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Provisional Peer-Reviewed Toxicity Values for

m-Nitrotoluene
(CASRN 99-08-1)

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Commonly Used Abbreviations

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

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR *m*-NITROTOLUENE (1-METHYL-3-NITROBENZENE) (CASRN 99-08-1)

Background

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

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

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

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

Disclaimers

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

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore,

users are strongly encouraged to read the entire PPRTV manuscript and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

Questions Regarding PPRTVs

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

INTRODUCTION

Neither the U.S. Environmental Protection Agency (U.S. EPA, 2008) Integrated Risk Information System (IRIS) nor the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006) included an entry for *m*-nitrotoluene. The Health Effects Assessment Summary Tables (HEAST; U.S. EPA, 1997) listed a chronic RfD of 1×10^{-2} mg/kg-day and a subchronic RfD of $1x10^{-1}$ mg/kg-day for *m*-nitrotoluene based on splenic lesions in rats gavaged with *o*-nitrotoluene at a dose of 200 mg/kg-day, 5 days/week, for 6 months (Ciss et al., 1980). Because similar effects also were noted in rats gavaged with *m*-nitrotoluene at a dose of 300 mg/kg-day (Ciss et al., 1980), the US EPA (1986) Health and Environmental Effects Profile (HEEP) for nitrotoluenes used results for *o*-nitrotoluene as the basis for an average daily intake (ADI) that would be protective for all three isomers (*o*-, *m*-, and *p*-nitrotoluene). The HEAST did not include RfC or cancer risk values for *m*-nitrotoluene. The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1991a, 1994) did not include any relevant documents other than the HEEP (U.S. EPA, 1986). Toxicological review documents for nitrotoluenes were not available from the Agency for Toxic Substances and Disease Registry (ATSDR, 2008) or the World Health Organization (WHO, 2008). California EPA had not derived oral public health goals for drinking water (CalEPA, 2008) or inhalation reference exposure levels (CalEPA, 2005) for m-nitrotoluene.

The National Institute for Occupational Safety and Health (NIOSH, 2005) listed a worker time-weighted average (TWA) recommended exposure limit of 2 ppm (11 mg/m^3) with a skin notation, indicating potential for skin absorption, for all isomers of nitrotoluene. The American Conference of Governmental Industrial Hygienists (ACGIH, 2001, 2007) adopted the same value (2 ppm; 11 mg/m^3) as a threshold limit value (TLV)-TWA, by analogy to aniline, to protect against methemoglobinemia and resulting anoxia and cyanosis, and included a BEI (biological exposure index) notation indicating the availability of a biological exposure index, based on methemoglobinemia (ACGIH, 2006). The Occupational Safety and Health Administration (OSHA, 2008) established a permissible exposure limit-TWA of 5 ppm (30 mg/m^3) for *m*-nitrotoluene with a skin notation, based on the previous TLV, which originally had been adopted in 1950 (ACGIH, 2001).

An International Agency for Research on Cancer (IARC) monograph on nitrotoluenes (IARC, 1996) included the assignment of *m*-nitrotoluene to Group 3, not classifiable as to its carcinogenicity in humans, based on inadequate evidence (no data) for carcinogenicity in humans and animals and largely negative results in genotoxicity assays. The National Toxicology Program (NTP) had not tested the carcinogenicity of *m*-nitrotoluene (NTP, 2008a, 2008b), although it had completed carcinogenicity assays for the para and ortho isomers in 2002.

Literature searches for studies relevant to the derivation of provisional toxicity values for *m*-nitrotoluene (CASRN 99-08-1) were conducted in PUBMED, TOXLINE special and DART/ETIC (1960s–June 2007); BIOSIS (August 2000–June 2007); TSCATS/TSCATS2, RTECS, CCRIS, HSDB and GENETOX (not date limited); and Current Contents (January-June 2007). Literature searches were spot-checked through July 2008, revealing no additional, relevant data. A review on the health effects of aromatic nitro- and amino- compounds (Weisburger and Hudson, 2001) also was consulted for relevant information.

REVIEW OF PERTINENT DATA

Human Studies

Human occupational exposure studies from the Russian literature (Makotchenko, 1974; Nemtseev and Smolyaninova, 1976; Kleiner and Stovpivskaya, 1981) were reviewed in the HEEP (U.S. EPA, 1986). Kleiner and Stovpivskaya (1981) studied a group of 38 women, between the ages of 30–45 years with "chronic nitrotoluene poisoning," who had been occupationally exposed to toluene nitro-derivatives for a minimum of 5 years. Exposure estimations were not specified except that they were 3–6 times above the MAC (maximum allowable concentration; level not specified). "A group of 24 healthy subjects who had not been exposed to harmful industrial factors was simultaneously examined." No additional information about the control group was provided. Both clinical and biochemical laboratory studies were conducted to assess intestinal function. Clinical signs in the exposed workers included shooting pains around the navel (32/38), constipation (30/38), distention of the intestine (18/38), intolerance to milk (16/38), and variable stool (5/38). Laboratory analyses showed alterations in some intestinal enzyme concentrations or activities and absorption of a carbohydrate. Radiological examinations of the subjects indicated signs of spastic dyskinesia of the small intestine in approximately 1/3 of the subjects. The authors concluded that mild changes in intestinal activity occurred in patients with chronic nitrotoluene poisoning. The lack of exposure data for *m*-nitrotoluene, the likelihood of exposure to a mixture of compounds, and the lack of reporting of study details limit the usefulness of this study.

Nemtseev and Smolyaninova (1976) detected signs of toxic neuropathy of the optic pathway, including constriction of visual field, in persons working in contact with mixed isomers of nitrotoluene. Makotchenko (1974) compared the results of clinical and biochemical studies of 130 patients with chronic poisoning from nitrogen-containing toluene compounds with the results of 55 lead-poisoned and 29 healthy individuals. The authors attributed dysfunction of the adrenal cortex to nitrotoluene "poisoning."

No studies were located that reported toxicity or carcinogenicity in humans following repeated exposure to known oral or inhalation doses of *m*-nitrotoluene alone.

Animal Studies

Oral Exposure

In a 14-day range-finding study, NTP (1992) fed groups of male and female F344/N rats (5/gender/group) diets containing 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm of *m*-nitrotoluene (purity >96%). "Occasional" batches for the low dose were formulated to 675 ppm rather than 625 ppm; this dose group was referred to as 625/675 ppm by NTP (1992). The doses were reported as 0, 61, 108, 259, 431 or 881 mg/kg-day for males and 0, 58, 114, 215, 420, or 754 mg/kg-day for females. Rats were observed twice daily for mortality and moribundity and once a week for clinical signs, body weight, and food consumption. At termination, all animals were necropsied and histopathological examinations were conducted for the liver, testis, epididymis, uterus, and representative portions of gross lesions. Treatment had no effect on survival or the incidence of clinical signs. The authors attributed reduced average feed consumption at the highest dose in both genders to poor palatability of the diet. A 9–25% reduction in body weight gain relative to controls was observed in treated males at ≥2,500 ppm and a 7–14% reduction in females at ≥5,000 ppm. At the highest feed concentration (10,000 ppm), the testes and uterus were reduced in size, with males showing mild-to-moderate degeneration of the testes and the presence of abnormal (syncytial) spermatids in the seminiferous tubules and epididymis and the females showing thinner uterine walls and less development of the endometrium. The LOAEL for pathology of reproductive organs in both genders of rats in this study was 10,000 ppm or 754 and 881 mg/kg-day in females and males, respectively.

Using the same study protocol, NTP (1992) exposed groups of male and female B6C3F$_1$ mice (5/gender/dose) to *m*-nitrotoluene (purity >96%) at dietary concentrations of 0, 388, 625, 1,250, 2,500, or 5,000 ppm for 14 days. "Occasional" batches for the low dose were formulated to 675 ppm rather than 625 ppm; NTP (1992) referred to this dose group as 625/675 ppm. NTP estimated the doses to be 66, 113, 212, 409, or 779 mg/kg-day for the males and 92, 164, 297, 543, or 901 mg/kg-day for the females. Treatment had no effect on survival in either gender. Both genders demonstrated reduced body weight gains at ≥5,000 ppm. In the males, there was a dose-related decrease in food consumption (10–22%) in all treated groups relative to controls. Females at ≥2,500 ppm showed a 10–27% decrease in food consumption. As in rats, NTP (1992) attributed the reduced feed consumption to poor palatability of the diet. Relative liver weights were increased for both males (≥2,500 ppm) and females (≥625/675 ppm), but no chemically-related gross or microscopic lesions were observed. Increases in liver weights relative to body weights, without histological alterations, might have suggested adaptive physiological changes that were not toxicologically adverse. Therefore, the highest dose tested in both genders, 5,000 ppm (779–901 mg/kg-day), is considered the NOAEL for mice in this study.

In the 13-week rat study (NTP, 1992; Dunnick et al., 1994), groups of male and female F344/N rats (10/gender/group) were given *m*-nitrotoluene (purity >96%) daily in feed at concentrations of 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm. "Occasional" batches for the low dose were formulated to 675 ppm rather than 625 ppm; NTP (1992) referred to this dose group as the 625/675 ppm group. Based on feed consumption, NTP calculated the doses as 0, 46, 86,

171, 342, or 661 mg/kg-day in males and 0, 48, 87, 172, 336, or 638 mg/kg-day in females. Rats were monitored twice daily for mortality and moribundity and once a week for body weight, clinical signs, and food consumption. Complete hematologic and serum chemistry analysis was performed on additional groups of male and female rats (10/gender/group) at weeks 1 and 3 and in the core group at termination. At termination, all animals were necropsied and organ weights were recorded for heart, liver, lungs, right kidney, thymus, and right testicle; organs and tissues were examined for gross lesions. Complete histopathological examinations were conducted on all control animals, all animals dying prematurely, and all animals in the highest-dose group with 60% survivors for the following tissues: any gross lesions observed at necropsy, tissue masses or suspect tumors, regional lymph nodes, and 35 other tissues including the nasal cavity and nasal turbinates. Target tissues were examined in the next lower-dose group until a no-effect dose was determined. At necropsy, NTP (1992) evaluated epididymal sperm count, concentration, morphology, and motility; and left testicular spermatid count in the male control and ≥2,500 ppm groups. For the 12 days prior to study termination, the females in the above groups underwent vaginal lavage to determine estrous cycle length.

Exposure to *m*-nitrotoluene for 13 weeks had no effect on survival in F344/N rats. Tables 1 and 2 present details of feed consumption, estimated daily *m*-nitrotoluene doses, body weight effects, and selected results of hematology, clinical chemistry, and histopathological examinations. Feed consumption and terminal body weights were decreased in males at 10,000 ppm and in females at ≥5,000 ppm. No treatment-related clinical signs were observed in either gender. Hematological changes observed at Week 1 included increases in hematocrit, hemoglobin and erythrocytes, and decreases in reticulocytes in both genders, primarily at 10,000 ppm. Subsequently, significant decreases relative to controls were observed in erythrocytes, hemoglobin, and hematocrit, and increases relative to controls in reticulocytes, in both genders at Weeks 3 and 13, primarily at 10,000 ppm. Similar effects also were seen in the 5,000-ppm females at Week 13 and sporadically in all dose groups in males at Week 3. There were statistically significant dose-related increases in methemoglobin in both genders at 10,000 ppm at Week 3 and in males at ≥2,500 ppm and females at 10,000 ppm at Week 13. There were no consistent or persistent effects on leukocyte count or differential in either gender. Clinical chemistry results were unremarkable, except for statistically significant mild-to-moderate increases in bile acids in males and females, primarily at 5,000 and 10,000 ppm. Noteworthy changes in organ weights were dose-related increases in relative, but not absolute, kidney weights in male rats at ≥2,500 ppm and large (30–40%) decreases in both absolute and relative testes weights in males at 10,000 ppm. Changes in weights of other organs (heart, liver, lungs, thymus) were secondary to effects on body weight, comprising small decreases in absolute weight or increases in relative weights at doses (10,000 ppm in males and ≥5,000 ppm in females) that caused decreased feed consumption and depressed body weights.

Treatment-related histopathology was observed in the kidneys and testes of males and in the spleen in both genders. Hyaline droplet nephropathy of minimal severity, but with the number of droplets increasing with dose, occurred with high incidence in all treated groups of males. NTP (1992) attributed the hyaline droplet nephropathy in male rats to α_{2u}-globulin accumulation, which does not occur in humans. Thus, it is not considered an appropriate endpoint for the evaluation of systemic toxicity in humans (U.S. EPA, 1991b). Dose-related increases in the incidence of hemosiderin pigment (minimal-to-mild severity) occurred in the spleens of males treated with ≥2,500 ppm and in females of all exposure levels. Minimal spleen

Table 1. Selected Results of Histopathological Examinations, Hematology and Clinical Chemistry from Male Rats Administered *m*-Nitrotoluene in the Feed for 13 Weeks[a]

Concentration (ppm)	0	625/675	1,250	2,500	5,000	10,000
Dose (mg/kg-day)	**0**	**46**	**86**	**171**	**342**	**661**
Average feed intake (g/day) (% relative to controls)	16.3	16.5 (101)	16.1 (98)	16.3 (100)	15.8 (96)	13.4 (82)
Final body weight (kg) (% relative to controls)	0.346	0.354 (103)	0.342 (99)	0.353 (102)	0.338 (98)	0.281 (81)
Lesions, incidence (severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked)						
Kidney Hyaline droplet	0	9 (1.0)	10 (1.0)	10 (1.0)	10 (1.0)	10 (1.0)
Spleen Hemosiderin pigment Congestion	0 1 (1.0)	1 (1.0) 0	0 0	2 (1.0) 1 (1.0)	5 (1.0) 0	10 (1.4) 9 (1.0)
Testes Degeneration	0	0	0	0	0	10 (2.2)
Hematology and Clinical Chemistry						
Hematocrit (%) Week 1 Week 3 Week 13	42.2 ± 0.5[b] 47.9 ± 0.7 45.3 ± 0.3	43.2 ± 0.4 45.6 ± 0.4[c] 46.6 ± 0.5	43.2 ± 0.4 45.6 ± 0.4[c] 45.9 ± 0.3	43.8 ± 0.4[c] 45.3 ± 0.3[d] 46.7 ± 0.3	43.0 ± 0.4 47.0 ± 0.3 44.1 ± 0.5	45.7 ± 0.4[d] 44.9 ± 0.4[d] 45.7 ± 0.4
Hemoglobin (g/dL) Week 1 Week 3 Week 13	14.3 ± 0.2 16.3 ± 0.3 15.6 ± 0.1	14.7 ± 0.2 15.4 ± 0.1[c] 16.1 ± 0.2	14.7 ± 0.2 15.3 ± 0.1[c] 15.8 ± 0.1	14.9 ± 0.1[c] 15.3 ± 0.1[d] 16.0 ± 0.1	14.5 ± 0.1 15.8 ± 0.1 14.9 ± 0.2	15.4 ± 0.2[d] 14.8 ± 0.2[d] 15.2 ± 0.1
Erythrocytes (10^6/µL) Week 1 Week 3 Week 13	7.08 ± 0.12 8.10 ± 0.13 8.84 ± 0.07	7.23 ± 0.07 7.64 ± 0.08[c] 9.09 ± 0.08	7.22 ± 0.08 7.61 ± 0.07[c] 8.95 ± 0.06	7.36 ± 0.07[c] 7.56 ± 0.07[d] 9.07 ± 0.05	7.26 ± 0.09 7.95 ± 0.08[c] 8.48 ± 0.09	7.76 ± 0.08[d] 7.37 ± 0.09[d] 8.32 ± 0.08[d]
Reticulocytes (10^6/µL) Week 1 Week 3 Week 13	0.25 ± 0.03 0.15 ± 0.01 0.32 ± 0.02	0.29 ± 0.03 0.18 ± 0.02 0.36 ± 0.04	0.29 ± 0.02 0.16 ± 0.02 0.32 ± 0.02	0.27 ± 0.02 0.18 ± 0.02 0.32 ± 0.03	0.25 ± 0.01 0.17 ± 0.02 0.35 ± 0.03	0.12 ± 0.01[d] 0.29 ± 0.03[d] 0.44 ± 0.03[d]
Methemoglobin (%) Week 3 Week 13	2.00 ± 0.23 3.19 ± 0.14	2.04 ± 0.19 3.64 ± 0.31	1.98 ± 0.24 3.14 ± 0.18	2.22 ± 0.21 3.71 ± 0.18[c]	2.31 ± 0.16 3.89 ± 0.24[c]	3.28 ± 0.22[d] 4.56 ± 0.36[d]
Bile acids (µmol/L) Week 1 Week 3 Week 13	11.67 ± 2.40 9.60 ± 1.85 4.00 ± 0.99	14.20 ± 5.52 6.10 ± 1.39 4.60 ± 1.33	19.10 ± 4.75 5.40 ± 0.73 5.40 ± 1.44	16.90 ± 4.42 6.30 ± 1.76 4.50 ± 1.39	19.30 ± 7.03 6.90 ± 2.10 15.00 ± 1.59[d]	22.44 ± 3.81 37.00 ± 6.80 23.90 ± 5.14[d]

[a]NTP, 1992
[b]Mean and standard error for groups of 10 animals (occasional measurements included only 8 or 9 animals/group)
[c]Significantly different ($p \leq 0.05$) from control group by Dunn's or Shirley's test
[d]Significantly different ($p \leq 0.01$) from control group by Dunn's or Shirley's test

Table 2. Selected Results of Histopathological Examinations, Hematology and Clinical Chemistry from Female Rats Administered *m*-Nitrotoluene in the Feed for 13 Weeks[a]

Concentration (ppm)	0	625/675	1,250	2,500	5,000	10,000
Dose (mg/kg-day)	**0**	**48**	**87**	**172**	**336**	**638**
Feed intake (g/day) (% relative to controls)	10.8	10.7 (99)	10.3 (95)	10.2 (95)	9.4 (88)	8.4 (79)
Final body weight (kg) (% relative to controls)	0.194	0.199 (103)	0.194 (100)	0.195 (101)	0.177 (91)	0.166 (86)
Lesions, incidence (severity: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked)						
Spleen Hemosiderin pigment Congestion	 1 (1.0) 0	 9 (1.1) 0	 10 (1.1) 0	 10 (1.2) 0	 8 (1.5) 2 (1.0)	 10 (1.2) 9 (1.0)
Hematology and Clinical Chemistry						
Hematocrit (%) Week 1 Week 3 Week 13	 44.4 ± 0.8[b] 46.5 ± 0.8 45.6 ± 0.4	 43.8 ± 0.5 46.5 ± 0.5 46.3 ± 0.3	 43.8 ± 0.4 46.7 ± 0.3 45.3 ± 0.3	 44.2 ± 0.5 47.2 ± 0.3 44.7 ± 0.4	 45.6 ± 0.7 47.3 ± 0.4 44.6 ± 0.3	 46.5 ± 0.4[c] 43.6 ± 0.7[c] 43.6 ± 0.5[d]
Hemoglobin (g/dL) Week 1 Week 3 Week 13	 15.4 ± 0.3 15.9 ± 0.3 15.6 ± 0.2	 15.2 ± 0.2 16.0 ± 0.2 15.9 ± 0.1	 15.3 ± 0.2 16.1 ± 0.1 15.6 ± 0.1	 15.4 ± 0.2 16.1 ± 0.1 15.4 ± 0.1	 15.6 ± 0.3 16.0 ± 0.1 15.1 ± 0.1[c]	 15.8 ± 0.2 14.4 ± 0.2[d] 14.4 ± 0.2[d]
Erythrocytes (10^6/μL) Week 1 Week 3 Week 13	 7.68 ± 0.14 7.79 ± 0.16 8.42 ± 0.08	 7.50 ± 0.10 7.77 ± 0.11 8.54 ± 0.06	 7.49 ± 0.06 7.77 ± 0.06 8.40 ± 0.05	 7.61 ± 0.09 7.90 ± 0.08 8.27 ± 0.07	 7.79 ± 0.13 7.97 ± 0.06 8.06 ± 0.06[d]	 8.02 ± 0.08[c] 7.23 ± 0.10[c] 7.53 ± 0.06[d]
Reticulocytes (10^6/μL) Week 1 Week 3 Week 13	 0.20 ± 0.02 0.11 ± 0.01 0.20 ± 0.03	 0.23 ± 0.03 0.13 ± 0.02 0.21 ± 0.02	 0.21 ± 0.01 0.09 ± 0.01 0.21 ± 0.02	 0.20 ± 0.03 0.10 ± 0.01 0.23 ± 0.02	 0.18 ± 0.02 0.11 ± 0.01 0.29 ± 0.03[c]	 0.08 ± 0.01[d] 0.25 ± 0.04[d] 0.35 ± 0.03[d]
Methemoglobin (%) Week 3 Week 13	 1.66 ± 0.20 3.09 ± 0.28	 2.50 ± 0.33 3.15 ± 0.27	 1.97 ± 0.24 3.48 ± 0.24	 2.14 ± 0.25 3.46 ± 0.39	 2.09 ± 0.23 3.82 ± 0.22	 3.00 ± 0.21[d] 5.00 ± 0.35[d]
Bile acids (μmol/L) Week 1 Week 3 Week 13	 9.11 ± 1.39 20.22 ± 3.64 9.00 ± 2.31	 19.33 ± 7.85 11.17 ± 5.24 6.80 ± 1.02	 14.89 ± 6.88 11.33 ± 1.69 17.20 ± 3.04[c]	 10.57 ± 2.92 16.50 ± 5.83 12.70 ± 1.78	 17.33 ± 2.09[c] 15.33 ± 3.27 16.30 ± 3.71	 37.88 ± 6.37[d] 49.17 ± 4.83 49.30 ± 9.65[d]

[a]NTP, 1992
[b]Mean and standard error for groups of 10 animals (occasional measurements included only 6–9 animals/group)
[c]Significantly different ($p \leq 0.05$) from control group by Dunn's or Shirley's test
[d]Significantly different ($p \leq 0.01$) from control group by Dunn's or Shirley's test

congestion was observed in 90% of males and females treated with 10,000 ppm, compared to ≤10% of controls. Mild testicular degeneration, evidenced by smaller testis and epididymes, decreased numbers of germ cells and spermatids in the seminiferous tubules, and cellular debris in the epididymis occurred in 10/10 males at 10,000 ppm, but no control or lower-dose animals. Spermatid head count, sperm motility, and sperm concentration were decreased by 70–80% in the 10,000 ppm males. The length of the estrous cycle was significantly increased from 5 to 6–6.5 days at 5,000 and 10,000 ppm, with only 9 and 3 female rats, respectively, clearly cycling. There was a dose-related increase in the diestrous phase and dose-related decrease in the estrous phase at ≥2,500 ppm, although the length of the cycle remained the same at 2,500 ppm. Alterations in the estrous cycle could lead to decreased fertility, although no such indices were examined.

NTP (1992) concluded that the alterations in hematologic parameters were consistent with hemoconcentration at Week 1, followed by the slow onset of mild regenerative anemia. They postulated the mechanism for these hematologic alterations to be oxidative damage to hemoglobin, possibly leading to Heinz body formation and decreased erythrocyte survival. Hemosiderin deposition, splenic congestion, and increased methemoglobin generally are consistent with this mechanism. The dietary concentration of 625/675 ppm (46 mg/kg-day in males and 48 mg/kg-day in females), the lowest dose tested in this study, was a LOAEL in male rats for hematological effects (decreased hematocrit, hemoglobin and erythrocyte count during Week 3) and in female rats due to the associated splenic effects (increased hemosiderin deposition). A NOAEL was not identified.

NTP (1992; Dunnick et al., 1994) also administered 0, 625, 12,50, 2,500, 5,000, or 10,000 ppm of *m*-nitrotoluene daily in feed to B6C3F$_1$ mice (10/gender/group) for 13 weeks. "Occasional" batches for the low dose were formulated to 675 ppm rather than 625 ppm; NTP (1992) referred to this dose group as 625/675 ppm. Based on food consumption data, NTP estimated the doses as 0, 114, 208, 398, 743, or 1,422 mg/kg-day in males and 0,139, 254, 493, 884, or 1,550 mg/kg-day in females. Mice were evaluated according to the same protocol as described above for 13-week rats, with the exception that liver weights included the gall bladder, and no clinical chemistry or hematology assays were conducted. Treatment had no effect on survival or the incidence of clinical signs in either gender. There were decreases compared to controls in feed consumption and terminal body weight at ≥5,000 ppm in both genders. In both genders of mice, relative liver weights were significantly increased in all treated groups in a dose-related manner; absolute liver weights were significantly increased in females at 1,250 and 2,500 ppm (see Table 3). Changes in weights of other organs (heart, kidneys, lungs, testes, and thymus) occurred primarily in the 5,000 and 10,000 ppm groups and appeared to be secondary to reduced body weights in these groups. No histopathological lesions were noted in either gender. Treatment had no effect on spermatid numbers or spermatozoal motility or numbers in males, or on length of estrous cycle or stages in females. The increases in liver weights, in the absence of histopathology at any dose, suggested that this response was physiologically adaptive rather than adverse. Thus, the LOAEL for mice in this study is 5000 ppm (743 mg/kg-day in males and 884 mg/kg-day in females) based on decreased body weight gain. The NOAEL is 2,500 ppm (398 mg/kg-day in males and 493 mg/kg-day in females).

In a study of all 3 nitrotoluene isomers, Ciss et al. (1980) administered 0 (vehicle) or 300 mg/kg-day of *m*-nitrotoluene (purity 99%) to groups of 10 male and 10 female Wistar rats by

gavage in olive oil, 5 days/week for 6 months. Adjusting for frequency of administration, the average dose was calculated to have been 214 mg/kg-day. After 3 months, the animals were mated, as follows: 5 pairs of treated rats, 5 pairs of controls, 5 treated males with 5 control females and 5 control males with 5 treated females. Treatment presumably continued throughout gestation and lactation, although experimental details were lacking and it is not clear whether the pups were treated postnatally. Behavior, mortality, and the number and vitality of the pups were recorded. Hematology and serum chemistry were conducted, presumably on all animals at termination; study details were not well reported. Animals that died or were sacrificed *in extremis* were autopsied. At necropsy, gross examinations were conducted, organs were weighed, and histological evaluations were conducted on the major organs.

Table 3. Terminal Body and Selected Organ Weights in Male and Female Mice Administered *m*-Nitrotoluene in the Feed for 13 Weeks[a]

Concentration (ppm)	0	625/675	1,250	2,500	5,000	10,000
Males						
Dose (mg/kg-day)	**0**	**114**	**208**	**398**	**743**	**1422**
Feed intake (g/day) (% of controls)	4.7	4.7 (100)	4.6 (98)	4.4 (94)	3.8 (81)	3.4 (72)
Final body weight (g) (% of controls)	36.3	34.8 (96)	34.6 (96)	34.7 (96)	31.0 (88)	27.7 (76)
Liver weight[b] relative (% of controls)	45.2 ± 0.8	48.5 ± 1.0[c] (107)	50.0 ± 0.9[d] (111)	51.5 ± 0.7[d] (114)	53.9 ± 0.6[d] (119)	57.9 ± 0.9[d] (128)
Females						
Dose (mg/kg-day)	**0**	139	254	493	884	1550
Feed intake (g/day) (% of controls)	5.1	5.2 (102)	5.1 (100)	4.9 (96)	4.2 (82)	3.4 (67)
Final body weight (g) (% of controls)	33.4	34.3 (103)	34.2 (102)	32.6 (98)	29.6 (88)	24.3 (72)
Liver weight[b] absolute relative (% of controls)	 1430 ± 40 42.2 ± 0.7	 1630 ± 30 46.9 ± 0.7[d] (112)	 1820 ± 60[d] 52.6 ± 0.9[d] (125)	 1830 ± 30[d] 55.0 ± 0.7[d] (130)	 1610 ± 40 54.1 ± 0.9[d] (128)	 1360 ± 30 55.9 ± 1.0[d] (132)

[a] NTP, 1992
[b]Mean ± standard error; absolute organ weight (mg); relative organ weight (mg organ weight/g body weight)
[c]Significantly different ($p \leq 0.05$) from control group by William's or Dunnett's test
[d]Significantly different ($p \leq 0.01$) from control group by William's or Dunnett's test

Alopecia was prominent in the females throughout the treatment period (Ciss et al., 1980). Except for a slight decrease in hemoglobin concentration, the authors reported that variations in hematological indices were not significant physiologically. Several of the hematologic and serum chemistry endpoints measured, including number of white blood cells, alkaline phosphatase, cholinesterase, and creatine phosphokinase appeared to be elevated in treated animals. While the authors acknowledged the "rather significant variations" in some of these endpoints, they stated that it was difficult to affirm their relation to the treatment. It is not clear how the authors arrived at their conclusions, and statistical analyses were not reported. An

increase in the weight and size of the spleens and splenic microscopic "modification" were noted in the males. Litter size and growth rate of pups were unaffected. The spleens of pups from dams treated with *m*-nitrotoluene showed microscopic alterations. The authors noted that *m*-nitrotoluene affected the testicles, although the basis was not stated, and that the compound might be transferred to the fetus. Because the results of this study were not adequately described, it is not possible to determine whether the tested dose was a NOAEL or LOAEL.

As described by the U.S. EPA (1986), reports by Kovalenko (1973) and Vasilenko and Kovalenko (1975) appeared to describe the same experiment examining the hemotoxicity of mono-, di-, and tri-nitrotoluenes with respect to isomerism and the number of nitro groups, in 30-day and 3-month experiments. These reports did not adequately describe the experimental design or results. White rats, mice, and rabbits (number, strain and gender not specified) were administered 20% of the LD_{50} of *m*-nitrotoluene by gavage daily for 30 days or 3 days/week for 3 months (vehicle not specified). A control group was used, however, no details were provided. Several hematological indices were examined, including methemoglobin, sulfhemoglobin, and clotting function. Only the results for the rats were presented. U.S. EPA (1986) calculated the dose of *m*-nitrotoluene to be 318 mg/kg-day in rats. During the 30-day study, *m*-nitrotoluene produced an increase in sulfhemoglobin, an increase in methemoglobin, increased reticulocytes, and symptoms of anemia, including decreased hemoglobin concentration and red blood cell (RBC) count. During the 3-month study, *m*-nitrotoluene produced an increase in sulfhemoglobin, an initial increase in Heinz bodies, a decrease in free SH groups in whole blood, prolongation of clotting times, and an increase in fibrinogen concentrations. Methemoglobin was not measured. Due to reporting deficiencies, the reliability of these data is uncertain.

Burns et al. (1994) assessed the immunotoxic response of female $B6C3F_1$ mice (3–16/group; usually 7–8/group) gavaged daily for 14 days with 0 (corn oil vehicle), 200, 400, or 600 mg/kg of *m*-nitrotoluene (purity not specified). On day 15, animals were evaluated for several immunological and hematological parameters and challenged with infectious or tumorigenic agents to assess host resistance. Mice were weighed on days 1, 8, and 15. Examination for gross pathology was conducted at necropsy. Histopathologic evaluations were performed on the brain, liver, thymus, spleen, lungs, kidneys, and lymph nodes. Dose-related increases in absolute and relative (organ-to-body or brain-weight ratio) liver and kidney weights (statistically significant relative to vehicle controls at 600 mg/kg) and decreases in absolute and relative thymus weight (significant relative to vehicle controls at 400 and 600 mg/kg) were noted. Histopathologic examination showed reversible, mild-to-moderate hepatocyte swelling in all treated groups. There was a statistically significant increase in total number and percentage of reticulocytes at 400 and 600 mg/kg; no alterations were reported for other hematological indices (erythrocyte and leukocyte numbers, hemoglobin, hematocrit, and mean corpuscular volume). The following statistically significant immunologic test results were reported: a dose-related decrease in the number of spleen IgM antibody-forming cells (≥400 mg/kg group; significant positive trend); a decreased response to the T-cell mitogens, phytohemagglutinin and Concavalin A (significant linear trend); a decrease in the stimulation indices for leukocyte response (data not shown); a decreased ability to produce a delayed hypersensitivity response to Keyhole limpet hemocyanin (600 mg/kg); an elevated serum complement levels (≥400 mg/kg); decreased percent of peritoneal lymphocytes (≥400 mg/kg); an increased number of chicken erythrocytes adhering to macrophages (all groups); and an increase in natural killer cell activity (600 mg/kg). Host resistance to *Listeria monocytogenes* was decreased, as indicated by a

dose-related increase in mortality (75–100%, statistically significant ≥200 mg/kg) relative to controls (13–58% depending on CFU density), but it was not altered when challenged with *Streptococcus pneumoniae*. A decrease in PYB6 fibrosarcoma incidence and a reduction in the number of B16F10 melanoma nodules (600 mg/kg) relative to controls was observed. While the alterations in host resistance to *Listeria monocytogenes*, decreased numbers of splenic IgM antibody-forming cells and changes in response to T-cell mitogens were statistically significant at ≥400 mg/kg-day, the statistically significant dose-related positive linear trends in the occurrence of these alterations suggested a biological effect at 200 mg/kg-day. Burns et al. (1995) suggested that the T-cell might be the primary cellular target. Therefore, the LOAEL for immunologic effects is 200 mg/kg-day for mice in this study.

Inhalation Exposure

No studies were located regarding the toxic effects of chronic or subchronic inhalation exposure to *m*-nitrotoluene in animals.

Other Studies

Acute and In Vitro Studies

Allenby et al. (1991) conducted *in vitro* and *in vivo* studies to evaluate the testicular toxicity of *m*-nitrotoluene. Adult male Sprague-Dawley rats (70 days old) were gavaged with single doses of 1,000, 1,500, or 2,000 mg/kg of *m*-nitrotoluene (98% purity) in corn oil; controls received the vehicle. All rats in the two higher-dose groups died. The 3 rats receiving 1,000 mg/kg were sacrificed at 1 or 3 days post-treatment. Their testes were weighed and their testicular interstitial fluid was collected for measurement of immunoactive inhibin. There was no effect on testes weight or spermatogenesis (data not shown) at 1 or 3 days post-treatment in animals receiving 1000 mg/kg.

Allenby et al. (1991) administered *m*-nitrotoluene *in vitro* to Sertoli cell-germ cell co-cultures, Sertoli cell cultures (from 28-day old rats), and seminiferous tubule cultures (from untreated adult SD male rats) at concentrations of 10^{-7}, 10^{-5}, or 10^{-3} moles/liter (M) for 24 hours. Immunoactive inhibin was measured in the culture media by radioimmunoassay. Addition of 10^{-3} M *m*-nitrotoluene to cultures of adult seminiferous tubule cells or immature Sertoli cells exerted a small stimulatory effect on the secretion of inhibin under basal conditions, i.e., without addition of FSH or cAMP, while lower doses did not.

In an *in vitro* study using Dorset sheep erythrocytes, French et al. (1995) reported that 2.5–10 M *m*-nitrotoluene led to a dose-dependent enhancement of methemoglobin formation both with and without a bioactivation system (NADP). Sabbioni (1994) demonstrated that *m*-nitrotoluene formed a hydrolyzable adduct with hemoglobin isolated from female Wistar rats treated 24 hours earlier.

Metabolism Studies

The comparative hepatic metabolism of nitroaromatic compounds, including mononitrotoluenes, has been extensively examined by Rickert and colleagues (DeBethizy and Rickert, 1984; Chism et al., 1984; Rickert et al., 1984; Chism and Rickert, 1985; Rickert et al., 1986; Rickert, 1987) in an effort to explain differential target organ toxicity. Of the 80% of ingested *m*-nitrotoluene (200 mg/kg) that was recovered, male F344 rats excreted 68% in the urine, including the following major metabolites:

- 3-nitrohippuric acid (24%)
- 3-nitrobenzoic acid (21%)
- 3-acetamidobenzoic acid (12%)

The remainder was excreted in the feces and expired air (Chism et al., 1984). These data indicated that *m*-nitrotoluene is hydroxylated at the methyl group first to the nitrobenzyl alcohol, which can subsequently undergo further metabolism via three possible pathways. These pathways include the following: (1) oxidization to nitrobenzoic acid, conjugation with glycine, and excretion into the urine; (2) conjugation with glucuronic acid, excretion into the gut via the bile, nitro reduction by the gut microflora to an aminobenzoic acid with subsequent resorption and further metabolism; (3) and conjugation with glutathione. Chism and Rickert (1985) postulated that nitro group reduction occurred in the intestinal contents after biliary excretion in rats. Excretion of 4–11% of a 200 mg/kg dose was in the bile after 12 hours elapsed; 28% of the bile-excreted materials in males and 16% in females was 3-nitrobenzyl glucuronide, indicating gender differences in the quantitative metabolism of *m*-nitrotoluene (Chism and Rickert, 1985; Doolittle et al., 1983). Bile duct cannulation decreased hepatic macromolecular covalent binding, particularly in the males (Chism and Rickert, 1985). In isolated rat hepatocytes, DeBethizy and Rickert (1984) demonstrated that *m*-nitrotoluene was metabolized via P450-dependent enzymatic processes to 3-nitrobenzoic acid (56%), the primary metabolite, via 3-nitrobenzyl alcohol (29%); 3-nitrobenzyl alcohol glucuronide was a minor metabolite (13%). *m*-Nitrotoluene has been shown to bind covalently with hepatic macromolecules, but not to DNA isolated from the rat livers (Rickert et al., 1984; Chism and Rickert, 1985). Whether the parent compound or the metabolite(s) are responsible for the systemic toxicity remains unclear.

Genotoxicity Studies

As summarized in review documents (U.S. EPA, 1986; IARC, 1996), genotoxicity results for *m*-nitrotoluene were primarily negative. All assays for reverse mutation in *Salmonella typhimurium* (strains TA92, TA94, TA98, TA100, TA1535, TA1537, and TA1538) yielded negative results, with or without metabolic activation. Without metabolic activation, assays for unscheduled DNA synthesis in cultured rat primary hepatocytes, pachytene spermatocytes or round spermatids were negative. No chromosomal aberrations were detected in cultured Chinese hamster ovary cells, with or without exogenous metabolic activation, or in cultured Chinese hamster liver cells without activation. According to the review documents, a single study reported positive results for sister chromatid exchanges in cultured Chinese hamster ovary cells without, but not with, metabolic activation. *In vivo* studies in male rats exposed orally to *m*-nitrotoluene reported negative results for unscheduled DNA synthesis in hepatocytes and reported binding to protein, but not to DNA, in the liver.

DERIVATION OF SUBCHRONIC ORAL p-RfD FOR *m*-NITROTOLUENE

The 13-week dietary studies in rats and mice provided the most comprehensive multiple-dose assessments of *m*-nitrotoluene toxicity via the oral exposure route (NTP, 1992; Dunnick et al., 1994). At the lowest dietary level 625/675 ppm (46 and 48 mg/kg-day in male and female rats, respectively), male rats exhibited transient decreased hematocrit, hemoglobin, and erythrocyte counts (Week 3 but not Week 13), and hyaline droplet nephropathy; female rats

showed a high incidence of hemosiderin pigment deposition in the spleen. The authors attributed the hyaline droplet nephropathy in male rats to alpha-$_{2u}$ globulin accumulation, which does not occur in humans and, therefore, is not an appropriate endpoint for evaluation of systemic toxicity in humans (U.S. EPA, 1991b). The alterations in hematologic parameters in the male rats were consistent with hemoconcentration at Week 1, followed by the slow onset of mild regenerative anemia. The NTP (1992) postulated that the mechanism for these hematologic alterations consisted of oxidative damage to hemoglobin that led to Heinz body formation and decreased erythrocyte survival. The observations of hemosiderin deposition, spleen congestion, and increased methemoglobin generally are consistent with this mechanism.

In the female rats, minimal-to-mild hemosiderin deposition, an indicator of damage to erythrocytes, was observed at comparable severity throughout the range of *m*-toluene doses (48–638 mg/kg-day). NTP (1992) considered this effect to be minimally adverse at the lower dose levels because additional signs of hematological effects, including decreases in erythrocytes, hemoglobin, and hematocrit; splenic congestion; and increased methemoglobin were seen only at the highest 2 dose levels (336 and 638 mg/kg-day). The minimal hemosiderin deposition at the lowest dose levels was considered a sensitive indicator of effects at the higher dose levels.

Splenic hemosiderin deposition may be a result of extravascular hemolysis within the spleen, explaining why hematological parameters were not affected at lower doses in female rats. In the absence of a species-specific mechanism for the hemolytic substance, hemosiderosis and its related findings in experimental animals can be considered predictive for spleen toxicity in humans, and representative of irreversible adverse effects (Muller et al., 2006).

In mice, there were no significant treatment-related histopathological lesions in the spleen or other tissues, and hematological endpoints were not evaluated. The only treatment-related changes were decreases in body weight in the 2 high dose groups (≥743 mg/kg-day) of both genders and significant increases in relative liver weight in all treated groups of male and female mice (≥114 mg/kg-day in males and ≥139 mg/kg-day in females). Increased liver weights, in the absence of histopathology at any dose, suggested this response was physiologically adaptive rather than adverse.

Identification of the blood and spleen as sensitive targets of *m*-nitrotoluene toxicity (NTP,1992; Dunnick et al., 1994) in rats was supported by the limited available database. Subchronic single-dose-level studies in rats reported splenic lesions at 214 mg/kg-day (Ciss et al., 1980) and hematological alterations at 318 mg/kg-day (Kovalenko, 1973; Vasilenko and Kovalenko, 1975), although the reliability of these data are uncertain due to inadequate reporting of methods and results. Identification of the hematopoietic system as a target organ is somewhat supported by the limited *in vitro* studies that showed *m*-nitrotoluene-hemoglobin adduct formation and methemoglobinemia. However, it should be noted that, while hemoglobin adduction is commonly used as a biomarker of internal dose, it is not necessarily a sign of hemotoxicity. NTP (1992) also reported methemoglobinemia at higher doses in rats.

Based on available repeated-dose oral studies, the subchronic LOAEL of 46–48 mg/kg-day for hematological effects, including hemosiderin deposition in the spleen of female rats identified in the NTP (1992) study, was considered a potential point of departure for

deriving subchronic and chronic p-RfDs for *m*-nitrotoluene. Hematological changes in low-dose male rats were slight but statistically significant during treatment Week 3, but not at treatment end (Week 13). Although significant changes in hematological parameters indicative of anemia were seen only at the highest two dose levels in female rats, hemosiderin deposition was observed in the spleen of 80–100% of the female rats at all dose levels. Such hemosiderin deposition typically results from RBC damage and eventually would lead to anemia and spleen necrosis.

Benchmark Dose (BMD) Software (U.S. EPA, 2000) v.1.4.1b methods were applied to the NTP (1992) data for incidence of spleen hemosiderin deposition in female rats exposed to dietary *m*-nitrotoluene for 13 weeks. Appendix B provides the BMD curves and summary data. Because the lowest dose (48 mg/kg-day) resulted in 90% incidence and the next higher dose (87 mg/kg-day) resulted in 100% incidence, only data for these treated groups and controls were used in the BMD calculations. Four of the six BMD models that demonstrated acceptable fit, calculated a $BMDL_{10}$ of 1.1 mg/kg-day (see Table B-1). This BMDL of 1.1 mg/kg-day was chosen as the point of departure (POD) for deriving the p-RfD, because it represents a more sensitive endpoint than the LOAEL of 46 mg/kg-day from the same data.

A composite uncertainty factor (UF) of 1,000, composed of the following, was applied to calculate the subchronic p-RfD:

- An UF of 10 for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans
- An UF of 10 for intraspecies differences to account for potentially susceptible individuals, in the absence of information on the variability of response in humans
- An UF of 10 for uncertainty in the database. The database included comprehensive 13-week assessments for *m*-nitrotoluene toxicity in two orally exposed animal species, including toxicity to reproductive organs and tissues (NTP, 1992; Dunnick et al., 1994; Ciss et al., 1980). However, adequate developmental toxicity and multigenerational reproductive toxicity studies were lacking. Although no explicit reproductive studies were identified in the literature, the changes in estrous cycle observed in rats indicated that *m*-nitrotoluene could potentially affect fertility. Also, although the Ciss et al. (1980) study was lacking in many details of experimental design and results necessary to determine a NOAEL or useful LOAEL, it did present developmental effects for pups from treated dams (i.e., microscopic splenic alternations). The fact that none of these studies demonstrated a NOAEL for oral m-nitrotoluene and the NTP (1992) LOAEL dose resulted in a 90% incidence of the critical effect suggested that repeated studies at lower doses might identify a lower POD. Thus, the full database UF of 10 was applied.

Subchronic p-RfD = BMDL ÷ UF
= (1.1 mg/kg-day) ÷ 1,000 = 0.0011 mg/kg-day
= **1×10^{-3} mg/kg-day**

Confidence in the key study is medium because, although the study was well conducted and reported, and included both genders of two species, a NOAEL was not identified in the rats and a postulated target system (hematopoietic) was not evaluated in the mice. Confidence in the database is low-to-medium because supporting subchronic studies were of uncertain reliability,

and adequate developmental toxicity and multigeneration reproductive toxicity studies were missing. Overall, confidence in the subchronic p-RfD value is low-to-medium.

Derivation of a chronic p-RfD was attempted. However, the additional UF of 10 for use of subchronic data resulted in a composite UF of 10,000 and no supportable rationale for reducing the UF was identified. Consequently, no chronic oral toxicity value is derived. However, Appendix A of this document contains a "screening value" that might be useful in certain instances. Please see the attached Appendix for details.

FEASIBILITY OF DERIVING SUBCHRONIC AND CHRONIC INHALATION p-RfCs FOR *m*-NITROTOLUENE

Subchronic and chronic p-RfC values could not be derived for *m*-nitrotoluene due to inadequate human data, the lack of subchronic or chronic inhalation toxicity data for animals, and insufficient toxicokinetic data to inform inter-route extrapolation.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR *m*-NITROTOLUENE

Weight-of-Evidence Descriptor

No human or animal data were available regarding the carcinogenicity of *m*-nitrotoluene. Available genotoxicity results for *m*-nitrotoluene were primarily negative. Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the descriptor, “inadequate information to assess carcinogenic potential” is assigned to *m*-nitrotoluene.

Quantitative Estimates of Carcinogenic Risk

The lack of data precludes the derivation of quantitative estimates of cancer risk following exposure to *m*-nitrotoluene.

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APPENDIX A. DERIVATION OF A SCREENING CHRONIC RfD FOR *m*-NITROTOLUENE

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

The 13-week $BMDL_{10}$ of 1.1 mg/kg-day (see Table B-1) for incidence of spleen hemosiderin deposition in female rats, reported by NTP (1992; Dunnick et al., 1994), was selected as the POD and a composite uncertainty factor (UF) of 10,000, composed of the following, was applied to derive a chronic screening RfD:

- An UF of 10 for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between rats and humans
- An UF of 10 for human variability to account for potentially susceptible individuals, in the absence of information on the variability of response in humans
- An UF of 10 for use of a subchronic (13-week) POD to derive a chronic toxicity value
- An UF of 10 for uncertainty in the database. The database included comprehensive 13-week assessments for *m*-nitrotoluene toxicity in two orally exposed animal species, including toxicity to reproductive organs and tissues (NTP, 1992; Dunnick et al., 1994; Ciss et al., 1980). However, adequate developmental toxicity and multigenerational reproductive toxicity studies were lacking. Although no explicit reproductive studies were identified in the literature, the changes in estrous cycle observed in rats indicated that *m*-nitrotoluene could potentially affect fertility. Also, although the Ciss et al. (1980) study was lacking in many details of experimental design and results necessary to determine a NOAEL or useful LOAEL, it did present developmental effects for pups from treated dams (i.e., microscopic splenic alternations). The fact that none of these studies demonstrated a NOAEL value for oral m-nitrotoluene and the NTP (1992) LOAEL dose resulted in a 90% incidence of the critical effect suggested that repeated studies at lower doses might identify a lower POD. Thus, the full database UF of 10 was applied.

Chronic screening RfD = BMDL ÷ UF
= 1.1 mg/kg-day ÷ 10,000 = 0.00011 mg/kg-day
= **1×10^{-4} mg/kg-day**

Confidence in the key study is medium because, although the study was well conducted and reported, and included both genders of two species, a NOAEL was not identified in the rats and a postulated target system (hematopoietic) was not evaluated in the mice. Confidence in the database is low-to-medium because supporting subchronic studies were of uncertain reliability, and adequate developmental toxicity and multigeneration reproductive toxicity studies were missing. Overall, confidence in the chronic screening p-RfD value is low-to-medium.

APPENDIX B. BENCHMARK DOSE MODELING

Table B-1. Benchmark Dose Modeling Summary Data, Sorted by Akaike Information Criterion (AIC), for Incidence of Spleen Hemosiderin Deposition in Female Rats Fed *m*-Nitrotoluene, Using Data for the Two Lowest Doses and Controls[a]

	P	Chi^2	AIC	BMD (mg/kg-day)	BMDL (mg/kg-day)	BMDU (mg/kg-day)
Log-logistic	**0.9960**	0	17.0034	37.8491	- - - - - -	
Gamma	**0.9928**	**0**	**17.0035**	**26.4152**	**1.14956**	
Probit	**0.9506**	0	17.0108	7.55406	4.05126	
Multistage	**0.9353**	**0.01**	**17.0161**	**10.4665**	**1.14771**	**14.8886**
Log-normal	**0.8600**	0.03	17.0619	8.14094	3.72076	
Quantal Linear & Multistage (1-degree poly)	**0.6847**	**0.16**	**17.2789**	**2.09725**	**1.11095**	**3.85887**
Log-Probit	NA	0	19.0033	34.232	- - - - - -	
Weibull	**NA**	**0**	**19.0033**	**19.6295**	**1.14958**	
Multi-stage, low dose only	NA	0	17.0033	2.30168	1.14867	4.9894

[a]LOAEL = 48 mg/m^3, 9/10 incidence; Controls: 1/10 incidence

BMD curves for m-Nitrotoluene female rat hemosiderin data

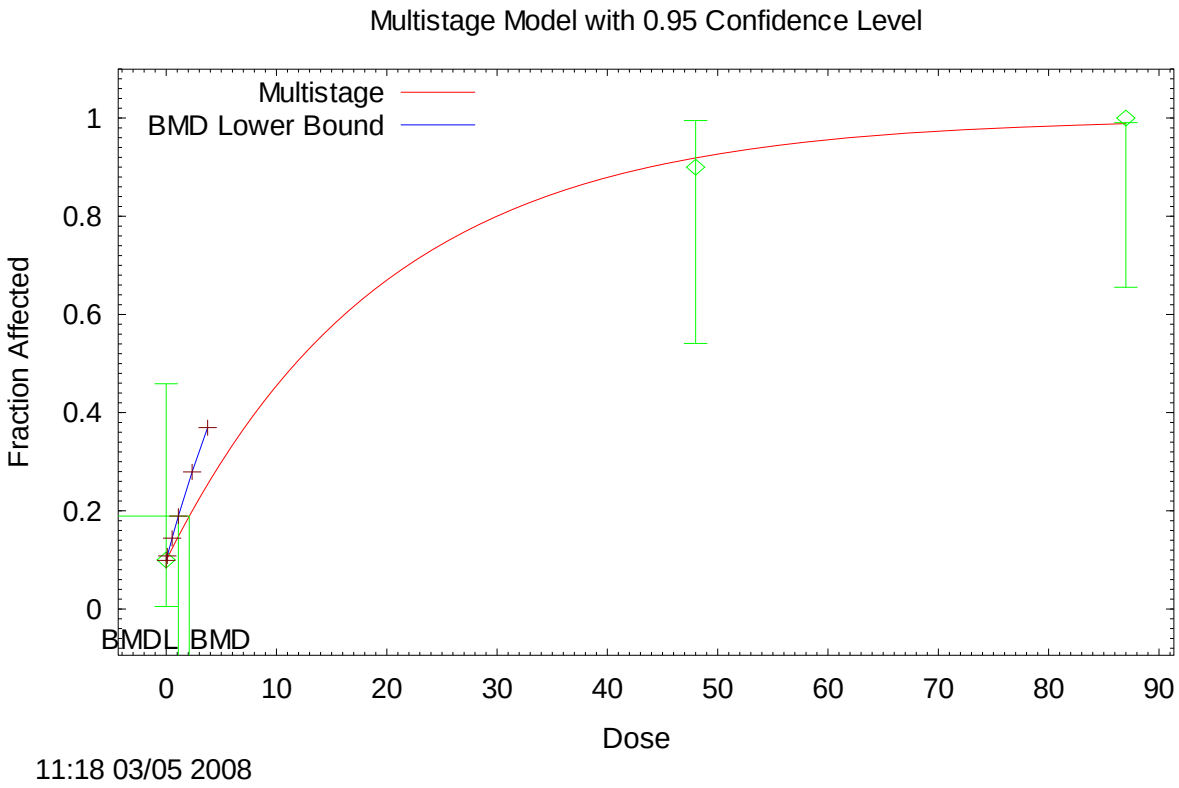

Figure B-1. BMD Curve for Multistage Model (noncancer) 1-Degree Poly and Quantal-Linear (BMDL 1.1)

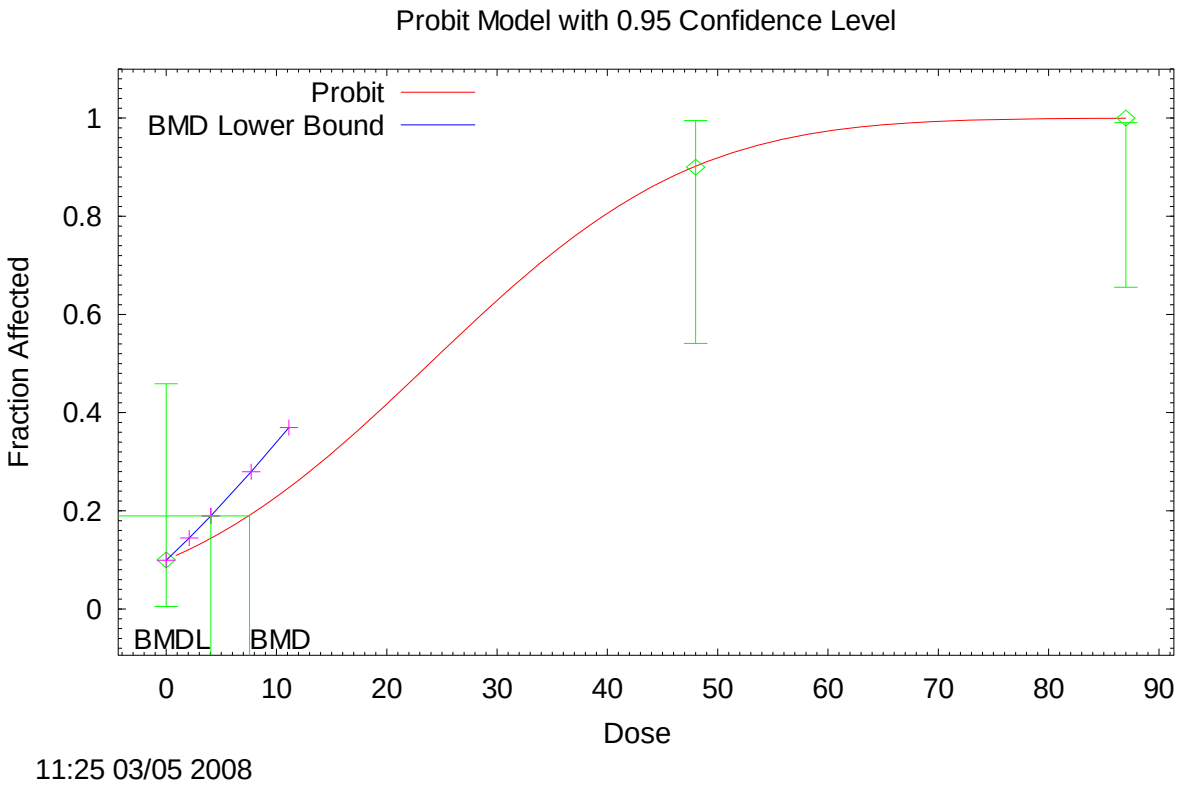

Figure B-2. BMD Curve for Probit Model (BMDL 4.0)

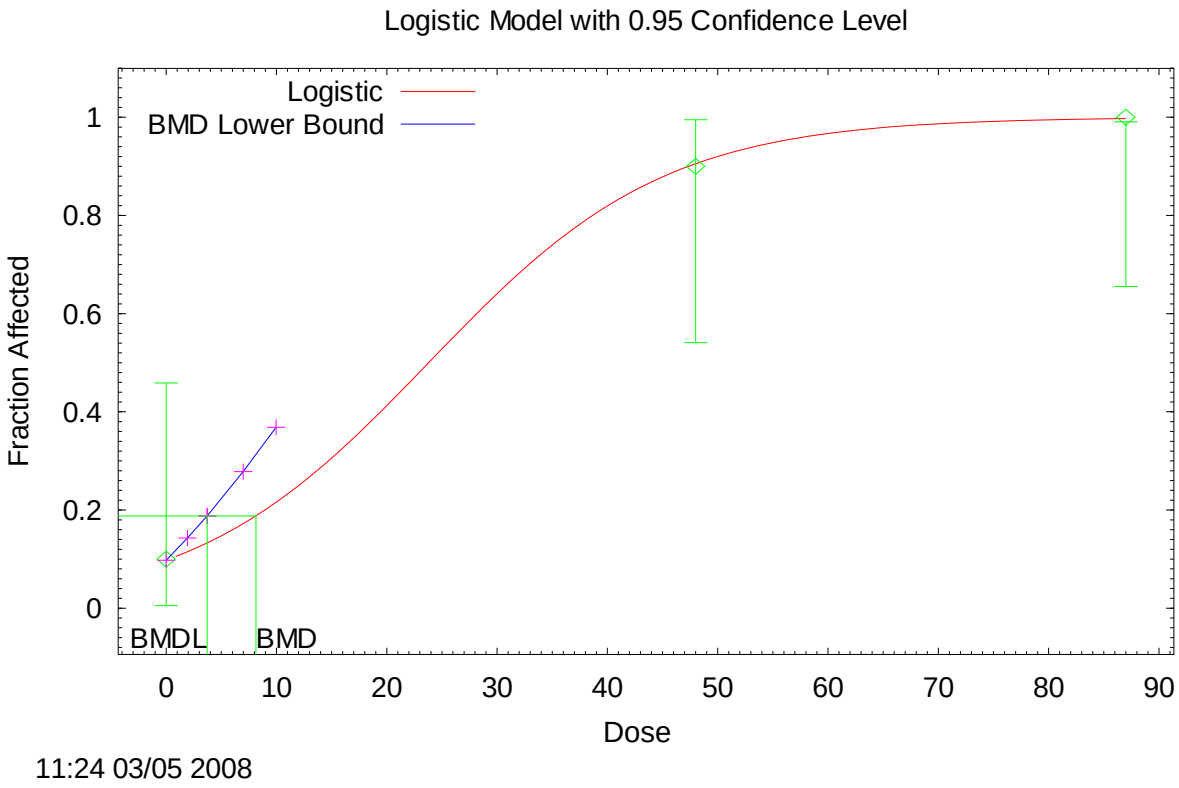

Figure B-3. BMD Curve for Logistic Model (BMDL 3.7)

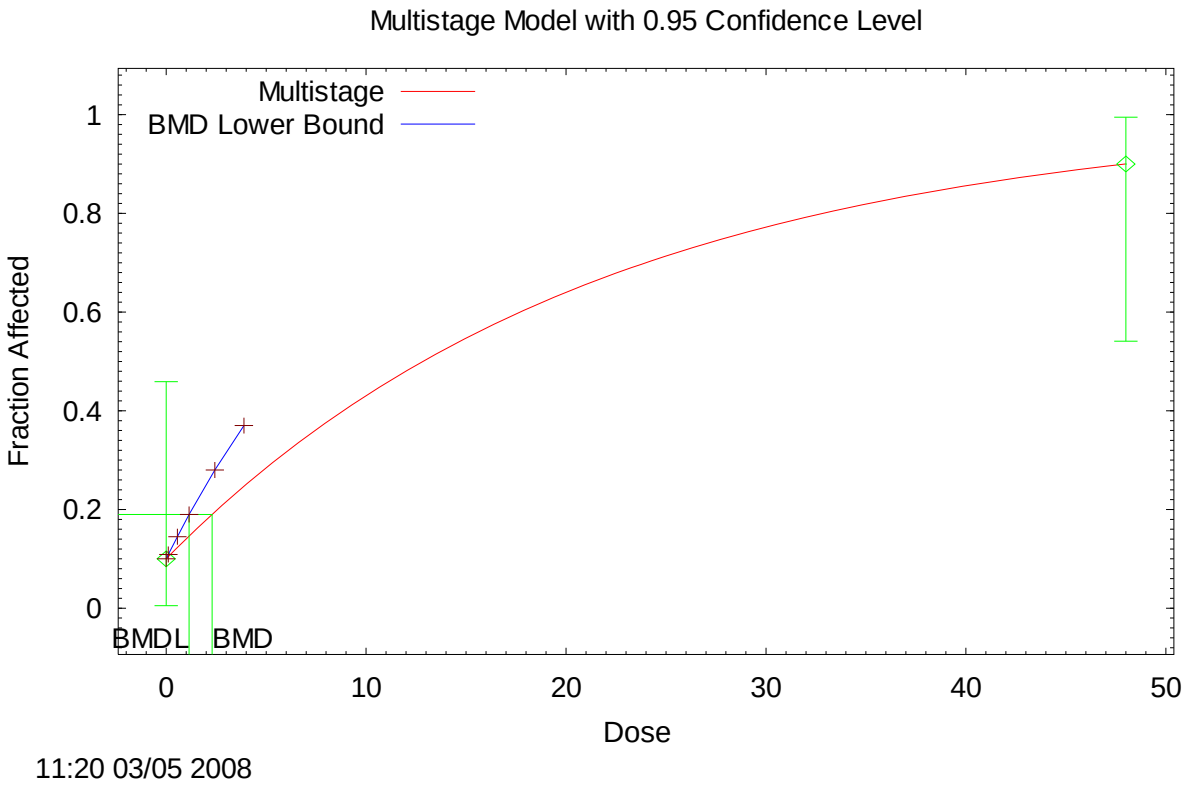

Figure B-4. BMD Curve for Multistage Model (noncancer) Low Dose Only (1-Degree Poly)
(BMDL 1.1)

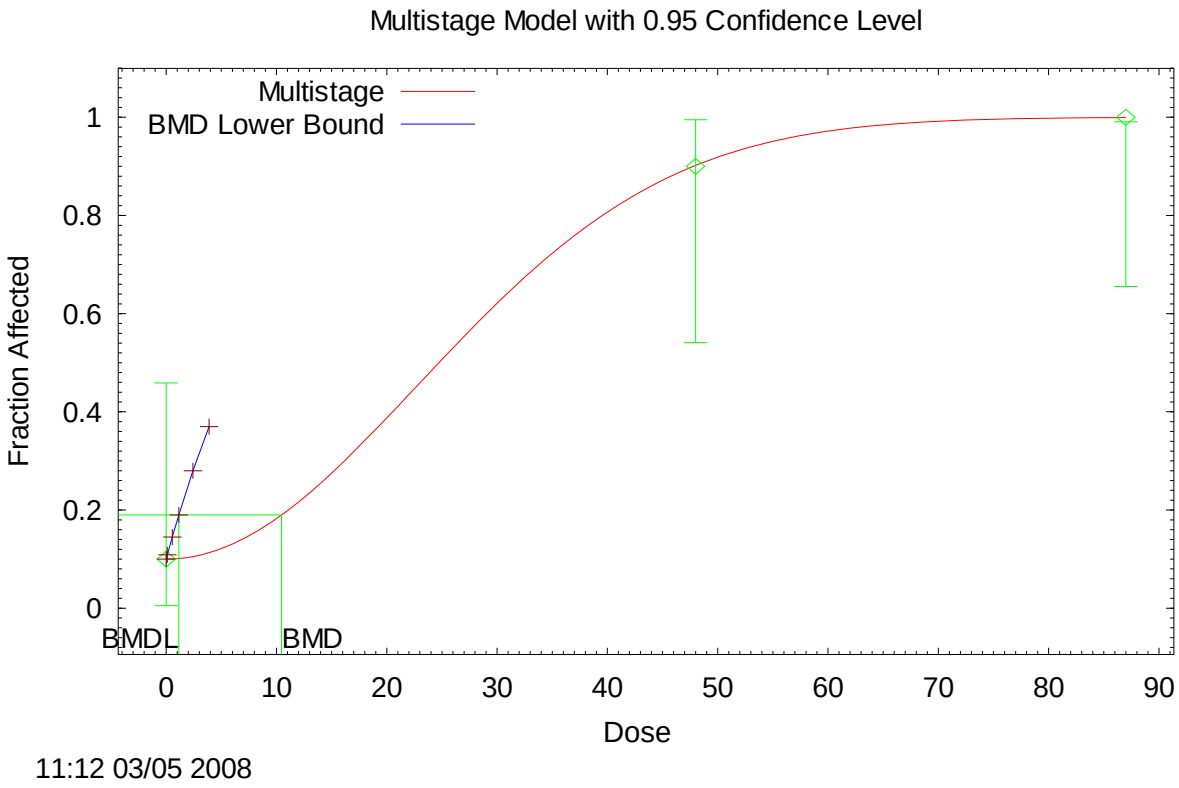

Figure B-5. BMD Curve for Multistage Model (noncancer) 2-Degree Poly (BMDL 1.1)

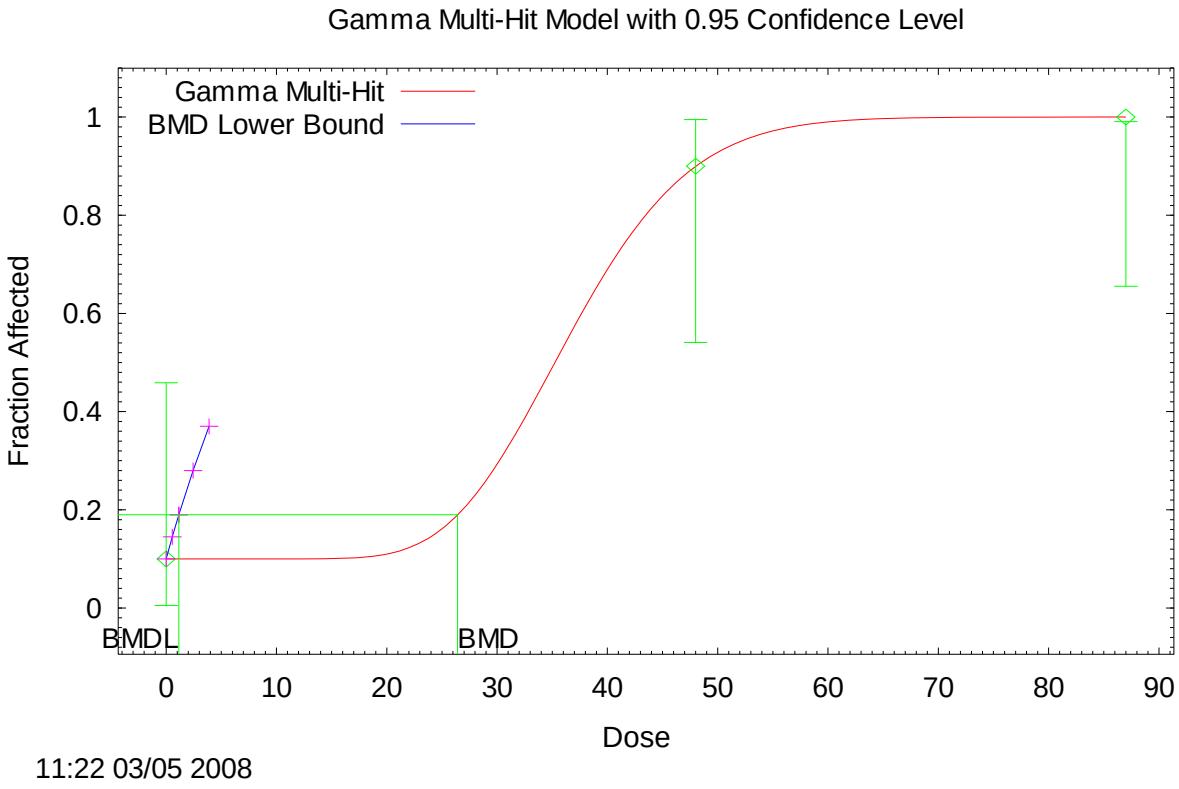

Figure B-6. BMD Curve for Gamma Model (BMDL 1.1)

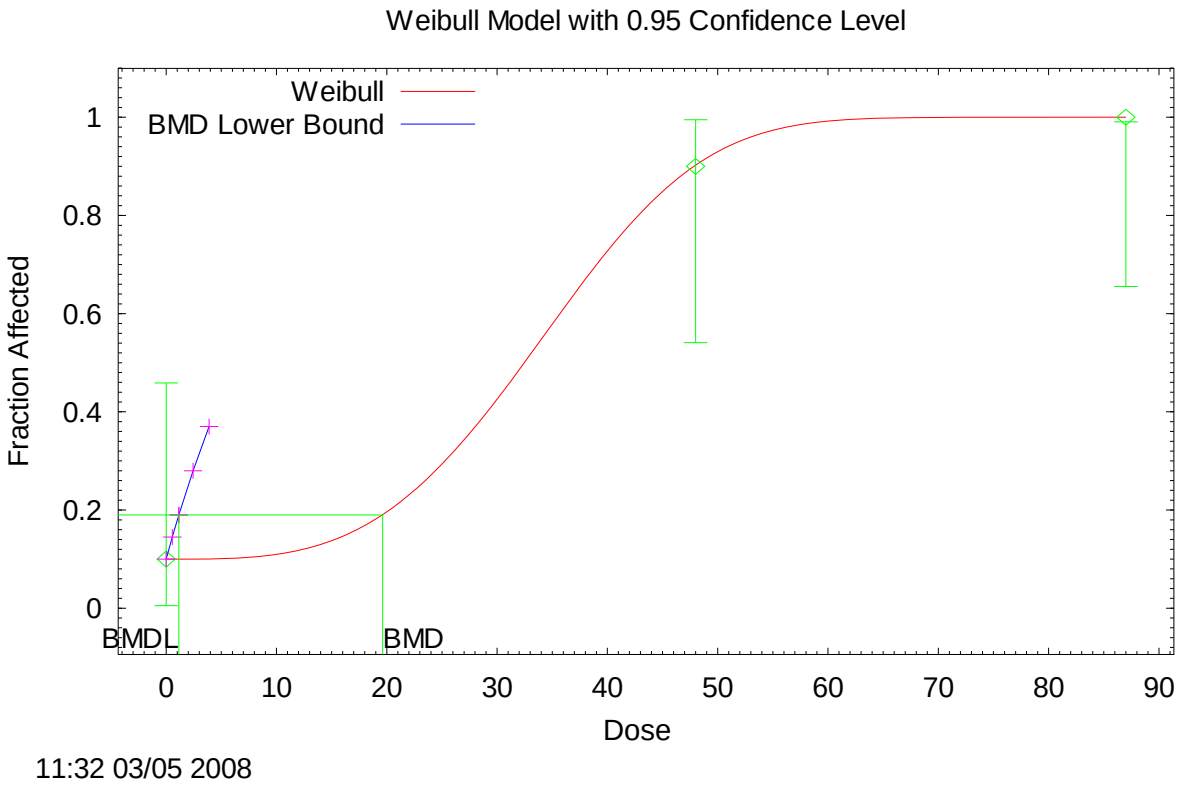

Figure B-7. BMD Curve for Weibull Model (BMDL 1.1)