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Provisional Peer-Reviewed Toxicity Values for

p-Chloronitrobenzene
(CASRN 100-00-5)

Superfund Health Risk Technical Support Center
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COMMONLY USED ABBREVIATIONS AND ACRONYMS

α2u-g	alpha 2u-globulin
ACGIH	American Conference of Governmental Industrial Hygienists
AIC	Akaike's information criterion
ALD	approximate lethal dosage
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CAS	Chemical Abstracts Service
CASRN	Chemical Abstracts Service Registry Number
CBI	covalent binding index
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CNS	central nervous system
CPN	chronic progressive nephropathy
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DEN	diethylnitrosamine
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GSH	glutathione
GST	glutathione-S-transferase
Hb/g-A	animal blood-gas partition coefficient
Hb/g-H	human blood-gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
IVF	in vitro fertilization
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose
NAG	N-acetyl-β-D-glucosaminidase
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
OCT	ornithine carbamoyl transferase
ORD	Office of Research and Development
PBPK	physiologically based pharmacokinetic
PCNA	proliferating cell nuclear antigen
PND	postnatal day
POD	point of departure
POD_{ADJ}	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RBC	red blood cell
RDS	replicative DNA synthesis
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	glutamic oxaloacetic transaminase, also known as AST
SGPT	glutamic pyruvic transaminase, also known as ALT
SSD	systemic scleroderma
TCA	trichloroacetic acid
TCE	trichloroethylene
TWA	time-weighted average
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_H	intraspecies uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
UF_D	database uncertainty factor
U.S.	United States of America
WBC	white blood cell

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR *p*-CHLORONITROBENZENE (CASRN 100-00-5)

BACKGROUND

A Provisional Peer-Reviewed Toxicity Value (PPRTV) is defined as a toxicity value derived for use in the Superfund Program. PPRTVs are derived after a review of the relevant scientific literature using established Agency guidance on human health toxicity value derivations. All PPRTV assessments receive internal review by a standing panel of National Center for Environment Assessment (NCEA) scientists and an independent external peer review by three scientific experts.

The purpose of this document is to provide support for the hazard and dose-response assessment pertaining to chronic and subchronic exposures to substances of concern, to present the major conclusions reached in the hazard identification and derivation of the PPRTVs, and to characterize the overall confidence in these conclusions and toxicity values. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of this substance.

The PPRTV review process provides needed toxicity values in a quick turnaround timeframe while maintaining scientific quality. PPRTV assessments are updated approximately on a 5-year cycle for new data or methodologies that might impact the toxicity values or characterization of potential for adverse human health effects and are revised as appropriate. It is important to utilize the PPRTV database (http://hhpprtv.ornl.gov) to obtain the current information available. When a final Integrated Risk Information System (IRIS) assessment is made publicly available on the Internet (http://www.epa.gov/iris), the respective PPRTVs are removed from the database.

DISCLAIMERS

The PPRTV document provides toxicity values and information about the adverse effects of the chemical and the evidence on which the value is based, including the strengths and limitations of the data. All users are advised to review the information provided in this document to ensure that the PPRTV used is appropriate for the types of exposures and circumstances at the site in question and the risk management decision that would be supported by the risk assessment.

Other U.S. Environmental Protection Agency (EPA) programs or external parties who may choose to use PPRTVs are advised that Superfund resources will not generally be used to respond to challenges, if any, of PPRTVs used in a context outside of the Superfund program.

This document has been reviewed in accordance with U.S. EPA policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

QUESTIONS REGARDING PPRTVs

Questions regarding the contents and appropriate use of this PPRTV assessment should be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

INTRODUCTION

p-Chloronitrobenzene, CASRN 100-00-5, is a widely used chemical intermediate in the production and synthesis of various compounds, including dyes, drugs, and chemicals. Although *p*-chloronitrobenzene is solid at room temperature, the vapor pressure of this chemical is sufficiently high to result in a significant exposure via inhalation (NIOSH/OSHA, 1978). The chemical structure of *p*-chloronitrobenzene is depicted in Figure 1 and its molecular formula is $C_6H_4ClNO_2$. Table 1 shows the physicochemical properties of *p*-chloronitrobenzene.

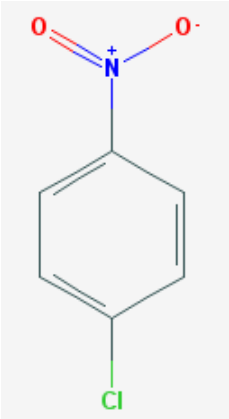

Figure 1. Chemical Structure of *p*-Chloronitrobenzene

Table 1. Physicochemical Properties of *p*-Chloronitrobenzene (CASRN 100-00-5)

Property (unit)	Value
Boiling point (°C)	242[a]
Melting point (°C)	83.5[a]
Density (g/cm^3)	1.52[b]
Vapor pressure (mm Hg at 25°C)	2.19×10^{-2}[b]
Log octanol-water partition coefficient (unitless)	2.39[a]
Henry's law constant (atm-m^3/mol)	4.89×10^{-6}[a]
pH (unitless)	Neutral[b]
Solubility in water (mg/L at 20°C)	225[a]
Relative vapor density (air = 1)	5.44[b]
Molecular weight (g/mol)	157.56[b]

[a]ChemIDplus (2014).
[b]HSDB (2014).

A summary of available toxicity values for *p*-chloronitrobenzene from U.S. EPA and other agencies/organizations is provided in Table 2.

Table 2. Summary of Available Toxicity Values for *p*-Chloronitrobenzene (CASRN 100-00-5)

Source/ Parameter[a]	Value (applicability)	Notes	Reference
Noncancer			
ACGIH	8-hr TLV−TWA = 0.1 ppm (0.64 mg/m^3)	Established to protect against methemoglobinemia and resulting anoxia and cyanosis.	ACGIH (2015)
	BEI = 1.5% of hemoglobin	Listed as methemoglobin inducer.	ACGIH (2015)
ATSDR	NV	NA	ATSDR (2015)
Cal/EPA	NV	NA	Cal/EPA (2011); Cal/EPA (2015b); Cal/EPA (2015a)
OSHA	8-hour PEL−TWA = 1 mg/m^3	NA	OSHA (2011); OSHA (2006)
IRIS	NV	NA	U.S. EPA (2015)
DWSHA	NV	NA	U.S. EPA (2012a)
HEAST	NV	NA	U.S. EPA (2011a)
NIOSH	NV	NA	NIOSH (2015)
HEED	Subchronic p-RfD = 1×10^{-3} mg/kg-d	Draft proposed to use the chronic p-RfD as the provisional subchronic p-RfD because subchronic oral data were available only in abstract form at the time and could not be fully evaluated.	SRC (1992); U.S. EPA (1985)
	Chronic p-RfD = 1×10^{-3} mg/kg-d	Based upon a hematological endpoint (methemoglobinemia) in rats.	
	Subchronic p-RfC = 2×10^{-2} mg/m^3	Based on a preliminary report of a subchronic inhalation assay by the NTP (1993).	
	Chronic p-RfC = 2×10^{-3} mg/m^3	Applied an additional UF of 10 (for the use of a subchronic-duration study) to the subchronic p-RfC.	
CARA HEEP	NV	NA	U.S. EPA (1985)
NTP	Subchronic p-RfC LOAEL = 9.67 mg/m^3	Based on preliminary report of a subchronic inhalation assay. This study reported anemia, methemoglobinemia, and liver effects in rats exposed to *p*-chloronitrobenzene vapor.	ChemIDplus (2015); Travlos et al. (1996)
	NV	NA	NTP (2014)
WHO	NV	NA	WHO (2015)

Table 2. Summary of Available Toxicity Values for *p*-Chloronitrobenzene (CASRN 100-00-5)

Source/ Parameter[a]	Value (applicability)	Notes	Reference
Cancer			
ACGIH	Category 3A, *"Confirmed Animal Carcinogen with Unknown Relevance to Humans"*	NA	ACGIH (2015)
IRIS	NV	NA	U.S. EPA (2015)
HEAST	Group B2, *"Possible Human Carcinogen"*	Based on no evidence in humans and positive evidence in mice (U.S. EPA, 1985). The classification was based on the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005).	U.S. EPA (2011a)
	OSF = 1.8×10^{-2} $(mg/kg\text{-}d)^{-1}$		
	OUR = 5.1^{-7} $(\mu g/L)^{-1}$		
	NV	NA	U.S. EPA (2011a)
HEED	WOE: Group C, *"Possible Human Carcinogen"*	Based on no evidence in humans and positive evidence in mice. The classification was based on the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986).	SRC (1992)
	OSF = 1.2×10^{-2} $(mg/kg\text{-}d)^{-1}$	This derivation employed the cube root of the BW ratio for scaling from animal to human doses (U.S. EPA, 1986).	
CARA HEEP	Human Slope Factor = 1.8×10^{-2} $(mg/kg\text{-}d)^{-1}$	Based on incidences of vascular tumors in male mice (compared to matched-controls) exposed to *p*-chloronitrobenzene in the diet for 18 months (Weisburger et al., 1978).	U.S. EPA (1985)
NTP	NV	NA	ChemIDplus (2015)
IARC	Group 3, *"Not Classifiable as to Its Carcinogenicity to Humans"*	Based on an absence of data in humans and inadequate data in animals.	IARC (1996)
NIOSH	REL = "Ca"	NIOSH did not list a REL for *p*-chloronitrobenzene because of reported carcinogenicity in exposed animals (vascular and hepatic tumors) (skin exposure).	NIOSH (2015)

Table 2. Summary of Available Toxicity Values for *p*-Chloronitrobenzene (CASRN 100-00-5)

Source/ Parameter[a]	Value (applicability)	Notes	Reference
Cal/EPA	*"Known to the State [of California] to Cause Cancer"*	Listed under Proposition 65 (Added 10-29-1999).	Cal/EPA (2015a)
	NV	NA	Cal/EPA (2011); Cal/EPA (2015b)

[a]Sources: ACGIH = American Conference of Governmental Industrial Hygienists; ATSDR = Agency for Toxic Substances and Disease Registry; Cal/EPA = California Environmental Protection Agency; CARA = Chemical Assessments and Related Activities; DWSHA = Drinking Water Standards and Health Advisories; HEAST = Health Effects Assessment Summary Tables; HEED = Health and Environmental Effects Document; HEEP = Health and Environmental Effects Profile; IARC = International Agency for Research on Cancer; IRIS = Integrated Risk Information System; NIOSH = National Institute for Occupational Safety and Health; NTP = National Toxicology Program; OSHA = Occupational Safety and Health Administration; WHO = World Health Organization.

BEI = Biological Exposure Indices; BW = body weight; "Ca" = potential occupational carcinogen; LOAEL = lowest-observed-adverse-effect level; NA = not applicable; NV = not available; OSF = Oral Slope Factor; OUR = Oral Unit Risk; PEL = permissible exposure level; p-RfC = provisional inhalation reference concentration; p-RfD = provisional oral reference dose; REL = recommended exposure level; TLV = threshold limit value; TWA = time-weighted average; UF = uncertainty factor; WOE = weight of evidence.

Literature searches were conducted on sources published from 1900 through October 2014 for studies relevant to the derivation of provisional toxicity values for *p*-chloronitrobenzene (CASRN 100-00-5). The following databases were searched by chemical name, synonyms, or CASRN: ACGIH, ANEUPL, ATSDR, BIOSIS, Cal/EPA, CCRIS, CDAT, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HERO, HMTC, HSDB, IARC, INCHEM IPCS, IPA, ITER, IUCLID, LactMed, NIOSH, NTIS, NTP, OSHA, OPP/RED, PESTAB, PPBIB, PPRTV, PubMed (toxicology subset), RISKLINE, RTECS, TOXLINE, TRI, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA HEEP, U.S. EPA OW, and U.S. EPA TSCATS/TSCATS2. The following databases were searched for relevant health information: ACGIH, ATSDR, Cal EPA, U.S. EPA IRIS, U.S. EPA HEAST, U.S. EPA HEEP, U.S. EPA OW, U.S. EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS. An Organization for Economic Co-operation and Development Screening Information Datasets (OECD SIDS) submission from Bayer (OECD, 2002) and toxicity reviews on aromatic nitro, amino, and nitro-amino compounds and their halogenated derivatives (Weisburger and Hudson, 2001; Woo and Lai, 2001) also were consulted for relevant information.

REVIEW OF POTENTIALLY RELEVANT DATA (NONCANCER AND CANCER)

Tables 3A and 3B provide an overview of the relevant database for *p*-chloronitrobenzene and include all potentially relevant repeated-dose short-term-, subchronic-, and chronic-duration studies. Principal studies are identified in bold. The phrase "statistical significance," used throughout the document, indicates a p-value < 0.05, unless otherwise indicated.

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Chloronitrobenzene (CASRN 100-00-5)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/BMCL[a]	LOAEL[a]	Reference (comments)	Notes[b]
Human								
1. Oral (mg/kg-d)[a]								
ND								
2. Inhalation (mg/m^3)[a]								
Acute	8 M/0 F, occupational retrospective cohort, no exposure duration information reported	NV	Nausea, headache, faintness, cyanosis, and anemia due to methemoglobinemia	NV	NA	NV	Yoshida et al. (1987); SRC (1992) (Absorbed dose was estimated from the metabolite excreted data)	PR
Short-term	23 subjects (1 hr/d for 14 d), 39 subjects (1.5 hr/d for 15 d), 6 subjects (8 hr/d for 16 d), sex not specified in any of the groups, occupational retrospective cohort	8.6, 19.6, or 22.3 mg/m^3	Increased methemoglobin, appearance of Heinz bodies, headache, vertigo, and eczema were reported in an unknown number of subjects/exposure group	NV	NA	NV	ACGIH (2001); Pacseri et al. (1958) (Skin absorption could not be excluded)	PR
Subchronic	12 (sex not specified), occupational retrospective cohort, intermittently for 0.5−1 hr/d for several m	6.44 to 393.09 mg/m^3 (average = 88.28 mg/m^3)	Tiredness, loss of appetite, headache, and afternoon fatigue	NV	NA	NV	NIOSH (1994); Watrous and Schultz (1950)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Chloronitrobenzene (CASRN 100-00-5)								
Category	**Number of Male/Female, Strain, Species, Study Type, Study Duration**	**Dosimetry[a]**	**Critical Effects**	**NOAEL[a]**	**BMDL/BMCL[a]**	**LOAEL[a]**	**Reference (comments)**	**Notes[b]**
Chronic	36 M/2 F, occupational retrospective cohort, 1−16 yr with a median of 6 yr	NV	NV	NV	NA	NV	Jones et al. (2007)	PR
Animal								
1. Oral (mg/kg-d)[a]								
Subchronic	10 M/10 F per group, S-D (Crl:COBS CD [SD]BR), rat, diet, 7 d/wk for 4 wk	ADD: 0, 12.6, 33.1, 66.8, 97.6, or 223.5 (M) 0, 14.2, 34.8, 73.1, 112.4, or 257.1 (F)	Splenomegaly, and abnormal coloration of the spleen (M and F)	NA	NDr	12.6 (M) 14.2 (F)	Monsanto (1994c)	TR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Chloronitrobenzene (CASRN 100-00-5)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/BMCL[a]	LOAEL[a]	Reference (comments)	Notes[b]
Subchronic	**20 M/20 F per group, S-D (Crl:COBS CD [SD]BR), rat, gavage, 7 d/wk for 90 d**	**ADD: 0, 3, 10, or 30 (M and F)**	**Male: hematology effects (methemoglobinemia, decreased erythrocyte counts and hemoglobin concentration, increased reticulocyte counts) and splenic effects (increased relative spleen weight, splenic hematopoiesis, and splenic hemosiderosis)** **Female: hematology effects (methemoglobinemia, decreased erythrocyte counts and hemoglobin concentration, increased reticulocyte counts) and splenic effects (increased relative spleen weight, and splenic hematopoiesis)**	**NA**	**Male: methemoglobinemia = 0.084; decreased hemoglobin = 0.24; increased splenic hematopoiesis = 0.060** **Female: decreased erythrocyte counts = 0.59; decreased hemoglobin = 0.20**	**3**	**Monsanto (1994b)**	**TR, PS**
	10 M/10 F per group, F344/DuCrj, rat, diet, 7 d/wk for 13 wk	Dietary target doses: 0, 24.7, 74.1, 222, 667, or 2,000 ppm ADD: 0, 1.2, 3.4, 11.8, 38.8, or 122.8 (M) 0, 1.4, 4.1, 13.8, 45.0, or 145.0 (F)	Male: hematology effects (decreased erythrocyte counts, and hemoglobin concentration) and splenic lesions (increased hemosiderin, congestion, and extramedullary hematopoiesis) Female: decreased erythrocyte counts	1.2 (M) NA (F)	Male: decreased erythrocyte counts = 0.59; increased extramedullary hematopoiesis = 0.81 Female: decreased erythrocyte counts = 1.43	3.4 (M) 1.4 (F)	Matsumoto et al. (2006b) (LOAEL with unknown biological significance in females)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Chloronitrobenzene (CASRN 100-00-5)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/BMCL[a]	LOAEL[a]	Reference (comments)	Notes[b]
Subchronic	10 M/10 F per group, Crj:BDF_1, mouse, diet, 7 d/wk for 13 wk	Dietary target doses: 0, 74.1, 222, 667, 2,000, or 6,000 ppm ADD: 0, 7.8, 27.4, 86.8, 280.1, or 659.5 (M) 0, 10.5, 36.7, 120.5, 334.3, or 856.5 (F)	Male: hemosiderin deposition and increased extramedullary hematopoiesis in the spleen Female: hemosiderin deposition in the spleen	27.4 (M) 10.5 (F)	NDr	86.8 (M) 36.7 (F)	Matsumoto et al. (2006b)	PR
Chronic	60 M/60 F per group, CD (S-D-derived), rat, daily gavage for 24 m	ADD: 0, 0.1, 0.7, or 5.0 (M and F)	Methemoglobinemia (M and F)	0.1 (M and F)	0.13 (M) 0.12 (F)	0.7 (M and F)	Bio Dynamics (1985)	TR, PS
	50 M/50 F per group, F344/DuCrj, rat, diet for 2 yr	Dietary target doses: 0, 40, 200, or 1,000 ppm ADD: 0, 1.5, 7.7, or 41.2 (M) 0, 1.9, 9.8, or 53.8 (F)	Male: splenic effects (increased splenic nodules, splenic capsule hyperplasia, splenic fibrosis and splenic fatty metamorphosis), increased relative liver weight and increased relative kidney weight Female: hematology effects (decreased erythrocyte counts and hemoglobin concentration) and splenic effects (increased splenic capsule hyperplasia, splenic fibrosis and splenic fatty metamorphosis)	1.5 (M) 1.9 (F)	NDr	7.7 (M) 9.8 (F)	Matsumoto et al. (2006a) (Both sexes affected at same dietary concentration)	PR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Chloronitrobenzene (CASRN 100-00-5)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/BMCL[a]	LOAEL[a]	Reference (comments)	Notes[b]
Chronic	50 M/50 F per group, Crj:BDF_1, mouse, diet for 2 yr	Dietary target doses: 0, 125, 500, or 2,000 ppm ADD: 0, 15.3, 60.1, or 240.1 (M) 0, 17.6, 72.6, or 275.2 (F)	Male: decreased erythrocyte counts and extramedullary hematopoiesis in the spleen Female: hematology effects (decreased erythrocyte counts and hematocrit percentage)	15.3 (M) 17.6 (F)	NDr	60.1 (M) 72.6 (F)	Matsumoto et al. (2006a) (Both sexes affected at same dietary concentration)	PR
Reproductive/ Developmental Toxicity	15 M/30 F per group, S-D CD, rat, gavage during premating, mating, gestation, and lactation periods for two generations. F0 adults were treated for 167 d (including 100 d premating), and F1 adults were treated for 217−219 d (including 120 d from birth to mating)	ADD: 0, 0.1, 0.7, or 5.0 (M and F)	Testicular effects (oligospermia, degeneration and reduced fertility) in F0 males	NA	NDr	5.0	Bio Dynamics (1984) (Failure to perform histological exam on F0 males at low and mid doses precludes identification of NOAEL)	TR
	20 M/20 F per group, Swiss CD-1, mouse, gavage 7 d precohabitation and 98 d during cohabitation	ADD: 0, 62.5, 125, or 250 (M and F)	Reduced body weight in F1 male pups	NA	NDr	62.5	Chapin et al. (1997); Gulati et al. (1991); NTP (1993)	PR, TR

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Chloronitrobenzene (CASRN 100-00-5)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/BMCL[a]	LOAEL[a]	Reference (comments)	Notes[b]
Reproductive/ Developmental Toxicity	0 M/24 F pregnant per group, S-D, rat, gavage GDs 6−19	ADD: 0, 5, 15, or 45	Maternal: increased absolute spleen weight Fetal: decreased fetal weight and increased skeletal anomalies and resorptions	Maternal: NA Fetal: 15	Maternal: increased absolute spleen weight = 1.43 Fetal: NDr	Maternal: 5 Fetal: 45	Bio Dynamics (1980); Nair et al. (1985)	TR, PR
	0 M/18 F pregnant per group, New Zealand white, rabbit, gavage GDs 7−19	ADD: 0, 5, 15, or 40	Maternal mortality and spontaneous abortions	Maternal: 15 Fetal: 15	NDr	Maternal: 40 (FEL) Fetal: NA	Bio Dynamics (1982) Due to high mortality in the high dose group, the treatment was terminated.	TR
2. Inhalation (mg/m^3)[a]								
Short-term	10 M/10 F per group, S-D, rat, inhalation, 6 hr/day, 5 d/wk for 4 wk	0, 5, 16, or 46 HEC: 0, 0.89, 2.86, or 8.21 (M and F)	Male: methemoglobinemia Female: reduced hematocrit (increased splenic hemosiderosis reported but no actual data shown)	NA (M and F)	NDr (M and F)	0.89 (M and F) [HEC equivalent = 0.89]	Nair et al. (1986) (Co-exposure to 2-ethoxyethanol vehicle)	PR
Subchronic	**10 M/10 F per group, F344/N, rat, inhalation, 6 hr/d, 5 d/wk for 13 wk**	**0, 1.5, 3, 6, 12, or 24 ppm** **HEC: 0, 1.7, 3.4, 6.9, 13.8, or 27.5 (M and F)**	**Hematology effects (methemoglobinemia, decreased erythrocyte counts and hematocrit and increased reticulocyte counts) and splenic effects (increased congestion and hemosiderin deposition) (M and F)**	**NA (M and F)**	**Male: decreased erythrocyte counts = 0.83; decreased hematocrit = 0.50** **Female: decreased hematocrit = 0.18**	**1.7 (M and F)** [HEC equivalent = 1.7]	**NTP (1993); Travlos et al. (1996)**	**TR, PR, PS**

Table 3A. Summary of Potentially Relevant Noncancer Data for *p*-Chloronitrobenzene (CASRN 100-00-5)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/BMCL[a]	LOAEL[a]	Reference (comments)	Notes[b]
Subchronic	10 M/10 F per group, $B6C3F_1$, mouse, inhalation, 6 hr/d, 5 d/wk for 13 wk	0, 1.5, 3, 6, 12, or 24 ppm HEC: 0, 1.7, 3.4, 6.9, 13.8, or 27.5 (M and F)	Male: splenic effects (increased absolute and relative weights, increased hemosiderin deposition and hematopoietic cell proliferation) and increased absolute liver weight Female: splenic effects (increased absolute and relative weights, increased hemosiderin deposition and hematopoietic cell proliferation) and increased absolute and relative liver weights	6.9 (M and F) [HEC equivalent = 6.9]	NDr	13.8 (M and F) [HEC equivalent = 13.8]	NTP (1993); Travlos et al. (1996)	TR, PR
Chronic	ND							
Developmental	ND							
Reproductive	ND							

[a]Dosimetry: Values are presented as Adjusted Daily Dose (ADD, in mg/kg-day) for oral noncancer effects and as Human Equivalent Concentration (HEC, in mg/m^3) for inhalation noncancer effects; HEC = (ppm × MW ÷ 24.45) × (hours per day exposed ÷ 24) × (days exposed ÷ total days observed) × blood-air partition coefficient (U.S. EPA, 1994).

[b]Notes: PS = principal study; PR = peer reviewed; TR = technical report. Treatment/exposure duration, unless otherwise noted: Short-term = repeated exposure for >24 hours ≤30 days (U.S. EPA, 2002); Long-term (Subchronic) = repeated exposure for >30 days ≤10% lifespan for humans (more than 30 days up to approximately 90 days in typically used laboratory animal species) (U.S. EPA, 2002); Chronic = repeated exposure for >10% lifespan for humans (more than approximately 90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2002).

F = female; GD = gestation day; M = male; NA = not applicable; ND = no data; NDr = not determined; NV = not available; S-D = Sprague-Dawley.

Table 3B. Summary of Potentially Relevant Cancer Data for *p*-Chloronitrobenzene (CASRN 100-00-5)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (comments)	Notes[b]
Human								
1. Oral (mg/kg-d)[a]								
ND								
2. Inhalation (mg/m^3)[a]								
ND								
Animal								
1. Oral (mg/kg-d)[a]								
Carcinogenicity	25 M/25 F, CD, rat, diet for 18 m	Dietary time weighted average doses: 0, 722, or 1,444 mg/kg HED: 0, 15, or 29	No significant effects were observed	NA	NDr	NA	Weisburger et al. (1978)	PR
	25 M/25 F per group, CD-1, mouse, diet for 18 m	Dietary target doses: 0, 3,000, or 6,000 mg/kg HED: 0, 75.3, or 150.3 (M); 0, 71.8, or 143.7 (F)	Unspecified vascular tumors (M and F)	NA	NDr	NA	Weisburger et al. (1978)	PR

Table 3B. Summary of Potentially Relevant Cancer Data for *p*-Chloronitrobenzene (CASRN 100-00-5)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a]	Reference (comments)	Notes[b]
Carcinogenicity	**50 M/50 F per group, F344/DuCrj, rat, diet for 2 yr**	**Dietary target doses: 0, 40, 200, or 1,000 ppm** **HED: 0, 0.41, 2.1, or 11.1 (M); 0, 0.49, 2.5, or 13.2 (F)**	**Male: splenic fibroma, fibrosarcoma, osteosarcoma, sarcoma not otherwise specified (NOS), and hemangiosarcoma** **Female: splenic fibrosarcoma and adrenal pheochromocytoma**	**NA**	**Male: fibroma = 3.64; fibrosarcoma = 3.70; osteosarcoma = 6.01; sarcoma NOS = 5.55; hemangiosarcoma = 3.60 hemangiosarcoma = 1.56 (with high-dose data dropped from BMD analysis)** **Female: fibrosarcoma = 5.82; pheochromocytoma = 3.15** **Spleen fibrosarcoma and adrenal glands pheochromocytoma = 2.94 (MS combo)**	**NA**	**Matsumoto et al. (2006a)**	**PR, PS**
	50 M/50 F per group, Crj:BDF_1, mouse, diet for 2 yr	Dietary target doses: 0, 125, 500, or 2,000 ppm HED: 0, 2.27, 8.99, or 35.64 (M); 0, 2.51, 10.4, or 39.26 (F)	Male: no significant effects were observed Female: hepatic hemangiosarcoma	NA	NDr	NA	Matsumoto et al. (2006a)	PR
2. Inhalation (mg/m^3)[a]								
ND								

[a]Dosimetry: The units for oral exposures are expressed as human equivalent dose (HED, mg/kg-day). HED = ADD × default dosimetric adjustment factor (U.S. EPA, 2011b).
[b]PS = principal study; PR = peer reviewed.

F = female; M = male; NA = not applicable; ND = no data; NDr = not determined.

HUMAN STUDIES

Oral Exposures

No data were located regarding oral exposure of humans to *p*-chloronitrobenzene.

Inhalation Exposures

Yoshida et al. (1987); SRC (1992)

In acute or subchronic-duration occupational exposures to *p*-chloronitrobenzene, the chemical was thought to have been absorbed by inhalation and transdermally. Following a combined accidental inhalation-dermal exposure to *p*-chloronitrobenzene, eight dock workers were hospitalized with headache, nausea, faintness, cyanosis, and anemia due to methemoglobinemia. Collected excreted metabolite data indicated that the workers absorbed a total of 12−76 mg/kg of *p*-chloronitrobenzene (SRC, 1992; Yoshida et al., 1987).

ACGIH (2001); Pacseri et al. (1958)

Increased methemoglobin, Heinz bodies, headache, vertigo, and eczema were observed in workers exposed to *p*-chloronitrobenzene at average concentrations of 8.6 mg/m^3 (1 hour/day for 14 days), 19.6 mg/m^3 (1.5 hours/day for 15 days), and 22.3 mg/m^3 (8 hours/day for 16 days). Because skin absorption could not be determined with any degree of precision, the study authors concluded that in addition to inhalation, absorption through the skin may have played a role in the development of these changes (ACGIH, 2001; Pacseri et al., 1958).

NIOSH (1994); Watrous and Schultz (1950)

A group of 12 workers exposed intermittently to *p*-chloronitrobenzene for 0.5−1 hour/day over several months at concentrations ranging from 6.44−393.09 mg/m^3 (average = 88.28 mg/m^3) reported symptoms of tiredness, loss of appetite, headache, and afternoon fatigue (NIOSH, 1994; Watrous and Schultz, 1950).

Jones et al. (2007)

Jones et al. (2007) studied the relationship of chronic inhalation exposure to chloronitrobenzenes with urinary metabolites and its health effects in human workers. Mercapturic acid *N*-acetyl-S-(4-nitrophenyl)-L-cysteine (NANPC), 4-chloroaniline (4CA), and 2-chloro-5-nitrophenol (CNP) were the most prevalent metabolites detected in all the exposed workers (but were absent in controls). However, the prediction of health effects using the urinary concentrations was ambiguous. The metabolite levels of 4CA and NANPC correlated well with hemoglobin adduct levels, but no other information about adducts were provided in the study.

ANIMAL STUDIES

Oral Exposures

Subchronic-Duration Studies

The subchronic-duration oral database includes three studies: a 4-week range-finding study in rats (Monsanto, 1994c), a 90-day study in rats (Monsanto, 1994b), and a 13-week study in rats and mice (Matsumoto et al., 2006b).

Monsanto (1994c)

In the 4-week range-finding study, Monsanto (1994c) fed 10 Sprague-Dawley (S-D) (Crl:COBS CD> [SD]BR) rats/sex/group *p*-chloronitrobenzene (99.4% purity in the diet at measured doses of 0, 12.6, 33.1, 66.8, 97.6, or 223.5 mg/kg-day for males and 0, 14.2, 34.8, 73.1,

112.4, or 257.1 mg/kg-day for females (main contaminants, *ortho*- and *mono*-nitrochlorobenzene were present at levels considered to be too low to interfere with the study). Animals were observed daily for mortality and obvious signs of toxicity. Body weight and food consumption were recorded weekly. Gross necropsy was performed in animals that died spontaneously and in all the animals that survived to the end of the study period. Deaths occurred only in females of the highest-dose group (3/10) during the third week of the study. By the end of the study, body-weight gain was significantly (>10%) reduced in males at ≥66.8 mg/kg-day and in females at ≥112.4 mg/kg-day. Decreases in food consumption were dose related in males with the highest-dose group being the most affected (>10%). Female dose groups showed a slightly different pattern, with decreases in food consumption (>10%) at 73.1 mg/kg-day (Week 1), 112.4 mg/kg-day (Weeks 1 and 2) and 257.1 mg/kg-day throughout the study. Increasing severity of paleness of eyes, ears, and feet was observed in both sexes at ≥12.6 mg/kg-day. This paleness advanced to cyanosis by the fourth week in males at the highest-dose group and in females at ≥112.4 mg/kg-day. Monsanto (1994c) considered other signs of physical deterioration (emaciation, squinting eyes, and piloerection) to be secondary to cyanosis. All treated groups in both sexes—but no control rats—exhibited splenomegaly with abnormal colors in both the spleen (statistically significantly increased in all groups of both sexes) and kidneys (not dose dependent but significant at higher doses) (see Table B-1). These discolorations were attributed to anemia, bilirubinemia from erythrocyte destruction, and methemoglobinemia. Testicular atrophy occurred at the two highest doses. The lowest dose of 12.6 mg/kg-day was a 4-week lowest-observed-adverse-effect level (LOAEL) for splenomegaly and abnormal coloration of spleen in male rats; a no-observed-adverse-effect level (NOAEL) was not identified for this study.

Monsanto (1994b)

Monsanto (1994b) is selected as the principal study for the derivation of the subchronic and chronic provisional reference doses (p-RfDs). Monsanto (1994b) administered 0, 3, 10, or 30 mg/kg-day of 99.12% purity *p*-chloronitrobenzene (main contaminants, *ortho*- and *meta*-isomers of chloronitrobenzene were present at levels considered to be too low to interfere with the study) to groups of S-D (Crl:COBS CD [SD]BR) rats (20/sex/group) by gavage in corn oil daily for 90 days. Animals were observed daily for mortality and obvious signs of toxicity. Body weight and food consumption were recorded weekly. At Days 42 and 43 (Week 7), and 84 and 85 (Week 13), 10 rats/sex/group were subjected to hematological (erythrocyte count, leukocyte count, hemoglobin concentration [Hgb], hematocrit [Hct], mean corpuscular volume [MCV], mean corpuscular hemoglobin concentration [MCHC], mean corpuscular hemoglobin [MCH], and methemoglobin concentration) and serum chemistry (serum glutamic pyruvic transaminase [SGPT], serum alkaline phosphatase [SAP], blood urea nitrogen [BUN], total bilirubin, glucose, total protein, sodium, and potassium) analysis. All rats surviving to Day 90 were subjected to complete gross necropsy, at which time organ weights were recorded for the brain, heart, adrenals, pituitary, kidneys, liver, testis, and spleen. Organs and tissues (i.e., brain, heart, adrenals, pituitary, kidneys, liver, testis/ovary, spleen, aorta, eye, trachea, stomach, skin, pancreas, large intestine, duodenum, jejunum, ileum, lung, mesenteric lymph node, muscle, prostrate/uterus, bone marrow of femur, bone, thyroids, and urinary bladder) containing lesions or abnormal masses in every animal were retained for microscopic examination. Histologic evaluations were performed on all of the above mentioned tissues from the control and high-dose animals. Spleen, liver, and kidney tissues from animals in the high-dose group, which showed statistically significant alterations from the controls, were examined in low- and mid-dose animals, as well. The study

authors observed no compound-related effects on mortality or body weight. Statistically significant dose-related increases in food consumption were observed in males at >10 mg/kg-day after 2 weeks of treatment; whereas no consistent pattern for food consumption was observed in females. The only treatment-related clinical sign was general paleness observed immediately after dosing in males of the high-dose group and females at >10 mg/kg-day.

As a percentage of total hemoglobin, methemoglobin concentrations showed a statistically significant elevation ($p < 0.01$) in all dose groups in both sexes following 90 days of exposure (see Table B-2). Statistically significant decreases in Hgb, Hct, and erythrocyte counts, and increases in reticulocyte counts and urinary urobilinogen were observed in all treatment groups of both sexes in Week 13. Discoloration of the kidneys and spleen in both sexes was observed at the high dose. Statistically significant increases were observed in relative spleen weights at all doses and absolute spleen weights in the mid- and high-dose groups (≥10 mg/kg-day) in both sexes. Absolute and relative weights in the kidney in males at ≥10 mg/kg-day, in the liver in both sexes, and in the heart of females in the high-dose group also showed statistically significant increases. Gross and histopathological changes observed in the spleen included statistically significantly increased hemosiderosis and hematopoiesis (≥3 mg/kg-day), congestion (≥10 mg/kg-day), and vacuolization of red pulp (≥30 mg/kg-day) in both sexes, as well as splenomegaly in the mid- and high-dose groups (≥10 mg/kg-day) of both sexes. At all doses of both sexes, an increase in hemosiderosis (but only statistically significant in mid-dose females) and extramedullary hematopoiesis (but only statistically significant in mid-dose males and mid- and high-dose females) in the liver were observed. A statistically significant increase in hemosiderosis in the kidney of both sexes and hyperplasia of the bone marrow, only in males, was observed at the highest dose. For this 90-day toxicity study, 3 mg/kg-day was a LOAEL for effects on erythrocytes (i.e., methemoglobinemia, decreased erythrocyte counts and Hgb, and increased reticulocyte counts), increased relative spleen weight in male and female rats, splenic hematopoiesis in males and females, and splenic hemosiderosis in males. A NOAEL was not identified for this study.

Matsumoto et al. (2006b)

In a third subchronic-duration study, Matsumoto et al. (2006b) evaluated the subchronic toxicity of *p*-chloronitrobenzene (>99% purity) incorporated into the diets of F344/DuCrj rats and Crj:BDF_1 mice. Groups of 10 animals/sex/group were treated for 13 weeks with *p*-chloronitrobenzene at the dietary concentrations of 0, 24.7, 74.1, 222, 667, or 2,000 ppm (rats) and 0, 74.1, 222, 667, 2,000, or 6,000 ppm (mice). Daily clinical observations were made, and body weight and food consumption were measured weekly. Based on the body weight and food consumption measurements, along with measured concentrations of *p*-chlorobenzene in the diet, the study authors estimated daily intakes of 0, 1.2, 3.4, 11.8, 38.8, or 122.8 mg/kg-day in male rats; 0, 1.4, 4.1, 13.8, 45.0, or 145.0 mg/kg-day in female rats; 0, 7.8, 27.4, 86.8, 280.1, or 659.5 mg/kg-day in male mice; and 0, 10.5, 36.7, 120.5, 334.3, or 856.5 mg/kg-day in female mice. At the termination of treatment, blood samples were collected for hematology (erythrocyte count, Hgb, Hct, and MCV) and serum chemistry (aspartate aminotransferase [AST], alanine aminotransferase [ALT], and total bilirubin). The animals were sacrificed, necropsied, and liver and spleen weights were recorded. Comprehensive histopathology evaluations were performed in accordance with OECD (1998) guidelines for 90-day subchronic-duration rodent studies (32 tissues). The OECD guidelines call for histopathology assessment of all tissues in the control and high-dose groups as well as target organs in remaining dose groups. In this study, the spleen, liver, and bone marrow were assessed in all animals.

In rats, the study authors observed visible evidence of anemia, including discolored and pale skin, eyes, and ears, in both sexes at the two highest doses but no deaths were reported. Body-weight data were not reported, but the study authors indicated that no statistically significant decreases were observed in any group of rats treated with *p*-chloronitrobenzene. Statistically significant decreases in hematologic parameters consistent with anemia occurred at ≥3.4 mg/kg-day in male rats (i.e., decreased erythrocyte count, Hgb, and Hct) and at ≥1.4 mg/kg-day in female rats (i.e., decreased erythrocyte count) (see Table B-3).

A statistically significant increase in bilirubin, likely indicative of erythrocyte destruction, was observed at the two highest doses in both sexes. AST increased by about 20% ($p < 0.01$) at the highest dose in male rats; whereas, ALT decreased by about 20% ($p < 0.01$) in males at ≥38.8 mg/kg-day and in females at the highest dose only (see Table B-3). Statistical analysis of the organ weights was not reported. However, organ-weight data presented graphically indicated a steep dose-dependent increase in relative spleen weights in both male and female rats, and a modest treatment-related increase in relative liver weight in both sexes. At the highest dose, the relative spleen weights increased by about 9-fold over the control weights in males and about 12-fold in females, while the maximum increase in liver weight was less than 50% over controls. Table B-4 summarizes histopathology findings in both sexes, including a statistically significant increase in erythropoiesis in the bone marrow (≥11.8 mg/kg-day) in addition to spleen findings of congestion, hemosiderin deposition, and increased extramedullary hematopoiesis (≥3.4 mg/kg-day) and capsule hyperplasia (≥11.8 kg-day). In the livers of the treated male rats, there was a statistically significant increase in hemosiderin deposition (≥11.8 mg/kg-day), increased extramedullary hematopoiesis (≥38.8 mg/kg-day), and centrilobular hypertrophy (≥122.8 mg/kg-day). Similar hepatic lesions were observed in female rats except that increased extramedullary hematopoiesis was only found at 145.0 mg/kg-day and there was no centrilobular hypertrophy. For this 13-week rat study, the study authors identified a LOAEL of 3.4 mg/kg-day based on hematology (i.e., decreased erythrocyte count and Hgb) and splenic lesions (i.e., increased hemosiderin, congestion, and extramedullary hematopoiesis) with a NOAEL of 1.2 mg/kg-day in male rats. However, in female rats, the LOAEL may be considered 1.4 mg/kg-day based on a modest but statistically significant dose-related decrease in erythrocyte count (see Table B-3); a NOAEL could not be determined.

In the 13-week subchronic-duration mouse study, one female mouse died during Week 7 after the highest-dose treatment. As with the rats, signs of anemia (i.e., pale or discolored skin, eye, and ears) were noted in mice of both sexes at the two highest doses. Body weights were decreased by at least 10% at the highest dose in male mice but not at other doses or in females at any dose (data not shown). Hematology changes consistent with anemia, including statistically significantly decreased erythrocyte counts and Hct, were also observed in mice (see Table B-5). However, these changes occurred at higher doses in mice compared to rats. Statistically significant increases in total bilirubin, AST, and ALT were recorded for male and female mice only at the highest dose. At the highest dose in male mice, the increases in AST and ALT were marked (3.7-fold and 6-fold higher than controls, respectively), indicating hepatocellular toxicity. Dose-dependent increases in relative liver weight (approximately twofold higher than controls at the highest dose for both sexes, based on visual examination of data presented graphically) provided further indication of liver toxicity. As with rats, marked increases in relative spleen weights (more than 10-fold) were observed in both male and female mice. Statistical analysis of the organ-weight data was not provided. Histopathology changes in mice were similar to those in rats and were confined to the spleen, liver, and bone marrow

(see Table B-6). These changes in male mice included statistically significant increase in erythropoiesis and hemosiderin deposition in the bone marrow (≥280.1 kg/kg-day); congestion (≥280.1 mg/kg-day), hemosiderin deposition, and increased extramedullary hematopoiesis in the spleen (≥86.8 mg/kg-day); and hemosiderin deposition, increased extramedullary hematopoiesis (≥280.1 mg/kg-day), and centrilobular hypertrophy (≥659.5 mg/kg-day) in the liver. Except for hemosiderin deposition in the spleen at ≥36.7 mg/kg-day, congestion in spleen at ≥120.5 mg/kg-day, and centrilobular hypertrophy in liver at ≥334.3 mg/kg-day, similar lesions were observed in female mice. Histopathology changes occurred at higher doses in mice compared to rats; in male mice, the lowest dose associated with statistically significant histopathology changes (i.e., hemosiderin deposition and increased extramedullary hematopoiesis in the spleen) was 86.8 mg/kg-day, while in females, it was 36.7 mg/kg-day (i.e., hemosiderin deposition in the spleen). For the mouse-specific portion of the study, the authors identified a 13-week LOAEL of 36.7 mg/kg-day and NOAEL of 10.5 mg/kg-day based on hemosiderin deposition in the spleen of female mice.

Chronic-Duration Studies

Two chronic-duration studies assessed multiple endpoints: one in rats (Bio Dynamics, 1985) and the other in both rats and mice (Matsumoto et al., 2006a). Additionally, Weisburger et al. (1978) and Matsumoto et al. (2006a) studied carcinogenicity in both rats and mice.

Bio Dynamics (1985)

Bio Dynamics (1985) administered 0, 0.1, 0.7, or 5.0 mg/kg-day of *p*-chloronitrobenzene (>99% purity) to groups of CD (S-D-derived) rats (60 rats/sex/group) by gavage in corn oil 7 days/week for 24 months. Rats were examined twice daily for mortality and obvious signs of toxicity and given a thorough physical examination weekly, including palpation for tissue masses. Ophthalmoscopic examinations were given before testing and at 3, 12, and 24 months. Body weights and food consumption were recorded before testing, twice weekly for the first 14 weeks and biweekly thereafter. No apparent differences in food consumption were observed over the duration of the study. Before treatment and after 6, 12, 18, and 24 months of treatment, 10 rats/sex/group were evaluated for hematology (i.e., erythrocyte count, leukocyte count, erythrocyte morphology, Hgb, Hct, platelets, and methemoglobin), clinical chemistry (i.e., SGPT, SAP, BUN, serum glutamic oxaloacetic acid transaminase [SGOT], total bilirubin, glucose, total protein, sodium, calcium, potassium, and lactic acid dehydrogenase), and urinalysis (i.e., specific gravity, gross appearance, pH, protein, glucose, ketones, bilirubin, urobilinogen, occult blood, and microscopic analysis); hematology also was analyzed at 10 months. All rats were given a complete gross necropsy, at which time, selected organ weights were recorded (i.e., brain, heart, adrenals, kidneys, liver, testes, ovaries, and spleen), and tissues (i.e., adrenals, bone and bone marrow [sternum], brain, epididymis, esophagus, eyes, gonads, heart, intestines [duodenum, ileum, colon], kidneys, liver, lungs, lymph node [mesenteric and pulmonary], mammary gland, right sciatic nerve, pancreas, parathyroid, pituitary, prostate, submandibular salivary gland, seminal vesicles, biceps femoris skeletal muscle, skin [with mammary gland], spinal cord [cervical and thoracic], spleen, stomach, thymus, thyroid, trachea, urinary bladder, and uterus) were retained for microscopic examination. Lesions or abnormal masses in all animals, all body tissues posterior to the head in all control and high-dose animals, and the testes, epididymides, and spleens in all low- and mid-dose animals were examined histopathologically. *p*-Chloronitrobenzene had no consistent effect on mortality, body weight, food consumption, ophthalmoscopic examination, clinical chemistry, or urinalysis. Statistically significant increases in blood methemoglobin concentrations were observed in the high-dose

group beginning at 6 months and in mid-dose group beginning at 10 months; significant ($p < 0.01$) elevations were maintained in these groups at all subsequent time points. Table B-7 shows the methemoglobin concentrations at the end of the 24-month treatment period. Slight, but statistically significant anemia, evidenced by reduced Hgb, Hct, and erythrocyte counts, was observed in both sexes at the high dose (5.0 mg/kg-day) from 6−18 months. At 24 months, indices of anemia in males at the high dose were not statistically different from the control values; the authors indicated that mean control values were abnormally low because of anemia in three control males. At 24 months, erythrocyte and Hgb counts were still significantly reduced in the high-dose females. Reticulocytes in both sexes and platelet counts in females were significantly elevated at the high dose at 12 and 18 months and are indicative of anemic compensation.

Animals at the high dose had elevated spleen weights and a slight increase in incidence and/or severity of hemosiderin accumulation. All treated male groups had statistically non-significant increases in absolute (>17−26%) and relative (>18−27%) testicular weights compared to controls. The incidence of interstitial cell tumors of the testes (1/60, 4/59, 5/60, and 6/60 in the control and treated groups, respectively) was elevated in males compared to controls; however, the incidence of these tumors at the high dose (10%) was nearly identical to the historical control mean (9.8%), and the statistical significance appeared to be related to an atypically low incidence in the concurrent control group (1.7%, compared to the historical range of 3.4−23.4%). A 2-year LOAEL of 0.7 mg/kg-day and a NOAEL of 0.1 mg/kg-day are identified based on methemoglobinemia in male and female rats.

Matsumoto et al. (2006a)

Matsumoto et al. (2006a) is selected as the principal study for the derivation of the provisional oral slope factor (p-OSF). Matsumoto et al. (2006a) conducted a chronic toxicity and carcinogenicity bioassay of *p*-chloronitrobenzene using F344/DuCrj rats and Crj:BDF_1 mice administered *p*-chloronitrobenzene in the diets. Groups of 50 animals/sex of each species were treated with dietary concentrations of 0, 40, 200, or 1,000 ppm (rats) or 0, 125, 500, or 2,000 ppm (mice) *p*-chloronitrobenzene (>99.9% purity) for 2 years. Daily observations for mortality and obvious signs of toxicity were performed. Food consumption and body weights were measured weekly for the first 14 weeks and biweekly thereafter. Based on measured food intakes, body weights, and concentrations of *p*-chloronitrobenzene, the study authors estimated the adjusted daily doses to be 0, 1.5, 7.7, and 41.2 mg/kg-day (equivalent to HEDs of 0, 0.41, 2.1, and 11.1 mg/kg-day) in male rats; 0, 1.9, 9.8, and 53.8 mg/kg-day (equivalent to HEDs of 0, 0.49, 2.5, and 13.2 mg/kg-day) in female rats; 0, 15.3, 60.1, and 240.1 mg/kg-day (equivalent to HEDs of 0, 2.27, 8.99, and 35.64 mg/kg-day) in male mice; and 0, 17.6, 72.6, and 275.2 mg/kg-day (equivalent to HEDs of 0, 2.51, 10.4, and 39.26 mg/kg-day) in female mice. The toxicological evaluations were performed at the end of the treatment period and included hematology (i.e., erythrocyte count, Hgb, Hct, and MCV) and serum chemistry (i.e., total bilirubin), gross necropsy, selected organ weights (i.e., liver, spleen, and kidney), and comprehensive histopathology for all 32 tissues mentioned in the OECD (1998) guidelines.

Survival was reduced in the high-dose male rats compared with controls, and the study authors attributed this reduction in survival to deaths from splenic tumors. No other clinical signs were reported. A statistically significant treatment-related reduction in terminal body weight, without any concomitant change in food consumption, was observed in the high-dose males (12% less than controls) and females (21% less than controls) and in mid-dose females

(11% less than controls). A statistically significant decrease in erythrocyte counts and Hct along with an increase in MCV was observed in high-dose males and in mid- and high-dose females (see Table B-8). In addition, a statistically significant decrease ($p < 0.01$) in Hgb was observed in mid-dose females (no data could be collected from high-dose males and females due to hemolysis of blood). The only serum chemistry parameter that appeared to be affected by treatment was total bilirubin, which was significantly ($p < 0.01$) increased in high-dose males and mid- and high-doses females (see Table B-8). As was observed in the subchronic-duration toxicity study conducted by the same investigators, both relative spleen and relative liver weights were increased in a dose-dependent manner in both sexes with statistically significant increases at the mid- and high-doses (see Table B-8). Spleen weights were 11-fold higher than controls in high-dose males and 7-fold higher in high-dose females, while liver weights were increased by 39% in high-dose males and 50% in high-dose females. Relative kidney weights also were increased in mid- and high-dose males (11 and 19% higher than controls, respectively) and in high-dose females (35%). Gross necropsy revealed an increased incidence of splenomegaly, but the incidence was not dose related. However, incidences of splenic nodules increased with dose in both sexes (see Table B-8). Histopathology findings were consistent with the gross necropsy results and included both nonneoplastic and neoplastic splenic lesions, as well as adrenal lesions (see Table B-9). Nonneoplastic lesions in the spleen included statistically significant increase in the incidences of splenic capsular fibroblast hyperplasia, fibrosis, fatty metamorphosis, and increased extramedullary hematopoiesis in the mid- and high-dose animals of both sexes. Apart from the spleen, the only nonneoplastic lesion noted was adrenal medullary hyperplasia in low-dose males and in high-dose females. A 2-year LOAEL in rats of 7.7 mg/kg-day and a NOAEL of 1.5 mg/kg-day were identified based on splenic effects, and relative liver and kidney weights in males.

Statistically significant increases in the incidences of splenic tumors in high-dose male and female rats were observed (see Table B-9). In males, the incidences of splenic fibroma, fibrosarcoma, osteosarcoma, sarcoma (not otherwise specified [NOS]), and hemangiosarcoma were statistically significantly increased at the high dose tested, whereas in females, only the incidence of fibrosarcoma was statistically significantly increased over controls. However, trend tests conducted by the study authors indicated statistically significant positive trends for fibroma, osteosarcoma, and hemangiosarcoma in females. The only splenic tumor type that was statistically significantly increased at the mid dose was hemangiosarcoma in males. In addition to the increases in splenic tumors, the incidence of adrenal pheochromocytoma was statistically significantly increased in the high-dose females ($p < 0.01$), and significant dose-related trends were observed for both males and females.

As with rats, the high-dose male mice exhibited reduced survival compared with controls; the decline was attributed to tumor-related deaths (Matsumoto et al., 2006a). Neither food consumption nor body weights were affected by treatment. Table B-10 shows the statistically significant ($p < 0.01$) hematology findings including reduced erythrocyte count and Hct, as well as increased MCV in both sexes at the high dose, reduced erythrocytes ($p < 0.05$) in males, and reduced erythrocytes and Hct ($p < 0.05$) in the mid-dose females. No specific serum chemistry findings were noted. In the high-dose mice of both sexes, relative liver and kidney weights were statistically significantly ($p < 0.01$) increased over controls (20−25% higher than controls for liver; <5% higher kidney weights); relative spleen weights were statistically significantly increased (75%) in the high-dose males only. Splenomegaly and splenic nodules were observed upon gross necropsy only in female mice with a significant increase in the high-dose animals

(see Table B-10). Histopathologic evaluations revealed nonneoplastic lesions in the spleen of the mid- and high-dose male mice and in the high-dose female mice. The lesions included congestion, extramedullary hematopoiesis, hemosiderin deposition, and ossification. The study authors reported no splenic tumors in mice at any dose. However, a significant increase in hepatic hemangiosarcoma was observed in the high-dose females. A 2-year LOAEL in mice of 60.1 mg/kg-day and a NOAEL of 15.3 mg/kg-day were identified based on reduced erythrocytes and extramedullary hematopoiesis in the spleen of males.

Weisburger et al. (1978)

There were two carcinogenicity studies available for *p*-chloronitrobenzene. In the first, a carcinogenicity study of 21 aromatic compounds, Weisburger et al. (1978) fed groups of male CD rats (25/group) diets containing 0, 2,000, or 4,000 mg/kg-diet of *p*-chloronitrobenzene (97−99% purity) for 3 months, then diets containing 0, 250, or 500 mg/kg-diet for 2 months, followed by diets containing 0, 500, or 1,000 mg/kg-diet for 13 months, and finally control diets for an observation period of 6 months. For comparative purposes, the time-weighted-average (TWA) dietary concentrations over the 18-month treatment period were 0, 722, or 1,444 mg/kg-diet. Using reference values for rat food consumption and body weight (U.S. EPA, 1988), the calculated TWA doses were 0, 50, or 99 mg/kg-day (equivalent to HEDs of 0, 15, and 29 mg/kg-day). Rats that died during the first 6 months were discarded without necropsy. Remaining rats' lung, liver, spleen, kidney, adrenal glands, heart, urinary bladder, stomach, intestines, reproductive organs, and pituitaries were examined for histopathology. Information on survival, body-weight gain, or nonneoplastic lesions was not reported. No tumor increase was observed in treated rats compared to matched or "pooled" controls, which included all 111 control male rats used during the period in which the 21 chemicals were tested.

Weisburger et al. (1978) also fed groups of CD-1 mice (25/sex/group) diets containing 0, 3,000, or 6,000 mg/kg-diet of *p*-chloronitrobenzene (97−99% purity) for 18 months, followed by control diets for an observation period of 3 months. Using reference values for mouse food consumption and body weight (U.S. EPA, 1988), the calculated doses during the 18-month treatment period were 0, 515, or 1,029 mg/kg-day (equivalent to HEDs of 0, 75.3, and 150.3 mg/kg-day) for males, and 0, 518, or 1,036 mg/kg-day (equivalent to HEDs of 0, 71.8, and 143.7 mg/kg-day) for females. 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 (i.e., lungs, liver, spleen, kidneys, 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 incidence of hepatocellular carcinomas in male mice (1/14, 4/14, and 0/14 in control, low-, and high-dose groups, respectively) was not significantly increased by treatment compared to matched controls, but the incidence in the low-dose group was significantly higher than "pooled" male controls (7/99). Hepatocellular carcinoma was not observed in female mice. The incidences of unspecified vascular tumors were increased in the treated animals of both sexes (0/14, 2/14, and 4/14 in the males, and 0/15, 2/20, and 7/18 in the females of the matched control, low-, and high-dose groups, respectively); these findings were statistically significantly increased in the high-dose groups of both sexes compared to current or pooled (5/99 males and 9/102 females) controls.

Reproductive/Developmental Toxicity Studies

Bio Dynamics (1984)

Several studies evaluated the reproductive toxicity of *p*-chloronitrobenzene in orally exposed rodents. In the first study, Bio Dynamics (1984) conducted a two-generation reproductive toxicity study in S-D CD rats. Groups of 15 males and 30 females in the F0 and F1 generations were administered 0, 0.1, 0.7, or 5.0 mg/kg-day (99.43% purity) *p*-chloronitrobenzene in corn oil by gavage during premating, mating, gestation, and lactation periods. F0 adults were exposed for 167 days (including100 days premating), and F1 adults were exposed for 217−219 days (including 120 days from birth to mating). Adults and litters were examined twice daily for mortality and obvious signs of toxicity. All generations received a weekly physical examination. Body weights were recorded weekly; in addition, female body weights were recorded on Days 0, 6, 15, and 20 of gestation and Days 0, 4, 14, and 21 of lactation. Food consumption was monitored weekly except during the mating period. On Days 0, 4, 14, and 21 of lactation, pup mortality, number of live pups by sex, live pup body weights, and external sex criteria (anogenital distance) were recorded for each litter. F0 adults were sacrificed after weaning of the last F1 offspring, and F1 adults were sacrificed 5 weeks after weaning of the last F2 offspring; F2 offspring were sacrificed at weaning. Complete gross necropsies were conducted for all animals and included uterine implantation sites and internal sex determination for weanlings. Selected tissues were preserved for all F0 and F1 adults and 40 randomly selected weanlings (5/sex/group) from both the F1 and F2 generations. The testes and epididymides were evaluated for the control and high-dose F0 males (15/group). Complete histopathological evaluations were performed for 40 F1 weanlings (5/sex/group), 80 F1 adults (10/sex/group), and 40 F2 weanlings (5/sex/group).

No consistent treatment-related effects were observed on survival, adult body weights, food consumption, gestation length, litter size, pup survival, or pup weights. Slight decreases (not statistically significant) in male fertility, female mating index, and pregnancy rate were observed in high-dose F0 animals. A statistically significant decrease in F1 pup survival at the high dose was attributed to the loss of two whole litters; similarly, two low-dose litters were lost among F2 offspring. The investigators did not consider these results treatment related because no dose-response relationship was evident. Examination of tissues from the high-dose F0 males revealed oligospermia in the epididymides (3/15), degenerated seminal product in the tubular lumen (2/15), bilateral degeneration of the testicular germinal epithelium in (2/15), and bilateral maturation arrest involving the germinal epithelium (1/15). Fertility of three males with oligospermia was reduced (one impregnated female out of five planned female matings in total from these three males). No testicular lesions were noted in control males, and the historical incidence of testicular lesions from five studies (of similar strain and age conducted in the same lab) were 1/71 for bilateral degeneration of the testicular germinal epithelium (range: 0/15−1/15) and 1/71 for bilateral maturation arrest involving the germinal epithelium (range: 0/15−1/15). However, the toxicological significance of these findings in the testes and potential effect, if any, on fertility is not known. No histological examination was performed on F0 males from the low- and mid-dose groups so it is unknown if any F0 males from these dose groups had similar lesions. All examined F1 adults (control and treated) exhibited hemosiderosis of reticuloendothelial cells and extramedullary hematopoiesis in the spleen, but the intensity appeared to be higher in the high-dose animals. Whether the testicular lesions observed in three high-dose F0 males were related to treatment is not clear because no similar lesions were observed in adult F1 males. In this rat study, a LOAEL of 5.0 mg/kg-day is identified for treatment-related testicular effects (oligospermia, degeneration, and reduced fertility) in

F0 males. However, the next lower dose of 0.7 mg/kg-day may not be a NOAEL because the F0 males from this group were not examined for testicular histopathology.

Chapin et al. (1997); Gulati et al. (1991); NTP (1993)

NTP (1993) evaluated the effects of *p*-chloronitrobenzene (~99% purity) on fertility and reproduction in Swiss CD-1 mice following gavage exposure. In a 2-week range-finding study, groups of 8 mice/sex were treated with 0, 40, 80, 160, 320, or 640 mg/kg-day of *p*-chloronitrobenzene in corn oil by gavage. Clinical signs, body weights, and water consumption data were recorded. All mice in the 640 mg/kg-day group died or were sacrificed moribund; other deaths in treatment and control groups were attributed to gavage trauma. Treatment had no adverse effect on terminal body weights. Decreased water consumption was observed in both sexes receiving 640 mg/kg-day, in females administered 320 mg/kg-day during Week 1, and in females administered 40 mg/kg-day during Week 2. Mice administered 160 or 320 mg/kg-day became cyanotic. On the basis of these results, doses between 62.5 and 250 mg/kg-day were selected for the continuous breeding study.

In the continuous breeding study, groups of 20 breeding pairs of Swiss CD-1 mice (F0 generation) were administered daily doses of 0, 62.5, 125, or 250 mg/kg of *p*-chloronitrobenzene (~99% pure) by gavage in corn oil, and a group of 40 control breeding pairs with only corn oil vehicle (Chapin et al., 1997; NTP, 1993; Gulati et al., 1991). After the beginning of dosing, the mice were housed separately for 7 days and then were housed in breeding pairs for 98 days while being dosed. Reproductive endpoints included average numbers of litters/pair, average number of live pups per litter, the proportion of pups born alive, the sex ratio of pups, and pup body weight. Adult endpoints included body weights (recorded after each delivery and at termination) and water consumption. Excluding deaths from gavage trauma or fight injuries, treatment-related changes in mortality in F0 mice (not reported by sex) were not statistically significant (1/80, 2/40, 1/40, and 5/40 in the control, low-, mid-, and high-dose groups, respectively). The final mean body weights of F0 dams were statistically significantly greater in the mid- and high-dose groups than in controls. Water consumption was significantly reduced in the high-dose mice. Five sets of litters were produced during the cohabitation period. The fertility index (fertile pairs/cohabiting pairs) was reduced in all groups, including controls, by the time of the last litter. The fertility index of the third (12 fertile pairs/14 cohabiting pairs) and fourth (11 fertile pairs/14 cohabiting pairs) sets of litters was significantly reduced in the high-dose group compared to controls. Treatment had no effect on the cumulative days to litter, the average number of live pups per breeding pair, or the sex ratios of pups. Reductions in male and female pup weights were statistically significant in all five sets of litters from the mid- (12% reduction) and high-dose (21% reduction) groups, and reductions in male pup weights (less than 10% below controls) were statistically significant in the second and fourth sets of litters in the low-dose group. Overall, the average male pup weight from litters 1−5 was statistically significantly reduced in all *p*-chloronitrobenzene-treated groups. For the final F1 set of litters, total pup survival (to Postnatal Day [PND] 21) was reduced in the mid- and high-dose groups. There were no clinical signs of toxicity.

Following the continuous breeding of F0 mice, the final F1 litters of the control and high-dose pairs were raised with the same treatment as the parents. After weaning of the F1 mice, nonsiblings were housed for mating for 7 days and housed singly through delivery of F2 pups; the same reproductive endpoints were monitored. F1 adult endpoints included body weight, water consumption, sperm morphology, and vaginal cytology at termination; F1 adults

were necropsied, and selected organ weights (i.e., liver, kidney, and reproductive organs) were recorded. Treatment of F1 mice at 250 mg/kg-day had no effect on water consumption or body weight at the time of mating, but most animals were cyanotic. At necropsy, absolute and relative liver weights were significantly greater than in controls. Spleens were not weighed but were observed to be enlarged and darker in color compared to the controls. Average estrous cycle length was significantly increased in treated F1 females [data not shown in NTP (1993)], but treatment had no effect on sperm morphology. Treatment had no effect on mating, pregnancy, fertility indices, average days to litter, or mean dam weight at delivery. There was no statistically significant effect on survival or sex ratios of F2 pups, but the total number of live pups per breeding pair (11% reduction) and male (12% reduction) and female pup weights (12% reduction) was significantly reduced ($p < 0.05$) at the high dose (250 mg/kg-day). Treated F2 pups showed no clinical signs of toxicity. Although methemoglobin concentrations were not measured in this study, the NTP study authors attributed the reduced pup weights as secondary to methemoglobinemia-related hypoxia, based on previous observations (Chapin et al., 1997; NTP, 1993; Gulati et al., 1991). In this study, 62.5 mg/kg-day is identified as a LOAEL for reduced body weight of F1 male mouse pups; no NOAEL was identified in this study.

Bio Dynamics (1980); Nair et al. (1985)

In a developmental toxicity study, pregnant S-D rats were exposed to *p*-chloronitrobenzene (>99% purity) in corn oil via gavage at doses of 0, 5, 15, or 45 mg/kg-day on GDs 6−19 (24 pregnant rats/group) (Nair et al., 1985; Bio Dynamics, 1980). Observations for mortality and obvious signs of toxicity were made daily, and maternal body weight was measured at regular (unspecified) intervals. Dams were sacrificed on GD 20 for examination of uterine contents, gross necropsy, and measurement of spleen weight. Numbers of viable and nonviable fetuses, as well as numbers of early and late resorptions, were recorded. Viable fetuses were weighed and examined for external malformations; half were then prepared for skeletal examination, and the remainder was prepared for visceral examination. None of the rats died during treatment. The high-dose dams exhibited pale eye color (incidence not reported) and reduced body-weight gain (40% less than controls, $p < 0.01$). Absolute spleen weight was statistically significantly increased in dams at all doses (43% higher at 5 mg/kg-day to more than 4-fold higher at 45 mg/kg-day), but relative spleen weight was statistically significantly increased only at the mid- and high-doses (2-fold and 4.5-fold higher, respectively). Uterine parameters were affected only at the high dose; a statistically significant increase ($p < 0.01$) in the number of resorptions (with a concomitant decrease in number of live fetuses) occurred at this dose (5.6 ± 5.8 vs. 0.5 ± 0.7 resorptions per dam in controls). Fetal body weight was also statistically significantly reduced ($p < 0.01$) at the high dose (16% lower than controls in males, and 17% in females, $p < 0.01$). While the incidences of external and visceral malformations were comparable among all groups, including controls, the incidence of skeletal anomalies was statistically significantly increased ($p < 0.01$) in the high-dose group, whether assessed on a litter basis or on an individual fetus basis. The most common skeletal anomalies were angulated ribs (2/24, 0/21, 0/24, and 9/15 affected litters in the control through the high-dose group, respectively) and with or without misshapen forelimbs (0/24, 0/21, 0/24, and 6/15 litters). The incidence of fetuses with at least one ossification variation was also increased at the high dose (data not shown). These data indicate a maternal LOAEL of 5 mg/kg-day for increased absolute spleen weights in rats; a maternal NOAEL was not identified. The developmental toxicity LOAEL is 45 mg/kg-day, with a NOAEL of 15 mg/kg-day, based on fetal resorptions, decreased fetal weight, and increased skeletal anomalies in rats.

Bio Dynamics (1982)

In a developmental toxicity study in rabbits, groups of 18 pregnant New Zealand White rabbits were gavaged with 0, 5, 15, or 40 mg/kg-day of *p*-chloronitrobenzene (99.43% purity) in corn oil on GDs 7−19 (Bio Dynamics, 1982). Does were examined twice daily for mortality and obvious signs of toxicity; they also received a detailed physical examination on GDs 0, 7, 10, 15, 19, 25, and 30. Body weights were recorded on GDs 0, 7, 19, and 30. Surviving does were sacrificed on GD 30 and given a complete gross necropsy during which spleen weights and uterine implantation data were recorded. All fetuses were evaluated for external malformations, and half for soft tissue or skeletal malformations. Treatment at 40 mg/kg-day resulted in statistically significant mortality (eight does died, and two had aborted their pregnancies during GDs 13−22); treatment was terminated, and no internal or fetal data were collected for this dose group. Treatment at 5 or 15 mg/kg-day had no statistically significant effect on maternal mortality, pregnancy rate, body weight, or mean spleen weight. No compound-related effect was noted at the 5 or 15 mg/kg-day doses in uterine implantation data (numbers of implants, fetuses, resorptions, mean percentage of resorptions/fetuses to implants), fetal weight, sex ratio, the incidence of external or soft tissue malformations, or fetal ossification variation data. However, during skeletal evaluation, the incidences of fetuses with malformation and litters containing affected fetuses were increased in the low- and mid-dose groups but were not statistically significant. In particular, the incidence of fused sternebrae was slightly increased in low- and mid-dose groups. The study authors reported that this minor skeletal malformation is historically seen in this rabbit strain and was also noted at low incidence in the control group. Based on this study, the mid dose of 15 mg/kg-day is identified as a maternal NOAEL for maternal effects in rabbits does, and 40 mg/kg-day is a frank effect level (FEL) for mortality and spontaneous abortion. The mid-dose of 15 mg/kg-day also is a NOAEL for fetal/developmental effects.

Inhalation Exposures

The database for inhalation toxicity of *p*-chloronitrobenzene is less extensive than for the oral route. There are short-term and subchronic-duration toxicity studies but no chronic-duration, developmental toxicity, or reproductive toxicity inhalation studies available.

Short-Term-Duration Studies

Nair et al. (1986)

In the short term study, (Nair et al., 1986) exposed the whole body of S-D rats (10/sex/group) to *p*-chloronitrobenzene vapor (99.43% purity) in ethylene glycol monoethyl ether (2-ethoxyethanol) at concentrations of 0, 5, 16, or 46 mg/m^3(equivalent to a daily average concentration of 0, 0.89, 2.86, or 8.21 $^{mg/}$m^3) for 6 hours/day, 5 days/week, for 4 weeks. The concentrations of the 2-ethoxyethanol carrier were 1,409, 1,353, or 1,296 mg/m^3 (equivalent to a daily average concentration of 251.6, 241.6, or 231.4 mg/m^3) at the low, medium, and high exposure concentrations, respectively. Control animals were exposed to 2,000 mg/m^3 (equivalent to a daily average concentration of 357.1 mg/m^3) of the 2-ethoxyethanol carrier; there was no air-only group. Rats were examined twice daily for signs of toxicity and mortality and given complete physical examinations weekly; body weights were recorded weekly. All rats were given an ophthalmoscopic examination before initiation of exposure and again before termination of the study. Before euthanasia, blood was harvested from 10 rats/sex/group for hematology (i.e., Hgb, Hct, erythrocyte count, methemoglobin, clotting time, red cell morphology, total, and differential leucocytes), and clinical chemistry (i.e., BUN, serum SGPT, SAP, glucose, albumin, total protein, globulin, sodium, potassium, chloride, calcium, and phosphorus) measurements. In addition, hematology parameters and

methemoglobin concentrations were determined for 5 rats/sex/group after 2 weeks of exposure. At termination, all surviving animals were necropsied, and organ weights were recorded for brain, testes/ovaries, kidneys, liver, lungs, pituitary, and spleen. Microscopic examinations of gross lesions and tissues (i.e., abdominal aorta, adrenals, bone and bone marrow, brain, esophagus, eyes, heart, cecum, colon, duodenum, ileum, jejunum, kidneys, liver, lungs, lymph nodes [mediastinal and mesenteric], mammary gland, nasal turbinates, pancreas, pituitary, prostrate, salivary gland [mandibular], sciatic nerve, seminal vesicle, skin, spinal cord [cervical], spleen, stomach, testes, thymus, thyroid/parathyroid, trachea, urinary bladder, uterus, and vagina) were conducted for 10 rats/sex from the control and high-exposure groups; spleens from the low- and mid-concentration rats were also examined for histopathology.

There were no exposure-related effects on mortality, body weight, clinical chemistry, or in the ophthalmologic examination. The study authors reported cyanosis in all exposed groups, increasing in intensity with concentration. Hematological analyses demonstrated a statistically significant increase in methemoglobinemia within 2 weeks of exposure at the two highest concentrations in females and only at the highest concentration in males. Statistically significant changes in hematological parameters at 4 weeks included (see Table B-11).

- At ≥0.89 mg/m^3, increased methemoglobin in males and decreased hematocrit in females.
- At ≥2.86 mg/m^3, increased methemoglobin in females and reduced erythrocytes in both sexes.
- At 8.21 mg/m^3, reduced in males and reduced Hgb and increased WBC count in both sexes. In addition, absolute and relative weights of both spleen and liver were elevated in males; the same was true for females, with the exception of absolute liver weight.

The study authors reported an increased incidence of splenic hemosiderosis at all concentration levels. Increased incidence of congestion, and extramedullary hematopoiesis in the spleen was also reported at 8.21 mg/m^3 (data not shown). At the lowest exposure concentration of 0.89 mg/m^3 in both sexes, Nair et al. (1986) observed toxicity in erythrocytes (methemoglobinemia in males and in females) and splenic hemosiderosis. A 4-week LOAEL of 0.89 mg/m^3 (corresponding HEC is 0.89 mg/m3) for hematological effects in rats may be appropriate. However, these results are confounded by the presence of the 2-ethoxyethanol carrier, which also induces hematological effects. These effects are discussed below in "Other Exposures."

Subchronic-Duration Studies

NTP (1993); *Travlos et al. (1996)*

NTP (1993) (data also reported by (Travlos et al., 1996) is selected as the principal study for the derivation of the subchronic and chronic provisional inhalation reference concentrations (p-RfCs). In the NTP study, the whole body of F344/N rats (10/sex/group) were exposed to 0, 1.5, 3, 6, 12, or 24 ppm (equivalent to daily average concentration of 0, 1.7, 3.4, 6.9, 13.8, or 27.5 mg/m^3) of *p*-chloronitrobenzene vapor (~99% purity), 6 hours/day, 5 days/week, for 13 weeks (Travlos et al., 1996; NTP, 1993). Supplemental groups of 10 rats/sex/group were designated for clinical pathology testing at interim time points (Days 3 and 23). Body weights were recorded weekly and at termination. Blood was collected on Days 3 and 23 from the supplemental group of rats and at the end of the study from rats in the

base study. Hematology parameters evaluated included Hct, Hgb, erythrocyte count, reticulocyte count, MCV, MCH, MCHC, platelet count, white blood cell (WBC) count, and methemoglobin concentration. Clinical chemistry parameters evaluated included blood urea nitrogen, creatinine, total protein, albumin, globulin, ALT, SAP, creatinine kinase (CK), sorbitol dehydrogenase (SDH), and bile acids. Animals in the 0 (control), 6.9, 13.8, and 27.5 mg/m^3 groups were examined for the following reproductive parameters: spermatid morphology, spermatozoan motility, and weights of reproductive organs in males; vaginal cytology, estrous cycle duration, and estrous cycle stage lengths in females. At termination, all surviving rats were necropsied for gross lesions, and weights of heart, right kidney, liver, lungs, spleen, right testis, and thymus were recorded. Lesions or abnormal masses and tissues (i.e., adrenal glands, brain, clitoral glands, esophagus, eyes, femur and marrow, heart, kidneys, large intestine [cecum, colon, and rectum], larynx, liver, lungs, lymph nodes [bronchial, mandibular, mediastinal, and mesenteric], mammary gland, nasal cavity and turbinates, ovaries, pancreas, parathyroid glands, pharynx, pituitary gland, preputial glands, prostate gland, salivary gland, seminal vesicle, small intestine [duodenum, jejunum, and ileum], spinal cord/sciatic nerve, spleen, stomach, testes, thigh muscle, thymus thyroid gland, trachea, urinary bladder, uterus, and vagina) from all animals were processed for histological examination. Complete histopathological examination was performed on all rats in the control and high-concentration groups and all rats that died prior to study termination. Additionally, target organs including bone marrow, harderian gland, kidneys, liver, mediastinal lymph node, spleen, and testes were identified and examined in all animals of the lower concentration groups.

There were no exposure-related effects on survival, body weight, or the incidence of clinical signs (Travlos et al., 1996; NTP, 1993). Statistically significant exposure-related hematological changes were observed in all exposed groups. Methemoglobinemia was first observed in both sexes at ≥1.7 mg/m^3 on Day 3. The methemoglobin concentrations in both sexes of all exposure groups were significantly different from the control ($p < 0.01$) at all observation times. Other hematological effects including changes in Hct, Hgb, erythrocyte counts, reticulocyte counts, nucleated erythrocyte counts, MCV, MCHC, platelets, lymphocytes, and leucocytes were significantly different from the control at the highest concentrations (27.5 mg/m^3) on Day 23. Among these parameters, Hct, Hgb, and erythrocyte counts were significantly decreased from the control ($p < 0.01$) in all exposure groups in both sexes at Week 13 (see Table B-12). Clinical chemistry changes indicative of liver damage or altered liver function were also observed. In both sexes, sorbitol dehydrogenase was significantly increased ($p < 0.01$) on Day 3 at ≥6.9 mg/m^3 and on Day 23 only at higher concentrations (male ≥27.5 mg/m^3; female ≥13.8 mg/m^3); values were similar to controls at Week 13. Serum alkaline phosphatase was statistically significantly ($p < 0.01$) decreased at all time points in males exposed to ≥13.8 mg/m^3 and in females exposed to ≥6.9 mg/m^3. Bile acids were statistically significantly increased in males exposed to ≥3.4 mg/m^3 at all 3 time points; in females, bile acids were statistically significantly increased in nearly all exposure groups (except 3.4 mg/m^3) on Day 3 and at ≥13.8 mg/m^3 on Day 23 and were not different from the controls at Week 13. Although clinical chemistry parameters are statistically significantly increased at different time points and at different concentrations, no consistent concentration- or time-dependent changes were observed with any of the parameters. Absolute spleen weight in both sexes, relative spleen weight in males, and absolute liver weight in females were statistically significantly increased at ≥3.4 mg/m^3; relative weights of spleen and liver in females were statistically significantly increased at ≥6.9 mg/m^3; absolute and relative thymus weights in females were statistically significantly increased at ≥13.8 mg/m^3; absolute and relative thymus weights in males and

absolute and relative right kidney weights in both sexes were statistically significantly increased at 27.5 mg/m^3. Among these, only absolute and relative spleen weight in both sexes and absolute and relative liver weight in females showed a concentration-dependent increase compared to controls. In reproductive tissues, there were statistically significant reductions in the weights of the right and left testis, left epididymis and left cauda epididymis, and also in the number of spermatid heads per testis and spermatid count in males following exposure to 27.5 mg/m^3. Atrophy of the testes characterized by reduced cellularity of seminiferous tubules was also observed at 27.5 mg/m^3. The length of the estrous cycle was statistically significantly increased in females exposed to ≥6.9 mg/m^3 (the lower exposure groups were not analyzed). As summarized in Table B-12, statistically significant increase in histopathological lesions of the spleen in both sexes included congestion and hemosiderin deposition at ≥1.7 mg/m^3, hematopoietic cell proliferation at ≥3.4 mg/m^3, and capsular fibrosis at ≥6.9 mg/m^3. Hematopoietic cell proliferation was also observed in the bone marrow in females at ≥3.4 mg/m^3 and in males at ≥6.9 mg/m^3. Kidney lesions included hyaline droplet nephropathy in males at ≥1.7 mg/m^3 as well as renal tubule pigmentation in females at ≥6.9 mg/m^3 and in males at ≥13.8 mg/m^3. In males, the protein-positive hyaline droplets were confirmed using Mallory-Heidenhain stain. However, the presence of alpha 2u-globulin protein within the hyaline droplets was not confirmed immunohistochemically and the pathological sequence of lesions associated with alpha 2u-globulin nephropathy was not demonstrated in this study. Thus, the hyaline droplet nephropathy observed in male rats at 1.7 mg/m^3 is of relevance to humans (U.S. EPA, 1991a). Hemosiderin deposition in Kupffer cells of the liver was observed in females at ≥3.4 mg/m^3 and in males at ≥13.8 mg/m^3. Chronic inflammation of the Harderian gland was observed in females at ≥13.8 mg/m^3 and in males at 27.5 mg/m^3. Hyperplasia/hypertrophy of the respiratory epithelium was not observed. The lowest exposure concentration of 1.7 mg/m^3 is identified as a LOAEL based on erythrocyte effects (methemoglobinemia, erythrocyte count, hematocrit, and reticulocyte count) and spleen effects (congestion and hemosiderin deposition) in both sexes of rats, and the corresponding HEC is 1.7 mg/m^3. A NOAEL is not identified in this study.

NTP (1993) and Travlos et al. (1996) also evaluated the subchronic-duration inhalation toxicity of *p*-chloronitrobenzene in B6C3F$_1$ mice exposed under the same conditions as rats discussed above. The analysis of toxicity in mice was the same as for rats, except that no hematology or clinical chemistry data were collected. There were no exposure-related effects on mortality, body weight, or the incidence of clinical signs. Statistically significant increases in absolute right kidney weight were observed in males at ≥1.7 mg/m^3 and in females at ≥3.4 mg/m^3 (but not concentration dependent); relative kidney weight was statistically significantly increased in females only at 27.5 mg/m^3. Absolute and relative liver weights were statistically significantly increased in males at ≥13.8 and 27.5 mg/m^3, respectively, and in females at ≥13.8 and 6.9 mg/m^3, respectively (see Table B-13). These changes were greater than 10% at these exposure concentrations. Absolute and relative spleen weights were statistically significantly increased in both sexes at ≥13.8 mg/m^3. Among these, only relative liver weight in females showed a concentration-dependent increase relative to the control. Exposure-related gross lesions included an enlarged and darkened spleen in males at 27.5 mg/m^3 and females at ≥13.8 mg/m^3. Histopathological lesions of the spleen in both sexes included statistically significant increase in the incidences of hemosiderin deposition and hematopoietic cell proliferation at ≥13.8 mg/m^3 and congestion at 27.5 mg/m^3. In the liver, hemosiderin deposition in both sexes and single cell necrosis and centrilobular cytoplasmic basophilia only in males were observed at 27.5 mg/m^3. Other statistically significant increases in lesions observed

at 27.5 mg/m^3 included squamous cell hyperplasia, hemosiderin deposition, and erythrocyte fragments in the bone marrow in both sexes and hyperplasia of the forestomach in females. The estrous cycle length was significantly increased in females exposed to 27.5 mg/m^3, but no reproductive effects were observed in males. Hyperplasia/hypertrophy of the respiratory epithelium was not observed; however, there was squamous cell hyperplasia of the forestomach epithelium in 7/10 females and 1/10 males in the high-exposure group with no incidence in the control group. In this 13-week study, a LOAEL of 13.8 mg/m^3 (HEC of 13.8 mg/m^3) and a NOAEL of 6.9 mg/m^3 (HEC of 6.9 mg/m^3) are identified based on increased absolute and relative weights, increased hemosiderin deposition and hematopoietic cell proliferation in spleen of both sexes, increased absolute liver weight in male mice, and increased absolute and relative liver weights in female mice exposed to *p*-chloronitrobenzene.

OTHER DATA (SHORT TERM TESTS, OTHER EXAMINATIONS)

Tables 4A and 4B summarize other studies conducted with *p*-chloronitrobenzene that are not appropriate for selection of a point of departure (POD) for derivation of a provisional RfD (p-RfD), provisional RfC (p-RfC) and oral slope factor (OSF) but provide supportive data.

Table 4A. Summary of Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute/ short-term studies	Acute study: Male BDF1 mice (24/group) received 0 or 300 mg/kg *p*-chloronitrobenzene by a single intraperitoneal (i.p.) or subcutaneous (s.c.) injection. Short-term study: Male BDF1 mice (15/group) received 0 or 30 mg/kg *p*-chloronitrobenzene by i.p. three times a wk for 4 wk.	Acute study: Number of B, T, NK, and subsets of T (CD4 and CD8) cells were statistically significantly reduced after D 5 in both i.p and s.c. *p*-chloronitrobenzene injected mice. Short-term study: NK cell activity, cytotoxic T-lymphocyte activity, and LPS-stimulated splenocyte proliferation were statistically significantly reduced from Wk 3 after the first dose of *p*-chloronitrobenzene.	*p*-Chloronitrobenzene is immunotoxic in mice in acute/short term studies.	Li et al. (1999); Li et al. (1998)
	Male F344 rats (5/group) received 0 or 157.56 mg/kg of *p*-chloronitrobenzene by a single i.p. injection.	*p*-Chloronitrobenzene produced statistically significant increases in methemoglobin after 48 hr and in urinary *N*-acetyl-beta-D-glucosaminidase (NAG) after 24 hr.	*p*-Chloronitrobenzene is nephrotoxic in rats in acute study.	Yoshida et al. (1989)
	Rabbits were injected subcutaneously with a single dose of 0 or 500 mg/kg of *p*-chloronitrobenzene (Number of rabbits and the duration of the experiment was not given).	*p*-Chloronitrobenzene produced an increase in methemoglobin and Heinz bodies and a decrease in catalase activity in the blood.	*p*-Chlorobenzene is hematotoxic in rats in acute study.	Hasegawa and Sato (1963)

Table 4A. Summary of Other Studies

Test	Materials and Methods	Results	Conclusions	References
Acute/ short term studies	Male S-D [Crl:CD [SD]BR] rats (16/group) were exposed to 0,50, 290, or 640 mg/m^3 of mixed *p*-chloronitrobenzene (99.2% purity) vapors and aerosols for 6 hr/d, 5 d/wk for 2 wk.	At ≥50 mg/m^3: Statistically significant adverse effects on erythrocytes (i.e., methemoglobinemia, decreased erythrocyte count, and hematocrit) and the spleen (i.e., splenomegaly, hemosiderosis, congestion, hyperplastic red pulp, and increased erythropoiesis). At ≥290 mg/m^3: Statistically significant increase in relative liver and kidney weights; decreased mean testes weight, degenerated spermatic contents of the epididymis, and increased degeneration of seminiferous tubules.	*p*-Chloronitrobenzene produces hematology, splenic and testicular changes in rats in short-term study.	Haskell Laboratories (1984)
Metabolism/ Toxicokinetics	Six male human subjects who had been accidentally exposed to *p*-chloronitrobenzene in an occupational setting were subjected to toxicokinetic evaluation using urine samples.	The metabolites identified in the urine were 2-chloro-5-nitrophenol, *N*-acetyl-S-(4-nitrophenyl)-L-cysteine, *p*-chloroaniline, 2,4-dichloroaniline, 2-amino-5-chlorophenol, *p*-chloroacetanilide, 4-chloro-2-hydroxyacetanilide, and *p*-chloro-oxanilic acid.		Yoshida et al. (1993)
	Thirty six male human subjects who had been accidentally exposed to *p*-chloronitrobenzene for 1−16 yr with a median of 6 yr in an occupational setting were subjected to toxicokinetic evaluation using urine samples.	*N*-acetyl-S-(4-nitrophenyl)-L-cysteine (NANPC), 4-chloroaniline (4CA), and 2-chloro-5-nitrophenol (CNP) were the metabolites identified.	NANPC is the most appropriate biomarker in the urine and it is the most prevalent metabolite detected in all the exposed workers.	Jones et al. (2007)

Table 4A. Summary of Other Studies

Test	Materials and Methods	Results	Conclusions	References
Metabolism/ Toxicokinetics	Male Fischer 344 rats (number of rats not given) were administered with a single dose of 0, 0.65, 6.5, or 65 mg/kg of radioactive *p*-chloronitrobenzene dermally.	51−62% of radioactive *p*-chloronitrobenzene was absorbed from the skin within 72 hr. 43−45% was excreted in urine; 5−12% was excreted in the feces.	The dermal absorption and urinary and fecal excretion were linear over 0.65−6.5 mg/kg and nonlinear at 65 mg/kg.	Nomeir et al. (1992)
	Female Wistar rats (2/group) were administered with a single dose of 0 or 79 mg/kg of *p*-chloronitrobenzene via gavage.	After 24 hr, *p*-chloronitrobenzene treated group form Hgb adducts.	The extent of hemoglobin binding increases with the reducibility of the nitro group.	Sabbioni (1994)
	Male F344 rats (3−4/group) were administered with a single oral dose of 0, 2, 20, 65, or 200 mg/kg of *p*-chloronitrobenzene via gavage. In a separate study, male S-D rats received a single oral dose of 0, or 200 mg/kg of *p*-chloronitrobenzene via gavage.	Approximately 86−93% of *p*-chloronitrobenzene was absorbed. 68−74.6% was excreted in urine and 12.3−20.5% in feces after 72 hr. Nitrochlorophenol and NANPC were the two metabolites identified in this study.	Nitrochlorophenol appeared to be the most promising candidate for a urinary monitoring program.	NTP (1993); Monsanto (1994a)

Table 4B. Summary of *p*-Chloronitrobenzene (CASRN 100-00-5) Genotoxicity Studies

Endpoint	Test System	Dose/Concentration	Results		Comments	References
			Without Activation[a]	With Activation[a]		
Genotoxicity studies in prokaryotic organisms						
Reverse Mutation (Ames test)	*Salmonella typhimurium* strains TA 98, 100, 1532, 1535, 1537, 1950, 1975, 1978, and G 46 in the presence or absence of S9	1−2,000 µg/plate	–	–	No positive results were observed	Gilbert et al. (1980); IARC (1996)
	Salmonella typhimurium strains TA 98, 100, 1535, and 1537 in the presence or absence of S9	1−3,000 µg/plate	–	+ (TA 100, 1535); – (TA 98, 1537)	Positive results observed in TA 100 from 500 µg/plate; and in TA 1535 at 3,000 µg/plate with S9 activation	Haworth et al. (1983); NTP (1993); IARC (1996)
	Salmonella typhimurium strains TA 98, 100, 98 NR, and 98NR/1,8-DNP_6 in the presence or absence of S9 and norharman	1−300 µg/plate	–	+ (TA 98, 98 NR and 98NR/1,8-DNP_6); – (TA 100)	Positive results observed in the presence of 200 µg/plate norharman (CASRN 244-63-3) along with S9 at 300 µg/plate	(Suzuki et al. (1987); Suzuki et al. (1983)); IARC (1996)
	Salmonella typhimurium strains TA 98, 100, 1535, 1537, and 1538 in the presence or absence of S9	25.6−3,276.8 µg/plate	+ (TA 100 and 1535); – (TA 98, 1537, and 1538)	–	Positive results seen in TA 100 and 1,535 from 25.6 µg/plate without S9 activation (If the tests were positive without activation system, no further tests with activation system is carried out)	Shimizu et al. (1983); (IARC (1996); NTP (1993))

Table 4B. Summary of *p*-Chloronitrobenzene (CASRN 100-00-5) Genotoxicity Studies

Endpoint	Test System	Dose/Concentration	Results		Comments	References
			Without Activation[a]	With Activation[a]		
DNA damage (SOS chromotest)	*E. Coli* PQ 37 in the presence or absence of S9	15,756 µg/mL (This is noted as the highest experiment dose. Other doses were not given)	–	–	No positive results were observed	von der Hude et al. (1988); IARC (1996)
Genotoxicity studies in nonmammalian eukaryotic cells—in vivo						
Mutation	Drosophila melanogaster	0−100 mg/kg via feed or injection. Males were mated after 72 hr (feed) and 24 hr (injection)	–	NV	No sex linked lethal mutation was observed in both larvae and adult treated *Drosophila melanogaster*	Zimmering et al. (1985); Zimmering et al. (1989); IARC (1996)
Genotoxicity studies in mammalian eukaryotic cells—in vitro						
Sister Chromatid Exchange (SCE)	Chinese hamster cells in vitro	0−500 µg/mL	–	+	Increased SCE observed from 250 µg/ml	NTP (1993); Galloway et al. (1987); IARC (1996)
Chromosomal Aberrations	Chinese hamster cells in vitro	0−5,000 µg/mL	+	+	Increased chromosomal aberrations observed from 600 µg/mL (with activation) and from 900 µg/mL (without activation)	
	Human peripheral lymphocytes in vitro	157,560 µg/mL (This is noted as highest experiment dose. Other doses are not given)	–	NV	No chromosomal aberrations were observed	Huang et al. (1996)

Table 4B. Summary of *p*-Chloronitrobenzene (CASRN 100-00-5) Genotoxicity Studies						
			Results			
Endpoint	**Test System**	**Dose/Concentration**	**Without Activation[a]**	**With Activation[a]**	**Comments**	**References**
DNA single strand breaks and repair	Male Wistar rat hepatocytes in vitro	5 and 50 µg/mL	+	NV	Single strand DNA damage was seen after 3 hr of exposure at both doses. But this DNA damage is completely repaired within 24 hr	Cesarone et al. (1984); NTP (1993); IARC (1996)
Genotoxicity studies in mammalian eukaryotic cells—in vivo						
DNA single strand breaks	Liver, kidney, and brain of *p*-chloronitrobenzene injected Swiss CD1 male mice in vivo	30, 60, 180, or 1,000 mg/kg; single i.p. injection; (12/group)	+	NV	Single strand DNA damage was seen in liver, kidney and brain after 4 hr of *p*-chloronitrobenzene injection in Swiss CD1 male mice in vivo	Cesarone et al. (1984); NTP (1993); IARC (1996)

[a](+) = positive; (–) = negative; NV = not available.

Li et al. (1999); Li et al. (1998)

Li et al. (1999) and Li et al. (1998) reported that BDF1 male mice exhibited immunotoxic responses with a decrease in B cells, T cells, NK cells, and subsets of T cells (CD4 and CD8) following a single intraperitoneal (i.p.) or subcutaneous (s.c.) injection of 300 mg/kg *p*-chloronitrobenzene (purity unknown) in olive oil, or a reduction in NK cell activity, cytotoxic T-lymphocyte activity, and LPS-stimulated splenocyte proliferation following 3 times/week i.p. injections of 30 mg/kg *p*-chloronitrobenzene over 4 weeks.

Yoshida et al. (1989)

Yoshida et al. (1989) investigated the renal toxicity of *p*-chloronitrobenzene and several other nitro-amino compounds in rats. Male F344 rats ($n = 5$) received a single intraperitoneal injection of 0 or 157.56 mg/kg of *p*-chloronitrobenzene (purity unknown). Control rats were injected with corn oil. Urine was collected for 24 hours after injection. Blood was collected and the kidneys were removed for histopathological examination after 48 hours of injection. Significant increase in methemoglobin and urinary *N*-acetyl-beta-D-glucosaminidase (NAG) levels was observed in *p*-chloronitrobenzene treated rats. These results indicate that *p*-chloronitrobenzene is nephrotoxic.

Hasegawa and Sato (1963)

Hasegawa and Sato (1963) reported formation of methemoglobin and Heinz bodies, as well as reduced catalase activity, in the blood of rabbits subcutaneously injected with 500 mg/kg of *p*-chloronitrobenzene (purity unknown). They concluded that *p*-chloronitrobenzene or its metabolites combined irreversibly with the hemoglobin molecule and functionally increased the molecule's oxygen affinity, suggesting acute hematotoxicity.

Haskell Laboratories (1984)

In a 2-week study, Haskell Laboratories (1984) exposed groups of 16 male S-D (Crl:CD [SD]BR) rats (nose/head only) to mean measured concentrations of 0, 50, 290, or 640 mg/m^3 of mixed *p*-chloronitrobenzene (99.2% purity) vapors and aerosols for 6 hours/day, 5 days/week; controls were exposed to air only. Using an 8-stage cascade impactor with a cyclone preseparator, analysis of the mid- and high-exposure concentrations revealed mass median aerodynamic diameter (MMAD) sizes of 10.3 and 22.6 μm and respirable fractions of 65.4 and 33.5%, respectively. Treatment had no effect on survival or body-weight gain. Stained fur, pallor, and alopecia were observed in the ≥290-mg/m^3 groups and hyperactivity in the 640-mg/m^3 group. A statistically significant increase in methemoglobinemia was observed in all exposed groups. Exposure-dependent trends were observed for increased splenic weight and decreased testes weight; relative liver and kidney weights were statistically significantly increased in the ≥290-mg/m^3 groups. Statistically significant increases in splenic effects (i.e., splenomegaly, dark coloration, hyperplastic red pulp, congestion, increased erythropoiesis, and hemosiderosis) were observed in all exposed groups. Rats exposed to ≥290 mg/m^3 had statistically significant decreased mean testes weight, degenerated spermatic contents of the epididymis, and increased degeneration of seminiferous tubules; the ratio of myeloid to erythroid bone marrow was also decreased in these rats. The lowest concentration, 50 mg/m^3, was a 6 hours/day, 5 days/week, 2-week LOAEL for effects on erythrocytes (i.e., methemoglobinemia and anemia), and the spleen (i.e., splenomegaly, hemosiderosis, congestion, hyperplastic red pulp, and increased erythropoiesis) in male rats.

Toxicokinetics

Yoshida et al. (1993); Nomeir et al. (1992)

p-Chloronitrobenzene (depicted as Chemical I in Figure 2) is metabolized similarly in rats and humans through eight metabolites, as shown in Figure 2 (Yoshida et al., 1993; Nomeir et al., 1992). The pathways include hydroxylation to 2-chloro-5-nitrophenol (Chemical II), glutathione conjugation to *N*-acetyl-S-(4-nitrophenyl)-L-cysteine (Chemical III), or reduction to *p*-chloroaniline (Chemical IV). The *p*-chloroaniline metabolite (Chemical IV) can undergo additional reactions to form three additional metabolites: chlorination to 2,4-dichloroaniline (Chemical V), acetylation to *p*-chloroacetanilide (Chemical VII), and hydroxylation to 2-amino-5-chlorophenol (Chemical VI). 2-Amino-5-chlorophenol (Chemical VI) can be further acetylated to 4-chloro-2-hydroxyacetanilide (Chemical VIII). *p*-Chloroacetanilide (Chemical VII) is then converted to *p*-chloro-oxanilic acid (Chemical IX) (Yoshida et al., 1993). These compounds are excreted via the urine in rats and humans. In addition, Nomeir et al. (1992) reported that, while 43−45% of radioactive *p*-chloronitrobenzene applied to rat skin was excreted in urine, 5−12% was excreted in the feces.

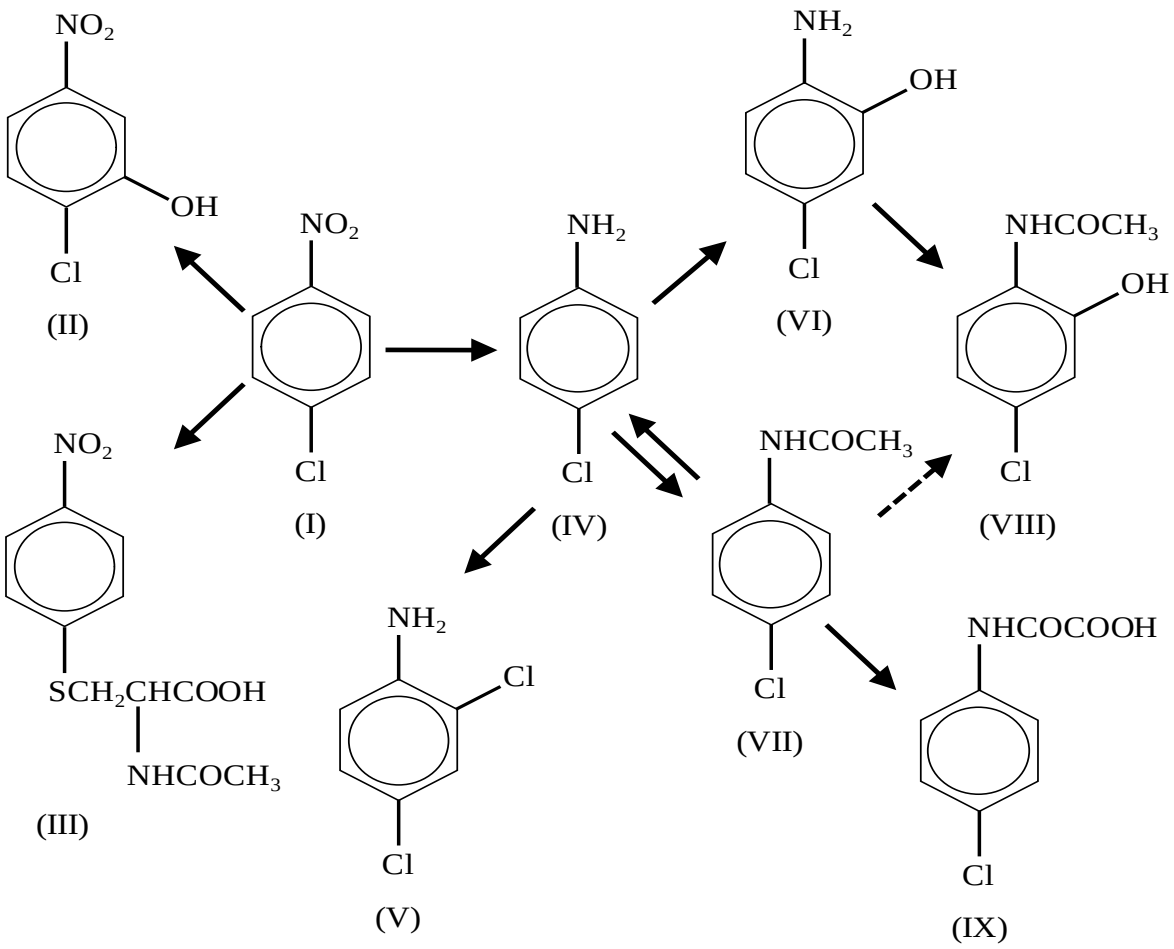

Figure 2. Metabolic Pathway of *p*-Chloronitrobenzene (Yoshida et al., 1993)
The compounds denoted by Roman numerals in this figure are as follows: *p*-Chloronitrobenzene (I); 2 chloronitrophenol (II); N acetyl S (4 nitrophenyl) L cysteine (III); *p*-chloroaniline (IV); 2,4 dichloroaniline (V); 2 amino 5 chlorophenol (VI); *p*-chloroacetanilide (VII); 4-chloro-2 hydroxyacetanilide (VIII); p-chloro-oxanilic acid (IX)

Jones et al. (2007)

Jones et al. (2007) identified the major metabolites in the urine of *p*-chloronitrobenzene exposed workers. Three conjugated metabolites were identified in exposed workers; *N*-acetyl-S-(4-nitrophenyl)-L-cysteine was the only metabolite detected in nonhydrolyzed urine, accounting for approximately 51% of the total metabolites detected. *p*-Chloroaniline and 2-chloro-5-nitrophenol were identified as cleavage products in hydrolyzed urine, accounting for approximately 18 and 30% of the metabolites detected, respectively.

Sabbioni (1994)

p-Chloronitrobenzene (0 or 79 mg/kg; 99% purity) administered by gavage to female Wistar rats (2/group) was found to form Hgb adducts at a high rate compared to other nitroarenes, but less than its *p*-chloroaniline metabolite, after 24 hours (Sabbioni, 1994). The Hgb binding index ([mmol compound/mol hemoglobin] ÷ [mmol compound/kg body weight])

was 215.4 ± 5.0 for *p*-chloronitrobenzene and 569.0 for *p*-chloroaniline. This activity was attributed to the reducibility of the nitro group.

NTP (1993); Monsanto (1994a)

In pharmacokinetic studies in male F344 rats (3−4/group), approximately 86−93% of a single oral dose of 0, 2, 20, 65, or 200 mg/kg (97−99% pure) *p*-chloronitrobenzene was absorbed (NTP, 1993). In male F344 or S-D rats (3−4/group) given a single oral dose of radiolabeled *p*-chloronitrobenzene (200 mg/kg), urinary excretion was the main route of elimination (Monsanto, 1994a; NTP, 1993); at 72 hours, recovery of label was 68−74.6% in urine and 12.3−20.5% in feces. Only 0.4% was recovered in expired air in the Monsanto (1994a) study. In both studies, the highest amount of radiolabel in tissues was recovered in fat, whole blood/blood cells, and the spleen.

Genotoxicity and Mutagenicity

IARC (1996); U.S. EPA (1985); NTP (1993); Huang et al. (1996)

IARC (1996) summarized available genotoxicity and mutagenicity data; doses and other test details are available in Table 3B of the IARC (1996) report. *p*-Chloronitrobenzene did not induce reverse mutations in *Salmonella typhimurium* strains TA98, TA1530, TA1537, TA1538, TA1532, TA1950, TA1975, TA1978, or G46 with or without metabolic activation, or in strain TA98NR with activation, and yielded conflicting results in strains TA100 and TA1535 with or without metabolic activation (IARC, 1996; NTP, 1993; Suzuki et al., 1987; U.S. EPA, 1985; Haworth et al., 1983; Shimizu et al., 1983; Suzuki et al., 1983; Gilbert et al., 1980). *p*-Chloronitrobenzene gave negative results in the *Escherichia coli* SOS chromotest (IARC, 1996; von der Hude et al., 1988). It did not induce heritable sex-linked recessive lethal mutations in *Drosophila melanogaster* when administered in feed to larvae or adults or when injected into adults (IARC, 1996; NTP, 1993; Zimmering et al., 1989; Zimmering et al., 1985). *p*-Chloronitrobenzene induced sister chromatid exchange (SCE) in cultured Chinese hamster ovary (CHO) cells in the presence of S9 (IARC, 1996; NTP, 1993). With or without S9, *p*-chloronitrobenzene induced chromosomal aberrations in CHO cells but only at cytotoxic concentrations (IARC, 1996; NTP, 1993; Galloway et al., 1987). It did not induce chromosomal aberrations in peripheral blood obtained from a human male donor (Huang et al., 1996). *p*-Chloronitrobenzene induces DNA single strand breaks, but followed up with DNA repair in hepatocytes isolated from male Wistar rats (IARC, 1996; NTP, 1993; Cesarone et al., 1984). When intraperitoneally injected into Swiss CD-1 mice, *p*-chloronitrobenzene induced DNA single-strand breaks in the liver, kidney, and brain (IARC, 1996; NTP, 1993; Cesarone et al., 1983). In summary, genotoxicity and mutagenicity assays for *p*-chloronitrobenzene were primarily negative in bacteria but were more often positive in mammalian systems, possibly reflecting a requirement for bioactivation.

DERIVATION OF PROVISIONAL VALUES

Tables 5 and 6 presents the summaries of noncancer and cancer provisional reference values, respectively.

Table 5. Summary of Noncancer Reference Values for *p*-Chloronitrobenzene (CASRN 100-00-5)

Toxicity Type (units)	Species/Sex	Critical Effect	p-Reference Value	POD Method	POD	UF_C	Principal Study
Subchronic p-RfD (mg/kg-d)	Rat/M	Methemoglobinemia	7×10^{-4}	$BMDL_{1SDHED}$	0.02	30	Monsanto (1994b)
Chronic p-RfD (mg/kg-d)	Rat/M	Methemoglobinemia	7×10^{-4}	$BMDL_{1SDHED}$	0.02	30	Monsanto (1994b)
Subchronic p-RfC (mg/m^3)	Rat/M+F	Methemoglobinemia	6×10^{-3}	$LOAEL_{HEC}$	1.7	300	NTP (1993); Travlos et al. (1996)
Chronic p-RfC (mg/m^3)	Rat/M+F	Methemoglobinemia	2×10^{-3}	$LOAEL_{HEC}$	1.7	1,000	NTP (1993); Travlos et al. (1996)

BMDL = lower confidence limit (95%) on the benchmark dose; F = female; HEC = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; M = male ; POD = point-of-departure; UF_c = composite uncertainty factor.

Table 6. Summary of Cancer Values for *p*-Chloronitrobenzene (CASRN 100-00-5)

Toxicity Type	Species/Sex	Tumor Type	Cancer Value	Principal Study
p-OSF $(mg/kg\text{-}d)^{-1}$	Rat/M	Splenic hemangiosarcoma	6×10^{-2}	Matsumoto et al. (2006a)
p-IUR	NDr			

M = male; NDr = not determined.

DERIVATION OF PROVISIONAL ORAL REFERENCE DOSES

The available animal studies provide sufficient information for the derivation of subchronic and chronic p-RfDs for *p*-chloronitrobenzene. The oral toxicity database consists of three subchronic-duration studies in rats and one subchronic-duration study in mice, two chronic-duration studies in rats and one chronic-duration study in mice, two reproductive toxicity studies (one in rats and one in mice), and two developmental toxicity studies (one in rats and one in rabbits). Table 3A summarizes the noncancer dose-response data from the available oral studies.

The database strongly supports methemoglobinemia as the most sensitive effect following oral exposure to *p*-chloronitrobenzene, and that this effect is a key precursor to subsequent hematotoxic and related organ tissue outcomes. Although no human oral data were located, humans are known to be susceptible to methemoglobinemia following inhalation or dermal exposure to *p*-chloronitrobenzene (ACGIH, 2001; SRC, 1992; Yoshida et al., 1987; Pacseri et al., 1958). Subchronic-duration oral studies in rats reported methemoglobinemia or anemia (reduced hemoglobin, hematocrit, and erythrocyte counts) and splenic effects (hemosiderosis, extramedullary hematopoiesis, congestion, splenomegaly, and increased relative spleen weight) as the most sensitive and consistently observed effects following oral exposure to *p*-chloronitrobenzene at doses of 3−257.1 mg/kg-day (Matsumoto et al., 2006b; Monsanto, 1994b, c). With increasing doses to rats, continued destruction of erythrocytes resulted in effects in other organs besides the spleen: extramedullary hematopoiesis and hemosiderosis in the liver, hemosiderosis in the kidney, and hyperplasia of the bone marrow (Matsumoto et al., 2006b; Monsanto, 1994b, c). Outward signs of methemoglobinemia, including paleness of extremities and cyanosis, were observed in rats at doses of 12.6−257.1 mg/kg-day (Monsanto, 1994b). In one subchronic-duration study in mice, related effects, including anemia, and splenic effects were identified, albeit at higher doses (≥36.7 mg/kg-day) than in rats (Matsumoto et al., 2006b). Chronic-duration studies (Matsumoto et al., 2006a; Bio Dynamics, 1985) further supported the finding of methemoglobinemia (at doses of 0.7−53.8 mg/kg-day) and related splenic effects (at doses of 7.7−53.8 mg/kg-day) as the primary response to *p*-chloronitrobenzene exposure in rats, and also confirmed the greater sensitivity of rats than mice to this effect.

Splenic effects also were observed in a developmental toxicity study in rats (at doses of 5−45 mg/kg-day) while developmental effects (decreased fetal weight and increased skeletal anomalies) were observed at higher doses of 45 mg/kg-day (Nair et al., 1985; Bio Dynamics, 1980). In mice, decreased fetal body weight was observed albeit at higher doses (≥62.5 mg/kg-day) than in rats (Chapin et al., 1997). Death and spontaneous abortions were observed in rabbits exposed during gestation to similarly higher doses of 40 mg/kg-day (Bio Dynamics, 1982). There was some evidence for testicular effects (oligospermia, degeneration, and reduced fertility) in F0 male rats at a dose of 5.0 mg/kg-day in a two-generation reproduction study; however, testicular effects were not observed in F1 males (Bio Dynamics, 1984).

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

The subchronic-duration studies (Matsumoto et al., 2006b; Monsanto, 1994b, c), reproductive toxicity studies (Chapin et al., 1997; Bio Dynamics, 1984) and developmental toxicity studies (Bio Dynamics, 1982, 1980) are considered as potential principal studies on which to base the subchronic p-RfD for *p*-chloronitrobenzene. Among the subchronic-duration, reproductive toxicity, and developmental toxicity studies shown in Table 3A, the lowest LOAEL is 1.4 mg/kg-day for decreased erythrocyte count in female rats in the 90-day dietary study

(Matsumoto et al., 2006b). However, the biological significance of a modest reduction in erythrocytes (e.g., 4% decrease at the LOAEL) is unclear. And, although Matsumoto et al. (2006b) calculated a benchmark dose lower confidence limit ($BMDL_{10}$) of 0.177 mg/kg-day using the Hill model for this endpoint, it is uncertain what a biologically relevant benchmark response (BMR) level for reduced erythrocyte counts should be. As such, a default BMR of 1 standard deviation (SD) from the control mean was used to model this endpoint (U.S. EPA, 2012b).

The following data were subjected to BMD modeling using the U.S. EPA's Benchmark Dose Software (BMDS) (Version 2.2.1): (1) the Monsanto (1994b) methemoglobin, erythrocyte count, hemoglobin concentration, reticulocyte count, relative spleen weight, and splenic hematopoiesis data from male and female rats, as well as splenic hemosiderosis in males; (2) the Matsumoto et al. (2006b) erythrocyte count data in female rats, as well as erythrocyte count, hemoglobin concentration and splenic hemosiderin, congestion and extramedullary hematopoiesis data in males; and (3) the Bio Dynamics (1980) maternal spleen weight. Appendix C describes the BMD results for data that were amenable to modeling. In the absence of a biologically relevant BMR level, a BMR of 1 SD from the control mean is used for each of the continuous endpoints. Candidate PODs from the subchronic-duration oral exposure studies are summarized in Table 7.

The $BMDL_{1SD}$ of 1.43 mg/kg-day, calculated for reduced erythrocyte counts in female rats, is based on a more appropriate BMR than the $BMDL_{10}$ of 0.177 mg/kg-day reported by Matsumoto et al. (2006b). Therefore, the $BMDL_{1SD}$ of 1.43 mg/kg-day is considered the POD for erythrocyte reduction in rats. Efforts to model the Monsanto (1994b) methemoglobin concentration and splenic hematopoiesis data in females, erythrocyte counts in males, reticulocyte counts and relative spleen weight in both sexes, as well as the hemoglobin concentration, splenic hemosiderin and congestion in male rats from Matsumoto et al. (2006b), were not successful; thus only NOAELs and/or LOAELs are available for these data (see Table 7).

Of the toxicological effects observed in rats and mice from the subchronic-duration, reproductive toxicity, and developmental toxicity studies, the most sensitive POD is a $BMDL_{10}$ of 0.060 mg/kg-day for splenic hematopoiesis in male rats (Monsanto, 1994b) (see Table 7). Although this BMDL is slightly more sensitive than the $BMDL_{1SD}$ of 0.084 mg/kg-day for methemoglobinemia in male rats (Monsanto, 1994b), it is appropriate to select methemoglobinemia as the critical effect because methemoglobinemia is likely the first step in a progression of the other hematopoietic and splenic effects; the chronic-duration study (Bio Dynamics, 1985) conducted on the same strain of rat also supports methemoglobinemia as the most sensitive effect. The selection of increased methemoglobinemia in male rats as the critical effect is supported by treatment-dependent hematotoxicity (i.e., reduced hemoglobin, hematocrit, and erythrocyte counts) and splenic effects (i.e., hemosiderosis, extramedullary hematopoiesis, and congestion) in rats and mice of both sexes. Thus, the $BMDL_{1SD}$ of 0.084 mg/kg-day for methemoglobinemia in male rats from the Monsanto (1994b) study is selected as the POD for derivation of the subchronic p-RfD.

Table 7. Candidate PODs for Multiple Noncancer Effects in Rats Following Subchronic-Duration Oral Exposure to *p*-Chloronitrobenzene (CASRN 100-00-5)

Effect	Dose (mg/kg-day)					Reference
	NOAEL	LOAEL	BMR	BMD	BMDL	
Methemoglobinemia	NV (M and F)	3 (M and F)	1 SD	0.12 (M) NF (F)	0.084 (M) NF (F)	Monsanto (1994b)
Reduced hemoglobin concentration			1 SD	0.41 (M) 0.49 (F)	0.24 (M) 0.20 (F)	
Reduced erythrocyte counts			1 SD	NF (M) 0.83 (F)	NF (M) 0.59 (F)	
Increased reticulocyte counts			1 SD	NF (M and F)	NF (M and F)	
Increased splenic hematopoiesis			10%	0.11 (M) NF (F)	0.060 (M) NF (F)	
Increased relative spleen weight			1 SD	NF (M and F)	NF (M and F)	
Increased hemosiderin in spleen	NV (M)	3 (M)	10%	NF	NF	
Reduced erythrocyte counts	1.2 (M) NV (F)	3.4 (M) 1.4 (F)	1 SD	1.23 (M) 2.00 (F)	0.59 (M) 1.43 (F)	Matsumoto et al. (2006b)
Reduced hemoglobin concentration	1.2 (M)	3.4 (M)	1 SD	NF	NF	
Increased extramedullary hematopoiesis in spleen			10%	1.20	0.81	
Increased hemosiderin in spleen			10%	NF	NF	
Increased congestion in spleen			10%	NF	NF	
Increased maternal absolute spleen weight	NV (F)	5 (F)	1 SD	1.76	1.43	Bio Dynamics (1980)

BMD input data are presented in Appendix B. The resulting curves and BMD output text are provided in Appendix C.

F = female; M = male; NV = not available; NF= no acceptable model fitSD = standard deviation;

The U.S. EPA endorses a hierarchy of approaches to derive human equivalent oral exposures from data from laboratory animal species, with the preferred approach being physiologically based toxicokinetic modeling. Another approach may include using some chemical-specific information without a complete physiologically based toxicokinetic model. In lieu of chemical-specific models or data to inform the derivation of human equivalent oral exposures, U.S. EPA endorses body-weight scaling to the 3/4 power (i.e., $BW^{3/4}$) as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory

animals to humans for the purpose of deriving an RfD under certain exposure conditions U.S. EPA (2011b). More specifically, the use of $BW^{3/4}$ scaling for deriving an RfD is recommended when the observed effects are associated with the parent compound or a stable metabolite but not for portal-of-entry effects. A validated human physiologically based pharmacokinetic model for *p*-chloronitrobenzene is not available for use in extrapolating doses from animals to humans. In addition, the selected POD of 0.084 mg/kg-day is based on increased methemoglobin, which is not a portal-of-entry or developmental effect. Therefore, scaling by $BW^{3/4}$ is relevant for deriving human equivalent doses (HEDs) for this effect.

Following U.S. EPA (2011b) guidance, the POD for the subchronic-duration rat study is converted to an HED through the application of a dosimetric adjustment factor (DAF^{1}) derived as follows:

$$DAF = (BW_a^{1/4} \div BW_h^{1/4})$$

where

DAF = dosimetric adjustment factor
BW_a = animal body weight
BW_h = human body weight

Using a BW_a of 0.25 kg for rats and a default BW_h of 70 kg for humans (U.S. EPA, 1988), the resulting DAF is 0.24. Applying this DAF to the $BMDL_{1SD}$ obtained from modeling the methemoglobinemia data from males in the Monsanto (1994b) study yields a $BMDL_{1SDHED}$ as follows:

$$BMDL_{1SDHED} = BMDL_{1SD}\ (mg/kg\text{-}day) \times DAF$$
$$= 0.084\ mg/kg\text{-}day \times 0.24$$
$$= 0.020\ mg/kg\text{-}day$$

The subchronic p-RfD for *p*-chloronitrobenzene is derived by applying a composite uncertainty factor (UF_C) of 30 to the $BMDL_{1SDHED}$ of 0.020 mg/kg-day as follows:

$$\textbf{Subchronic p-RfD} = BMDL_{1SDHED} \div UF_C$$
$$= 0.020\ mg/kg\text{-}day \div 30$$
$$= \mathbf{7 \times 10^{-4}\ mg/kg\text{-}day}$$

Table 8 summarizes the uncertainty factors for the subchronic p-RfD for *p*-chloronitrobenzene.

[1]As described in detail in *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* U.S. EPA (2011b), rate-related processes scale across species in a manner related to both the direct ($BW^{1/1}$) and allometric scaling ($BW^{3/4}$) aspects such that $BW^{3/4} \div BW^{1/1} = BW^{-1/4}$, converted to a $DAF = BW_a^{1/4} \div BW_h^{1/4}$.

Table 8. Uncertainty Factors for the Subchronic p-RfD for *p*-Chloronitrobenzene (CASRN 100-00-5)

UF	Value	Justification
UF_A	3	Methemoglobin reductase activity in rodents has been reported to be approximately 5−9.5 times higher than in humans (Bolyai et al., 1972; Smith et al., 1967; Stolk and Smith, 1966). Thus, humans may potentially be more susceptible to *p*-chloronitrobenzene-induced methemoglobinemia than rodents. A UF_A of 3 ($10^{0.5}$) is applied to account for remaining uncertainty such as the toxicodynamic differences between rats and humans following oral *p*-chloronitrobenzene exposure. The toxicokinetic uncertainty has been accounted for by calculation of a HED through application of a DAF as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	1	A UF_D of 1 is applied because the database includes one acceptable 2-generation reproductive toxicity study in rats (Bio Dynamics, 1984), and 2 acceptable developmental toxicity studies ―1 in rats (Nair et al., 1985; Bio Dynamics, 1980) and 1 in rabbits (Bio Dynamics, 1982)―via the oral route.
UF_H	10	A UF_H of 10 is applied for intraspecies variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of *p*-chloronitrobenzene in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL.
UF_S	1	A UF_S of 1 is applied because a subchronic-duration study was selected as the principal study.
UF_C	30	$UF_C = UF_A \times UF_D \times UF_H \times UF_L \times UF_S$

BMDL = lower confidence limit (95%) on the benchmark dose; DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point-of-departure.

The confidence in the subchronic p-RfD for *p*-chloronitrobenzene is high as explained in Table 9.

Table 9. Confidence Descriptors for the Subchronic p-RfD for *p*-Chloronitrobenzene (CASRN 100-00-5)

Confidence Categories	Designation[a]	Discussion
Confidence in principal study	H	Confidence in the principal study is high. Monsanto (1994b) utilized an adequate number of animals of both sexes, examined a large number of endpoints, including suspected targets of toxicity (hematologic, spleen), and the study was well-designed and well-documented.
Confidence in database	H	Confidence in the database is high because it includes 3 subchronic-duration studies in rats, a subchronic-duration study in mice, 2 chronic-duration studies in rats, a chronic-duration study in mice, reproductive toxicity studies in rats and mice, and developmental toxicity studies in rats and rabbits.
Confidence in subchronic p-RfD	H	The overall confidence in the subchronic p-RfD is high.

[a]H = high.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

The available chronic-duration studies (Matsumoto et al., 2006a; Bio Dynamics, 1985), reproductive toxicity studies (Chapin et al., 1997; Bio Dynamics, 1984), and developmental toxicity studies (Bio Dynamics, 1982, 1980) are considered as potential principal studies on which to base the chronic p-RfD for *p*-chloronitrobenzene. Among these studies, the lowest LOAEL was 0.7 mg/kg-day for methemoglobinemia in male and female rats in a 24-month gavage study (Bio Dynamics, 1985); the corresponding NOAEL was 0.1 mg/kg-day. NOAELs and LOAELs from the chronic-duration dietary study in rats, which did not measure blood methemoglobin concentrations, were approximately 10-fold higher (Matsumoto et al., 2006a).

The following data were subjected to BMD modeling using the U.S. EPA's BMDS (Version 2.2.1): (1) the Bio Dynamics (1985) data on methemoglobin concentrations in male and female rats, measured at the end of the 24-month exposure period and (2) the Bio Dynamics (1980) maternal splenic weight. Candidate PODs from the chronic-duration oral exposure studies are presented in Table 10.

Table 10. Candidate PODs for Multiple Noncancer Effects in Rats Following Chronic-Duration Oral Exposure to *p*-Chloronitrobenzene (CASRN 100-00-5)

Effect	Dose (mg/kg-day)					Reference
	NOAEL	LOAEL	BMR	BMD	BMDL	
Methemoglobinemia	0.1 (M and F)	0.7 (M and F)	1SD	0.16 (M) 0.15 (F)	0.13 (M) 0.12 (F)	Bio Dynamics (1985)
Increased maternal absolute spleen wt	NV (F)	5.0 (F)	1SD	1.76 (F)	1.43 (F)	Bio Dynamics (1980)

M and F in the parentheses denotes males and females, respectively.
BMD input data are presented in Appendix B. The resulting curves and BMD output text are provided in Appendix C.

BMDL = lower confidence limit (95%) on the benchmark dose; BMD = benchmark dose; BMR = benchmark response; F = female; LOAEL = lowest-observed-adverse-effect level; M = male; NOAEL = no-observed-adverse-effect level; NV = not available; SD = standard deviation; wt = weight.

Of the toxicological effects observed in rats and mice in the chronic-duration oral toxicity studies, as well as reproductive and developmental toxicity studies (see derivation of subchronic p-RfD above), the most sensitive POD is the $BMDL_{1SD}$ of 0.12 mg/kg-day for increased methemoglobin in female rats from the 24-month gavage study (Bio Dynamics, 1985). Appendix C describes the BMD modeling results for methemoglobinemia. In the absence of a biologically relevant BMR level, a default BMR of 1 SD above the control mean is used to estimate the BMD, as recommended by U.S. EPA (2012b). $BMDL_{1SD}$ values of 0.12 mg/kg-day (female rats) or 0.13 mg/kg-day (male rats) are essentially identical to the NOAEL of 0.1 mg/kg-day from this data set and are more robust indicators of a POD because they are based on the entire data set. The selection of increased methemoglobin in female rats as the critical effect is supported by treatment-dependent hematotoxicity (i.e., reduced hemoglobin, hematocrit, and erythrocyte counts) and splenic effects (i.e., capsular fibroblast hyperplasia, fibrosis, fatty metamorphosis, increased extramedullary hematopoiesis, and splenic nodules) in rats of both

sexes; treatment-dependent hematotoxicity (i.e., reduced hematocrit and erythrocyte counts) in mice of both sexes, and splenic effects in mice (i.e., congestion in both sexes, extramedullary hematopoiesis in males, hemosiderin deposition, splenic ossification, and splenic nodules in females).

Similar to the derivation shown for the subchronic-duration studies, the BMDLs are converted to HEDs following "*Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose*" (U.S. EPA, 2011b).

$$\begin{aligned} BMDL_{1SDHED} &= BMDL_{1SD}\ (mg/kg\text{-}day) \times DAF \\ &= BMDL_{1SD}\ (mg/kg\text{-}day) \times 0.24 \\ &= 0.12\ mg/kg\text{-}day \times 0.24 \\ &= 0.029\ mg/kg\text{-}day \end{aligned}$$

It should be noted that methemoglobinemia has the most sensitive POD for both subchronic- and chronic-duration studies in the same strain of rats (S-D). In addition, both subchronic- and chronic-duration studies are well-conducted. However, the POD from the subchronic-duration study ($BMDL_{1SDHED}$ = 0.020 mg/kg-day) is slightly more sensitive than that of the chronic-duration study ($BMDL_{1SDHED}$ = 0.029 mg/kg-day). Additionally, the available data indicate that at approximately equivalent orally administered doses, the severity of methemoglobinemia does not increase in magnitude with increasing duration of *p*-chloronitrobenzene exposure in rats (see Figure 3). As such, the $BMDL_{1SDHED}$ of 0.020 mg/kg-day from the subchronic-duration study (Monsanto, 1994b) is used as the POD to derive the chronic p-RfD.

A chronic p-RfD for *p*-chloronitrobenzene is derived by applying a UF_C of 30 to the $BMDL_{1SDHED}$ of 0.020 mg/kg-day as follows:

$$\begin{aligned} \textbf{Chronic p-RfD} &= BMDL_{1SDHED} \div UF_C \\ &= 0.020\ mg/kg\text{-}day \div 30 \\ &= \mathbf{7 \times 10^{-4}\ mg/kg\text{-}day} \end{aligned}$$

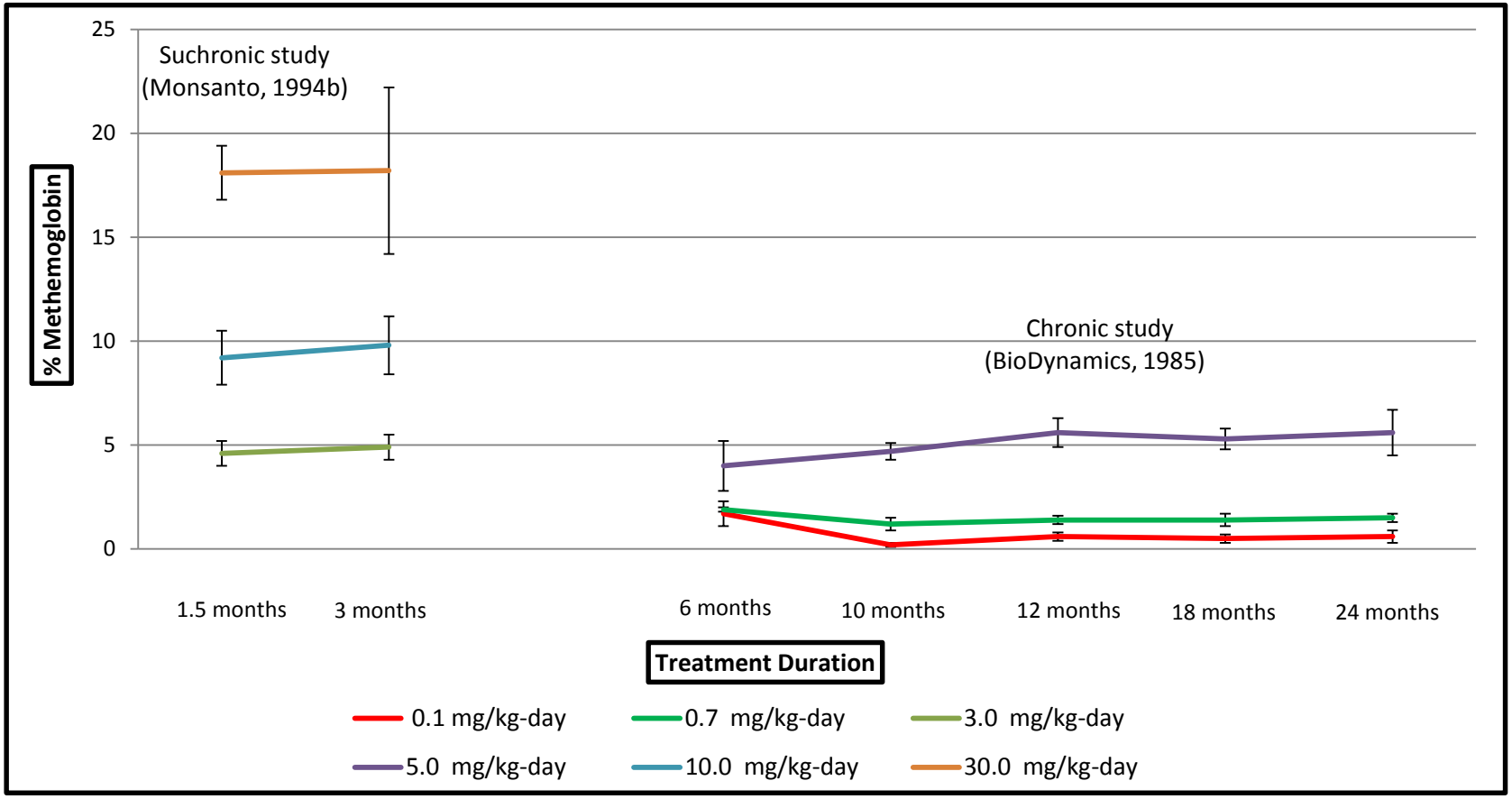

Figure 3. Percent Increase in Methemoglobin over Control Values at Various Time Points Following Oral Administration of *p*-Chloronitrobenzene to Female S-D Rats (Monsanto, 1994b; Bio Dynamics, 1985)

Table 11 summarizes the uncertainty factors for the chronic p-RfD for *p*-chloronitrobenzene.

Table 11. Uncertainty Factors for the Chronic p-RfD for *p*-Chloronitrobenzene (CASRN 100-00-5)

UF	Value	Justification
UF_A	3	Methemoglobin reductase activity in rodents has been reported to be approximately 5−9.5 times higher than in humans (Bolyai et al., 1972; Smith et al., 1967; Stolk and Smith, 1966). Thus, humans may potentially be more susceptible to *p*-chloronitrobenzene-induced methemoglobinemia than rodents. A UF_A of 3 ($10^{0.5}$) is applied to account for remaining uncertainty such as the toxicodynamic differences between rats and humans following oral *p*-chloronitrobenzene exposure. The toxicokinetic uncertainty has been accounted for by calculation of a HED through application of a DAF as outlined in the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
UF_D	1	A UF_D of 1 is applied because the database includes 1 acceptable two-generation reproductive toxicity study in rats (Bio Dynamics, 1984) and 2 acceptable developmental toxicity studies—1 in rats (Nair et al., 1985; Bio Dynamics, 1980) and 1 in rabbits (Bio Dynamics, 1982)—via the oral route.
UF_H	10	A UF_H of 10 is applied for intraspecies variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of *p*-chloronitrobenzene in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL.
UF_S	1	A UF_S of 1 is applied because there is no increase in the magnitude of methemoglobinemia severity beyond subchronic-duration *p*-chloronitrobenzene oral exposure and the database included both subchronic- and chronic-duration studies. Additionally, methemoglobinemia is the most sensitive effect observed in both subchronic- and chronic-duration oral studies in the same strain of rats.
UF_C	30	$UF_C = UF_A \times UF_D \times UF_H \times UF_L \times UF_S$

BMDL = lower confidence limit (95%) on the benchmark dose; DAF = dosimetric adjustment factor; HED = human equivalent dose; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point-of-departure.

The confidence in the chronic p-RfD for *p*-chloronitrobenzene is high as explained in Table 12.

Table 12. Confidence Descriptors for the Chronic p-RfD for *p*-Chloronitrobenzene (CASRN 100-00-5)

Confidence Categories	Designation[a]	Discussion
Confidence in principal study	H	Confidence in the principal study is high. Monsanto (1994b) utilized an adequate numbers of animals of both sexes, examined a large number of endpoints, including suspected targets of toxicity (hematologic, spleen), and the study was well-designed and well-documented.
Confidence in database	H	Confidence in the database is high because it includes 3 subchronic-duration studies in rats, a subchronic-duration study in mice, 2 chronic-duration studies in rats, a chronic-duration study in mice, reproductive toxicity studies in rats and mice, and developmental toxicity studies in rats and rabbits.
Confidence in chronic p-RfD	H	The overall confidence in the chronic p-RfD is high.

[a]H = high.

DERIVATION OF PROVISIONAL INHALATION REFERENCE CONCENTRATIONS

The available animal studies provide sufficient information for the derivation of subchronic and chronic p-RfCs for *p*-chloronitrobenzene. The inhalation toxicity database consists of one short-term study in rats, one subchronic-duration study in rats, and one subchronic-duration study in mice. No inhalation studies examining chronic-duration, reproductive, or developmental toxicity are available. Table 3A summarizes the noncancer exposure-response data from available inhalation studies.

Humans are known to be susceptible to methemoglobinemia from combined inhalation and dermal exposure to *p*-chloronitrobenzene (ACGIH, 2001; SRC, 1992; Yoshida et al., 1987; Pacseri et al., 1958), although no quantitative data are available for long-term inhalation exposures. Short-term and subchronic-duration inhalation exposure studies in rats reported methemoglobinemia or anemia (i.e., reduced Hgb, Hct, and erythrocyte counts) and splenic effects (i.e., hemosiderosis and congestion) as the most sensitive and consistently observed effects following inhalation exposure to *p*-chloronitrobenzene at concentrations of 0.89−27.5 mg/m^3 (Travlos et al., 1996; NTP, 1993; Nair et al., 1986).

With increasing inhalation exposure concentrations of *p*-chloronitrobenzene to rats, a concentration-dependent reduction in erythrocyte count was observed that actuates effects in other organs, including increased hematopoietic cell proliferation in the bone marrow and increased hemosiderin deposition in the liver (Travlos et al., 1996; NTP, 1993). A concentration-dependent increase in cyanosis, an apparent clinical sign of methemoglobinemia, was also observed in rats (Nair et al., 1986). In addition, related hematologic effects in the spleen (i.e., hemosiderin deposition and hematopoietic cell proliferation) and in the liver (hemosiderin deposition) were also observed in mice, but at higher exposure concentrations (≥13.8 mg/m^3) than in rats (≥1.7 mg/m^3) (Travlos et al., 1996; NTP, 1993). Inhalation-induced effects of *p*-chloronitrobenzene on hematology and related effects in other organs (i.e., liver, spleen, and bone marrow) are also observed following oral administration of *p*-chloronitrobenzene to rats and mice (as described in the p-RfD section above).

Derivation of Subchronic Provisional RfC (Subchronic p-RfC)

The subchronic-duration (Travlos et al., 1996; NTP, 1993) and 4-week (Nair et al., 1986) rat and mouse inhalation toxicity studies are considered as potential principal studies on which to base the subchronic p-RfC for *p*-chloronitrobenzene. No NOAELs were identified in any of these studies. A LOAEL of 1.7 mg/m^3 was identified from the subchronic-duration inhalation study by the NTP based on methemoglobinemia, hematology effects (erythrocyte counts, Hct, and reticulocyte counts), and splenic lesions (congestion and hemosiderosis) in male and female rats (Travlos et al., 1996; NTP, 1993). A subchronic-duration inhalation study in mice (Travlos et al., 1996; NTP, 1993) indicated that mice are less sensitive to *p*-chloronitrobenzene than rats, with LOAELs of 13.8 mg/m^3 in both sexes for both splenic effects (increased absolute and relative weights, hemosiderosis and hematopoietic cell proliferation) and increased relative liver weight. Because methemoglobin concentrations were not analyzed in mice the identification of NOAELs of 6.9 mg/m^3 in both sexes is uncertain. Nair et al. (1986) identified a 4-week LOAEL of 0.89 mg/m^3 for similar hematological effects in rats (i.e., methemoglobinemia in males, and reduced hematocrit in females). However, interpretation of the Nair et al. (1986) data is confounded by coexposure to high concentrations of 2-ethoxyethanol, a carrier used for *p*-chloronitrobenzene that also has been associated with hematologic effects (U.S. EPA, 1991b; Doe, 1984). Thus, it cannot be determined if the hematological effects observed in the Nair et al. (1986) study may be due the combined effects of *p*-chloronitrobenzene and its 2-ethoxyethanol carrier. Hence, the data from the Nair et al. (1986) study are not considered suitable for derivation of a p-RfC.

The following data from the NTP (1993) and Travlos et al. (1996) studies were subjected to BMD modeling using the U.S. EPA's BMDS (Version 2.2.1): methemoglobin concentrations, erythrocyte counts, reticulocyte counts, hematocrit, and splenic congestion and hemosiderin from both sexes of rats. Appendix D describes the BMD results for data that were amenable to modeling. In each case, a 1 SD default BMR was used to calculate the benchmark concentration (BMC) and benchmark concentration lower confidence limit (BMCL) in the absence of information to indicate a more suitable biological response level.

From the NTP (1993) study, efforts to model the splenic lesion data, methemoglobin concentration data, and reticulocyte counts in both sexes, as well as erythrocyte counts in female rats, were not successful. A model fit was not achieved with any model, even after dropping up to three of the highest-exposure groups. Adequate model fits were provided by the Hill model for the NTP (1993) erythrocyte data in males (with nonconstant variance) and hematocrit data (with constant variance and the high-concentration data dropped to achieve a better fit) in both sexes. Candidate PODs from the subchronic-duration inhalation exposure studies are summarized in Table 13.

To provide a common basis for comparing the available data, the NOAELs and LOAELs from the studies were converted to human equivalent concentrations ($NOAEL_{HEC}$s and $LOAEL_{HEC}$s) based on guidance provided in U.S. EPA (1994) *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry*. No portal-of-entry adverse effects were observed in any of the studies. Each effect level was first adjusted to an equivalent continuous exposure concentration (see example calculation below using the data from the NTP (1993) and Travlos et al. (1996) studies):

$$LOAEL_{ADJ} = (LOAEL) \times (hours \div 24\ hours) \times (days \div 7\ days)$$
$$= (9.7\ mg/m^3) \times (6\ hours \div 24\ hours) \times (5\ days \div 7\ days)$$
$$= 1.7\ mg/m^3$$

Human equivalent concentrations were then calculated using the appropriate dosimetric adjustment (U.S. EPA, 1994). As methemoglobinemia, hematologic effects, and splenic lesions are extrarespiratory effects, *p*-chloronitrobenzene was treated as a Category 3 gas, and the ratio of blood:gas partition coefficients was used to make the dosimetric adjustment (U.S. EPA, 1994). In the absence of blood:gas partition coefficients for *p*-chloronitrobenzene, the default ratio of 1.0 was used; an example calculation is shown below:

$$LOAEL_{HEC} = (LOAEL_{ADJ}) \times [(H_{b/g})_A \div (H_{b/g})_H]$$
$$= 1.7\ mg/m^3 \times 1$$
$$= 1.7\ mg/m^3$$

where

$(H_{b/g})_A \div (H_{b/g})_H$ = Rat-to-human ratio of blood:gas partition coefficients

Table 3A shows the $NOAEL_{HEC}$s and $LOAEL_{HEC}$s calculated for each of the studies.

Table 13. Candidate PODs for Multiple Noncancer Effects Following Subchronic-Duration Inhalation Exposure to p-Chloronitrobenzene (CASRN 100-00-5)

Effect	Concentration in HEC (mg/m^3)					Reference
	NOAEL	LOAEL	BMR	BMC	BMCL	
Decreased erythrocyte counts	NV (M and F)	1.7 (M and F)	1 SD	0.99 (M) NF (F)	0.83 (M) NF (F)	NTP (1993); Travlos et al. (1996)
Decreased hematocrit				0.66 (M) 0.24 (F)	0.50 (M) 0.18 (F)	
Increased methemoglobin				NF (M and F)	NF (M and F)	
Increased reticulocyte counts				NF (M and F)	NF (M and F)	
Increased congestion in spleen			10%	NF (M and F)	NF (M and F)	
Increased hemosiderin in spleen				NF (M and F)	NF (M and F)	

BMD input data are presented in Appendix B. The resulting curves and BMD output text are provided in Appendix D.

F = female; M = male; NV = not available; NF = no acceptable model fit

Of all the toxicological effects observed, the effect with the lowest benchmark concentration lower confidence limit (BMCL) is decreased hematocrit in females with a

$BMCL_{1SDHEC}$ of 0.18 mg/m^3. However, considering the logical causal pathway of *p*-chlorobenzene-induced blood effects, it is not certain that the selection of the $BMCL_{1SDHEC}$ of 0.18 mg/m^3 for decreased hematocrit as the POD would protect against methemoglobinemia because 1) it is likely that methemoglobinemia is the first step in the progression of other hematopoietic and splenic effects, and 2) the methemoglobinemia data from both males and females could not be modeled and does not have a NOAEL. Thus, it is appropriate to select methemoglobinemia as the critical effect with a $LOAEL_{HEC}$ of 1.7 mg/m^3 as the POD that would most likely protect against other hematopoietic and splenic effects. Methemoglobinemia as the critical effect is also supported by oral subchronic- and chronic-duration studies.

The subchronic p-RfC for *p*-chloronitrobenzene is derived by applying a UF_C of 300 to the $LOAEL_{HEC}$ of 1.7 mg/m^3 for methemoglobinemia in both male and female rats as follows:

$$\textbf{Subchronic p-RfC} = LOAEL_{HEC} \div UF_C$$
$$= 1.7 \text{ mg/m}^3 \div 300$$
$$= \mathbf{6 \times 10^{-3} \text{ mg/m}^3}$$

Table 14 summarizes the uncertainty factors for the subchronic p-RfC for *p*-chloronitrobenzene.

Table 14. Uncertainty Factors for the Subchronic p-RfC for *p*-Chloronitrobenzene (CASRN 100-00-5)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for remaining uncertainty such as the toxicodynamic differences between rats and humans following inhalation exposure to *p*-chloronitrobenzene. The toxicokinetic uncertainty has been accounted for by calculation of a HEC as described in the RfC methodology (U.S. EPA, 1994).
UF_D	1	A UF_D of 1 is applied for database deficiencies. Although the database for inhaled *p*-chloronitrobenzene is limited to subchronic-duration studies in rats and mice, the available data identified the same toxicological endpoint (hematologic effects) as the oral studies, and there are well-designed subchronic- and chronic-duration oral studies in rats and mice, as well as oral reproductive toxicity studies in rats and mice and oral developmental toxicity studies in rats and rabbits that support hematologic effects as the most sensitive health outcome by both the inhalation and oral exposure routes.
UF_H	10	A UF_H of 10 is applied for intraspecies variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of *p*-chloronitrobenzene in humans.
UF_L	10	A UF_L of 10 is applied for LOAEL-to-NOAEL extrapolation because the POD is a LOAEL.
UF_S	1	A UF_S of 1 is applied because a subchronic-duration study was selected as the principal study.
UF_C	300	$UF_C = UF_A \times UF_D \times UF_H \times UF_L \times UF_S$

HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point-of-departure.

The confidence in the subchronic p-RfC for *p*-chloronitrobenzene is medium as explained in Table 15.

Table 15. Confidence Descriptors for the Subchronic p-RfC for *p*-Chloronitrobenzene (CASRN 100-00-5)

Confidence Categories	Designation[a]	Discussion
Confidence in principal study	H	Confidence in the principal study (Travlos et al., 1996; NTP, 1993) is high. The study was peer-reviewed, well-conducted and well-reported, used adequate numbers of animals of both sexes, tested multiple exposure concentrations, and examined methemoglobinemia effects in addition to standard endpoints. In addition, the critical effects, methemoglobinemia and other hematological effects, are known to be relevant to humans.
Confidence in database	H	Confidence in the database is high. Although the database for inhalation toxicity is limited, a substantial oral toxicity database supports selecting methemoglobinemia and related effects as the critical effect(s) regardless of exposure route.
Confidence in subchronic p-RfC	H	The overall confidence in the subchronic p-RfC is high.

[a]H = high.

Derivation of Chronic Provisional RfC (Chronic p-RfC)

There are no chronic-duration, reproductive toxicity, or developmental toxicity studies available for inhaled *p*-chloronitrobenzene. The database for inhalation toxicity is limited to only subchronic-duration studies in rats and mice. However, toxicological endpoints (i.e., hematologic and splenic effects) identified in the subchronic-duration studies are the same as observed in oral subchronic-duration and chronic-duration studies for *p*-chloronitrobenzene. Methemoglobinemia is the critical effect in both inhalation (subchronic-duration) and oral (subchronic- and chronic-duration) toxicity studies. Furthermore, no duration-dependent increase in the severity of methemoglobinemia was observed in the oral toxicity studies (see Figure 3). Therefore, the subchronic-duration rat inhalation toxicity study (Travlos et al., 1996; NTP, 1993) is considered as the principal study, and methemoglobinemia is considered as the critical effect for the derivation of the chronic p-RfC for *p*-chloronitrobenzene.

A provisional chronic RfC is derived for *p*-chloronitrobenzene by applying a UF_C of 1,000 to the $LOAEL_{HEC}$ of 1.7 mg/m^3 from the subchronic-duration rat inhalation toxicity study (Travlos et al., 1996; NTP, 1993) as follows:

$$\textbf{Chronic p-RfC} = LOAEL_{HEC} \div UF_C$$
$$= 1.7\ mg/m^3 \div 1{,}000$$
$$= \mathbf{2 \times 10^{-3}\ mg/m^3}$$

Table 16 summarizes the uncertainty factors for the chronic p-RfC for *p*-chloronitrobenzene.

Table 16. Uncertainty Factors for the Chronic p-RfC for *p*-Chloronitrobenzene (CASRN 100-00-5)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for remaining uncertainty such as the toxicodynamic differences between rats and humans following inhalation exposure to *p*-chloronitrobenzene. The toxicokinetic uncertainty has been accounted for by calculation of a human equivalent Concentration (HEC) as described in the RfC methodology (U.S. EPA, 1994).
UF_D	1	A UF_D of 1 is applied for database deficiencies. Although the database for inhaled *p*-chloronitrobenzene is limited to subchronic-duration studies in rats and mice, the available data identified the same toxicological endpoint (hematologic effects) as the oral studies, and there are well-designed subchronic- and chronic-duration oral studies in rats and mice, as well as oral reproductive toxicity studies in rats and mice and oral developmental toxicity studies in rats and rabbits that support hematologic effects as the most sensitive health outcome by both the inhalation and oral exposure routes.
UF_H	10	A UF_H of 10 is applied for intraspecies variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of *p*-chloronitrobenzene in humans.
UF_L	10	A UF_L of 10 is applied for LOAEL-to-NOAEL extrapolation because the POD is a LOAEL.
UF_S	3	A UF_S of 3 ($10^{0.5}$) to account for subchronic-to-chronic extrapolation is applied because the chronic p-RfC is derived from a 13-wk study in rats. The UF_S is reduced from the default of 10 because the oral database for *p*-chloronitrobenzene demonstrated that there is no increase in the magnitude of methemoglobinemia severity beyond subchronic-duration *p*-chloronitrobenzene oral exposure. However, other toxicity endpoints may result from chronic oral exposure due to route-specific differences in metabolism, pharmacokinetics, and/or toxicodynamics that were not observed in the subchronic-duration inhalation studies.
UF_C	1,000	$UF_C = UF_A \times UF_D \times UF_H \times UF_L \times UF_S$

HEC = human equivalent concentration; LOAEL = lowest-observed-adverse-effect level; NOAEL = no-observed-adverse-effect level; POD = point-of-departure.

The confidence in the chronic p-RfC for *p*-chloronitrobenzene is medium as explained in Table 17.

Table 17. Confidence Descriptors for Chronic p-RfC for *p*-Chloronitrobenzene (CASRN 100-00-5)

Confidence Categories	Designation[a]	Discussion
Confidence in principal study	H	Confidence in the principal study (Travlos et al., 1996; NTP, 1993) is high. The study was peer-reviewed, well-conducted and well-reported, used adequate numbers of animals of both sexes, tested multiple exposure concentrations, and examined methemoglobinemia effects in addition to standard endpoints. In addition, the critical effects, methemoglobinemia and other hematological effects, are known to be relevant to humans.
Confidence in database	H	Confidence in the database is high. Although the database for inhalation toxicity is limited, a substantial oral toxicity database supports using methemoglobinemia and related effects as the critical effect(s) regardless of exposure route.
Confidence in chronic p-RfC	H	The overall confidence in the chronic p-RfC is high.

[a]H = high.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR (WOE)

No data are available to assess the carcinogenicity of *p*-chloronitrobenzene in humans. Although there are no reports of human carcinogenicity resulting from exposure to *p*-chloronitrobenzene, humans and experimental animals appear to share a common sensitivity to hematotoxic effects (e.g., methemoglobinemia) (U.S. EPA, 1985). Furthermore, *p*-chloronitrobenzene is readily absorbed and follows similar metabolic pathways in humans and rats (Monsanto, 1994a; Yoshida, 1994; NTP, 1993; Yoshida et al., 1993, 1992; Yoshida et al., 1991).

Weisburger et al. (1978) reported a dose-dependent increase in vascular tumors in albino CD-1 mice, with a statistically significant increase in males that received 1,029 mg/kg-day and females that received 1,036 mg/kg-day, by diet for 18 months. Male mice receiving 515 mg/kg-day showed a statistically significant increase in hepatocellular carcinomas, but no increase was observed in the high-dose group (1,029 mg/kg-day).

Daily exposure to low doses of *p*-chloronitrobenzene (0.1−5 mg/kg-day) by gavage for 2 years did not induce tumors in male or female CD (S-D-derived) rats (Bio Dynamics, 1985). However, in a more recent study of F344/DuCrj rats (employing doses of 1.5−53.8 mg/kg-day) and Crj:BDF_1 mice (employing doses of 15.3−275.2 mg/kg-day) following dietary administration of *p*-chloronitrobenzene for 2 years, Matsumoto et al. (2006a) reported statistically significant increases in the incidences of several splenic tumors (i.e., fibroma, fibrosarcoma, osteosarcoma, sarcoma NOS, and hemangiosarcoma) and adrenal pheochromocytoma in male F344/DuCrj rats exposed to doses of 41.2 mg/kg-day, as well as splenic fibrosarcoma and adrenal pheochromocytoma in females at 53.8 mg/kg-day. As shown in Table B-9, the incidence of splenic hemangiosarcoma was statistically significantly increased in males exposed to 7.7 mg/kg-day. In a similar study in mice (Matsumoto et al., 2006a), a significant increase in hepatic hemangiosarcoma was observed in the high-dose females. However, there were no splenic tumors observed in mice at any dose.

As stated in the U.S. EPA's Cancer Guidelines (U.S. EPA, 2005), one of the examples for a chemical to be considered *likely to be carcinogenic to humans* is*: "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans.*" Based on these guidelines and the carcinogenicity data from available animal studies, the weight-of-evidence (WOE) descriptor of *likely to be carcinogenic to humans* is appropriate for *p*-chloronitrobenzene.

Table 18 identifies the cancer WOE descriptor for *p*-chloronitrobenzene.

Table 18. Cancer WOE Descriptor for *p*-Chloronitrobenzene (CASRN 100-00-5)[a]

Possible WOE Descriptor	Designation	Route of Entry (oral, inhalation, or both)	Comments
"Carcinogenic to humans"	NS	NA	There are no human data available.
"Likely to be carcinogenic to humans"	**Selected**	**Oral**	**A study by Matsumoto et al. (2006a) reported significant dose-related increases in splenic tumors (males and females) and adrenal tumors (females) in rats (see Table B-9), and liver tumors in female mice (see Table B-10). Additionally, a significant dose-related increase in vascular tumors in male and female mice was reported by Weisburger et al. (1978).**
"Suggestive evidence of carcinogenic potential"	NS	NA	The evidence from oral animal data is more than suggestive of carcinogenicity, which raises a concern for carcinogenic effects and is judged sufficient for a stronger conclusion.
"Inadequate information to assess carcinogenic potential"	Selected	Inhalation	Adequate information is available to assess the carcinogenic potential of *p*-chloronitrobenzene via the oral route exposure but not via the inhalation route of exposure.
"Not likely to be carcinogenic to humans"	NS	NA	Evidence of the carcinogenic potential of *p*-chloronitrobenzene is available in animals via the oral exposure route.

[a]Bold text indicates choice of cancer WOE descriptor.

NS = not selected; NA = not applicable.

MODE-OF-ACTION DISCUSSION

The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) defines mode of action (MOA) as a sequence of key events and processes, starting with interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer. Examples of possible modes of carcinogenic action for any given chemical include mutagenicity, mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation, and immunologic suppression.

Information to support mutagenicity as a putative MOA for *p*-chloronitrobenzene carcinogenicity is limited but includes evidence of sister chromatid exchanges in CHO cells cultured with the compound in the presence of S9 (IARC, 1996) and DNA single-strand breaks in the liver, kidney, and brain of mice exposed to *p*-chloronitrobenzene via i.p. injection (IARC, 1996). A major metabolite of *p*-chloronitrobenzene (namely, *p*-chloroaniline) has exhibited some evidence of mutagenicity (Martin et al., 2000; IARC, 1997; Zeiger, 1990; Corbett et al., 1989; NTP, 1989; Caspary et al., 1988; Dunkel et al., 1988; Garberg et al., 1988; Sakagami et al., 1988; Wangenheim and Bolcsfoldi, 1988; Rashid et al., 1987; U.S. EPA, 1987) and DNA damage (Sasaki et al., 1999a, b). The similarity of metabolism in rats and humans (Yoshida, 1994) argues to the relevance of this information for human risk assessment. However, given the limited available data, it is uncertain whether the mode(s) of action by which *p*-chloronitrobenzene induces splenic and adrenal tumors in rats or liver and vascular tumors in mice involves a genotoxic/mutagenic key event.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor (p-OSF)

The data on tumors in rats reported by Matsumoto et al. (2006a) provide the most appropriate data set for p-OSF derivation, as splenic and adrenal tumors were observed at doses of 7.7 and 53.8 mg/kg-day (HEDs = 2.1 and 13.2 mg/kg-day) in male and female rats, respectively, well below those at which vascular tumors were observed in mice (>1,000 mg/kg-day; HED >143 mg/kg-day) (Weisburger et al., 1978). Matsumoto et al. (2006a) reported increased incidences ($p < 0.05$ compared with controls) in a variety of splenic tumors in male rats exposed to 41.2 mg/kg-day (HED = 11.1 mg/kg-day) and female rats exposed to 53.8 mg/kg-day (HED = 13.2 mg/kg-day). In addition, Matsumoto et al. (2006a) reported splenic hemangiosarcomas at increased incidences ($p < 0.05$) in male rats at doses of 7.7 mg/kg-day (HED = 2.1 mg/kg-day), and an increased incidence ($p < 0.01$) of adrenal pheochromocytomas in female rats at 53.8 mg/kg-day (HED = 13.2 mg/kg-day).

A POD for p-OSF derivation was determined using the incidence data for tumor types that were significantly increased over control in at least one dose group, and these data were dose-response modeled using the multistage-cancer model in the U.S. EPA's BMDS (Version 2.2.1). The tumor types modeled include splenic fibroma, fibrosarcoma, osteosarcoma, sarcoma NOS, and hemangiosarcoma in male rats, and splenic fibrosarcoma and adrenal pheochromocytoma in female rats. Although significant dose-related trends were reported for splenic fibroma, osteosarcoma, and hemangiosarcoma in female rats and adrenal pheochromocytoma in male rats, increases in tumor incidences versus controls were not statistically significant at any dose, and these data were not modeled. Appendix E describes the modeling approach and results. HED values (see footnote of Table B-9 for calculation) are used in the cancer BMD analysis. The $BMDL_{10HED}$ (lower bound on a dose estimated to produce a 10% increase in tumor incidence over background) was estimated using the U.S. EPA (2012b) benchmark dose methodology.

Because treatment with *p*-chloronitrobenzene produced multiple types of tumors (fibrosarcoma in the spleen and pheochromocytoma in the adrenal glands of female rats), the overall tumor dose response in female rats was modeled based on the incidence data for combined fibrosarcoma in the spleen and pheochromocytoma in the adrenal glands by assuming that different tumor types are independent from each other. The overall tumor incidence was fit with the MS_Combo multiple tumor model (BMDS Version 2.2.2), and the estimated

$BMDL_{10HED}$ is 2.94 mg/kg-day. Due to a lack of combined splenic tumor incidence data, and the lack of clarity on whether the different types of tumors observed in the spleen of male rats are related tumorigenic processes, the most sensitive tumor response (i.e., hemangiosarcoma) in the spleen of male rats was used to estimate a splenic tumor BMDL. In comparison to the estimated $BMDL_{10HED}$ for female combined tumor data, the $BMDL_{10HED}$ of 1.56 mg/kg-day for male splenic hemangiosarcoma represents a lower POD, therefore, this $BMDL_{10\ HED}$ was used to calculate the p-OSF.

Table 19 presents the BMD_{10HED}s and $BMDL_{10HED}$s estimated from the best fitting models for the various tumor types.

Table 19. BMD_{10}s and $BMDL_{10}$s for Splenic and Adrenal Tumors in Male and Female Rats Exposed to *p*-Chloronitrobenzene (CASRN 100-00-5)[a,b]

Sex	Target Organ	Tumor Type	BMD_{10HED} (mg/kg-day)	$BMDL_{10HED}$ (mg/kg-day)
M	Spleen	Fibroma	5.86	3.64
		Fibrosarcoma	5.54	3.70
		Osteosarcoma	8.37	6.01
		Sarcoma NOS	9.74	5.55
		Hemangiosarcoma[c]	**2.17**	**1.56**
F	Spleen	Fibrosarcoma	8.35	5.82
	Adrenal glands	Pheochromocytoma	9.33	3.15
F	Multiple tumor combination analysis	Female spleen fibrosarcoma and adrenal glands pheochromocytoma	6.97	2.94

[a]Matsumoto et al. (2006a).
[b]See Appendix E for details of modeling.
[c]High-dose data dropped from analysis to improve model fit.

F = female; M = male; NOS = not otherwise specified.

The MOA for hemangiosarcomas produced by *p*-chloronitrobenzene is unknown; thus, a linear low-dose extrapolation was conducted. Using the $BMDL_{10HED}$ of 1.56 mg/kg-day for splenic hemangiosarcomas in male rats as the POD, a p-OSF is calculated as follows:

$$\textbf{p-OSF} = 0.1 \div BMDL_{10HED}$$
$$= 0.1 \div 1.56 \text{ mg/kg-day}$$
$$= \mathbf{6.4 \times 10^{-2}\ (mg/kg\text{-}day)^{-1}}$$

Derivation of Provisional Inhalation Unit Risk (p-IUR)

There are no human or animal inhalation carcinogenicity data from which to derive a p-IUR for *p*-chloronitrobenzene.

APPENDIX A. SCREENING PROVISIONAL VALUES

No screening values are presented.

APPENDIX B. DATA TABLES

Table B-1. Selected Gross Necropsy Changes in S-D (Crl:COBS CD> [SD]BR) Rats Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 4 Weeks[a]

Males						
	Control	**12.6 mg/kg-d**	**33.1 mg/kg-d**	**66.8 mg/kg-d**	**97.6 mg/kg-d**	**223.5 mg/kg-d**
Number of animals	10	10	10	10	10	10
Spleen						
Splenomegaly	[b]0/10	7/10[c]	10/10[c]	10/10[c]	10/10[c]	10/10[c]
Abnormal color	0/10	5/10[d]	8/10[c]	9/10[c]	7/10[c]	9/10[c]
Kidney						
Abnormal color—left	0/10	1/10	2/10	4/10	3/10	9/10[c]
Abnormal color—right	0/10	1/10	2/10	4/10	3/10	9/10[c]
Females						
	Control	**14.2 mg/kg-d**	**34.8 mg/kg-d**	**73.1 mg/kg-d**	**112.4 mg/kg-d**	**257.1 mg/kg-d**
Number of animals	10	10	10	10	10	10
Spleen						
Splenomegaly	0/10	10/10[c]	10/10[c]	10/10[c]	10/10[c]	7/7[c]
Abnormal color	0/10	7/10[c]	9/10[c]	10/10[c]	8/10[c]	5/7[c]
Kidney						
Abnormal color—left	0/10	1/10	6/10[d]	8/10[c]	10/10[c]	7/7[c]
Abnormal color—right	0/10	1/10	6/10[d]	8/10[c]	10/10[c]	7/7[c]

[a]Monsanto (1994c). Number of animals = 10/group except for 257.1 mg/kg-day females; where 3 animals were dead during the third week of study.
[b]Number affected/number examined.
[c]Significantly different from the control at $p < 0.01$.
[d]Significantly different from the control at $p < 0.05$.

Table B-2. Selected Changes in S-D (Crl:COBS CD® [SD]BR) Rats Treated by Gavage with *p*-Chloronitrobenzene (CASRN 100-00-5) for 90 Days[a]

Endpoint	Exposure Concentration (mg/kg-d)			
	0	3	10	30
Males				
Number of animals	20	20	20	20
Methemoglobin (% of Hgb)	0.9 ± 0.17[b]	4.5 ± 0.63[c]	9.0 ± 1.04[c]	14.2 ± 3.12[c]
Erythrocyte count (10^6/µL)	9.07 ± 0.44	8.03 ± 0.49[c]	7.47 ± 0.37[c]	5.90 ± 0.41[c]
Hemoglobin (g/dL)	17.70 ± 0.5	15.62 ± 0.6[c]	15.10 ± 0.6[c]	14.45 ± 0.6[c]
Hematocrit (%)	50.9 ± 1.6	46.9 ± 1.3[c]	48.7 ± 3.0[d]	44.5 ± 1.7[c]
Reticulocytes (10^6/µL)	0.72 ± 0.28	7.62 ± 1.18[c]	21.49 ± 2.42[c]	36.04 ± 2.60[c]
Spleen				
Relative spleen weight (%)	0.14 ± 0.02	0.16 ± 0.03[d]	0.29 ± 0.04[d]	0.53 ± 0.08[d]
Hemosiderin	0/20[e]	19/20[c]	20/20[c]	18/20[c]
Hematopoiesis	0/20	19/20[c]	20/20[c]	20/20[c]
Females				
Number of animals	20	20	20	20
Methemoglobin (% of Hgb)	1.0 ± 0.18	4.9 ± 0.57[c]	9.8 ± 1.34[c]	18.2 ± 3.85[c]
Erythrocyte count (10^6/µL)	8.88 ± 0.38	7.67 ± 0.37[c]	6.56 ± 0.34[c]	5.58 ± 0.58[c]
Hemoglobin (g/dL)	18.78 ± 0.7	16.14 ± 1.0[c]	15.55 ± 0.8[c]	15.47 ± 0.9[c]
Hematocrit (%)	54.1 ± 2.2	47.7 ± 2.9[c]	46.6 ± 2.0[c]	47.0 ± 2.9[c]
Reticulocytes (10^6/µL)	0.59 ± 0.28	7.74 ± 1.07[c]	25.4 ± 3.16[c]	39.8 ± 2.93[c]
Spleen				
Relative spleen weight (%)	0.19 ± 0.02	0.24 ± 0.04[d]	0.37 ± 0.07[d]	0.93 ± 0.14[d]
Hemosiderin	0/20	20/20[c]	20/20[c]	5/20
Hematopoiesis	0/20	17/20[c]	20/20[c]	17/20[c]

[a]Monsanto (1994b).
[b]Mean ± SD.
[c]Significantly different from the control at $p < 0.01$.
[d]Significantly different from the control at $p < 0.05$.
[e]Number affected/number examined.

Table B-3. Selected Hematology and Serum Chemistry Changes in F344/DuCrj Rats Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 13 Weeks[a]

Males						
	Control	**24.7 ppm (1.2 mg/kg-d)**	**74.1 ppm (3.4 mg/kg-d)**	**222 ppm (11.8 mg/kg-d)**	**667 ppm (38.8 mg/kg-d)**	**2,000 ppm (122.8 mg/kg-d)**
Number of animals	10	10	10	10	10	10
Hematology						
Erythrocyte count (10^6/µL)	9.45 ± 0.29[b]	9.31 ± 0.29	8.90 ± 0.28[c]	8.36 ± 0.25[c]	7.18 ± 0.20[c]	5.42 ± 0.20[c]
Hemoglobin (g/dL)	16.0 ± 0.5	15.7 ± 0.2	15.3 ± 0.3[c]	14.8 ± 0.4[c]	14.3 ± 0.2[c]	14.2 ± 0.4[c]
Hematocrit (%)	43.3 ± 1.5	42.8 ± 1.4	41.7 ± 1.4[d]	40.8 ± 1.3[c]	39.4 ± 1.0[c]	40.7 ± 1.4[c]
Mean corpuscular volume (fL)	45.8 ± 0.4	46.0 ± 0.2	46.9 ± 0.4	48.8 ± 0.6[c]	54.9 ± 1.3[c]	75.2 ± 2.3[c]
Clinical chemistry						
Total bilirubin (mg/dL)	0.15 ± 0.02	0.14 ± 0.02	0.14 ± 0.01	0.16 ± 0.02	0.23 ± 0.03[d]	0.47 ± 0.06[c]
AST (IU/L)	64 ± 8	65 ± 10	65 ± 9	64 ± 12	60 ± 8	77 ± 13[c]
ALT (IU/L)	21 ± 2	22 ± 3	22 ± 2	20 ± 4	16 ± 2[c]	17 ± 2[c]
Females						
	Control	**24.7 ppm (1.4 mg/kg-d)**	**74.1 ppm (4.1 mg/kg-d)**	**222 ppm (13.8 mg/kg-d)**	**667 ppm (45.0 mg/kg-d)**	**2,000 ppm (145.0 mg/kg-d)**
Number of animals	9	10	10	10	10	10
Hematology						
Erythrocyte count (10^6/µL)	8.75 ± 0.38	8.42 ± 0.34[d]	7.94 ± 0.16[c]	7.29 ± 0.22[c]	5.93 ± 0.18[c]	4.58 ± 0.26[c]
Hemoglobin (g/dL)	15.9 ± 0.6	15.6 ± 0.5	15.0 ± 0.3	14.3 ± 0.4[c]	13.9 ± 0.3[c]	13.8 ± 0.2[c]
Hematocrit (%)	42.7 ± 1.8	41.8 ± 1.6	40.5 ± 1.0	38.9 ± 1.1[c]	36.4 ± 4.3[c]	40.6 ± 1.1
Mean corpuscular volume (fL)	48.8 ± 0.3	49.6 ± 0.2	51.0 ± 0.4	53.3 ± 0.5[c]	61.2 ± 6.4[c]	88.8 ± 0.4[c]

Table B-3. Selected Hematology and Serum Chemistry Changes in F344/DuCrj Rats Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 13 Weeks[a]

Females						
	Control	**24.7 ppm (1.4 mg/kg-day)**	**74.1 ppm (4.1 mg/kg-day)**	**222 ppm (13.8 mg/kg-day)**	**667 ppm (45.0 mg/kg-day)**	**2,000 ppm (145.0 mg/kg-day)**
Clinical chemistry						
Total bilirubin (mg/dL)	0.19 ± 0.02	0.20 ± 0.04	0.19 ± 0.04	0.23 ± 0.02	0.36 ± 0.06[c]	0.58 ± 0.05[c]
AST (IU/L)	58 ± 4	65 ± 11	62 ± 9	60 ± 5	61 ± 11	67 ± 4
ALT (IU/L)	18 ± 3	22 ± 6	21 ± 6	18 ± 4	15 ± 3	13 ± 1[c]

[a]Matsumoto et al. (2006b). Number of animals = 10/group except for control females; a shortage of blood volume precluded analysis for one control.
[b]Mean ± SD; when number of animals <10, it is due to a blood volume shortage.
[c]Significantly different from the control at $p < 0.01$.
[d]Significantly different from the control at $p < 0.05$.

Notes: mg/kg-day was calculated from ppm using the following formula:

mg/kg-day = ppm × food intake factor

where:

Food intake factor = food intake (kg/day) ÷ BW (kg)

BW = body weight of animal

Table B-4. Selected Histopathology Changes in F344/DuCrj Rats Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 13 Weeks[a]

Males						
	Control	**24.7 ppm (1.2 mg/kg-d)**	**74.1 ppm (3.4 mg/kg-d)**	**222 ppm (11.8 mg/kg-d)**	**667 ppm (38.8 mg/kg-d)**	**2,000 ppm (122.8 mg/kg-d)**
Number of animals	10	10	10	10	10	10
Bone marrow						
Increased erythropoiesis	0/10[b]	0/10	0/10	6/10[c]	10/10[d]	10/10[d]
Spleen						
Congestion	0/10	0/10	10/10[d]	10/10[d]	10/10[d]	10/10[d]
Hemosiderin deposition	0/10	0/10	8/10[d]	10/10[d]	9/10[d]	10/10[d]
Increased extramedullary hematopoiesis	0/10	1/10	10/10[d]	10/10[d]	10/10[d]	10/10[d]
Capsule hyperplasia	0/10	0/10	0/10	7/10[d]	10/10[d]	9/10[d]
Liver						
Hemosiderin deposition	0/10	0/10	0/10	8/10[d]	10/10[d]	10/10[d]
Increased extramedullary hematopoiesis	0/10	0/10	0/10	1/10	4/10[c]	10/10[d]
Centrilobular hepatocyte hypertrophy	0/10	0/10	0/10	0/10	0/10	10/10[d]
Females						
	Control	**24.7 ppm (1.4 mg/kg-d)**	**74.1 ppm (4.1 mg/kg-d)**	**222 ppm (13.8 mg/kg-d)**	**667 ppm (45.0 mg/kg-d)**	**2,000 ppm (145.0 mg/kg-d)**
Number of animals	10	10	10	10	10	10
Bone marrow						
Increased erythropoiesis	0/10	1/10	0/10	10/10[d]	10/10[d]	10/10[d]
Spleen						
Congestion	0/10	0/10	10/10[d]	10/10[d]	10/10[d]	10/10[d]
Hemosiderin deposition	0/10	1/10	10/10[d]	10/10[d]	10/10[d]	10/10[d]

Table B-4. Selected Histopathology Changes in F344/DuCrj Rats Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 13 Weeks[a]

Females						
	Control	**24.7 ppm (1.4 mg/kg-d)**	**74.1 ppm (4.1 mg/kg-d)**	**222 ppm (13.8 mg/kg-d)**	**667 ppm (45.0 mg/kg-d)**	**2,000 ppm (145.0 mg/kg-d)**
Increased extramedullary hematopoiesis	0/10	0/10	6/10[d]	10/10[d]	10/10[d]	10/10[d]
Capsule hyperplasia	0/10	0/10	0/10	10/10[d]	9/10[d]	4/10[c]
Liver						
Hemosiderin deposition	0/10	0/10	0/10	4/10[c]	10/10[d]	10/10[d]
Increased extramedullary hematopoiesis	0/10	0/10	0/10	0/10	1/10	10/10[d]

[a]Matsumoto et al. (2006b).
[b]Number affected/number examined.
[c]Significantly different from the control at $p < 0.05$.
[d]Significantly different from the control at $p < 0.01$.

Table B-5. Selected Hematology and Serum Chemistry Changes in Crj:BDF$_1$ Mice Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 13 Weeks[a]

Males						
	Control	**74.1 ppm (7.8 mg/kg-d)**	**222 ppm (27.4 mg/kg-d)**	**667 ppm (86.8 mg/kg-d)**	**2,000 ppm (280.1 mg/kg-d)**	**6,000 ppm (659.5 mg/kg-d)**
Number of animals	10	8	8	10	10	9
Hematology						
Erythrocyte count (10^6/µL)	10.68 ± 0.33[b]	10.55 ± 0.35	10.64 ± 0.30	10.34 ± 0.47	9.12 ± 0.28[c]	6.58 ± 0.92[c]
Hemoglobin (g/dL)	15.0 ± 0.3	14.8 ± 0.4	14.9 ± 0.4	16.3 ± 0.8	ND[d]	ND[d]
Hematocrit (%)	44.0 ± 1.2	43.3 ± 1.6	43.7 ± 1.0	43.4 ± 1.7	39.2 ± 1.3[c]	38.8 ± 3.9[c]
Mean corpuscular volume (fL)	41.2 ± 0.5	41.0 ± 0.5	41.0 ± 0.5	42.0 ± 0.7	42.9 ± 0.4[c]	59.3 ± 3.7[c]
Clinical chemistry						
Total bilirubin (mg/dL)	0.28 ± 0.03	0.31 ± 0.04	0.27 ± 0.05	0.29 ± 0.04	0.30 ± 0.13	0.46 ± 0.05[c]
AST (IU/L)	36 ± 3	37 ± 4	36 ± 5	40 ± 7	38 ± 7	134 ± 71[c]
ALT (IU/L)	9 ± 2	9 ± 1	10 ± 2	11 ± 5	9 ± 2	54 ± 46[c]
Females						
	Control	**74.1 ppm (10.5 mg/kg-d)**	**222 ppm (36.7 mg/kg-d)**	**667 ppm (120.5 mg/kg-d)**	**2,000 ppm (334.3 mg/kg-d)**	**6,000 ppm (856.5 mg/kg-d)**
Number of animals	10	10	10	10	10	9
Hematology						
Erythrocyte count (10^6/µL)	10.42 ± 0.34	10.51 ± 0.47	10.40 ± 0.44	9.83 ± 0.38[e]	8.69 ± 0.30[c]	6.18 ± 0.54[c]
Hemoglobin (g/dL)	14.9 ± 0.4	15.1 ± 0.6	14.9 ± 0.5	15.0 ± 0.4	ND[d]	ND[d]
Hematocrit (%)	43.4 ± 1.4	44.0 ± 2.2	43.7 ± 1.7	42.2 ± 1.8	37.6 ± 1.5[c]	36.8 ± 3.6[c]
Mean corpuscular volume (fL)	41.6 ± 0.4	41.8 ± 0.5	42.0 ± 0.3	42.9 ± 0.5[c]	43.2 ± 0.8[c]	59.5 ± 2.2[c]

Table B-5. Selected Hematology and Serum Chemistry Changes in Crj:BDF$_1$ Mice Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 13 Weeks[a]

Females						
	Control	**74.1 ppm (10.5 mg/kg-day)**	**222 ppm (36.7 mg/kg-day)**	**667 ppm (120.5 mg/kg-day)**	**2,000 ppm (334.3 mg/kg-day)**	**6,000 ppm (856.5 mg/kg-day)**
Clinical chemistry						
Total bilirubin (mg/dL)	0.32 ± 0.03	0.33 ± 0.06	0.33 ± 0.07	0.29 ± 0.04	0.30 ± 0.05	0.50 ± 0.10[c]
AST (IU/L)	48 ± 8	47 ± 9	50 ± 9	46 ± 9	50 ± 8	79 ± 15[c]
ALT (IU/L)	11 ± 2	11 ± 2	11 ± 2	11 ± 2	12 ± 2	17 ± 2[c]

[a]Matsumoto et al. (2006b).
[b]Mean ± SD; when number of animals <10, it is due to a blood volume shortage or death (one female in the highest dose group).
[c]Significantly different from the control at $p < 0.01$.
[d]No data due to incomplete hemolysis of blood.
[e]Significantly different from the control at $p < 0.05$.

ND = no data.

Table B-6. Selected Histopathology Changes in Crj:BDF$_1$ Mice Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 13 Weeks[a]

Males						
	Control	**74.1 ppm (7.8 mg/kg-d)**	**222 ppm (27.4 mg/kg-d)**	**667 ppm (86.8 mg/kg-d)**	**2,000 ppm (280.1 mg/kg-d)**	**6,000 ppm (659.5 mg/kg-d)**
Number of animals	10	10	10	10	10	10
Bone marrow						
Hemosiderin deposition	0/10[b]	0/10	0/10	0/10	10/10[c]	10/10[c]
Increased erythropoiesis	0/10	0/10	0/10	0/10	7/10[c]	8/10[c]
Spleen						
Congestion	0/10	0/10	0/10	2/10	10/10[c]	9/10[c]
Hemosiderin deposition	0/10	0/10	0/10	6/10[c]	10/10[c]	9/10[c]
Increased extramedullary hematopoiesis	0/10	0/10	1/10	9/10[c]	10/10[c]	9/10[c]
Liver						
Hemosiderin deposition	0/10	0/10	0/10	0/10	9/10[c]	10/10[c]
Increased extramedullary hematopoiesis	0/10	0/10	0/10	0/10	3/10[d]	10/10[c]
Centrilobular hepatocyte hypertrophy	0/10	0/10	0/10	0/10	1/10	10/10[c]
Females						
	Control	**74.1 ppm (10.5 mg/kg-d)**	**222 ppm (36.7 mg/kg-d)**	**667 ppm (120.5 mg/kg-d)**	**2,000 ppm (334.3 mg/kg-d)**	**6,000 ppm (856.5 mg/kg-d)**
Number of animals	10	10	10	10	10	10
Bone marrow						
Hemosiderin deposition	0/10	0/10	0/10	2/10	10/10[c]	10/10[c]
Increased erythropoiesis	0/10	0/10	0/10	0/10	8/10[c]	10/10[c]

Table B-6. Selected Histopathology Changes in Crj:BDF$_1$ Mice Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 13 Weeks[a]

Females						
	Control	**74.1 ppm (10.5 mg/kg-d)**	**222 ppm (36.7 mg/kg-d)**	**667 ppm (120.5 mg/kg-d)**	**2,000 ppm (334.3 mg/kg-d)**	**6,000 ppm (856.5 mg/kg-d)**
Spleen						
Congestion	0/10	0/10	0/10	8/10[c]	10/10[c]	9/10[c]
Hemosiderin deposition	0/10	0/10	6/10[c]	10/10[c]	10/10[c]	10/10[c]
Increased extramedullary hematopoiesis	0/10	0/10	0/10	7/10[c]	10/10[c]	9/10[c]
Liver						
Hemosiderin deposition	0/10	0/10	0/10	0/10	10/10[c]	10/10[c]
Increased extramedullary hematopoiesis	0/10	0/10	0/10	0/10	8/10[c]	10/10[c]
Centrilobular hepatocyte hypertrophy	0/10	0/10	0/10	0/10	9/10[c]	9/10[c]

[a]Matsumoto et al. (2006b).
[b]Number affected/number examined. One female in the highest dose group died during Week 7.
[c]Significantly different from the control at $p < 0.01$.
[d]Significantly different from the control at $p < 0.05$.

Table B-7. Percent Methemoglobin in CD (S-D-derived) Rats Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) by Gavage for 24 Months[a]

	Control	0.1 MG/kg-d	0.7 MG/kg-d	5.0 MG/kg-d
Number of animals	10	10	10	10
Male	0.4 ± 0.2[b]	0.4 ± 0.3	1.5 ± 0.3[c]	6.0 ± 2.8[d]
Female	0.4 ± 0.2	0.6 ± 0.3	1.5 ± 0.2[d]	5.6 ± 1.1[c]

[a]BIO DYNAMICS (1985).
[b]Means ± SD (number of rats evaluated).
[c]Significantly different from the control, $p \leq 0.05$.
[d]Significantly different from the control at $p \leq 0.01$.

Table B-8. Selected Changes in F344/DuCrj Rats Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 2 Years[a]

Males				
	Control	**40 ppm (1.5 mg/kg-d)**	**200 ppm (7.7 mg/kg-d)**	**1,000 ppm (41.2 mg/kg-d)**
Number of animals treated	**50**	**50**	**50**	**50**
Number of animals examined for hematology, clinical chemistry, and organ weights	43 (41 for hematology and serum chemistry)	46	42	12
Terminal body weight	420 ± 46[b]	413 ± 45 (−1.66%)[g]	412 ± 51 (−1.90%)	369 ± 23[c] (−12.1%)
Hematology				
Erythrocyte count (10^6/μL)	8.79 ± 1.97	9.28 ± 1.57	8.38 ± 0.91	6.26 ± 1.48[c]
Hemoglobin (g/dL)	15.8 ± 3.1	16.5 ± 2.7	15.3 ± 1.4	ND[d]
Hematocrit (%)	44.1 ± 8.6	45.9 ± 7.4	43.0 ± 4.1	38.8 ± 6.5[c]
Mean Corpuscular Volume (fL)	51.1 ± 5.2	49.4 ± 1.7	51.4 ± 2.5	64.1 ± 10.7[c]
Clinical chemistry				
Total bilirubin (mg/dL)	0.31 ± 0.26	0.23 ± 0.07	0.24 ± 0.05	0.54 ± 0.65[c]
Organ weights				
Spleen/body weight (%)	0.320 ± 0.245	0.261 ± 0.085 (−18.4%)	0.423 ± 0.074[c] (32.2%)	3.471 ± 3.443[c] (985%)
Liver/body weight (%)	2.797 ± 0.613	2.879 ± 0.417 (2.93%)	3.114 ± 0.436[c] (11.3%)	3.891 ± 0.752[c] (39.1%)
Kidney/body weight (%)	0.673 ± 0.102	0.712 ± 0.160 (5.79%)	0.744 ± 0.152[e] (10.5%)	0.804 ± 0.080[c] (19.5%)
Gross necropsy findings				
Splenomegaly	11/50[f]	7/50	28/50[c]	15/50
Splenic nodules	0/50	0/50	7/50[c]	29/50[c]
Females				
	Control	**40 ppm (1.9 mg/kg-d)**	**200 ppm (9.8 mg/kg-d)**	**1,000 ppm (53.8 mg/kg-d)**
Number of animals treated	**50**	**50**	**50**	**50**

Table B-8. Selected Changes in F344/DuCrj Rats Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 2 Years[a]

Number of animals examined for hematology, clinical chemistry, and organ weights	36	41	38	28
Terminal body weight	314 ± 30	323 ± 31 (2.87%)	280 ± 60[e] (−10.8%)	247 ± 43[c] (−21.4%)
Hematology				
Erythrocyte count (10^6/μL)	8.13 ± 0.73	8.08 ± 0.51	6.88 ± 1.48[c]	4.94 ± 0.80[c]
Hemoglobin (g/dL)	15.6 ± 1.3	15.6 ± 0.8	14.2 ± 2.1[c]	ND[d]
Hematocrit (%)	43.4 ± 3.6	43.6 ± 2.2	39.8 ± 6.3[c]	36.7 ± 4.7[c]
Mean corpuscular volume (fL)	53.5 ± 3.3	54.0 ± 1.7	59.6 ± 9.7[c]	75.1 ± 7.8[c]
Clinical chemistry				
Total bilirubin (mg/dL)	0.22 ± 0.04	0.24 ± 0.10	0.37 ± 0.44[c]	1.13 ± 3.52[c]
Organ weights				
Spleen/body weight (%)	0.260 ± 0.249	0.213 ± 0.054 (−18.1%)	0.777 ± 0.722[c] (199%)	1.774 ± 1.750[c] (582%)
Liver/body weight (%)	2.464 ± 0.331	2.429 ± 0.252 (−1.42%)	2.908 ± 0.849[c] (18.0%)	3.688 ± 0.427[c] (49.7%)
Kidney/body weight (%)	0.656 ± 0.085	0.637 ± 0.065 (−2.90%)	0.764 ± 0.264 (16.4%)	0.883 ± 0.180[c] (34.6%)
Gross necropsy findings				
Splenomegaly	10/50	3/50	42/50[c]	32/50[c]
Splenic nodules	1/50	0/50	3/50	28/50[c]

[a]Matsumoto et al. (2006a).
[b]Mean ± SD.
[c]Significantly different from the control at $p < 0.01$.
[d]No data due to incomplete hemolysis of blood.
[e]Significantly different from the control at $p < 0.05$.
[f]Number affected/number examined.
[g]Percentage change compared to control.

ND = no data.

Table B-9. Histopathology Findings in F344/DuCrj Rats Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 2 Years[a]

Males				
	Control	**1.5 mg/kg-d (0.41 mg/kg-d)[b]**	**7.7 mg/kg-d (2.1 mg/kg-d)**	**41.2 mg/kg-d (11.1 mg/kg-d)**
Number of animals	50	50	50	50
Nonneoplastic lesions				
Splenic capsule hyperplasia	0/50[c]	0/50	43/50[d]	47/50[d]
Splenic fibrosis	0/50	1/50	40/50[d]	47/50[d]
Splenic fatty metamorphosis	0/50	0/50	14/50[d]	24/50[d]
Splenic extramedullary hematopoiesis	4/50	4/50	18/50[d]	13/50[d]
Hemosiderin deposition	4/50	5/50	6/50	0/50
Adrenal medullary hyperplasia	11/50	26/50[d]	14/50	16/50
Neoplastic lesions				
Splenic fibroma[e]	0/50	0/50	1/50	15/50[d]
Splenic fibrosarcoma[e]	0/50	1/50	0/50	29/50[d]
Splenic osteosarcoma[e]	0/50	0/50	0/50	11/50[d]
Splenic sarcoma NOS[e]	0/50	0/50	1/50	6/50[f]
Splenic Hemangiosarcoma[e]	0/50	0/50	5/50[f]	7/50[f]
Adrenal pheochromocytoma[e]	7/50	7/50	6/50	16/50
Females				
	Control	**1.9 mg/kg-d (0.49 mg/kg-d)[b]**	**9.8 mg/kg-d (2.5 mg/kg-d)**	**53.8 mg/kg-d (13.2 mg/kg-d)**
Number of animals	50	50	50	50
Nonneoplastic lesions				
Splenic capsule hyperplasia	0/50	0/50	42/50[d]	46/50[d]
Splenic fibrosis	2/50	3/50	31/50[d]	43/50[d]
Splenic fatty metamorphosis	0/50	0/50	6/50[f]	9/50[d]
Splenic extramedullary hematopoiesis	8/50	16/50[f]	25/50[d]	20/50[d]
Hemosiderin deposition	7/50	8/50	10/50	6/50
Adrenal medullary hyperplasia	8/50	6/50	9/50	22/50[d]

NOS = not otherwise specified.

Table B-9. Histopathology Findings in F344/DuCrj Rats Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 2 Years[a]

Females				
	Control	**1.9 mg/kg-d (0.49 mg/kg-d)[b]**	**9.8 mg/kg-d (2.5 mg/kg-d)**	**53.8 mg/kg-d (13.2 mg/kg-d)**
Neoplastic lesions				
Splenic fibroma[e]	0/50	0/50	1/50	3/50
Splenic fibrosarcoma[e]	0/50	0/50	0/50	17/50[d]
Splenic osteosarcoma[e]	0/50	0/50	0/50	3/50
Splenic sarcoma NOS	0/50	0/50	0/50	1/50
Splenic hemangiosarcoma[e]	0/50	0/50	2/50	4/50
Adrenal pheochromocytoma[e]	3/50	6/50	4/50	16/50[d]

[a]Matsumoto et al. (2006a).
[b]Adjusted daily dose (corresponding HED).
[c]Number affected/number examined.
[d]Significantly different from the control at $p < 0.01$.
[e]Significant dose-related trend, $p < 0.05$.
[f]Significantly different from the control at $p < 0.05$.

HEDs were calculated using the equation adapted from *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005).

$$BMDL_{10HED} = (Dose_A) \times (BW_A \div BW_H)^{1/4}$$

$Dose_A$ = Dose of chemical used in animal
BW_A = Body weight of animal (terminal body weight of animal is used in the above calculation)
BW_H = Default body weight of human (70 kg)

NOS = not otherwise specified.

Table B-10. Selected Changes in Crj:BDF$_1$ Mice Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 2 Years[a]

Males				
	Control	**15.3 mg/kg-d (2.27 mg/kg-d)[b]**	**60.1 mg/kg-d (8.99 mg/kg-d)**	**240.1 mg/kg-d (35.64 mg/kg-d)**
Hematology (number of animals examined)	45	48	41	37
Terminal body weight	35.3 ± 5.0	34.0 ± 3.8	34.8 ± 3.9	33.5 ± 3.8
Erythrocyte count (10^6/μL)	10.03 ± 0.82[c]	10.02 ± 0.75	9.60 ± 1.02[d]	7.25 ± 1.14[e]
Hematocrit (%)	43.8 ± 2.4	43.4 ± 3.1	42.1 ± 4.5	34.3 ± 4.7[e]
MCV (fL)	43.8 ± 3.0	43.3 ± 1.4	43.8 ± 1.1	47.6 ± 2.8[e]
Organ weights (number of animals examined)	47	49	42	38
Spleen/body weight (%)	0.227 ± 0.182	0.256 ± 0.389	0.209 ± 0.115	0.397 ± 0.444[e]
Liver/body weight (%)	3.591 ± 1.207	3.695 ± 1.035 (2.89%)[h]	3.681 ± 2.023 (2.51%)	4.498 ± 1.312[e] (25.3%)
Kidney/body weight (%)	1.658 ± 0.453	1.615 ± 0.163 (−2.60%)	1.636 ± 0.487 (−1.33%)	1.724 ± 0.144[e] (3.98%)
Nonneoplastic lesions				
Splenic congestion	0/50[f]	0/50	0/50	29/50[e]
Splenic extramedullary hematopoiesis	1/50	2/50	6/50[d]	9/50[e]
Neoplastic lesions				
Hepatocellular carcinoma	1/50	3/50	1/50	6/50
Malignant lymphoma[g]	2/50	2/50	1/50	8/50
Females				
	Control	**17.6 mg/kg-d (2.51 mg/kg-d)[b]**	**72.6 mg/kg-d (10.4 mg/kg-d)**	**275.2 mg/kg-d (39.26 mg/kg-d)**
Hematology (number of animals examined)	30	33	35	28
Terminal body weight	27.7 ± 4.4	28.9 ± 3.4	28.8 ± 3.9	28.8 ± 3.3
Erythrocyte count (10^6/μL)	10.17 ± 1.10	9.46 ± 2.09	9.05 ± 1.23[e]	6.83 ± 0.75[e]
Hematocrit (%)	43.7 ± 4.9	40.9 ± 7.5	39.5 ± 6.1[d]	33.9 ± 2.6[e]
MCV (fL)	43.0 ± 1.9	44.0 ± 4.9	43.6 ± 2.9	49.8 ± 3.4[e]
Organ weights (number of animals examined)	32	35	35	29
Spleen/body weight (%)	0.620 ± 0.662	0.669 ± 0.558 (7.9%)	0.963 ± 1.439 (55.3%)	0.819 ± 1.033 (32.1%)

Table B-10. Selected Changes in Crj:BDF$_1$ Mice Treated with *p*-Chloronitrobenzene (CASRN 100-00-5) in the Diet for 2 Years[a]

Liver/body weight (%)	4.859 ± 3.299	5.052 ± 2.813 (3.97%)	4.476 ± 1.428 (−7.88%)	5.900 ± 1.895[e] (21.4%)
Kidney/body weight (%)	1.537 ± 0.770	1.935 ± 3.349 (25.9%)	1.746 ± 1.808 (13.6%)	1.579 ± 0.384[e] (2.73%)
Gross necropsy findings				
Splenomegaly	6/50	14/50	13/50	15/50[d]
Splenic nodules	1/50	1/50	5/50	9/50[e]
Nonneoplastic lesions				
Splenic congestion	0/50	1/50	0/50	26/50[e]
Splenic hemosiderin deposition	0/50	0/50	3/50	9/50[e]
Splenic ossification	0/50	0/50	0/50	6/50[d]
Neoplastic lesions				
Hepatocellular carcinoma	2/50	0/50	2/50	5/50
Hepatic hemangiosarcoma[g]	0/50	1/50	0/50	5/50[d]

[a]Matsumoto et al. (2006a).
[b]Adjusted daily dose (corresponding HED).
[c]Mean ± SD.
[d]Significantly different from the control at $p < 0.05$.
[e]Significantly different from the control at $p < 0.01$.
[f]Number affected/number examined.
[g]Significant dose-related trend, $p < 0.05$.
[h]Percentage change compared to control.

Table B-11. Selected Changes in S-D Rats Exposed to *p*-Chloronitrobenzene (CASRN 100-00-5) in 2-Ethoxyethanol via Inhalation for 4 Weeks[a]

Endpoint	Daily Average Exposure Concentration, mg/m^3			
	0	**0.89**	**2.86**	**8.21**
Males				
Number of animals	10	10	10	10
Methemoglobin (% of Hgb)	0.9 ± 0.9[b]	3.1 ± 1.7[c]	3.1 ± 1.2[c]	7.7 ± 2.6[d]
Hemoglobin (g/dL)	16.6 ± 1.0	15.8 ± 0.7	16.0 ± 0.9	15.4 ± 0.6[c]
Hematocrit (%)	47.1 ± 2.1	45.5 ± 1.7	46.3 ± 1.7	43.9 ± 1.4[d]
Erythrocyte count (10^6/µL)	7.8 ± 0.3	7.6 ± 0.3	7.3 ± 0.3[d]	6.9 ± 0.2[d]
White blood cell count (10^6/µL)	10.6 ± 2.3	14.3 ± 4.1	13.1 ± 1.7	17.1 ± 5.3[c]
Absolute liver weight (g)	8.33 ± 0.46	8.68 ± 0.47 (4.20%)[e]	8.34 ± 0.83 (0.12%)	9.13 ± 0.75[c] (9.60%)
Liver/body weight (%)	2.69 ± 0.12	2.73 ± 0.13 (1.49%)	2.77 ± 0.11 (2.97%)	2.97 ± 0.18[d] (10.4%)
Absolute spleen weight (g)	0.63 ± 0.13	0.67 ± 0.10	0.71 ± 0.11	1.17 ± 0.28[d]
Spleen/body weight (%)	0.20 ± 0.05	0.21 ± 0.03	0.24 ± 0.03	0.38 ± 0.09[d]
Females				
Number of animals	10	10	10	10
Methemoglobin (% of Hgb)	1.8 ± 1.5	2.1 ± 1.6	5.0 ± 1.0[c]	12.3 ± 4.0[d]
Hemoglobin (g/dL)	15.8 ± 0.6	14.9 ± 0.6	14.8 ± 0.5	14.4 ± 0.8[d]
Hematocrit (%)	45.6 ± 1.4	43.2 ± 1.9[c]	42.8 ± 1.6[d]	41.4 ± 2.3[d]
Erythrocyte count (10^6/µL)	7.4 ± 0.3	7.1 ± 0.3	6.8 ± 0.4[d]	6.3 ± 0.4[d]
White blood cell count (10^6/µL)	9.4 ± 1.6	9.1 ± 2.4	10.6 ± 2.4	14.5 ± 4.7[d]
Absolute liver weight (g)	5.64 ± 0.37	5.74 ± 0.36 (1.77%)	5.87 ± 0.74 (4.08%)	5.89 ± 0.39 (4.43%)
Liver/body weight (%)	2.92 ± 0.22	3.03 ± 0.13 (3.77%)	2.97 ± 0.22 (1.71%)	3.17 ± 0.21[c] (8.56%)
Absolute spleen weight (g)	0.48 ± 0.07	0.46 ± 0.08	0.56 ± 0.10	0.93 ± 0.17[d]
Spleen/body weight (%)	0.25 ± 0.03	0.24 ± 0.03	0.28 ± 0.04	0.50 ± 0.08[d]

[a]Nair et al. (1986).
[b]Mean ± SD.
[c]Significantly different from the control at $p < 0.05$.
[d]Significantly different from the control at $p < 0.01$.
[e]Percentage change compared to control.

Table B-12. Selected Changes in F344/N Rats Exposed to *p*-Chloronitrobenzene (CASRN 100-00-5) via Inhalation for 13 Weeks[a]

Endpoint	Daily Average Exposure Concentration, mg/m^3					
	0	**1.7**	**3.4**	**6.9**	**13.8**	**27.5**
Males						
Number of animals	10	10	10	10	10	10
Terminal body weight	342 ± 4[b]	354 ± 9 (3.51%)[g]	347 ± 4 (1.46%)	348 ± 6 (1.75%)	337 ± 5 (−1.46%)	346 ± 7 (1.17%)
Methemoglobin (% of Hgb)	0.16 ± 0.01	0.50 ± 0.01[c]	0.73 ± 0.01[c]	1.22 ± 0.04[c]	2.08 ± 0.06[c]	2.96 ± 0.05[c]
Erythrocyte count (10^6/µL)	9.00 ± 0.06	8.69 ± 0.04[c]	8.40 ± 0.04[c]	7.83 ± 0.04[c]	7.13 ± 0.06[c]	5.73 ± 0.07[c]
Hematocrit (%)	46.8 ± 0.3	44.9 ± 0.2[c]	43.8 ±0.3[c]	41.9 ± 0.3[c]	39.9 ± 0.2[c]	36.1 ± 0.5[c]
Hemoglobin (g/dL)	14.9 ± 0.1	14.2 ± 0.1[c]	13.9 ±0.1[c]	13.3 ± 0.1[c]	13.4 ± 0.1[c]	12.6 ± 0.2[c]
Reticulocytes (10^6/µL)	0.17± 0.01	0.27± 0.02[c]	0.30 ± 0.02[c]	0.42 ± 0.02[c]	0.59 ± 0.03[c]	0.91 ± 0.03[c]
Spleen						
Absolute spleen weight (g)	0.64 ± 0.02	0.72 ± 0.02	0.79 ± 0.04[d]	0.98 ± 0.02[c]	1.66 ± 0.66[c, e]	3.28 ± 0.08[c]
Relative spleen weight	1.86 ± 0.04	2.02 ± 0.02	2.28 ± 0.11[c]	2.82 ± 0.05[c]	4.92 ± 0.15[c, e]	9.48 ± 0.17[c]
Congestion	0/10[f]	10/10[c]	10/10[c]	10/10[c]	10/10[c]	10/10[c]
Hemosiderin	0/10	10/10[c]	10/10[c]	10/10[c]	10/10[c]	10/10[c]
Hematopoietic cell proliferation	0/10	0/10	10/10[c]	9/10[c]	10/10[c]	10/10[c]
Capsular fibrosis	0/10	0/10	4/10	8/10[c]	10/10[c]	10/10[c]
Liver						
Absolute liver weight (g)	12.06 ± 0.37	12.68 ± 0.45 (5.14%)	12.21 ± 0.28 (1.24%)	12.85 ± 0.36 (6.55%)	12.43 ± 0.23 (3.07%)	14.32 ± 0.37[c] (18.7%)
Relative liver weight	35.19 ± 0.86	35.79 ± 0.58 (1.71%)	35.18 ± 0.52 (−0.10%)	36.89 ± 0.68 (4.83%)	36.91 ± 0.40 (4.89%)	41.35 ± 0.45[c] (17.5%)
Hemosiderin	0/10	0/10	0/10	0/10	9/10[c]	10/10[c]
Bone marrow						
Hematopoietic cell proliferation	0/10	0/10	3/10	10/10[c]	10/10[c]	10/10[c]
Kidney						
Hyaline droplet nephropathy	0/10	8/10[c]	9/10[c]	10/10[c]	10/10[c]	10/10[c]
Tubule pigment	0/10	0/10	0/10	0/10	8/10[c]	10/10[c]

Table B-12. Selected Changes in F344/N Rats Exposed to *p*-Chloronitrobenzene (CASRN 100-00-5) via Inhalation for 13 Weeks[a]

Endpoint	Daily Average Exposure Concentration, mg/m^3					
	0	**1.7**	**3.4**	**6.9**	**13.8**	**27.5**
Females						
Number of animals	10	10	10	10	10	10
Terminal body weight	192 ± 5	195 ± 5 (1.56%)	202 ± 6 (5.21%)	196 ± 4 (2.08%)	197 ± 6 (2.60%)	200 ± 2 (4.17%)
Methemoglobin (% of Hgb)	0.16 ± 0.01	0.63 ± 0.03[c]	0.90 ± 0.03[c]	1.69 ± 0.04[c]	2.50 ± 0.09[c]	2.85 ± 0.07[c]
Erythrocyte count (10^6/µL)	8.68 ± 0.06	7.77 ± 0.10[c]	7.41 ± 0.05[c]	7.00 ± 0.06[c]	6.36 ± 0.08[c]	4.87 ± 0.09[c]
Hematocrit (%)	48.7 ± 0.3	44.2 ± 0.4[c]	42.6 ± 0.3[c]	41.6 ± 0.3[c]	39.8 ± 0.3[c]	34.5 ± 0.5[c]
Hemoglobin (g/dL)	15.4 ± 0.1	14.2 ± 0.1[c]	13.6 ± 0.1[c]	13.7 ± 0.1[c]	13.7 ± 0.1[c]	12.3 ± 0.2[c]
Reticulocytes (10^6/µL)	0.17 ± 0.02	0.21 ± 0.01[e]	0.38 ± 0.02[c]	0.54 ± 0.03[c]	0.81 ± 0.07[c]	1.51 ± 0.07[c]
Spleen						
Absolute spleen weight (g)	0.39 ± 0.01	0.45 ± 0.01[e]	0.56 ± 0.02[d,e]	0.84 ± 0.03[c]	1.49 ± 0.06[c]	3.10 ± 0.09[c]
Relative spleen weight	2.06 ± 0.05	2.31 ± 0.07[e]	2.80 ± 0.10[e]	4.32 ± 0.14[c]	7.61 ± 0.34[c]	15.55 ± 0.53[c]
Congestion	0/10	10/10[c]	10/10[c]	10/10[c]	10/10[c]	10/10[c]
Hemosiderin	0/10	10/10[c]	10/10[c]	10/10[c]	10/10[c]	10/10[c]
Hematopoietic cell proliferation	0/10	0/10	9/10[c]	10/10[c]	9/10[c]	10/10[c]
Capsular fibrosis	0/10	0/10	2/10[c]	10/10[c]	10/10[c]	10/10[c]
Liver						
Absolute liver weight (g)	5.92 ± 0.24	6.21 ± 0.20[e] (4.90%)	6.71 ± 0.28[d,e] (13.3%)	6.79 ± 0.24[c] (14.7%)	6.84 ± 0.23[c] (15.5%)	7.70 ± 0.13[c] (30.1%)
Relative liver weight	30.89 ± 0.87	31.96 ± 0.95[e] (3.46%)	33.19 ± 0.86[e] (7.45%)	34.65 ± 0.72[c] (12.2%)	34.80 ± 0.82[c] (12.7%)	38.63 ± 0.74[c] (25.1%)
Hemosiderin	0/10	0/10	7/10[c]	10/10[c]	10/10[c]	10/10[c]
Bone marrow						
Hematopoietic cell proliferation	0/10	0/10	9/10[c]	9/10[c]	10/10[c]	10/10[c]

Table B-12. Selected Changes in F344/N Rats Exposed to *p*-Chloronitrobenzene (CASRN 100-00-5) via Inhalation for 13 Weeks[a]

Endpoint	Daily Average Exposure Concentration, mg/m^3					
	0	**1.7**	**3.4**	**6.9**	**13.8**	**27.5**
Kidney						
Hyaline droplet nephropathy	0/10	0/10	0/10	0/10	0/10	0/10
Tubule pigment	0/10	0/10	0/10	10/10[c]	10/10[c]	10/10[c]

[a]NTP (1993); Travlos et al. (1996).
[b]Mean ± SE.
[c]Significantly different from the control at $p < 0.01$.
[d]Significantly different from the control at $p < 0.05$.
[e]$n = 9$.
[f]Number affected/number examined.
[g]Percentage change compared to control.

Body weight and organ weights are given in grams. Relative organ weights are given as mg organ weight/g body weight.

HECs were calculated using the equation adapted from *Methods for Derivation of Inhalation Reference Concentrations (RfCs) and Application of Inhalation dosimetry* (U.S. EPA, 1994):

$$LOAEL_{HEC} = (LOAEL_{ADJ}) \times [(H_{b/g})_A \div (H_{b/g})_H]$$

where:

$(H_{b/g})_A \div (H_{b/g})_H$ = Rat-to-human ratio of blood:gas partition coefficients

Table B-13. Selected Changes in B6C3F$_1$ Mice Exposed to *p*-Chloronitrobenzene (CASRN 100-00-5) via Inhalation for 13 Weeks[a]

Endpoint	Daily Average Exposure Concentration, mg/m^3					
	0	**1.7**	**3.4**	**6.9**	**13.8**	**27.5**
Males						
Number of animals	10	10	10	10	10	10
Terminal body weight (g)	34.9 ± 0.8[b]	36.7 ± 1.0 (5.16%)[f]	36.7 ± 0.9 (5.16%)	35.3 ± 1.4 (1.15%)	35.8 ± 0.5 (2.58%)	36.8 ± 0.7 (5.44%)
Spleen						
Absolute spleen weight (g)	0.07 ±0.00	0.07 ± 0.00	0.07 ± 0.00	0.06 ± 0.00	0.08 ± 0.00[c]	0.16 ± 0.01[c]
Relative spleen weight	1.87 ± 0.07	1.85 ± 0.05	1.83 ± 0.06	1.83 ± 0.07	2.21 ± 0.06[d]	4.36 ± 0.19[c]
Congestion	0/1[e]	0/10	0/10	0/10	1/10	10/10[c]
Hemosiderin	0/10	0/10	0/10	0/10	10/10[c]	10/10[c]
Hematopoietic cell proliferation	0/10	0/10	0/10	0/10	7/10[c]	10/10[c]
Liver						
Absolute liver weight (g)	1.60 ± 0.05	1.68 ± 0.05 (5.00%)	1.73 ± 0.04 (8.13%)	1.70 ± 0.06 (6.25%)	1.76 ± 0.05[d] (10.0%)	1.87 ± 0.05[c] (16.9%)
Relative liver weight	45.81 ± 1.24	45.77 ± 1.05 (−0.09%)	47.32 ± 1.09 (3.30%)	48.44 ± 1.40 (5.74%)	49.21 ± 1.26 (7.42%)	50.82 ± 1.27[c] (10.9%)
Hemosiderin	0/10	0/10	0/10	0/10	0/10	10/10[c]
Necrosis	0/10	0/10	0/10	0/10	1/10	5/10[d]
Cytoplasmic basophilia	0/10	0/10	0/10	0/10	0/10	4/10
Females						
Number of animals	10	10	10	10	10	10
Terminal body weight (g)	31.5 ± 0.9	32.3 ± 1.1 (2.54%)	33.5 ± 1.3 (6.35%)	31.1 ± 0.8 (−1.27%)	33.1 ± 0.8 (5.08%)	33.0 ± 0.4 (4.76%)
Spleen						
Absolute spleen weight (g)	0.09 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.13 ± 0.01[c]	0.25 ± 0.01[c]
Relative spleen weight	2.98 ± 0.16	2.95 ± 0.10	2.93 ± 0.15	3.20 ± 0.09	3.94 ± 0.16[c]	7.68 ± 0.27[c]
Congestion	0/10	0/10	0/10	0/10	0/10	10/10[c]
Hemosiderin	0/10	0/10	0/10	0/10	10/10[c]	10/10[c]
Hematopoietic cell proliferation	0/10	1/10	1/10	2/10	9/10[c]	10/10[c]

Table B-13. Selected Changes in B6C3F$_1$ Mice Exposed to *p*-Chloronitrobenzene (CASRN 100-00-5) via Inhalation for 13 Weeks[a]

Endpoint	Daily Average Exposure Concentration, mg/m^3					
	0	1.7	3.4	6.9	13.8	27.5
Females						
Liver						
Absolute liver weight (g)	1.47 ± 0.03	1.54 ± 0.05 (4.76%)	1.62 ± 0.05 (10.2%)	1.55 ± 0.05 (5.44%)	1.76 ± 0.06[c] (19.7%)	1.89 ± 0.02[c] (28.6%)
Relative liver weight	46.8 ± 0.89	47.7 ± 1.00 (1.92%)	48.5 ± 1.02 (3.63%)	50.1 ± 1.34[d] (7.05%)	53.1 ± 0.97[c] (13.5%)	57.3 ± 0.82[c] (22.4%)
Hemosiderin	0/10	0/10	0/10	0/10	0/10	10/10[c]

[a]NTP (1993); Travlos et al. (1996).
[b]Mean ± SE.
[c]Significantly different from the control at $p < 0.01$.
[d]Significantly different from the control at $p < 0.05$.
[e]Number affected/number examined.
[f]Percentage change compared to control.

Body weight and organ weights are given in grams. Relative organ weights are given as mg organ weight/g body weight.

APPENDIX C. BENCHMARK DOSE MODELING RESULTS FOR THE SUBCHRONIC p-RfD AND CHRONIC p-RfD

MODEL-FITTING PROCEDURE FOR DICHOTOMOUS DATA

The benchmark dose (BMD) modeling of dichotomous data was conducted with the U.S. EPA's Benchmark Dose Software (BMDS) (Version 2.2.1). For these data, all of the dichotomous models (i.e., Gamma, Multistage, Logistic, Log-logistic, Probit, Log-probit, and Weibull models) available within the software were fit using a default benchmark response (BMR) of 10% extra risk based on the U.S. EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012b). Adequacy of model fit was judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. Among all models providing adequate fit, the lowest benchmark dose lower confidence limit (BMDL) was selected if the BMDLs estimated from different models varied greater than three-fold; otherwise, the BMDL from the model with the lowest Akaike's information criterion (AIC) was selected as a potential point of departure (POD) from which to derive a provisional oral reference dose (p-RfD).

In addition, data from exposures much higher than the study lowest-observed-adverse-effect level (LOAEL) do not provide reliable information regarding the shape of the response curve at low doses. However, such exposures can have a strong effect on the shape of the fitted model in the low-dose region of the dose-response curve in some cases. Thus, if lack of fit in the low-dose region is due to characteristics associated with dose-response data for high doses, then the U.S. EPA's *Benchmark Dose Technical Guidance Document* allows for data to be adjusted by eliminating high-dose groups (U.S. EPA, 2012b).

MODEL-FITTING PROCEDURE FOR CONTINUOUS DATA

The BMD modeling of continuous data was conducted with the U.S. EPA's BMDS (Version 2.2.1). For these data, all continuous models available within the software were fit using a default BMR of 1 standard deviation (SD) extra risk in the absence of a biologically relevant BMR level for these endpoints. An adequate fit was judged based on the goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination was made as to whether the variance across dose groups was constant. If a constant variance model was deemed appropriate based on the statistical test provided in BMDS (i.e., Test 2), the final BMD results were estimated from a constant variance model. If the test for homogeneity of variance was rejected ($p < 0.1$), the model was run again while modeling the variance as a power function of the mean to account for this nonconstant variance. If this nonconstant variance model did not adequately fit the variance data (i.e., Test 3; $p < 0.1$), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different models varied greater than three-fold; otherwise, the BMDL from the model with the lowest AIC was selected as a potential POD from which to derive a p-RfD.

INCREASED METHEMOGLOBIN IN MALE S-D (CRL:COBS CD> [SD]BR) RAT TREATED WITH *p*-CHLORONITROBENZENE FOR 90 DAYS (Monsanto, 1994b)

All available continuous models in U.S. EPA BMDS (Version 2.2.1) were fit to the increased methemoglobin data from male S-D rats treated with *p*-chloronitrobenzene for 90 days

(Monsanto, 1994b) **(**see Table B-2). For increased methemoglobin, a default BMR of 1 SD from the control mean was used (U.S. EPA, 2012b). As assessed by the χ^2 goodness-of-fit statistic, AIC score, and visual inspection, the Exponential Models 4 and 5, using nonhomogeneous variance and restricted power, adequately provided the best model fit based on the lowest AIC and BMDL (see Table C-1 and Figure C-1). Estimated doses associated with a 1 SD BMR and the 95% lower confidence limit on these doses (BMD_{1SD} and $BMDL_{1SD}$ values, respectively) were 0.12 and 0.084 mg/kg-day.

Table C-1. Model Predictions for Increased Methemoglobin In Male S-D Rats[a]

Model	BMD_{1SD} (mg/kg-d)	$BMDL_{1SD}$ (mg/kg-d)	*p*-Value Test 2[b]	*p*-Value Test 3[b]	Goodness-of-Fit *p*-Value[b]	AIC	Conclusion
Exponential (M2)	10.55	8.27	<0.0001	0.107	<0.0001	124.6	Goodness-of-fit *p*-value <0.1
Exponential (M3)	10.55	8.27	<0.0001	0.107	<0.0001	124.6	Goodness-of-fit *p*-value <0.1
Exponential (M4)	**0.12**	**0.084**	**<0.0001**	**0.107**	**0.168**	**31.02**	**Lowest AIC and BMDL**
Exponential (M5)	**0.12**	**0.084**	**<0.0001**	**0.107**	**0.168**	**31.02**	**Lowest AIC and BMDL**
Hill	0.11	NA	<0.0001	0.107	0.513	29.54	No BMDL calculated
Linear	0.20	0.13	<0.0001	0.107	<0.0001	89.19	Goodness-of-fit *p*-value <0.1
Polynomial	0.83	0.091	<0.0001	0.107	<0.0001	89.19	Goodness-of-fit *p*-value <0.1
Power	0.83	0.091	<0.0001	0.107	<0.0001	81.39	Goodness-of-fit *p*-value <0.1

[a]Monsanto (1994b).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.

AIC = Akaike's information criteria; BMD = Benchmark dose; BMDL = Lower confidence limit (95%) on the benchmark dose; NA = not applicable; SD = standard deviation.

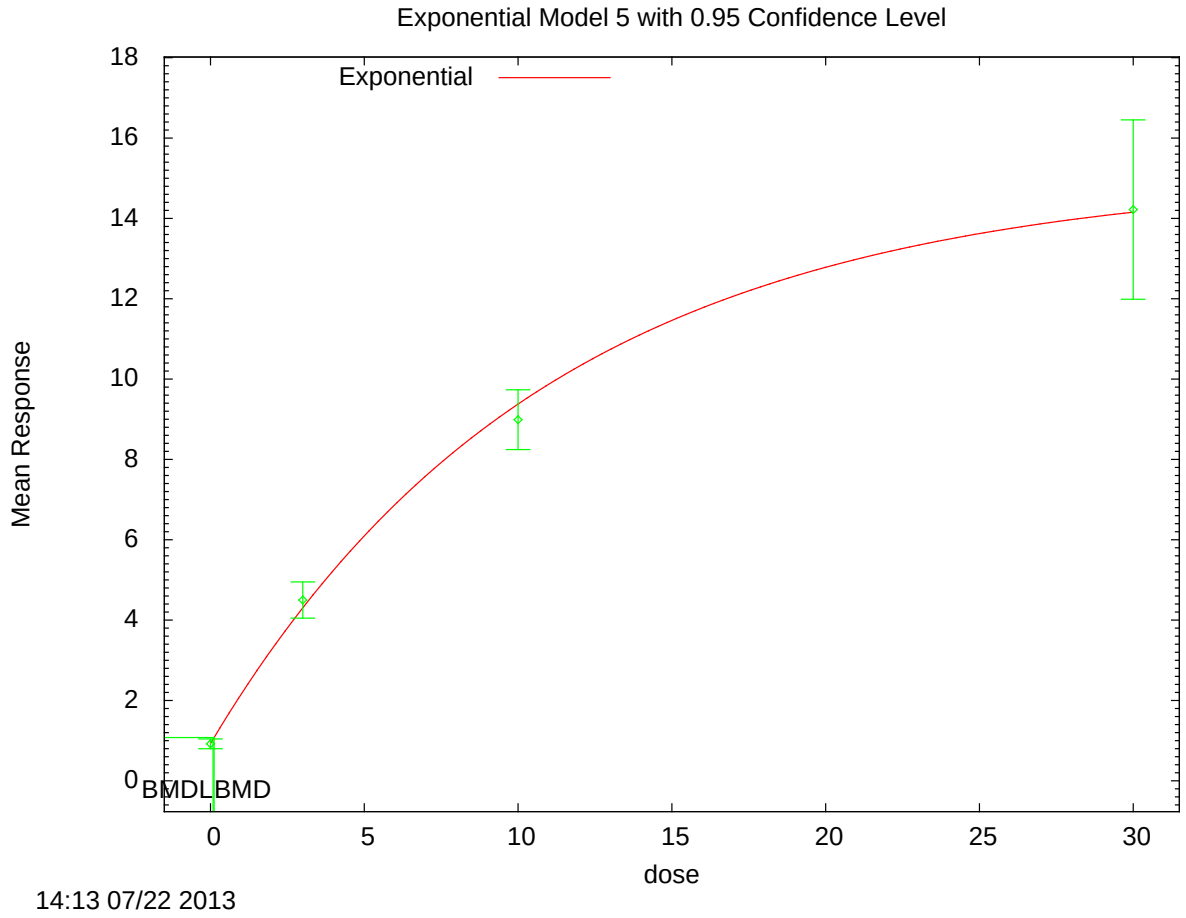

Figure C-1. Fit of Exponential Model with Nonhomogeneous Variance and Restricted Power to Data on Methemoglobin in Male Rats (Monsanto, 1994b)

Text Output for Exponential BMD Model for Methemoglobin in Male Rats (Monsanto, 1994b)

```
 ================================================================================
        Exponential Model. (Version: 1.7;  Date: 12/10/2009)
        Input Data File: C:/US EPA/BMDS220/Data/SessionFiles/p-CNB/exp_p-CNB -
Monsanto, 1994b methemoglobin M rats_Setting.(d)
        Gnuplot Plotting File:
                                                  Mon Jul 22 14:13:45 2013
 ================================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
```

```
        sign = +1 for increasing trend in data;
        sign = -1 for decreasing trend.

     Model 2 is nested within Models 3 and 4.
     Model 3 is nested within Model 5.
     Model 4 is nested within Model 5.


  Dependent variable = Mean
  Independent variable = Dose
  Data are assumed to be distributed: normally
  Variance Model: exp(lnalpha +rho *ln(Y[dose]))
  The variance is to be modeled as Var(i) = exp(lalpha + log(mean(i)) * rho)

  Total number of dose groups = 4
  Total number of records with missing values = 0
  Maximum number of iterations = 250
  Relative Function Convergence has been set to: 1e-008
  Parameter Convergence has been set to: 1e-008

  MLE solution provided: Exact
```

Initial Parameter Values

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	-3.59877	-3.59877	-3.59877	-3.59877
rho	1.95827	1.95827	1.95827	1.95827
a	2.24132	2.24132	0.874	0.874
b	0.0707721	0.0707721	0.0981502	0.0981502
c	--	--	17.0835	17.0835
d	--	1	--	1

Parameter Estimates by Model

Variable	Model 2	Model 3	Model 4	Model 5
lnalpha	1.10641	1.10641	-3.65433	-3.65433
rho	0.445359	0.445359	2.02733	2.02733
a	3.74685	3.74685	0.928059	0.928059
b	0.0458881	0.0458881	0.0908906	0.0908906
c	--	--	16.2502	16.2502
d	--	1	--	1

Table of Stats From Input Data

Dose	N	Obs Mean	Obs Std Dev
0	10	0.92	0.17
3	10	4.5	0.63
10	10	8.99	1.04
30	10	14.22	3.12

Estimated Values of Interest

Model	Dose	Est Mean	Est Std	Scaled Residual
2	0	3.747	2.333	-3.831

	3	4.3	2.406	0.2631
	10	5.929	2.584	3.746
	30	14.84	3.171	-0.6218
3	0	3.747	2.333	-3.831
	3	4.3	2.406	0.2631
	10	5.929	2.584	3.746
	30	14.84	3.171	-0.6218
4	0	0.9281	0.1491	-0.1709
	3	4.306	0.7066	0.8691
	10	9.378	1.555	-0.7886
	30	14.16	2.361	0.08704
5	0	0.9281	0.1491	-0.1709
	3	4.306	0.7066	0.8691
	10	9.378	1.555	-0.7886
	30	14.16	2.361	0.08704

Other models for which likelihoods are calculated:

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

Model R: Yij = Mu + e(i)
Var{e(ij)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	DF	AIC
A1	-38.55968	5	87.11936
A2	-7.323404	8	30.64681
A3	-9.558295	6	31.11659
R	-86.1603	2	176.3206
2	-58.28778	4	124.5756
3	-58.28778	4	124.5756
4	-10.50805	5	31.01611
5	-10.50805	5	31.01611

Additive constant for all log-likelihoods = -36.76. This constant added to the above values gives the log-likelihood including the term that does not depend on the model parameters.

Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does Model 2 fit the data? (A3 vs. 2)

Test 5a: Does Model 3 fit the data? (A3 vs 3)
Test 5b: Is Model 3 better than Model 2? (3 vs. 2)

Test 6a: Does Model 4 fit the data? (A3 vs 4)
Test 6b: Is Model 4 better than Model 2? (4 vs. 2)

Test 7a: Does Model 5 fit the data? (A3 vs 5)
Test 7b: Is Model 5 better than Model 3? (5 vs. 3)
Test 7c: Is Model 5 better than Model 4? (5 vs. 4)

Tests of Interest

Test	-2*log(Likelihood Ratio)	D. F.	p-value
Test 1	157.7	6	< 0.0001
Test 2	62.47	3	< 0.0001
Test 3	4.47	2	0.107
Test 4	97.46	2	< 0.0001
Test 5a	97.46	2	< 0.0001
Test 5b	-7.816e-013	0	N/A
Test 6a	1.9	1	0.1681
Test 6b	95.56	1	< 0.0001
Test 7a	1.9	1	0.1681
Test 7b	95.56	1	< 0.0001
Test 7c	-1.705e-013	0	N/A

The p-value for Test 1 is less than .05. There appears to be a difference between response and/or variances among the dose levels, it seems appropriate to model the data.

The p-value for Test 2 is less than .1. A non-homogeneous variance model appears to be appropriate.

The p-value for Test 3 is greater than .1. The modeled variance appears to be appropriate here.

The p-value for Test 4 is less than .1. Model 2 may not adequately describe the data; you may want to consider another model.

The p-value for Test 5a is less than .1. Model 3 may not adequately describe the data; you may want to consider another model.

Degrees of freedom for Test 5b are less than or equal to 0. The Chi-Square test for fit is not valid.

The p-value for Test 6a is greater than .1. Model 4 seems to adequately describe the data.

The p-value for Test 6b is less than .05. Model 4 appears to fit the data better than Model 2.

The p-value for Test 7a is greater than .1. Model 5 seems to adequately describe the data.

The p-value for Test 7b is less than .05. Model 5 appears to fit the data better than Model 3.

Degrees of freedom for Test 7c are less than or equal to 0. The Chi-Square test for fit is not valid.

Benchmark Dose Computations:

Specified Effect = 1.000000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

BMD and BMDL by Model

Model	BMD	BMDL
2	10.5504	8.27379
3	10.5504	8.27379
4	0.116556	0.0838429
5	0.116556	0.0838429

INCREASED METHEMOGLOBIN IN FEMALE CD (S-D-DERIVED) RATS TREATED WITH p-CHLORONITROBENZENE FOR 24 MONTHS (Bio Dynamics, 1985)

All available continuous models in U.S. EPA BMDS (Version 2.2.1) were fit to the increased methemoglobin data from female S-D rats treated with *p*-chloronitrobenzene for 24 months (Bio Dynamics, 1985) (see Table B-7). For increased methemoglobin, a default BMR of 1 SD from the control mean was used (U.S. EPA, 2012b). As assessed by the χ^2 goodness-of-fit statistic, AIC score, and visual inspection, neither the homogenous nor nonhomogeneous variance models provided adequate fit to the data for females when all dose groups were included. With the high-dose group from each data set dropped, the Linear, Polynomial, and Power models (using homogenous variance) adequately provided the best model fit based on the lowest AIC and BMDL (see Table C-2 and Figure C-2). Estimated doses associated with a 1 SD BMR and the 95% lower confidence limit on these doses (BMD_{1SD} and $BMDL_{1SD}$ values, respectively) were 0.15 and 0.12 mg/kg-day.

Table C-2. Model Predictions for Methemoglobinemia in Female Rats[a]							
Model	**BMD_{1SD} (mg/kg-d)**	**$BMDL_{1SD}$ (mg/kg-d)**	***p*-Value Test 2[b]**	***p*-Value Test 3[b]**	**Goodness-of-Fit *p*-Value[b]**	**AIC**	**Conclusion**
Without high-dose group							
Exponential (M2)	0.24	0.20	0.310	0.310	0.259	−52.00	NA
Exponential (M3)	0.24	0.20	0.310	0.310	0.259	−52.00	NA
Exponential (M4)	0.11	0.06	0.310	0.310	NA	−51.28	Goodness of fit not available
Exponential (M5)	0.35	0.24	<0.0001	<0.0001	0.888	1.237	NA
Linear	**0.15**	**0.12**	**0.310**	**0.310**	**0.651**	**−53.07**	**Lowest AIC and BMDL**
Polynomial	**0.15**	**0.12**	**0.310**	**0.310**	**0.651**	**−53.07**	**Lowest AIC and BMDL**
Power	**0.15**	**0.12**	**0.310**	**0.310**	**0.651**	**−53.07**	**Lowest AIC and BMDL**

[a]Bio Dynamics (1985).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.

AIC = Akaike's information criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; NA = not applicable; SD = standard deviation.

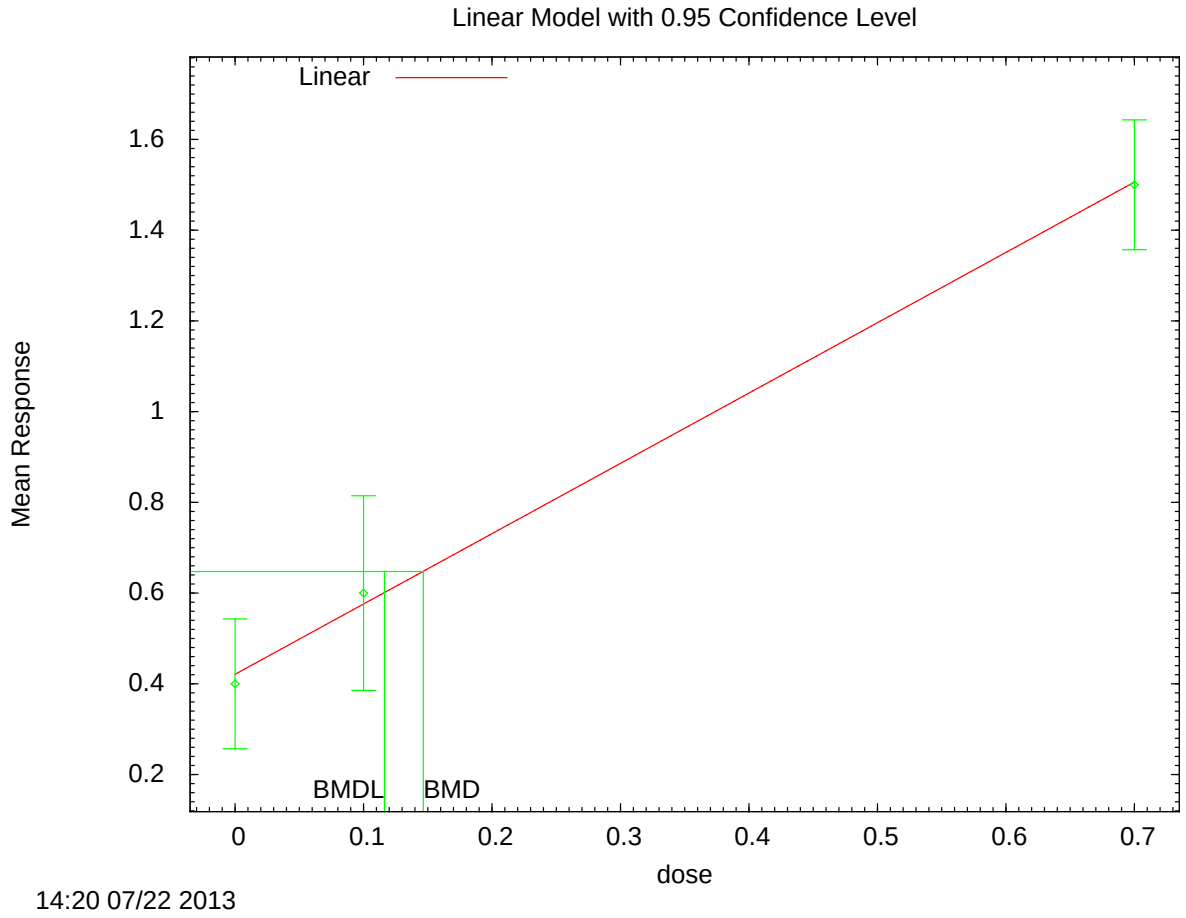

Figure C-2. Fit of Linear Model with Homogenous Variance to Data (without high dose) on Methemoglobinemia in Female Rats (Bio Dynamics, 1985)

Text Output for Linear Model to Data on Methemoglobinemia in Female Rats (Bio Dynamics, 1985)

```
 ================================================================
        Polynomial Model. (Version: 2.16;  Date: 05/26/2010)
        Input Data File: C:/US EPA/BMDS220/Data/SessionFiles/p-CNB/lin_p-CNB -
Biodynamics, 1985 methemoglobin_Opt.(d)
        Gnuplot Plotting File:  C:/US EPA/BMDS220/Data/SessionFiles/p-CNB/lin_p-CNB -
Biodynamics, 1985 methemoglobin_Opt.plt
                                              Mon Jul 22 14:20:47 2013
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = beta_0 + beta_1*dose + beta_2*dose^2 + ...


   Dependent variable = Mean
```

```
   Independent variable = Dose
   rho is set to 0
   Signs of the polynomial coefficients are not restricted
   A constant variance model is fit

   Total number of dose groups = 3
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                    Default Initial Parameter Values
                            alpha =    0.0566667
                              rho =            0   Specified
                           beta_0 =      0.42093
                           beta_1 =      1.54651


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                    alpha       beta_0       beta_1

     alpha              1    -5.7e-009     7.2e-009

    beta_0    -5.7e-009            1        -0.65

    beta_1     7.2e-009        -0.65            1


                                  Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha        0.0513488        0.0132582           0.0253632
0.0773345
         beta_0          0.42093        0.0546388             0.31384
0.52802
         beta_1          1.54651         0.133837              1.2842
1.80883


     Table of Data and Estimated Values of Interest

 Dose       N    Obs Mean     Est Mean   Obs Std Dev  Est Std Dev   Scaled Res.
------     ---   --------     --------   -----------  -----------   ----------

    0    10        0.4        0.421          0.2        0.227         -0.292
  0.1    10        0.6        0.576          0.3        0.227          0.341
  0.7    10        1.5          1.5          0.2        0.227        -0.0487


 Model Descriptions for likelihoods calculated
```

```
 Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

 Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

 Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2
     Model A3 uses any fixed variance parameters that
     were specified by the user

 Model  R:         Yi = Mu + e(i)
            Var{e(i)} = Sigma^2


                       Likelihoods of Interest

            Model      Log(likelihood)   # Param's      AIC
              A1          29.638945            4     -51.277889
              A2          30.808894            6     -49.617788
              A3          29.638945            4     -51.277889
          fitted          29.536695            3     -53.073390
               R           4.100439            2      -4.200877


                    Explanation of Tests

 Test 1:  Do responses and/or variances differ among Dose levels?
          (A2 vs. R)
 Test 2:  Are Variances Homogeneous? (A1 vs A2)
 Test 3:  Are variances adequately modeled? (A2 vs. A3)
 Test 4:  Does the Model for the Mean Fit? (A3 vs. fitted)
 (Note:  When rho=0 the results of Test 3 and Test 2 will be the same.)

                     Tests of Interest

   Test    -2*log(Likelihood Ratio)  Test df        p-value

   Test 1              53.4169          4          <.0001
   Test 2               2.3399          2          0.3104
   Test 3               2.3399          2          0.3104
   Test 4               0.2045          1          0.6511

The p-value for Test 1 is less than .05.  There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1.  A homogeneous variance
model appears to be appropriate here


The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


              Benchmark Dose Computation

Specified effect =            1
```

```
Risk Type        =     Estimated standard deviations from the control mean

Confidence level =          0.95

             BMD =       0.146525


            BMDL =       0.116233
```

APPENDIX D. BENCHMARK DOSE MODELING RESULTS FOR THE SUBCHRONIC p-RfC AND CHRONIC p-RfC

MODEL-FITTING PROCEDURE FOR DICHOTOMOUS DATA

The benchmark dose (BMD) modeling of dichotomous data was conducted with the U.S. EPA's Benchmark Dose Software (BMDS) (Version 2.2.1). For these data, all of the dichotomous models (i.e., Gamma, Multistage, Logistic, Log-logistic, Probit, Log-probit, and Weibull models) available within the software were fit using a default benchmark response (BMR) of 10% extra risk based on the U.S. EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012b). Adequacy of model fit was judged based on the χ^2 goodness-of-fit *p*-value ($p > 0.1$), magnitude of scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. Among all models providing adequate fit, the lowest benchmark concentration lower confidence limit (BMCL) was selected if the BMCLs estimated from different models varied greater than three-fold; otherwise, the BMCL from the model with the lowest Akaike's information criterion (AIC) was selected as a potential point of departure (POD) from which to derive a provisional inhalation reference concentration (p-RfC).

In addition, data from exposures much higher than the study lowest-observed-adverse-effect level (LOAEL) do not provide reliable information regarding the shape of the response curve at low doses. However, such exposures can have a strong effect on the shape of the fitted model in the low-dose region of the dose-response curve in some cases. Thus, if lack of fit in low-dose region is due to characteristics associated with dose-response data for high doses, then the U.S. EPA's *Benchmark Dose Technical Guidance Document* allows for data to be adjusted by eliminating high-dose groups (U.S. EPA, 2012b).

MODEL-FITTING PROCEDURE FOR CONTINUOUS DATA

The BMD modeling of continuous data was conducted with the U.S. EPA's BMDS (Version 2.2.1). For these data, all continuous models available within the software were fit using a default BMR of 1 standard deviation (SD) extra risk. An adequate fit was judged based on the goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination was made as to whether the variance across dose groups was constant. If a constant variance model was deemed appropriate based on the statistical test provided in BMDS (i.e., Test 2), the final BMD results were estimated from a constant variance model. If the test for homogeneity of variance was rejected ($p < 0.1$), the model was run again while modeling the variance as a power function of the mean to account for this nonconstant variance. If this nonconstant variance model did not adequately fit the variance data (i.e., Test 3; $p < 0.1$), the data set was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMCL was selected if the BMCLs estimated from different models varied greater than three-fold; otherwise, the BMCL from the model with the lowest AIC was selected as a potential POD from which to derive a p-RfC.

DECREASED HEMATOCRIT IN FEMALE F344/N RAT TREATED WITH *p*-CHLORONITROBENZENE FOR 13 WEEKS (Travlos et al., 1996; NTP, 1993)

All available continuous models in U.S. EPA's BMDS (Version 2.2.1) were fit to the decreased hematocrit data from female F344/N rats treated with *p*-chloronitrobenzene for 13 weeks (Travlos et al., 1996; NTP, 1993) (see Table B-12). For decreased hematocrit, a

default BMR of 1 SD from the control mean was used (U.S. EPA, 2012b). As assessed by the χ^2 goodness-of-fit statistic, AIC score, and visual inspection, neither the homogenous nor nonhomogeneous variance models provided adequate fit to the data for females when all concentration groups were included. With the high-concentration group from each data set dropped, the Hill model using homogenous variance and restricted power, adequately provided the best model fit based on the lowest AIC and BMCL (see Table D-1 and Figure D-1). Estimated concentration associated with a 1 SD BMR and the 95% lower confidence limit on these concentrations (BMC_{1SD} and $BMCL_{1SD}$ values, respectively) were 0.24 and 0.18 mg/m^3.

Table D-1. Model Predictions for Hematocrits in Female Rats[a]

Model	BMC_{1SDHEC} (mg/m^3)	$BMCL_{1SDHEC}$ (mg/m^3)	*p*-Value Test 2[b]	*p*-Value Test 3[b]	Goodness-of-Fit *p*-Value[b]	AIC	Conclusion
Without high-dose group							
Exponential (M3)	3.09	2.48	0.649	0.649	<0.0001	114.1	Goodness-of-fit *p*-value <0.1
Exponential (M4)	0.36	0.27	0.649	0.649	0.007	62.08	Goodness-of-fit *p*-value <0.1
Exponential (M5)	0.36	0.27	0.649	0.649	0.007	62.08	Goodness-of-fit *p*-value <0.1
Hill	**0.24**	**0.18**	**0.649**	**0.649**	**0.170**	**55.58**	**Lowest AIC and BMCL**
Linear	−9,999.00	149.1	0.649	0.649	<0.001	169.1	Goodness-of-fit *p*-value <0.1
Polynomial	−9,999.00	149.1	0.649	0.649	<0.001	169.1	Goodness-of-fit *p*-value <0.1
Power	3.48	2.81	0.649	0.649	<0.001	116.6	Goodness-of-fit *p*-value <0.1

[a]NTP (1993); Travlos et al. (1996).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.

AIC = Akaike's information criteria; BMC = benchmark concentration; BMCL = lower confidence limit (95%) on the benchmark concentration; HEC = human equivalent dose; SD = standard deviation.

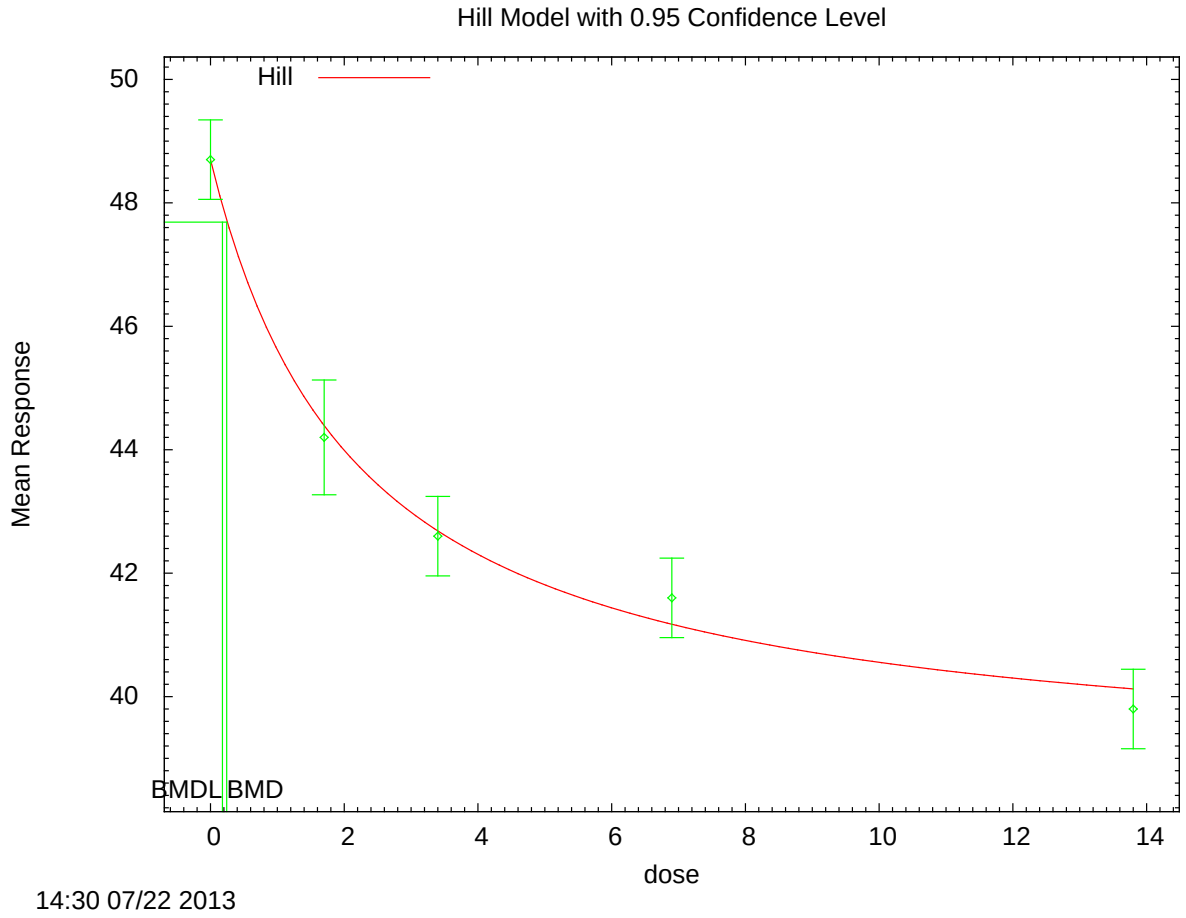

Figure D-1. Fit of Hill Model with Homogenous Variance and Restricted Power to Data on Hematocrit in Female Rats (Travlos et al., 1996; NTP, 1993)

Text Output for Hill Model Homogenous Variance to Data on Hematocrit in Female Rats (Travlos et al., 1996; NTP, 1993)

```
 ================================================================
        Hill Model. (Version: 2.16;  Date: 04/06/2011)
        Input Data File: C:/US EPA/BMDS220/Data/SessionFiles/p-CNB/hil_p-CNB-NTP1993a
Hematocrit F rat_Opt.(d)
        Gnuplot Plotting File:  C:/US EPA/BMDS220/Data/SessionFiles/p-CNB/hil_p-CNB-
NTP1993a Hematocrit F rat_Opt.plt
                                              Mon Jul 22 14:30:31 2013
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the response function is:

   Y[dose] = intercept + v*dose^n/(k^n + dose^n)


   Dependent variable = Mean
```

```
   Independent variable = Dose
   rho is set to 0
   Power parameter restricted to be greater than 1
   A constant variance model is fit

   Total number of dose groups = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                          alpha =        0.986
                            rho =            0   Specified
                      intercept =         48.7
                              v =         -8.9
                              n =       1.3629
                              k =      1.68111


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -rho    -n
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                    alpha    intercept            v            k

     alpha              1     1.5e-009     8.7e-009    -7.4e-008

 intercept     1.5e-009             1        -0.41        -0.43

         v     8.7e-009        -0.41            1        -0.56

         k    -7.4e-008        -0.43        -0.56            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
          alpha         0.952677         0.190535             0.579234
1.32612
      intercept          48.6638         0.309222              48.0578
49.2699
              v         -9.95681          0.55018             -11.0351            -
8.87848
              n                1               NA
              k          2.22928         0.419966              1.40616
3.0524

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.



     Table of Data and Estimated Values of Interest
```

Dose	N	Obs Mean	Est Mean	Obs Std Dev	Est Std Dev	Scaled Res.
0	10	48.7	48.7	0.9	0.976	0.117
1.7	10	44.2	44.4	1.3	0.976	-0.505
3.4	10	42.6	42.7	0.9	0.976	-0.162
6.9	10	41.6	41.1	0.9	0.976	1.5
13.8	10	39.8	40.1	0.9	0.976	-0.945

Model Descriptions for likelihoods calculated

Model A1: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2

Model A2: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma(i)^2

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = Sigma^2
Model A3 uses any fixed variance parameters that
were specified by the user

Model R: Yi = Mu + e(i)
Var{e(i)} = Sigma^2

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-22.013514	6	56.027028
A2	-20.775209	10	61.550418
A3	-22.013514	6	56.027028
fitted	-23.788015	4	55.576030
R	-82.567127	2	169.134254

Explanation of Tests

Test 1: Do responses and/or variances differ among Dose levels?
(A2 vs. R)
Test 2: Are Variances Homogeneous? (A1 vs A2)
Test 3: Are variances adequately modeled? (A2 vs. A3)
Test 4: Does the Model for the Mean Fit? (A3 vs. fitted)
(Note: When rho=0 the results of Test 3 and Test 2 will be the same.)

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	123.584	8	<.0001
Test 2	2.47661	4	0.6488
Test 3	2.47661	4	0.6488
Test 4	3.549	2	0.1696

The p-value for Test 1 is less than .05. There appears to be a
difference between response and/or variances among the dose levels
It seems appropriate to model the data

The p-value for Test 2 is greater than .1. A homogeneous variance
model appears to be appropriate here

```
The p-value for Test 3 is greater than .1.  The modeled variance appears
 to be appropriate here

The p-value for Test 4 is greater than .1.  The model chosen seems
to adequately describe the data


        Benchmark Dose Computation

Specified effect =             1

Risk Type        =     Estimated standard deviations from the control mean

Confidence level =           0.95

             BMC =       0.242284

            BMCL =      0.178808
```

APPENDIX E. BENCHMARK DOSE MODELING RESULTS FOR THE ORAL SLOPE FACTOR

MODEL-FITTING PROCEDURE FOR CANCER INCIDENCE DATA

The model-fitting procedure for dichotomous cancer incidence data is as follows. The Multistage-Cancer model in the EPA's Benchmark Dose Software (BMDS) (Version 2.2.1) is fit to the incidence data using the extra risk option. The Multistage-Cancer model is run for all polynomial degrees up to $n - 1$ (where n is the number of dose groups including control). An adequate model fit is judged by three criteria: (1) goodness-of-fit p-value ($p > 0.1$), (2) visual inspection of the dose-response curve, and (3) scaled residual at the data point (except the control) closest to the predefined benchmark response (BMR). Among all the models providing adequate fit to the data, the benchmark dose lower confidence limit (BMDL) from the best fitting Multistage-Cancer model as judged by the goodness-of-fit p-value, is selected as the point of departure (POD). In accordance with U.S. EPA (2012b) guidance, benchmark doses (BMDs) and BMDLs associated with an extra risk of 10% are calculated. In addition, multiple tumor combination analyses were also run if different tumors were identified in different organs/tissues within the same study.

INCREASED SPLENIC HEMANGIOSARCOMA IN MALE F344/DuCrj RAT TREATED WITH *p*-CHLORONITROBENZENE FOR 2 YEARS (Matsumoto et al., 2006a)

The above modeling procedure was applied to the Matsumoto et al. (2006a) splenic hemangiosarcoma data in male rat (see Table B-9). The initial BMD analysis of the hemangiosarcoma data using the multistage cancer model results in a p-value of 0.1099. However, visual inspection indicated that the dose-response curve does not adequately fit the data at the response level close to BMR of 10%. Consequently, the analysis was repeated with the data at the highest dose dropped, and the 2-degree Multistage-Cancer model provided an adequate fit (goodness-of-fit p-value > 0.1; see Table E-1 and Figures E-1A and E-1B). The estimated BMD_{10HED} value is 2.17 mg/kg-day with a $BMDL_{10HED}$ of 1.56 mg/kg-day.

Table E-1. Model Predictions for Splenic Hemangiosarcoma in Male Rats[a]

Model	Degrees of Freedom	χ^2	χ^2 Goodness-of-Fit p-Value[b]	AIC	$BMD_{10\ HED}$ (mg/kg-d)	$BMDL_{10\ HED}$ (mg/kg-d)
Multistage-cancer (degree = 3)[c]	3	6.04	0.11	80.08	5.59	3.60
Multistage-cancer (degree = 2)[c]	3	6.04	0.11	80.08	5.59	3.60
Multistage-cancer (degree = 1)[c]	3	6.04	0.11	80.08	5.59	3.60
Multistage-cancer (degree = 2)[c] (highest dose dropped)	**2**	**0.20**	**0.91**	**34.89**	**2.17**	**1.56**

[a]Matsumoto et al. (2006a).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[c]Betas restricted to ≥0.

AIC = Akaike's information criteria; BMD = benchmark dose; BMDL = lower confidence limit (95%) on the benchmark dose; HED = human equivalent dose.

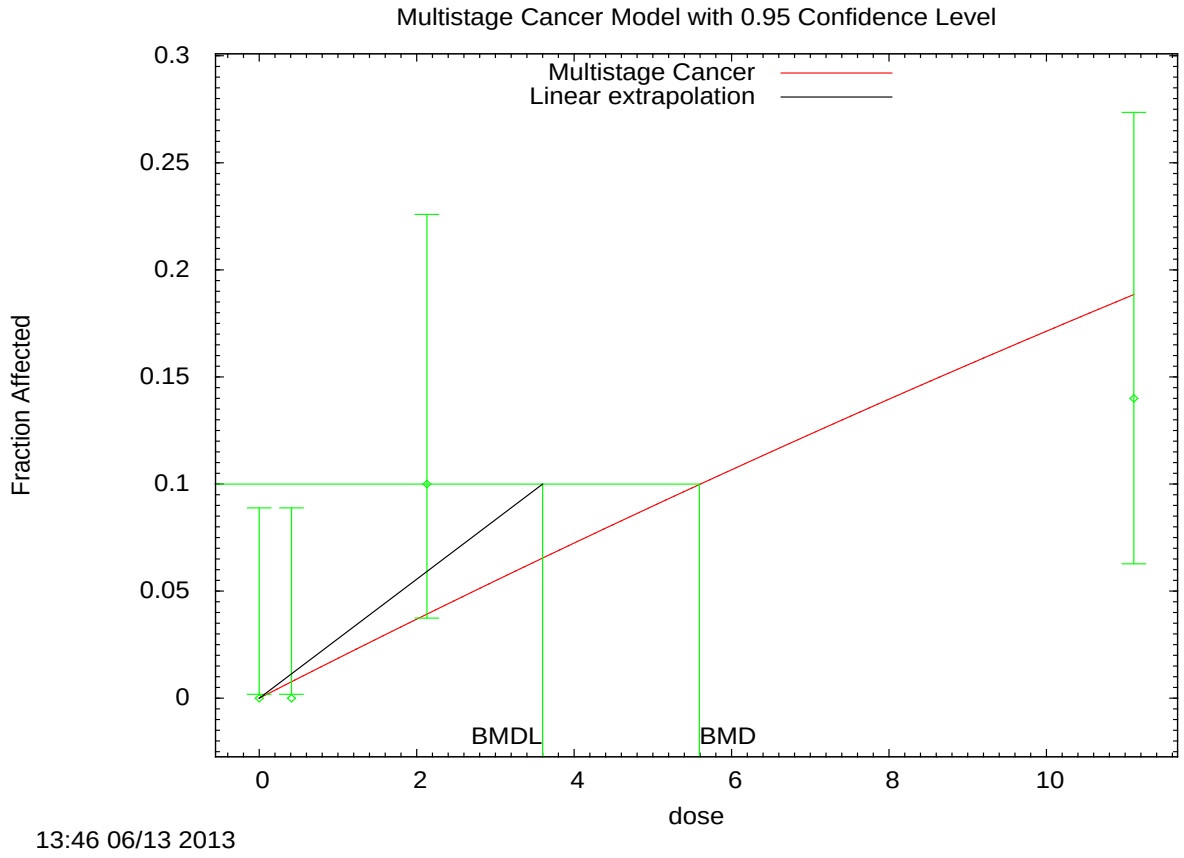

Figure E-1A. Fit of Multistage (2-Degree) Model to Data on Splenic Hemangiosarcomas in Male Rats (Matsumoto et al., 2006a)

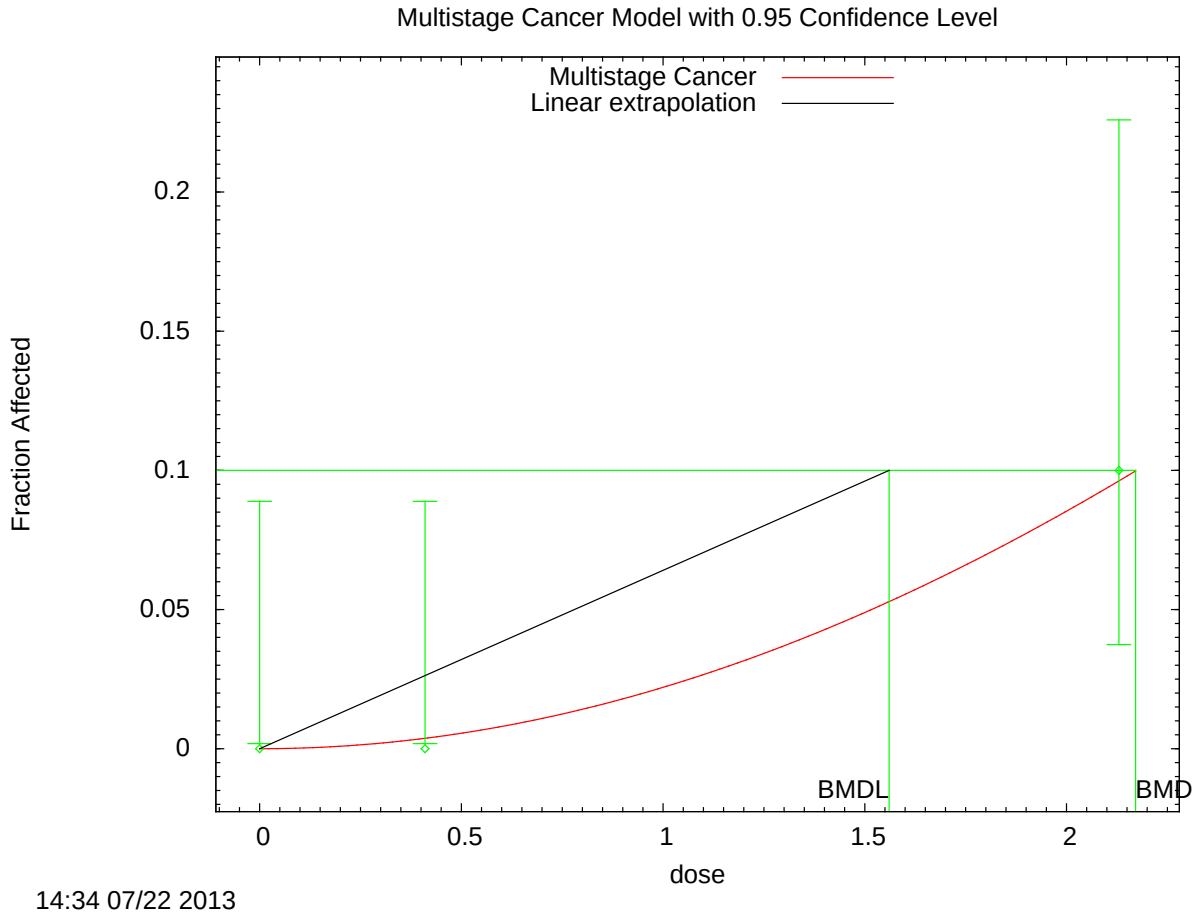

Figure E-1B. Fit of Multistage (2-Degree) Model to Data on Splenic Hemangiosarcomas in Male Rats with the Highest-Dose Data Dropped from the Analysis (Matsumoto et al., 2006a)

Text Output for Multistage (2-Degree) Model to Data on Splenic Hemangiosarcomas in Male Rats with the Highest-Dose Data Dropped from the Analysis (Matsumoto et al., 2006a)

```
 ================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:\US EPA\BMDS220\Data\SessionFiles\p-CNB\msc_p-CNB -
Matsumoto 2006b spleen hemangiosarcoma in M rats_Opt.(d)
        Gnuplot Plotting File: C:\US EPA\BMDS220\Data\SessionFiles\p-CNB\msc_p-CNB -
Matsumoto 2006b spleen hemangiosarcoma in M rats_Opt.plt
                                          Mon Jul 22 14:34:33 2013
 ================================================================

 BMDS_Model_Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(
                 -beta1*dose^1-beta2*dose^2)]
```

```
   The parameter betas are restricted to be positive


   Dependent variable = Effect
   Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 3
 Total number of specified parameters = 0
 Degree of polynomial = 2


 Maximum number of iterations = 250
 Relative Function Convergence has been set to: 1e-008
 Parameter Convergence has been set to: 1e-008



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =            0
                        Beta(2) =    0.0236361


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(1)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(2)

   Beta(2)           1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0           *               *                   *
        Beta(1)                0           *               *                   *
        Beta(2)        0.0223498           *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -16.2541         3
   Fitted model        -16.4456         1      0.382945      2          0.8257
  Reduced model        -21.9217         1       11.3351      2        0.003456

           AIC:         34.8912


                                Goodness  of  Fit
                                                        Scaled
```

Dose	Est._Prob.	Expected	Observed	Size	Residual
0.0000	0.0000	0.000	0.000	50	0.000
0.4100	0.0037	0.187	0.000	50	-0.434
2.1000	0.0964	4.821	5.000	50	0.086

Chi^2 = 0.20 d.f. = 2 P-value = 0.9069

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 2.17121

BMDL = 1.56032

BMDU = 4.41287

Taken together, (1.56032, 4.41287) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0640894

APPENDIX F. REFERENCES

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