United States
Environmental Protection
Agency

EPA/690/R-10/003F
Final
9-30-2010

Provisional Peer-Reviewed Toxicity Values for

4-Chloro-2-methylaniline
(CASRN 95-69-2)

Superfund Health Risk Technical Support Center
National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

TABLE OF CONTENTS

COMMONLY USED ABBREVIATIONS

BMC	Benchmark Concentration
BMD	Benchmark Dose
BMCL	Benchmark Concentration Lower bound 95% confidence interval
BMDL	Benchmark Dose Lower bound 95% confidence interval
HEC	Human Equivalent Concentration
HED	Human Equivalent Dose
IRIS	Integrated Risk Information System
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
POD	point of departure (oral)
RfC	reference concentration (inhalation)
RfD	reference dose
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 4-CHLORO-2-METHYLANILINE (CASRN 95-69-2)

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

4-Chloro-2-methylaniline (also referred to as 4-chloro-*o*-toluidine) and its hydrochloride salt can serve as a component in various dyes and pigments—including azo dyes used for coloring fabrics—and was used from the 1960s though the 1980s in the production of chlordimeform, an acaricide and insecticide (IARC, 1990). The empirical formula for 4-chloro-2-methylaniline is C_7H_8ClN (see Figure 1). A table of chemico-physical properties is provided below (see Table 1). In this document, unless otherwise noted, "statistically significant" denotes a p-value < 0.05.

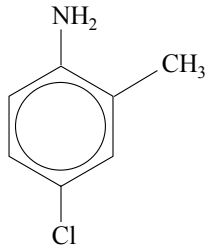

Figure 1. 4-Chloro-2-Methylaniline Structure

Table 1. Physical Properties Table (4-Chloro-2-Methylaniline)[a]	
Property (unit)	**Value**
Boiling point (ºC)	241
Melting point (ºC)	29−30
Density (g/cm^3)	1.19
Vapor pressure (Pa at 25 ºC)	5.5 mm Hg
pH (unitless)	Not available
Solubility in water (g/100 mL at 25 ºC)	0.095
Relative vapor density (air = 1)	4.9
Molecular weight (g/mol)	141.6
Flash point (°C)	99
Octanol/water partition coefficient (unitless)	2.27 (Log K_{ow})

[a]National Institute for Occupational Safety and Health (NIOSH, 2003).

The U.S. Environmental Protection Agency (U.S. EPA) IRIS database (U.S. EPA, 2009) does not list a chronic oral reference dose (RfD), a chronic inhalation reference concentration (RfC), or a cancer assessment for 4-chloro-2-methylaniline. Subchronic or chronic RfDs or RfCs for 4-chloro-2-methylaniline are not listed in the HEAST (U.S. EPA, 2003) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). The HEAST (U.S. EPA, 2003) reports a cancer weight-of evidence (WOE) classification of Group B2 (*Probable Human Carcinogen*) and oral slope factors (OSFs) of 5.8×10^{-1} mg/kg-day 4-chloro-2-methylaniline and 4.6×10^{-1} mg/kg-day 4-chloro-2-methylaniline hydrochloride, with corresponding unit risk factors of 1.6×10^{-5} µg/L and 1.3×10^{-5} µg/L, respectively, based on increased incidence of vascular tumors in male and female mice treated with 4-chloro-2-methylaniline hydrochloride (Weisburger et al., 1978). Due to use of the salt form in this study, the OSF of 4.6×10^{-1} mg/kg-day from treatment with 4-chloro-2-methylaniline hydrochloride was converted to the free-base OSF by multiplying the molecular weight ratio of the salt to the base to get 5.8×10^{-1} mg/kg-day. The 1994 CARA list (U.S. EPA, 1994a) includes a Health and Environmental Effects Profile (HEEP) for 4-chloro-2-methylaniline, detailing a Reportable Quantity (RQ) value of 5000 for both 4-chloro-2-methylaniline and 4-chloro-2-methylaniline hydrochloride, as well as carcinogen potency factors for oral exposure of 0.58 mg/kg-day and 0.46 mg/kg-day, respectively. No occupational exposure limits for 4-chloro-2-methylaniline have been derived by the American Conference of Governmental Industrial Hygienists (ACGIH, 2009), the National Institute of Occupational Safety and Health (NIOSH, 2003), or the Occupational Safety and Health Administration (OSHA, 1998). The toxicity of 4-chloro-2-methylaniline has not been reviewed by the ATSDR (2008) or the World Health Organization (WHO, 2010). The International Agency for Research on Cancer (IARC, 2000) has published a toxicological review on 4-chloro-2-methylaniline, and the National Toxicology Program (NTP, 2005) management status and health and safety reports for 4-chloro-2-methylaniline were consulted for relevant information.

Literature searches were conducted on sources published from 1900 through August 2010, for studies relevant to the derivation of provisional toxicity values for

4-chloro-2-methylaniline, CAS No. 95-69-2. Searches were conducted using EPA's Health and Environmental Research Online (HERO) evergreen database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, 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: ACGIH, 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 provides information for all of the potentially relevant toxicity studies. Entries for the principal studies are bolded.

Table 2. Summary of Potentially Relevant Data for 4-Chloro-2-Methylaniline (CASRN 95-69-2)								
Category	**Number Male/Female, Species, Study Type, Study Duration**	**Dosimetry[a]**	**Effects Observed**	**NOAEL[a]**	**BMDL/ BMCL[a]**	**LOAEL[a,b]**	**Reference (Comments)**	**Notes[c]**
Human								
1. Oral (mg/kg-day)[a]								
None								
2. Inhalation (mg/m^3)[a]								
Subchronic	None							
Chronic	None							
Developmental	None							
Reproductive	None							
Carcinogenic	116/0, occupational, median exposure duration = 25.5 years	Not reported	Bladder cancer.	None	Not run	None	(Stasik, 1988)	
	49/0, occupational, duration of exposure ranged from 3 to 956 days	Not reported	Bladder cancer.	None	Not run	None	(Popp et al., 1992)	
	342 employees (sex not reported), occupational, exposure duration reported as 1 to 5+ years	Not reported	Malignant neoplasms causing death.	None	Not run	None	(Ott and Langner, 1983)	

Table 2. Summary of Potentially Relevant Data for 4-Chloro-2-Methylaniline (CASRN 95-69-2)								
Category	**Number Male/Female, Species, Study Type, Study Duration**	**Dosimetry[a]**	**Effects Observed**	**NOAEL[a]**	**BMDL/ BMCL[a]**	**LOAEL[a,b]**	**Reference (Comments)**	**Notes[c]**
Animal								
			1. Oral (mg/kg-day)[a]					
Subchronic	**5/5, F344 rat, diet, 7 d/wk, for 7 wks**	**Male ADJ: 25, 50, 100, 200, 400, 600, 620, 650, 700, 800, 1000 Female ADJ: 113, 282, 339, 452, 677, 700, 734, 790, 903, 1130, 1410, 2820, 5650**	**10% decrease in mean body weight in females.**	**677**	**Not run**	**700**	**(NCI, 1979)**	**PS**
	5/5, B6C3F1 mouse, diet, 7 d/wk for 7 wks	Male ADJ: 361, 722, 902, 1350, 1800, 2710 Female ADJ: 2930, 3410, 3900	10% decrease in mean body weight in males and females.	1800	Not run	2710	(NCI, 1979)	
Chronic	30/30, Sprague-Dawley rat, diet, 7 d/wk for 94 and 104 wks for males and females, respectively	Male ADJ: 1.38, 6.88, 34.4 Female ADJ: 1.64, 8.20, 41.0	Increased liver weights in males and females.	8.20	Not run	34.4	(Ciba-Geigy, 1992a)	NPR
	30/30, ICR mouse, diet, 7 d/wk for 80 wks	**Male ADJ: 3.60, 18.0, 89.9 Female ADJ: 3.69, 18.4, 92.2**	**Decreased total serum protein in males and females; increased blood urea nitrogen (BUN) in females; increased serum glutamic pyruvic transaminase (SGPT) in females; mortality in males and females.**	**3.69**	**Not run**	**18.0 (FEL)**	**(Ciba-Geigy, 1992b)**	**PS, NPR**
Developmental	None							
Reproductive	125/0, NMRI/SPF mouse, gavage, 7 d/wk for 7 wks	Male ADJ: 200	Reproductive performance in the F0 and F1 males.	200	Not run	None	(Lang and Adler, 1982)	

Table 2. Summary of Potentially Relevant Data for 4-Chloro-2-Methylaniline (CASRN 95-69-2)								
Category	**Number Male/Female, Species, Study Type, Study Duration**	**Dosimetry[a]**	**Effects Observed**	**NOAEL[a]**	**BMDL/ BMCL[a]**	**LOAEL[a,b]**	**Reference (Comments)**	**Notes[c]**
Carcinogenic	50/50, F344 rat, diet, 7 d/wk for 107 wks	Male HED: 26.8, 107 Female HED: 27.4, 110	Increased adenomas of the pituitary in males and females.	26.8	No fit	107	(NCI, 1979)	
	25/0, CD rat, diet, 7 d/wk for 18 mos	Male HED: 35.4, 70.8	No effects observed.	70.8	Not run	None	(Weisburger et al., 1978)	
	30/30, Sprague-Dawley rat, diet, 7 d/wk, 94 and 104 wks for males and females, respectively	Male HED: 0.405, 2.02, 10.1 Female HED: 0.421, 2.11, 10.5	Increased liver tumors (benign and malignant) in males and females.	None	Not run	0.405	(Ciba-Geigy, 1992a)	NPR
	50/50, B6C3F1 mice, diet, 7 d/wk for 92−99 wks	Male HED: 98.4, 393 Female HED: 32.8, 131	Increased hemangiomas and hemangiosarcomas in males and females.	None	50.87 for males only	32.8	(NCI, 1979)	
	25/25, HaM/ICR mouse, diet, 7 d/wk for 18 mos	**Male HED: 19.7, 39.4 Female HED: 52.5, 105**	**Increased vascular tumors (hemangiomas and hemangiosarcomas) and total tumors in males and females.**	**None**	**2.24 for males only**	**19.7**	**(Weisburger et al., 1978)**	**PS**
	30/30, ICR mouse, diet, 7 d/wk for 80 wks	Male HED: 0.525, 2.62, 13.1 Femal HED: 0.525, 2.62, 13.1	Increased reticulum cell sarcomas and unclassified malignant tumors.	None	Not run	0.525	(Ciba-Geigy, 1992b)	NPR
2. Inhalation (mg/m^3)[a]								
None								

[a]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/m^3) units. Noncancer oral data are only adjusted for continuous exposure.
[b]Not reported by the study author but determined from the data.
[c]Notes: IRIS = Utilized by IRIS, date of last update; PS = Principal study, NPR = Not peer reviewed.

HUMAN STUDIES

Oral Exposures

No studies investigating the effects of subchronic or chronic oral exposure to 4-chloro-2-methylaniline in humans have been identified.

Inhalation Exposures

No studies investigating the effects of subchronic or chronic inhalation exposure to 4-chloro-2-methylaniline in humans have been identified.

Other Exposures

Little information is available regarding occupational exposure of humans to 4-chloro-2-methylaniline, although three retrospective studies analyzing outcomes of workers exposed during manufacturing are presented below.

Stasik (1988) reported that 116 male workers in a 4-chloro-2-methylaniline production and processing plant (Hoechst AG; Frankfurt, Germany), employed before protective industrial hygiene improvements were made to the plant in 1970, had a significantly higher incidence of bladder cancer. The exposure of this historical subcohort was presumed to be high, although no measurements of exposure were available. Within this subcohort of 116 workers, 8 individuals developed carcinomas of the urinary bladder. The median exposure duration pre-1970 for the eight individuals with bladder cancers was 14 years, while the median total exposure (pre- and post-1970) was 25.5 years. The authors used sex- and age-specific population data from the nearby German state of Saarland for 1983 to calculate the expected incidence rate of bladder cancer among the subcohort. The expected incidence was calculated to be 0.11 among the 116 workers. When compared to the actual incidence rate, the authors reported a standardized incidence rate that was 72.7 times higher than expected for bladder cancer in a comparable population. Confounders such as smoking and exposure to other contaminants are not taken into account in this study, and the sample size limits the statistical power of the study.

Similarly, Popp et al. (1992) described seven cases of bladder cancer among 49 male workers involved in the synthesis of chlordimeform from 4-chloro-2-methylaniline in Germany. The study authors reported that exposure duration ranged from three to 956 days, and for $n = 39$ workers, an average of 18 years had passed since the start of exposure by the end of 1990; however, the typical production period was 8−12 weeks per year. No exposure measurements were taken. Using similar statistical analysis to Stasik (1988), the incidence rate of bladder tumors in the cohort was compared to the standard incidence rates of populations from the former German Democratic Republic (1978 to 1982), Saarland (1988), and Denmark (1978 to 1982). The incidence rate of bladder cancer with the cohort was calculated to be 89.7, 53.8, and 35.0 times higher than the comparable populations in the former German Democratic Republic, Saarland, and Denmark, respectively.

A retrospective cohort of 342 employees (sex not reported) in a plant in the United States from 1914 and 1958, engaged in the production of dyes that involved the use and uncharacterized exposure to 4-chloro-2-methylaniline, was studied by Ott and Langner (1983) for increased mortality or cancer incidence that would have been reported in U.S. census data as a cause of death. After removing groups of employees for separate analysis due to confounding

factors, 275 individuals were found to have a standard mortality ratio from total malignant neoplasms of 1.3 and a standard mortality ratio of 1.8 from malignant neoplasms in the digestive tract.

While these studies provide important data that taken together support the possibility of increased cancers from exposure to 4-chloro-2-methylaniline, they are limited by the small populations analyzed, as well as the scope of outcomes and analyses available. Furthermore, no measurement or estimation of dose, form, or route of exposure was made. Coexposure to other potential carcinogens in the workplace (e.g., aromatic amines) and lack of controlling for tobacco usage also confounds the interpretation of the human data. Thus, these studies do not support the derivation of a provisional toxicity value.

ANIMAL STUDIES

Oral Exposures

The effects of oral exposure of animals to 4-chloro-2-methylaniline have been evaluated in subchronic-duration (NCI, 1979), chronic-duration (NCI, 1979; Weisburger et al., 1978; Ciba-Geigy, 1992a,b), and reproductive studies (Lang and Adler, 1982). NCI (1979) is a report of the bioassay for possible carcinogenicity, which includes four studies. To make the differentiation between the studies, a designation using the study type and species will be made (i.e., subchronic-duration mouse study) for each of the four studies, and the reference, NCI (1979), will be used throughout this document. Similarly, Weisburger et al. (1978) is an article that reports results from two chronic-duration studies. To make the differentiation between the studies, they will be designated using the species (i.e., rats or mice), but the reference for the entire Weisburger et al. (1978) study will be used in all cases. Ciba-Geigy sponsored two chronic-duration toxicity studies in 1992, which will be referred two as Ciba-Geigy (1992b) and Ciba-Geigy (1992a) to differentiate. Lang and Adler (1982) conducted a reproduction study using oral exposure in male mice. No studies investigating the developmental toxicity of 4-chloro-2-methylaniline have been identified.

Short-term Studies—No studies could be located regarding the effects of short-term oral exposure to 4-chloro-2-methylaniline.

Subchronic-duration Studies—The study by NCI **(1979)** is selected as the principal study for deriving the subchronic p-RfD. NCI (1979) reported an 8-week study in which groups of five F344 male rats were administered 4-chloro-2-methylaniline hydrochloride (purity >99%, based on liquid chromatography analysis) at 0, 250, 500, 1000, 2000, or 4000 ppm (0, 25, 50, 100, 200, or 400 mg/kg-day average daily dose) in the diet in one test or 6000, 6200, 6500, 7000, 8000, or 10,000 ppm (600, 620, 650, 700, 800, or 1000 mg/kg-day average daily dose) in the diet in a separate test (see Appendix B, Table B.1). In the same study, five F344 female rats were also administered 4-chloro-2-methylaniline at 0, 1000, 2500, 3000, or 4000 ppm (0, 113, 282, 339, or 452 mg/kg-day average daily dose); 0, 6000, 6200, 6500, 7000, 8000, or 10,000 ppm (0, 677, 700, 734, 790, 903, or 1130 mg/kg-day average daily dose); and 0, 6200, 12,500, 25,000 or 50,000 ppm (0, 700, 1410, 2820, or 5650 mg/kg-day average daily dose) in the diet of 4-chloro-2-methylaniline in three separate tests as shown in Table B.1. All animals were treated 7 days per week, for 7 weeks followed, by 1 week of observation after which all animals were sacrificed. The study authors recorded body weights before treatment, then biweekly during the

exposure period, and before termination, along with clinical observations. The study authors necropsied the rats at 8 weeks and conducted histopathological examinations.

A decrease in mean body weights when compared to controls was seen as doses increased, but female rats showed less of a dose-response relationship than males (see Table B.1). In the first and second experiments in male and female rats, there was a dose-response relationship between decrease in body weight and increasing doses of 4-chloro-2-methylaniline (see Table B.1). In the third experiment in female rats, the dose-response relationship between decrease in body weights and increasing doses of 4-chloro-2-methylaniline was more evident and noticeable (see Table B.1), with all treated animals exhibiting a greater than 10% decrease in body weight compared to the concurrent controls. In addition to decreases in body weight, an enlargement of the spleen was observed in the 1000- and 1130-mg/kg-day dose groups in males and females, respectively, as well as in males in the 650-mg/kg-day dose group (data not provided by study authors). This effect was reported to be produced by increased hematopoiesis and hyperemia (no data presented by study authors). Increases in marrow cellularity involving all cell types were also noted, although no data or statistics on the histopathology are provided in the report. No other treatment-related effects were seen during the clinical and histopathologic examinations. All female rats in the third experiment in the 5650-mg/kg-day dose group died during the exposure period, but the cause of death was not examined. Based on a 10% decrease in body weight in females from the third experiment (see Table B.1), a $LOAEL_{ADJ}$ of 700 mg/kg-day is identified, and a $NOAEL_{ADJ}$ of 677 mg/kg-day from the first experiment in female rats (see Table B.1) is identified (NCI, 1979).

In a study parallel to the subchronic-duration rat study described above, a subchronic-duration mouse study was conducted by NCI (1979). Groups of five B6C3F1 male mice were administered 0, 2000, 4000, 5000, 7500, 10,000, or 15,000 ppm of 4-chloro-2-methylaniline hydrochloride, and five B6C3F1 female mice were administered 0, 15,000, 17,500, or 20,000 ppm in the diet 7 days per week for 7 weeks. The corresponding adjusted daily doses are 0, 361, 722, 902, 1350, 1800, or 2710 mg/kg-day and 0, 2930, 3410, or 3900 mg/kg-day 4-chloro-2-methylaniline in the diet, for males and females, respectively. After 7 weeks of treatment and 1 week of observation, the study authors sacrificed all animals. Animals were evaluated in the same manner as the 7-week NCI (1979) subchronic-duration rat study.

Oral treatment of mice with 4-chloro-2-methylaniline in the diet did not affect the survival of any group. Similar to the NCI (1979) subchronic-duration rat study, calculated mean body weights on Day 49 decreased with increasing doses in both sexes (see Table B.2). The 2710- and 2930-mg/kg-day dose groups of male and female mice, respectively, showed a 10% body weight decrease relative to control. No other treatment-related effects were seen during the clinical and histopathologic examinations. Based on a 10% body weight decrease observed in males, a $LOAEL_{ADJ}$ of 2710 mg/kg-day and a $NOAEL_{ADJ}$ of 1800 mg/kg-day are identified for 4-chloro-2-methylaniline.

Chronic-Duration Studies—The study by Ciba-Geigy (1992b) is selected as the principal study for deriving the chronic p-RfD. The effects of chronic oral exposure to 4-chloro-2-methylaniline have been investigated in an 80-week study in ICR mice (Ciba-Geigy, 1992b).

Ciba-Geigy (1992b), in an unpublished study, treated groups of 30 ICR mice per sex per dose group with neat 4-chloro-2-methylaniline hydrochloride (purity not reported) in the diet at doses of 0, 20, 100, or 500 ppm daily for 80 weeks. The corresponding adjusted daily doses were 0, 3.60, 18.0, or 89.9 mg/kg-day in the male mice and 0, 3.69, 18.4, or 92.2 mg/kg-day in the female mice. For the cancer endpoints, the corresponding HEDs are 0, 0.525, 2.62, or 13.1 mg/kg-day for male and female mice. The study authors observed mice for mortality and clinical signs of toxicity at unreported intervals and monitored body weight and food consumption of the mice weekly. Urinalysis (pH, glucose, albumen, acetone, occult blood, urobilinogen, and bile pigments) and hematological examinations (hemoglobin, erythrocyte count, leukocyte count, and differential leukocyte count) were conducted on all mice prior to treatment and after 55 and 80 weeks of treatment. Bone marrow (differential bone marrow cell count), serum chemistry (total protein, urea nitrogen, glucose, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, alkaline phosphatase, and total cholesterol), tissue pathology (bone marrow, peripheral nerve, brain, small intestines, eyes, heart, testes, pituitary, lungs, ovaries, thyroid gland, spleen, adrenal glands, thymus, liver, lymph nodes, kidneys, pancreas, urinary bladder, stomach, and tumors), as well as the blood thrombocyte count were evaluated in all mice at 80 weeks. Macroscopic pathology and organ weights of the brain, heart, lung, liver, kidney, adrenal gland, pituitary gland, thyroid, thymus, testis, ovary, and eye after termination of exposure at 80 weeks were also analyzed.

Although the study authors did not report the statistical significance of the endpoints examined, independent significance tests were performed for this review and are presented in Table B.3 (statistically significantly different from control at $p < 0.05$). Treatment resulted in dose-related increased mortality in treated females, and to a lesser extent, treated males, with the female high-dose group showing 100% mortality by Week 67 (Ciba-Geigy, 1992b). Mortality was significantly increased in males and females at the mid- and high-dose groups (Fisher's Exact Test, $p < 0.05$). The weekly mean body weights for 4-chloro-2-methylaniline-treated animals sacrificed at the end of the study were similar to controls throughout the study, with the exception of the high-dose females, which reportedly had slightly depressed body weights (data not shown by study authors). Food intake did not vary according to exposure.

The study authors reported no marked treatment-related effects on any urinalysis parameters or hematological parameters in mice sacrificed at study termination. The only significant effect observed in the urinalysis is the urine pH in females exposed to 18.4 mg/kg-day. Relative organ weights exhibited a significant difference-with kidneys showing a decrease in males exposed in the 18.0-mg/kg-day dose group, thyroid gland (increase) in males exposed to 3.60 mg/kg-day, and spleen (increase) in females exposed to 18.4 mg/kg-day. Significant changes in the hematological values are decreases at all doses in thrombocyte cell counts in males, decreases in lymphocytes in all doses in males, an increase in neutrophils in males exposed to 3.60 mg/kg-day, increases in the white blood cell count in high-dose females, increases in neutrophils in the females exposed to 3.69 mg/kg-day, and decreases in lymphocytes in females exposed to 3.69 mg/kg-day. Bone marrow analysis indicated reduced erythroblast counts in male mice treated with 3.60 mg/kg-day of 4-chloro-2-methylaniline compared to the control group; however, the counts were normal in male mice treated with 18.0 and 89.9 mg/kg-day 4-chloro-2-methylaniline.

Table B.3 summarizes the changes in biochemical parameters in the 4-chloro-2-methylaniline exposure groups after 80 weeks of exposure. Decreases in total serum protein are found to be significant in the 3.60-mg/kg-day dose group for the males and in the 3.69- and 18.4-mg/kg-day dose groups in the sacrificed females; however, the biological relevance of this finding in and of itself is questionable. Other significant increases over control values included glucose in the males of the 3.60-mg/kg-day dose group, serum glutamic pyruvic transaminase (SGPT) in the females of the 18.4-mg/kg-day dose group, and BUN in the females of the 3.69-mg/kg-day dose group.

Table B.4 presents the percentage of specific tumor incidences in the exposed mice. Increases in hepatoma (probably benign), leukemia (includes lymphosarcomas), lung-adenomas, reticulum cell sarcomas, probably reticulum cell sarcomas, fibrosarcoma, and probably fibrosarcoma along with other benign and unclassified malignant tumors were reported using the Naïve method. The authors reported that there were no additional dose-related findings regarding macroscopic or histopathological analysis of any organs.

Based on the absence of biologically significant findings and significant frank effects (i.e., mortality) in mice at 3.69 mg/kg-day, this dose is identified as a $NOAEL_{ADJ}$ for 4-chloro-2-methylaniline. A frank effect level (FEL_{ADJ}) of 18.0 mg/kg-day is also identified. Though useful in derivation of a p-RfD, this study was not selected as the primary study for deriving a p-OSF because other peer-reviewed studies of equal or greater scientific merit were available.

In another unpublished chronic-duration study (94 weeks and 104 weeks, for males and females, respectively) conducted by Ciba-Geigy (1992a), groups of 30 Sprague-Dawley rats per sex per dose group were administered 0, 20, 100, or 500 ppm of 4-chloro-2-methylaniline hydrochloride (purity not specified) by diet. The corresponding adjusted daily doses were 0, 1.38, 6.88, or 34.4 mg/kg-day for the male rats and 0, 1.64, 8.20, or 41.0 mg/kg-day for female rats. The corresponding HEDs were 0, 0.405, 2.02, or 10.1 mg/kg-day for the male rats and 0, 0.421, 2.11, or 10.5 mg/kg-day for female rats. The study authors performed macroscopic pathology and organ weight analysis (brain, heart, lungs, liver, kidneys, adrenal glands, thyroid gland, pituitary gland, thymus, testes, ovaries, and eyes) following sacrifice. Tissues were preserved and stained for histopathology, (bone marrow, peripheral nerve, brain, heart, lungs, spleen, liver, kidneys, pancreas, small intestine, testes, ovaries, adrenal glands, lymph nodes, urinary bladder, eyes, pituitary gland, thyroid gland, thymus, stomach, and any tumors) and microscopic examination was conducted on the same tissues of all animals following the termination of the exposure duration.

Although the study authors did not report the statistical significance of the endpoints examined, independent significance tests are performed for this review (statistically different from control at $p < 0.05$). The 1.38-and 6.88-mg/kg-day dose groups in male rats are significantly different from the control in the heart, lung, adrenal, and brain weights. However, these organ weight increases did not appear to be dose related as none of them were significantly increased at the high-dose (34.4 mg/kg-day) level. The liver was the only organ whose weight was significantly increased over the control group at the 34.4-mg/kg-day dose in male rats. The only significant change in organ weights of female rats treated with 4-chloro-2-methylaniline was noted in liver, which showed an increase in weight in the 41.0-mg/kg-day dose group when

compared to the control group. Gross macroscopic findings included a significantly increased incidence of "tumor-like nodules" of the liver in the 34.4-mg/kg-day dose males, as well as the 8.2 and 41.0-mg/kg-day dose females. However, the biological significance of these "tumor-like nodules" is unclear because they are not well characterized pathologically, and it is also unknown whether they could be preneoplastic in nature. Therefore, it is not prudent to use this endpoint quantitatively in the derivation of a chronic p-RfD. Although significant changes in heart, lung, adrenal, and brain weights were seen in males at the lowest dose tested, these changes were not dose-related. Thus, based on increased liver weight observed in both males and females, a $LOAEL_{ADJ}$ of 34.4 mg/kg-day and a $NOAEL_{ADJ}$ of 6.88 mg/kg-day are identified.

Tumor incidences, which the study authors reported using a life-table method as well as the Naïve method (see Table B.5), increased in a dose-related manner in the liver (malignant and benign) and adrenal gland, with higher incidences noted in female rats as compared to controls. Tumors of the pituitary and mammary glands were also noted, but these tumors did not exhibit a dose-response trend, but like the liver and adrenal tumors, appeared at a higher incidence rate in female rats compared to male rats. Low tumor incidences were observed in other organs (thyroid, brain, kidneys, mammary glands, uteri, urinary bladder, vagina, and skin), but these incidences were reported not to be dose related. The authors reported no other dose-related histological and pathological findings in any of the organs examined, with the exception of tumor-like nodules in the liver of both male and female rats as stated above.

NCI (1979) conducted a 2-year chronic carcinogenicity peer-reviewed study in mice and rats. In the 2-year chronic carcinogenicity study in rats conducted by NCI (1979), the study authors administered 4-chloro-2-methylaniline hydrochloride (purity >99%) in the diet to 50 F344 rats per sex per dose group at doses of 1250 or 5000 ppm for 107 weeks. The corresponding control group fed diet alone consisted of 20 F344 rats/sex. The corresponding HEDs are 0, 26.8, or 107 mg/kg-day for males and 0, 27.4, or 110 mg/kg-day for females. Rats were evaluated as described for the subchronic-duration rat study (NCI, 1979) with the following changes. The study authors recorded body weights monthly, except for Weeks 50−90 and 96−104, in which no body weight data were collected. All animals were examined twice per day, and observations were also made for moribund and sick, tumor-bearing animals. Clinical examination and palpations for masses were conducted monthly during the duration of the study, and at termination. All surviving animals were sacrificed at the end of treatment and were necropsied. A gross and microscopic examination on major tissues, major organs, and all gross lesions was also performed. Microscopic examination was performed on skin, lungs and bronchi, trachea, femur bone marrow, spleen lymph nodes, thymus, heart, salivary glands, liver pancreas, esophagus, stomach, small and large intestines, kidneys, urinary bladder, pituitary, adrenal glands, thyroid, parathyroid, testes, prostate, mammary glands, uteri, ovaries, brain, and all tissue masses. When possible, peripheral blood smears were made.

Treatment with oral 4-chloro-2-methylaniline hydrochloride lead to reduced mean body weights in male and female rats exposed to the high dose as compared to controls (NCI, 1979). Treatment did not affect mortality in a dose-related manner in either male or female rats during the study. Other treatment-related clinical signs were not observed. Histopathology revealed a dose-related increase of adenomas of chromophobe cells of the pituitary gland in both sexes as compared to controls (see Table B.6). All of these adenomas were found to be benign, although in nine rats, compression of the hypothalamus was observed. On page 23 of the report, the study

authors noted that this effect "may be considered to be compound related on the basis of this study," but they note that this type of tumor is common (21%) in controls rats of this strain (NCI, 1979). A number of inflammatory and degenerative lesions were found in controls and dosed rats but were mild in nature and not considered compound related. The results of the statistical analysis of these effects were equivocal. Analysis of the incidence of chromophobe adenomas of the pituitary in male rats showed the increase to be significant by the Cochran-Armitage test but was not significant by the Fisher's exact test. In female rats, the incidence of this tumor type was not significant by the Cochran-Armitage test, but it was significant at the high-dose level comparison against controls by the Fisher's exact test. Based on the histopathological findings, the study authors conclude that 4-chloro-2-methylaniline was not carcinogenic to F344 rats in the conditions used for this assay. Based on increased of pituitary adenomas in both males and females, a $LOAEL_{HED}$ of 107 mg/kg-day and a $NOAEL_{HED}$ of 26.8 mg/kg-day are identified. This study is not selected to support the development of a p-OSF because rats appear to be a less sensitive model of the effects of 4-chloro-2-methylaniline administered orally, as compared to mice.

In a study parallel to the chronic-duration rat study, NCI (1979) conducted a chronic-duration/carcinogenicity mouse study. The study authors administered neat 4-chloro-2-methylaniline hydrochloride in the diet to groups of 50 B6C3F1 mice per sex per dose group with doses of 3750 or 15,000 ppm and 1250 or 5000 ppm of 4-chloro-2-methylaniline, respectively (purity >99%) in the diet for 99 weeks, except for the high-dose females, which were exposed for 92 weeks (NCI, 1979). The corresponding control group fed the diet alone consisted of 20 mice/sex. The corresponding HEDs were 0, 98.4, or 393 mg/kg-day for males and 0, 32.8, or 131 mg/kg-day for females. Mice were evaluated as described for the rat chronic-duration study (NCI, 1979) with the following changes: The study authors recorded body weights, clinical findings, and palpations for masses monthly for the duration of the study, and at termination.

Treatment with oral 4-chloro-2-methylaniline hydrochloride led to reduced body weights throughout the study duration in male and female mice at the two administered doses when compared to the control group, with females exhibiting a more notable effect (NCI, 1979). Treatment significantly affected mortality in both male and female mice over the course of the study. Histopathology revealed a dose-related significant increase of hemangiosarcoma in both sexes (see Table B.7). Furthermore, the combined incidence of hemangiosarcomas and hemangiomas were dose related and significantly higher than control, which is consistent with the findings of the Weisburger et al. (1978) mouse study (see below). Tumor morphology was highly variable. The study authors reported the bulk of the hemangiosarcoma to be composed of large hematomatous masses of necrotizing extravasated blood, with tumor tissue at the periphery. These lesions were associated with hemorrhage of the peritoneal cavity and variable enlargement of the spleen, which appeared to be produced by an increase of extramedullary hemapoiesis from the continued hemorrhaging from the tumors.

Benign hemangiomas in the genital fat were discovered in one male and one female mouse in the low-dose groups. Hemangiomas in other organs and tissues were found at a low rate of incidence. A high incidence rate of hemosiderin deposition in the renal tube epithelia was found in concurrence with the hemangiosarcoma (43/119 mice), while a lesser concurrence of hydronephrosis with the hemangiosarcoma (10/119 mice) was noted and assumed to be from the

compression of the ureters by the tumor. Inflammatory and degenerative lesions were seen at a low rate of incidence, seemingly due to the hemangiosarcoma. Additionally, pulmonary metastasis was found at a low rate of incidence (5/119 mice), but this tumor, when present, proved to be lethal (75%), resulting from hemorrhage in the peritoneal cavity and the space-consuming nature of the lesions (NCI, 1979). Increased vascular tumors reached statistical significance at the lowest dose in females and support a $LOAEL_{HED}$ of 32.8 mg/kg-day. Because the increase in tumor incidence was seen in the lowest dose-level group, a NOAEL cannot be identified. This study, while scientifically acceptable and well conducted, does not support the development of a p-OSF due to the lack of a dose response in the incidence of the tumors.

The study by Weisburger et al. (1978) is selected as the principal study for deriving the p-OSF. Weisburger et al. (1978) conducted a study on the carcinogenicity of 21 aromatic amines and amine derivatives, including 4-chloro-2-methylaniline hydrochloride (97−99% pure), on CD rats and HaM/ICR-derived CD-1 mice. The study authors administered 0, 750, or 1500 mg/kg neat material in the diet to 25 males per dose and 0, 2000, or 4000 mg/kg in the diet of 25 females per dose for 7 days per week, for 18 months (Weisburger et al., 1978). The corresponding HEDs were 0, 19.7, or 39.4 mg/kg-day and 0, 52.5, or 105 mg/kg-day for males and females, respectively. Following 18 months of treatment, all animals were maintained on a control diet for an additional 3 months. Simultaneous controls of 14 male mice and 15 female mice were fed an untreated diet over the same period. Pooled controls included the simultaneous controls, as well as the simultaneous controls from the studies of other chemicals presented in this paper. The study authors conducted necropsies on all animals that died after 6 months of exposure, and all surviving animals were sacrificed at the termination of the study. Examination of all sacrificed animals included histopathological examination of all abnormal organs, tumor masses, lungs, spleen, liver, kidneys, adrenal glands, heart, bladder, stomach, intestines, and reproductive organs.

The study authors reported that exposures produced statistically significant (Fisher's Exact Test, $p < 0.05$) increases in vascular tumor incidence (hemangiosarcoma and hemangioma) among male and female mice at all dose levels as compared to simultaneous controls (see Table B.8). Weisburger et al. (1978) noted that these tumors arose primarily in the spleen and subcutaneous or subperitoneal fat (data not provided). Incidences of the multiple tumors were significant in male mice in the low-dose group when compare to the pooled controls. Based on increased vascular tumor incidence, a LOAEL of 19.7 mg/kg-day is identified. A NOAEL is not identified because the increase in tumor incidence was seen in the lowest dose level group. The study supports the development of a p-OSF because it has been peer reviewed and performed according to Good Laboratory Practice (GLP) principles and meets the standards of study design and performance.

In a study parallel to the chronic-duration mouse study, Weisburger et al. (1978) administered neat 4-chloro-2-methylaniline hydrochloride (purity not reported) in the diet of male CD rats at 0, 3000, or 6000 mg/kg for 3 months followed by 0, 500, or 1000 mg/kg for 15 months. The calculated HEDs are 0, 35.4, and 70.8 mg/kg-day. After 15 months of treatment, all rats were fed a control diet for an additional 6 months prior to study termination. Animals that died during the first 6 months of the study were not necropsied; however, animals that died or were sacrificed at study termination received a complete necropsy. Histopathology of all sacrificed animals included examination of all abnormal organs, tumor masses, lungs,

spleen, liver, kidneys, adrenal glands, heart, bladder, stomach, intestines, pituitary gland, and reproductive organs. The study authors reported that, in general, 4-chloro-2-methylaniline was inactive in rats; however, one male rat in the high-dose (6000-mg/kg) group exhibited a mesothelioma that showed a large amount of involvement of the pleural and pericardial surfaces. The authors concluded that this tumor was likely not due to exposure to 4-chloro-2-methylanaline. This study is not selected to support the development of a p-OSF due to the generally inactive response in male rats exposed to 4-chloro-2-methylaniline.

Developmental and Reproduction Studies—4-Chloro-2-methylaniline was tested for reproductive toxicity in 125 male F0 NMRI/SPF mice by Lang and Alder (1982). The study authors administered 200 mg/kg-day aqueous 4-chloro-2-methylaniline by gavage 7 days per week, for 7 weeks. The study authors weighed the animals at the termination of exposure and placed them in cages with two untreated females for 7 days. Litter sizes were reported at birth and at weaning. The study authors evaluated the reproductive performance of F1 males at 3−8 months of age using the sequential decision procedure on litter sizes.

The study authors reported no significant reproductive effects in the F0 or F1 generation from F0 4-chloro-2-methylaniline exposure (Lang and Adler, 1982). Using mean litter size, as compared to simultaneous positive (Tretamine) and negative (vehicle) controls, the authors concluded that there was no dominant lethality in the F1 generation in treated groups. The frequency of observed matings was not reported to have changed compared to controls. The study authors concluded, and the data support that 4-chloro-2-methylanlinline does not produce adverse reproductive effects under these conditions. A NOAEL of 200 mg/kg-day is identified.

Inhalation Exposures

Short-term Studies—No studies could be located regarding the effects of short-term oral exposure to 4-chloro-2-methylaniline.

Subchronic-Duration Studies—No studies could be located regarding the effects of subchronic inhalation exposure of animals to 4-chloro-2-methylaniline.

Chronic-Duration Studies—No studies could be located regarding the effects of chronic inhalation exposure of animals to 4-chloro-2-methylaniline.

Developmental and Reproductive Studies—No studies could be located regarding the effects of inhaled 4-chloro-2-methylaniline on reproduction and fetal development.

Other Exposures

No studies could be located regarding the effects of exposure of animals via other routes to 4-chloro-2-methylaniline.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

A few studies on the toxicokinetics of 4-chloro-2-methylaniline are available (Bentley et al., 1986; Hill et al., 1979; Struck et al., 1978; Leslie et al., 1988). Results of available studies indicate that 4-chloro-2-methylaniline undergoes distribution to primarily the liver and kidney in rodents, but the specific distribution seems to be species dependent. These studies also indicate that 4-chloro-2-methyalniline is metabolized in rodents, and this

metabolism, though not completely understood, seems to be responsible for the observed toxicity. Furthermore, 4-chloro-2-methylaniline or its metabolites are capable of binding macromolecules in rodent liver and kidney; in the case of the rat liver in vitro, the binding to DNA is NADPH-dependent and involves the cytochrome P-450 pathway.

The genotoxicity of 4-chloro-2-methylaniline has been tested in several studies using in vitro test systems (McGregor et al., 1988; Galloway et al., 1987; Göggelmann et al., 1996; IARC, 2000). These test results generally indicate that 4-chloro-2-methylaniline does not have mutagenic activity when tested in bacteria, while the majority of mammalian tests indicate some genotoxicity, with unclear results from chromosome aberration tests. Although there is only one study investigating the genotoxic potential of 4-chloro-2-methylaniline in vivo, the results demonstrate that oral exposure can induce DNA damage in the tissues of mice and rats (Sekihashi et al., 2002), although no heritable translocations were found in spermatocytes from F1 mice (Lang and Adler, 1982). The literature on the mutagenic action of 4-chloro-2-methylaniline is equivocal, and further investigations are needed before a conclusive mechanism of action can be established.

Table 3 summarizes the toxicokinetics and genotoxicity studies.

Table 3. Other Studies				
Tests	**Materials & Methods**	**Results**	**Conclusions**	**References**
Toxicokinetic	Six male S-D rats were administered 10 or 100 mg/kg 4-chloro-2-methylaniline in corn oil by intraperitoneal injection for 7 consecutive days. Animals sacrificed 24 hours after the final dose, and liver microsomes, along with proteins, were isolated.	Significantly ($p < 0.05$)-induced cytochrome P-450, ethoxyresorfin-*O*-deethylase (18-fold increase at 100 mg/kg), ethoxycoumarin-*O*-deethylase, epoxide hydrolase, and glutathione *S*-transferase. Aminopyrine *N*-demethylase was not affected. Increased 7α, 16β (3-fold), and 16α (1.6-fold) hydroxylase. Testosterone decreased. SDS-PAGE of liver microsomes showed protein increase in treated animals at MW 54 kD.	4-chloro-2-methylaniline appears to be metabolized in microsomes through the P-450c and P-450d pathways, as marked by increases in ethoxyresorufin-*O*-deethylase and ethoxycoumarin-*O*-deethylase, notably at the low dose as well as the high dose. Induction of P-450c and P-450d supported by results of SDS-PAGE.	(Leslie et al., 1988)
Toxicokinetic	Five Osborne-Mendel rats (sex not reported) were administered 14 mg/kg of 4-chloro-2-[*methyl*-^{14}C] methylaniline hydrochloride in 0.9% sodium chloride solution by intraperitoneal injection for 24 hours. Following sacrifice at 24 hours, macromolecules, DNA, RNA, and proteins were isolated.	4-chloro-2-[*methyl*-^{14}C]-methylaniline bound most highly in the liver, where more radioactivity was measured than in all other organs combined. In vitro, liver microsomes showed NADPH-dependent binding that increased with phenobarbital pretreatment. Two soluble products were identified by mass spectrometry and chemical synthesis from the microsomes: 5-chloro-2-hydroxylaminotoluene and 4,4'-dichloro-2,2'-dimethylazobenzene.	Results of the in vitro portion of the study indicate binding is catalyzed by liver microsomes and is irreversible. Results of pretreatment with phenobarbital, a cytochrome P-450 inducer, suggest that cytochrome P-450 pathway is in the binding process.	(Hill et al., 1979; Struck et al., 1978)

Table 3. Other Studies				
Tests	**Materials & Methods**	**Results**	**Conclusions**	**References**
Toxicokinetic	Male mice and Sprague-Dawley rats were administered 25 mg/kg 4-[^{14}C] chloro-2-methylaniline hydrochloride by gavage in one dose and were sacrificed 18 or 37 hours later, or were treated for 14 days and were sacrificed 18 hours after last treatment. Following sacrifice, DNA, RNA, and protein fractions were isolated from the livers. Single cell suspensions were prepared from mouse livers. In vitro, liver supernatant fractions were combined with 4.8 mg calf thymus DNA and 11 mM 4-[^{14}C] chloro-2-methylaniline hydrochloride and were incubated for 30 minutes.	4-[^{14}C] chloro-2-methylaniline hydrochloride bound DNA in mice and rats ($p < 0.01$), with binding decreasing from 12 to 68 hours, but at all time points, mouse liver DNA bound to a greater extent than rat liver DNA (roughly 2-fold). Binding was greater in RNA and protein fractions, than in DNA, and was greater in rats than mice. Isolated mouse liver cells showed more binding in nonparenchymal cell DNA than in whole liver DNA at early time points, but the trend reversed at later time points. Mouse liver supernatant fractions were more successful in binding calf thymus DNA than rat fractions.	Hepatic mouse DNA bound more 4-[^{14}C] chloro-2-methylaniline hydrochloride than rat, with amounts decreasing in both species over time attributable to DNA repair. Binding was found to be proportional to the total administered dose and showed mouse liver DNA to be a much more potent binder, per dose, than rat liver DNA. Similarly, in vitro studies showed mouse liver microsomal fractions more efficiently catalyzed metabolites, which bound to the calf thymus DNA, than did the rat fractions, with the reverse observed for protein binding. This result implies that two species may produce different metabolic intermediates, or that binding of proteins may protect DNA in rat livers. Results in nonparenchymal cells could not support susceptibility of mice to tumor induction in blood vessel endothelial cells. It was suggested that 4-chloro-2-methylaniline may be preferentially activated at that target.	(Bentley et al., 1986)
Genotoxicity	L5178Y tk +/- mouse lymphoma cell forward mutation assay, with and without metabolic activation.	Test results indicate that 4-chloro-2-methylaniline has no mutagenic activity, with or without metabolic activation.	Result suggests that in this in vitro assay, 4-chloro-2-methylaniline is not genotoxic.	(McGregor et al., 1988)

Table 3. Other Studies				
Tests	**Materials & Methods**	**Results**	**Conclusions**	**References**
Genotoxicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) with and without metabolic activation using TA1535, TA1537, TA98, and TA100 strains. TA100 was exposed to a 100-µg/plate. TA1535 and TA1537 were exposed to a 1500-µg/plate. TA98 was exposed to a 375-µg/plate. Chromosomal aberrations and sister chromatid exchange were observed in human lymphocytes. Induction of spindle disturbances in V79 Chinese hamster ovary (CHO) cells was observed.	No mutations were found in *Salmonella typhimurium* strains without metabolic activation. With S9 metabolic activation revertants observed in TA100 and TA98 at rates 2-fold over control. No structural or numerical changes were observed in mammalian cells, with or without metabolic activation.	In presence of metabolic (S9) activation, 4-chloro-2-methylaniline active in TA100 (base substitutions) and TA98 (frameshift mutations). Differences between this result and other negative published Ames test results are thought to be due to protocol differences and dose ranges. Other results indicate that standard mammalian test did not show genotoxicity.	(Göggelmann et al., 1996)
Genotoxicity	125 F0 NMRI/SPF male mice administered 200 mg/kg 4-chloro-2-methylaniline by gavage 7 days/week, for 7 weeks, then bred with untreated females. Resulting F1 males tested for cytogenicity.	Spermatocytes from sterile, partially sterile, or unclassifiable (1025) F1 mice did not show a significant increase in heritable translocation events.	Results from negative and positive controls in two-generation study were as expected, but no cytogenicity was found in 4-chloro-2-methylaniline-exposed male offspring.	(Lang and Adler, 1982)
Genotoxicity	Four mice or rats were administered 600 mg/kg 4-chloro-2-methylaniline in olive oil orally. Stomach, colon, liver, kidney, bladder, lung, brain, and bone marrow were then sampled at 3, 8, and 24 hours following exposure for a Comet Assay of DNA damage.	DNA damage ($p < 0.01$) in mice found in liver, bladder, lung, and brain 24 hours following last exposure to 4-chloro-2-methylaniline. DNA damage in brain was also found at significant levels 3 hours following last dose. In rat, significant DNA damage was found in the liver at all time points and in the kidney after 24 hours.	4-chloro-2-methylaniline was positive in both mice and rats in a Comet Assay. Comparisons of chemicals tested showed certain organs in a given species may be more sensitive to genotoxicity. Kidney cells were found to be more sensitive in rats than mice, while the liver was very sensitive in mice.	(Sekihashi et al., 2002)

Table 3. Other Studies				
Tests	**Materials & Methods**	**Results**	**Conclusions**	**References**
Genotoxicity	CHO cells exposed to 50 µg/mL and examined for sister chromatid exchange or exposed to 400 µg/mL and tested for chromosomal aberrations.	Test was positive for sister chromatid exchange events, with and without metabolic activation, but only positive for chromosomal aberrations with metabolic activation, and negative without.	4-chloro-2-methylaniline was positive for cytogenicity. Chromosomal aberrations depend on the metabolic fraction in this assay.	(Galloway et al., 1987)
Genotoxicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) with and without metabolic activation using TA1535, TA1537, TA98, and TA100 strains. TA100 exposed to 333 µg/plate, with and without metabolic activation, while other strains exposed to 1000 µg/plate, with and without metabolic activation.	Test was negative for reverse mutations in all strains, under all conditions.	Negative result for mutagenicity in the Ames assay.	The original source of Haworth et al.,1983 was unavailable for review at this time. (IARC, 2000).

DERIVATION OF PROVISIONAL VALUES

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

DERIVATION OF ORAL REFERENCE DOSES

Derivation of Subchronic Provisional RfD

The subchronic-duration rat study by NCI (1979) is selected as the principal study for derivation of a subchronic p-RfD. The critical endpoint is a 10% decrease in body weight in female rats. This study is a range-finding study in a peer-reviewed report conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, in Bethesda, Maryland, has been performed according to GLP principles, and meets the standards of study design and performance with regards to the numbers of animals, and the examination of potential toxicity. Details are provided in the ***Review of Potentially Relevant Data*** section. BMD modeling is not possible with these data because the body-weight data were provided by the study authors in the form of mean percent decrease from control, with no raw data or means with standard deviations. Among the available acceptable studies, the NCI (1979) study represents the lowest credible point-of-departure (POD) for deriving a subchronic p-RfD.

Table 4. Summary of Noncancer Reference Values for 4-Chloro-2-Methylaniline (CASRN 95-69-2)							
Toxicity type (units)[a]	**Species/Sex**	**Critical Effect**	**p-Reference Value**	**POD Method**	**POD**	**UF_C**	**Principal Study**
Subchronic p-RfD (mg/kg-day)	Rat/F	10% decrease in body weight	**5×10^{-1}**	NOAEL	677	1000	(NCI, 1979)
Screening chronic p-RfD[b] (mg/kg-day)	Mouse/F	Absence of biologically significant liver effects and significant frank effects	**3×10^{-3}**	NOAEL	3.69	1000	(Ciba-Geigy, 1992b)
Subchronic p-RfC (mg/m^3)	None						
Chronic p-RfC	None						

[a]All the reference values obtained from IRIS are indicated with the latest review date.
[b]A screening value is provided in Appendix A of this document.

Table 5. Summary of Cancer Values for 4-Chloro-2-Methylaniline (CASRN 95-69-2)				
Toxicity Type	**Species/Sex**	**Tumor Type**	**Cancer Value**	**Principal Study**
p-OSF	Mouse/F	Hemangiomas or hemangiosarcomas (vascular tumors)	**1×10^{-1} per mg/kg-day**	(Weisburger et al., 1978)
p-IUR	None			

Of the two subchronic-duration studies considered for the derivation of the subchronic p-RfD, the NCI (1979) subchronic-duration study in rats with decreased body-weight changes in females gives the lowest LOAEL (700 mg/kg-day) and NOAEL (677 mg/kg-day). The NCI (1979) subchronic-duration study in mice provides a LOAEL more than four times higher for the same endpoint, thereby supporting the selection of the NCI (1979) study as the principal study. Because the study administered 4-chloro-2-methylaniline hydrochloride, the p-RfD is adjusted to reflect the molecular weight difference between 4-chloro-2-methylaniline and the salt form.

The POD in this study is a NOAEL of 677 mg/kg-day in female rats from principal study data (i.e., NCI, 1979]).

Adjusted points for daily exposure:

The following dosimetric adjustments were made for each dose in the principal study for diet treatment in adjusting for daily exposure.

$$\begin{aligned} NOAEL_{ADJ} &= NOAEL_x \times \text{Food Consumption per Day} \\ &\quad \times (1 \div \text{Body Weight}) \times (\text{Days Dosed} \div \text{Total Days}) \\ &= 6000 \text{ mg/kg} \times 0.014 \text{ kg/day} \times (1 \div 0.124 \text{ kg}) \\ &\quad \times (49 \text{ days dosed} \div 49 \text{ total days}) \\ &= 84 \text{ mg/day} \times 8.06 \text{ kg}^{-1} \times 1 \\ &= 677 \text{ mg/kg-day} \times 1 \\ &= 677 \text{ mg/kg-day} \end{aligned}$$

A subchronic p-RfD is developed as follows:

$$\begin{aligned} \text{Subchronic p-RfD}_{salt} &= NOAEL_{ADJ} \div UF_C \\ &= 677 \text{ mg/kg-day} \div 1000 \\ &= 0.68 \text{ mg/kg-day or } 7 \times 10^{-1} \text{ mg/kg-day} \end{aligned}$$

$$\begin{aligned} \textbf{Subchronic p-RfD}_{\textbf{base}} &= \textbf{MW of base} \div \textbf{MW of salt} \times \textbf{p-RfD}_{\textbf{salt}} \\ &= \textbf{141.6} \div \textbf{178.07} \times \textbf{0.68 mg/kg-day} \\ &= \textbf{0.80} \times \textbf{0.68 mg/kg-day} \\ &= \textbf{0.54 mg/kg-day or } \mathbf{5 \times 10^{-1}} \textbf{ mg/kg-day} \end{aligned}$$

Tables 6 and 7 summarize the uncertainty factors (UFs) and the confidence descriptors for the 4-chloro-2-methylaniline subchronic p-RfD, respectively.

Table 6. Uncertainty Factors for Subchronic p-RfD of 4-Chloro-2-Methylaniline[a]		
UF	**Value**	**Justification**
UF_A	10	A UF_A of 10 is applied for interspecies 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 subchronic toxicity of 4-chloro-2-methylaniline.
UF_D	10	A UF_D of 10 is selected because there are no acceptable two-generation reproduction studies or developmental studies, and there is no indication of any other studies that may be relevant for the database uncertainty factor.
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 in humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a NOAEL.
UF_S	1	A UF_S of 1 is applied because a subchronic study (NCI [1979]) was utilized as the principal study.
$UF_C \leq 3000$	1000	

[a]NCI (1979).

The confidence of the subchronic p-RfD for 4-chloro-2-methylaniline is medium as explained in Table 7.

Table 7. Confidence Descriptor for Subchronic p-RfD for 4-Chloro-2-Methylaniline		
Confidence Categories	**Designation[a]**	**Discussion**
Confidence in study	M	Confidence in the key study is medium. NCI (1979) was a preliminary range-finding study for a longer-term study to follow, but assessed 10% decrease in body weight in an appropriate number of animals. A NOAEL is identified, and the key study is supported by similar observations in the chronic-duration study in rats conducted by NTP (1979).
Confidence in database	M	The database includes subchronic- and chronic-duration toxicity studies in two species (rats and mice), no developmental toxicity studies, and a two-generation reproduction study.
Confidence in subchronic p-RfD[b]	M	The overall confidence in the subchronic p-RfD is medium.

[a]L = Low, M = Medium, H = High.
[b]The overall confidence cannot be greater than the lowest entry in the table.

Table 8 summarizes the relevant oral systemic toxicity studies for 4-chloro-2-methylaniline.

Table 8. Summary of Oral Systemic Toxicity Studies for 4-Chloro-2-Methylaniline

References	# Sex (M/F), Species	Exposure (mg/kg-day)	Frequency/ Duration	$NOAEL_{ADJ}$[a] (mg/kg-day)	$LOAEL_{ADJ}$[b] (mg/kg-day)	Critical Endpoints
NCI (1979)	5/5, rat	Male ADJ: 25, 50, 100, 200, 400, 600, 620, 650, 700, 800, 1000 Female ADJ: 113, 282, 339, 452, 677, 700, 734, 790, 903, 1130, 1410, 2820, 5650	7 d/wk for 7 wks in the diet	677	700	10% decrease in mean body weight in females
Ciba-Geigy (1992a)	30/30, rat	Male ADJ: 1.38, 6.88, 34.3 Female ADJ: 1.64, 8.20, 41.0	7 d/wk for 94 and 104 wks for males and females, respectively, in the diet	8.20	34.4	Increased liver weight in males and females
NCI (1979)	5/5, mouse	Male ADJ: 361, 722, 902, 1350, 1800, 2710 Female ADJ: 2930, 3410, 3900	7 d/wk for 7 wks in the diet	1800	2710	10% decrease in mean body weight in males and females
Ciba-Geigy (1992b)	30/30, mouse	Male ADJ: 3.60, 18.0, 89.9 Female ADJ: 3.69, 18.4, 89.9	7 d/wk for 18 mos in the diet	3.69	18.0 (FEL)	Increased mortality in males and females; increased serum glutamic pyruvic transaminase (SGPT) in females.

[a]$NOAEL_{ADJ}$ = NOAEL × (average food intake) × (1/body weight) × (feeding schedule).
[b]$LOAEL_{ADJ}$ = LOAEL × (average food intake) × (1/body weight) × (feeding schedule).

Derivation of Chronic Provisional RfD

No chronic p-RfD can be derived for the following reason: a nonpeer-reviewed study is selected as the principal study for the chronic p-RfD; however, Appendix A of this document contains a screening value that may be useful in certain instances. Please see the attached Appendix A for details.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

Derivation of Subchronic Provisional RfC

For the reasons noted in the main document, it is inappropriate to derive a provisional subchronic RfC for 4-chloro-2-methylaniline. No quantitative human or animal studies examining the effects of subchronic inhalation exposure to 4-chloro-2-methylaniline have been located. Derivation of a screening value is precluded.

Derivation of Chronic Provisional RfC

For the reasons noted in the main document, it is inappropriate to derive a provisional chronic p-RfC for 4-chloro-2-methylaniline. No quantitative human or animal studies examining the effects of chronic inhalation exposure to 4-chloro-2-methylaniline have been located. Derivation of a screening value is precluded.

CANCER WEIGHT-OF-EVIDENCE DESCRIPTOR

Table 9 identifies the cancer WOE descriptor for 4-chloro-2-methylaniline.

Table 9. Cancer WOE Descriptor for 4-Chloro-2-Methylaniline			
Possible WOE Descriptor	**Designation[a]**	**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"	X	Oral administration by diet only	Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the available evidence for oral exposure to 4-chloro-2-methylaniline implies likely carcinogenicity based on evidence of carcinogenicity in mice in the NCI (1979) study and the Weisburger et al. (1978) oral bioassay, as well as limited human data. Results of the Weisburger et al. (1978) bioassay show significant increases over the ranges for historical controls and significant positive trends for vascular tumors observed mainly in the spleen or adipose tissue (hemangiomas and hemangiosarcomas combined) in male and female mice treated orally for 18 months (see Table B.8). In addition, the occurrence of hemangiosarcomas and hemangiomas and hemangiosarcomas originating from adipose tissue in the NCI (1979) study shows a similar significant positive trend during a 2-year period and also increases over ranges for historical controls. Thus, 4-chloro-2-methylaniline is included in the *11th Report on Carcinogens*, which concludes that it is "*Reasonably Accepted to be a Human Carcinogen*" (NTP, 2005). Exposure-related tumors have not been observed in male or female rats exposed to oral 4-chloro-2-methylaniline for 18 months and 2 years (Weisburger et al., 1978; NCI, 1979). Studies evaluating the carcinogenic potential of inhaled 4-chloro-2-methylaniline animals were not located. Occupational studies indicate carcinogenic potential, although the doses and routes were not controlled or measured (Stasik, 1988; Popp et al., 1992; Ott and Langner, 1983).
"Suggestive Evidence of Carcinogenic Potential"	N/A	N/A	The evidence from human and animal data is more than suggestive of carcinogenicity, which raises a concern for carcinogenic effects, and is judged sufficient for a stronger conclusion.
"Inadequate Information to Assess Carcinogenic Potential"	N/A	N/A	There is adequate available information to assess carcinogenic potential.
"Not Likely to Be Carcinogenic to Humans"	N/A	N/A	No strong evidence of noncarcinogenicity in humans is available.

MODE-OF-ACTION DISCUSSION

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

The mechanism of 4-chloro-2-methyaniline-induced carcinogenicity has not yet been determined; however, available evidence suggests that vascular tumors (hemangiomas and hemangiosarcomas) observed in mice following oral exposure to 4-chloro-2-methylaniline may arise from genetic mechanisms. Other potential modes of action for 4-chloro-2-methylaniline-induced hemangiomas and hemangiosarcomas have not yet been identified.

Mutagenic Mode of Action

Key Events—Numerous studies using in vitro test systems provide evidence that 4-chloro-2-methylaniline has mutagenic activity in mammalian systems in vitro, although evidence of genotoxic activity in vivo is lacking. In bacteria, conflicting results have been reported with 4-chloro-2-methylaniline, both in the presence and in the absence of metabolic activators (IARC, 1990). At least one study author believes these inconsistencies may reflect sensitivity to the particular metabolic system used in the assay (Göggelmann et al., 1996). In mammalian cells, 4-chloro-2-methylaniline-induced sister chromatid exchanges in Chinese hamster ovary (CHO) cells (Galloway et al., 1987), and unscheduled DNA synthesis in primary rat hepatocytes (IARC, 1990). The Galloway et al. (1987) reported positive results for chromosomal aberrations in cultured CHO cells in the presence of metabolic activation and negative results in the absence of metabolic activation. Bentley et al. (1986) attempted to look at the susceptibility of nonparenchymal cells, but results were equivocal. Studies evaluating the genotoxicity of 4-chloro-2-methylaniline in cells of vascular origin or in vivo in humans are lacking.

Strength, Consistency, Specificity of Association—Although NCI (1979) reported equivocal evidence of the potential of oral 4-chloro-2-methylaniline to induce hemangiomas and hemangiosarcomas in mice, evidence demonstrating that 4-chloro-2-methylaniline can induce mutagenic changes in vascular cells is lacking. Thus, data are not available to link results of genotoxicity studies to the development of hemangiomas and hemangiosarcomas reported by NCI (1979). There is evidence in the literature for 4-chloro-2-methylaniline and/or a metabolite binding to macromolecules in rodents involving one or more cytochrome P-450 isomers (Leslie et al., 1988; Hill et al., 1979; Struck et al., 1978; Bentley et al., 1986).

Dose-Response Concordance—A dose-response concordance has not been established between the development of hemangiomas and hemangiosarcomas and mutagenesis, because in vivo evidence of mutagenicity for 4-chloro-2-methylanilineis not available. Furthermore, evidence is lacking on the mutagenic potential of 4-chloro-2-methylanilinein vascular cells following in vitro or in vivo exposure.

Temporal Relationships—Hemangiosarcomas (74% males and 78% females) and combined hemangiomas and hemangiosarcomas at all sites (82% males and 78% females) have

been observed in mice exposed to 4-chloro-2-methylaniline for 2 years (NCI, 1979). In a similar study, Weisburger et al. (1978) noted combined hemangiomas and hemangiosarcomas incidence at 65% and 94% in male and female mice, respectively, treated with 4-chloro-2-methylaniline for 18 months. However, due to the lack of data on the mutagenic potential of 4-chloro-2-methylaniline in vascular cells, the temporal relationship between possible mutagenic mechanisms and the development of hemangiomas and hemangiosarcomas could not be assessed.

Biological Plausibility and Coherence—As mentioned previously, although several studies provide evidence that 4-chloro-2-methylaniline is metabolized through the cytochrome P-450 pathway and binds DNA in vivo and in vitro, no evidence is available linking mutagenesis in vascular cells to the development of hemangiomas and hemangiosarcomas.

Conclusions—Evidence does not clearly support a mutagenic mode of action for 4-chloro-2-methylaniline tumorigenicity. Although in vitro studies provide evidence that 4-chloro-2-methylaniline is capable of eliciting genotoxic effects in mammalian cells, two key uncertainties remain (1) data evaluating the genotoxic potential of 4-chloro-2-methylaniline in vivo are lacking, and (2) no evidence linking mutagenesis to the development of vascular cell tumors is available. Therefore, a default linear approach is applied.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

Derivation of Provisional Oral Slope Factor

The study by Weisburger et al. (1978) is selected as the principal study. The critical endpoint is the incidence of vascular tumors (hemangiosarcoma or hemangioma, all sites) in male 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 an investigator at the National Cancer Institute, has been performed according to GLP principles, and meets the standards of study design and performance with numbers of animals, examination of potential toxicity endpoints, and presentation of information. Details are provided in the ***Selection of Potentially Relevant Studies*** section. Among the available, acceptable studies, this study represents the highest OSF from selected studies in the database.

The Weisburger et al. (1978) 18-month carcinogenicity study yielded unequivocal evidence in male and female mice that 4-chloro-2-methylaniline significantly induced hemangiomas or hemangiosarcomas (vascular tumors) in both sexes at both doses tested (19.7 and 39.4 mg/kg-day for males; 52.5 and 105 mg/kg-day for females). However, because of the nonmonotonic dose-response relationship of vascular tumor incidence in the female mice (see Table B.8) the data set did not pass the *p*-value criteria test following BMD modeling. Because tumorigenicity at the low-dose range is of the highest interest, the high-dose group data were removed from the female data set, and BMD modeling was re-run. This model run provided an OSF of 0.09 per mg/kg-day. Although an adequate model fit was achieved using both low and high doses for the male vascular tumor data set from the Weisburger et al. (1978) study due to the monotonic nature of the dose response (see Table B.8), it provided a lower OSF than the female data set (0.05 per mg/kg-day; see Appendix C). Additionally, when only the low dose was used in the BMD modeling, the male data set still provided a lower OSF than the female data set (0.07 per mg/kg-day vs. 0.09 per mg/kg-day, respectively; see Appendix C). The NCI (1979) study also provided statistically and biologically significant data of increased vascular

tumors in mice, and combined incidence of hemangiosarcomas or hemangiomas in male mice resulted in a model that passed the *p*-value criteria test. However, the modeling of the male data from the NCI (1979) study resulted in a lower OSF (0.002 per mg/kg-day) compared to the female vascular tumor data from Weisburger et al. (1978) (Appendix C). Similar to the Weisburger et al. (1978) study, the female data set from the NCI (1979) study did not pass the *p*-value criteria test following BMD modeling because of the nonmonotonic dose-response relationship of tumor incidence (see Table B.7). When the high-dose group data were removed, the model run provided an OSF of 0.09 per mg/kg-day, which is identical to that derived from the female data set from the Weisburger et al. (1978) study (see Appendix C).

The estimated OSF of 0.09 per mg/kg-day based on data on vascular tumor incidence (hemangiomas or hemangiosarcomas) in female mice was selected for oral exposure to 4-chloro-2-methylaniline (Weisburger et al., 1978). Because the Weisburger et al. (1978) study, like the NCI (1979) study, administered 4-chloro-2-methylaniline hydrochloride, the p-OSF is adjusted to reflect the molecular weight difference between 4-chloro-2-methylaniline and the salt form.

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

$\mathbf{DOSE_{ADJ,\,HED}}$ = **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, 1997])

BW_A = 0.0317 kg (average body weight for male mice [U.S. EPA, 1994b])

Body-weight adjustment = $(0.0317/70)^{1/4} = 0.146$

$\mathbf{(DOSE_{ADJ,\,HED})}$ = $(\text{Dose})_n \times (0.0057 \text{ kg/day}) \times (1 \div 0.0317 \text{ kg}) \times (560 \text{ days}/560 \text{ days}) \times 0.146$
= $2000 \text{ mg/kg} \times (0.0057 \text{ kg/day}) \times (31.55 \text{ kg}^{-1}) \times 1 \times 0.146$
= $11.4 \text{ mg/day} \times 31.55 \text{ kg}^{-1} \times 1 \times 0.146$
= $359.67 \text{ mg/kg-day} \times 0.146$
= **52.5 mg/kg-day**

Table 10 presents BMD input data for incidence of vascular tumors in female mice exposed to 4-chloro-2-methylaniline in the diet for 18 months.

Table 10. BMD Input for Incidence of Vascular Tumors in Female HaM/ICR Mice Exposed to 4-Chloro-2-Methylaniline in the Diet for 18 Months[a]			
			Response
$(Dose)_n$ (mg/kg-day)	$(DOSE_{ADJ,HED})_n$ (mg/kg-day)	Number of Subjects	Vascular Tumors, All Sites[b,c]
0	0	15	0(0)
359.67	52.5	19	18(95)[d]

[a]Weisburger et al. (1978).
[b]Number of mice with tumors, () = percentage of mice with lesions or tumors.
[c]Statistically significant positive trend.
[d]Statistically significant in pairwise test versus control.

Table 11 shows the modeling results. Adequate model fit is obtained for the vascular tumor incidence data using the multistage cancer model. The BMD modeling results for vascular tumors yield a BMD_{10HED} of 1.879 mg/kg-day and a $BMDL_{10HED}$ of 1.070 mg/kg-day (see Table 11).

Table 11. Goodness-of-Fit Statistics, BMD_{10HED}, and $BMDL_{10HED}$ Values for the Multistage Cancer Model for Vascular Tumors (All Sites) in Female Mice Exposed to 4-Chloro-2-Methylaniline in the Diet for 18 Months[a]				
Model	Goodness-of-Fit *p*-Value[b]	AIC	BMD_{10HED} (mg/kg-day)	$BMDL_{10HED}$ (mg/kg-day)
Multistage Cancer[c]	0.996	9.835	1.879	1.070

[a]Weisburger et al. (1978).
[b]Values >0.1 meet conventional goodness-of-fit criteria.
[c]Betas restricted to ≥ 0.

$$\begin{aligned} p\text{-}OSF_{salt} &= 0.1 \div BMDL_{10HED} \\ &= 0.1 \div 1.070 \text{ mg/kg-day} \\ &= 0.093 \text{ (mg/kg-day)}^{-1} \text{ or } 9 \times 10^{-2} \text{ per mg/kg-day} \end{aligned}$$

$$\begin{aligned} \mathbf{p\text{-}OSF_{base}} &= \mathbf{MW \text{ of salt} \div MW \text{ of base} \times p\text{-}OSF_{salt}} \\ &= \mathbf{178.07 \div 141.6 \times 0.093 \text{ mg/kg-day}} \\ &= \mathbf{1.26 \times 0.093 \text{ (mg/kg-day)}^{-1}} \\ &= \mathbf{0.117 \text{ (mg/kg-day)}^{-1} \text{ or } 1 \times 10^{-1} \text{ per mg/kg-day}} \end{aligned}$$

Derivation of Provisional Inhalation Unit Risk

No human or animal studies examining the carcinogenicity of 4-chloro-2-methylaniline following inhalation exposure have been located. Therefore, derivation of an inhalation unit risk is precluded.

APPENDIX A. PROVISIONAL SCREENING VALUES

DERIVATION OF SCREENING PROVISIONAL ORAL REFERENCE DOSES

Derivation of Screening Chronic Provisional RfD

For reasons noted in the main PPRTV document, it is inappropriate to derive provisional toxicity values for 4-chloro-2-methylaniline. However, information is available for this chemical which, although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

The study by Ciba-Geigy (1992b) is selected as the principal study for the derivation of a screening chronic p-RfD. Based on the data from the Ciba-Geigy (1992b) study, general biological trends can be found, and the liver appears to be a sensitive target organ of 4-chloro-2-methylaniline. Although mice exposed to 4-chloro-2-methylaniline do not exhibit dose-related pathological or weight changes in the liver, levels of SGPT—an enzyme that is released into the blood following liver damage—were increased and reached statistical significance at the 18.0 mg/kg-day dose level in male mice, which is also a FEL. Additionally, serum glutamic oxaloacetic transaminase levels were also increased in female mice by 50% and 60% in the 3.69 and 18.4 mg/kg-day dose groups, respectively; however, these changes did not reach statistical significance. Although total serum protein level was significantly decreased at a lower dose than SGPT (3.69 mg/kg-day), the biological significance and adversity of this endpoint in and of itself is questionable. Additional support for liver as a sensitive target organ is exhibited by data from the Ciba-Geigy (1992a) rat study in which liver weights were significantly increased in males and females at the highest dose tested, and gross macroscopic liver pathological findings were also present at the mid- and high-dose in females.

The Ciba-Geigy (1992b) study is unpublished, but it is a report submitted to EPA under Toxic Substances Control Act Section 8ECP, and it has been performed according to GLP principles, and it meets the standards of study design and performance with numbers of animals, examination of potential toxicity endpoints, and presentation of information. Details are provided in the ***Review of Potentially Relevant Data*** section. Among the available acceptable studies, this study represents the lowest and most appropriate POD for developing a chronic p-RfD. Because the study administered 4-chloro-2-methylaniline hydrochloride, the p-RfD is converted to reflect the molecular weight difference between 4-chloro-2-methylaniline and the salt form.

From the Ciba-Geigy (1992b) study, based on the absence of biologically significant liver effects and significant frank effects (i.e., mortality) in mice at 3.69 mg/kg-day, this dose is

identified as a $NOAEL_{ADJ}$ for 4-chloro-2-methylaniline, and this $NOAEL_{ADJ}$ was selected as the POD for derivation of the subchronic p-RfD.

Adjusted points for daily exposure:

The following dosimetric adjustments were made for each dose in the principal study for treatment in the diet in adjusting for daily exposure.

$$\begin{aligned} \mathbf{NOAEL_{ADJ}} &= \mathbf{NOAEL_x \times \text{Food Consumption per Day} \times (1 \div \text{Body Weight}) \times (\text{Days Dosed} \div \text{Total Days})} \\ &= 20 \text{ ppm} \times 0.0053 \text{ kg/day} \times (1 \div 0.02875 \text{ kg}) \times (560 \text{ days dosed} \div 560) \\ &= 0.106 \text{ mg/day} \times 34.78 \text{ kg}^{-1} \times 1 \\ &= 3.69 \text{ mg/kg-day} \times 1 \\ &= 3.69 \text{ mg/kg-day} \end{aligned}$$

A screening chronic p-RfD is developed as follows:

$$\begin{aligned} \text{Screening Chronic p-RfD}_{salt} &= NOAEL_{ADJ} \div UF_C \\ &= 3.69 \text{ mg/kg-day} \div 1000 \\ &= 0.004 \text{ mg/kg-day or } 4 \times 10^{-3} \text{ mg/kg-day} \end{aligned}$$

$$\begin{aligned} \mathbf{\text{Screening Chronic p-RfD}_{base}} &= \mathbf{\text{MW of base} \div \text{MW of salt} \times \text{p-RfD}_{salt}} \\ &= \mathbf{141.6 \div 178.07 \times 0.004 \text{ mg/kg-day}} \\ &= \mathbf{0.80 \times 0.004 \text{ mg/kg-day}} \\ &= \mathbf{0.003 \text{ mg/kg-day or } 3 \times 10^{-3} \text{ mg/kg-day}} \end{aligned}$$

Table A.1 summarizes the UFs for the screening chronic p-RfD for 4-chloro-2-methylaniline.

Table A.1. Uncertainty Factors for Screening Chronic p-RfD of 4-Chloro-2-Methylaniline[a]

UF	Value	Justification
UF_A	10	A UF_A of 10 is applied for interspecies 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 mice to the chronic toxicity of 4-chloro-2-methylaniline.
UF_D	10	A UF_D of 10 is selected because there are no acceptable two-generation reproduction studies or developmental studies, and there is no indication of any other studies that may be relevant for the database UF.
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 in humans.
UF_L	1	A UF_L of 1 is applied because the POD was developed using a NOAEL.
UF_S	1	A UF_S of 1 is applied because a chronic-duration study (Ciba-Geigy [1992b]) was utilized as the principal study.
UF_C ≤3000	1000	

[a]Ciba-Geigy (1992b).

APPENDIX B. DATA TABLES

Table B.1. Survival and Mean Body-Weight Parameters in F344 Rats Exposed to 4-Chloro-2-Methylaniline in the Diet for 7 Weeks[a]

Male			Female		
Exposure Group, ppm (Adjusted Daily Dose, mg/kg-day)	**Survival[b]**	**Mean Body Weight, Day 49 (% of Control)[c]**	**Exposure Group, ppm (Adjusted Daily Dose, mg/kg-day)**	**Survival[b]**	**Mean Body Weight, Day 49 (% of Control)[c]**
First experiment					
0	5/5 (100)	100			
250 (25)	5/5 (100)	93			
500 (50)	5/5 (100)	94			
1000 (100)	5/5 (100)	95			
2000 (200)	5/5 (100)	94			
4000 (400)	5/5 (100)	92			
Second experiment			**First experiment**		
0	5/5 (100)	100	0	5/5 (100)	100
6000 (600)	5/5 (100)	91	6000 (677)	5/5 (100)	92
6200 (620)	5/5 (100)	98	6200 (700)	5/5 (100)	90
6500 (650)	5/5 (100)	99	6500 (734)	5/5 (100)	93
7000 (700)	5/5 (100)	92	7000 (790)	5/5 (100)	89
8000 (800)	5/5 (100)	89	8000 (903)	5/5 (100)	91
10,000 (1000)	5/5 (100)	92	10,000 (1130)	5/5 (100)	90
			Second experiment		
			0	5/5 (100)	100
			1000 (113)	5/5 (100)	103
			2500 (282)	5/5 (100)	101
			3000 (339)	5/5 (100)	98
			4000 (452)	5/5 (100)	101
			Third experiment		
			0	5/5 (100)	100
			6200 (700)	5/5 (100)	81
			12,500 (1410)	5/5 (100)	67
			25,000 (2820)	5/5 (100)	55
			50,000 (5650)	0/5 (0)	--

[a]NCI (1979).
[b]Number of surviving animals per number of animals exposed, () = percent of total.
[c]Incidence, percentage of control, and independent statistics could not be provided due to a lack of information in the study report.

Table B.2. Survival and Mean Body Weight Parameters in B6C3F1 Mice Exposed to 4-Chloro-2-Methylaniline in the Diet for 7 Weeks[a]

Exposure Group, ppm (Adjusted Daily Dose, mg/kg-day)	Survival[b]	Mean Body Weight, Day 49 (% of Control)[c]
Male		
0	5/5 (100)	100
2000 (361)	5/5 (100)	103
4000 (722)	5/5 (100)	96
5000 (902)	5/5 (100)	99
7500 (1350)	5/5 (100)	97
10,000 (1800)	5/5 (100)	98
15,000 (2710)	5/5 (100)	89
Female		
0	5/5 (100)	100
15,000 (2930)	5/5 (100)	90
17,500 (3410)	5/5 (100)	90
20,000 (3900)	5/5 (100)	78

[a]NCI (1979).
[b]Number of surviving animals per number of animals exposed, () = percent of total.
[c]Incidence, percentage of control, and independent statistics could not be provided due to a lack of information in the study report.

Table B.3. Selected Biochemistry Parameters in ICR Mice Exposed to 4-Chloro-2-Methylaniline in the Diet for 80 Weeks[a]

Parameter	Exposure Group (Adjusted Daily Dose, mg/kg-day)			
	0 ppm	**20 ppm (3.60)**	**100 ppm (18.0)**	**500 ppm (89.9)**
Male mouse				
Mortality	8/29 (28%)	11/30 (37%)	20/30 (67%)[e]	29/30 (97%)[e]
Sample size	21	19	10	1
Total serum protein (g/dL)[b]	7.23 ±1.10	5.93± 0.73 (82)[c,d]	6.51 ± 0.96 (90)	5.20 --- (72)
Blood urea nitrogen[b] (mg/dL)	17.21 ± 3.97	21.36 ± 7.46 (124)[c]	19.51 ± 8.59 (113)	53.00 --- (308)
Glucose (g/dL)[b]	135.8 ± 39.5	164.4 ± 23.9 (121)[c,d]	155.6 ± 24.0 (115)	190.0 --- (140)
Serum glutamic oxaloacetic transaminase (mU)[b]	100.1 ± 32.6	99.2 ± 26.0 (99)	94.7 ±15.0 (95)	92.0 --- (92)
Serum glutamic pyruvic transaminase (mU)[b]	24.4 ± 14.9	33.8 ± 28.1 (139)	36.1 ±21.6 (148)	28.0 --- (115)
Serum alkaline phosphatase (mU)[b]	22.8 ± 10.7	20.2 ±12.4 (89)	24.3 ± 16.6 (107)	23.0 --- (101)
Total Cholesterol (mg/dL)[b]	177.5 ± 68.7	138.4 ± 47.1 (78)[c]	139.9 ± 60.0 (79)	61.0 --- (34)
Parameter	**Exposure Group (Adjusted Daily Dose, mg/kg-day)**			
	0 ppm	**20 ppm (3.69)**	**100 ppm (18.4)**	**500 ppm (92.2)**
Female mouse				
Mortality	13/29 (44%)	19/30 (63%)	24/29 (83%)[e]	28/28 (100%)[e]
Sample size	16	11	5	0
Total serum protein (g/dL)[b]	6.57 ± 0.41	6.24 ± 0.37 (95)[c,d]	5.78 ± 0.36 (88)[c,d]	-
Blood urea nitrogen (mg/dL)[b]	15.69 ± 2.96	34.53 ± 28.91 (220)[c,d]	20.40 ± 15.36 (130)	-
Glucose (g/dL)[b]	117.0 ± 20.8	140.5 ± 44.9 (120)	137.8 ± 49.8 (118)	-
Serum glutamic oxaloacetic transaminase (mU)[b]	112.6 ± 28.0	168.3 ± 167.8 (150)	179.6 ± 201.8 (160)	-
Serum glutamic pyruvic transaminase (mU)[b]	16.4 ± 9.8	21.3 ± 7.6 (130)	35.4 ± 35.2 (216)[c,d]	-
Serum alkaline phosphatase (mU)[b]	26.3 ± 9.6	25.5 ± 10.2 (97)	28.4 ± 16.8 (108)	-
Total Cholesterol (mg/dL)[b]	99.1 ± 15.4	123.4 ± 53.0 (125)[c]	97.6 ± 40.8 (99)	-

[a]Ciba-Geigy (1992b).
[b]Means ± SD, () = percent of control.
[c]Statistically significantly different from control by independent Standard *t*-Test ($p < 0.05$) performed for this review.
[d]Statistically significantly different from control by independent Dunnett's Multiple Comparisons Test ($p < 0.05$) performed for this review.
[e]Statistically significantly different from control by independent Fisher's Exact Test ($p < 0.05$) performed for this review.

Table B.4. Incidence of Selected Tumor in ICR Mice Exposed to 4-Chloro-2-Methylaniline in the Diet for 80 Weeks[a]

Parameter	Exposure Group (Human Equivalency Dose, mg/kg-day)			
	0 ppm	**20 ppm (0.525)**	**100 ppm (2.62)**	**500 ppm (13.1)**
Male mouse				
Sample size	30	30	30	30
Liver: hepatoma and adenoma[b]	48.5 (26.7)	56.3 (30.0)	53.3 (27.6)	63.9 (20.7)
Lung: adenoma[b]	38.7 (20.0)	41.4 (20.0)	8.7 (3.4)	61.4 (17.2)
Leukemia and lymphosarcoma [b]	20.1 (16.7)	3.4 (3.3)	19.9 (10.3)	0.0 (0.0)
Reticulum cell sarcoma[b]	7.7 (3.3)	3.8 (3.3)	0.0 (0.0)	6.2 (3.4)
Probably reticulum cell sarcoma[b]	0.0 (0.0)	8.3 (3.3)	41.8 (24.1)	84.6 (20.7)
Unclassified malignant tumor[b]	0.0 (0.0)	23.1 (10.0)	72.8 (48.3)	62.2 (48.3)
Tumor incidence[b]	72.7 (56.7)	83.3 (60.0)	96.1 (86.2)	100.0 (86.2)
Parameter	**Exposure Group (Human Equivalency Dose, mg/kg-day)**			
	0 ppm	**20 ppm (0.525)**	**100 ppm (2.62)**	**500 ppm (13.1)**
Female mouse				
Sample size	30	30	30	30
Liver: hepatoma and adenoma[b]	14.8 (6.9)	7.4 (3.3)	0.0 (0.0)	18.8 (10.7)
Lung: adenoma[b]	14.8 (6.9)	37.5 (20.0)	59.0 (13.8)	10.6 (7.1)
Leukemia and lymphosarcoma [b]	38.0 (24.1)	17.3 (10.0)	32.8 (20.7)	3.6 (3.6)
Reticulum cell sarcoma[b]	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Probably reticulum cell sarcoma[b]	0.0 (0.0)	17.3 (17.3)	14.3 (6.9)	84.9 (21.4)
Unclassified malignant tumor[b]	0.0 (0.0)	70.0 (46.7)	70.8 (41.4)	87.0 (64.3)
Tumor incidence[b]	56.3 (37.9)	85.5 (70.0)	93.6 (79.3)	100.0 (92.9)

[a]Ciba-Geigy (1992b).
[b]Percentages calculated by study authors using the modified life table method, () = calculated by Naïve method.

Table B.5. Selected Tumor Incidence in Sprague-Dawley Rats Exposed to 4-Chloro-2-Methylaniline in the Diet for 94 or 104 Weeks[a]

Parameter	Exposure Group (Human Equivalency Dose, mg/kg-day)			
	0 ppm	**20 ppm (0.405)**	**100 ppm (2.02)**	**500 ppm (10.1)**
Male mouse				
Sample size	30	30	30	30
Liver tumor[b]	0.0 (0.0)	11.1 (3.3)	45.1 (16.7)	80.3 (43.3)
Malignant and probably malignant hepatoma[b]	0.0 (0.0)	11.1 (3.3)	12.5 (3.5)	45.5 (16.7)
Probably benign hepatoma and adenomatous hyperplasia[b]	0.0 (0.0)	0.0 (0.0)	36.8 (13.3)	56.1 (26.7)
Adrenal gland adenoma and adenomatous hyperplasia[b]	33.3 (13.3)	47.8 (20.0)	45.1 (16.7)	38.4 (20.0)
Pituitary gland adenoma[b]	44.7 (23.3)	71.8 (36.7)	50 (16.7)	43.2 (20.0)
Total tumor incidence[b]	74.8 (46.7)	86.1 (56.7)	86.2 (43.3)	92.5 (56.7)
Parameter	**Exposure Group (Human Equivalency Dose, mg/kg-day)**			
	0 ppm	**20 ppm (0.421)**	**100 ppm (2.11)**	**500 ppm (10.5)**
Female mouse				
Sample size	30	30	30	30
Liver tumor[b]	0.0 (0.0)	25.9 (10.0)	81.8 (31.0)	91.8 (66.7)
Malignant and probably malignant hepatoma[b]	0.0 (0.0)	0.0 (0.0)	14.5 (3.4)	48.7 (16.7)
Probably benign hepatoma and adenomatous hyperplasia[b]	0.0 (0.0)	25.9 (10.0)	76.2 (27.6)	78.0 (50.0)
Adrenal gland adenoma and adenomatous hyperplasia[b]	58.0 (27.6)	72.6 (40.0)	87.2 (55.2)	82.7 (53.3)
Pituitary gland adenoma[b]	83.9 (58.6)	86.6 (60.0)	80.4 (55.2)	90.5 (60.0)
Total tumor incidence[b]	93.9 (82.8)	98.0 (83.3)	-[c]	100.0 (96.7)

[a]Ciba-Geigy(1992a).
[b]Percentages calculated by study authors using the modified life table method, ().= calculated by Naïve method.
[c]Values could not be determined in the study report.

Table B.6. Selected Incidence of Neoplasms in F344 Rats Exposed to 4-Chloro-2-Methylaniline in the Diet for 107 Weeks[a]

Parameter	Exposure Group (Human Equivalency Dose, mg/kg-day)					
	0 ppm (0)		1250 ppm (26.8)		5000 ppm (107)	
	Tumor Incidence[b]	Weeks until First Tumor	Tumor Incidence[b]	Weeks until First Tumor	Tumor Incidence[b]	Weeks until First Tumor
Male rat						
Integumentary system: fibroma	1/20 (5)	107	4/50 (8)	107	2/50 (4)	107
Lung: alveolar/bronchiolar carcinoma or adenoma	1/20 (5)	107	6/50 (12)	107	2/49 (4)	107
Hematopoietic system: lymphoma or leukemia	6/20 (30)	89	1/50 (2)	92	1/50 (2)	73
Liver: hepatocellular carcinoma or adenoma	0/20 (0)	-	5/50 (10)	100	4/50 (8)	107
Pituitary: chromophobe adenoma	2/19 (11)	107	6/48 (13)	92	15/47 (32)[d]	84
Adrenal: pheochromocytoma	0/20 (0)	-	0/49 (0)	-	4/49 (8)	107
Thyroid: follicular-cell carcinoma or adenoma	1/19 (5)	107	0/49 (0)	-	4/49 (8)	62
Testis: interstitial-cell tumor	16/20 (80)	93	39/48 (81)	92	42/50 (84)	62
Tunica vaginalis: mesothelioma, NOS	2/20 (10)	107	0/50 (0)	-	0/50 (0)	-
Parameter	**Exposure Group (Human Equivalency Dose, mg/kg-day)**					
	0 ppm (0)		**1250 ppm (27.4)**		**5000 ppm (110)**	
	Tumor Incidence[b]	**Weeks until First Tumor**	**Tumor Incidence[b]**	**Weeks until First Tumor**	**Tumor Incidence[b]**	**Weeks until First Tumor**
Female rat						
Hematopoietic system: lymphoma or leukemia	3/20 (15)	72	4/50 (8)	88	1/50 (2)	107
Pituitary: chromophobe adenoma	1/19 (5)	107	13/47 (28)	92	15/48 (31)[c]	88
Mammary gland: adenoma, NOS	0/20 (0)	-	6/50 (12)	103	1/50 (2)	107
Mammary gland: fibroadenoma	4/20 (20)	102	10/50 (20)	107	6/50 (12)	107
Uterus: endometrial stromal polyp	5/19 (26)	107	5/49 (10)	107	8/49 (16)	107

[a]NCI (1979).
[b]Number of animals with tumors/number of animals examined, () = percent of total.
[c]Statistically significantly different from control by Fisher's Exact Test ($p < 0.05$) performed by the researchers.
[d]Statistically significantly different from control by Cochran-Armitage Test ($p < 0.05$) performed by the researchers.

Table B.7. Selected Incidence of Neoplasms in B6C3F1 Mice Exposed to 4-Chloro-2-Methylaniline in the Diet for 99 Weeks[a]

Parameter	Exposure Group (Human Equivalency Dose, mg/kg-day)					
	0 ppm (0)		3750 ppm (98.4)		15,000 ppm (393.0)	
	Tumor Incidence[b]	Weeks until First Tumor	Tumor Incidence[b]	Weeks until First Tumor	Tumor Incidence[b]	Weeks until First Tumor
Male mouse						
Lung: alveolar/ bronchiolar carcinoma	2/20 (10)	99	7/46 (15)	96	1/48 (2)	95
Lung: alveolar/ bronchiolar carcinoma or adenoma	4/20 (20)	99	14/46 (30)	96	3/48 (6)	95
Hematopoietic system: lymphoma	1/20 (5)	99	3/50 (6)	99	1/50 (2)	99
All sites: hemangioma	0/20 (0)	-	3/50 (6)	99	5/50 (10)	65
All sites: hemangiosarcoma	0/20 (0)	-	3/50 (6)	87	37/50 (74)[d]	66
All sites: hemangiosarcoma or hemangioma	0/20 (0)	-	6/50 (12)	87	41/50 (82)[d]	65
Liver: hepatocellular carcinoma	4/20 (20)	99	5/50 (10)	99	7/50 (14)	77
Liver: hepatocellular carcinoma or adenoma	4/20 (20)	99	7/50 (14)	99	10/50 (20)	77
Parameter	**Exposure Group (Human Equivalency Dose, mg/kg-day)**					
	0 ppm (0)		**1250 ppm (32.8)**		**5000 ppm (131)[c]**	
	Tumor Incidence[b]	**Weeks until First Tumor**	**Tumor Incidence[b]**	**Weeks until First Tumor**	**Tumor Incidence[b]**	**Weeks until First Tumor**
Female mouse						
Lung: alveolar/ bronchiolar adenoma	0/18 (0)	-	2/47 (4)	99	3/48 (6)	64
Hematopoietic system: lymphoma or leukemia	1/18 (6)	99	6/49 (12)	72	1/50 (2)	82
All sites: hemangioma	1/18 (6)	99	6/49 (12)	65	0/50 (0)	-
All sites: hemangiosarcoma	0/18 (0)	-	40/49 (82)[d]	43	39/50 (78)[d]	66
All sites: hemangiosarcoma or hemangioma	1/18 (6)	99	44/49 (90)[d]	43	39/50 (78)[d]	66
Liver: hepatocellular carcinoma or adenoma	1/18 (6)	99	4/49 (8)	96	0/49 (0)	-

[a]NCI (1979).
[b]Number of animals with tumors/number of animals examined, () = percent of total.
[c]Female mice in the high-dose group were only exposed for 92 weeks.
[d]Statistically significantly different from control by Fisher's Exact Test ($p < 0.05$) performed by the researchers.

Table B.8. Tumor Incidence in HaM/ICR Mice Exposed to 4-Chloro-2-Methylaniline in the Diet for 18 Months[a]

Parameter	Exposure Group (Human Equivalency Dose, mg/kg-day)			
	Simultaneous Control (0)	**Pooled Control (0)**	**750 mg/kg (19.7)**	**1500 mg/kg (39.4)**
Male mouse				
Vascular tumors[b]	0/14 (0)	5/99 (5)	12/20 (60)[c]	13/20 (65)[c]
Multiple tumors[b]	1/14 (7)	14/99 (14)	7/20 (35)[d]	6/20 (30)
Parameter	**Exposure Group (Human Equivalency Dose, mg/kg-day)**			
	Simultaneous Control (0)	**Pooled Control (0)**	**2000 mg/kg (52.5)**	**4000 mg/kg (105)**
Female mouse				
Vascular tumors[b]	0/15 (0)	9/102 (9)	18/19 (95)[c]	12/16 (75)[c]

[a]Weisburger et al. (1978).
[b]Number of animals with tumors/number of animals examined, () = percent of total.
[c]Statistically significantly different from all controls by Fisher's Exact Test ($p < 0.05$) performed by the researchers.
[d]Statistically significantly different from pooled controls only by Fisher's Exact Test ($p < 0.05$) performed by the researchers.

APPENDIX C. BMD MODELING OUTPUTS FOR 4-CHLORO-2-METHYANILINE

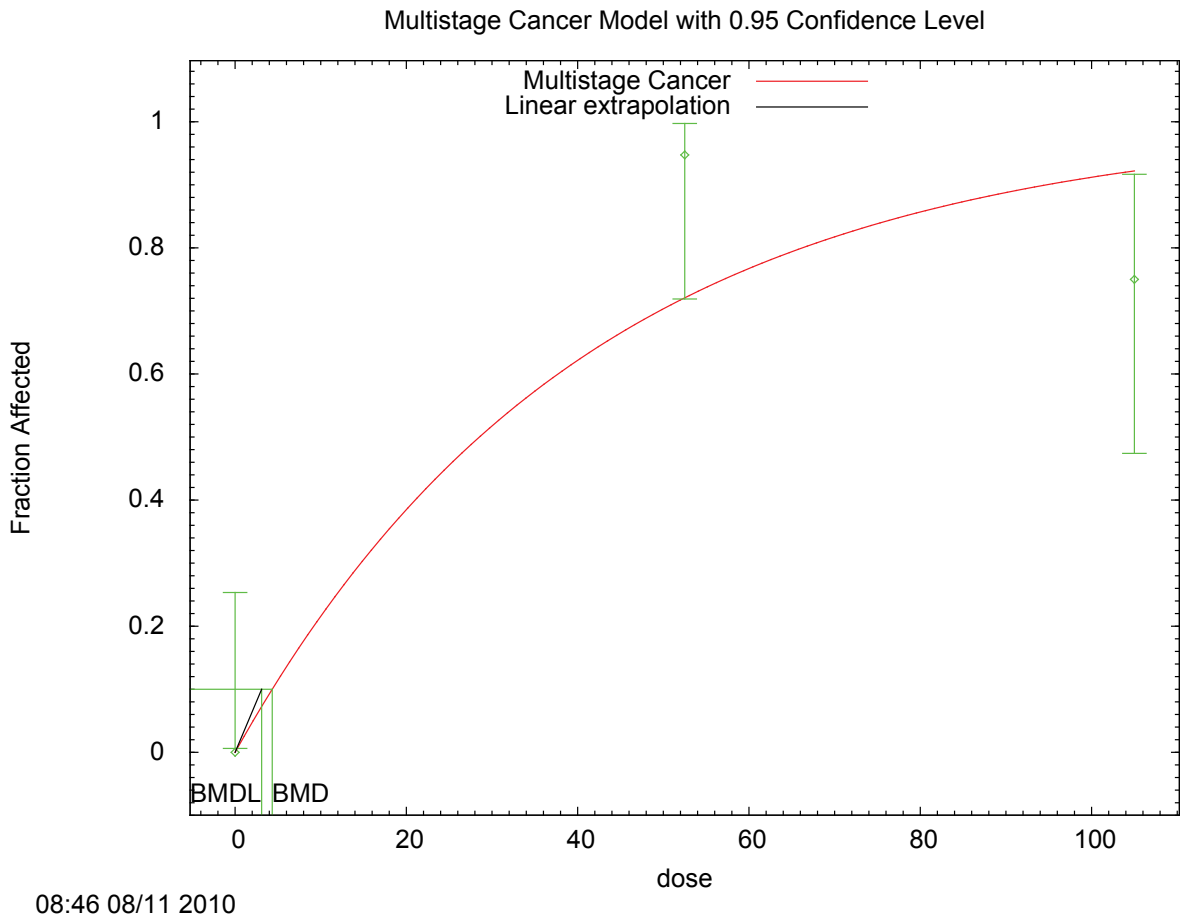

Figure C.1. Multistage Cancer BMD Model for Female Vascular Tumor Incidence (Weisburger et al., 1978)

Text Output for Multistage Cancer BMD Model for Female Vascular Tumor Incidence Data (Weisburger et al., 1978)

```
================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS21/Data/msc_4C2M_Weisburger1978_F_Msc2-
BMR10.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS21/Data/msc_4C2M_Weisburger1978_F_Msc2-
BMR10.plt
                                                Wed Aug 11 08:26:17 2010
 ================================================================

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

   The form of the probability function is:

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

```
   The parameter betas are restricted to be positive


   Dependent variable = Incidence
   Independent variable = Dose

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


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



                  Default Initial Parameter Values
                     Background =     0.527837
                        Beta(1) =    0.0132028
                        Beta(2) =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(2)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(1)

   Beta(1)            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0           *               *                   *
        Beta(1)        0.0243031           *               *                   *
        Beta(2)                0           *               *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -12.915         3
   Fitted model        -18.3494         1       10.8688      2        0.004364
  Reduced model        -33.6506         1       41.4711      2         <.0001

           AIC:         38.6989


                                Goodness  of  Fit
                                                      Scaled
```

Dose	Est._Prob.	Expected	Observed	Size	Residual
0.0000	0.0000	0.000	0.000	15	0.000
52.5000	0.7208	13.696	18.000	19	2.201
105.0000	0.9221	14.753	12.000	16	-2.567

Chi^2 = 11.44 d.f. = 2 P-value = 0.0033

Benchmark Dose Computation

Specified effect = 0.1

Risk Type = Extra risk

Confidence level = 0.95

BMD = 4.33528

BMDL = 3.10321

BMDU = 6.39484

Taken together, (3.10321, 6.39484) is a 90 % two-sided confidence interval for the BMD

Multistage Cancer Slope Factor = 0.0322247

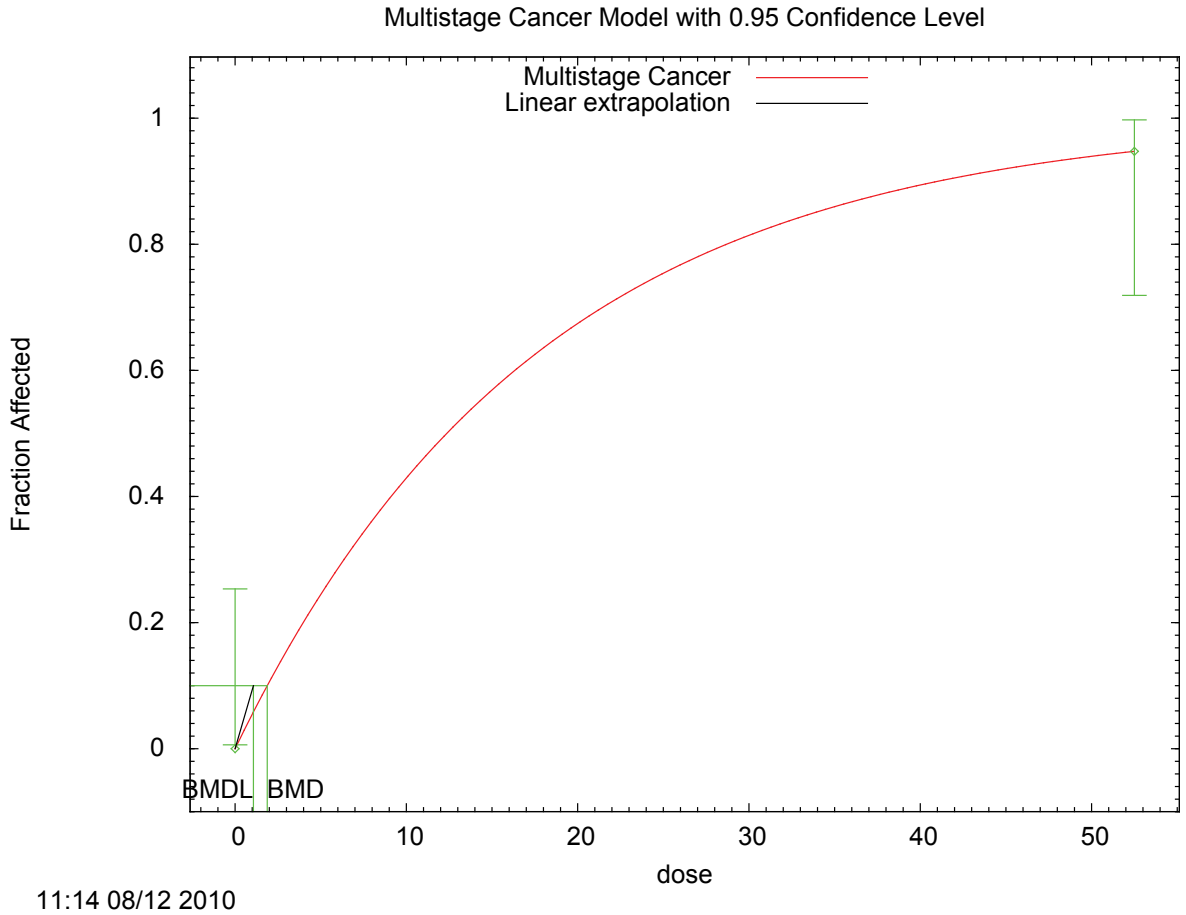

Figure C.2. Multistage Cancer BMD Model for Female Vascular Tumor Incidence Data without the High-Dose Data (Weisburger et al., 1978)

Text Output for Multistage Cancer BMD Model for Female Vascular Tumor Incidence Data without the High-Dose Data (Weisburger et al., 1978)

```
================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS21/Data/msc_4C2M_Weisburger1978_F_Msc1-
BMR10.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS21/Data/msc_4C2M_Weisburger1978_F_Msc1-
BMR10.plt
                                                Thu Aug 12 11:14:34 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 = Incidence
```

```
   Independent variable = Dose

 Total number of observations = 2
 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
                        Beta(1) =    0.0560846


           Asymptotic Correlation Matrix of Parameter Estimates

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

                Beta(1)

   Beta(1)            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0            *                *                   *
        Beta(1)        0.0560846            *                *                   *

* - Indicates that this value is not calculated.



                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -3.91765         2
   Fitted model        -3.91768         1        6e-005      1          0.9938
  Reduced model        -23.5081         1        39.181      1         <.0001

           AIC:         9.83536


                               Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          15       -0.005
   52.5000     0.9474        18.000    18.000          19       -0.000

 Chi^2 = 0.00      d.f. = 1        P-value = 0.9956
```

```
   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =         1.8786

           BMDL =        1.07041

           BMDU =        3.21519

Taken together, (1.07041, 3.21519) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0934223
```

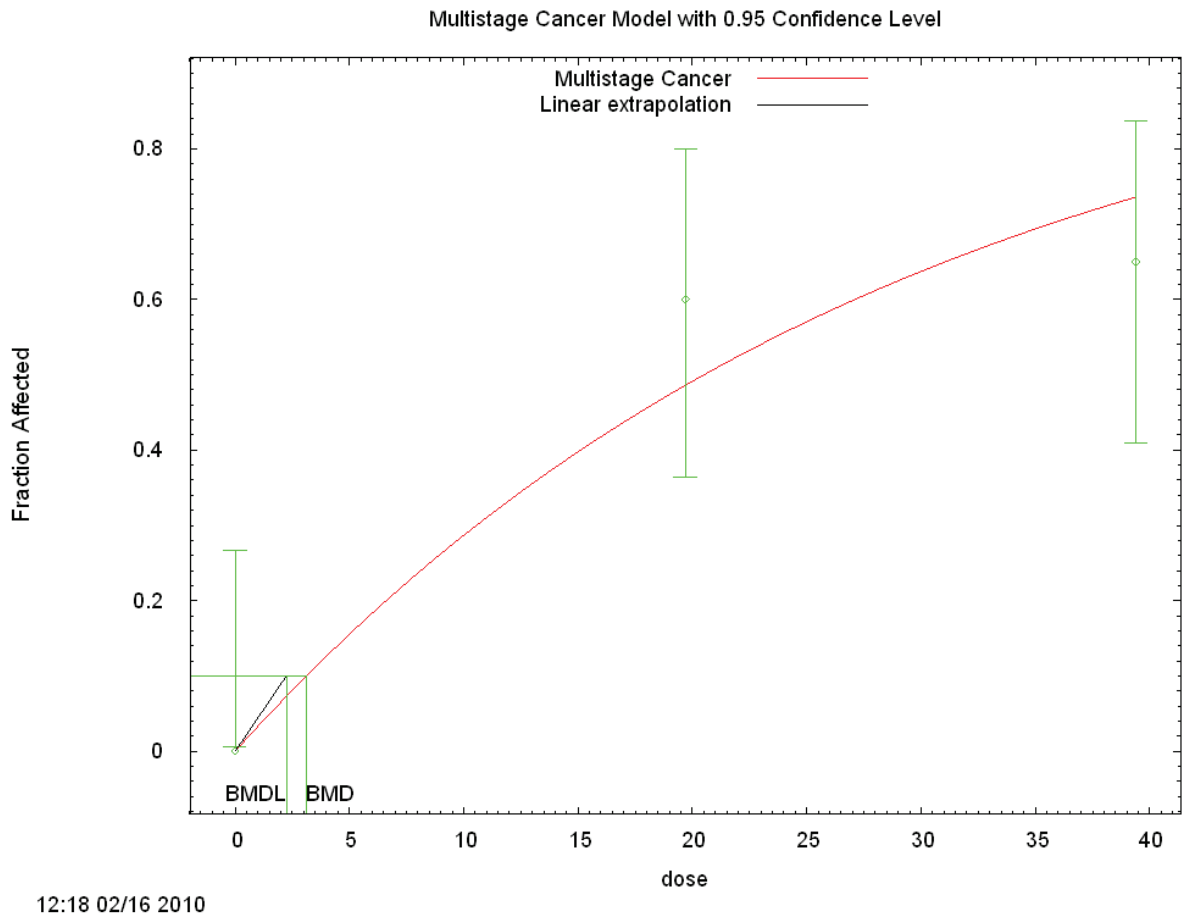

Figure C.3. Multistage Cancer BMD Model for Male Vascular Tumor Incidence (Weisburger et al., 1978)

Text Output for Multistage Cancer BMD Model for Male Vascular Tumor Incidence Data (Weisburger et al., 1978)

```
 ======================================================================
        Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
        Input Data File: C:\2\Weisburger_1978a_18mo_vasctumor_M_MultiCanc_1.(d)
        Gnuplot Plotting File:
C:\2\Weisburger_1978a_18mo_vasctumor_M_MultiCanc_1.plt
                                              Thu Feb 18 17:11:47 2010
 ======================================================================

 TableB4_vascular_tumor_incidence_male
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive
```

```
   Dependent variable = DichPerc
   Independent variable = Dose

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


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



                  Default Initial Parameter Values
                     Background =     0.122308
                        Beta(1) =    0.0266452
                        Beta(2) =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Background    -Beta(2)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

                Beta(1)

   Beta(1)            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0            *                *                   *
        Beta(1)        0.0338098            *                *                   *
        Beta(2)                0            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -26.4092         3
   Fitted model        -27.2885         1       1.75876      2           0.415
  Reduced model        -37.2817         1        21.745      2         <.0001

           AIC:         56.5771


                                Goodness  of  Fit
                                                                      Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------------
   0.0000     0.0000         0.000     0.000          14        0.000
```

```
   19.7000     0.4863         9.725    12.000          20        1.018
   39.4000     0.7361        14.722    13.000          20       -0.873

 Chi^2 = 1.80      d.f. = 2        P-value = 0.4069


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        3.11627

            BMDL =        2.24215

            BMDU =        5.75997

Taken together, (2.24215, 5.75997) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0446001
```

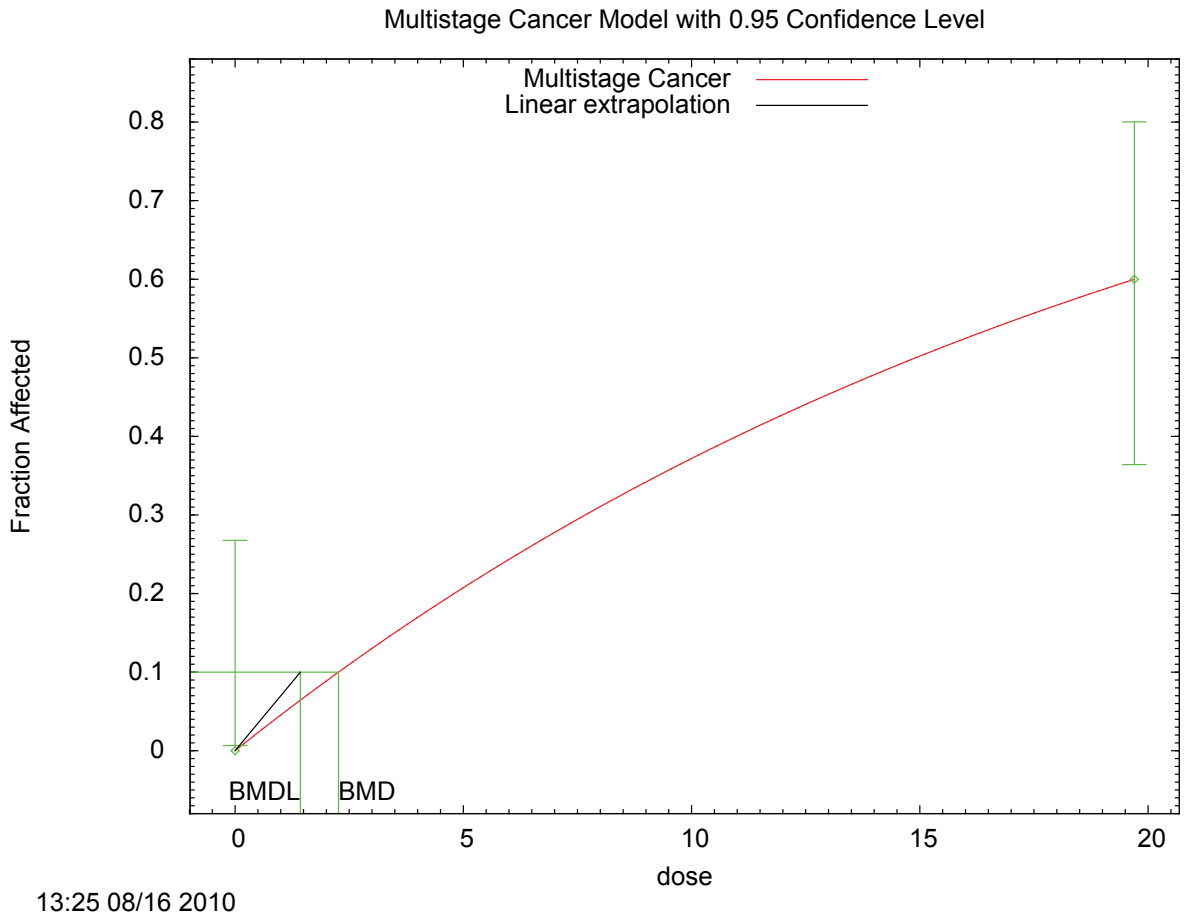

Figure C.4. Multistage Cancer BMD Model for Male Vascular Tumor Incidence Data without the High-Dose Data (Weisburger et al., 1978)

Text Output for Multistage Cancer BMD Model for Male Vascular Tumor Incidence Data without the High-Dose Data (Weisburger et al., 1978)

```
=================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS21/Data/msc_4C2M_Weisburger1978_M_Msc1-
BMR10.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS21/Data/msc_4C2M_Weisburger1978_M_Msc1-
BMR10.plt
                                                Mon Aug 16 13:25:40 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 = Incidence
   Independent variable = Dose

 Total number of observations = 2
 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
                        Beta(1) =    0.0465122


           Asymptotic Correlation Matrix of Parameter Estimates

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

                Beta(1)

   Beta(1)            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0            *                *                   *
        Beta(1)        0.0465122            *                *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -13.4602         2
   Fitted model        -13.4602         1  3.55271e-015      1              1
  Reduced model        -22.0744         1       17.2284      1         <.0001

           AIC:         28.9205


                               Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size        Residual
  ----------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          14        0.000
   19.7000     0.6000        12.000    12.000          20        0.000
```

```
 Chi^2 = 0.00      d.f. = 1        P-value = 1.0000


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        2.26522

            BMDL =        1.43027

            BMDU =        3.84714

Taken together, (1.43027, 3.84714) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =      0.069917
```

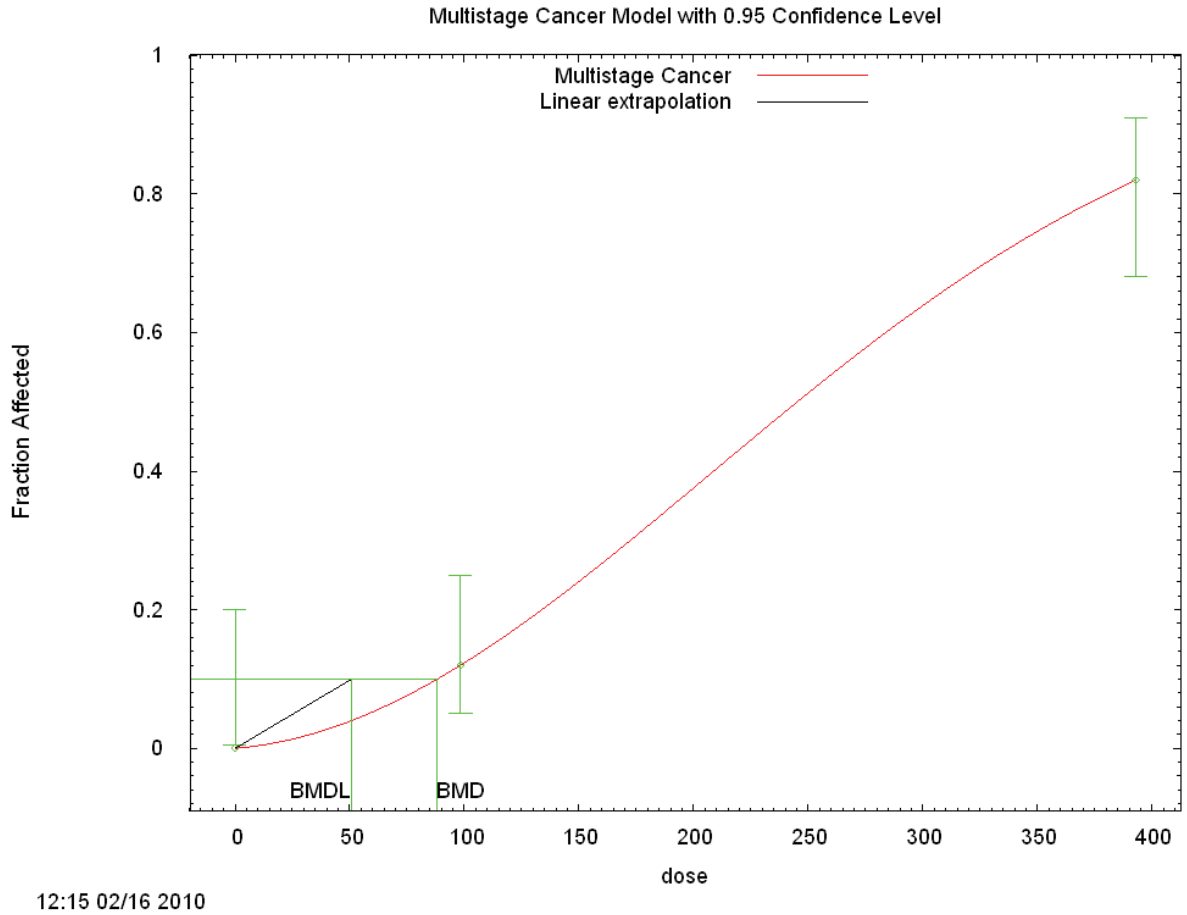

Figure C.5. Multistage Cancer BMD Model for Male Hemangiosarcoma or Hemangioma Tumor Incidence Data (NCI, 1979)

Text Output for Multistage Cancer BMD Model for Male Hemangiosarcoma or Hemangioma Tumor Incidence Data (NCI, 1979)

```
=================================================================
             Multistage Cancer Model. (Version: 1.7;  Date: 05/16/2008)
             Input Data File: C:\2\NCI_1979c_99wk_sarc_ang_M_MultiCanc_1.(d)
             Gnuplot Plotting File:  C:\2\NCI_1979c_99wk_sarc_ang_M_MultiCanc_1.plt
                                          Tue Feb 16 12:15:48 2010
 =================================================================

 TableB2_all_sites_hemangiosarcoma_or_hemangioma_male_mouse
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = DichPerc
   Independent variable = Dose
```

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


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



                  Default Initial Parameter Values
                     Background =            0
                        Beta(1) =  0.000275628
                        Beta(2) = 1.04013e-005


           Asymptotic Correlation Matrix of Parameter Estimates

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

                Beta(1)      Beta(2)

   Beta(1)            1        -0.93

   Beta(2)        -0.93            1



                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background                0            *               *                   *
        Beta(1)      0.000275585            *               *                   *
        Beta(2)     1.04015e-005            *               *                   *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -41.9159         3
   Fitted model        -41.9159         2  4.27222e-009      1          0.9999
  Reduced model        -80.3385         1       76.8452      2         <.0001

           AIC:         87.8318


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          20       -0.000
```

```
   98.4000     0.1200         6.000     6.000          50        0.000
  393.0000     0.8200        41.000    41.000          50       -0.000

 Chi^2 = 0.00      d.f. = 1        P-value = 0.9999


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        88.2652

            BMDL =        50.8704

            BMDU =        110.309

Taken together, (50.8704, 110.309) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =    0.00196578
```

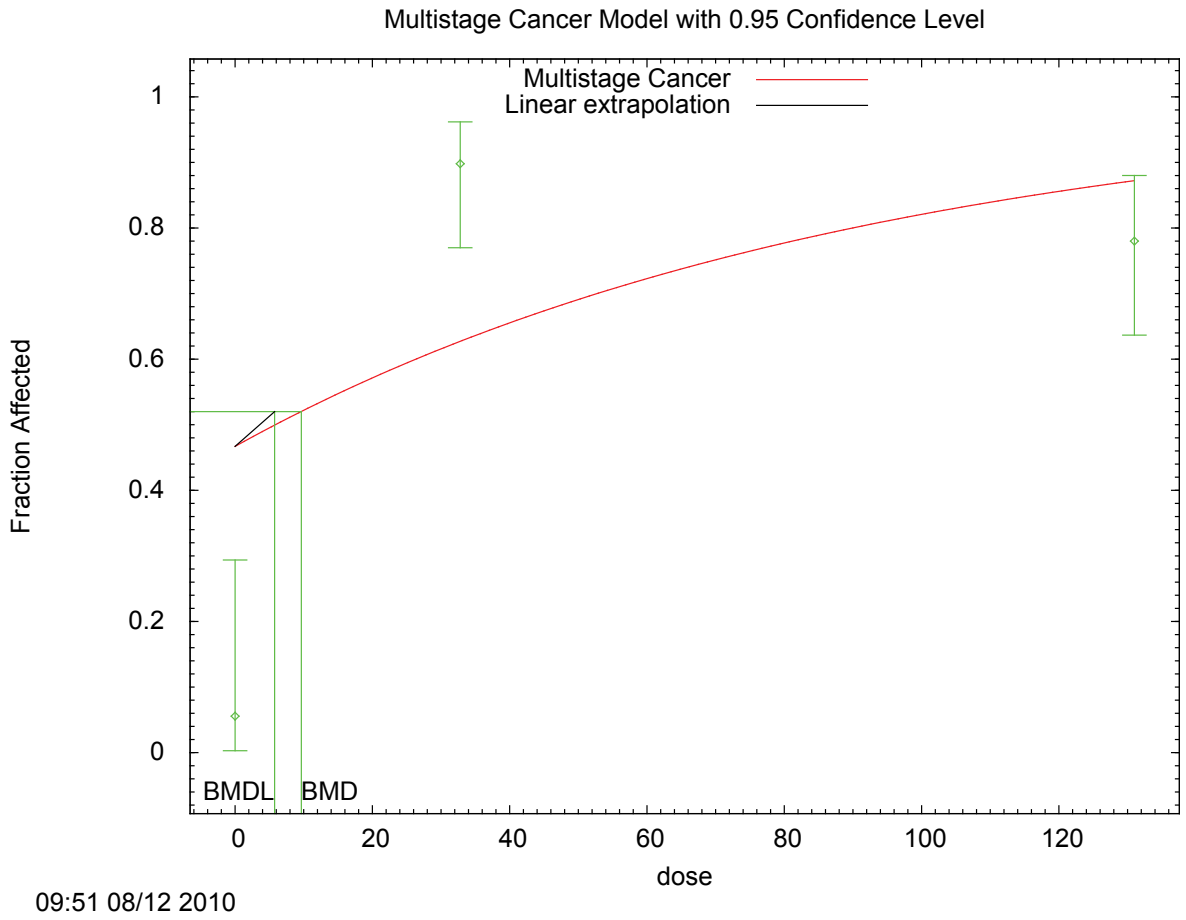

Figure C.6. Multistage Cancer BMD Model for Female Hemangiosarcoma or Hemangioma Tumor Incidence Data (NCI, 1979)

Text Output for Multistage Cancer BMD Model for Female Hemangiosarcoma or Hemangioma Tumor Incidence Data (NCI, 1979)

```
================================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS21/Data/msc_4C2M_NCI1979_F_Msc2-BMR10.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS21/Data/msc_4C2M_NCI1979_F_Msc2-
BMR10.plt
                                                  Thu Aug 12 09:51:22 2010
 ================================================================================

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

   The form of the probability function is:

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

   The parameter betas are restricted to be positive


   Dependent variable = Incidence
   Independent variable = Dose
```

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


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



                  Default Initial Parameter Values
                     Background =     0.599716
                        Beta(1) =   0.00675779
                        Beta(2) =            0


           Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -Beta(2)
                 have been estimated at a boundary point, or have been specified by
the user,
                 and do not appear in the correlation matrix )

             Background      Beta(1)

Background            1         -0.8

   Beta(1)         -0.8            1



                                Parameter Estimates

                                                          95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background         0.466639            *                *                  *
        Beta(1)        0.0109191            *                *                  *
        Beta(2)                0            *                *                  *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -46.3551         3
   Fitted model        -64.8787         2       37.0472      1      1.1530338e-009
  Reduced model         -69.601         1       46.4917      2         <.0001

           AIC:         133.757


                                Goodness  of  Fit
                                                                      Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ----------------------------------------------------------------------
    0.0000     0.4666         8.399     1.000          18       -3.496
```

```
   32.8000     0.6272        30.733    44.000          49        3.920
  131.0000     0.8724        43.621    39.000          50       -1.959

 Chi^2 = 31.42     d.f. = 1        P-value = 0.0000


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        9.64917

            BMDL =        5.75827

            BMDU =        22.3152

Taken together, (5.75827, 22.3152) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0173663
```

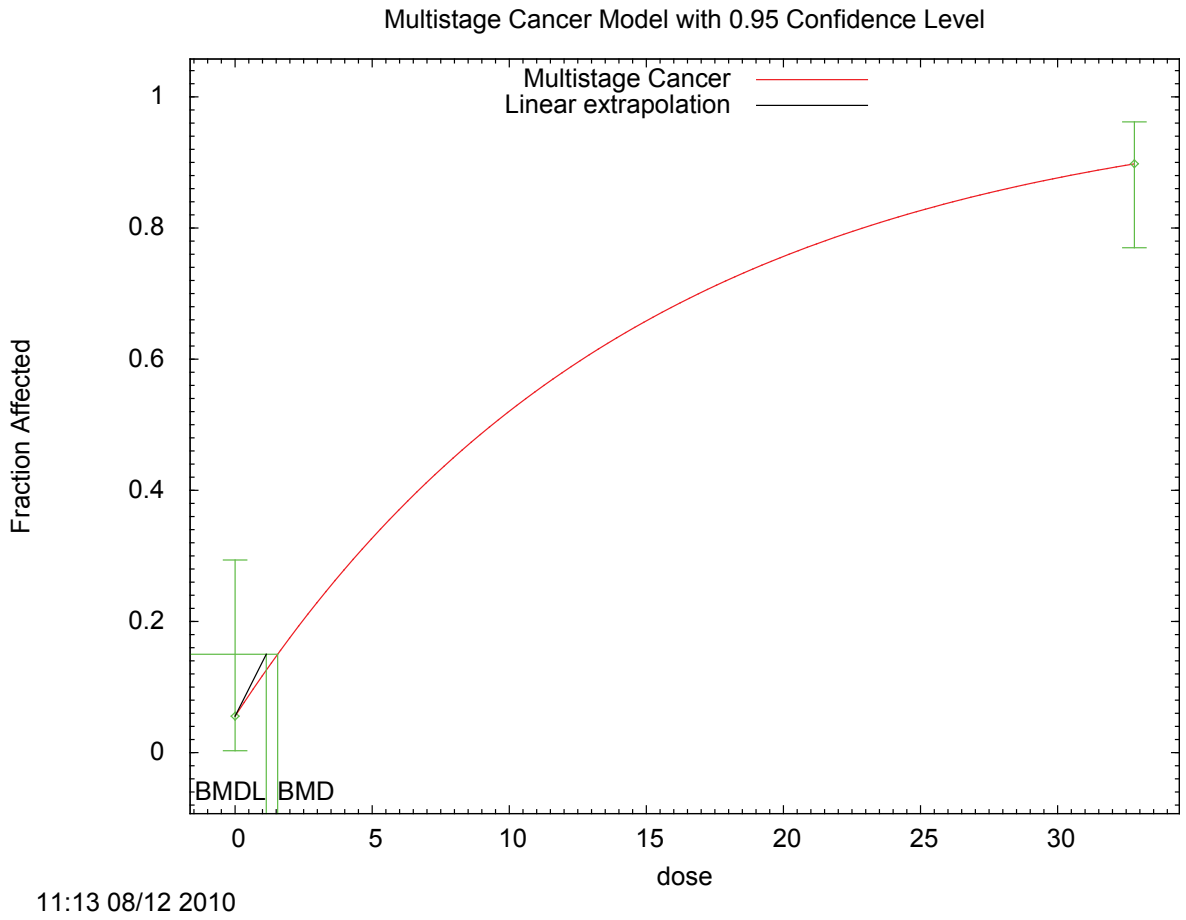

Figure C.7. Multistage Cancer BMD Model for Female Hemangiosarcoma or Hemangioma Tumor Incidence Data without the High-Dose Data (NCI, 1979)

Text Output for Multistage Cancer BMD Model for Female Hemangiosarcoma or Hemangioma Tumor Incidence Data without the High-Dose Data (NCI, 1979)

```
=================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File: C:/USEPA/BMDS21/Data/msc_4C2M_NCI1979_F_Msc1-BMR10.(d)
        Gnuplot Plotting File:  C:/USEPA/BMDS21/Data/msc_4C2M_NCI1979_F_Msc1-
BMR10.plt
                                                Thu Aug 12 11:13:00 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 = Incidence
  Independent variable = Dose

 Total number of observations = 2
 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 =    0.0555556
                        Beta(1) =    0.0678422


           Asymptotic Correlation Matrix of Parameter Estimates

             Background      Beta(1)

Background            1        -0.48

   Beta(1)        -0.48            1


                                Parameter Estimates

                                                        95.0% Wald Confidence
Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit   Upper Conf.
Limit
     Background        0.0555556            *                *                  *
        Beta(1)        0.0678422            *                *                  *

* - Indicates that this value is not calculated.

 Warning: Likelihood for the fitted model larger than the Likelihood for the full
model.
Error in computing chi-square; returning 2


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -20.0097         2
   Fitted model        -20.0097         2 -7.10543e-015      0         NA
  Reduced model        -42.4117         1       44.8039      1         <.0001

           AIC:         44.0195


                                Goodness  of  Fit
                                                                    Scaled
    Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0556         1.000     1.000          18       -0.000
   32.8000     0.8980        44.000    44.000          49        0.000
```

```
 Chi^2 = 0.00      d.f. = 0        P-value =     NA


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        1.55302

            BMDL =        1.13769

            BMDU =         2.1497

Taken together, (1.13769, 2.1497 ) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0878975
```

APPENDIX D. REFERENCES

ACGIH (American Conference of Governmental Industrial Hygienists). (2009) 2009 TLVs and BEIs: Threshold limit values for chemical substances and physical agents and biological exposure indices. Cincinnati, OH: American Conference of Governmental Industrial Hygienists. 594528

ATSDR (Agency for Toxic Substances and Disease Registry). (2008) Toxicological profile information sheet. Available online at http://www.atsdr.cdc.gov/toxpro2.html. Accessed on 2/25/2010. 595415

Bentley P; Bieri F; Muecke W; Waechter F; Stäubli W. (1986) Species differences in the toxicity of *p*-chloro-*o*-toluidine to rats and mice: Covalent binding to hepatic macromolecules and hepatic non-parenchymal cell DNA and an investigation of effects upon the incorporation of [3H] thymidine into capillary endothelial cells. Chem Biol Interact, 57:7−40. 201736

Ciba-Geigy Corporation. (1992a) Initial submission: Effects of 4-chloro-o-toluidine in oral prolonged administration to rat for 92(male) and 104(female) weeks with cover letter dated 073192. U.S. Environmental Protection Agency. Washington, DC. 201706

Ciba-Geigy Corporation. (1992b) Initial submission: Effects of 4-chloro-o-toluidine in oral prolonged administration to mice for 80 weeks (volume I-II) with cover letter dated 081492. U.S. Environmental Protection Agency. Washington, DC. 201707

Galloway SM; Armstrong MJ; Reuben C; Colman S; Brown B; Cannon C; Bloom AD; Nakamura F; Ahmed M; Duk S; Rimpo J; Margolin BH; Resnick MA; Anderson B; Zeiger E. (1987) Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. Environ Mol Mutagen, 10:1−175. 007768

Göggelmann W; Bauchinger M; Kulka U; Schmid E. (1996) Genotoxicity of 4-chloro-o-toluidine in Salmonella typhimurium, human lymphocytes and V79 cells. Mutat Res, 370:39−47. 201731

Haworth S; Lawlor T; Mortelmans K; Speck W; Zeiger E. (1983) Salmonella mutagenicity test results for 250 chemicals. Environ Mutagen, 5(Suppl 1):3-142. 028947

Hill DL; Shih TW; Struck RF (1979). Macromolecular binding and metabolism of the carcinogen 4-chloro-2-methylaniline. Cancer Res, 39:2528−2531. 594404

IARC (International Agency for Research on Cancer). (1990) para-Chloro-ortho-toluidine and its strong acid salts. IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 48. Lyon, France: World Health Organization, pp. 123−137. 201829

IARC (International Agency for Research on Cancer). (2000) 4-Chloro-ortho-toluidine. IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. Vol. 77. Lyon, France: World Health Organization, pp.323−339. 201822

Lang R; Adler ID. (1982) Studies on the mutagenic potential of the pesticide chlordimeforme and its principal metabolites in the mouse heritable translocation assay. Mutat Res, 92:243−248. 201832

Leslie C; Reidy GF; Murray M; Stacey NH. (1988) Induction of xenobiotic biotransformation by the insecticide chlordimeform, a metabolite 4-chloro-O-toluidine and a structurally related chemical O-toluidine. Biochem Pharmacol, 37:2529−2535. 064629

McGregor DB; Brown A; Cattanach P; Edwards I; McBride D; Riach C; Caspary WJ. (1988) Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. Environ Mol Mutagen, 2:85−154. 184364

NIOSH (National Institute for Occupational Safety and Health). (2003) NIOSH pocket guide to chemical hazards. Atlanta, GA: Department of Health and Human Services, Centers for Disease Prevention and Control. 081445

NCI (National Cancer Institute). (1979) Bioassay of 4-chloro-o-toluidine hydrochloride for possible carcinogenicity (CAS no. 3165-93-3). National Cancer Institute. Bethesda, MD. 79−1721. 201662

NTP (National Toxicology Program). (2005) p-Chloro-o-Toluidine and p-Chloro-o-Toluidine Hydrochloride CAS Nos. 95-69-2 and 3165-93-3. National Toxicology Program. Washington, DC. Report on Carcinogens, 11th Edition. Available online at http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s043pcot.pdf. 201660

OSHA (1998). TABLE Z-1 limits for air contaminants. Available online at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9992. Accessed 2/22/2010. 595050

Ott MG; Langner RR. (1983) A mortality survey of men engaged in the manufacture of organic dyes. J Occup Med, 25:763−768. 201820

Popp W; Schmieding W; Speck M; Vahrenholz C; Norpoth K. (1992) Incidence of bladder cancer in a cohort of workers exposed to 4-chloro-o-toluidine while synthesising chlordimeform. Br J Ind Med, 49:529−531. 201732

Sekihashi K; Yamamoto A; Matsumura Y; Ueno S; Watanabe-Akanuma M; Kassie F; Knasmüller S; Tsuda S; Sasaki YF. (2002) Comparative investigation of multiple organs of mice and rats in the comet assay. Mutat Res, 517:53−75. 201821

Stasik MJ. (1988) Carcinomas of the urinary bladder in a 4-chloro-o-toluidine cohort. Int Arch Occup Environ Health, 60:21−24. 064637

Struck RF; Kirk MC; Shih TW; Hill DL. (1978) Liver microsomal metabolism of 4-chloro-2-methylaniline: A cancer suspect agent. Pharmacologist, 20:200. 202110

U.S. EPA (U.S. Environmental Protection Agency). (1994a) Chemical Assessments and Related Activities (CARA). Office of Health and Environmental Assessment, Office of Research and Development. Washington, DC. OHEA-I-127. 596444

U.S. EPA (1994b) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment. Washington, DC. EPA/600/8-90/066F. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=71993. 006488

U.S. EPA (U.S. Environmental Protection Agency). (1997) Exposure factors handbook (final report) 1997. U.S. Environmental Protection Agency. Washington, DC. EPA/600/P-95/002F a-c. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=12464. 594981

U.S. EPA (U.S. Environmental Protection Agency). (2003) Health Effects Assessment Summary Tables (HEAST). Available online at http://epa-heast.ornl.gov/. Accessed 2/25/2010. 595422

U.S. EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment, Final Report. Risk Assessment Forum, U.S. Environmental Protection Agency. Washington, DC. EPA/630/P-03/001F. Available online at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=116283. 086237

U.S. EPA (U.S. Environmental Protection Agency). (2006) 2006 Edition of the drinking water standards and health advisories. U.S. Environmental Protection Agency. Washington, Dc. EPA 822-R-06-013. Available online at http://www.epa.gov/waterscience/criteria/drinking/dwstandards.pdf. 091193

U.S. EPA (U.S. Environmental Protection Agency). (2009). Integrated risk information system (IRIS). Available online at http://cfpub.epa.gov/ncea/iris/index.cfm. Accessed 6/24/2009. 192196

Weisburger EK; Russfield AB; Homburger F; Weisburger JH; Boger E; Van Dongen CG; Chu KC. (1978) Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. J Environ Pathol Toxicol, 2:325−356. 064640

WHO (World Health Organization). (2010) Online catalogs for the Environmental Health Criteria Series. Available online at from http://www.who.int/ipcs/publications/ehc/en/. Accessed 2/25/2010. 595424