, whole body, to 0, 7.2, 14.7, 28.7, 57, or 115 mg/m^3 *o*-chloronitrobenzene vapor, 6 hours/day, 5 days/week, for 13 weeks. Animals were observed twice daily for mortality and clinical signs. Body weights were recorded weekly and at termination. Blood samples were collected at the end of treatment and analyzed for hematology (MetHgb, Hgb, Hct, MCV, MCH, MCHC, RBC counts, leukocyte counts, platelet counts, lymphocyte counts, and RBC morphology) and clinical chemistry (ALT, AP, sorbitol dehydrogenase [SDH], total protein, albumin, globulin, and bile acids); an additional 10 rats/gender/group were designated for analysis of hematology and clinical chemistry at interim time points (days 4 and 23). At the end of the treatment period, animals in the 0-, 28.7-, 57-, and 115-mg/m^3 groups were examined for reproductive parameters:spermatid counts and morphology, spermatozoan motility, and weights of left reproductive organs in males and vaginal cytology and estrous cycle duration and stage lengths in females. At termination, all surviving rats were necropsied for gross lesions and organ weights of hearts, right kidneys, livers, lungs, spleens, right testes, and thymus were recorded. Histopathological examinations of 32 tissues were performed on all rats in the control and highest-exposure groups and all rats that died prior to study termination. 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, tissue masses, heart, kidneys, large intestine (cecum, colon, rectum), larynx, liver, lungs, lymph nodes (bronchial, mandibular, mediastinal, and mesenteric), mammary gland, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland,

preputial glands, prostate gland, salivary gland, seminal vesicle, small intestine (duodenum, jejunum, ileum), spinal cord/sciatic nerve (if neurologic signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thigh muscle, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only) . Gross lesions in rats and mice from all lower exposure groups also were examined.

There were no exposure-related effects on survival, body weight, or the incidences of clinical signs in rats (NTP, 1993; Travlos et al., 1996). Exposure-related hematological changes indicative of methemoglobinemia and subsequent anemia and compensatory erythropoiesis were observed in higher-exposure groups after only 4 days of exposure and in the lower-exposure groups as the study progressed. Significantly increased methemoglobin was the most sensitive effect, observed at $\geq$14.7 mg/m^3 in both genders on day 4 and at $\geq$7.2 mg/m^3 in males on day 23 and in both genders at 13 weeks (see Table 16). In general, methemoglobinemia increased in severity in all exposed groups during the course of the study. Reductions in hemoglobin and hematocrit were observed at $\geq$57 mg/m^3 on day 4 and at $\geq$14.7 mg/m^3 after 13 weeks.

Erythrocyte counts were reduced in males at $\geq$57 mg/m^3 on day 4 and at $\geq$28.7 mg/m^3 after 13 weeks; significant erythrocyte reductions were not observed in females until week 13, at which time all groups except for the 14.7-mg/m^3 group were affected. Abnormal erythrocyte morphology (nucleated erythrocytes) was observed in males exposed to $\geq$57 mg/m^3 and females exposed to 115 mg/m^3. Elevated ALT and SDH were observed in males and females at various time points (see Table 16). The most pronounced changes occurred in males and females in the 115 mg/m^3-groups on Day 4. However, at the end of the treatment period, SDH was increased only in males exposed to 115 mg/m^3 and females exposed to $\geq$57 mg/m^3, and ALT was either similar to or slightly decreased compared to control. Increased bile acid concentrations, indicative of cholestasis, occurred on day 4 in male rats exposed to $\geq$14.7 mg/m^3 and in females exposed to 115 mg/m^3; at the end of treatment, bile acid concentrations were increased only in males in the 14.7-mg/m^3 group.

Organ weight changes were observed in the livers, spleens, and kidneys of rats exposed to inhaled *o*-chloronitrobenzene (NTP, 1993; Travlos et al., 1996) (see Table 17). In males, absolute and relative liver weights were increased at $\geq$7.2 and 14.7 mg/m^3 respectively; absolute and relative spleen weights were increased at 115 mg/m^3; and relative kidney weights were increased at $\geq$57 mg/m^3. In females, absolute and relative liver weights were increased at $\geq$14.7 and 28.7 mg/m^3 respectively; absolute and relative spleen weights were increased at $\geq$28.7 mg/m^3; and absolute and relative kidney weights were increased at $\geq$115 mg/m^3. At 115 mg/m^3, there were significant reductions in spermatid counts and in the absolute weights of the left cauda epididymis in males; no reproductive effects were observed in females. The following increased dose-related histopathological lesions were observed (see Table 17):

- At $\geq$7.2 mg/m^3, hyperplasia of nasal respiratory epithelium in both genders
- At $\geq$28.7 mg/m^3, regeneration and pigmentation of kidney tubules in males
- At $\geq$57 mg/m^3, cytoplasmic basophilia of the liver in both genders and pigmentation of the kidney tubules in females

Table 16. Selected Hematology and Clinical Chemistry Parameters in F344 Rats Exposed Whole-Body to Airborne *o*-Chloronitrobenzene 6 hours/day, 5 days/week, for 13 weeks[a]

Parameter	Time	Exposure Group (mg/m^3)					
		0	7.2	14.7	28.7	57	115
Males							
MetHgb (g/dL)	4 days	0.09 ± 0.01[b]	0.11 ± 0.01	0.12 ± 0.01[d]	0.17 ± 0.01[d]	0.24 ± 0.01[d]	1.14 ± 0.09[d]
	23 days	0.14 ± 0.02	0.15 ± 0.01[c]	0.19 ± 0.01[d]	0.23 ± 0.02[d]	0.41 ± 0.02[d]	0.55 ± 0.01[d]
	13 weeks	0.15 ± 0.01	0.21 ± 0.01[d]	0.26 ± 0.01[d]	0.36 ± 0.01[d]	0.55 ± 0.01[d]	0.87 ± 0.02[d]
ALT (IU/L)	4 days	44 ± 1	48 ± 1	50 ± 2[c]	53 ± 2[d]	57 ± 3[d]	212 ± 19[d]
	23 days	35 ± 1	33 ± 1	36 ± 1	33 ± 1	39 ± 2	47 ± 3[d]
	13 weeks	62 ± 5	57 ± 3	60 ± 4	54 ± 3	49 ± 1[c]	55 ± 2
SDH (IU/L)	4 days	10 ± 1	12 ± 0[d]	14 ± 1[d]	15 ± 1[d]	16 ± 1[d]	34 ± 4[d]
	23 days	9 ± 0	9 ± 0	10 ± 0	10 ± 0	14 ± 1[d]	16 ± 1[d]
	13 weeks	20 ± 2	20 ± 2	21 ± 3	21 ± 3	22 ± 1	28 ± 2[d]
Females							
MetHgb (g/dL)	4 days	0.09 ± 0.01	0.11 ± 0.01	0.14 ± 0.01[d]	0.17 ± 0.01[d]	0.25 ± 0.01[d]	1.04 ± 0.08[d]
	23 days	0.16 ± 0.01	0.18 ± 0.01	0.22 ± 0.01[d]	0.30 ± 0.01[d]	0.47 ± 0.02[d]	0.71 ± 0.04[d]
	13 weeks	0.19 ± 0.01	0.22 ± 0.01[d]	0.28 ± 0.01[d]	0.35 ± 0.01[d]	0.51 ± 0.01[d]	0.79 ± 0.03[d]
ALT (IU/L)	4 days	41 ± 2	40 ± 1	38 ± 1	35 ± 1[c]	38 ± 1	137 ± 20
	23 days	33 ± 1	33 ± 1	36 ± 1	36 ± 4	35 ± 1	45 ± 3[d]
	13 weeks	58 ± 4	50 ± 2	58 ± 3	53 ± 2	47 ± 2[c]	41 ± 2[d]
SDH (IU/L)	4 days	9 ± 1	9 ± 0	9 ± 1	9 ± 0	12 ± 1[d]	20 ± 1[d]
	23 days	11 ± 1	12 ± 1	12 ± 1	11 ± 1	14 ± 1[d]	23 ± 2[d]
	13 weeks	19 ± 1	18 ± 1	23 ± 1	22 ± 1	24 ± 1[c]	26 ± 2[d]

[a]NTP, 1993; Travlos et al., 1996
[b]Means ± SE
[c]Significantly different from control ($p \leq 0.05$)
[d]Significantly different from control ($p \leq 0.01$)

Table 17. Organ Weights and Incidence of Lesions in Groups of 10 F344 Rats Exposed Whole-Body to Airborne *o*-Chloronitrobenzene 6 hours/day, 5 days/week, for 13 Weeks[a]

Parameter	Exposure group (mg/m^3)					
	0	**7.2**	**14.7**	**28.7**	**57**	**115**
Males						
Absolute liver weight (g)	11.820 ± 0.356[b]	13.102 ± 0.401[d]	12.987 ± 0.356[d]	14.126 ± 0.576[e]	14.160 ± 0.398[e]	15.543 ± 0.346[e]
Relative liver weight (mg/g body weight)	35.33 ± 0.58	37.48 ± 1.26	37.81 ± 0.48[d]	40.40 ± 0.84[e]	41.67 ± 0.61[e]	48.07 ± 0.68[e]
Absolute spleen weight (g)	0.631 ± 0.016	0.680 ± 0.014	0.650 ± 0.020	0.659 ± 0.018	0.669 ± 0.012	0.753 ± 0.018[e]
Relative spleen weight (mg/g body weight)	1.89 ± 0.03	1.93 ± 0.04	1.89 ± 0.03	1.89 ± 0.02	1.97 ± 0.02	2.33 ± 0.04[e]
Absolute kidney weight (g)	1.124 ± 0.032	1.153 ± 0.063	1.184 ± 0.032	1.242 ± 0.032	1.218 ± 0.029	1.225 ± 0.029
Relative kidney weight (mg/g body weight)	3.36 ± 0.04	3.28 ± 0.15	3.45 ± 0.04	3.56 ± 0.04	3.59 ± 0.04[d]	3.79 ± 0.04[e]
Cytoplasm basophilia (liver)	0[c]	0	0	0	10 (1.0)	10 (1.0)
Congestion (spleen)	8 (1.4)	9 (1.6)	10 (1.5)	10 (1.6)	10 (1.4)	10 (1.9)
Tubule pigment (kidney)	0	0	0	4 (1.0)	4 (1.0)	10 (1.0)
Tubule regeneration (kidney)	1 (1.0)	4 (1.0)	6 (1.0)	9 (1.2)	8 (1.0)	10 (1.3)
Respiratory epithelial hyperplasia (nasal cavity)	4 (1.0)	9 (1.0)	8 (1.0)	10 (1.2)	10 (1.4)	9 (1.1)
Females						
Absolute liver weight (g)	6.658 ± 0.191[b]	6.751 ± 0.124	7.397 ± 0.203[d]	7.610 ± 0.221[e]	8.594 ± 0.273[e]	9.773 ± 0.362[e]
Relative liver weight (mg/g body weight)	34.86 ± 0.75	36.00 ± 0.68	36.91 ± 0.61	39.29 ± 0.72[e]	43.73 ± 0.54[e]	50.67 ± 1.07[e]
Absolute spleen weight (g)	0.422 ± 0.006	0.420 ± 0.009	0.440 ± 0.012	0.463 ± 0.008[d]	0.468 ± 0.010[e]	0.538 ± 0.020[e]

Table 17. Organ Weights and Incidence of Lesions in Groups of 10 F344 Rats Exposed Whole-Body to Airborne *o*-Chloronitrobenzene 6 hours/day, 5 days/week, for 13 Weeks[a]

Parameter	Exposure group (mg/m^3)					
	0	**7.2**	**14.7**	**28.7**	**57**	**115**
Relative spleen weight (mg/g body weight)	2.21 ± 0.04	2.24 ± 0.04	2.20 ± 0.03	2.40 ± 0.05[d]	2.39 ± 0.05[d]	2.80 ± 0.09[e]
Absolute kidney weight (g)	0.641 ± 0.009	0.641 ± 0.014	0.720 ± 0.054	0.666 ± 0.017	0.696 ± 0.016	0.739 ± 0.028[d]
Relative kidney weight (mg/g body weight)	3.36 ± 0.02	3.42 ± 0.08	3.57 ± 0.21	3.44 ± 0.07	3.55 ± 0.05	3.83 ± 0.09[e]
Cytoplasm basophilia (liver)	0[c]	0	0	0	6 (1.0)	8 (1.0)
Congestion (spleen)	4 (1.0)	4 (1.0)	7 (1.0)	3 (1.0)	9 (1.0)	10 (1.0)
Tubule pigment (kidney)	0	0	0	0	10 (1.0)	10 (3.0)
Tubule regeneration (kidney)	0	0	0	0	0	0
Respiratory epithelial hyperplasia (nasal cavity)	0	8 (1.0)	9 (1.0)	10 (1.1)	9 (1.1)	6 (1.2)

[a]NTP, 1993; Travlos et al., 1996
[b]Means ± SE
[c]Number of rats with lesion; ()=average severity; 1=minimal, 2 = mild, 3 = moderate, 4 = marked; *n* = 10/group; statistical analysis not conducted by NTP (1993) for nonneoplastic lesions
[d]Significantly different from control ($p \leq 0.05$)
[e]Significantly different from control ($p \leq 0.01$)

The severity of spleen congestion was slightly increased in 115-mg/m^3 males and the incidence increased in females at this exposure concentration. The lowest exposure concentration of 1.1 ppm (7.2 mg/m^3 or average daily concentration of 1.3 mg/m^3) of *o*-chloronitrobenzene was a LOAEL for methemoglobinemia and nasal respiratory epithelium hyperplasia in male and female rats exposed to *o*-chloronitrobenzene for 13 weeks; a NOAEL was not identified.

In the NTP (1993; Travlos et al., 1996) subchronic inhalation study in mice, groups of B6C3F$_1$ mice (10/gender/group) were exposed to 0, 7.2, 14.7, 28.7, 57, or 115 mg/m^3 *o*-chloronitrobenzene vapor, 6 hours/day, 5 days/week, for 13 weeks. Mice were analyzed for the same systemic and reproductive endpoints as rats, except that no hematology or clinical chemistry data were collected. During treatment week 12, 2 of 10 males exposed to 115 mg/m^3 died (cause of death not reported); no other mortalities occurred. Treatment with *o*-chloronitrobenzene had no adverse effect on the incidence of clinical signs or on body weight, although body weight was increased by 6 to 12% (statistical significance not reported) compared to controls in females in all *o*-chloronitrobenzene groups. Absolute and relative liver and kidney weights generally increased with dose in male and female mice and were statistically significant at exposures as low as 7.2 mg/m^3 (absolute liver weights in female mice). However, the changes from control were small (less than 10%) at $\leq$28.7 mg/m^3 (see Table 18). Lesions of the liver and spleen were observed at concentrations $\geq$57 mg/m^3 (see Table 18). Hepatic changes were observed at $\geq$57 mg/m^3 (cytomegaly in males) and at 115 mg/m^3 (necrosis, mineralization, and chronic inflammation). Severe sinusoidal congestion associated with hepatocellular degeneration and necrosis was noted in the two male mice that died prematurely (115 mg/m^3), although study reports did not indicate whether this might have been the cause of observed deaths. Hematopoietic cell proliferation of the spleen occurred in males at 115 mg/m^3 and in females at $\geq$57 mg/m^3. Sperm motility was significantly decreased in males exposed to $\geq$28.7 mg/m^3 (lower exposure groups were not evaluated). No significant changes were observed in female reproductive parameters. A LOAEL of 28.7 mg/m^3 (average daily concentration of 5.1 mg/m^3) was identified for reduced sperm motility in male mice exposed to *o*-chloronitrobenzene for 13 weeks; since lower exposure groups were not examined for this endpoint, a NOAEL could not be determined. Although NTP (1993) reported statistically significant liver and kidney weight increases at lower exposures (Table 18) these increases were very small and, thus, had questionable biological significance.

Chronic Exposure—No studies regarding the effects of chronic inhalation exposure of animals to *o*-chloronitrobenzene were located.

Developmental and Reproduction Studies—No studies regarding the reproductive or developmental effects of airborne exposure of animals to *o*-chloronitrobenzene were located.

Table 18. Organ Weights and Incidence of Lesions in Groups of 10 B6C3F$_1$ Mice Exposed Whole-Body to Airborne *o*-Chloronitrobenzene 6 hours/day, 5 days/week, for 13 Weeks[a]

Parameter	Exposure group (mg/m^3)					
	0	7.2	14.7	28.7	57	115
Males						
Absolute liver weight (g)	1.713 ± 0.039[b]	1.835 ± 0.057	1.816 ± 0.059	1.794 ± 0.044	2.025 ± 0.066[e]	2.279 ± 0.103[e]
Relative liver weight (mg/g body weight)	46.75 ± 0.76	49.30 ± 1.33	50.24 ± 1.22[d]	51.46 ± 0.82[e]	54.92 ± 0.83[e]	63.51 ± 1.46[e]
Absolute kidney weight (g)	0.318 ± 0.010	0.335 ± 0.007	0.344 ± 0.007[d]	0.348 ± 0.012[d]	0.353 ± 0.005[e]	0.354 ± 0.009[e]
Relative kidney weight (mg/g body weight)	8.69 ± 0.27	9.01 ± 0.16	9.54 ± 0.21[d]	10.00 ± 0.34[e]	9.62 ± 0.21[e]	9.91 ± 0.22[e]
Cytomegaly (liver)	0[c]	0	0	0	10 (1.0)	10 (1.7)
Necrosis/mineralization (liver)	0	0	0	0	0	8 (1.9)
Sinusoidal congestion (liver)	0	0	0	0	0	2 (4.0)
Chronic inflammation (liver)	0	0	0	0	0	5 (2.0)
Hematopoietic cell proliferation (spleen)	0	0	0	0	0	4 (1.0)
Females						
Absolute liver weight (g)	1.472 ± 0.040	1.625 ± 0.042[d]	1.768 ± 0.050[e]	1.723 ± 0.052[e]	1.933 ± 0.048[e]	2.234 ± 0.065[e]
Relative liver weight (mg/g body weight)	49.00 ± 1.25	49.93 ± 0.97	51.32 ± 0.86	52.31 ± 1.24	56.00 ± 0.97[e]	66.37 ± 2.15[e]
Absolute kidney weight (g)	0.205 ± 0.008	0.223 ± 0.005	0.239 ± 0.003[e]	0.231 ± 0.004[e]	0.244 ± 0.012[e]	0.237 ± 0.003[e]
Relative kidney weight (mg/g body weight)	6.81 ± 0.24	6.87 ± 0.18	6.97 ± 0.20	7.04 ± 0.21	7.09 ± 0.38	7.07 ± 0.24
Cytomegaly (liver)	0	0	0	0	0	10 (2.0)
Necrosis/mineralization (liver)	0	0	0	0	1 (1.0)	4 (1.2)
Sinusoidal congestion (liver)	0	0	0	0	0	0
Chronic inflammation (liver)	0	0	0	0	0	1 (1.0)
Hematopoietic cell proliferation (spleen)	3 (1.0)	0	0	0	10 (1.0)	8 (1.2)

[a]NTP, 1993; Travlos et al., 1996
[b]Means ± SE, n = 10/group (8/group for high dose males)
[c]Number of mice with lesion; ()=average severity; 1 = minimal, 2 = mild, 3 = moderate, 4 = marked; n = 10/group; statistical analysis not conducted by NTP (1993) for nonneoplastic lesions
[d]Significantly different from control ($p \leq 0.05$)
[e]Significantly different from control ($p \leq 0.01$)

Other Studies

Toxicokinetics Studies

NTP (1993) investigated the toxicokinetics of oral and dermal *o*-chloronitrobenzene in male F344/N rats. Results of oral administration studies indicated that *o*-chloronitrobenzene was well absorbed from the gastrointestinal tract and rapidly metabolized and excreted. Following administration of single oral doses (2–200 mg/kg), 86–93% of the administered radioactivity was recovered in urine and feces within 4 days. Urinary excretion was the main route of elimination. At 72 hours, 60–73% of the absorbed dose of *o*-chloronitrobenzene was recovered in urine and 7–28.2% in feces. A minor biliary excretory component also was identified. Up to 23 metabolites of *o*-chloronitrobenzene were identified in urine. After 72 hours, ≤3.9% of the administered dose was retained in tissues. Fat, the liver, and the kidneys retained the highest amounts (≤1.18, 2.25, and 0.50% of the administered dose, respectively). NTP (1993) did not estimate an elimination half-life ($t_{½}$) for *o*-chloronitrobenzene. The results of the dermal studies indicated that *o*-chloronitrobenzene was dermally absorbed (NTP, 1993). Following dermal application of 0.65, 6.5, or 65 mg/kg of radio-labeled *o*-chloronitrobenzene to male F344/N rats under nonocclusive conditions, absorption ranged from 33 and 40% of the administered dose within 72 hours. Results of an *in vitro* study of hepatocytes isolated from male F344 rats showed that *o*-chloronitrobenzene was converted to 2-chloroaniline and 2-chloroaniline-*N*-glucuronide. Reduction of the nitro group to the amine was dependent upon microsomal cytochrome P-450.

Genotoxicity Studies

Genotoxicity assays of *o*-chloronitrobenzene primarily were negative in bacteria, but they were positive more often in mammalian systems; positive results appeared to be associated with bioactivation. *o*-Chloronitrobenzene did not induce mutations in *Salmonella typhimurium* strains TA1530, TA1535, TA1537, TA1532, TA1950, TA1978, or G46 with or without activation; in strains TA1538 and TA98NR with activation; or in strain TA100 without activation (U.S. EPA, 1985; NTP, 1993; IARC, 1996). Conflicting results were observed in strain TA98 with or without activation, in TA100 with activation, and in TA1538 without activation (U.S. EPA, 1985; NTP, 1993; IARC, 1996). *o*-Chloronitrobenzene gave negative results in the *Escherichia coli* SOS chromotest (IARC, 1996). *o*-Chloronitrobenzene induced sister chromatid exchanges (SCE) and chromosomal aberrations in Chinese hamster ovary cells (CHO) *in vitro* (NTP, 1993; IARC, 1996). Heritable gender-linked lethal mutations were not induced in *Drosophila melanogaster* administered *o*-chloronitrobenzene in feed to larvae or adults, or when injected into adults (IARC, 1996). When intraperitoneally injected into Swiss CD-1 mice, *o*-chloronitrobenzene induced DNA single-strand breaks in the liver, kidney, and brain (IARC, 1996). However, following administration of *o*-chloronitrobenzene by gavage to female Wister rats, no DNA adducts were found in the liver (Jones and Sabbioni, 2003).

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RfDs FOR *o*-CHLORONITROBENZENE

Subchronic p-RfD

No studies investigating the effects of subchronic oral exposure of humans to *o*-chloronitrobenzene were identified. Subchronic toxicity studies in animals included a 13-week dietary study in rats and mice (Matsumoto et al., 2006a), a 5-week dietary study in mice (Bayer, 1991, 1993) that was available only in a secondary source (OECD/SIDS, 2001), a continuous breeding (98-day) study in mice (NTP, 1993), and a developmental study in rats (Monsanto,

1986). Based on the available data, adverse hematological effects including methemoglobin-induced anemia resulting in compensatory erythropoiesis in bone marrow and spleen, and hepatotoxicity evidenced by elevated hepatic serum enzymes and nonneoplastic lesions were identified as possible critical effects for derivation of the subchronic p-RfD.

Oral exposure of rats for 13 weeks and mice for 5 or 13 weeks to *o*-chloronitrobenzene resulted in anemia and compensatory erythropoiesis (Matsumoto et al., 2006a; Bayer, 1991, 1993). Although blood methemoglobin concentrations were not measured in the 13-week study in rats and mice (Matsumoto et al., 2006a), exposure of animals to chloronitrobenzene compounds has been shown to increase methemoglobin concentrations in several animal models (NTP, 1993). Furthermore, elevated methemoglobin was reported in male and female mice exposed to dietary *o*-chloronitrobenzene for 5 weeks (Bayer, 1991, 1993), in the 2-generation reproduction study in mice (NTP, 1993), and in the chronic dietary study in rats (Matsumoto et al., 2006b). Thus, methemoglobinemia was considered to be the most likely cause of *o*-chloronitrobenzene-induced anemia observed in rats (see Table 1) and mice (see Table 3) exposed to dietary *o*-chloronitrobenzene for 13 weeks.

Methemoglobin differs from normal hemoglobin in that the oxygen-carrying ferrous iron of the heme groups is oxidized to ferric iron. Ferric iron cannot bind oxygen, resulting in functional anemia and tissue hypoxia. In addition, ferric iron oxidizes the globin groups of hemoglobin, leading to denatured hemoglobin molecules that precipitate within the erythrocyte to form Heinz bodies. Due to the presence of Heinz bodies and precipitated hemoglobin, erythrocytes are prematurely removed from blood by the spleen, resulting in hemolytic anemia (NTP, 1993). As a compensatory response to methemoglobin-induced functional and hemolytic anemia, hematopoiesis is increased. Effects observed in subchronic animal studies on hematological parameters (decreased Hgb, Hct, and RBC count), spleen (congestion, hemosiderin deposition and extramedullary hematopoiesis), and bone marrow (increased erythropoiesis) were consistent with *o*-chloronitrobenzene-induced methemoglobinemia, followed by anemia and compensatory erythropoiesis. Female rats were more sensitive than male rats or male or female mice to the adverse hematological effects of *o*-chloronitrobenzene, based on the LOAEL of 4.0 mg/kg-day; a NOAEL was not established (Matsumoto et al., 2006a).

Dietary exposure of rats and mice to *o*-chloronitrobenzene also produced hepatotoxic effects, characterized by necrosis and degeneration, release of hepatocellular enzymes into serum, and increased liver weight (Matsumoto et al., 2006a). Hepatotoxicity was observed in mice exposed to dietary *o*-chloronitrobenzene for 5 or 13 weeks and rats exposed for 13 weeks (Matsumoto et al., 2006a; Bayer, 1991, 1993). Lesions in mice were the most sensitive adverse liver effect, observed at doses of 43.6 and 49.5 mg/kg-day in males and females, respectively (see Table 4; Matsumoto et al., 2006a). Since *o*-chloronitrobenzene-induced anemia in female rats was a more sensitive effect, hepatotoxicity was not considered as the critical effect for derivation of the subchronic p-RfD.

Results of the 2-generation reproduction study in mice revealed no adverse effects on fertility or reproduction at doses up to 160 mg/kg-day (NTP, 1993). Minor fetal skeletal variations (7^{th} cervical rib) and early resorptions were observed in the developmental study in Sprague Dawley (Crl:CD[SD]BR) rats, with NOAEL and LOAEL values of 25 and 75 mg/kg-day, respectively. Developmental effects were observed in the presence of clinical signs of maternal toxicity (Monsanto, 1986). However, effects were not reproduced in a

different strain of Sprague-Dawley rats (Crl:COBS-CD) at doses up to 100 mg/kg-day (Monsanto, 1986).

Anemia in female rats (Matsumoto et al., 2006a) was identified as the most sensitive effect of subchronic oral exposure to *o*-chloronitrobenzene and, therefore, was selected as the basis for the subchronic p-RfD. To determine the point of departure (POD) for derivation of the subchronic p-RfD, data sets for RBC counts and hemoglobin concentration (see Table 19) were evaluated for suitability for benchmark dose (BMD) modeling using the U.S. EPA Benchmark Dose Software (BMDS) version 1.4.1b (U.S. EPA, 2000). If data could be modeled by BMD analysis, the POD would be identified as the lowest BMDL (e.g., lower confidence limit [95%] on the benchmark dose) for the best fitting model. If data were not suitable for BMD analysis, the POD would be based on a NOAEL/LOAEL approach. Initial results of BMD analysis showed that data sets for RBC count and hemoglobin concentration in female rats were not suitable for BMD modeling (Appendix B). However, using the unrestricted power model provided a good fit, calculating a $BMDL_{1SD}$ of 1.7 mg/kg-day.

Table 19. Hematology Parameters in Female F344 Rats Exposed to Dietary *o*-Chloronitrobenzene for 13 Weeks[a]

Parameter	Dose (mg/kg-day)					
	0	**4.0**	**15.5**	**63.9**	**133.3**	**249.3**
RBC count ($10^6/\mu L$)	8.84 ± 0.22	8.54 ± 0.16[c]	8.48 ± 0.14[c]	8.03 ± 0.21[c]	7.67 ± 0.23[c]	7.20 ± 0.18[c]
Hgb (g/dL)	16.1 ± 0.4	15.5 ± 0.3[c]	15.3 ± 0.3[c]	14.3 ± 0.4[c]	13.7 ± 0.4[c]	13.4 ± 0.4[c]

[a]Matsumoto et al., 2006a
[b]Means ± SD, n = 10/group
[c]Significantly different from control ($p \leq 0.01$)

An uncertainty factor of 100, composed of the following, was applied to the subchronic $BMDL_{1SD}$ POD of 1.7 mg/kg-day (Matsumoto et al., 2006a) for decreased RBC count and decreased hemoglobin in female rats.

- A UF of 10 for intraspecies differences was applied to account for potentially susceptible individuals in the absence of quantitative information on the variability of response in humans. For example, individuals with pre-existing anemia or hematopoietic disorders might be more susceptible to oral *o*-chloronitrobenzene.
- A UF of 10 was applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans.
- No UF for database deficiencies was applied. Well designed oral subchronic and chronic studies in two species are available, as well as developmental and reproduction studies that demonstrated developmental effects only at higher doses (75 mg/kg-day) and in the presence of maternal toxicity. So, additional data seemed unlikely to identify a lower subchronic oral POD.

Subchronic p-RfD = $BMDL_{1SD} \div UF$
= 1.7 mg/kg-day ÷ 100
= 0.017 mg/kg-day or **2×10^{-2} mg/kg-day**

Confidence in the critical effect was high. Anemia has been observed in rats and mice in subchronic oral toxicity studies, and it has been observed in humans exposed via inhalation to other isomers of chloronitrobenzene (ACGIH, 2001a). Confidence in the key study was high. Matsumoto et al. (2006a) assessed comprehensive endpoints in an appropriate number of animals. Although a NOAEL was not identified, the data exhibited a clear dose-response trend that was successfully modeled with unrestricted Power model in the BMD software, providing a good fit to the data. Confidence in the database was high. Subchronic and chronic oral toxicity studies had been conducted in rats and mice; in addition, an oral 2-generation reproduction study and an oral developmental study were available. High confidence in the subchronic p-RfD resulted.

Chronic p-RfD

No studies investigating the effects of chronic oral exposure of humans to *o*-chloronitrobenzene were identified. Matsumoto et al. (2006b) investigated the chronic toxicity of *o*-chloronitrobenzene in a 2-year dietary study in rats and mice. Weisburger et al. (1978) conducted an 18-month carcinogenicity study in rats; however, noncancer endpoints were not evaluated. Results of the Matsumoto et al. (2006b) study showed that chronic exposure of rats and mice to *o*-chloronitrobenzene produced adverse effects to the following organ systems:

- Hematological system: methemoglobin-induced anemia resulting in compensatory erythropoiesis.
- Liver: elevated serum enzymes, centrilobular hypertrophy, pre-neoplastic lesions, and liver tumors.
- Kidney: chronic progressive nephropathy.
- Spleen: nonneoplastic lesions secondary to anemia and compensatory erythropoiesis.

Holder (1999) suggested that the progressive nephropathy from exposure to nitrobenzenes probably resulted from resorption of chlorobenzene residues and acetylated amines, from reduction of the *o*-chloronitrobenzene nitro group.

Dietary exposure of rats for 2 years to *o*-chloronitrobenzene resulted in anemia, possibly secondary to elevated methemoglobin (Matsumoto et al., 2006b). Elevated methemoglobin and decreased blood hemoglobin were observed in male rats at a dose of 19 mg/kg-day and in female rats at doses $\geq$22 mg/kg-day (see Table 5). Although methemoglobinemia and anemia were not observed in mice exposed chronically to *o*-chloronitrobenzene, reticulocyte counts (indicative of erythropoiesis) were elevated in males and females exposed to $\geq$54 and $\geq$69 mg/kg-day, respectively (see Table 8). Furthermore, elevated methemoglobin was reported in male and female mice exposed to dietary *o*-chloronitrobenzene, 1120 mg/kg-day in males and 1310 mg/kg-day in females, for 5 weeks (Bayer, 1991, 1993) and in the 2-generation reproduction study in mice at 160 mg/kg-day, the lowest dose evaluated for methemoglobin (NTP, 1993). Based on the available data, rats appeared more sensitive than mice to adverse hematological effects of *o*-chloronitrobenzene. The lowest doses at which *o*-chloronitrobenzene-induced hematological effects (elevated methemoglobin and decreased hemoglobin) occurred were 19 and 22 mg/kg-day in male and female rats, respectively (see Table 5; Matsumoto et al., 2006b). Although MCV and MCH were slightly decreased in male rats exposed to 4 mg/kg-day (see Table 5), the biological significance of these observations was uncertain, since changes in MCV and MCH were not accompanied by decreases in RBC counts or blood hemoglobin.

Nonneoplastic lesions of the spleen observed in rats (hemosiderin deposition, capsule hyperplasia, and extramedullary hematopoiesis) and mice (hemosiderin deposition and extramedullary hematopoiesis) were consistent with hemolytic anemia and compensatory erythropoiesis (Matsumoto et al., 2006b). In rats, statistically significant increases in hemosiderin deposition were observed at ≥19 and ≥22 mg/kg-day in male and female rats, respectively (see Tables 6A and 6B). In mice, increases in the incidence of hemosiderin deposition were observed at all doses (≥11 mg/kg-day) in males and ≥69 mg/kg-day in females (see Table 9). The lowest dose at which histopathological effects of the spleen were observed was 11 mg/kg-day in male mice. Based on the results of the Matsumoto et al. (2006b) study, hemosiderin deposition of the spleen in male mice is considered to have been the most sensitive effect associated with *o*-chloronitrobenzene-induced anemia and compensatory erythropoiesis, and it is subsequently used as the critical effect for derivation of the chronic p-RfD.

The renal pathology observed in the Matsumoto et al. (2006b) study was similar to the chronic progressive nephropathy (CPN) that spontaneously occurred in rats and has been characterized by glomerular dysfunction and tubular proliferative regeneration (Hard and Khan, 2004; Matsumoto et al., 2006b). Therefore, the effect of *o*-chloronitrobenzene on the kidney has been described as an exacerbation of spontaneous CPN (Matsumoto et al., 2006b). Dose-dependent increases in the incidences of CPN were observed in rats, but not mice, fed diets containing *o*-chloronitrobenzene for 2 years (Matsumoto et al., 2006b). The incidences of chronic progressive nephropathy were increased at all doses (≥4 mg/kg-day in male and females), with statistically significant increases at ≥4 mg/kg-day in females and ≥19 mg/kg-day in males (see Tables 6A and 6B). The incidence of spontaneous CPN has been shown to vary across rat strains and to be affected by a variety of factors, including diet (e.g., high protein, Hard and Khan, 2004). The F344 strain has exhibited a particularly high incidence of spontaneous CPN (Matsumoto et al., 2006b) and, therefore, may be more sensitive than other strains to chemically induced chronic nephropathy. However, no data were available that allowed a comparison of the dose-response relationships for *o*-chloronitrobenzene-induced nephropathy in the F344 strain to other rat strains. Although the pathology of spontaneous CPN in rats has had no strict representation in humans, features of CPN have occurred in human disease, including glomerular nephritis, glomerulosclerosis, tubular nephrosis, and interstitial fibrosis. Several authors (Falk et al., 2000; Kelly and Neilson, 2000; Mitch and Walser, 2000) also have identified associations between the progression of renal disease and dietary protein intake. Therefore, the observed *o*-chloronitrobenzene dose-related chronic nephropathy and exacerbation of spontaneous CPN in the rat was a toxicity endpoint considered potentially relevant to humans. The occurrence of spontaneous CPN in rodents was a potential complication in the interpretation of chemically-induced renal tumors that occurred with exacerbation of spontaneous CPN (Hard and Khan, 2004).

Chronic exposure of rats and mice to dietary *o*-chloronitrobenzene produced hepatotoxicity (Matsumoto et al., 2006b). Chronic oral exposure produced liver tumors (adenomas, carcinomas, hepatoblastomas), histopathological changes (pre-neoplastic lesions, including acidophilic and basophilic cell foci), elevated serum liver enzymes (γ-GGT, LDH, ALT), and increased relative liver weight. Additional discussion of liver tumors has been provided in the provisional carcinogenicity assessment of this document. Except for an increase in relative liver weight in male rats at 4 mg/kg-day and an increase in the incidence of centrilobular hypertrophy in male mice at 11 mg/kg-day, histopathological changes and elevated serum liver enzymes were observed at higher doses (≥19 mg/kg-day in male rats, ≥22 mg/kg-day in female rats, ≥54 mg/kg-day in male mice, and ≥69 mg/kg-day in female mice). Since

pre-neoplastic liver lesions, elevated liver enzymes, and increased liver weight were most likely related to tumor development, the only liver lesion selected as a possible critical effect for derivation of the chronic p-RfD was centrilobular hypertrophy in male mice (see Table 9). However, the relatively flat dose-response curve appeared not to be amenable to BMD analysis, so the potential POD for this effect was the LOAEL of 11 mg/kg/day, at which male mice exhibited a 64% incidence (32/50 compared with 0/50 among controls) of centrilobular hypertrophy.

Incidence of chronic progressive nephropathy (CPN) in female rats and hemosiderin deposition of the spleen in male and female mice provided the most sensitive measures of effect for chronic oral exposure to *o*-chloronitrobenzene (Matsumoto et al., 2006b). To determine the POD for derivation of the chronic p-RfD, data sets for hemosiderin deposition of the spleen in mice and CPN in female rats (see Table 20) were modeled using the U.S. EPA BMD Software (BMDS) version 1.4.1b (U.S. EPA, 2000). Appendix C summarizes of the results of the BMD analysis. For chronic progressive nephropathy in female rats, only the log-logistic model provided adequate fit to the data (see Table B-2 and Figure B-1), calculating a $BMDL_{10}$ of 0.30 mg/kg-day. For hemosiderin deposition in male mice, adequate fits to the data were observed for several models (gamma, log-logistic, multi-stage, quantal-linear, and Weibull); several of the model fits were identical. Comparing across models, the log-logistic model provided the better fit, as indicated by a lower AIC (U.S. EPA, 2000), predicting a $BMDL_{10}$ of 7.86 mg/kg-day (see Table B-2 and Figure B-2). Incidence data for hemosiderin deposition in female mice were adequately fit by all available dichotomous models (gamma, logistic, log-logistic, multi-stage, probit, log-probit, quantal-linear, quantal-quadratic, and Weibull). Several models provided the smaller AIC value (gamma, multi-stage, quantal-linear, and Weibull), predicting a $BMDL_{10}$ of 15.93 mg/kg-day (see Table B-2 and Figure B-3). Chronic progressive nephropathy was selected as the critical effect for derivation of the chronic p-RfD because it resulted in the lowest $BMDL_{10}$—0.30 mg/kg-day.

Table 20. Nonneoplastic Lesions in Rats and Mice Exposed to Dietary *o*-Chloronitrobenzene for 2 Years[a]

Species/Gender	Parameter	Daily Exposure and Incidence of Lesion			
Rats/Female	Dose (mg/kg-day)	0	4	22	117
	Chronic progressive nephropathy	20/50[b]	33/50[d]	45/50[d]	49/50[d]
Mice/Male	Dose (mg/kg-day)	0	11	54	329
	Hemosiderin deposition in the spleen	9/50[b]	20/50[c]	21/50[d]	40/50[d]
Mice/Female	Dose (mg/kg-day)	0	14	69	396
	Hemosiderin deposition in the spleen	17/50[b]	23/50	27/50[c]	45/50[d]

[a]Matsumoto et al., 2006b
[b]Number of animals with lesion/number of animals examined
[c]Significantly different from control ($p \leq 0.05$)
[d]Significantly different from control ($p \leq 0.01$)

A UF of 100, composed of the following, was applied to the chronic $BMDL_{10}$ POD of 0.30 mg/kg-day for chronic progressive nephropathy in female rats (Matsumoto et al., 2006a).

- A UF of 10 for intraspecies differences was applied to account for potentially susceptible individuals in the absence of quantitative information on the variability of response in humans. Individuals with pre-existing renal disorders might be more susceptible to oral *o*-chloronitrobenzene.
- A UF of 10 was applied for interspecies extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans.
- No UF for database deficiencies was applied. Well designed subchronic and chronic studies in two species are available, as well as developmental and reproduction studies that demonstrated developmental effects only at higher doses (75 mg/kg-day) and in the presence of maternal toxicity. So, additional data seemed unlikely to identify a lower chronic oral POD.

Chronic p-RfD = $BMDL_{10} \div UF$
= 0.30 mg/kg-day ÷ 100
= 0.003 mg/kg-day or **3×10^{-3} mg/kg-day**

Confidence in the key study is medium-to-high. Matsumoto et al. (2006b) assessed comprehensive endpoints in an appropriate number of animals, but a NOAEL is not identified. Confidence in the critical effect is medium. Matsumoto et al. (2006b) observed chronic progressive nephropathy in rats, but not in mice; furthermore, no evidence of renal toxicity has been reported in subchronic oral exposure studies in rats or mice; Matsumoto et al. (2006b) observed nephrotoxicity in a subchronic inhalation study in rats. Confidence in the database is high. Subchronic and chronic oral toxicity studies have been conducted in rats and mice; in addition, an oral 2-generation reproduction study and an oral developmental study are available. Medium-to-high confidence in the chronic p-RfD results.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfCs FOR *o*-CHLORONITROBENZENE

Subchronic p-RfC

Two studies reporting the effects of human exposure to airborne *o*-chloronitrobenzene were located (Renshaw and Ashcroft, 1926; Jones et al., 2006). The available data indicates that inhalation of *o*-chloronitrobenzene may produce methemoglobinemia and anemia. However, the data are not suitable for use in derivation of the subchronic RfC due to concomitant exposure to *p*-chloronitrobenzene and inadequate reporting.

Available subchronic inhalation studies in animals include 2- and 4-week studies in rats (Haskell Laboratories, 1984; Nair et al., 1986), and a 13-week study in rats and mice (NTP, 1993). Results identified the upper respiratory tract (nasal epithelial hyperplasia), blood (methemoglobinemia and anemia), spleen (hemosiderin deposition), liver (nonneoplastic lesions), and male reproductive system as targets for adverse effects of subchronic airborne exposure to *o*-chloronitrobenzene. Airborne exposure of rats for 2, 4, or 13 weeks to *o*-chloronitrobenzene produced methemoglobinemia. Effects on hematological parameters, including decreased Hgb and RBC count, spleen congestion, and increased severity of hemosiderin deposition are consistent with *o*-chloronitrobenzene-induced methemoglobinemia, followed by anemia and compensatory erythropoiesis. Male and female rats experienced the most sensitive effects associated with *o*-chloronitrobenzene-induced anemia and compensatory

erythropoiesis: Methemoglobin in rats exposed to an average daily concentration of 1.3 mg/m^3 for 13 weeks (NTP, 1993; Travlos et al., 1996; see Table 16) and increased hemosiderin deposition of the spleen in rats exposed to an average daily concentration of 1.8 mg/m^3 for 4 weeks (Nair et al., 1986).

Adverse effects to the upper respiratory tract (nasal respiratory epithelial hyperplasia) were observed in male and female rats exposed to inhaled *o*-chloronitrobenzene for 13 weeks (NTP, 1993; Travlos et al., 1996; see Table 17). Nasal epithelial hyperplasia, which may represent an irritant effect, was observed in all treatment groups ($\geq$7.2 mg/m^3, average daily concentration of 1.3 mg/m^3). Because no other histopathological changes to the upper respiratory tract were observed, nasal epithelial hyperplasia was considered a minimal effect. Nonneoplastic lesions of the liver were observed in male rats exposed to an average daily concentration of $\geq$29 mg/m^3 for 2 weeks (Haskell Laboratories, 1984) and in rats (see Table 17) and mice (see Table 18) exposed to a daily average of $\geq$10 mg/m^3 for 13 weeks (NTP, 1993; Travlos et al., 1996). However, because hepatic effects occurred only following exposures to much higher concentrations than those leading to blood and upper respiratory effects, hepatotoxicity was not considered to be the critical effect for derivation of the subchronic p-RfC. Reduced sperm motility was observed in mice exposed for 13 weeks (NTP, 1993; Travlos et al., 1996). However, sperm motility was assessed only at the two highest exposure concentrations (daily average of $\geq$5.1 mg/m^3) and endpoints that appeared to be more sensitive were identified (e.g., methemoglobinemia and nasal irritation); thus, reduced sperm motility in mice was not selected as the critical effect for derivation of the subchronic p-RfC. Mild elevations in liver, spleen, and kidney weights were observed in all subchronic inhalation studies in animals; however, because the changes in organ weights were slight and not accompanied by histopathological lesions or functional changes, increased organ weights were not considered as adverse effects.

The most sensitive effects identified as possible critical effects for derivation of the subchronic p-RfC are methemoglobinemia, hemosiderin deposition in the spleen, and nasal epithelial hyperplasia. Increased severity of hemosiderin deposition in rat spleens was observed in a 4-week study (Nair, 1986) at average daily exposure concentration >1.8 mg/m^3. This concentration is slightly greater than the LOAEL (1.3 mg/m^3) for other effects observed in rats. Because the data are presented only graphically, they could not be modeled using BMD, so this endpoint for derivation of the subchronic p-RfC was not considered. To determine the most sensitive endpoint for derivation of the subchronic p-RfC, human equivalent concentration (HEC) conversions were calculated for systemic effects (methemoglobinemia) and upper respiratory tract effects (nasal epithelial hyperplasia). As shown in see Table 17, the incidence of nasal lesions in female rats (NTP, 1993; Travlos et al., 1996) increased from 0% in the control group to 80% in the lowest *o*-chloronitrobenzene group (7.2 mg/m^3; average daily concentration of 1.3 mg/m^3). Therefore, these data are not suitable for BMD modeling and a NOAEL/LOAEL approach to derive the subchronic p-RfC was employed.

The LOAEL value of 7.2 mg/m^3 for methemoglobinemia and nasal epithelial hyperplasia for continuous exposure was adjusted ($LOAEL_{[ADJ]}$) as shown below:

$$LOAEL_{[ADJ]} = LOAEL \times 6/24 \times 5/7$$
$$LOAEL_{[ADJ]} = 7.2 \text{ mg/m}^3 \times 6/24 \times 5/7$$
$$LOAEL_{[ADJ]} = 1.3 \text{ mg/m}^3$$

The $LOAEL_{[ADJ]}$ of 1.3 mg/m^3 was used to calculate the HEC values. For methemoglobinemia, an extrarespiratory effect, *o*-chloronitrobenzene was treated as a category 3 gas, as defined in U.S. EPA (1994b). The corresponding human equivalent exposure concentration ($LOAEL_{[HEC]}$) of 1.3 mg/m^3 for methemoglobinemia was calculated as follows (see Table 21):

$$LOAEL_{[HEC]} = LOAEL_{[ADJ]} \times (H_{b/g})_A/(H_{b/g})_H$$
$$LOAEL_{[HEC]} = 1.3 \text{ mg/m}^3 \times 1$$
$$LOAEL_{[HEC]} = 1.3 \text{ mg/m}^3$$

where $(H_{b/g})_A$ and $(H_{b/g})_H$ are the blood/gas partition coefficients for *o*-chloronitrobenzene in the animal (i.e., rat) and human, respectively. Because the partition coefficients for *o*-chloronitrobenzene are unknown, the default value of 1.0 for the ratio of $(H_{b/g})_A/(H_{b/g})$ (U.S. EPA, 1994b) was used in the calculation.

Table 21. Human Equivalent Concentrations (HEC) from LOAELs for Extrarespiratory Effects and Extra-thoracic Respiratory Effects in Male and Female Rats Reported by NTP, 1993 and Travlos et al., 1996

Effect (Gender)	$LOAEL_{[ADJ]}$ (mg/m^3)	$RGDR_{ET}$	$LOAEL_{[HEC]}$ (mg/m^3)
Methemoglobinemia (M)	1.3	1	1.3
Methemoglobinemia (F)	1.3	1	1.3
Nasal epithelial hyperplasia (M)	1.3	0.151	0.19
Nasal epithelial hyperplasia (F)	1.3	0.105	0.14

For nasal epithelial hyperplasia, an extra-thoracic respiratory effect, *o*-chloronitrobenzene was treated as a category 1 gas, as defined in U.S. EPA (1994b). Using the average body weights for control male and female rats reported by NTP (1993; Travlos et al., 1996) and default values for humans (U.S. EPA, 1994b) (see Table 21), the $LOAEL_{[HEC]}$ was calculated as follows:

$$LOAEL_{[HEC]} = (LOAEL_{[ADJ]})\,(RGDR_{ET})$$

$$RGDR_{ET} = \frac{(Dose_{ET})_A}{(Dose_{ET})_H} = \frac{\left((V_E)/SA_{ET}\right)_A}{\left((V_E)/SA_{ET}\right)_H}$$

where:

$(V_E)_A$ = 156.4 cm^3/min [minute volume for male rat (0.211 kg body weight)]; 109.0 cm^3/min [minute volume for female rat (0.136 kg body weight)]
$(V_E)_H$ = minute volume for 70 kg human (13,800 cm^3/min)
$(SA_{ET})_A$ = surface area of extra-thoracic region for rat (15 cm^2)
$(SA_{ET})_H$ = surface area of extra-thoracic region for 70 kg human (200 cm^2)

Values for $(V_E)_H$, $(SA_{ET})_H$ and $(SA_{ET})_A$ are recommended reference values for humans and rats, while values for $(V_E)_A$ are based on a recommended scaling algorithm for rats (U.S. EPA, 1994b):

$$\ln(V_E)_A = -0.578 + 0.821 \cdot \ln(BW)$$

Based on the lower $LOAEL_{[HEC]}$ values of 0.19 and 0.14 in male and female rats, respectively (see Table 21), nasal epithelial hyperplasia was selected as the critical effect for derivation of the subchronic p-RfC for *o*-chloronitrobenzene. Since the $LOAEL_{[HEC]}$ for females was less than that for males, the $LOAEL_{[HEC]}$ 0.14 in female rats was selected as the POD for derivation of the subchronic p-RfC. Although most of the database for *o*-chloronitrobenzene points toward hematological or liver effects, using the data for nasal lesions to derive the p-RfC should be protective for those systemic effects, as well, because the resulting POD is nearly 10 times lower than that for the most sensitive (hematologic) effects (see Table 21).

A UF of 1000 is applied to the LOAEL of 0.14 mg/m^3 for nasal lesions in female rats (NTP, 1993; Travlos et al., 1996):

- A 10-fold UF for intraspecies differences is used to account for potentially susceptible individuals in the absence of quantitative information or information on the variability of response in humans. Individuals with pre-existing upper respiratory disorders may be more susceptible to inhaled *o*-chloronitrobenzene.
- A partial UF factor of 3 ($10^{0.5}$) is applied to account for potential toxicodynamic differences between rats and humans. A full UF of 10 is not necessary because the toxicokinetic differences were accounted for by calculating a HEC value as the POD.
- An UF of 10 is applied for use of a LOAEL. Although the effect might have had minimal adversity, the fact that it occurred in 80% of rats at the LOAEL (NTP, 1993; Travlos et al., 1996) resulted in substantial uncertainties, so the full UF of 10 was applied.
- A partial UF of 3 ($10^{0.5}$) is included for database insufficiencies. The database lacked developmental and multi-generation reproduction studies for inhaled *o*-chloronitrobenzene. Although the available oral developmental toxicity and 2-generation reproduction studies showed that the reproductive system and developing fetus were not sensitive endpoints, adverse effects on sperm were noted in mice in the subchronic NTP inhalation study. Although a NOAEL for this effect was not identified, observations for this effect were made only in the most highly exposed mice (NTP 1993; Travlos et al., 1996), so it is unclear whether this effect might occur at lower exposure concentrations. However, the spectrum of the toxicity seen in inhalation studies is similar to oral studies. Therefore, it seems unlikely the sperm toxicity was more sensitive than the other effects, such as changes in methemoglobin. Because of the uncertainties inherent in these assumptions and in extrapolation of oral developmental data to inhalation, the partial UF of 3 is included in this derivation.

$$\textbf{Subchronic p-RfC} = LOAEL_{[HEC]} \div UF$$
$$= 0.14\ mg/m^3 \div 1000$$
$$= 0.00014\ mg/m^3 \text{ or } \mathbf{1 \times 10^{-4}\ mg/m^3}$$

Confidence in the key study was medium-to-low. The NTP (1993; Travlos et al., 1996) study was well-conducted and well-reported, used adequate numbers of both genders in two species and tested multiple exposure concentrations, but a NOAEL was not identified. In addition, the critical effect was observed in 80% of female rats exposed to the lowest concentration, so there is low confidence that this LOAEL/10 approximates a NOAEL for this effect. In addition, the animals were exposed whole-body to the contaminated air, resulting in likely concomitant dermal and oral exposures that were not accounted for. Confidence in the critical effect was medium. Nasal lesions were observed in male and female rats, although not in

mice exposed to the same concentrations. Confidence in the database was medium due to lack of reproductive and developmental toxicity studies by the inhalation route, although developmental and reproduction studies by the oral route were available. The absence of an inhalation reproduction study was a particular problem because effects on sperm were noted in mice in the subchronic NTP study and a NOAEL for this effect was not identified. However, oral data indicated that reproductive toxicity occurred only at higher doses than those resulting in methemoglobinemia (NTP, 1993). Medium confidence in the provisional subchronic p-RfC resulted.

Chronic p-RfC

The added uncertainty in developing a chronic value using the same data as the subchronic precludes presenting a value. However, the Appendix of this document contains a Screening Value that may be useful in certain instances. Please see the attached Appendix for details.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR *o*-CHLORONITROBENZENE

Weight-of-Evidence Descriptor

Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the available evidence suggests that oral exposure to *o*-chloronitrobenzene is "*Likely to be Carcinogenic to Humans*" based on significant dose-related increases in liver tumors in male and female mice (Matsumoto et al., 2006b; see Table 10), equivocal increases in liver tumors in male and female rats (Matsumoto et al., 2006b; see Table 7), significant increases in liver tumors in male and female mice (Weisburger et al., 1978; see Table 11), and a significant increase in the number of animals bearing multiple tumors (types not specified) in male rats (Weisburger et al., 1978). No studies were located that evaluated the carcinogenic potential in humans exposed to oral *o*-chloronitrobenzene, or that were suitable for evaluation of the carcinogenic potential for inhaled *o*-chloronitrobenzene.

Mode of Action Discussion

Limited evidence supported the mutagenic mode of action for *o*-chloronitrobenzene tumorigenicity. Available studies provided evidence that *o*-chloronitrobenzene is capable of eliciting genotoxic effects in mammalian cells *in vitro* and in the livers of mice following parenteral exposure of mice to *o*-chloronitrobenzene. In the absence of evidence for other potential modes of action for carcinogenicity of oral *o*-chloronitrobenzene, a linear approach was applied to calculate the OSF (U.S. EPA, 2005).

Quantitative Estimates of Carcinogenic Risk

Carcinogenic Risk from Oral Exposure

Evidence of hepatic carcinogenicity was observed in rats and mice exposed chronically to oral *o*-chloronitrobenzene in studies conducted by Matsumoto et al. (2006b; see Table 22) and in mice in the study conducted by Weisburger et al. (1978; see Table 23). Data from the Weisburger cancer bioassay was not selected as the basis for the oral slope factor because the study was conducted in a small number of animals and daily doses were much higher than those used in the Matsumoto et al. (2006b) study. The results of the Matsumoto et al. (2006b) study showed that mice were more sensitive than rats to *o*-chloronitrobenzene-induced hepatotoxicity.

In male and female mice, significant increases in the incidence of hepatocellular adenomas were observed in all *o*-chloronitrobenzene groups and for hepatocellular carcinomas and hepatoblastomas in the mid- and high-dose groups. The incidence of hepatoblastomas was significantly increased in males and females in the mid- and high-dose groups; in male mice, the incidence of hepatoblastomas in the low-dose groups was increased relative to control, although differences from control did not reach statistical significance. The maximum incidence of adenomas or carcinomas in male and female mice, carcinomas in female mice, and hepatoblastomas in male mice were identified as possible effects for derivation of the OSF. Hepatoblastomas in combination with the other liver tumors were not considered because they appeared to have occurred in different liver tissues.

Table 22. Liver Tumors in F344 Rats and CD-1 Mice Exposed to Dietary *o*-Chloronitrobenzene for 2 Years[a]

Species (Gender)	Parameter	Daily Dose and Tumor Incidence			
Rats (M)	**Daily dose (mg/kg-day)**	**0**	**4**	**19**	**99**
	Hepatocellular adenoma	2[b,e]	3	7[g]	1
	Hepatocellular carcinoma	0[f]	0	3[g]	1
Rats (F)	**Daily dose (mg/kg-day)**	**0**	**4**	**22**	**117**
	Hepatocellular adenoma	0[f]	0	2	20[d]
	Hepatocellular carcinoma	0[f]	0	0	4
Mice (M)	**Daily dose (mg/kg-day)**	**0**	**11**	**54**	**329**
	Hepatocellular adenoma	19[b,f]	29[c]	30[c]	34[d]
	Hepatocellular carcinoma	15[f]	14	20	35[d]
	Liver tumors (adenoma or carcinoma)[g]	19	29	30	35
	Hepatoblastoma	1[f]	6	35[d]	44[d]
Mice (F)	**Daily dose (mg/kg-day)**	**0**	**14**	**69**	**396**
	Hepatocellular adenoma	8[f]	22[d]	48[d]	38[d]
	Hepatocellular carcinoma	0[f]	3	14[d]	48[d]
	Liver tumors (adenoma or carcinoma)[g]	8	22	48	48
	Hepatoblastoma	0[f]	0	9[d]	28[d]

[a]Matsumoto et al., 2006b
[b]Number of animals with tumor; 50 animals examined in each treatment group
[c]Significantly different from control ($p \leq 0.05$), Fisher's exact test
[d]Significantly different from control ($p \leq 0.01$), Fisher's exact test
[e]Significant trend ($p \leq 0.05$), Peto's trend test
[f]Significant trend ($p \leq 0.01$), Peto's trend test
[g]Matsumoto et al. (2006b) reported the incidence of liver adenomas and carcinomas, but not the maximum incidence of adenomas or carcinomas (i.e., incidence of animals having an adenoma, carcinoma, or both). The highest incidence of either tumor type in each dose group was used to reflect a minimum estimate of the combined tumor type incidence. This approach may have introduced a bias in the estimate of the maximum incidence for each dose to the extent that adenomas and carcinomas occurred in different animals.

Table 23. Liver Tumors in Male and Female CD-1 Mice Exposed to Dietary *o*-Chloronitrobenzene for 18 Months[a]

Gender	Parameter	Daily Dose and Tumor Incidence		
M	Daily dose (mg/kg-day)	0	372	743
	Tumor incidence[b]	3/18[b]	7/17[c]	3/16
F	Daily dose (mg/kg-day)	0	375	749
	Tumor incidence[b]	0/20	5/22[c,d]	5/19[c,d]

[a]Weisburger et al., 1978
[b]Number of animals with tumor/number of animals examined
[c]Significantly different from incidence in pooled controls (Males: 7/99; Females: 1/102), $p < 0.025$
[d]Significantly different from matched controls, $p < 0.05$

To determine the POD for derivation of the OSF, BMD modeling using BDMS version 1.4.1b (U.S. EPA, 2000) was conducted on data sets for the maximum incidence of hepatocellular adenoma or carcinoma in male and female mice, carcinoma in female mice, and hepatoblastoma in male mice. Appendix Table D-1 summarizes the results of the BMD modeling for adenoma or carcinoma in male and female mice, carcinoma in female mice, and hepatoblastoma in male mice. The multi-stage model of the female mouse liver tumor data for incidence of either adenoma or carcinoma, with highest dose data dropped, has adequate fit and provides the lowest $BMDL_{10}$ of 2.1 mg/kg-day. Dropping the high-dose data is justified because the tumor incidence approached 100% (48/50) at both the middle and high doses.The human equivalent dose (HED) of the mouse $BMDL_{10}$ of 0.32 mg/kg-day was calculated as follows:

$$\begin{aligned} BMDL_{10\,HED} &= BMDL_{10} \times (W_{animal}/W_{human})^{1/4} \\ &= 2.11 \text{ mg/kg-day} \times (0.037 \text{ kg} / 70 \text{ kg})^{1/4} \\ &= 2.11 \text{ mg/kg-day} \times 0.15 \\ &= 0.32 \text{ mg/kg-day} \end{aligned}$$

where

W_{human} = 70 kg (human reference body weight)
W_{animal} = 0.037 kg (terminal body weight for control female mice, Matsumoto et al., 2006b)

In the absence of a known mode of action for carcinogenicity of oral *o*-chloronitrobenzene, a linear approach was assumed to calculate the p-OSF (U.S. EPA, 2005). To extrapolate cancer risks linearly from the $BMDL_{10\,HED}$ to the origin, a p-OSF was calculated as the ratio $0.1/BMDL_{10\,HED}$. Taking the $BMDL_{10\,HED}$ of 0.3 mg/kg-day for the maximum incidence of adenoma or carcinoma in female mice as the POD was calculated as follows:

$$\begin{aligned} \textbf{p-OSF} &= 0.1 / BMDL_{10\,HED} \\ &= 0.1 / 0.32 \text{ mg/kg-day} \\ &= 0.3 \text{ or } \mathbf{3 \times 10^{-1}\ (mg/kg\text{-}day)^{-1}} \end{aligned}$$

Estimates of continuous lifetime exposure to *o*-chloronitrobenzene that corresponded to specified risk levels (i.e., 1×10^{-4}, 1×10^{-5}, 1×10^{-6}) are shown in Table 24. It should be noted, however, that this method of combining incidence data for two types of tumors might have underestimated cancer risk because it does not consider the possibility that both tumor types might have occurred in the same experimental animal.

Table 24. Continuous Lifetime Exposure Estimates Corresponding to Specified Cancer Risk for Oral Doses of *o*-Chloronitrobenzene	
Risk[a]	**Dose**
1×10^{-4} Risk	3.2×10^{-4} mg/kg-day
1×10^{-5} Risk	3.2×10^{-5} mg/kg-day
1×10^{-6} Risk	3.2×10^{-6} mg/kg-day

[a]Extra risk due to *o*-chloronitrobenzene exposure

Carcinogenic Risk from Inhalation Exposure

No human or animal studies examining the carcinogenicity of *o*-chloronitrobenzene following airborne exposure were located, precluding derivation of an inhalation unit risk.

REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). 2001a. *p*-Nitrochlorobenzene. In Documentation of the Threshold Limit Values for Chemical Substances (7th edition). ACGIH, Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 2001b. Methemoglobin inducers. In Documentation of the Biological Exposure Indices (7th edition). ACGIH, Cincinnati, OH.

ACGIH (American Conference of Governmental Industrial Hygienists). 2007. 2007 Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices. ACGIH, Cincinnati, OH.

ATSDR (Agency for Toxic Substances and Disease Registry). 2008. Toxicological Profile Information Sheet. Online. http://www.atsdr.cdc.gov/toxpro2.html.

Bayer, A.G. 1991. Data Report No. 20209(F): Enzymhistochemisch darstellbare Veränderungen des Kohlenhydratstoffwechsels der Mausleber nach Gabe von o-Chlornitrobenzol, May/6/1991. (Cited in OECD/SIDS, 2001).

Bayer, A.G. 1993. Data Report No. 22240: o-Chlornitrobenzol: Subakute Toxizitätsstudie an B6C3F1-Mäusen - Schwerpunkt Leberdiagnostic - (Verabreichung im Futter bis zu 5 Wochen), May/7/1993. (at the request of BG-Chemie, Heidelberg) (Cited in OECD/SIDS, 2001).

Falk, R.J., J.C. Jennette and P.H. Nachman. 2000. Primary glomerular disease. In: The Kidney, 6th Edition. Brenner, B.M., ed. W.B. Saunders, Philadelphia, PA. p. 1263–1349.

Hard, G.C. and K.N. Khan. 2004. A contemporary overview of chronic progressive nephropathy in the laboratory rat, and its significance for human risk assessment. Toxicol. Pathol. 32(2):171–180.

Haskell Laboratories. 1984. Subchronic inhalation toxicity study of o-chloronitrobenzene (OCNB) in rats. Produced 12/17/84. Submitted 8/10/92 by Du Pont Chemical Co. to U.S. EPA under TSCA Section 8ECP. EPA Doc. No. 88-920010177. Fiche No. OTS0546562. TSCATS 433873.

Holder, J.W. 1999. Nitrobenzene carcinogenicity in animals and human hazard evaluation. Toxicology and Industrial Health 15: 445–457.

IARC (International Agency for Research on Cancer). 1996. 2-Chloronitrobenzene, 3-chloronitrobenzene, and 4-chloronitrobenzene. Printing Processes and Printing Inks, Carbon Black and Some Nitro Compounds. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Lyon, France. Vol. 65, p. 263-296. Online. http://monographs.iarc.fr/ENG/Monographs/vol65/volume65.pdf.

Jones, C.R. and G. Sabbioni. 2003. Identification of DNA adducts using HPLC/MS/MS following *in vitro* and *in vivo* experiments with arylamines and nitroarenes. Chem. Res. Toxicol. 16:1251–1263.

Jones, C.R., Y.-Y. Liu, O. Sepai et al. 2006. Internal exposure, health effects, and cancer risk of humans exposed to chloronitrobenzene. Environ. Sci. Technol. 40:387-394. Online. http://pubs.acs.org/cgi-bin/article.cgi/esthag/2006/40/i01/pdf/es050693p.pdf.

Kelly, C.J. and E.G. Neilson. 2000. Tubulointerstitial disease. In: The Kidney, 6th Edition. Brenner, B.M., ed. W.B. Saunders, Philadelphia, PA. p. 1509–1536.

Matsumoto, M., Y. Umeda, H. Arito, K. Nagano, S. Yamamoto and T. Matsushima. 2006a. Thirteen-week oral toxicity study of para- and ortho-chloronitrobenzene in rats and mice. J. Toxicol. Sci. 31(1):9-22. Online. http://www.jstage.jst.go.jp/article/jts/31/1/9/_pdf.

Matsumoto, M., Y. Umeda, H. Senoh et al. 2006b. Two-year feed study of carcinogenicity and chronic toxicity of ortho-chloronitrobenzene in rats and mice. J. Toxicol. Sci. 31(3):247-267. Online. http://www.jstage.jst.go.jp/article/jts/31/3/247/_pdf.

Mitch, W.E. and M. Walser. 2000. Nutritional therapy in renal disease. In: The Kidney, 6th Edition. Brenner, B.M., ed. W.B. Saunders, Philadelphia, PA. pp. 2298–2340.

Monsanto. 1986. A teratology study in rats with o-nitrochlorobenzene and a modified teratology study in rats. Produced 7/18/86. Submitted 5/8/90 to U.S. EPA under TSCA Section 8E. EPA Doc. No. 88-900000146. Fiche No. OTS0524332. TSCATS 408750.

Nair, R.S., F.R. Johannsen, G.J. Levinskas et al. 1986. Assessment of toxicity of *o*-nitrochlorobenzene in rats following a 4-week inhalation exposure. Fund. Appl. Toxicol. 7:609–614.

NIOSH (National Institute for Occupational Safety and Health). 2005. Online NIOSH Pocket Guide to Chemical Hazards. Online. http://www.cdc.gov/niosh/npg/npgsyn-a.html.

NTP (National Toxicology Program). 1993. Toxicity studies of 2-chloronitrobenzene and 4-chloronitrobenzene administered by inhalation to F344/N rats and B6C3F1 mice. NTP-Tox 33. NIH Publ. No. 93-3382. Online. http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/TOX33.pdf.

NTP (National Toxicology Program). 2005. Report on Carcinogens, Eleventh Edition; U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. Online. http://ntp.niehs.nih.gov/index.cfm?objectid=32BA9724-F1F6-975E-7FCE50709CB4C932.

OECD/SIDS (Organization for Economic Cooperation and Development/Screening Information Data Set). 2001. 1-Chloro-2-Nitrobenzene. Screening Information Data Set (SIDS) Initial Assessment Report of OECD High Production Volume Chemicals Programme. Online. http://www.inchem.org/documents/sids/sids/CHLORONITROB.pdf.

OSHA (Occupational Safety and Health Administration). 2008. OSHA Standard 1910.1000 Table Z-1. Part Z, Toxic and Hazardous Substances. Online. http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992.

Renshaw, A. and G.V. Ashcroft. 1926. Four cases of poisoning by monochlorobenzene and one by acetanilide, occurring in a chemical works: with an explanation of the toxic symptoms produced. J. Ind. Hyg. 8:67–73.

Travlos, G.S., J. Mahler, H.A. Ragan et al. 1996. Thirteen-week inhalation toxicity of 2- and 4-chloronitrobenzene in F344/N rats and B6C3F1 mice. Fund. Appl. Toxicol. 30:75–92.

U.S. EPA. 1984. Proposed Guidelines for Carcinogen Risk Assessment; Notice. Federal Register. 49(227):46294–46301.

U.S. EPA. 1985. Health and Environmental Effects Profile for Chloronitrobenzenes (o-, m-, p-). Prepared by the Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

U.S. EPA. 1988. Recommendations for and Documentation of Biological Values for Use in Risk Assessment. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH. NTIS PB88-17874. Online. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855.

U.S. EPA. 1991. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. April.

U.S. EPA. 1994a. Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Washington, DC. December.

U.S. EPA. 1994b. Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry. Office of Research and Development, Washington, DC. October. EPA/600/8-90/066F. Online. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=71993.

U.S. EPA. 1997. Health Effects Assessment Summary Tables. FY-1997 Update. Prepared for the Office of Research and Development, National Center for Environmental Assessment, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC. July. EPA/540/R-97/036. NTIS PB 97-921199.

U.S. EPA. 2000. Benchmark Dose Technical Guidance Document [external review draft]. EPA/630/R-00/001. Online. http://www.epa.gov/nceawww1/pdfs/bmds/BMD-External_10_13_2000.pdf.

U.S. EPA. 2005. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, Washington, DC; EPA/630/P-03/001F. Federal Register 70(66):17765–17817. Online. http://www.epa.gov/iris/cancer032505-final.pdf.

U.S. EPA. 2006. 2006 Edition of the Drinking Water Standards and Health Advisories. Office of Water, Washington, DC. EPA 822-R-06-013. Online. http://www.epa.gov/waterscience/drinking/standards/dwstandards.pdf.

U.S. EPA. 2008. Integrated Risk Information System (IRIS). Office of Research and Development, National Center for Environmental Assessment, Washington, DC. Online. http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showSubstanceList.

WHO (World Health Organization). 2008. International Programme on Chemical Safety Environmental Health Criteria. Online. http://www.who.int/ipcs/publications/ehc/ehc_alphabetical/en/index.html.

Weisburger, E.K., A.B. Russfield, F. Homburger et al. 1978. Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity of carcinogenicity. J. Environ. Pathol. Toxicol. 2:325–356.

Weisburger, E.K. and V.W. Hudson. 2001. Aromatic nitro and amino compounds. In: Patty's Toxicology. Volume 4, 5th ed. E. Bingham, B. Cohrssen and C.H. Powell, Ed. John Wiley and Sons, Inc., New York. p. 817–968.

Woo, Y.-T. and D.Y. Lai. 2001. Aromatic amino and nitro-amino compounds and their halogenated derivatives. In: Patty's Toxicology. Volume 4, 5th ed. E. Bingham, B. Cohrssen and C.H. Powell, Ed. John Wiley and Sons, Inc., New York. p. 969–1099.

APPENDIX A. DERIVATION OF A SCREENING VALUE FOR o-CHLORONITROBENZENE

For reasons noted in the main PPRTV document, it is inappropriate to derive provisional toxicity values for *o*-chloronitrobenzene. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an Appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

In the absence of chronic inhalation toxicity data in humans or animals, the chronic screening RfC was based on the same POD as that used for derivation of the subchronic p-RfC, the $LOAEL_{[HEC]} = 0.14$ mg/m^3 for nasal lesions in female rats (NTP, 1993; Travlos et al., 1996).

A composite UF of 10,000 was applied to the subchronic $LOAEL_{[HEC]}$ of 0.14 mg/m^3 for nasal lesions in female rats (NTP, 1993; Travlos et al., 1996):

- A full UF of 10 is applied for use of subchronic study.
- A full 10-fold UF for intraspecies differences is applied to account for potentially susceptible individuals in the absence of quantitative information or information on the variability of response in humans. Individuals with pre-existing upper respiratory disorders may be more susceptible to inhaled *o*-chloronitrobenzene.
- A partial UF factor of 3 ($10^{0.5}$) is applied to account for potential toxicodynamic differences between rats and humans. A full UF of 10 is not necessary because the toxicokinetic differences were accounted for by calculating a HEC value as the POD.
- A UF of 10 is applied for use of a LOAEL. Although the effect might have had minimal severity, the fact that it occurred in 80% of rats at the LOAEL resulted in substantial uncertainties, so the full UF of 10 was applied.
- A partial UF of 3 ($10^{0.5}$) is included for database insufficiencies. The database lacked developmental and multi-generation reproduction studies for inhaled *o*-chloronitrobenzene. Although the available oral developmental toxicity and 2-generation reproduction studies showed that the reproductive system and developing fetus were not sensitive endpoints, adverse effects on sperm were noted in mice in the NTP (1993; Travlos et al., 1996) subchronic study of airborne exposure and a NOAEL for this effect was not identified.

Screening p-RfC = $LOAEL_{[HEC]} \div UF$
= 0.14 mg/m^3 ÷ 10,000
= 0.00001 mg/m^3 or **1×10^{-5} mg/m^3 (1×10^{-5} mg/m^3)**

Confidence in the key study was low. The NTP study (1993; Travlos et al., 1996) was well-conducted and well-reported, used adequate numbers of both genders in two species and tested multiple exposure concentrations. However, the exposure duration was less than chronic and a NOAEL was not identified. In addition, the critical effect was observed in 80% of female rats exposed to the lowest concentration, so there is little confidence that LOAEL/10 approximated a NOAEL for this effect. The animals were exposed whole-body to the contaminated air, resulting in likely concomitant dermal and oral exposures that were not accounted for. Confidence in the critical effect was medium. Nasal lesions were observed in male and female rats, although not in mice exposed to the same concentrations. Confidence in the database was medium due to lack of chronic reproductive and developmental toxicity studies by the inhalation route, although chronic developmental and reproduction studies by the oral route were available. The absence of an inhalation reproductive study was a particular problem because effects on sperm were noted in mice in the subchronic NTP study and a NOAEL for this effect was not identified. However, oral data indicated that reproductive toxicity occurred only at higher doses than those resulting in methemoglobinemia (NTP 1993). Medium-to-low confidence in the chronic p-RfC resulted.

APPENDIX B. DETAILS OF BENCHMARK DOSE ANALYSIS OF ORAL DATA FOR HEMATOLOGICAL EFFECTS IN FEMALE RATS FOR DERIVATION OF SUBCHRONIC p-RFD

The model fitting procedure for continuous data involves first applying the simplest model (linear) to the data while assuming constant variance. If the data are consistent with the assumption of constant variance, then the fit of the linear model to the means is evaluated. If the linear model adequately fits the means (goodness of fit $p \geq 0.1$), then it is selected as the model for BMD derivation. If the linear model does not adequately fit the means, then the more complex models are fit to the data while assuming constant variance. Among the models providing adequate fit to the means (goodness of fit $p \geq 0.1$), the one with the lowest AIC for the fitted model is selected for BMD derivation. If the test for constant variance is negative, the linear model is run again while applying the power model integrated into the BMDS to account for nonhomogenous variance. If the nonhomogenous variance model provides an adequate fit ($p \geq 0.1$ in test 3) to the variance data, then the fit of the linear model to the means is further evaluated. If the linear model does not provide adequate fit (goodness of fit $p \geq 0.1$) to the means while the nonhomogenous variance model is applied, then the polynomial, power and Hill models are fit to the data and evaluated while the variance model is applied. Among those providing adequate fit to the means (goodness of fit $p \geq 0.1$), the one with the lowest AIC for the fitted model is selected for BMD derivation. If the test for constant variance is negative and the nonhomogenous variance model does not provide an adequate fit to the variance data, then the data set is considered unsuitable for modeling.

Following the above procedure, continuous-variable models in the EPA BMDS (version 1.4.1b) were fit to the data shown in Table A-1 for RBC count and blood hemoglobin concentration in female rats (Matsumoto et al., 2006a). As recommended by U.S. EPA (2000), a default value of 1 standard deviation (SD) above the control mean was used as the benchmark response (BMR) level, because an adverse concentration of methemoglobin or RBC counts in experimental animals have not been established. Statistics for benchmark dose modeling were summarized in Table B-2. With all doses included, the variance data for female rats were fit by the constant variance model [$p = 0.60$ for RBC, $p = 0.84$ for Hgb in variances homogeneous test indicating constant variance (test 2)]. With the homogeneous variance model applied, the linear model did not provide an adequate fit to the means (goodness of fit $p < 0.1$). Further, none of the remaining restricted models provided adequate fit to the data. In an attempt to achieve model fit, the three high-dose groups were dropped one-by-one from the analysis. With the reduced data sets, the homogenous variance model again did not fit the variance data adequately. With the homogeneous variance model applied, the linear model did not provide an adequate fit to the means ($p < 0.1$). Further, none of the remaining models provided adequate fit to the data. In the absence of adequate fit using any of the standard, restricted power models, the use of the Power model was attempted with unrestricted power on the RBC count data. Following careful review of the fit statistics (see Table B-2) and the visual fit of the curve to the data (see Figure B-1), it was concluded that this model provided a good fit to the data and would be the best source for the subchronic POD for reduced RBC count in female rats.

Table B-1. Hematology Parameters in Female F344 Rats Exposed to Dietary *o*-Chloronitrobenzene for 13 Weeks[a]

Parameter	Dose (mg/kg-day)					
	0	**4.0**	**15.5**	**63.9**	**133.3**	**249.3**
RBC count (10^6/μL)	8.84 ± 0.22	8.54 ± 0.16[c]	8.48 ± 0.14[c]	8.03 ± 0.21[c]	7.67 ± 0.23[c]	7.20 ± 0.18[c]
Hgb (g/dL)	16.1 ± 0.4	15.5 ± 0.3[c]	15.3 ± 0.3[c]	14.3 ± 0.4[c]	13.7 ± 0.4[c]	13.4 ± 0.4[c]

[a]Matsumoto et al., 2006a
[b]Means ± SD, n = 10/group
[c]Significantly different from control ($p \leq 0.01$)

Table B-2. Summary Statistics for Benchmark Dose Analysis of Hematological Effects in Female Rats Exposed to Dietary *o*-Chloronitrobenzene for 13 Weeks[a]

Model (constant variance)	Variance homogeneity *p* value[b]	Mean model *p* value[c]	AIC for fitted model	BMD_{1SD} (mg/m^3)	$BMDL_{1SD}$ (mg/m^3)
RBC Count – All Doses Included					
Linear[d]	0.6056	<0.0001	NA	NA	NA
Polynomial (2nd degree)[d]	0.6056	<0.0001	NA	NA	NA
Polynomial (3rd degree)[d]	0.6056	<0.0001	NA	NA	NA
Polynomial (4th degree)[d]	0.6056	<0.0001	NA	NA	NA
Power[e]	0.6056	<0.0001	NA	NA	NA
Power (unrestricted)[f]	0.6047	0.3846	130.387	4.10126	1.68526
RBC Count – 2 Lowest Doses and Controls Only[g]					
Linear[d]	0.3269	0.004162	NA	NA	NA
Hemoglobin Concentration – All Doses Included					
Linear[d]	0.8422	<0.0001	NA	NA	NA
Polynomial (2nd degree)[d]	0.8422	<0.0001	NA	NA	NA
Polynomial (3rd degree)[d]	0.8422	<0.0001	NA	NA	NA
Polynomial (4th degree)[d]	0.8422	<0.0001	NA	NA	NA
Power[e]	0.8422	<0.0001	NA	NA	NA
Hemoglobin Concentration – 2 Lowest Doses and Controls Only[g]					
Linear[d]	0.5593	0.00426	NA	NA	NA

[a]Matsumoto et al., 2006a
[b]Values <0.05 were defined as not meeting conventional variance criteria
[c]Values <0.10 fail to meet conventional goodness-of-fit criteria
[d]Betas restricted to ≤ 0
[e]Power restricted to ≥ 1
[f]Power unrestricted
[g]Insufficient degrees of freedom to run polynomial and power models

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; NA = not available (BMD software could not generate a model output)

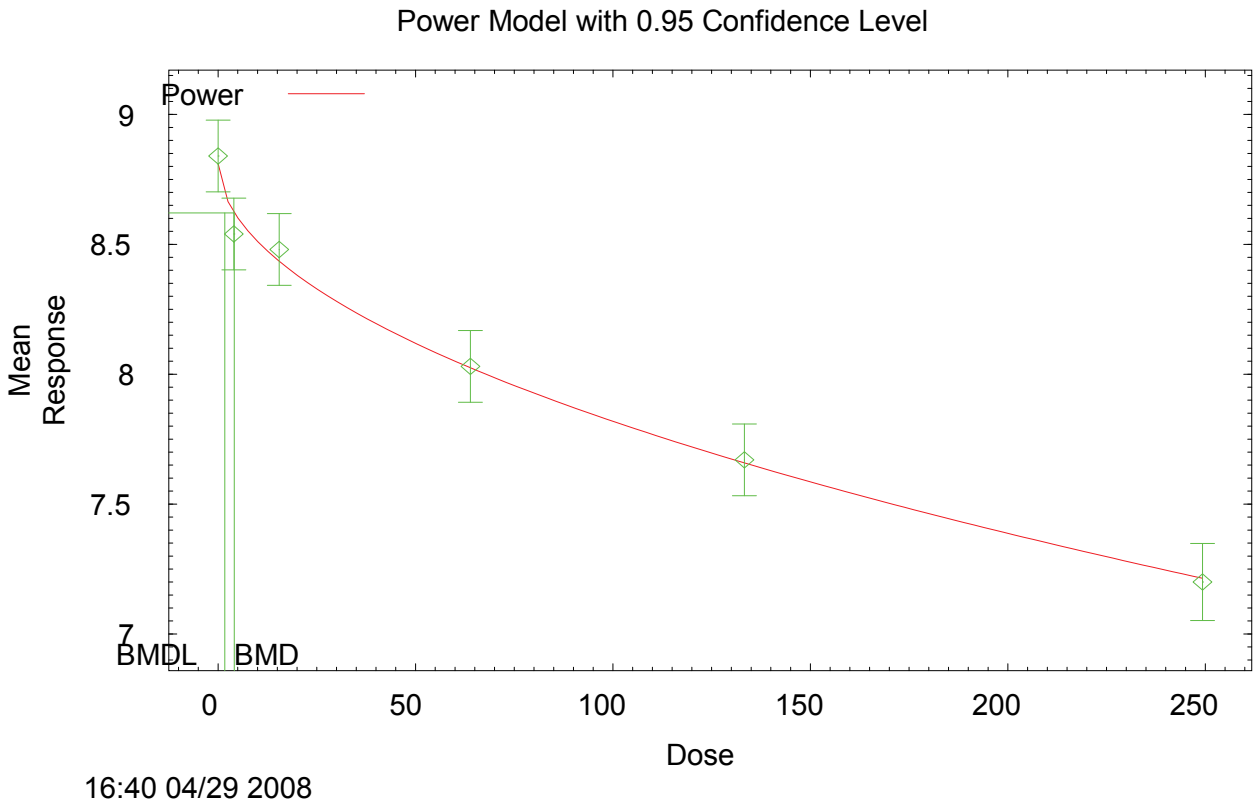

Figure B-1. Plot of Unrestricted Power Model of Subchronic Red Blood Cell Count Data in Female F344 Rats (Matsumoto, et al., 2006a)

APPENDIX C. BENCHMARK DOSE ANALYSIS OF ORAL DATA FOR CHRONIC PROGRESSIVE NEPHROPATHY IN FEMALE RATS AND HEMOSIDERIN DEPOSITION OF THE SPLEEN IN MICE FOR DERIVATION OF CHRONIC p-RFD

Chronic progressive nephropathy in female rats and hemosiderin deposition of the spleen in male and female mice provided the most sensitive measures of effect for chronic oral exposure to *o*-chloronitrobenzene (Matsumoto et al., 2006b). To determine the POD for derivation of the chronic p-RfD, data sets for chronic progressive nephropathy in female rats and hemosiderin deposition of the spleen in mice (see Table C-1) were modeled using Benchmark Dose Modeling Software (BMDS; Version 1.4.1b) developed by the National Center for Environmental Assessment (U.S. EPA, 2000). In accordance with the U.S. EPA (2000) BMD methodology, the default benchmark response (BMR) of a 10% increase in extra risk was used as the basis for the BMD (BMD_{10}), with the $BMDL_{10}$ represented by the 95% lower confidence limit on the BMD_{10}. All available dichotomous models were fit to the incidence data for chronic progressive nephropathy and hemosiderin deposition of the spleen (see Table C-1). Goodness-of-fit was evaluated using the Chi-square statistic calculated by the BMDS program. Acceptable global goodness-of-fit was a Chi-square *p*-value greater than or equal to 0.1. Models that did not meet this criterion were eliminated from consideration. Local fit was evaluated visually on the graphic output, by comparing the observed and estimated results at each data point. The model with the smaller Akaike's information criteria (AIC) was considered to provide a superior fit; it fit the low doses as well as other adequately-fitting models.

Table C-1. Nonneoplastic Lesions in Rats and Mice Exposed to Dietary *o*-Chloronitrobenzene for 2 Years[a]

Species/Gender	Parameter	Daily Exposure and Incidence of Lesion			
Rats/Female	Dose (mg/kg-day)	0	4	22	117
	Chronic progressive nephropathy	20/50[b]	33/50[d]	45/50[d]	49/50[d]
Mice/Male	Dose (mg/kg-day)	0	11	54	329
	hemosiderin deposition in the spleen	9/50[b]	20/50[c]	21/50[d]	40/50[d]
Mice/Female	Dose (mg/kg-day)	0	14	69	396
	hemosiderin deposition in the spleen	17/50[b]	23/50	27/50[c]	45/50[d]

[a]Matsumoto et al., 2006b
[b]Number of animals with lesions/number of animals examined
[c]Significantly different from control ($p \leq 0.05$)
[d]Significantly different from control ($p \leq 0.01$)

Results of the BMDS modeling for chronic progressive nephropathy in female rats and hemosiderin deposition of the spleen in male and female mice are summarized in Table C-2. For chronic progressive nephropathy in female rats, only the log-logistic model provided adequate fit to the data (see Figure C-1), with a $BMDL_{10}$ of 0.30 mg/kg-day. For hemosiderin deposition in male mice, adequate fits to the data were observed for several models (gamma, log-logistic, multi-stage, quantal-linear and Weibull). Comparing across models, the log-logistic model provided the better fit, as indicated by a lower AIC (U.S. EPA, 2000), predicting a $BMDL_{10}$ of 7.9 mg/kg-day for hemosiderin deposition in male mice (see Figure C-2). Incidence data for hemosiderin deposition in female mice was adequately fit by all available dichotomous models (gamma, logistic, log-logistic, multi-stage, probit, log-probit, quantal-linear, quantal-quadratic and Weibull). Several models provided the lesser AIC value (gamma, multi-stage, quantal-linear

and Weibull), predicting a $BMDL_{10}$ of 16 mg/kg-day for hemosiderin deposition in female mice (see Figure C-3).

Table C-2. Summary Benchmark Dose Statistics for Chronic Progressive Nephropathy in Female Rats and Spleen Hemosiderin Deposition in Male and Female Mice Exposed to Dietary *o*-Chloronitrobenzene for 2 Years[a]

Model	Degrees of Freedom	χ^2 Test Statistic	χ^2 *p*-Value[b]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Female Rats – Chronic Progressive Nephropathy						
Gamma[c]	2	18.53	0.0001	185.954	2.04788	1.35341
Logistic	2	29.17	0.0000	189.868	3.17534	2.16688
Log-Logistic[d,e]	**1**	**<0.01**	**0.9562**	**179.72**	**0.672183**	**0.2993**
Multi-Stage 1-Degree[f,g]	2	18.52	0.0001	185.954	2.04813	1.35341
Probit	2	17.39	0.0002	193.908	5.06211	3.56996
Log-Probit[e]	2	7.97	0.0186	182.271	2.44043	1.52378
Quantal-Linear	2	18.52	0.0001	185.954	2.04813	1.35341
Quantal-Quadratic	2	23.75	0.0000	203.276	19.499	13.6986
Weibull[c]	2	18.52	0.0001	185.954	2.04813	1.35341
Male Mice –Hemosiderin Deposition (Spleen)						
Gamma[c]	2	4.38	0.1122	240.94	25.6369	18.3178
Logistic	2	5.37	0.0683	242.129	45.3373	35.61
Log-Logistic[d,e]	**2**	**3.82**	**0.1483**	**240.258**	**13.4733**	**7.85512**
Multi-Stage 1-Degree[f,g]	2	4.38	0.1122	240.94	25.6369	18.3178
Probit	2	5.37	0.0683	242.125	45.4201	36.5531
Log-Probit[e]	2	6.06	0.0482	242.803	48.4312	32.9702
Quantal-Linear	2	4.38	0.1122	240.94	25.6367	18.3178
Quantal-Quadratic	2	7.23	0.0269	244.18	95.7591	79.8498
Weibull[c]	2	4.38	0.1122	240.94	25.6367	18.3178
Female Mice –Hemosiderin Deposition (Spleen)						
Gamma[c,d]	**2**	**0.70**	**0.7052**	**239.299**	**22.5995**	**15.9287**
Logistic	2	1.18	0.5546	239.791	36.383	27.6452
Log-Logistic[e]	2	1.06	0.3039	241.655	27.3802	7.18472
Multi-Stage 1-Degree[d,f,g]	**2**	**0.70**	**0.7052**	**239.299**	**22.5994**	**15.9287**
Probit	2	1.28	0.5284	239.89	38.6289	30.5752
Log-Probit[e]	2	1.38	0.5026	239.981	40.4395	26.9305
Quantal-Linear[d]	**2**	**0.70**	**0.7052**	**239.299**	**22.5994**	**15.9287**
Quantal-Quadratic	2	3.10	0.2119	241.742	96.3315	79.3042
Weibull[c,d]	**2**	**0.70**	**0.7052**	**239.299**	**22.6018**	**15.9287**

[a]Matsumoto et al., 2006b
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria
[c]Power restricted to ≥1
[d]Best-fitting model(s)
[e]Slope restricted to ≥1
[f]Betas restricted to ≥0
[g]Model output presented for the lowest degree polynomial with adequate fit

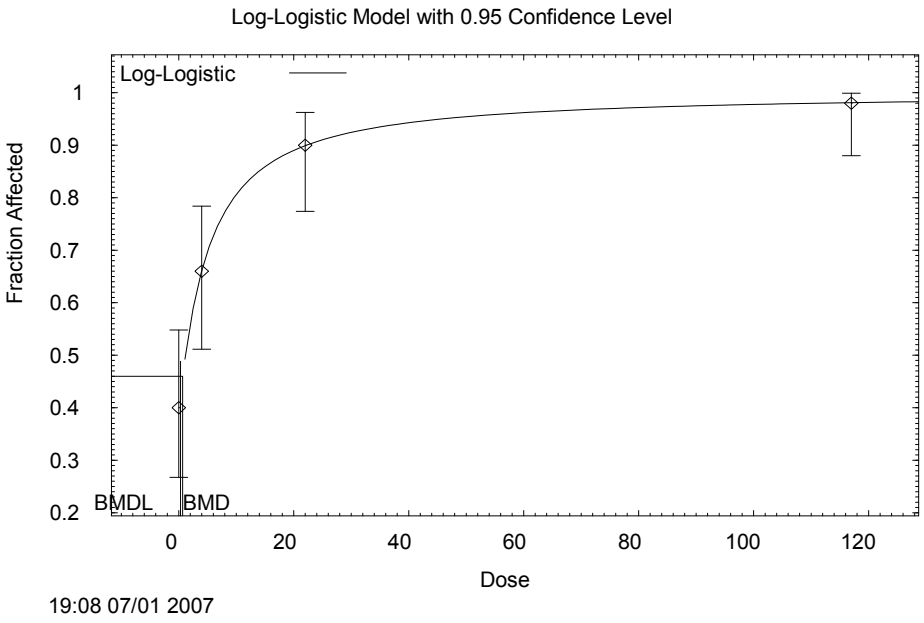

Figure C-1. Observed and Predicted Incidences of Chronic Progressive Nephropathy in Female Rats Exposed to Dietary *o*-Chloronitrobenzene for 2 Years (Log-Logistic Model) (Matsumoto et al., 2006b)

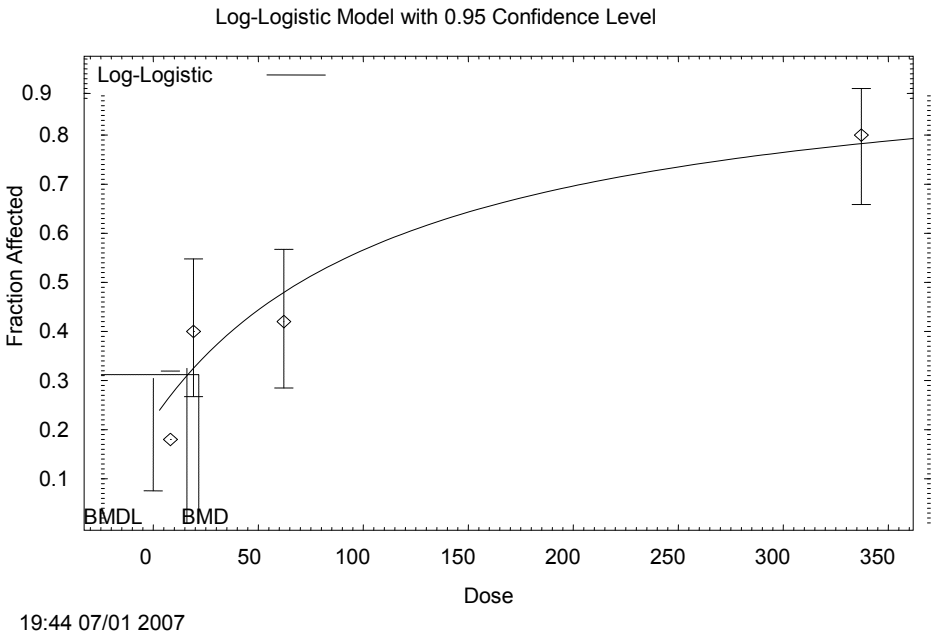

Figure C-2. Observed and Predicted Incidences of Hemosiderin Deposition of the Spleen in Male Mice Exposed to Dietary *o*-Chloronitrobenzene for 2 Years (Log-Logistic Model) (Matsumoto et al., 2006b)

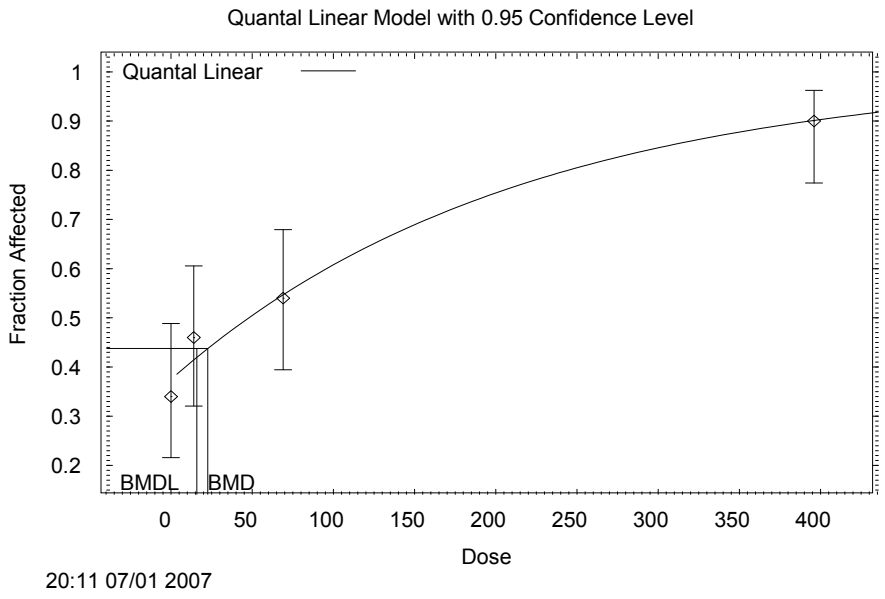

Figure C-3. Observed and Predicted Incidences of Hemosiderin Deposition of the Spleen in Female Mice Exposed to Dietary *o*-Chloronitrobenzene for 2 Years (Quantal-Linear Model) (Matsumoto et al., 2006b)

APPENDIX D. BENCHMARK DOSE ANALYSIS OF ORAL DATA FOR LIVER TUMORS IN MALE AND FEMALE MICE FOR DERIVATION OF THE CANCER ORAL SLOPE FACTOR

Description of Model Fitting Procedure for Dichotomous Data

The model fitting procedure for dichotomous data is as follows. All available dichotomous models in the EPA BMDS (version 1.4.1c) are fit to the incidence data using the extra risk option. The multistage model is run for all polynomial degrees up to *n*-1 (where *n* is the number of dose groups including control). Goodness-of-fit is assessed by the χ^2 test. When several models provide adequate fit to the data (χ^2 $p \geq 0.1$), models are compared using the Akaike Information Criterion (AIC). The model with the lowest AIC is considered to provide the best fit to the data. When several models have the same AIC, the model resulting in the lowest BMDL is selected. In accordance with U.S. EPA (2000) guidance, benchmark doses (BMDs) and lower bounds on the BMD (BMDLs) associated with an extra risk of 10% are calculated for all models. If after these attempts, no model provides an adequate fit to the data, the highest dose is dropped, if appropriate, and the entire procedure is repeated. Dose dropping continues until: (1) adequate fit is obtained; (2) there are only controls and two dose groups remaining. If no fit is obtained following application of this procedure, than the data set is not considered to be amenable to BMD modeling.

Results of Model Fitting for Datasets of Interest

The Matsumoto et al. (2006b) dataset was modeled for chronic oral exposure to *o*-chloronitrobenzene. Table D-1 presents the results of BMD modeling for female and male mice using adenoma or carcinoma, carcinoma only, or hepatoblastoma endpoints. As shown in the table, dropping the high dose in both the adenoma and carcinoma and in the hepatoblastoma endpoints was required to provide an adequate fit. A graph of the multistage model for incidences of adenoma or carcinoma in female mice is shown in Figure D-1.

Table D-1. Summary Benchmark Dose Statistics for Liver Tumors in Male and Female Mice Exposed to Dietary *o*-Chloronitrobenzene for 2 Years[a]

Model	Degrees of Freedom	χ^2 Test Statistic	χ^2 *p*-Value[b]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Male Mice – Adenoma or Carcinoma (All Doses Included)						
Multi-Stage 1-Degree[c]	2	4.56	0.1025	271.419	60.2705	33.6746
Female Mice – Adenoma or Carcinoma (All Doses Included)						
Multi-Stage 1-Degree[c]	2	224.76	0.0000	178.002	5.43928	4.05897
Female Mice – Adenoma or Carcinoma (High Dose Dropped)						
Multi-Stage 1-Degree[c]	**1**	**1.18**	**0.2783**	**134.588**	**2.7762**	**2.10539**
Female Mice – Carcinoma (All Doses Included)						
Multi-Stage 1-Degree[c]	3	2.73	0.4346	103.86	16.7123	13.1623
Male Mice –Hepatoblastoma (All Doses Included)						
Multi-Stage 1-Degree[c]	2	25.58	0.0000	169.195	10.2258	8.09239
Male Mice –Hepatoblastoma (High Dose Dropped)						
Multi-Stage 1-Degree[c]	1	2.73	0.0983	114.588	5.54602	4.25843

[a]Matsumoto et al., 2006b
[b]Values <0.1 fail to meet conventional goodness-of-fit criteria
[c]Betas restricted to ≥0

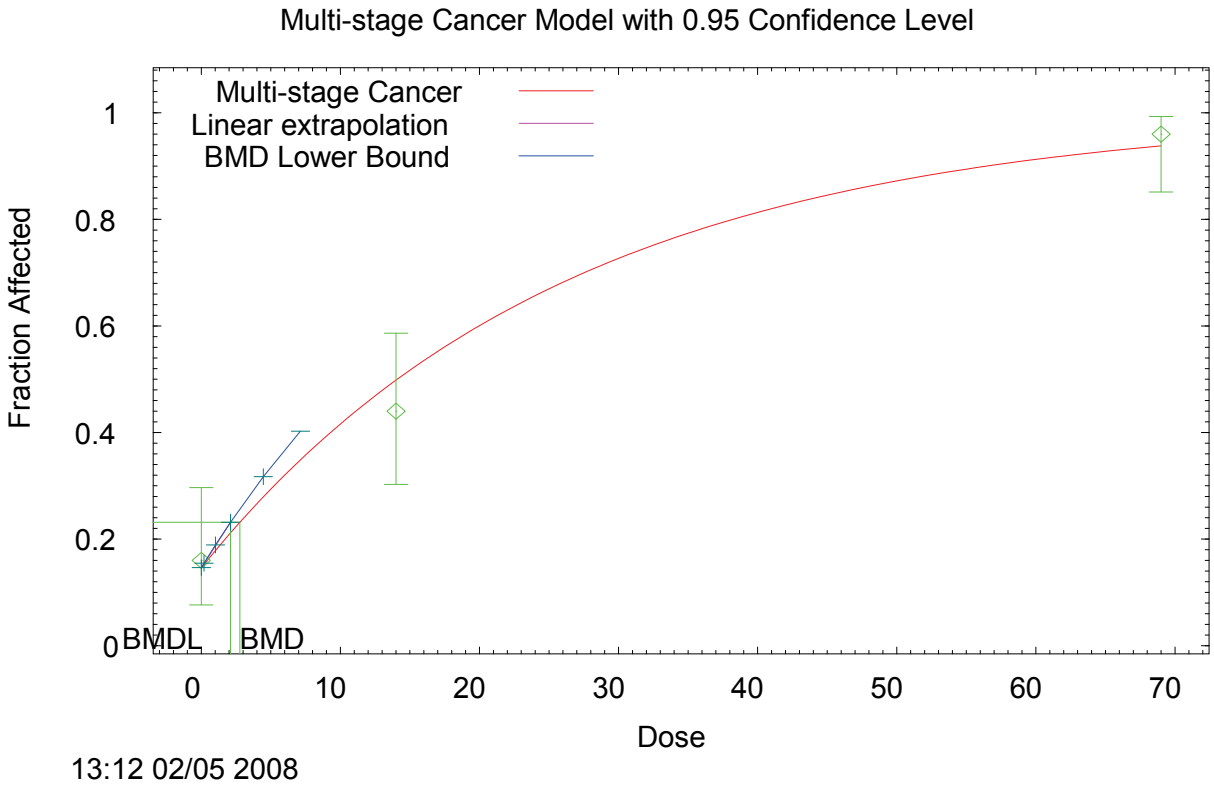

Figure D-1. Multi-stage Cancer Model of Observed and Predicted Incidences of Adenoma or Carcinoma in Female Mice Exposed to Dietary *o*-Chloronitrobenzene for 2 Years, with High Dose Dropped (Matsumoto et al., 2006b)