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Provisional Peer-Reviewed Toxicity Values for

2,4-Dimethylaniline
(CASRN 95-68-1)

Superfund Health Risk Technical Support Center
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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower bound 95% confidence interval
BMD	benchmark dose
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
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
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 2,4-DIMETHYLANILINE (CASRN 95-68-1)

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

Dimethylaniline, 2,4- (also called 2,4-xylidine) is a colorless to yellow or dark brown liquid used as an intermediate for pesticides, pharmaceuticals, dyes, wood preservatives, wetting agents for textiles, frothing agents for ore dressing, metal complexes, special lacquers, and photographic chemicals (HSDB, 2009; OSHA, 2009b). The empirical formula for 2,4-dimethylaniline is $C_8H_{11}N$, and its structure are shown in Figure 1, and Table 1 provides several physicochemical properties for this compound. In this document, "statistically significant" denotes a *p*-value of <0.05.

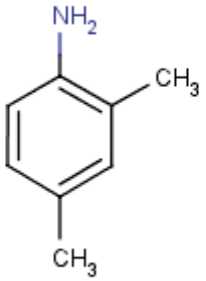

Figure 1. Structure of 2,4-Dimethylaniline

Table 1. Physicochemical Properties Table (2,4-Dimethylaniline)	
Property (unit)	**Value**
Boiling point (ºC)	214[a]
Melting point (ºC)	14.3[b]
Density (g/cm^3)	0.9723[b]
Vapor pressure (mm Hg at 25ºC)	0.133 mm Hg[b]
pH (unitless)	Data not available
Solubility in water (g/L at 20ºC)	5 (slightly soluble)[c]
Relative vapor density (air = 1)	Data not available
Molecular weight (g/mol)	121.18[a]
Flash point (°C)	90[a]
Octanol/water partition coefficient (unitless) at pH of 7.5	47.86 (log K_{ow} = 1.68)[c]

[a]Columbia Analytical Services (2010).
[b]Values from NTP (2009).
[c]ChemBlink (2010).

No reference dose (RfD), reference concentration (RfC), or cancer assessment for 2,4-dimethylaniline (or 2,4-xylidine) is included in the EPA IRIS database (U.S. EPA, 2010a) or on the Drinking Water Standards and Health Advisories List (U.S. EPA, 2009a). No acute exposure guideline levels (AEGLs) for 2,4-dimethylaniline have been derived by the EPA's Office of Pollution Prevention and Toxics (U.S. EPA, 2009b). No assessments were reported on the Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1994a).

The EPA has published a Health and Environmental Effects Profile (HEEP) for 2,4-dimethylaniline and 2,4-dimethylaniline hydrochloride. The human carcinogen potency factor (q1*) for 2,4-dimethylaniline is 0.75 $(mg/kg\text{-}day)^{-1}$ for oral exposure, and the Reportable Quantity (RQ) value is 1000 pounds under CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act) (U.S. EPA, 1987). The HEAST lists an oral unit risk for 2,4-dimethylaniline of 2.1×10^{-5} $(\mu g/L)^{-1}$ based on mouse lung tumors (Weisburger et al., 1978). HEAST classifies 2,4-dimethylaniline as a Group C carcinogen ("possibly carcinogenic to humans: agents with limited animal evidence and little or no human data") (U.S. EPA, 2010b).

The International Agency for Research on Cancer (IARC) reviewed the carcinogenic potential of 2,4-dimethylaniline and concluded that no adequate human data existed and inadequacies of animal studies did not allow for an evaluation of carcinogenicity (IARC, 1978). An IARC update subsequently classified the chemical in Group 3 ("not classifiable as to carcinogenicity to humans") (IARC, 1987). In addition, the Health Council of the Netherlands (2002) has concluded that there is insufficient information to classify 2,4-dimethylaniline for carcinogenicity.

CalEPA has not derived toxicity values for exposure to 2,4-dimethylaniline nor have they derived quantitative estimates of the carcinogenic potential of 2,4-dimethylaniline (CalEPA, 2008, 2009a,b,c). 2,4-Dimethylaniline is not included in the *11th Report on Carcinogens* (NTP,

2005). The toxicity of 2,4-dimethylaniline has not been reviewed by ATSDR or the World Health Organization (WHO) (ATSDR, 2009; WHO,1986).

No occupational exposure limits or guidelines have been derived by the Occupational Safety and Health Administration (OSHA), National Institute of Occupational Safety and Health (NIOSH), or the American Conference of Governmental Industrial Hygienists (ACGIH) for 2,4-dimethylaniline (ACGIH, 2001; NIOSH, 2009; OSHA, 2009a). However, exposure limits have been derived for mixed xylidine isomers (CASRN 1300-73-8). For mixed xylidine isomers (including 2,4-dimethylaniline), the OSHA permissible exposure limit (PEL) time-weighted average (TWA) is 2 ppm (10 mg/m^3) [skin], the NIOSH recommended exposure limit (REL) TWA is also 2 ppm (10 mg/m^3) [skin], and the ACGIH threshold limit value (TLV) is 0.5 ppm (2.5 mg/m^3) as a TWA (inhalable vapor; skin) (NIOSH, 1994; OSHA, 2009b; ACGIH, 2001; RTECS, 2009). The NIOSH Immediately Dangerous to Life or Health (IDLH) concentration is 50 ppm for mixed xylidine isomers, but this is stated to be possibly conservative due to the lack of relevant acute toxicity data for human workers (NIOSH, 1996). The ACGIH (ACGIH, 2008) has classified mixed xylidine isomers in Group A3 ("confirmed animal carcinogen with unknown relevance to humans") (HSDB, 2009).

Literature searches were conducted on sources published from 1900 through October 2010 for studies relevant to the derivation of provisional toxicity values for 2,4-dimethylaniline (CAS No. 95-68-1). Searches were conducted using EPA's Health and Environmental Research Online (HERO) evergreen database of scientific literature. HERO searches the following databases: GRICOLA; 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, Multi-Database Search, NIOSH, NTIS, PESTAB, PPBIB, RISKLINE, TRI; and TSCATS; Virtual Health Library; Web of Science (searches Current Content database among others); World Health Organization; and Worldwide Science. The following databases outside of HERO were searched for risk assessment values: CGIH, ATSDR, CalEPA, EPA IRIS, EPA HEAST, EPA HEEP, EPA OW, EPA TSCATS/TSCATS2, NIOSH, NTP, OSHA, and RTECS.

REVIEW OF POTENTIALLY RELEVANT DATA (CANCER AND NONCANCER)

Table 2 contains information on all the potentially relevant studies, and the principal study (PS) has been bolded.

Table 2. Summary of Potentially Relevant Data for 2,4-Dimethylaniline (CASRN 95-68-1)

Notes[a]	Category	Number of Male/Female Species, Study Type, and Duration	Dosimetry[b]	Critical Effects	NOAEL[b,c]	BMDL/ BMCL[b]	LOAEL[b,c]	Reference	Comments
Human									
				1. Oral					
				None					
				2. Inhalation					
				None					
Animal									
				1. Oral					
PS	**Chronic**	**10 M/10 F Osborne-Mendel rats per group, oral 2,4-dimethylaniline, 6 months**	**Male ADJ: 18, 36, 148, 329, or 1137 mg/kg-day Female ADJ: 26, 55, 209, 511, or 1304 mg/kg-day**	**Increased relative liver and kidney weights observed at all doses in a dose-related manner; cholangiofibrosis, bile duct proliferation, occasional necrosis and foci of hyperplastic cells in liver; in the kidney, tubuli, edema, papillary necroses and casts; relative kidney and liver weights increased at all doses**	**None**	**18.87 mg/kg-day (increased relative kidney wt., females)**	**18 mg/kg-day (males), 26 mg/kg-day (females)**	**Lindstrom et al. (1963)**	
	Short-term	5/5 Sprague-Dawley rats, gavage, 4 wks	Male/Female ADJ: 475 mg/kg-day	Hepatomegaly and enlargement of hepatocytes; decreased liver glycogen and glucose-6-phosphatase activity with occasional necrotic cells; increased absolute and relative liver weights, decreased body weight in male rats; elevated glucuronyl transferase concentration	N/A	Not run	475 mg/kg-day	Magnusson et al. (1979)	Dose of 400 mg/kg-day adjusted by authors after 1 week to 500 mg/kg-day; LOAEL identified by causing 10% increase in absolute and relative liver weight considered to be biologically significant
		5/5 Sprague-Dawley rats per group, gavage, 4 wks	Male/Female ADJ: 20, 100, or 600 mg/kg-day	Increased liver and kidney weights at all doses; at highest dose, bile duct proliferation and liver cell necrosis, with decreased hematocrit and hemoglobin levels	100 mg/kg-day	Not run	600 mg/kg-day	Magnusson et al. (1971)	High dose of 500 mg/kg-day adjusted by authors after 2 weeks to 700 mg/kg-day; LOAEL identified by causing 10% increase in liver and kidney weight considered to be biologically significant

Table 2. Summary of Potentially Relevant Data for 2,4-Dimethylaniline (CASRN 95-68-1)

Notes[a]	Category	Number of Male/Female Species, Study Type, and Duration	Dosimetry[b]	Critical Effects	NOAEL[b,c]	BMDL/ BMCL[b]	LOAEL[b,c]	Reference	Comments
		1/1 Beagles per group, gavage, 4 wks	Male/Female ADJ: 2, 10, 50 mg/kg-day	Highest dose resulted in increased liver weight, and fatty degeneration; two highest doses induced emesis, body wt. reduction and increased liver to body wt. ratio	N/A	Not run	N/A	Magnusson et al. (1971)	No statistical significance tests performed in study
		10 F344 rats per duration of 5,10, or 20 d	Male ADJ: 117 mg/kg-day	Liver lesions in rats: extensive cloudy swelling and necrosis, early periacinar necrosis with connective tissue proliferation, biliary hyperplasia for the shortest duration; at the longest duration, periacinar vacuolar degeneration with occasional discrete foci; liver and kidney weights elevated in all duration groups	N/A	Not run	117 mg/kg-day	Short et al. (1983)	LOAEL based on significantly increased liver and kidney weights
	Carcinogenic	50 Sprague-Dawley male rats, oral, 2 yrs	Not known	Excess subcutaneous fibromas or fibrosarcomas in treated animals; excess hepatomas also occurred	N/A	Not run	N/A	IARC (1978) as cited in HSDB (2009)	Statistical analyses not available, data possibly from an abstract
		25 Sprague-Dawley male rats, oral, 24 months	Male HED: 10.9 and 22.1 mg/kg-day; duration adjusted over 24 mos	None; no effects observed even at highest dose	22.1 mg/kg-day	Not run	N/A	Weisburger et al. (1978)	Feed concentrations adjusted by authors
PS	**Carcinogenic**	**25/25 CD-1 HaM/ICR mice, oral, 21 months**	**Male HED: 2.8 and 5.6 mg/kg-day Female HED: 2.9 and 5.8 mg/kg-day; adjusted for 21 mos**	**None in males; lung tumors statistically significant at highest dose in females**	**2.9 mg mg/kg-day**	**Not run**	**5.8 mg/kg-day**	**Weisburger et al. (1978)**	

Table 2. Summary of Potentially Relevant Data for 2,4-Dimethylaniline (CASRN 95-68-1)

Notes[a]	Category	Number of Male/Female Species, Study Type, and Duration	Dosimetry[b]	Critical Effects	NOAEL[b,c]	BMDL/ BMCL[b]	LOAEL[b,c]	Reference	Comments
2. Inhalation									
	Subchronic	LAS and Swiss strain mice, rats (unknown strain), rabbits, cats, dogs, monkeys, and chicks; up to 40 wks	7 hrs/d, 5 d/wk, 223 mg/m^3 vapor (isomeric mixture)	Mortality (except monkeys and chicks) and liver damage (except chicks) in all species; cats, dogs, mice had elevated methemoglobin levels and increased numbers of Heinz bodies	N/A	Not run	223 mg/m^3 based on isomeric mixture	Von Oettingen et al. (1947)	Isomeric mixture used; dose not converted to HEC nor adjusted for study duration
		Unknown numbers of rats (unknown strain), guinea pigs, rabbits, cats, monkeys, unspecified duration	7 hrs/d, 5 d/wk, doses ranging from 36 mg/m^3 to 703 mg/m^3	Mortality, pneumonitis, degeneration of cells in heart, liver, kidneys	Not stated	Not run	Not stated	Treon et al. (1950)	Isomeric mixture used; dose not converted to HEC nor adjusted for study duration

[a]Notes: PS = Principal study.
[b]Dosimetry, NOAEL, BMDL/BMCL and LOAEL values are converted to Human Equivalent Dose (HED in mg/kg-day) or Human Equivalent Concentration (HEC in mg/m3) units. Noncancer oral data are only adjusted for continuous exposure. Dose = Feed Concentration × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days), where daily Food Consumption rates used were from EPA's (1988) default subchronic for Osborne Mendel rats [0.023 kg (males), 0.019 kg (females)]. Dose = Adjusted Dose, since both Days Dosed and Total Days were 182.
[c]Not reported by the study author, but determined from data.

HUMAN STUDIES

No data on the effects of 2,4-dimethylaniline in humans following inhalation or oral exposure were located in the literature searches. It has been noted that occupational hazards include burns to the skin and eyes, and that the chemical is toxic via inhalation, ingestion, and dermal absorption (HSDB, 2009). In addition, it was stated that a 1-hour exposure to 400 ppm or long-term exposure to 10 ppm of mixed methylaniline isomers would be lethal to humans, although no epidemiological or occupational information exists (ACGIH, 1988, as cited in OSHA, 2009b; ACGIH, 2001).

ANIMAL STUDIES

Oral Exposure

In a published peer-reviewed chronic-duration study, Lindstrom et al. (1963) administered dietary doses of 0, 375, 750, 2500, 5000, and 10,000 ppm (0, 18, 36, 148, 329, and 1137 mg/kg-day in males, 0, 26, 55, 209, 511, and 1304 mg/kg-day in females [the calculations for adjusted doses are shown in Table 2]) of 2,4-dimethylaniline (purity unknown) to groups of 10 Osborne-Mendel rats per sex, per group for 6 months (n = 120). **This study was selected as the principal study for derivation of the screening chronic and subchronic p-RfDs.** In this study, corn oil (3% in feed) was used as a vehicle even in control feed (Lindstrom et al., 1963). Food and water were provided ad libitum, and rats were weighed weekly. At the end of 3 months, 4/20 rats from each dose level were chosen for sacrifice; only high-dose rats were additionally given microscopic examinations of the liver, kidneys, and testes (males) or adrenals and spleen (females). After 6 months, the remaining 96 rats were sacrificed, and 26 rats (4 from each dose group, plus 6 controls) were chosen for sacrifice in an unbiased fashion (by order of animal number) for microscopic examination of liver, kidneys, and spleen. Another 8/20 rats were sacrificed from the high-dose group for examination of the pancreas, stomach, small intestine, colon, and adrenals. Also at the termination of the study at 6 months, blood analyses were collected from 10 animals per dose group, and organ-weight data were reported. In this study, the authors were not consistent with time period reporting; results were reported at 12 weeks or 13 weeks or 3 months, as well as 6 months or 26 weeks. Explanations were not given for the varying time periods reported.

A total of four rats (one from the control group and three from the 2,4-dimethylaniline groups) died before the completion of the study. However, no differences in mortality rates were observed, while statistically significant decreases in body-weight gain were observed at the three highest dose levels, both at 12 weeks and at 6 months in male and female rats. Data on body weight changes at 6 months are shown in Appendix C. At 6 months, target cell anemia was observed in a dose-related fashion, but statistical analyses were not shown by the authors.

After 6 months, relative (liver-to-body-weight) liver weight was statistically significantly increased in both males and females in a dose-related fashion (Lindstrom et al., 1963). Data on relative liver weight changes at the end of 6 months are shown in Appendix C. There were elevated relative liver weights observed even at the lowest dose, significant at the $p < 0.05$ level in males and females. Livers at the two highest doses (5000 and 10000 ppm) demonstrated pale foci ranging from 0.5 mm to >2 mm scattered throughout the parenchyma (presumed in both sexes). At the 2500 ppm dose, a half-dozen pinpoint-sized foci were observed, but no foci were observed below this dose. High-dose animals sacrificed at 3 months did not show the same gross liver and kidney effects observed in high-dose animals at 6 months, although milder changes (not described) were noted. On microscopic examination of animals sacrificed at 6 months, three of

the four (2 males, 1 female) highest dose animals showed large foci of 0.5 to 3.0 mm diameter of cholangiofibrosis (nonneoplastic bile duct proliferation) while one female rat had none. Ducts were irregular, relatively large, and often filled with necrotic debris; surrounding the ducts was considerable fibrosis. Of the four high-dose animals examined at 13 weeks, one (sex unspecified) showed early stages of this process. Rats at lower doses of treatment were not microscopically examined at 13 weeks.

In addition, in livers at the highest dose, there was a moderate amount of scattered new small bile duct formation; at 13 weeks, there was limited evidence of this at the highest dose, but it was less defined (Lindstrom et al., 1963). The authors also noted rounded foci of hepatic cell hyperplasia ranging from 0.5 to 4 mm in diameter, with some irregularly shaped foci. Occasional individual necrotic cells were seen at 6 months, but at 13 weeks, necrosis was more pronounced. At the highest dose at Week 13, liver damage was graded as slight (2/4) and moderate (2/4), while at 6 months, livers were graded as slight to moderate (1/4), moderate (1/4), or moderate to marked (2/4); there were no sex differences observed at either period. At the second highest dose of 5000 ppm, there was less liver damage than in the high dose—including an absence of cholangiofibrotic foci and reduction of new small bile duct formation. Hyperplastic foci were present but less well defined. Overall liver damage in this group was slight in the two males and slight-to-moderate in the two females. In the third dose group of 2500 ppm, livers appeared relatively normal, but slight formation of new bile ducts and a few poorly-defined hyperplastic hepatic cell foci were still evident in females. Overall liver damage was graded as minimal but definite in the two females, intermediate in one male rat, and almost normal in the other male rat. No liver abnormalities attributable to treatment could be determined in the two lower dose groups of 375 and 750 ppm.

Relative kidney weight (kidney-to-body weight) was statistically significantly increased in both males and females in a dose-related fashion (Lindstrom et al., 1963). Data on kidney-weight changes at the end of 6 months are shown in Appendix C. At the lowest dose, relative kidney weights were significantly increased at the $p < 0.05$ level in males and females. Gross pathology revealed slight or moderate irregular pitting or depressed scarring at the highest dose (presumed in both sexes), and microscopic examination revealed similar effects as observed in the livers. The effects included cortical foci of tubular atrophy and interstitial fibrosis with chronic inflammation progressing to depressed scar formation, as well as papillary changes (edema, cast formation in small looped tubules, progression to necrosis in the lower end of the papilla). In addition, there were less serious changes observed such as cystic dilation of tubular segments around the corticomedullary junction. Across all doses, kidney damage varied from little to moderate gradation among the animals and averaged at least slight. The same general changes were seen at 13 weeks as those seen at 6 months, though in earlier stages and less noticeable on gross examination. At the second highest dose, 5000 ppm, some kidney damage was evident, but it was so slight that the authors could not say whether it was due to treatment-related effects. Forestomachs of rats showed slight hyperkeratosis at the highest dose. All other rat organs at the highest dose were normal at 6 months, and similarly, no abnormalities were seen at the highest dose at 13 weeks. Rats at lower doses of treatment were not microscopically examined at 13 weeks.

This study supports the development of a p-RfD because of the well documented and scientifically acceptable nature of the publication. The LOAEL for Lindstrom et al. (1963) is

identified as 375 ppm (18 mg/kg-day [males]; 26 mg/kg-day [females]) for significantly increased liver and kidney weights at the lowest dose; no NOAEL is identified.

Inhalation Exposure

In one inhalation study, mice (6–29 weeks), rats (28 weeks), rabbits (23 weeks), cats (3 weeks), dogs (6.5 weeks), chicks (11 weeks), and monkeys (40 weeks) were exposed to 45 ppm (223 mg/m^3) of an isomeric mixture of xylidine vapor (purity not known) for 7 hours/day, 5 times/week, respectively (Von Oettingen et al., 1947). Strain (Swiss and LAS) was only mentioned for mouse. Mortality (except monkeys and chicks) and liver damage (except chicks) were reported in all species. Cats, dogs, and mice (but not rats, rabbits, chicks, or monkeys) had elevated methemoglobin levels and increased number of Heinz bodies. This study does not provide adequate information regarding the toxicity of 2,4-dimethyaniline due to the employment of an isomeric mixture of xylidines (Von Oettingen et al., 1947).

In a second study, monkeys, rats, guinea pigs, rabbits, and cats exposed to 2,4-dimethylaniline vapor at concentrations of 7.8 to 142 ppm (36 to 703 mg/m^3) for 7 hours/day, 5 times/week, for an unspecified duration had mortality, pneumonitis and degeneration of cells in the heart, liver, and kidneys (Treon et al., 1950). Strain was not mentioned for any of the experimental animals and it appears that there was no control group. All species except for the cat, which demonstrated liver toxicity, tolerated doses of 17.5 ppm (87 mg/m^3). One monkey (not mentioned previously) and two cats tolerated 92 exposures at 7.8 ppm (36 mg/m^3) without any effect. Animals were treated with an isomeric mixture of xylidines and the amount of 2,4-dimethyaniline in this mixture is unknown.

It was briefly reported in a third study that the NOEL, after repeated inhalation of 2,4-dimethylaniline as an aerosol-vapor mixture, was 6 ppm (30 mg/m^3), and that effects included chronic inflammation of the airways; methemoglobinemia; and damage to the liver, kidneys, and heart, which was detected by histopathological exams (Anonymous-German, 1993, as cited in HSDB, 2009). Study duration or species tested was unknown, and no other study details were reported; the original study was not available for review at this time and, therefore, it was not possible to determine whether this study referred to the previous study by Treon et al. (1950).

Chronic or Cancer Studies

Two carcinogenicity studies have been identified in the literature for 2,4-dimethylaniline. In the first, 50 male Charles River (Sprague-Dawley) rats were given 2,4-dimethylaniline (purity not known) in feed for 2 years. Thirty-nine percent (39%) of treated rats had subcutaneous fibromas or fibrosarcomas compared to only 16% of controls. The study also noted excess hepatomas in treated rats, but the original source (an abstract, according to the Health Council of the Netherlands, 2002) was not available for review at this time (IARC, 1978, as cited in HSDB, 2009).

In a published peer-reviewed carcinogenicity study, Weisburger et al. (1978) administered 2,4-dimethylaniline hydrochloride (97−99% purity) in the diet to male Charles River rats and male and female albino CD-1 HaM/ICR mice. The chemical was one of 21 aromatic amines or derivatives tested for carcinogenicity in rats and mice. The doses were administered in the diet at the maximum tolerated dose (MTD) and half of the known MTD; however, weight gain was monitored carefully such that if gains were equal or greater than

10% lower than in corresponding controls, or death occurred, doses were lowered. Control animals were observed simultaneously and received only laboratory chow. Complete histological examination was done for all grossly abnormal organs, and statistical analysis of tumors was performed using Fisher's exact test. Nonneoplastic degenerative or inflammatory lesions were recorded but were discussed only if they were considered to be compound related.

Twenty-five male rats per group were treated for 18 months, followed by 6 months of observation. Feed concentration correction was needed in rats during the study (Weisburger et al., 1978). The low concentration (administered in feed) was 2000 mg/kg for 3 months, 250 mg/kg for 2 months, and then 500 mg/kg for 13 months. High-dose rats received 4000 mg/kg for 3 months, 500 mg/kg for 2 months, and then 1000 mg/kg for 13 months in feed. Duration-adjusted for the 24-month study, controls received 0 mg/kg-day, low-dose rats received 542 mg/kg 2,4-dimethylaniline in feed per day, while high-dose rats received 1083 mg/kg 2,4-dimethylaniline in feed per day[1] (0, 37, and 75 mg/kg-day, respectively[2]). The corresponding Human Equivalent Doses (HEDs) were 0, 10.9, and 22.1 mg/kg-day[3], respectively. These HEDs are shown in Table 2 and have been calculated based on duration-adjusted doses during the 24 months of the study, using EPA (1988) default factors for Sprague-Dawley rats. A $NOAEL_{HED}$ of 22.1 mg/kg-day is identified based on no effects being observed at any dose in male Sprague-Dawley rats.

In the same carcinogenicity study, albino CD-1 HaM/ICR mice were administered 2,4-dimethylaniline hydrochloride in the diet according to a similar study protocol investigating 21 aromatic amines (Weisburger et al., 1978). Twenty-five mice per sex per dose group were treated for 18 months followed by 3 months of observation. **This mouse study by Weisburger et al., 1978 is selected as the principal study for deriving the provisional oral slope factor (p-OSF).** Concentrations administered were equivalent to 125 and 250 mg/kg in feed followed by 3 months of observation and were duration-adjusted for the 21 months of the study to 107 and 214 mg/kg in feed per day[4] (0, 19, and 39 mg/kg-day in males, 20 and 40 mg/kg-day in females[5]). The corresponding HEDs were 0, 2.8, and 5.6 mg/kg-day and 0, 2.9, and 5.8 mg/kg-day for males and females, respectively[3]. Male mice did not have tumor incidences in excess of controls. In females, however, lung tumors occurred in 28% of animals (5/18) at the low dose, and in 58% (11/19) at the high dose, compared to 23% in concurrent controls. Lung tumors in female mice occurred with a statistically significant positive trend (Cochran-Armitage trend test performed for this analysis, $p = 0.01$), and the tumor incidence was significant at the high dose compared to concurrent controls by Fisher's exact test ($p < 0.05$).

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[1]Calculated by averaging feed concentrations for the 24 months of study duration (3 months at the initial concentration, then 2 months at the next concentration, then 13 months at the last concentration, followed by 6 months of recovery).

[2]Adjusted dose = Average Feed Concentration during treatment × Food Consumption per Day × (1 ÷ Body Weight) × (Months Dosed ÷ Total Months), where body weights used were from EPA's (1994b) default chronic values for male Sprague-Dawley Rats (0.523 kg) and where feed intakes used were from EPA's (1988) default chronic values for male Sprague-Dawley Rats (0.036 kg); Months Dosed was 18, and Total Months was 24.

[3]Human Equivalent Dose = Adjusted dose × $[BW_{animal} \div BW_{human}]^{0.25}$ where BW_{animal} was obtained from EPA's (1994b) default chronic values for male Sprague-Dawley Rats (0.523 kg) and 'Other' mouse strains (0.0317 kg males, 0.02875 kg, females) and where BW_{human} (70 kg) was obtained from EPA's Exposure Factors Handbook (1997).

[4]Calculated by averaging feed concentrations for the 21 months of study duration (18 months of treatment followed by 3 months of recovery).

[5]Adjusted dose = Feed Concentration × Food Consumption per Day × (1 ÷ Body Weight) × (Months Dosed ÷ Total Months), where body weights used were from EPA's (1994) default chronic values for 'Other' mouse strains (0.0317 kg males, 0.02875 kg, females) and where feed intakes used were from EPA's (1988) default chronic values for 'Other' mouse strains (0.0057 kg males, and 0.0053 kg females); Months Dosed was 18, and Total Months was 21.

Based on increased lung tumor incidence, a $LOAEL_{HED}$ of 5.8 mg/kg-day is identified. A $NOAEL_{HED}$ is identified as 2.9 mg/kg-day. The study supports the development of a p-OSF because of the well documented and scientifically acceptable nature of the publication.

OTHER STUDIES

Short-term Oral Studies

In a 4-week study, five Sprague-Dawley derived CFY rats per sex (n = 10) were gavaged once daily with 0 or 400 mg/kg of 2,4-dimethylaniline during the first week and then 500 mg/kg for the following 3 weeks (thus having a duration-adjusted dose of 475 mg/kg-day for treated animals) (Magnusson et al., 1979). Control rats were given saline at the same dosage volumes as the treated group. Rats were observed daily and weighed once per week. Autopsies were performed on all rats regardless of when they died. Biochemical parameters were measured, including cytochrome P450, aniline hydroxylase, and glucuronyl transferase. Statistical analyses were performed using Bartlett's t-test to compare treated rats to controls.

No deaths were attributed to treatment, but there was a statistically significant decrease in male body weights (Magnusson et al., 1979). Both sexes had increased liver and liver-to-body weight ratios ($p < 0.05$). Enlargement of hepatocytes was observed, and this effect was statistically significant in females, primarily in the centrilobular regions. Occasional isolated necrotic cells were found, along with a centrilobular decrease in liver glycogen that was most pronounced in males. Glucose-6-phosphatase enzyme activity was statistically significantly decreased ($p < 0.05$) in the centrilobular region in male rats. In addition, proliferation of smooth endoplasmic reticulum was observed along with isolated degenerative hepatocytes containing vacuoles and inclusion bodies. There were also dilated bile canaliculi associated with loss or atrophy of microvilli and occasional pigmented Kupffer cells. Biochemical results demonstrated that the concentration of glucuronyl transferase was statistically significantly elevated in both male and female rats ($p < 0.05$). Hepatic microsomal protein content was statistically significantly increased in male rats but not significantly elevated in female rats. Other enzyme elevations were observed but were not statistically significant. The authors postulated that hypertrophy and hyperplasia may have occurred in the liver of treated rats, based on hepatocyte size and increased liver weights (Magnusson et al., 1979). LOAEL of 475 mg/kg-day is identified by causing 10% increase in absolute and relative liver weight considered to be biologically significant.

In a 4-week study, 10 young Sprague-Dawley rats, 5 of each sex per dose, were treated by gavage once per day (no dose adjustment needed) with 0, 20, 100, or 500 mg/kg 2,4-dimethylaniline (Magnusson et al., 1971). After 2 weeks of treatment, the dose in the highest dose group was increased to 700 mg/kg-day resulting in an adjusted dose of 600 mg/kg-day. Food and water were given ad libitum, and rats were observed daily for clinical effects. Hematology and blood chemistry were examined upon termination of the study. Liver and kidneys were examined microscopically. Tests of statistical significance were not shown or discussed by the authors in this study.

There were six mortalities that occurred during the study at the highest dose, from Days 6 to 25 (Magnusson et al., 1971). In females and in males at the highest dose, decreased weight gain (qualitative statement given) was observed. Clinical examination found decreased hemoglobin concentrations and hematocrit concentrations at the highest dose, particularly in females. Serum urea-nitrogen levels were normal, but increased values for ornithine

carbamyltransferase (OCT) were observed in 2/10, 1/10, and 4/4 rats at the low, mid-, and high dose, respectively. Hyperkeratosis of the forestomachs was observed at the highest dose, which likely represented irritation of the stomach. Gross pathology revealed liver enlargement and increased liver weights in treated rats at all doses; high-dose livers contained occasional reddish and greyish foci in sizes of 0.5−2 mm. These foci were most evident in rats that died, but otherwise, livers did not show any other biologically significant change.

Microscopic examination revealed necrosis and vacuolization of hepatocytes in all rat livers at the highest dose, with necrosis appearing as scattered foci in primarily the midzone of hepatic lobules (Magnusson et al., 1971). Necrotic foci were small and well defined, while large foci were more irregular in shape. Some necrotic areas had hemorrhage and cellular infiltration of histiocytes with some neutrophilic granulocytes. In the centrilobular areas, various sized vacuoles, either empty or with filamentous content, were observed in hepatic cells. Proliferation of bile ducts was observed at the highest dose as well. No fatty change in liver was evident, and the kidneys had a normal appearance. The authors postulated that focal hepatic necrosis likely resulted from insufficient nutrition of liver cells due to low blood pressure and reduced circulation, supported by the fact that the necrosis was most common in rats that died, and, thus, focal hepatic necrosis was not a toxic effect of the chemical itself. Although statistical significance was not discussed by authors, the identified NOAEL is 100 mg/kg-day, and the LOAEL is 600 mg/kg-day based on the biological significance of a 10% increase in relative liver and kidney weight in Sprague-Dawley rats.

The same authors conducted the same study in dogs; one male and one female beagle were administered 0, 2, 10, or 50 mg/kg-day of 2,4-dimethylaniline (purity not known) orally in capsules daily for 4 weeks (Magnusson et al., 1971). Tests of statistical significance were not possible given the small sample size used in this study. Dogs at the two highest doses vomited within the first 4 hours after treatment, with more vomiting seen at the highest dose. At the two highest doses, body weights were reduced, and liver-to-body-weights were increased. Values for clinical chemistry were within normal ranges. The highest dose showed enlarged, pale liver; microscopic pathology showed fatty degeneration at the highest dose level. The kidneys were not markedly affected by treatment.

In another study, male F344 rats were administered 2,4-dimethylaniline (98.7% purity) by gavage at doses of 117 mg/kg-day (25% of the LD_{50} determined by study authors) for either 0, 5, 10, or 20 days (Short et al., 1983). Ten animals in each dose group plus 30 controls were sacrificed at the appointed time ($n = 60$). Daily observations of body weight and food and water consumption were obtained. Histopathology of liver, spleen, thyroid, bladder, and kidneys was conducted. Analysis of body and organ weight was done using ANOVA and Dunnett's test, while scoring for lesions and mortality were analyzed using Fisher's exact test.

Two rats died in the mid-duration group, and one died from the longest-duration group; however, mortality was stated as not significant (Short et al., 1983). Clinical observation revealed thinness and rough hair coat. In addition, body weight was depressed at all durations of treatment. Liver weights and liver-to-body-weight ratios, as well as kidney weights (and kidney-to-body weight ratios) were statistically significantly increased ($p < 0.05$) in all duration groups. Histopathology revealed no significant treatment-related effects on spleen or bone marrow. In the liver, statistically significant toxicity was noted at the shortest duration (5 days) with extensive cloudy swelling and necrosis, early periacinar necrosis with connective tissue

proliferation, and biliary hyperplasia. At the longest duration (20 days), periacinar vacuolar degeneration with occasional discrete foci was observed. According to the researchers, the study demonstrated toxic hepatopathy as characterized by liver lesions in rats (Short et al., 1983). The LOAEL is identified to be 117 mg/kg-day based on significantly increased absolute kidney and liver weight in male Fischer 344 rats.

In an oral exposure study, 10 male and 10 female Charles River CD (Manston) rats were given 0 or 400 mg/kg-day 2,4-dimethylaniline (purity unknown) for 7 days in an oral saline solution (Gopinath et al., 1980). A control group was given an equal volume of saline; both groups were given feed and water ad libitum. Blood samples were then collected, and serum bile acid and enzyme concentrations were measured as an indicator of liver cell injury. Clinical biochemistry measures included alkaline phosphatase (AP), glutamate pyruvic transaminase (GPT), glutamic dehydrogenase (GDH), and total and conjugated bilirubin. Liver histopathology was examined as well. Statistical significance was not given, but rather results were shown by histograms, which demonstrated elevated GPT, GDH, and bile acids (but not AP) in treated animals. Treated male rats showed higher elevations than females. Examination of the liver revealed cell enlargement, occasional cell necrosis, and/or minimal bile duct hyperplasia and degeneration. Electron microscopy revealed dilated bile canaliculi with loss of microvilli and proliferation of smooth endoplasmic reticulum. There appeared to be an overall reduction in the canalicular ATPase in the treated rats. The authors concluded that treatment with 2,4-dimethylaniline induced hepatotoxicity and altered liver function (Gopinath et al., 1980).

Acute Oral Studies

An acute lethality study determined an LD_{50} of 470 mg/kg (ranging from 320−690 mg/kg) in rats and an LD_{50} of 250 mg/kg (ranging from 150−420 mg/kg) in mice (Vernot et al., 1977). Another acute study in rats determined the oral LD_{50} to be 1259 mg/kg (Lindstrom et al., 1969).

In a brief abstract, it was reported that Takahashi et al. (1974) administered a single oral dose of 157.6 mg/kg of 2,4-dimethylaniline HCl (purity not known) to mice (strain and number unspecified). Biochemical and morphological changes were observed, with acidophilic granules and bodies appearing 24 hours after dosing and increasing markedly by 48 hours after dosing. Microscopic examination revealed increased lysosomes, dilatation of endoplasmic reticulum, and autolysome and focal degeneration of hepatocytes at 24 hours. Glucose-6-phosphate dehydrogenase and lysosomal enzymes in liver soluble fractions were increased at 12 and 48 hours after dosing and did not recover by 72 hours after dosing. In addition, radioactive 2,4-dimethylaniline-^{3}H demonstrated highest radioactivity levels during a 72-hour period.

In rabbits (strain and number unspecified), it was stated that an isomeric mixture of an unknown impure composition consisting of 2,4-dimethylaniline dissolved in isooctane was fatal even at doses of 0.5 g/animal while 0.1 g/animal was tolerated (Anonymous-German, 1993, as cited in HSDB, 2009).

Acute Inhalation Studies

An acute inhalation lethality study determined an LC_{50} (4-hour) of 1.53 mg/L for the rat (strain and number unspecified); irritation of the eyes and snout were seen in addition to labored breathing. Furthermore, exhaustion, dyspnea, and terminal convulsions were evident (Anonymous-German, 1993, as cited in HSDB, 2009). The LC_{50} (7-hour) in mice (strain not

known) is reported to be 149 ppm or 738 mg/m^3 (von Oettingen et al., 1947, as cited in NIOSH, 1996).

Other Exposure Routes

Following a single intravenous injection of 20 mg, the blood methemoglobin content of rats (number or strain not discussed) increased from 1.5% to 3.5% after 1 hour (IARC, 1978 in HSDB, 2009). In another intravenous injection study in cats, 0.25 mmol/kg of mixed methylaniline isomers caused a 6.3%−38.3% increase in methemoglobin (McLean et al., 1969).

The compound is considered irritating to the skin and eyes of rabbits (Anonymous-German, 1993, as cited in HSDB, 2009). Mixed methylaniline isomers can be absorbed through the skin in rabbits to cause cyanosis and death (Proctor et al., 1988, as cited in OSHA, 2009b). Exposure to mixed methylaniline isomers caused injury to the rabbit cornea on a scale of 5/10, where 10/10 was the most severe (Grant, 1986, as cited in OSHA, 2009b).

Liver damage and effects on the blood were observed after repeated dermal application of an isomeric methylaniline mixture to dogs and cats (Anonymous-German, 1993, as cited in HSDB, 2009). No additional details were available.

In cats, dermal administration of 2000 mg/kg for 24 hours resulted in methemoglobin formation and increases in Heinz bodies in the blood. It was noted that the compound tested in these studies was an isomeric mixture of unknown composition rather than pure 2,4-dimethylaniline (Anonymous-German, 1993, as cited in HSDB, 2009).

In a study of unknown route, cats exposed to a mixed methylaniline isomer concentration of 138 ppm became uncoordinated, prostrate, and cyanotic before death. Duration of exposure was unknown, and study details were not available for review at this time. Autopsy revealed edema of the lungs, pneumonia, and damage to the liver and kidneys (Proctor et al., 1988, as cited in OSHA, 2009b).

Metabolism Studies

In a urine metabolite study, 117 mg/kg-day (25% of the LD_{50} determined by the study authors) of 2,4-dimethylaniline (>99% purity) was administered in a corn oil gavage for 10 days to 16 young male F344 rats (Short et al., 1989). Pooled 24-hour urine samples were collected on Days 1 and 10 and analyzed for the parent compounds and metabolites. Animals were weighed on Day 5, and doses were adjusted accordingly to maintain a constant mg/kg dose. A paired *t*-test was used to compare effect of length of treatment (Day 1 vs. 10) on urine excretion products within each dosing group. The study authors found that the chemical was excreted as *N*-acetyl-4-amino-3-methylbenzoic acid (AAMBA), the parent compound, and the sulfate or glucuronide conjugates of these compounds. There was no significant difference in the total excretion of either the parent compound or AAMBA between Days 1 and 10, thus demonstrating that the chemical did not induce its own metabolism. The authors hypothesized that the metabolite AAMBA and its conversion to *N*-hydroxy-2,4-dimethylaniline could be responsible for the liver toxicity observed in rats.

In the same study, five purebred beagle dogs received 25 mg/kg-day of 2,4-dimethylaniline (98.7% purity) for 10 days, administered orally in gelatin capsules with no vehicle (Short et al., 1989). Dogs were weighed every 5 days and doses adjusted. Twenty-four-

hour urine samples were collected on Days 1 and 10 and analyzed. The chemical was excreted as 6-hydroxy-2,4-dimethylaniline (6-HDMA), the parent compound, and 4-amino-3-methylbenzoic acid (4-AMBA). In both rats and dogs, *N*,2,4-trimethylamine was detected at low concentrations inadequate for quantitation. There were no marked differences in urine content of *N*,2,4-trimethylamine at either Day 1 or 10. The authors noted that 2,4-dimethylaniline was markedly less toxic in the dog than the rat, possibly due to rapid 6-hydroxylation of the parent compound or the diminished amount of 4-AMBA produced.

Genotoxicity

Results from genotoxicity tests are mixed but generally positive; results are shown in Tables 3 (in vitro) and 4 (in vivo). In the Ames mutagenicity assay, Zeiger et al. (1988) tested mutagenicity of 2,4-dimethylaniline with *Salmonella typhimurium* strains TA 97, TA 98, TA 100, TA 1535, and TA 1537. Strains TA 98 and TA 100 with both hamster and rat liver S9 metabolic activation resulted in positive tests for mutagenicity at concentrations of 10−1000 μg/plate, while only the hamster S9 mix tested positive in strain TA 97 at the same concentrations. Strains TA 97, TA 98, TA 100, and TA 1535 were tested with no metabolic activation and found to be negative at concentrations of 33−1666 μg/plate, and even with metabolic activation using hamster and rat liver S9 mix, strains TA 1535 and TA 1537 tested negative. Shimizu and Takemura (1983, as cited in CCRIS, 2005) also tested strains TA 98 and TA 100 in the Ames assay; TA 98 tested negative both with and without activation at 0−5000 μg/plate, while TA 100 was negative without activation but positive with S9 activation at 0−5000 μg/plate.

Table 3. Genotoxicity Studies of 2,4-Dimethylaniline In Vitro

Test System	Endpoint	Test Conditions	Results[a]		Dose[c]	Reference
			Without Activation	With Activation[b]		
Salmonella typhimurium TA 97, 98, TA 100	Reverse mutation	Plate incorporation assay	−	+	1666 µg/plate (non activation), 10−1000 µg/plate (activation)	Zeiger et al. (1988)
Salmonella typhimurium TA 1535	Reverse mutation	Plate incorporation assay	−	−	1666 µg/plate	Zeiger et al. (1988)
Salmonella typhimurium TA 1537	Reverse mutation	Plate incorporation assay	ND	−	1666 µg/plate	Zeiger et al. (1988)
Salmonella typhimurium TA 98	Reverse mutation	Plate incorporation assay	−	−	5000 µg/plate	Shimizu and Takemura (1983,) as cited in CCRIS (2005)
Salmonella typhimurium TA 100	Reverse mutation	Plate incorporation assay	−	+	0−5000 µg/plate	Shimizu and Takemura (1983), as cited in CCRIS (2005)
Salmonella typhimurium TA 98, 100	Reverse mutation	Plate incorporation assay	−	+	100 µL/plate	Nohmi et al. (1983)
Salmonella typhimurium TA 98, 100	Reverse mutation	Plate incorporation assay	−	+	5−50 nmol/plate	Nohmi et al. (1984)
Salmonella typhimurium TA 100	Reverse mutation	Plate incorporation assay	ND	+	5−1000 µg/plate	Chung et al. (1981), as cited in CCRIS (2005)
Salmonella typhimurium TA 100	Reverse mutation	Plate incorporation assay	ND	+	0−15 µmol/plate	Zimmer et al. (1980)
Salmonella typhimurium TA 98, 1537	Reverse mutation	Plate incorporation assay	−?[d]	−?	0−15 µmol/plate	Zimmer et al. (1980)
Salmonella typhimurium TA 100	Reverse mutation	Plate incorporation assay	ND	+	25 µg/plate	Anonymous-German (1993) as cited in HSDB (2009)
Salmonella typhimurium TA 100	Reverse mutation (presumed)	Plate incorporation assay	−	+	1 µmol/plate	Kimmel et al. (1986), as cited in RTECS (2009).
Chinese Hamster V79 fibroblasts	Alkaline elution	DNA breakage test	ND	−	1.0, 3.0 mM (2 hr and 4 hr)	Zimmer et al. (1980)
Bacillus subtilis	Transforming DNA activity	Loss of DNA transforming activity	−	ND	10 mM	Nohmi et al. (1984)

Table 3. Genotoxicity Studies of 2,4-Dimethylaniline In Vitro

Test System	Endpoint	Test Conditions	Results[a]		Dose[c]	Reference
			Without Activation	With Activation[b]		
Rat hepatocytes	Unscheduled DNA Synthesis	DNA Repair test	+	ND	1−1000 μmol	Yoshimi et al. (1988), as cited in CCRIS (2005)
Chinese Hamster Lung (CHL) cells	Chromosomal aberrations	DNA Repair test	−	+	0.013−0.2 mg/mL (6-hr exposure, 18-hr recovery)	Japan Chemical Industry Ecology (1996), as cited in CCRIS (2005)
Chinese Hamster Lung (CHL) cells	Chromosomal aberrations	DNA Repair test	+	ND	0.13−0.5 mg/mL (24-hr and 48-hr treatment)	Japan Chemical Industry Ecology (1996), as cited in CCRIS (2005)

[a]+ = positive, − = negative, ± = equivocal, ND = no data.
[b]Exogenous metabolic activation used.
[c]Lowest effective dose for positive results, highest dose tested for negative or equivocal results.
[d]? = Positive or negative results identified, but activation status unknown.

Table 4. Genotoxicity Studies of 2,4-Dimethylaniline In Vivo

Test System	Endpoint	Test Conditions	Results[a]	Dose[b]	Reference
B6C3F1 mouse bone marrow	DNA damage	Alkaline single cell gel electrophoresis (“comet”) assay	+	200 mg/kg	Przybojewska (1997)
B6C3F1 mouse liver cells	DNA damage	Alkaline single cell gel electrophoresis (“comet”) assay	+	100, 200 mg/kg	Przybojewska (1999)
Female Wistar rat liver	DNA adducts	Single oral gavage dose	−	0.5 M solution (0.1 mL/100 g body weight)	Jones and Sabbioni (2003)
Female Wistar rat hemoglobin	DNA adducts	Single oral gavage dose	+	0.5 M solution (0.1 mL/100 g body weight)	Jones and Sabbioni (2003)
Male mouse testicle	DNA synthesis	Oral application or intraperitoneal injection	+	200 mg/kg (p.o.) or 100 mg/kg (i.p.)	Seiler et al. (1977) as cited in ACGIH (2001) and RTECS (2009)

[a]+ = positive, − = negative
[b]Lowest effective dose for positive results, highest dose tested for negative or equivocal results.

In other microsome tests, *Salmonella typhimurium* strain TA 100 with S9 activation tested positive with concentrations of 25 µg/plate or higher (Anonymous-German, 1993, as cited in HSDB, 2009) and 5−1000 µg/plate (Chung et al., 1981 as cited in CCRIS, 2005), and was weakly mutagenic at 0−15 µmol/plate (Zimmer et al., 1980). The study authors noted that the chemical was not mutagenic in TA 98 and TA 1537 strains. In another study, mutations in *Salmonella typhimurium* TA 100 were observed with metabolic activation at concentrations of 1 µmol/plate (Kimmel et al., 1986, as cited in RTECS, 2009).

In the alkaline elution/DNA breakage test, 2,4-dimethylaniline did not induce DNA damage in Chinese hamster V79 lung fibroblasts with activation at 1.0 and 3.0 mM for 2-hour and 4-hour exposure periods, respectively (Zimmer et al., 1980). In three additional in vitro tests using Chinese hamster lung (CHL) cells, one test was found to be negative for chromosomal aberrations without activation at concentrations of 0.013−0.2 mg/mL (6-hour treatment, 18-hour recovery) while two tests were positive, either with no metabolic activation at concentrations of 0.13−0.5 mg/mL (24 and 48 hour continuous treatment) or with rat liver S9 activation at concentrations of 0.013−0.2 mg/mL (6-hour treatment, 18-hour recovery) (Japan Chemical Industry Ecology, 1996, as cited in CCRIS, 2005). Also, Yoshimi et al. (1988) examined unscheduled DNA synthesis (UDS) in rodent hepatocytes and found that 2,4-dimethylaniline elicited positive repair responses in the DNA repair test.

Nohmi et al. (1983) tested the mutagenicity of 2,4-dimethylaniline metabolites in the plate incorporation assay using *Salmonella typhimurium* strains TA 98 and 100. Out of several metabolites, 2,4-dimethylphenylhydroxylamine was identified as being directly mutagenic to TA 100 cells, whereas 2,4-dimethylaniline was only mutagenic in the presence of S9 mixture, with the TA 100 strain more sensitive than the TA 98 strain. In a second study, the authors again tested the mutagenicity of both 2,4-dimethylaniline as well as the *N*-hydroxy derivative of 2,4-dimethylaniline, 2,4-dimethylphenylhydroxylamine, in *S. typhimurium* strains TA 98 and 100 for the plate incorporation assay (Nohmi et al., 1984). The authors observed that 2,4-dimethylaniline was negative without metabolic activation at concentrations of 5−50 nmoles/plate but showed positive results with liver S9 mix, inducing less than 10 (to the power of 3) revertants per µmol; the *N*-hydroxy compound was mutagenic even in the absence of activation and induced more than 10 (to the power of 4) revertants per µmol.

In the Rec-assay using *Bacillus subtilis,* a metabolite of 2,4-dimethylaniline tested positive. Nohmi et al. (1984) tested the chemical and its *N*-hydroxy metabolite in the *Bacillus subtilis*-transforming DNA assay (thus giving an assessment of the reactivity of the chemical with DNA). The chemical 2,4-dimethylaniline exerted no marked effect on the transforming activity of the DNA, and remaining activity of transforming DNA was 98%; however, its phenylhydroxylamine derivative caused a decrease in the activity of transforming DNA by more than 50% during an incubation time of 30 minutes.

Several in vivo genotoxicity tests have also been conducted. Przybojewska (1997) tested the genotoxicity of 2,4-dimethylaniline using the alkaline single cell gel electrophoresis (or "comet") assay. A single intraperitoneal injection at the oral LD_{50} concentration (200 mg/kg), as determined by study authors, was given to six male B6C3F1 mice 16 hours prior to sacrifice. Bone marrow suspensions were then analyzed to detect the presence of DNA damage in individual cells (e.g., single-strand breaks). The single dose of 2,4-dimethylaniline resulted in an increased number of bone marrow cells with DNA damage as evidenced by an increased extent

of DNA migration in bone marrow cells of treated mice compared to controls. Przybojewska (1999, as cited in RTECS, 2009) also conducted another comet assay under alkaline conditions to measure the DNA damage in the liver cells of B6C3F1 mice following a single intraperitoneal injection of 2,4-dimethylaniline at doses of 100 or 200 mg/kg body weight, respectively. The chemical damaged DNA in the liver cells of the mice, but no further details could be obtained as the original study was not available for review at this time.

Jones and Sabbioni (2003) examined the formation of DNA adducts in two female Wistar rats, who were given a single oral gavage dose of 2-methylaniline in calf thymus DNA. DNA adducts were not detected in the liver but were detected in hemoglobin. Additionally, a presumed single oral application of 200 mg/kg or intraperitoneal injection of 100 mg/kg to male mice inhibited testicular DNA synthesis (Seiler et al., 1977, as cited in ACGIH, 2001 and RTECS, 2009). The original source was unavailable for review at this time.

DERIVATION OF PROVISIONAL VALUES

DERIVATION OF ORAL REFERENCE DOSE

Derivation of Chronic and Subchronic Provisional RfD

An evaluation of the available oral studies indicated that the 6-month chronic-duration toxicity study by Lindstrom et al. (1963) was identified as the principal study and deemed adequate for the derivation of a chronic and subchronic p-RfD. However, it was determined that the UF_C would be >3000. A screening subchronic and a chronic p-RfD is provided in Appendix A. The benchmark dose calculations for the screening subchronic and chronic p-RfD can be found in Appendix D.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

Derivation of Chronic and Subchronic Provisional RfC

There are two main inhalation studies identified in the database. The first study exposed mice, rats, rabbits, cats, dogs, chicks, and monkeys to a single dose of 45 ppm (223 mg/m^3) of an isomeric xylidine vapor mixture for 7 hours/day, 5 times/week, for up to 40 weeks (Von Oettingen et al., 1947). Some effects noted included mortality in all species (except monkeys and chicks), liver damage in all species except chicks and elevated methemoglobin levels and increased number of Heinz bodies in cats, dogs, and mice. Due to the use of a single dose, known data gaps in the study, and the use of an impure xylidine vapor mixture, it is not possible to derive a chronic or subchronic p-RfC from this study.

Similarly, in a second study, multiple species of animals (i.e., rats, guinea pigs, rabbits, cats, and monkeys) exposed to 2,4-dimethylaniline vapor at concentrations of 50 to 142 ppm (36 to 703 mg/m^3) for 7 hours/day, 5 days/week, for an unspecified duration (Treon et al., 1950) experienced increased mortality, pneumonitis, and degeneration of cells in the heart, liver, and kidneys. All species except for the cat, which demonstrated liver toxicity, tolerated doses of 17.5 ppm (86 mg/m^3). One monkey and two cats tolerated 92 exposures at 7.8 ppm (36 mg/m^3) without any effect. Due to the use of an impure xylidine vapor mixture, it is not possible to derive a chronic or subchronic p-RfC from this study.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 5 identifies the cancer weight-of-evidence descriptor for 2,4-dimethylaniline.

Table 5. Cancer WOE Descriptor for 2,4-Dimethylaniline

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), 2,4-dimethylaniline is considered to have **"S*uggestive Evidence of Carcinogenic Potential*"** for humans by the oral route of exposure. Previously, EPA classified 2,4-dimethylaniline as a Group C carcinogen ("*possibly carcinogenic to humans: agents with limited animal evidence and little or no human data"*) (U.S. EPA, 2010b), according to the 1986 guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1986). Although Weisburger et al. (1978) did not find excess incidence of tumors in male rats nor in male mice, there was a statistically significantly increased incidence of lung tumors in female mice ($p < 0.05$). Lung tumors in female mice also occurred with a statistically significant positive trend (Cochran-Armitage trend test, $p = 0.01$). Furthermore, an additional 2-year dietary study suggests neoplastic effects from exposure to 2,4-dimethylaniline. It was reported that a 23% excess incidence of subcutaneous fibromas or fibrosarcomas and hepatomas was observed in male Sprague-Dawley rats, but no other details could be obtained from the source, which was possibly from an abstract (Health Council of the Netherlands, 2002; IARC, 1978, in HSDB, 2009). EPA has previously published a HEEP for 2,4-dimethylaniline and 2,4-dimethylaniline hydrochloride. The human carcinogen potency factor (q1*) for 2,4-dimethylaniline is 0.75 $(mg/kg\text{-}day)^{-1}$ for oral exposure, and the EPA's HEAST lists an oral unit risk for 2,4-dimethylaniline of 2.1×10^{-5} $(\mu g/L)^{-1}$ based on mouse lung tumors as observed in Weisburger et al. (1978). Genotoxicity studies for 2,4-dimethylaniline have demonstrated mixed but generally positive results. Results from plate incorporation mutagenicity assays show positive results in *S. typhimurium* especially in the presence of metabolic activation (Zeiger et al., 1988; Shimizu and Takemura, 1983; Chung et al., 1981; Zimmer et al., 1980; Nohmi et al., 1983; Nohmi et al., 1984). Yoshimi et al. (1988) found positive results for unscheduled DNA synthesis in rodent hepatocytes at concentrations of 1−1000 µmols, and Przybojewska (1997, 1999 as cited in RTECS, 2009) found increased DNA damage in bone marrow cells and liver cells of B6C3F1 using the comet assay. Inhibition of testicular DNA synthesis was observed in an oral mouse study (Seiler et al., 1977, as cited in ACGIH, 2001 and HSDB, 2009). Some negative tests found that 2,4-dimethylaniline did not induce DNA damage in Chinese hamster V79 lung fibroblasts with activation (Zimmer et al., 1980), while Jones and Sabbioni (2003) did not find DNA adducts in liver, but did find adducts in hemoglobin. Nohmi et al. (1984) found that 2,4-dimethylaniline itself did not decrease *Bacillus subtilus* DNA-transforming activity but attributed mutagenic activity of 2,4-dimethylaniline to its *N*-hydroxy derivative.

Table 5. Cancer WOE Descriptor for 2,4-Dimethylaniline

Possible WOE Descriptor	Designation	Route of Entry (Oral, Inhalation, or Both)	Comments
"Inadequate Information to Assess Carcinogenic Potential"	N/A	N/A	Available data are judged inadequate to assess carcinogenic potential.
"Not Likely to Be Carcinogenic to Humans"	N/A	N/A	No strong evidence of noncarcinogenicity in humans is available.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUE

Derivation of Provisional Oral Slope Factor (p-OSF)

The mouse study by Weisburger et al. (1978) is selected as the principal study. The critical endpoint is the incidence of lung tumors in CD-1 HaM/ICR female mice. This study is generally well conducted, and the data from this study are able to support a quantitative cancer dose-response assessment. This study is a peer-reviewed technical report from the National Cancer Institute, has been performed according to GLP standards, and has an acceptable study design and performance with numbers of animals, examination of potential toxicity endpoints, and presentation of information. This study is the only available, acceptable study with a positive tumor response following 2,4-dimethylaniline oral exposure. A mode of action for this chemical to induce lung tumors cannot be clearly identified from the available studies (see Tables 3–5); therefore, a linear approach is appropriate to model these data.

The oral data are sufficient to derive a quantitative estimate of cancer risk using benchmark dose (BMD) modeling. The dose-response data for lung tumors in female mice (see Table 6) can be used to derive a p-OSF for 2,4-dimethylaniline. Statistical significance tests were conducted and the results indicate that lung tumors in female mice occurred with a statistically significant positive trend (Cochran-Armitage trend test, $p = 0.01$), and a statistically significant increase in tumor incidence was observed at the highest dose (Fisher's exact test, $p < 0.05$).

The following dosimetric adjustments were made for diet treatment in adjusting doses for derivation of a p-OSF:

$DOSE_{ADJ, HED}$	**=**	**Dose × Food Consumption per Day × (1 ÷ Body Weight) × (Days Dosed ÷ Total Days) × body-weight adjustment**
Body-weight adjustment	=	$(BW_A \div BW_H)^{1/4}$
BW_H	=	70 kg (human reference body (U.S. EPA , 2010b)
BW_A	=	0.02875 kg (average body weight for female mice (U.S. EPA, 1994)
Body-weight adjustment	=	$(0.02875 \div 70)^{1/4} = 0.142$
$(DOSE_{ADJ, HED})$	=	$(Dose)_n \times (0.0053 \text{ kg/day}) \times (1 \div 0.02875 \text{ kg}) \times (18 \text{ months} \div 21 \text{ months}) \times 0.142$
	=	$125 \text{ mg/kg} \times (0.0053 \text{ kg/day}) \times (34.78 \text{ kg}^{-1}) \times 0.857 \times 0.142$
	=	$0.663 \text{ mg/day} \times 34.78 \text{ kg}^{-1} \times 0.857 \times 0.142$
	=	23.04 mg/kg-day × 0.122
$(DOSE_{ADJ, HED})$	**=**	**2.9 mg/kg-day**

Table 6 presents BMD input data for incidence of lung tumors in female mice exposed to 2,4-dimethylaniline by diet for 21 months.

Table 6. BMD Input for Incidence of Lung Tumors in the Female CD-1 HaM/ICR Mouse Exposed to 2,4-Dimethylaniline by Diet for 21 Months[a]

$(Dose)_n$ (mg/kg-day)	$(DOSE_{ADJ,HED})_n$ (mg/kg-day)	Number of Subjects	Response
			Lung Tumors[b,c]
0	0	22	5(23)
20	2.9	18	5(28)
40	5.8	19	11(58)[d]

[a]Weisburger et al. (1978).
[b]Number of mice with tumors, () = percentage of mice with lung tumors.
[c]Statistically significant trend using Cochrane-Armitage test for dose-response relationship.
[d]Statistically significant in pairwise test versus control.

Table **7** shows the modeling results. Adequate model fit is obtained for the lung tumor incidence data using the 1-degree multistage-cancer model. The BMD modeling results for lung tumors yield a BMD_{10HED} of 1.241 mg/kg-day and a $BMDL_{10HED}$ of 0.674 mg/kg-day (see Table 7). The BMD output for increased incidence of lung tumors in female mice can be seen in Figure D-1.

Table 7. Goodness-of-Fit Statistics, BMD_{10HED}, and $BMDL_{10HED}$ Values for Dichotomous Models for Lung Tumors in the Female Mouse Exposed to 2,4-Dimethylaniline in Diet for 21 Months[a]

Multistage Cancer Model	Goodness-of-Fit *p*-Value[b]	AIC	BMD_{10HED} (mg/kg-day)	$BMDL_{10HED}$ (mg/kg-day)
Multistage Cancer	0.28	75.914	1.241	0.674

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

$$\textbf{p-OSF} = BMR \div BMDL_{10HED}$$
$$= 0.1 \div 0.674 \text{ mg/kg-day}$$
$$= \mathbf{0.148 \text{ or } 2 \times 10^{-1} \text{ (mg/kg-day)}^{-1}}$$

Derivation of Provisional Inhalation Unit Risk (p-IUR)

The available data are inadequate for the derivation of a quantitative cancer risk estimate from inhalation exposure to 2,4-dimethylaniline (i.e., all data are from exposure conditions employing isomeric mixtures of chemicals).

APPENDIX A. PROVISIONAL NONCANCER SCREENING VALUES

Considering the uncertainties in the 2,4-dimethylaniline database described below (see Table A-2), the total composite UF for the derivation of a provisional chronic p-RfD is 10,000, consisting of four areas of maximum uncertainty. In the report, *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) the RfD/RfC technical panel concluded that, in cases where maximum uncertainty exists in four or more areas of uncertainty, or when the total UF is 10,000 or more, it is unlikely that the database is sufficient to derive a reference value. Because of this uncertainty, a provisional chronic p-RfD for 2,4-dimethylaniline is not derived. However, information is available which, although insufficient to support derivation of provisional RfD values, 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 a supplemental appendix and develops a screening value. Appendices receive the same level of internal and external scientific peer review as the main document to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in a supplement to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of a supplement 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 Heath Risk Technical Support Center.

Table A-1. Benchmark Dose Modeling Results for Decreased Body-Weight Gain and Increased Relative Kidney Weights in Osborne-Mendel Rats (Lindstrom et al., 1963)

Endpoint	Species	Sex	Model	Homogeneity Variance *p*-Value	Goodness-of-Fit *p*-Value	AIC for Fitted Model	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)	Conclusions
Decreased Body-Weight Gain[a]	Rat	F	Continuous-Linear	0.0001383	0.1888	668.072	297.65	237.21	Lowest AIC Lowest BMDL
			Continuous-Polynomial	0.0001383	0.1888	668.072	297.65	237.21	Lowest AIC Lowest BMDL Maximum order beta = 0 β2 = 0, β3 = 0, β4 = 0
			Continuous-Power	0.0001383	0.1888	668.072	297.65	237.21	Lowest AIC Lowest BMDL
Increased Relative Kidney Weight	Rat	M	Continuous-Hill	0.0001248	0.3735	65.6072	44.81	19.55	Lowest AIC Lowest BMDL
	Rat	F	Continuous-Hill	0.002347	0.7339	60.3687	31.33	18.87	Lowest AIC Lowest BMDL

[a]Modeling for decreased body-weight gain was done using 1SD.

DERIVATION OF SCREENING ORAL REFERENCE DOSE

Derivation of Screening Chronic and Subchronic p-RfD

The 6-month chronic-duration toxicity study by Lindstrom et al. (1963) was identified as the principal study and deemed adequate for the derivation of a screening chronic and subchronic p-RfD. This study had five dose groups in addition to controls and tested 10 rats per sex per dose group (n = 120). Although this study reported some limited toxicological data for rats at 13 weeks, the study was primarily designed with the duration of 6 months in mind. Microscopic examination was performed in every dose group at 6 months and only in the highest dose group at 13 weeks as an indication of the types of effects that would be seen at 6 months (e.g., only four rats at the highest dose at 13 weeks, compared to four rats at each dose at 6 months). In addition, organ-weight data for kidneys, livers, and spleen were not provided at 13 weeks nor was hematological analysis performed. Therefore, the results obtained at 6 months were used to identify a point of departure (POD).

Decreases in body-weight gain were statistically significant in males and females at the three highest dose levels (Lindstrom et al., 1963). In addition, relative liver weight was statistically significantly increased at all dose levels in males and females. Relative kidney weight was also statistically significantly increased at all dose levels in males and females. Because these three endpoints were the most sensitive effects reported in this study, all of the common continuous models (i.e., Linear, Polynomial, Power, and Hill models) available in the EPA's Benchmark Dose Software (BMDS, version 2.1) were fit to the data. In general, model fit was assessed by a χ^2 goodness-of-fit test (i.e., models with $p < 0.1$ failed to meet the goodness-of-fit criterion) and the Akaike Information Criterion (AIC) value (i.e., a measure of the deviance of the model fit that allows for comparison across models for a particular endpoint).

The initial modeling of all the data including all dose groups failed to provide an adequate fit to the data, as assessed by the χ^2 goodness-of-fit test. After excluding the highest dose group, the Linear, Polynomial, and Power models adequately fit the body-weight gain data for female rats, and the Hill model adequately fit the male and female relative kidney weight data. No adequate model fits were achieved with the relative liver-weight data even when the three highest dose groups were excluded.

For the increase in relative kidney weight, the Hill model in female rats was considered most appropriate because it produced a slightly lower BMD_{10} and $BMDL_{10}$ of 31.33 and 18.87 mg/kg-day, respectively, compared to those from male rats. BMD outputs for increased relative kidney weights in male and female rats using the Hill model, can be seen in Figures C-1 and C-2. Because both male and female relative liver weights did not provide adequate model fits, the LOAEL of 18 mg/kg-day (male rats) was considered as an alternative POD. It is important to note that increased relative liver weight was quantitatively the more sensitive response compared to increased relative kidney weight based on the magnitude of change from control (see Figure A-1). Specifically, at the LOAEL of 18 mg/kg-day in male rats, a ~10% increase in relative kidney weight was observed whereas a ~40% increase in relative liver weight was observed (compared to the respective control values [see Figure A-1]). A similar trend was observed for relative liver weight in female rats. The general dose-response trend based on a 10% change modeled for both relative kidney and liver weight in male rats as assessed by the BMD (i.e., the maximum likelihood estimate not influenced by sample size) indicates that the relative liver-weight response is more sensitive (BMD_{10} = 6.56) than the relative kidney-weight response (BMD_{10} = 37.54). While the selection of the $BMDL_{10}$ from the relative kidney-weight

dataset as the POD would protect against kidney toxicity, it may not confer protection against the more sensitive endpoint of liver toxicity (i.e., increased relative liver weight). **Therefore, the LOAEL of 18 mg/kg-day based on increased relative liver weight in male rats (Lindstrom et al., 1963) was chosen as the POD to derive both a screening chronic and subchronic p-RfD.**

The screening chronic p-RfD for 2,4-dimethylaniline was derived as follows:

Screening Chronic p-RfD = LOAEL ÷ UF_C
= 18 mg/kg-day ÷ 10,000
= **0.0018 or 2×10^{-3} mg/kg-day**

The composite UF of 10,000 is estimated, as presented in Table A-2.

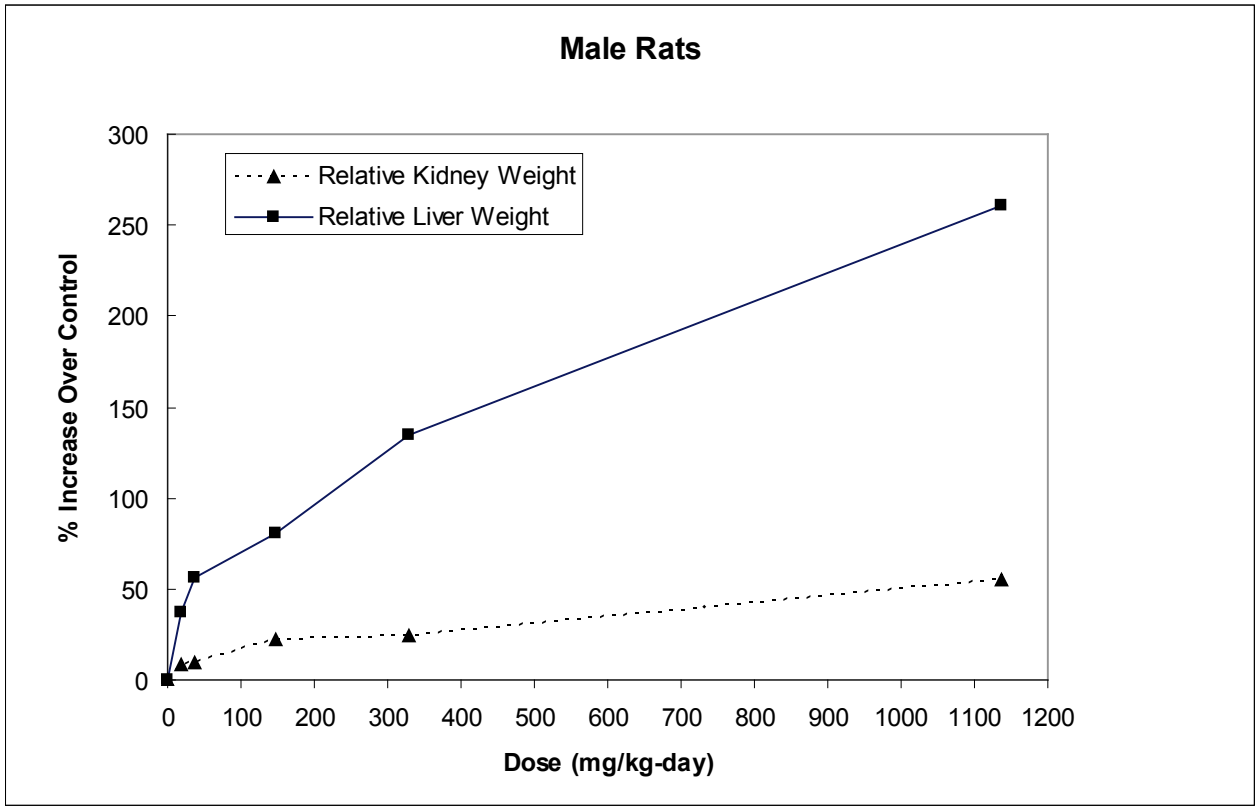

Figure A-1. Percent Increase Over Control for Relative Kidney Weight and Relative Liver Weight in Male Osborne-Mendel Rats Exposed to 2,4-Dimethyaniline in Diet for 6 Months[a]

[a]Lindstrom (1963).

Table A-2. Uncertainty Factors for Screening Chronic p-RfD for 2,4-Dimethylaniline		
UF	**Value**	**Justification**
UF_H	10	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 to humans.
UF_A	10	A UF_A of 10 is applied for animal-to-human extrapolation to account for potential toxicokinetic and toxicodynamic differences between rats and humans. There are no data to determine whether humans are more or less sensitive than rats to the kidney effects of 2,4-dimethylaniline.
UF_D	10	A UF_D of 10 is applied for database inadequacies because there are no acceptable two-generation reproductive studies or developmental studies, and there are no indications of any other studies that may be relevant for the database uncertainty factor.
UF_L	10	A UF_L of 10 is applied because the POD was developed using a LOAEL.
UF_S	1	A UF_S of 1 is applied because further adjustments for duration of exposure are not warranted when chronic toxicity data are used to develop a POD.
UF_C>3000	10,000	

A **screening subchronic p-RfD of 0.002 mg/kg-day** was derived by adopting the screening chronic p-RfD as the screening subchronic p-RfD, in the absence of relevant chronic data. There is low confidence in both the screening subchronic and screening chronic p-RfDs.

APPENDIX B. DATA TABLES

Table B-1. Mean Body-Weight Gains, Relative Liver Weights, and Relative Kidney Weights in Osborne-Mendel Rats Exposed to Oral 2,4-Dimethylaniline for 6 Months[a]

Adjusted Dose Group (mg/kg-day)	Number of Rats	Body-Weight Gains (assumed g) ± Std. Error [b]	Relative Liver Weights (g/kg body weight) ± Std. Error [b]	Relative Kidney Weights (g/kg body weight) ± Std. Error
Males				
0	16	425.6 ± 9.20	26.29 ± 0.46	6.68 ± 0.16
18	16	441.9 ± 23.89	35.98 ± 1.56*	7.3 ± 0.18*
36	16	437.6 ± 16.64	41.14 ± 1.51*	7.33 ± 0.17*
148	16	343.3 ± 19.15*	47.55 ± 0.97*	8.16 ± 0.22*
329	16	304.5 ± 13.83*	61.72 ± 1.29*	8.31 ± 0.41*
1137	16	157.3 ± 12.28*	94.79 ± 3.05*	10.33 ± 0.58*
Females				
0	16	235.9 ± 15.75	28.88 ± 0.76	7.47 ± 0.15
26	16	224.4 ± 12.61	38.12 ± 2.36*	8.21 ± 0.18*
55	16	211.9 ± 7.58	43.4 ± 1.36*	8.57 ± 0.16*
209	16	182.6 ± 8.45*	56.4 ± 2.52*	9.55 ± 0.32*
511	16	141 ± 5.18*	71.52 ± 2.26*	9.99 ± 0.30*
1304	16	100.7 ± 4.37*	115.66 ± 6.71*	12.55 ± 0.82*

[a]Values obtained from Lindstrom et al. (1963), measured at study termination (6 months).
[b]Std. error (S.E.) converted to std. Deviation (S.D.) using S.D = SE x √n.

*$p < 0.05$.

APPENDIX C. BENCHMARK DOSE CALCULATIONS FOR THE RFD

Modeling Procedure For Continuous Data

The BMD modeling of continuous data was conducted with EPA's BMDS (version 2.1 beta). For these data (e.g., increased relative kidney weight), all continuous models available within the software were fit using a default BMR of 10% extra risk. An adequate fit was judged based on the χ^2 goodness-of-fit p-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination was made as to whether the variance across dose groups was homogeneous. If a homogeneous variance model was deemed appropriate based on the statistical test provided in BMDS (i.e., Test 2), the final BMD results were estimated from a homogeneous variance model. If the test for homogeneity of variance was rejected ($p < 0.1$), the model was run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model did not adequately fit the data (i.e., Test 3; p-value < 0.1), the dataset was considered unsuitable for BMD modeling. Among all models providing adequate fit, the lowest BMDL was selected if the BMDLs estimated from different models varied greater than 3-fold; otherwise, the BMDL from the model with the lowest AIC was selected as a potential POD from which to derive the RfD.

In addition, in the absence of a mechanistic understanding of the biological response to a toxic agent, data from exposures much higher than the study LOAEL do not provide reliable information regarding the shape of the response at low doses. Such exposures, however, can have a strong effect on the shape of the fitted model in the low-dose region of the dose-response curve. Thus, if lack of fit is due to characteristics of the dose-response data for high doses, then the EPA Benchmark Dose Technical Guidance Document allows for data to be adjusted by eliminating the high-dose group (U.S. EPA, 2000). Because the focus of BMD analysis is on the low dose region of the response curve, eliminating high-dose groups is deemed reasonable. Modeling was performed without constant variance because initial analyses with constant variance models revealed poor model fit. Data outputs from the three modeled endpoints—after dropping the highest dose from the dataset—were evaluated, and the outputs from decreased body-weight gain and increased relative kidney weight (in female rats) were deemed valid and are provided in Table A-1.

Relative Kidney Weight in Male and Female Rats Exposed to 2,4-Dimethylaniline for 6 Months (Lindstrom et al., 1963)

Relative liver and kidney weights were determined to be the most sensitive endpoints and, therefore, all available continuous models in BMDS (version 2.1 beta) were fit to the relative kidney- and liver-weight data (see Table A-2) from Osborne-Mendel rats exposed to 2,4-dimethylaniline for 6 months (Lindstrom et al., 1963). However, data from relative liver weights failed to meet the modeling criteria. The initial modeling of the male and female rat relative kidney weights including all dose groups failed to provide an adequate fit to the data, as assessed by the χ^2 goodness-of-fit test. After excluding the highest dose (1304 mg/kg-day) group to provide better model fit, as described in EPA (2000), only the Hill continuous model adequately fit the data (see Tables C-1a and C-1b). Therefore, only the BMD modeling results based on the data without the highest dose group included are summarized in Tables C-2 and C-3. Initial tests determined that constant variance was invalid for modeling these data. Thus, all of the BMD modeling results shown in Tables C-2 and C-3 were obtained from nonconstant

variance models. Estimated doses associated with 10% extra risk and the 95% lower confidence limit on these doses (BMD_{10} values and $BMDL_{10}$ values, respectively) were 44.81 and 19.55 mg/kg-day in male rats and 31.33 and 18.87 mg/kg-day in female rats, respectively.

Table C-1a. Relative Kidney Weight in Male Osborne-Mendel Rats Exposed to 2,4-Dimethylaniline for 6 Months[a]

Dose (mg/kg-day)	0	18	36	148	329
Number	16	16	16	16	16
Relative kidney weight (g/kg body weight) ± SD	6.68 ± 0.64	7.3 ± 0.72[b]	7.33 ± 0.68[b]	8.16 ± 0.88[b]	8.31 ± 1.23[b]

[a]Lindstrom et al., (1963) Table 4.
[b]Relative kidney weight significantly increased compared to control ($p < 0.05$).

Table C-1b. Relative Kidney Weight in Female Osborne-Mendel Rats Exposed to 2,4-Dimethylaniline for 6 Months[a]

Dose (mg/kg-day)	0	26	55	209	511
Number	16	16	16	16	16
Relative kidney weight (g/kg body weight) ± SD	7.47 ± 0.6	8.21 ± 0.72[b]	8.57 ± 0.64[b]	9.55 ± 1.28[b]	9.99 ± 1.20[b]

[a]Lindstrom et al. (1963) Table 4.
[b]Relative kidney weight significantly increased compared to control ($p < 0.05$).

Table C-2. BMD Modeling Results on Increased Relative Kidney Weight in Male and Female Rats Exposed to 2,4-Dimethylaniline for 6 Months

Model	Test 2	Test 3	χ^2 *p*-Value	AIC	BMD_{10}	$BMDL_{10}$
Males						
Linear	0.0001	0.2411	0.1051	67.7751	128.39	91.95
Polynomial	0.0001	0.2411	0.1051	67.7751	128.39	91.95
Power	0.0001	0.2411	0.1051	67.7751	128.39	91.95
Hill	0.0001	0.2411	0.3735	65.6072	44.81	19.55
Females						
Linear	0.0023	0.5089	<0.0001	79.1454	154.48	116.51
Polynomial	0.0023	0.5089	<0.0001	79.1454	154.48	116.51
Power	0.0023	0.5089	<0.0001	79.1454	154.48	116.51
Hill	0.0023	0.5089	0.7339	60.3687	31.33	18.87

Table C-3. BMD Modeling Output Summary for 2,4-Dimethylaniline, Using Data from Lindstrom et al. (1963) with Nonconstant Variance and Dropping the Highest Dose Data Point

Endpoint	Species	Sex	Model	Homogeneity Variance *p*-Value	Goodness-of-Fit *p*-Value[a]	AIC for Fitted Model	BMD_{10} (mg/kg-day)[b]	$BMDL_{10}$ (mg/kg-day)	Conclusions
Decreased body-weight gain	Rat	M	Continuous-Hill	0.00544	0.3269	764.4763	140.69	-999.00	Invalid BMDL Poor variance model Observed to modeled std. dev. ratio > 1.5
			Continuous-Linear	0.0054	0.1387	765.0135	172.13	128.98	Poor variance model Observed to modeled std. dev. ratio > 1.5
			Continuous-Polynomial	0.0054	<.0001	1046.2256	-999.00	-999.00	Invalid BMD Invalid BMDL *p*-score 4 < 0.1 Poor variance model Observed to modeled std. dev. ratio > 1.5
			Continuous-Power	0.0054	0.1387	765.0134	172.13	128.98	Poor variance model Observed to modeled std. dev. ratio > 1.5
Decreased body-weight gain	Rat	F	Continuous-Hill	0.0001	0.3884	667.186	191.35	-999.00	Invalid BMDL
			Continuous-Linear	0.0001	0.1888	668.072	297.65	237.21	Lowest AIC Lowest BMDL
			Continuous-Polynomial	0.0001	0.1888	668.072	297.65	237.21	Lowest AIC Lowest BMDL Maximum order beta = 0 β2 = 0 β3 = 0 β4 = 0
			Continuous-Power	0.0001	0.1888	668.072	297.65	237.21	Lowest AIC Lowest BMDL

Table C-3. BMD Modeling Output Summary for 2,4-Dimethylaniline, Using Data from Lindstrom et al. (1963) with Nonconstant Variance and Dropping the Highest Dose Data Point

Endpoint	Species	Sex	Model	Homogeneity Variance *p*-Value	Goodness-of-Fit *p*-Value[a]	AIC for Fitted Model	BMD_{10} (mg/kg-day)[b]	$BMDL_{10}$ (mg/kg-day)	Conclusions
Increased relative liver weight	Rat	M	Continuous-Hill	0.0001	<.0001	355.4078	3.90	2.91	Lowest AIC Lowest BMDL *p*-score 4 < 0.1 Poor variance model Observed to modeled std. dev. ratio > 1.5
			Continuous-Linear	0.0001	<.0001	6	35.72	N/D	*p*-score 4 < 0.1 Poor variance model Observed to modeled std. dev. ratio > 1.5 Residual of interest >= 2
			Continuous-Polynomial	0.0001	<.0001	8	20.10	N/D	Invalid BMD Invalid BMDL *p*-score 4 < 0.1 Poor variance model Observed to modeled std. dev. ratio > 1.5
			Continuous-Power	0.0001	<.0001	373.7113	37.48	32.88	*p*-score 4 < 0.1 Poor variance model Observed to modeled std. dev. ratio > 1.5 Residual of interest >= 2
Increased relative liver weight	Rat	F	Continuous-Hill	<.0001	0.1075	410.5187	8.36	5.60	Lowest AIC, lowest BMDL, Poor variance model, Observed to modeled std. dev. ratio > 1.5
			Continuous-Linear	<.0001	<.0001	536.7464	-999.00	79.42	*p*-score 4 < 0.1 Poor variance model Observed to modeled std. dev. ratio > 1.5

Table C-3. BMD Modeling Output Summary for 2,4-Dimethylaniline, Using Data from Lindstrom et al. (1963) with Nonconstant Variance and Dropping the Highest Dose Data Point

Endpoint	Species	Sex	Model	Homogeneity Variance *p*-Value	Goodness-of-Fit *p*-Value[a]	AIC for Fitted Model	BMD_{10} (mg/kg-day)[b]	$BMDL_{10}$ (mg/kg-day)	Conclusions
			Continuous-Polynomial	<.0001	<.0001	8	20.84	N/D	Invalid BMD Invalid BMDL *p*-score 4 < 0.1 Poor variance model Observed to modeled std. dev. ratio > 1.5 Maximum order beta = 0 β1 = 0 β2 = 0 β3 = 0 β4 = 0
			Continuous-Power	<.0001	<.0001	433.6389	44.99	37.55	*p*-score 4 < 0.1 Poor variance model Observed to modeled std. dev. ratio > 1.5
Increased relative kidney weight	Rat	M	Continuous-Hill	0.0001	0.3735	65.6072	44.81	19.55	Lowest AIC Lowest BMDL
			Continuous-Linear	0.0001	0.1051	67.7751	128.39	91.95	
			Continuous-Polynomial	0.0001	0.1051	67.7751	128.39	91.95	Maximum order beta = 0 β2 = 0 β3 = 0 β4 = 0
			Continuous-Power	0.0001	0.1051	67.7751	128.39	91.95	
Increased relative kidney weight	Rat	F	Continuous-Hill	0.0023	0.7039	60.3687	31.33	18.87	Lowest AIC Lowest BMDL

Table C-3. BMD Modeling Output Summary for 2,4-Dimethylaniline, Using Data from Lindstrom et al. (1963) with Nonconstant Variance and Dropping the Highest Dose Data Point

Endpoint	Species	Sex	Model	Homogeneity Variance *p*-Value	Goodness-of-Fit *p*-Value[a]	AIC for Fitted Model	BMD_{10} (mg/kg-day)[b]	$BMDL_{10}$ (mg/kg-day)	Conclusions
			Continuous-Linear	0.0023	<.0001	79.1454	154.48	116.51	*p*-score 4 < 0.1
			Continuous-Polynomial	0.0023	<.0001	79.1454	154.48	116.51	*p*-score 4 < 0.1 Maximum order beta = 0 β2 = 0 β3 = 0 β4 = 0
			Continuous-Power	0.0023	<.0001	79.1454	154.48	116.51	*p*-score 4 < 0.1

[a]N/D = not determined.
[b]Body-weight gain was modeled using 1SD.

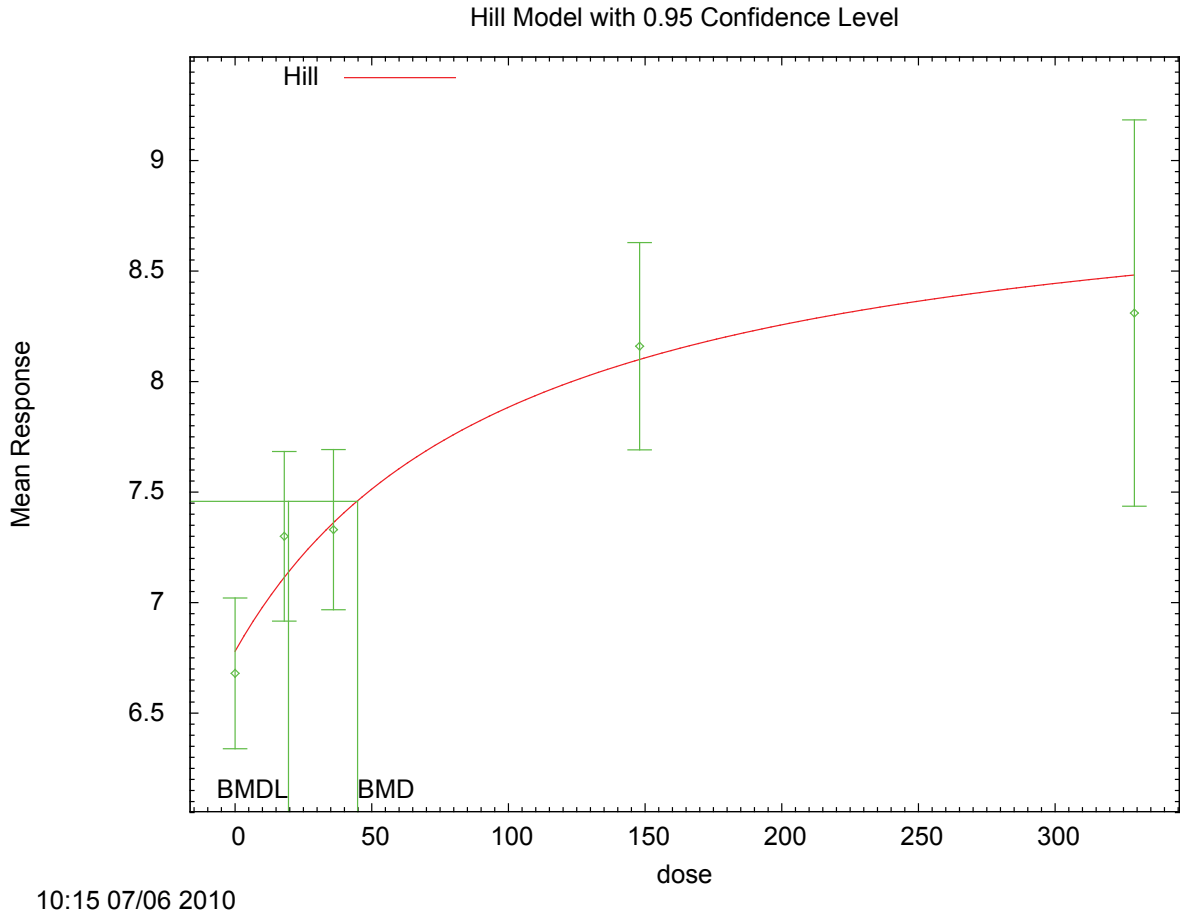

Figure C-1. Nonconstant Variance Hill BMD Model-Increased Relative Kidney Weights in Male Osborne-Mendel Rats after Dropping the Highest Dose (Lindstrom et al., 1963)

```
Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\USEPA\BMDS21\Data\hil_RelKid_Methylaniline_mnohd_Hil-
ModelVariance-BMR10-Restrict.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS21\Data\hil_RelKid_Methyaniline_mnohd_Hil-ModelVariance-BMR10-
Restrict.plt
                                                  Tue Jul 06 10:15:35 2010
 ================================================================================

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

   The form of the response function is:

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


   Dependent variable = mean
   Independent variable = dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))
```

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


                  Default Initial Parameter Values
                         lalpha =   -0.0295524
                            rho =            0
                      intercept =         6.68
                              v =         1.63
                              n =     0.135532
                              k =      237.735


           Asymptotic Correlation Matrix of Parameter Estimates

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

                 lalpha          rho    intercept            v            k

    lalpha            1           -1        -0.27         0.33        0.022

       rho           -1            1         0.27        -0.33       -0.025

 intercept        -0.27         0.27            1          0.2         0.64

         v         0.33        -0.33          0.2            1         0.79

         k        0.022       -0.025         0.64         0.79            1


                                 Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
         lalpha         -15.6119          4.14649            -23.7389            -
7.48496
            rho          7.57708          2.06157             3.53647
11.6177
      intercept          6.78014         0.153019             6.48023
7.08006
              v          2.22631         0.625923            0.999528
3.4531
              n                1               NA
              k          102.325          81.1928            -56.8103
261.46

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


     Table of Data and Estimated Values of Interest
```

Dose	N	Obs Mean	Est Mean	Obs Std. Dev	Est Std. Dev	Scaled Res.
0	16	6.68	6.78	0.64	0.574	-0.698
18	16	7.3	7.11	0.72	0.689	1.09
36	16	7.33	7.36	0.68	0.783	-0.151
148	16	8.16	8.1	0.88	1.12	0.226
329	16	8.31	8.48	1.64	1.34	-0.503

Model Descriptions for likelihoods calculated

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-36.236363	6	84.472726
A2	-24.721007	10	69.442013
A3	-26.818717	7	67.637434
fitted	-27.803623	5	65.607247
R	-49.675911	2	103.351823

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	49.9098	8	<.0001
Test 2	23.0307	4	0.0001248
Test 3	4.19542	3	0.2411
Test 4	1.96981	2	0.3735

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

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

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

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


        Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =     Relative risk

Confidence level =           0.95

             BMD =        44.8089

            BMDL =       19.5498
```

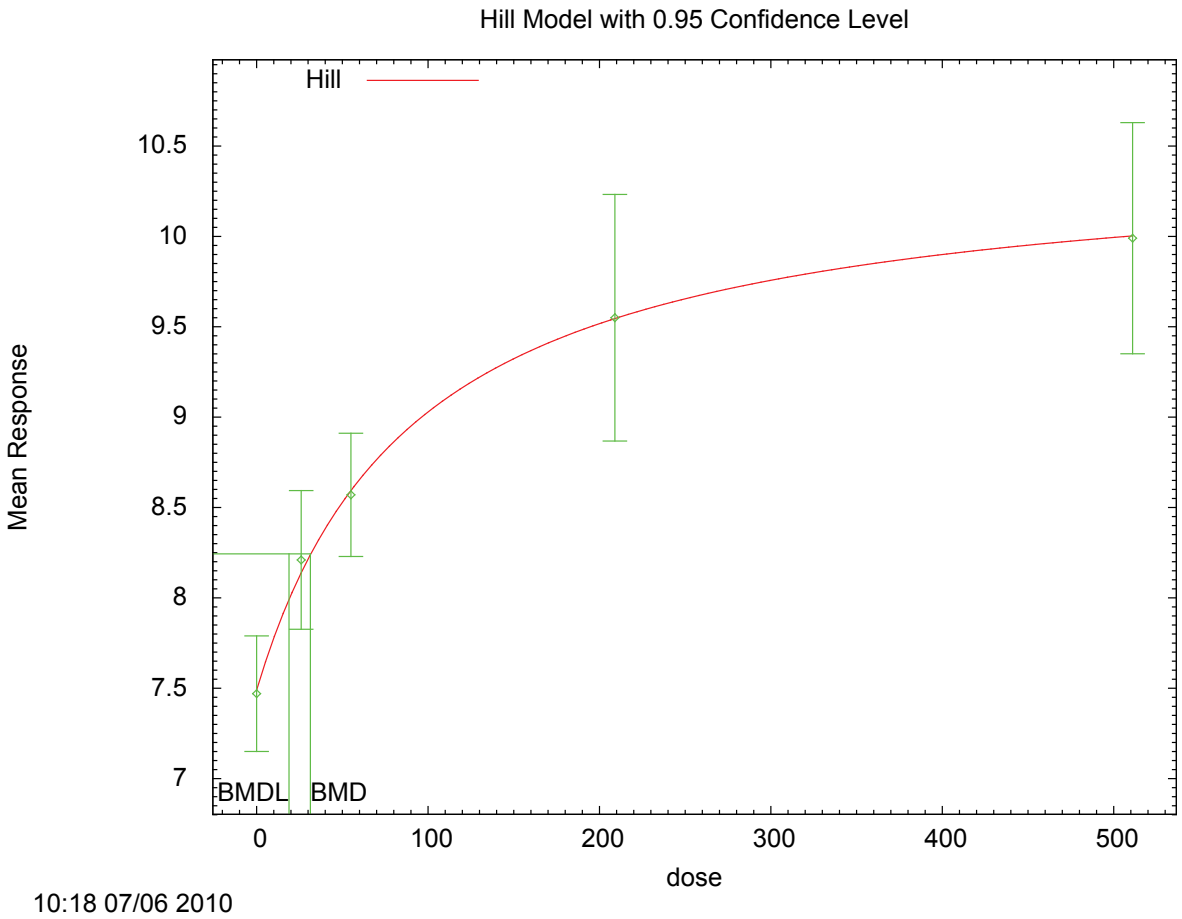

Figure C-2. Nonconstant Variance Hill BMD Model-Increased Relative Kidney Weights in Female Osborne-Mendel Rats after Dropping the Highest Dose (Lindstrom et al., 1963)

```
Hill Model. (Version: 2.14;  Date: 06/26/2008)
         Input Data File: C:\USEPA\BMDS21\Data\hil_Rel_Kid_Methya_females_nhd_Hil-
ModelVariance-BMR10-Restrict.(d)
         Gnuplot Plotting File:
C:\USEPA\BMDS21\Data\hil_Rel_Kid_Methya_females_nhd_Hil-ModelVariance-BMR10-
Restrict.plt
                                                  Tue Jul 06 10:18:49 2010
 ================================================================================

 BMDS Model Run
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   The form of the response function is:

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


   Dependent variable = mean
   Independent variable = dose
   Power parameter restricted to be greater than 1
   The variance is to be modeled as Var(i) = exp(lalpha  + rho * ln(mean(i)))

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

Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values

lalpha =	-0.135499
rho =	0
intercept =	7.47
v =	2.52
n =	0.160973
k =	337.857

Asymptotic Correlation Matrix of Parameter Estimates

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

	lalpha	rho	intercept	v	k
lalpha	1	-1	-0.21	0.28	0.052
rho	-1	1	0.2	-0.29	-0.053
intercept	-0.21	0.2	1	-0.13	0.48
v	0.28	-0.29	-0.13	1	0.67
k	0.052	-0.053	0.48	0.67	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
lalpha	-12.4275	3.34769	-18.9888	-5.86611
rho	5.56998	1.54805	2.53587	8.6041
intercept	7.49402	0.133614	7.23215	7.7559
v	2.97289	0.419397	2.15088	3.79489
n	1	NA		
k	92.9577	36.1888	22.029	163.886

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

Table of Data and Estimated Values of Interest

Dose	N	Obs Mean	Est Mean	Obs Std. Dev	Est Std. Dev	Scaled Res.
0	16	7.47	7.49	0.6	0.546	-0.176

26	16	8.21	8.14	0.72	0.689	0.385
55	16	8.57	8.6	0.64	0.801	-0.145
209	16	9.55	9.55	1.28	1.07	-0.00636
511	16	9.99	10	1.2	1.22	-0.0632

Model Descriptions for likelihoods calculated

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

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

Model A3: Yij = Mu(i) + e(ij)
Var{e(ij)} = exp(lalpha + rho*ln(Mu(i)))
Model A3 uses any fixed variance parameters that
were specified by the user

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

Likelihoods of Interest

Model	Log(likelihood)	# Param's	AIC
A1	-31.998497	6	75.996995
A2	-23.715497	10	67.430993
A3	-24.875027	7	63.750054
fitted	-25.184352	5	60.368704
R	-59.949377	2	123.898754

Explanation of Tests

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

Tests of Interest

Test	-2*log(Likelihood Ratio)	Test df	p-value
Test 1	72.4678	8	<.0001
Test 2	16.566	4	0.002347
Test 3	2.31906	3	0.5089
Test 4	0.61865	2	0.7339

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

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

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

The p-value for Test 4 is greater than .1. The model chosen seems

to adequately describe the data

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Relative risk

Confidence level = 0.95

BMD = 31.3304

BMDL = 18.8667

APPENDIX D. BENCHMARK DOSE CALCULATIONS FOR THE ORAL SLOPE FACTOR

Model-Fitting Procedure for Cancer Incidence Data

The model-fitting procedure for dichotomous cancer incidence data is as follows. The multistage-cancer model in the EPA benchmark dose software (BMDS) is fit to the incidence data using the extra risk option. The multistage-cancer model is run for all polynomial degrees up to *n*-1 (where *n* is the number of dose groups including control). An adequate model fit is judged by three criteria: goodness-of-fit *p*-value ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined benchmark response (BMR). Among all the models providing adequate fit to the data, the lowest bound of the BMD (BMDL) is selected as the point of departure when the difference between the BMDLs estimated from these models is more than 3-fold (unless it appears to be an outlier); otherwise, the BMDL from the model with the lowest (Akaike Information Criterion) AIC is chosen. In accordance with EPA (2000) guidance, BMDs and BMDLs associated with an extra risk of 10% are calculated.

Model-Fitting Results for Lung Tumors in HaM/ICR Derived CD-1 Female Mice (Weisburger et al., 1978)

Table 6 shows the dose-response data on lung tumors in HaM/ICR derived CD-1 female mice administered 2,4-dimethylaniline via diet for 21 months (Weisburger et al., 1978). Modeling was performed according to the procedure outlined above using BMDS version 2.1 with default parameter restrictions for females based on the duratio*n*- HEDs shown in Table 2. Model predictions are shown in Table 7. For female mice, the multistage-cancer model provided an adequate fit (goodness-of-fit *p*-value > 0.1). The 1-degree polynomial model yielded a BMD_{10HED} value of 1.241 mg/kg-day with an associated 95% lower confidence limit ($BMDL_{10HED}$) of 0.673 mg/kg-day. The fit of the 1-degree multistage-cancer model to the lung tumor incidence data for female mice is shown in Table 7.

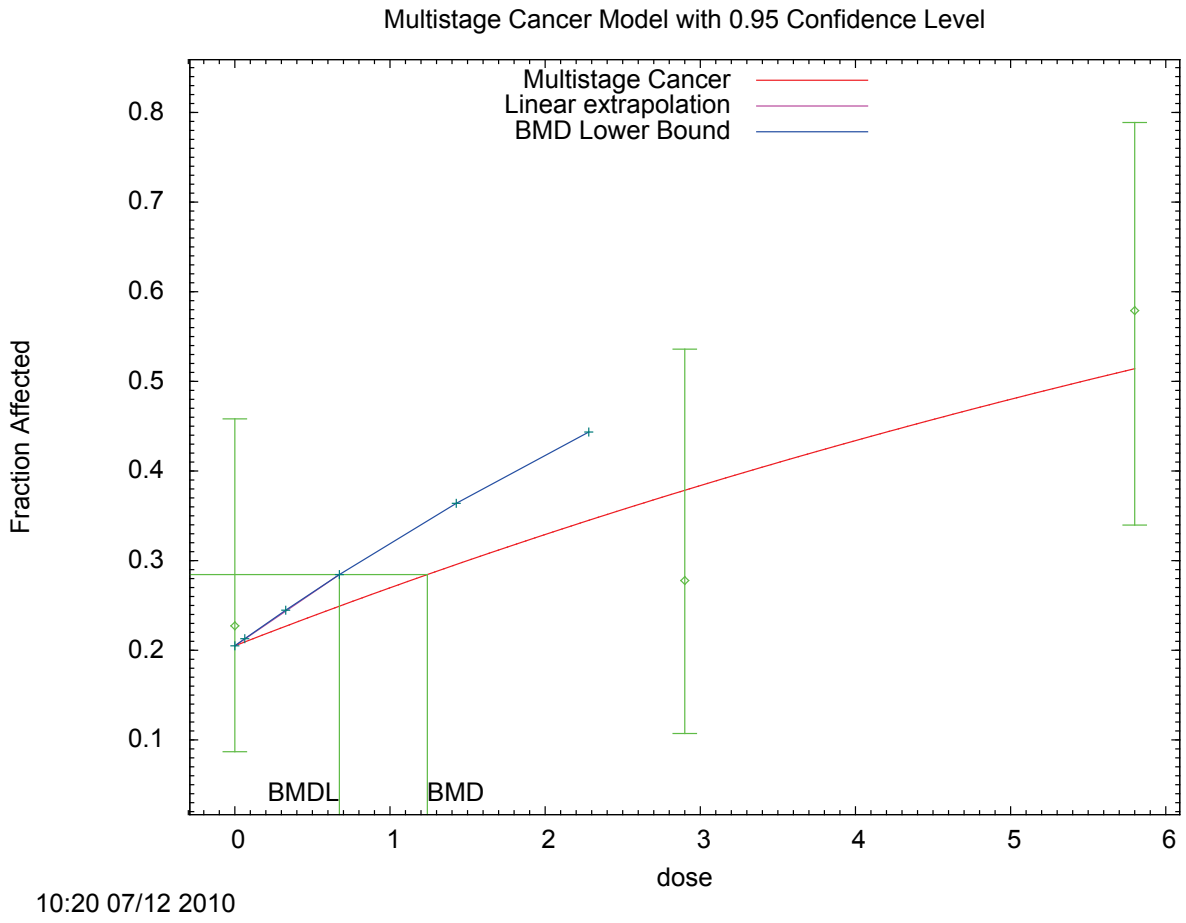

Figure D-1. Multistage Cancer BMD Model for Female Lung Tumor Incidence (Weisburger et al., 1978)

```
=================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\msc_Weisburger_et_al_1978_Msc1-
BMR10.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\msc_Weisburger_et_al_1978_Msc1-
BMR10.plt
                                          Mon Jul 12 10:20:40 2010
 =================================================================

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

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Response
```

```
  Independent variable = Dose

 Total number of observations = 3
 Total number of records with missing values = 0
 Total number of parameters in model = 2
 Total number of specified parameters = 0
 Degree of polynomial = 1


 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 =     0.164032
                       Beta(1) =     0.104684


          Asymptotic Correlation Matrix of Parameter Estimates

            Background      Beta(1)

Background            1        -0.67

   Beta(1)        -0.67            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.204962            *               *                   *
        Beta(1)        0.0849098            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -35.3582         3
   Fitted model        -35.9571         2       1.19763      1          0.2738
  Reduced model        -38.4115         1       6.10645      2         0.04721

           AIC:         75.9141


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size        Residual
  ---------------------------------------------------------------------
    0.0000     0.2050         4.509     5.000          22         0.259
    2.9000     0.3785         6.813     5.000          18        -0.881
    5.8000     0.5141         9.769    11.000          19         0.565

 Chi^2 = 1.16      d.f. = 1        P-value = 0.2809
```

```
   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        1.24085

            BMDL =       0.673865

            BMDU =        4.84519

Taken together, (0.673865, 4.84519) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =      0.148398
```

APPENDIX E. REFERENCES

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