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Provisional Peer-Reviewed Toxicity Values for

2-Methyl-5-Nitroaniline
(CASRN 99-55-8)

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Questions regarding the contents of this document may be directed to the U.S. EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center (513-569-7300).

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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMD	benchmark dose
BMCL	benchmark concentration lower bound 95% confidence interval
BMDL	benchmark dose lower bound 95% confidence interval
HEC	human equivalent concentration
HED	human equivalent dose
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 reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
POD	point of departure
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR 2-METHYL-5-NITROANILINE (CASRN 99-55-8)

BACKGROUND

HISTORY

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

1) EPA's Integrated Risk Information System (IRIS)
2) Provisional Peer-Reviewed Toxicity Values (PPRTVs) 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 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 a panel of six EPA scientists and external peer review by three independently selected scientific experts. PPRTVs differ from IRIS values in that PPRTVs do not receive the multiprogram consensus review provided for IRIS values. This is because IRIS values are generally intended to be used in all EPA programs, while PPRTVs are developed specifically for the Superfund Program.

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

DISCLAIMERS

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

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

2-Methyl-5-nitroaniline (CASRN 99-55-8) is an intermediate compound in the synthesis of a wide range of azo dyes and is an in vivo metabolic product of 2,4-dinitrotoluene. The empirical formula for 2-methyl-5-nitroaniline is $C_7H_8N_2O_2$, and its structure is shown in Figure 1. Synonyms for 2-methyl-5-nitroaniline include 5-nitro-*o*-toluidine, 4-nitro-2-aminotoluene, 2-amino-4-nitrotoluene, 6-methyl-3-nitro-aniline, and 2-methyl-5-nitro-benzenamine.

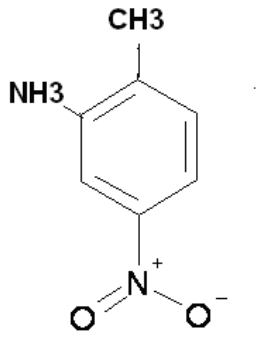

Figure 1. Chemical Structure of 2-Methyl 5-nitroaniline

No reference dose (RfD), reference concentration (RfC), or cancer assessments for 2-methyl-5-nitroaniline are included in the EPA's IRIS database (U.S. EPA, 2009a) nor on the Drinking Water Standards and Health Advisories List (U.S. EPA, 2009b). No acute exposure guideline levels (AEGLs) for 2-methyl-5-nitroaniline have been derived by the EPA's Office of Pollution Prevention and Toxics (U.S. EPA, 2009c).

In 1987, the EPA published a Health and Environmental Effects Profile (HEEP) for 2-methyl-5-nitroaniline and evaluated it as a carcinogen. The human carcinogen potency factor (q_1*) for 2-methyl-5-nitroaniline is 0.033 $(mg/kg\text{-}day)^{-1}$ for oral exposure (U.S. EPA, 1987). In addition, the Chemical Assessments and Related Activities (CARA) lists a Reportable Quantity Carcinogenicity document available for 2-methyl-5-nitroaniline (U.S. EPA, 1988a, 1994). The EPA's HEAST lists an oral unit risk for 2-methyl-5-nitroaniline of 9.4×10^{-7} $(\mu g/L)^{-1}$ based on mouse liver carcinomas and classifies 2-methyl-5-nitroaniline as a Group C carcinogen (*Possibly Carcinogenic to Humans*: agents with limited animal evidence, and little or no human data) (U.S. EPA, 2011).

The International Agency for Research on Cancer (IARC) reviewed the carcinogenic potential of 2-methyl-5-nitroaniline and noted that there was limited evidence for its carcinogenicity in experimental animals and classified 2-methyl-5-nitroaniline as *Not Classifiable as to its Carcinogenicity to Humans* (Group 3) (IARC, 1990).

In 1997, CalEPA prepared a preliminary evaluation of carcinogenicity and exposure data for 2-methyl-5-nitroaniline. It did not place the chemical on the Proposition 65 list but noted it as a 'medium high' level of carcinogenic concern (CalEPA, 1997a,b). In 2009, the Carcinogen Identification Committee recommended that the compound be placed on the 'low' priority list (CalEPA, 2009a,b). CalEPA has not derived quantitative estimates of the carcinogenic potential of 2-methyl-5-nitroaniline (CalEPA, 2008, 2009c,d, 2011).

2-Methyl-5-nitroaniline has not included in the *11th Report on Carcinogens* (NTP, 2005). The toxicity of 2-methyl-5-nitroaniline has not been reviewed by ATSDR (2009) nor the World Health Organization (WHO, 2009).

The American Conference of Governmental Industrial Hygienists (ACGIH, 2009) has classified the chemical in Group A3 (*Confirmed Animal Carcinogen with Unknown Relevance to Humans*) (HSDB, 2009), and a threshold limit value of 1 mg/m^3 is listed. An occupational exposure limit for 2-methyl-5-nitroaniline has not been derived by the National Institute of Occupational Safety and Health (NIOSH, 2009) nor the Occupational Safety and Health Administration (OSHA, 2009).

Genetic toxicity studies for 2-methyl-5-nitroaniline indicate generally positive results in reverse-mutation assays in *Salmonella typhimurium* and *Escherichia coli,* and in Syrian hamster embryo (SHE) cell transformation assays (Couch et al., 1987; Dunkel et al., 1985; Kerckaert et al., 1998); the compound is weaker in potency than other 2,4-dinitrotoluene metabolites (Mori et al., 1982; Sayama et al., 1991).

A literature search was conducted through October, 2010, for studies relevant to the derivation of provisional toxicity values for 2-methyl-5-nitroaniline (CAS No. 99-55-8) using EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications

available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, Multidatabase Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI; and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); WHO; and Worldwide Science. The following databases outside of HERO were searched for toxicity information: ACGIH, ATSDR, CalEPA, EPA IRIS, EPA HEAST, EPA HEEP, EPA OW, EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

In this document, the word significant means "statistically significant witha *p*-value of <0.05." If the *p*-value is different, then the correct *p*-value is stated.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 1 provides information for all of the potentially relevant toxicity studies. Entries for the principal studies are bolded and identified by the marking "PS."

Table 1. Summary of Potentially Relevant Data for 2-Methyl-5-Nitroaniline (CASRN 99-55-8)

Notes[a]	Category	Number of Male/Female Species, Study Type, and Duration	Dosimetry[b]	Critical effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b, c]	Reference (Comments)
Human								
			1. Oral					
		Human Occupational Exposures	None	Hepatic Failures	None	Not Run	None	Shimuzu et al. (2002)
			2. Inhalation					
		Human Occupational Exposure	None	Hepatic Injury	None	Not run	None	Shimuzu et al. (2002)
Animal								
			1. Oral					
	Subchronic	5 rats/gender/grp, oral, 7 d/wk, 3 wks	0, 9, 19, 37, 150 mg/kg-day (males); 0, 10.2, 21, 42, 79, 169 mg/kg-day (females)	Mortality in all dose groups; severe weight reduction in all dose groups	Not identified	Not run	Not identified	NCI (1978); dose-finding study; insufficient data to derive a p-RfD
		5 rats/gender/grp, oral, 7 d/wk, 4 wks followed by 2 wks of observation	0, 9, 19, 37, 150 mg/kg-day (males); 0, 10.2, 21, 42, 79, 169 mg/kg-day (females)	Decreased body weights observed; no other details reported	Not identified	Not run	Not identified	NCI (1978); dose-finding study; insufficient data to derive a p-RfD
		5 mice/gender/grp, oral, 7 d/wk, 4 wks followed by 2 wks of observation	0, 16.2, 34.3, 66.7, 128.7 mg/kg-day (males); 0, 17.6, 37.1, 72.2, 136.61 mg/kg-day (females)	Decreased body weights observed; no other details reported	Not identified	Not run	Not identified	NCI (1978); dose-finding study; insufficient data to derive a p-RfD

Table 1. Summary of Potentially Relevant Data for 2-Methyl-5-Nitroaniline (CASRN 99-55-8)

Notes[a]	Category	Number of Male/Female Species, Study Type, and Duration	Dosimetry[b]	Critical effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b, c]	Reference (Comments)
	Chronic	50 rats/gender/grp, oral, 7 d/wk, 78 wks with additional 20 wks of observation	0, 3.9, 7.9 mg/kg-day for (males); 0, 4.6, 9.2 mg/kg-day for (females)	No treatment-related effects in male and female rats	7.9 mg/kg-day for males 9.2 mg/kg-day for females	Not run	Not identified; NOAEL was highest dose tested	NCI (1978)
PS		50 mice/gender/grp, oral, 7 d/wk, 78 wks with additional 20 wks of observation	0, 206, 395 mg/kg-day for (males); 0, 207 and 397 mg/kg-day for (females)	Visual inspection of graphical presentation of data showed toxicologically significant effects (>20% relative to controls) in low- and high-dose females	None	Not run	207 mg/kg-day	NCI (1978); visual inspection noted significant body-weight depression in female rats from at least Week 40 onward, with no compensatory weight gain following treatment termination
	Carcinogenic	50 rats/gender/grp, oral, 7 d/wk, 78 wks with additional 20-wks of observation	0, 0.77, 1.6 mg/kg-day (males); 0, 0.79, 1.6 mg/kg-day (females)None	No statistically significant effects in either males or females; low incidence of hepatocellular carcinomas observed in high-dose males relative to concurrent controls; historical control data not presented	Not identified	Not run	Not identified	NCI (1978); due to insufficient data on nonneoplastic effects, a NOAEL or LOAEL in rats is not identified

Table 1. Summary of Potentially Relevant Data for 2-Methyl-5-Nitroaniline (CASRN 99-55-8)

Notes[a]	Category	Number of Male/Female Species, Study Type, and Duration	Dosimetry[b]	Critical effects	NOAEL[b]	BMDL/ BMCL[b]	LOAEL[b, c]	Reference (Comments)
PS		50 mice/gender/grp, oral, 7 d/wk, 78 wks with additional 20 wks of observation 50 rats/gender/grp, oral, 7 d/wk, 78 wks with additional 20 wks of observation	0, 25, 48 mg/kg-day (males); 0, 25, 47 mg/kg-day (females)0, 0.77, 1.6 mg/kg-day (males); 0, 0.79, 1.6 mg/kg-day (females)	Statistically dose-related increase in incidence of hepatocellular carcinomas in males and females; insignificant increases in combined hemangiomas and hemangiosarcomas in males and in hemangiosarcomas in females considered by authors to be treatment-related because of rarity of occurrence of these tumor types; tumors were scattered throughout the body at various sites. No toxicologically significant effects in either males or females; low incidence of hepatocellular carcinomas observed in high-dose males relative to concurrent controls; historical control data not presented	Not identified	Male BMDL = 10.08 Female BMDL = 10.75	Not identified	NCI (1978); benchmark dose (BMD) modeling performed for incidence of hepatocellular carcinomas in male and female mice NCI (1978); due to insufficient data on nonneoplastic effects, a NOAEL or LOAEL in rats not identified
2. Inhalation								
None								

[a]Notes: IRIS = Utilized by IRIS, date of last update; PS = Principal study, NPR = Not peer reviewed.
[b]Dosimetry, NOAEL, BMDL/BMCL, and LOAEL values are converted to human equivalent dose (HED in mg/kg-day).
[c]Not reported by the study author but determined from data.

HUMAN STUDIES

Oral Exposure

Shimuzu et al. (2002) reported on eight historical cases of 2-methyl-5-nitroaniline poisoning in Tokyo and Osaka in 1946. In the Tokyo incidents, at least six people who had accidentally ingested 2-methyl-5-nitroaniline as a sweetening agent died of liver failure. No quantitative estimates of fatal doses were available. Symptoms included high fever, nausea, vomiting, liver swelling, jaundice, and "bleeding tendencies." Three of the six cases presented with fulminant hepatic failure; pathological findings during autopsy included liver atrophy, centrilobular necrosis, formation of pseudo bile ducts, and thrombosis associated with endothelial cell injury. In one case, deposition of azo pigment in the stomach and liver was reported. In the Osaka poisonings, two patients ingested small quantities of 2-methyl-5-nitroaniline repeatedly over several weeks. Daily intake was reported as 500 mg over 20 days and 80 mg over 25 days. The patients survived these dosing regimes, and no reports of symptomatology were provided.

Methemoglobinemia has been reported to be a major toxic effect of excessive oral exposure to 2-methyl-5-nitroaniline (Hamblin, 1967 as cited in NCI, 1978). Symptoms of exposure have included bluish lips and/or fingernails, headache, nausea, and fatigue (Hamblin, 1967).

Inhalation Exposure

Shimuzu et al. (2002) discussed one historical case of occupational liver injury resulting from occupational exposure for 3 months to both *o*-toluidine and 2-methyl-5-nitroaniline in Osaka in 1976. The exposed worker developed fulminant hepatic failure. Air concentrations in the workplace were reported as 0.23–6.8 mg/m^3 *o*-toluidine, and 0.53 mg/m^3 2-methyl-5-nitroaniline, which was considered by authors to be a trace concentration. Dermal absorption of the chemicals was suspected as well.

In another occupational study, Shimuzu et al. (2002) investigated the association between liver injury and exposure to 2-methyl-5-nitroaniline, used as a raw material for the production of hair dyes, in a cohort of 15 workers. The factory had begun production of dyes using 2-methyl-5-nitroaniline approximately 18 days before the first patient developed symptoms. Four workers presented to physicians with multiple symptoms, and three were hospitalized immediately. Exposure was assessed by determining the frequency and duration of employee handling of 2-methyl-5-nitroaniline during the performance of daily work activities. For health evaluation, blood and urine were sampled from all workers. Hematology, clinical chemistry, and urinalysis were conducted immediately after specimen collection. The following endpoints were evaluated from blood in all subjects: aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin (Tbil), albumin (ALB), total cholesterol (TC), Hepatitis B antigen, and Hepatitis A and C antibodies. The three hospitalized patients were also assessed for prothrombin time (PT) and cytomegalovirus and Epstein-Barr virus antibodies. Liver biopsies were performed on all hospitalized patients at 1 month following symptoms onset, and liver morphology was monitored by ultrasonography in all subjects with abnormal liver function. Data on age, medical history, alcohol use, and prior liver function test results were also obtained for study participants.

All six habitually exposed workers showed clinical and biochemical signs of liver toxicity, which appeared about 2 weeks following initial exposures to 2-methyl-5-nitroaniline. Symptoms in these principally exposed workers included fatigue, loss of appetite, and upper abdominal discomfort. In addition, four workers (three of which were hospitalized) had clinical signs of illness and severely elevated liver enzymes indicative of liver dysfunction; two experienced high fever and joint pain, and three patients observed dark urine. Three of the remaining 11 asymptomatic workers, two of which were principal workers and one a substitute worker, were tested and found to have significantly elevated serum liver enzyme concentrations relative to normal ranges and were diagnosed with liver injury. Neither clinical signs nor serum abnormalities were found in the remaining eight workers. No differences in age, medical history, alcohol use, and serum markers for viral function or liver function were noted between the two groups.

No quantitative estimates of exposure were given in the occupational study (Shimuzu et al., 2002). Six of 15 workers employed at the factory were engaged in the manufacturing processes, which involved scooping 2-methyl-5-nitroaniline from barrels into machines where it was mixed with sulfuric acid. The remaining nine employees sometimes substituted for the most highly exposed workers in performing these tasks. The duration of exposure periods usually ranged from 4–5 hours. The frequency of handling the compound was about 6- to 12-fold higher in habitually exposed workers as compared to occasionally exposed workers; principal workers had 12 to 20 exposures, while backup workers had 1–3 exposures, respectively. Among affected workers, there was some evidence of a dose response as the severity of liver dysfunction increased with increasing frequency of handling 2-methyl-5-nitroaniline ($p < 0.01$). The study authors postulated that 15 hours of minimum exposure duration were capable of causing liver dysfunction under those working conditions. Following closure of the work site, all affected workers eventually recovered.

ANIMAL STUDIES

Oral Exposure

Subchronic-duration Studies

There is only one useful study on the health effects of oral exposure. This study (NCI, 1978) includes two initial range-finding experiments, and chronic-duration studies where oral carcinogenicity was studied in Fischer 344 (F344) rats and B6C3F1 mice. Range-finding studies were initially performed to provide a scientifically-defensible rationale for dose selection in the 2-year chronic-duration bioassays. In the first phase of the range-finding studies, 2-methyl-5-nitroaniline (purity unspecified) was administered in the diet to F344 rats (5/gender/group) at concentrations of 0, 0.009, 0.019, 0.037, 0.07, or 0.15%, 7 days a week, for 3 weeks. The adjusted doses would be 0, 9, 19, 37, 150 mg/kg-day (males); 0, 10.2, 21, 42, 79, 169 mg/kg-day (females).

Mortality was reported to occur in all dose groups, and severe weight reductions were observed in all dose groups relative to controls in the first 3 weeks. No further details of adverse effects were given. In the second phase, F344 rats and B6C3F1 mice (5/gender/species/group) were fed diets containing 2-methyl-5-nitroaniline at concentrations of 0, 0.009, 0.019, 0.037, and 0.07%, 7 days a week, for 4 weeks, followed by a recovery period of 2 weeks during which all animals were fed the control diet (NCI ,1978). No deaths were observed during the course of this study. Based upon decreased body weights relative to those of concurrent controls (details

not given), the following feed concentrations were initially selected for the chronic-duration bioassays: 0.0045 and 0.009% for the low- and high-dose rat groups, respectively; and 0.005 and 0.01% for the low- and high-dose mice groups, respectively.

Chronic-duration Studies

Following the subchronic-duration dose-finding studies, a 78-week study was undertaken. In the F344 rat study, males and females (50/gender/group) were exposed to 0, 0.009, and 0.0045, only for the first 8 weeks of treatment (NCI, 1978). At Week 9, the feed concentrations were increased to 0.005 and 0.01% for the low-and high-dose groups, respectively (reason unspecified). Treatment continued for an additional 69 weeks. Five rats/gender/group were sacrificed and necropsied after Week 78. The remaining rats at this time were switched to control diets and observed for an additional 20 weeks. Sacrifice and necropsy were subsequently performed. Untreated rat controls received the basal control diet for the entire study period. NCI (1978) calculated time-weighted average dietary concentrations at doses of 0.005% and 0.01% (equivalent to 50 and 100 mg of 2-methyl-5-nitroaniline per kilogram of feed) for the low- and high-dose groups, respectively, for both males and females, over the course of the treatment period. Using allometric values for F344 rats for body weight (0.380 kg for males and 0.229 kg for females) and food consumption rates (0.03 kg/day for males and 0.021 kg/day for females) for a chronic-duration study (U.S. EPA, 1988b), the doses calculated for this review were 0, 3.9, and 7.9 mg/kg-day for males and 0, 4.6, and 9.2 mg/kg-day for females in the control, low-, and high-dose groups, respectively.

All rats were inspected twice daily for mortality; clinical examination for the presence of tissue masses and/or lesions was performed monthly (NCI, 1978). Animals were weighed immediately prior to commencement of treatment, twice weekly for the first 12 weeks, and at monthly intervals thereafter. Food consumption was monitored for 7 consecutive days once a month for the first 9 months and for 3 consecutive days once a month thereafter. Drinking water consumption was not recorded. Necropsy was performed on all animals who were killed at the end of the study and on all those dying or sacrificed in extremis during the study. Gross pathology and histopathology were performed on all major tissues and organs and on gross lesions taken from terminally sacrificed animals and whenever possible on all animals found dead or sacrificed moribund.

Analysis of estimated probabilities of survival for rats of both genders using the Tarone and Cox tests did not shown any statistically significant association between dosage and mortality (NCI, 1978). Infrequent clinical signs of toxicity were not considered treatment related. Slight mean body-weight decreases were observed for high-dose male rats and for both high- and low-dose female rats relative to concurrent controls. Data were presented only in graphical form, and visual inspection did not suggest any statistically significant treatment-related effects on body weights. No hematology, clinical chemistry, urinalysis, or measurement of organ weights were conducted. Gross and microscopic pathology did not demonstrate any adverse nonneoplastic effects associated with compound administration. Thus, based on lack of statistically and toxicologically significant findings (albeit on a very limited set of nonneoplastic endpoints), the NOAELs for the study were identified as 9.2 mg/kg-day for females and 7.9 mg/kg-day for males. A LOAEL could not be determined.

Liver neoplastic nodules were observed in 5/47, 1/41, and 1/46 control, low-, and high-dose males, respectively; however, the low frequency of these findings compared to the control group indicated that these findings were not toxicologically significant (NCI, 1978). Hepatocellular carcinomas were observed in treated males at the following incidences: 0/47, 0/41, and 3/46 in the control, low-, and high-dose groups, respectively. However, the number of animals with these tumors was too small to permit a determination of whether the effect was compound related, and the Fisher's exact comparison test did not show statistical significance (NCI, 1978). Other neoplastic findings were similar in control and treated rats, and the incidences of these lesions were within the normal range of variation for F344 aging male rats. No treatment-related neoplastic effects were observed in female rats.

A similar 78-week oral toxicity study was conducted in B6C3F1 mice. **This study was selected as the principal study for deriving a screening chronic p-RfD and for the quantification of a p-OSF.** For mice, males and females (50/gender/group) were exposed to the initial dietary concentrations of 0.005% and 0.01% 2-methyl-5-nitroaniline (purity unspecified) only for the first 18 weeks of treatment (NCI, 1978). No mortality or body-weight changes were observed. At Week 19, feed concentrations of the test compound were increased to 0.15 and 0.3% for the low- and high-dose groups, respectively. No reasons for the dose changes were given. Animals were dosed for an additional 60 weeks for a total of 78 weeks.

Five mice/gender were sacrificed and necropsied from the high-dose and control groups after Week 78. The remaining animals were switched to control diets and observed for a period of up to 20 additional weeks. Untreated mouse controls received the basal diet for the entire study period. NCI (1978) calculated time-weighted average feed concentrations of 0.12 and 0.23% for low- and high-dose groups, respectively, for both males and females. NCI (1978) calculated time-weighted average dietary concentrations of 0.12 and 0.23% (equivalent to 1200 and 2300 mg of 2-methyl-5-nitroaniline per kilogram of feed) for the low- and high-dose groups, respectively, over the course of the treatment period. Using allometric values for B6C3F1 mice for body weight (0.0373 kg for males and 0.0353 kg for females) and food consumption rates (0.0064 kg/day for males and 0.0061 kg/day for females) for a chronic-duration study (U.S. EPA, 1988b), the doses calculated for this review were 0, 206, and 395 mg/kg-day for males and 0, 207, and 397 mg/kg-day for females in the control, low-, and high-dose groups, respectively.

Clinical observations, body-weight and food consumption measurements, and pathology and histopathology were the same as those for the rat study. No positive associations between treatment and survival were observed in either male or female mice (NCI, 1978). Neither gender showed any clinical signs of toxicity that were treatment related. Mean body weight was decreased in low- and high-dose males and females relative to controls throughout most of the study, with females showing a greater reduction than males. Body-weight data were presented only in graphical form; however, visual inspection showed that (1) the effect was statistically significant in treated females from at least Week 40 onward; (2) the reduction was greater than 20% relative to controls and is thus considered to be toxicologically significant; and (3) there was no compensatory body-weight gain or rebound during the observation period following termination of treatment at Study Week 78 (see Figure 2 of NCI 1978.). No hematology, clinical chemistry, urinalysis, or measurement of organ weights was conducted. Based on female

body-weight reductions during treatment, the study identified a LOAEL of 207 mg/kg-day—the lowest dose tested—and a NOAEL could not be determined.

Hepatocellular carcinomas were observed in 12/50, 12/44, and 29/45 control, low- and high-dose males, respectively, and in 2/47, 7/46, and 20/45 control, low- and high-dose females, respectively. These data are presented in Table 2. Trend tests were significant for both males and females ($p < 0.001$ using the Cochran-Armitage test), although the Fisher's exact test for pairwise comparisons was only statistically significant ($p < 0.001$) for the high-dose groups relative to controls. The first mouse dying with hepatocellular carcinoma was a high-dose male in Week 79; the first such female died during Week 97. In contrast, the first such death of liver tumor-bearing animals in the control group occurred during Week 94 for both males and females.

Table 2. Incidence of Treatment-Related Neoplastic Lesions in 2-Methyl-5-Nitroaniline Treated B6C3F1 Mice at Study Termination (NCI, 1978)

	Female			Male		
Dose Group	**Control**	**Low**	**High**	**Control**	**Low**	**High**
Hepatocellular Carcinoma	2/47[a]	7/46	20/45[b]	12/50	12/44	29/45[b]
Hemangiosarcoma or Hemangioma[c]	1/48	5/47[d]	3/47	1/50	0/47	4/48[e]

[a]Number of tumor-bearing animals/number of animals examined.
[b]$p < 0.05$ with Fisher's exact test for comparison of a treated group with the control group.
[c]Historical control rates for these tumors are 5/350 or 1.4% for each gender, markedly lower than observed rates.
[d]$p < 0.001$ using binomial distribution, based on probability of observing 5 or more mice with such tumors out of 47.
[e]$p < 0.005$ using binomial distribution, based on probability of observing 4 or more mice with such tumors out of 48.

No hepatic tumor was deemed benign, and hepatocellular carcinomas in two control animals had metastasized to the lungs (NCI, 1978). The carcinomas had invaded either a part or an entire lobe of the liver. Lobular architecture was distorted, area sinusoids were distended, pleomorphism in the size of neoplastic hepatocytes was observed, and nuclei were hyperchromatic. The cytoplasm was acidophilic and occasionally vacuolated, suggesting fatty infiltration. There were numerous mitotic figures.

Low incidences of hemangiomas and hemangiosarcomas (all sites combined) were also observed in male mice at the following incidences: 1/50, 0/47, and 4/48 in control, low-, and high-dose groups, respectively. No hemangiomas were noted in female mice, but the incidences of hemangiosarcomas (all sites combined) were 1/48, 5/47, and 3/47 in control, low-, and high-dose groups, respectively (see Table 2). None of the statistical tests showed significant trends for hemangiomas and hemangiosarcomas in male mice or for hemangiosarcomas in female mice. However, historical data from the NCI Carcinogenesis Testing Program laboratory (1978) showed that the background incidence of these tumors was very rare, approximately

5/350 per gender (1.4%) of either tumor at all body sites. The incidences in 2-methyl-5-nitroaniline-treated mice ranged from 6 to 11%, thus exceeding historical control rates. These findings were considered by NCI (1978) to be toxicologically significant and possibly related to treatment, even in the absence of statistical significance because of the rarity of spontaneous occurrence of these tumors in this strain of species. This provides limited support to the finding of hepatic tumors in mice. However, the data are not amenable to modeling because of the low incidences, statistical significance of only one of the two doses, and limited dose-response functions. No other tumors or nonneoplastic lesions were considered treatment-related under the conditions of this study.

Inhalation Exposure

No subchronic-duration, chronic-duration, reproductive, or developmental inhalation toxicity studies in animals have been conducted with 2-methyl-5-nitroaniline.

OTHER STUDIES

Acute Lethality Studies

The oral LD_{50} for 2-methyl-5-nitroaniline has been reported as 574 mg/kg for the rat (Lewis, 2004 as cited in HSDB, 2009).

Short-term Studies

Methemoglobinemia was detected in guinea pigs following a single-dose intraperitoneal (i.p.) injection of 600 to 700 mg/kg of 2-methyl-5-nitroaniline (purity unspecified) in a vegetable oil vehicle. In a similar experiment with cats, methemoglobinemia was detected at much lower doses—5 to 10 mg/kg (HSDB, 2009).

Several mouse studies using i.p. injection as the route of administration have been conducted as screening studies to investigate the potential for experimentally-induced chemical carcinogenicity of 2-methyl-5-nitroaniline (HSDB, 2009). In a study examining interlaboratory agreement, A/St female mice (20/group), aged 6–8 weeks, were given i.p. injections of 25, 50, or 100 mg/kg of 2-methyl-5-nitroaniline in tricaprylin vehicle at a dose rate of 3 times per week for 8 weeks (Maronpot et al., 1986). Body weights were recorded every 2 weeks. One control group (n = 60 females) received i.p. injections of only tricaprylin (vehicle control), and a second control group (n = 80 females) was untreated. All surviving animals were sacrificed at 16 weeks of age following treatment termination. Survival rates were 85% (17/20) for the lowest dose, 100% for the mid-dose, and 85% for the high-dose mice. Lung adenomas were found in survivors at each dose: 8% in untreated controls, 11% in tricaprylin-treated controls, 18% in low-dose animals, 30% in mid-dose animals, and 6% in high-dose animals.

In the second laboratory, A/J male mice (30/group), aged 68 weeks, were administered i.p. injections of either 40, 100, or 200 mg/kg-day of 2-methyl-5-nitroaniline (purity unspecified) in corn oil vehicle at a dose rate of 3 times per week for 8 weeks (Maronpot et al., 1986). Control groups either received i.p. injections of corn oil vehicle (n = 30) or were untreated (n = 20). The survival rates were 73%, 77%, and 30% in the low-, mid-, and high-dose groups, respectively. All surviving animals were sacrificed at 16 weeks after treatment was discontinued. The percentages of animals having tumors were 21%, 31%, 23%, 43%, and 33% for untreated controls, corn oil-treated controls, and low-, mid-, and high-dose mice,

respectively. There were no statistically significant differences in the percentage of survivors with lung adenomas as observed compared to vehicle controls.

Toxicokinetics

Mori et al. (1982) identified 2-methyl-5-nitroaniline as one of multiple urinary metabolites of 2,4-dinitrotoluene (2,4-DNT). It has been speculated that the toxicity, genotoxicity, and carcinogenicity of 2,4-DNT may be due, at least in part to, in vivo biotransformation to 2-methyl-5-nitroaniline and possibly to other metabolic products (Mori et al., 1982, 1985; HSDB, 2010). No information on the absorption, distribution, and metabolism of 2-methyl-5-nitroaniline has been identified in the literature. Based on toxicokinetic studies of 2,4-DNT, it is likely that urinary excretion is a major route of elimination of 2-methyl-5-nitroaniline from the body.

Genotoxicity

2-Methyl-5-nitroaniline has been tested in a number of bacterial and mammalian-cell assays and in one in vivo study. A summary of genotoxicity data is presented in Table 3. Many of the mutagenicity tests have been reported as positive, both with and without exogenous metabolic activation. However, these positive findings were frequently observed only at high millimolar (mM) plate concentrations of the test substance, suggesting that high-dose cytotoxicity or cell killing may have confounded the test results.

In bacterial mutagenicity assays, 2-methyl-5-nitroaniline was only weakly mutagenic in *Salmonella typhimurium* tester strains TA98 and TA100 and then only at high millimolar (mM) concentrations (Mori et al., 1982). Cytotoxicity at these concentrations was not reported by the study authors. In a repeat Ames study by the same authors using lower molar concentrations of 0 to 2000 µg/plate, 2-methyl-5-nitroaniline was not mutagenic in either TA98 or TA100, with or without S9 activation (Mori et al., 1985). Further studies using more specialized tester strains, TA98NR (nitroreductase-deficient) and TA98/1,8-DNP_6 *O*-acetylase-deficient) were also nonmutagenic in the presence or absence of a S9 mix (Sayama et al., 1991). Couch et al. (1987), using a quantitative reversion assay with *S. typhimurium* TA98, reported the occurrence of 2-methyl-5-nitroaniline mutagenicity, with and without metabolic activation; however, critical examination of these data showed that this effect only occurred at high mM concentrations, which may have been cytotoxic. 2-Methyl-5-nitroaniline, was not mutagenic in the *Escherichia Coli* WP2uvrA bacterial test system with or without exogenous metabolic activation (Dunkel et al., 1984).

In a number of in vitro cytogenicity assays in mammalian cells, 2-methyl-5-nitroaniline was reported to induce chromosomal aberrations only in the presence of metabolic activation (NTP, 1986). NTP (1986) also reported weakly positive and positive results for sister chromatid exchanges in two mammalian assays with metabolic activation, respectively, and "questionable" findings in the absence of exogenous activation in the same assay. Plate concentrations were as high as 5 mg/mL, so again, cytotoxic effects cannot be excluded. 2-Methyl-5-nitroaniline was reported to induce significant morphological transformations in the in vitro SHE cell transformation assay (Kerckaert et al., 1998), as interpreted by the study authors based on a statistically significant trend test (unstratified binomial exact permutation trend test, Cytel Software). The findings were reported as positive at 200- and 400-µg/ml plate concentrations of test substance, with 30% cytotoxicity occurring at the 300-µg/ml concentration (not considered positive) and a "slight precipitate" occurring at the 400-µg/ml dose.

Table 3. Available Genotoxicity Data on 2-Methyl-5-Nitroaniline

Type of Study	Species/Strain	Results[a]	Reference/Comments
Bacterial mutagenicity	*S. typhimurium* TA98 with/without S9	+/+	Mori et al. (1982). Reported as weakly mutagenic only at high mM concentrations. No cytotoxicity data.
Bacterial mutagenicity	*S. typhimurium* TA98 with/without S9	−/−	Mori et al. (1982). Not mutagenic at µM concentrations under the same conditions as the previous tests.
Bacterial mutagenicity	*S. typhimurium* TA100 with/without S9	+/+	Mori et al. (1982). Reported as weakly mutagenic only at high mM concentrations. No cytotoxicity data.
Bacterial mutagenicity	*S. typhimurium* TA100 with/without S9	−/−	Mori et al. (1982). Not mutagenic at µM concentrations under the same conditions as the previous tests.
Bacterial mutagenicity	*S. typhimurium* TA98NR with/without S9	−/−	Sayama et al. (1991). Specialized tester strain TA98 nonreductase.
Bacterial mutagenicity	*S. typhimurium* TA98/1,8-DNP_6, with/ without S9	−/−	Sayama et al. (1991). Specialized tester strain TA98 *O*-acetylase deficient.
Bacterial mutagenicity	*S. typhimurium* TA98 with/without S9	+/+	Couch et al. (1987). Positive results occurred at high mM concentrations. Possibly cytotoxic at these concentrations.
Bacterial mutagenicity	*S. typhimurium* TA98 with/without S9	+/+	NTP (1985); Dunkel et al. (1985).
Bacterial mutagenicity	*S. typhimurium* TA100 with/without S9	+/+	NTP (1985); Dunkel et al. (1985).
Bacterial mutagenicity	*E. coli* WP2uvrA with/without S9	−/−	HSDB (2009); Dunkel et al. (1985).
Bacterial mutagenicity	*S. typhimurium* TA100 with/without S9	+/+	Goeggelmann et al. (1989). Number of revertants similar with/without S9 mix. Findings reported in published abstract.
In vitro chromosomal aberrations	Human lymphocytes with/without S9	+/+	Goeggelmann et al. (1989). Findings reported in published abstract.
In vitro chromosomal aberrations	Chinese hamster ovary cells with/without S9	+/−	NTP (1986).
In vitro sister chromatid exchanges	Human lymphocytes with/without S9	+/+	Goeggelmann et al. (1989). Findings reported in published abstract.
In vitro sister chromatid exchanges	Chinese hamster ovary cells with/without S9	+/?	NTP (1986).
In vitro cell transformation assay	SHE cells	+	Kerckaert et al. (1998). Significant morphological transformations based on trend test (unstratified binomial exact permutation trend test, Cytel Software).
In vivo hemoglobin adduct formation	Female Wistar rats dosed by gavage	+	Zwirner-Baier et al. (1994). Covalently bound hydrolysable hemoglobin adducts observed.

[a]Notations: "–" = negative; "–/–" = negative with/without S9 activation; "+" = positive; "+/+" = positivewith/without S9 activation; "+/–" = positive with S9 activation/negative without activation; "+/?" = positive with S9 activation; "questionable" without activation.

As part of a study assessing blood markers of exposure and/or metabolism of amino- and nitro-substituted benzenes and toluenes, Zwirner-Baier et al. (1994) administered a single gavage dose of 0.5 mmol/kg of one such compound—2-methyl-5-nitroaniline—to female Wister rats. Blood was extracted, hydrolyzed, and analyzed for hemoglobin adducts 24 hours following dosing. The results showed that 2-methyl-5-nitroaniline formed in vivo covalently bound to hydrolyzable hemoglobin adducts, which might contribute to the blood toxicity (i.e., methemoglobinemia) of this substance. Similar findings were observed by Johnson et al. (1985) in studies assessing the inhibitory effects of 2-methyl-5-nitroaniline and other substituted nitrobenzenes on the in vitro activity of two enzymes important for the synthesis of heme: delta-aminolevulinic acid synthetase (ALAS) and ferrochelatase (FC).

DERIVATION OF PROVISIONAL VALUES

Table 4 below presents a summary of noncancer reference values. Table 5 presents a summary of cancer values. The toxicity values were converted to HED units, as detailed in the text. IRIS data are indicated in the table if available.

Table 4. Summary of p-Reference Values for 2-Methyl-5-Nitroaniline (CASRN 99-55-8)

Toxicity Type (units)	Species/ Gender	Critical Effect	p-Reference Value	POD Method	POD	UF_C	Principal Study
Subchronic p-RfD (mg/kg-day)	None	None	None	None	None	None	None
Chronic (Screening) p-RfD (mg/kg-day)[a]	Mice/F	Body weight reductions	2×10^{-2} mg/kg-day	LOAEL/UF	207	10,000	NCI (1978)
Subchronic p-RfC (mg/m^3)	None	None	None	None	None	None	None
Chronic p-RfC (mg/m^3)	None	None	None	None	None	None	None

[a]Note: this is a screening p-RfD. Please see Appendix A for details.

Table 5. Summary of Cancer Values for 2-Methyl-5-Nitroaniline (CASRN 99-55-8)

Toxicity Type	Species/Gender	Tumor Type	Cancer Value	Principal Study
p-OSF	Mouse/F	Hepatocellular carcinomas	9×10^{-3} $(mg/kg\text{-}day)^{-1}$	NCI (1978)
p-IUR	None	None	None	None

DERIVATION OF ORAL REFERENCE DOSE

Derivation of Subchronic p-RfD

A summary of the available data is shown in Table 1. Data on the oral subchronic toxicity of 2-methyl-5-nitroaniline in humans have not been located in the literature. Animal short-term studies exist in the form of 3 and 4-week studies performed for dose selection in the NCI carcinogenicity assay (NCI, 1978). In the first study, mortality occurred in all dose groups within 3 weeks, and no additional details of adverse effects were given. In the second study, decreased body weights were observed in treated animals, but no dose-response details, gross pathology, or histopathology were discussed. Due to the scarcity of data results and the nature of the short-term dose-response studies, these were not deemed appropriate for derivation of a subchronic p-RfD.

Derivation of Chronic p-RfD

Two animal studies were identified with possible utility for the development of a provisional chronic p-RfD (NCI, 1978). However, these studies were carcinogenicity bioassays and were conducted in the 1970s, when only limited data on systemic toxicity unrelated to tumor development were collected and analyzed. Specifically, the only information available for the 2-methyl-5-nitroaniline bioassays was mortality, clinical signs of toxicity, body weight, and gross and microscopic pathology. Further, body weights were presented only in graphical form without accompanying numerical data (i.e., means, standard deviations, statistical test results, and levels of significance). Nonneoplastic histopathology was summarized but not statistically evaluated. However, these studies were well conducted and peer reviewed, with sufficient information on gender, strains, and species employed in the study, the size of dose groups, dietary concentrations, treatment protocols, and some statistical analysis (NCI, 1978).

In the rat study (NCI, 1978), no treatment-related mortality, clinical signs of toxicity, body-weight changes, and pathological/histopathological findings were observed during the course of the study. It appeared from the results of the study that the Maximum Tolerated Dose (MTD) had not been reached. This is likely to have accounted for the lack of any effects. Therefore, neither NOAELs nor LOAELs were established that could be used for derivation of a chronic p-RfD. In the mouse study, visual inspection of the body-weight data graphs showed clearly that (1) the decrease in mean body weight in females would have been statistically significant had these findings been statistically analyzed and (2) the estimated magnitude of the change was greater than approximately 20% relative to controls during the second half of the study, indicating toxicological significance (see Figure 2 of NCI 1978). Further, no compensatory increase in body weight occurred during the approximately 20-week observation period following discontinuation of treatment at Week 78. Significant body-weight reductions were noted in females in both the low- and high-dose groups. Based on decreased body weight, a LOAEL of 207 mg/kg-day was identified, and a NOAEL could not be determined.

Consideration was given to developing a chronic p-RfD. However, in an extensive literature search, no additional data on the oral or inhalation subchronic or chronic toxicity of 2-methyl-5-nitroaniline were located. Similarly, reproductive and developmental toxicity studies have not been conducted. A NOAEL was not identified from the NCI (1978) female mouse study. Due to the paucity of repeat dose toxicity data, the composite uncertainty factor (UF_C) for p-RfD calculation was estimated to be 10,000, as summarized below in Table 6.

Table 6. Uncertainty Factors for Chronic p-RfD for 2-Methyl-5-Nitroaniline		
UF	**Value**	**Justification**
UF_A	10	A UF_A of 10 is applied for animal-to-human extrapolation to account for potential toxicokinetic and toxicodynamic differences between mice and humans. Little toxicokinetic or toxicodynamic data are available for this compound in either the mouse or the human.
UF_D	10	A UF_D is applied for database deficiencies due to the absence of any developmental or reproductive toxicity studies. Further, the limited set of noncancer endpoints examined in the NCI (1978) does not fully characterize the potential toxicity of 2-methyl-5-nitroaniline. Therefore, there are also database deficiencies pertaining to potential hematology, clinical chemistry, urinalysis, and organ-weight effects.
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of definitive information on the variability of response in humans.
UF_L	10	A UF_L of 10 is applied because insufficient data are available to conduct benchmark dose (BMD) modeling, and the LOAEL is used as the point of departure (POD) for deriving a chronic p-RfD. Consideration was given to the use of a UF_L of 3 because the critical effect was decreased body weight. However, the following factors argued against a reduction in this UF: (a) the body-weight decrease was greater than 20%, which is considered toxicologically significant, and the animals did not regain much weight during the observation period following termination of dosing; (b) other effects pertaining to the toxicity of nitroanilines, most specifically hematological effects, were not measured. These types of effects have been extensively reported in structurally similar compounds and those belonging to the same structural category. Methemoglobinemia associated with 2-methyl-5-nitroaniline was noted to occur in humans in one study, but no details were available. The possibility that blood effects could have been detected at the LOAEL had they been measured precluded decreasing the UF.
UF_S	1	A UF_S of 1 is applied because these data are from a chronic-duration study.
UF_C		10,000

In accordance with EPA guidance, substances with a UF of 10,000 are not considered amenable to the development of either a subchronic or a chronic p-RfD. However, Appendix A of this document contains a screening value (a screening chronic p-RfD), based on available data, which may be useful for certain applications. Please see Appendix A for further details.

Derivation of Inhalation Reference Concentrations

The only available data on human inhalation exposure to 2-methyl-5-nitroaniline is a single case study of an occupationally-exposed worker who developed fulminant hepatitis, and an observational study of a small cohort of exposed workers who showed evidence of liver dysfunction following several weeks of working with the compound in a hair dye manufacturing facility. Quantitative estimates of exposure and adverse liver effects were not available for these workers. No animal studies investigating the effects of inhalation exposure have been

conducted. Therefore, data are inadequate for the derivation of subchronic and chronic p-RfCs for 2-methyl-5-nitroaniline.

Cancer Weight-of-Evidence (WOE) Descriptor

Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), 2-methyl-5-nitroaniline is considered to have "*Suggestive Evidence of Carcinogenic Potential*" for humans by the oral route of exposure.

As detailed in Table 7, this classification is based upon a WOE analysis of the nature and extent of 2-methyl-5-nitroaniline's human carcinogenic potential.

Table 7. Cancer WOE Descriptor for 2-Methyl-5-Nitroaniline

Possible WOE Descriptor	Designation	Route of Entry (Oral, Inhalation, or Both)	Comments
"Carcinogenic to Humans"	N/A	N/A	No human cancer studies are available.
"Likely to Be Carcinogenic to Humans"	N/A	N/A	No strong animal cancer data are available.
"Suggestive Evidence of Carcinogenic Potential"	**X**	**Oral dietary administration**	**Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), available evidence for oral exposure to 2-methyl-5-nitroaniline is based mainly on clear evidence of liver carcinogenicity (hepatocellular carcinomas) in female and male B6C3F1 mice (NCI, 1978). No hepatic tumor was deemed benign, and hepatocellular carcinomas in two control animals had metastasized to the lungs. There was also a trend toward elevated incidence of combined hemangiomas and hemangiosarcomas in male mice and of hemangiosarcomas in female mice. These tumors were found at various sites throughout the body, not concentrated in one organ such as the liver. Although these findings were not statistically significant, the incidence in treated groups exceeded the historical control range for the laboratory. These latter results are considered equivocal evidence of carcinogenicity, although there was no clear dose response and the incidence rate in the concurrent control group was also elevated relative to historical controls. No tumors were observed in male and female rats in a 2-year dietary bioassay. Limited data are available on toxicokinetics and mode of action. Although a reasonable number of in vitro mutagenicity/genotoxicity tests have been conducted with the test compound, the findings are unclear because 2-methyl-5-nitroaniline was generally positive at high culture concentrations, which increased cell deaths in a manner suggestive of high-dose cytotoxicity.**
"Inadequate Information to Assess Carcinogenic Potential"	N/A	N/A	Adequate information to assess carcinogenic potential is available.
"Not Likely to Be Carcinogenic to Humans"	N/A	N/A	No strong evidence of noncarcinogenicity in humans is available.

1

Mode-of-Action Discussion

The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) define mode of action as a sequence of key events and processes starting with the interaction of an agent with a cell, proceeding through operational and anatomical changes, and resulting in cancer formation. Examples of possible modes of carcinogenic action include mutagenic, mitogenic, antiapoptotic (inhibition of programmed cell death), cytotoxic with reparative cell proliferation, and immune suppression.

The mechanism of 2-methyl-5-nitroaniline-induced liver carcinogenicity has not yet been determined. Mechanistic studies other than mutagenicity and some genotoxicity assays have not been conducted. These data are not sufficient to determine mode of carcinogenic action.

In an in vivo study, gavage administration of 2-methyl-5-nitroaniline to female Wistar rats resulted in the formation of covalently-bonded hemoglobin adducts (Zwirner-Baier et al., 1994). However, these findings were reported in rats, not mice, and may be consistent with compound-induced systemic hematotoxicity. Even if these findings were to be replicated in mice, it would be difficult to postulate a causal chain of mechanistic events leading from hemoglobin adduct formation to hepatic tumor development. However, methemoglobinemia has been reported to occur with exposure to 2-methyl-5-nitroaniline and rodent studies of other nitroanilines, and structurally similar compounds have observed that numerous adverse blood effects may occur, depending on the specific compound tested and the dose levels utilized in the study (e.g., HSDB, 2010). It has been suggested that some compounds affecting the hematopoietic system may induce hepatic tumors by nongenotoxic mechanisms, specifically sustained cytotoxicity and regenerative cell proliferation in the liver associated with clearance of red blood cell fragments such as porphyrin from damaged cells (Holsapple et al., 2006).

The data are insufficient at this time to provide any insight into an association between oral exposure to 2-methyl-5-nitroaniline and liver tumorigenicity, and a nongenotoxic mechanism has not been clearly demonstrated. A discussion of possible sequences of key events leading to carcinogenesis, concordance of findings, sensitivity and specificity of responses, dose-response assessments, biological plausibility, and reproducibility are not possible for 2-methyl-5-nitroaniline.

QUANTITATIVE ESTIMATES OF CARCINOGENIC RISK

Derivation of Provisional Oral Slope Factor (p-OSF)

Oral data are sufficient to derive a p-OSF for 2-methyl-5-nitroaniline. In the NCI (1978) study, male and female B6C3F1 mice were administered time-weighted average dietary concentrations of 0.12 and 0.23% of the test compound for 78 weeks and observed for up to 20 weeks prior to terminal sacrifice. Both sexes exhibited statistically significantly increased incidences of hepatocellular carcinomas relative to concurrent and historical controls ($p < 0.001$). The trend in hemangiosarcomas and hemangiomas further bolsters the case for the relevance of the mouse hepatocellular carcinomas for use in human health risk assessment.

The mode of action for liver carcinomas produced by 2-methyl-5-nitroaniline is not known. Available mutagenicity data are generally positive with and without exogenous metabolic activation, but the results of at least some of these assays may be confounded by high-concentration cytotoxicity. In the absence of data to inform the mode of action and the shape of the dose-response curve at low doses, a linear low-dose extrapolation was performed.

The following dosimetric adjustments were made to dietary doses given to male and female mice in the NCI (1978) study, in accordance to EPA (2005) *Guidelines for Carcinogen Risk Assessment*. Animal doses were first corrected for exposure duration and then converted to human equivalent doses (HEDs), using the appropriate cross-species scaling factor to adjust for differences in body weight between the human and the mouse, in accordance with EPA guidelines (U.S. EPA, 2005).

$(DOSE_{ADJ, HED})_n$ = $(Dose)_n$ × (correction to average daily dose) × (body-weight adjustment)
= $(Dose)_n$ × (no. weeks of treatment) ÷ (no. weeks of treatment + no. weeks of subsequent observation without treatment) × (body-weight adjustment)

Body-weight adjustment = $(BW_A \div BW_H)^{1/4}$

For female mice in the low-dose group, the following adjustments were performed:

BW_H = 70 kg (human reference body)

BW_A = 0.0353 kg (default body weight for female B6C3F1 mice in a chronic-duration study, as per U.S. EPA, 1988b)

Body-weight adjustment = $(0.0353 \div 70)^{1/4} = 0.15$
= (Dose) × 78 weeks ÷ 97 weeks × 0.15

$(DOSE_{ADJ, HED})_n$ = (Dose) × 0.12
= 207 mg/kg-day × 0.11
= 25 mg/kg-day (rounded to two significant digits)

Using the above formulae, HEDs were calculated for the other three groups of interest, using appropriate sex/species-specific body-weight adjustments (U.S. EPA, 1988b). The HEDs for all four groups of interest were calculated to be 25 mg/kg-day and 48 mg/kg-day for males at low and high doses, and 25 mg/kg-day and 47 mg/kg-day for females at the low and high doses, respectively.

Dose-response modeling of the data in Table 8 was performed to obtain the POD for a quantitative assessment of cancer risk. The POD is an estimated concentration (expressed in human-equivalent terms) near the lower end of the experimental range of observations that marks the starting point (or POD) for extrapolation to lower doses. Tumor incidences were modeled separately for each gender.

Table 8 shows the input data used for benchmark dose (BMD) modeling; and the results of BMD modeling are presented in Table 9. Adequate model fit is obtained for hepatocellular carcinomas in both male mice and female mice, using the multistage-cancer model, as evidenced by the acceptable goodness-of-fit *p*-value for both data sets. The AIC is lower for female mice as compared with the AIC for male mice, indicating a better fit for the female data set. Therefore, female hepatocellular carcinoma tumor data are selected for derivation of the final p-OSF. The BMD_{10} is 21.10 mg/kg-day, and the $BMDL_{10}$ is 10.75 mg/kg-day.

Table 8. BMD Input for Incidence of Hepatocellular Carcinomas in Male and Female B6C3F1 Mice (NCI, 1978)

DOSE (mg/kg-day)		($DOSE_{ADJ,HEC}$) (mg/kg-day)		Number of Animals Examined		Hepatocellular Carcinomas	
M	**F**	**M**	**F**	**M**	**F**	**M**	**F**
0	0	0	0	50	47	12	2
206	207	25	25	44	46	12	7
394	397	48	47	45	45	29	20

Table 9. Goodness-of-Fit Statistics, BMD_{10}, and $BMDL_{10}$ Values for Dichotomous Models for Hepatocellular Carcinomas in Male and Female B6C3F1 Mice[a]

	Multistage Cancer Model (mg/kg-day)	Goodness-of-Fit *p*-Value[b]	AIC	BMD_{10HEC} (mg/kg-day)	$BMDL_{10HEC}$ (mg/kg-day)
Male	Hepatocellular carcinomas	0.2082	170.868	18.57	10.08
Female	Hepatocellular carcinomas	0.6813	121.775	21.10	10.75

[a]NCI (1978).
[b]Values >0.1 meet conventional goodness-of-fit criteria.

$$\textbf{p-OSF} = 0.1 \div BMDL_{10HEC}$$
$$= 0.1 \div 10.75 \text{ mg/kg-day}$$
$$= \textbf{0.0093 (mg/kg-day)}^{-1} \textbf{ or } \mathbf{9 \times 10^{-3}} \textbf{ per mg/kg-day (rounded to one significant digit)}$$

The p-OSF is 0.009 per mg/kg-day from the BMD program.

Using a p-OSF to calculate risks greater than, or approaching the p-OSF (0.009), is generally inappropriate because of the nature of the p-OSF derivation (i.e., the dose-response slope is calculated based on the experimental POD linearized to the origin by default). An examination of Figures C-1 and C-2 shows that the slope above the POD falls within the standard deviation of the observed tumor incidences, and, hence, its uncertainty has some actual measure as opposed to the low-dose slope. Generally, however, the slope of the line close to and above the POD is not reliable; thus, the risk calculated at this point provides too much uncertainty.

Derivation of Provisional Inhalation Unit Risk (p-IUR)

No human or animal studies examining the carcinogenicity of 2-methyl-5-nitroaniline following inhalation have been located. Therefore, a p-IUR was not derived.

APPENDIX A. DERIVATION OF A SCREENING CHRONIC RFD

For reasons noted in the main PPRTV document, it is inappropriate to derive a screening chronic p-RfD for 2-methyl-5-nitroaniline. However, some information is available for this chemical, which, although insufficient to support the 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.

DERIVATION OF SCREENING ORAL REFERENCE DOSES

Screening Provisional Reference Dose (p-RfD)

Data on the oral subchronic or chronic toxicity of 2-methyl-5-nitroaniline in humans have not been located in the literature. Chronic-duration animal bioassays in F344 rats and B6C3F1 mice were conducted by NCI (1978) to assess the carcinogenicity of 2-methyl-5-nitroaniline, and these studies were critically reviewed in order to determine their suitability for the development of a chronic p-RfD. It should be noted that carcinogenicity bioassays conducted in the 1970s collected and analyzed only a limited data set on systemic toxicity unrelated to the tumorigenic process.

In these study reports, available information was restricted to mortality, clinical signs of toxicity, body weights, and gross and microscopic pathology. Body weights were presented only in graphical form without accompanying numerical data (i.e., means, standard deviations, statistical tests, and levels of significance). Nonneoplastic histopathology was summarized in appendices but neither statistically evaluated nor assessed by an independent pathologist (as is currently done in these types of bioassays).

In the rat bioassay (NCI, 1978), no treatment-related mortality, body-weight changes, and gross or microscopic pathology were observed during the course of the study. Therefore, no data were available that could be used for derivation of a chronic p-RfD. In the mouse study, no treatment-related mortality or gross and microscopic pathology unrelated to tumor formation were noted. However, visual inspection of the body-weight data graphs in the NTP (1978) study report showed clearly that (1) the decrease in mean body weight in females would have been statistically significant had these findings been statistically analyzed and (2) the estimated magnitude of the change was ≥20% relative to concurrent controls during the second half of the study, indicating toxicological significance (see Figure 2 of NCI 1978). Further, no compensatory increase in body weight occurred during the approximately 20-week observation period, which occurred following termination of treatment at Week 78 and prior to animal sacrifice at approximately Weeks 96–98. Based on significant body-weight reductions occurring among females in both dosed groups, a LOAEL of 207 mg/kg/day was identified, and a NOAEL could not be determined.

No additional data on the oral subchronic or chronic toxicity of 2-methyl-5-nitroaniline were identified in the published or unpublished literature. Reproductive and developmental toxicity studies have not been conducted. Due to lack of sufficient toxicity data, the UF_C for p-RfD calculation is 10,000. In accordance with EPA guidance, a UF of this magnitude precludes derivation of either a subchronic or chronic p-RfD. Based on available data, a screening value (a screening chronic p-RfD) was derived, which may be useful in certain situations.

Screening Chronic p-RfD = LOAEL(POD) ÷ UF_C
= 207 mg/kg-day ÷ 10,000
= **0.02 mg/kg-day**

The UF_C of 10,000 is composed of the following individual UFs:

- A UF_A of 10 is applied for interspecies extrapolation to account for potential pharmacokinetic and pharmacodynamic differences between mice and humans.
- A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response in humans.
- A UF_D of 10 is applied for uncertainty in the database. No developmental or reproductive toxicity studies have been conducted.
- A UF_L of 10 is applied for the use of a LOAEL instead of a NOAEL as the point of departure (POD) for the development of the screening chronic p-RfD.
- A UF_S of 1 is utilized for exposure duration because no adjustment is needed for a chronic-duration study.

Confidence in the principal study is low. Although the NTP (1978) study was well conducted, a comprehensive evaluation of endpoints for nonneoplastic effects was not performed. Of specific concern is the potential for hematological effects as 2-methyl-5-nitroaniline and its structurally-similar analogs have been associated with the induction of methemoglobinemia and with the formation of hemoglobin adducts (HSDB, 2009; Zwirner-Baier et al., 1994). Confidence in the database is low. No reproductive or developmental toxicity studies are available, and as previously noted above, very limited data are available on the nature and extent of systemic toxicity. Therefore, low confidence in the screening chronic p-RfD follows.

APPENDIX B. BMD MODELING OUTPUT FOR THE OSF

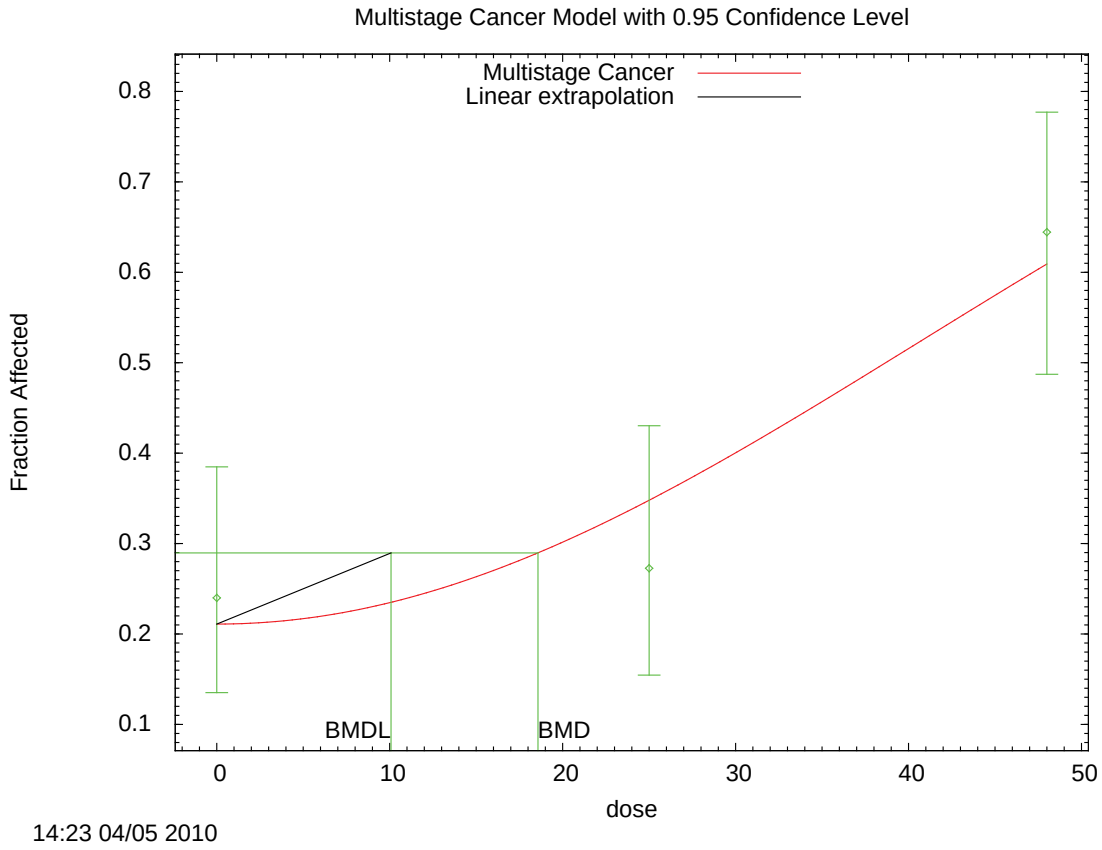

Figure C-1. Male Mouse Hepatocellular Carcinoma Data (NCI, 1978)

Text Output for Multistage BMD Model for Male Hepatocellular Carcinoma Data (NCI, 1978)

```
 ======================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\mscDaxSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\mscDaxSetting.plt
                                                Mon Apr 05 14:23:09 2010
 ======================================================================

 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 = Incidence
   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 = 0.18196
Beta(1) = 0
Beta(2) = 0.000349755

Asymptotic Correlation Matrix of Parameter Estimates

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

	Background	Beta(2)
Background	1	-0.59
Beta(2)	-0.59	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.210786	*	*	*
Beta(1)	0	*	*	*
Beta(2)	0.000305392	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-82.6227	3			
Fitted model	-83.434	2	1.62255	1	0.2027
Reduced model	-92.3925	1	19.5396	2	<.0001

AIC: 170.868

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
25.0000	0.3479	15.308	12.000	44	-1.047
48.0000	0.6095	27.428	29.000	45	0.480

```
    0.0000     0.2108        10.539    12.000          50        0.506

 Chi^2 = 1.58      d.f. = 1        P-value = 0.2082

   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =        18.5742

             BMDL =        10.0771

             BMDU =        24.3265

Taken together, (10.0771, 24.3265) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00992352
```

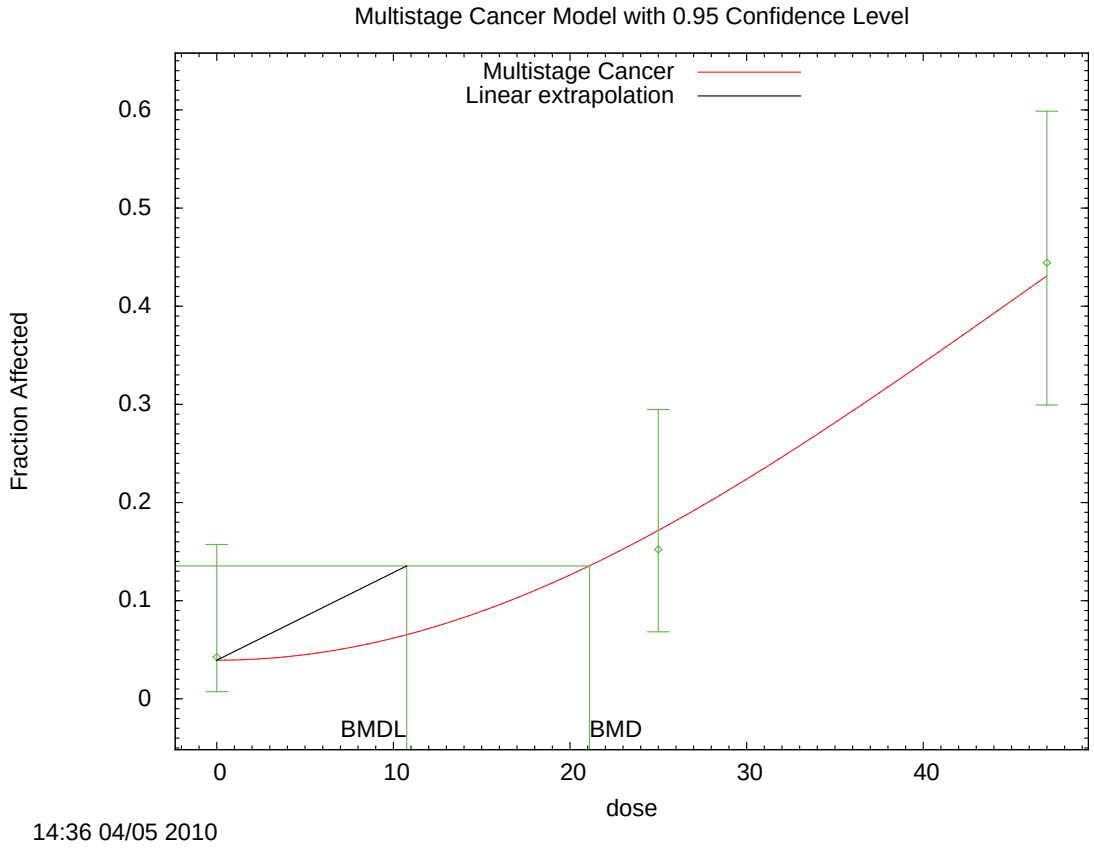

Figure C-2. Female Mouse Hepatocellular Carcinoma Data (NCI, 1978)

Text Output for Multistage BMD Model for Female Hepatocellular Carcinoma Data (NCI, 1978)

```
 ======================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\mscDaxSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\mscDaxSetting.plt
                                                Mon Apr 05 14:36:14 2010
 ======================================================================

 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 = Incidence
   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 =    0.0284952
                        Beta(1) =            0
                        Beta(2) =  0.000250397


           Asymptotic Correlation Matrix of Parameter Estimates

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

             Background      Beta(2)

Background            1        -0.64

   Beta(2)        -0.64            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0394377            *               *                   *
        Beta(1)                0            *               *                   *
        Beta(2)      0.000236596            *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -58.8013         3
   Fitted model        -58.8874         2      0.172143      1          0.6782
  Reduced model        -70.9525         1       24.3024      2         <.0001

           AIC:         121.775


                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size          Residual
  ----------------------------------------------------------------------
    0.0000     0.0394         1.854     2.000          47          0.110
   25.0000     0.1715         7.888     7.000          46         -0.347
```

```
   47.0000     0.4304        19.369    20.000          45        0.190

 Chi^2 = 0.17      d.f. = 1        P-value = 0.6813


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

              BMD =        21.1026

             BMDL =        10.7537

             BMDU =        26.2881

Taken together, (10.7537, 26.2881) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00929914
```

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