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Provisional Peer-Reviewed Toxicity Values for

o-Toluidine
(CASRN 95-53-4)

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

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COMMONLY USED ABBREVIATIONS

BMC	benchmark concentration
BMCL	benchmark concentration lower bound 95% confidence interval
BMD	benchmark dose
BMDL	benchmark dose lower bound 95% confidence interval
HEC	human equivalent concentration
HED	human equivalent dose
IUR	inhalation unit risk
LOAEL	lowest-observed-adverse-effect level
$LOAEL_{ADJ}$	LOAEL adjusted to continuous exposure duration
$LOAEL_{HEC}$	LOAEL adjusted for dosimetric differences across species to a human
NOAEL	no-observed-adverse-effect level
$NOAEL_{ADJ}$	NOAEL adjusted to continuous exposure duration
$NOAEL_{HEC}$	NOAEL adjusted for dosimetric differences across species to a human
NOEL	no-observed-effect level
OSF	oral slope factor
p-IUR	provisional inhalation unit risk
POD	point of departure
p-OSF	provisional oral slope factor
p-RfC	provisional reference concentration (inhalation)
p-RfD	provisional reference dose (oral)
RfC	reference concentration (inhalation)
RfD	reference dose (oral)
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	incomplete-to-complete database uncertainty factor
UF_H	interhuman uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
WOE	weight of evidence

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR *o*-TOLUIDINE (95-53-4)

BACKGROUND

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

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

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

DISCLAIMERS

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

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

QUESTIONS REGARDING PPRTVs

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

INTRODUCTION

ortho-Toluidine (*o*-toluidine; also referred to as 2-methylaniline or 2-aminotoluene) is a synthetic aromatic amine with amine and methyl groups in an *ortho* (1,2) configuration. *o*-Toluidine is used as an intermediate in the production of dyes, pigments, and rubbers (IARC, 2000). The empirical formula for *o*-toluidine is C_7H_9N (see Figure 1). A table of physicochemical properties is provided below (see Table 1).

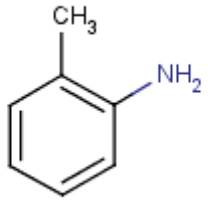

Figure 1. *o*-Toluidine Structure

Table 1. Selected Physicochemical Properties Table (*o*-Toluidine)[a]

Property (unit)	Value
Boiling point (ºC)	200.3
Melting point (ºC)	−16.3
Density (g/cm^3)	0.9984
Vapor pressure (kPa at 20 ºC)	0.013
Solubility in water (mg/L at 25ºC)	15
Relative vapor density (air = 1)	3.72
Molecular weight (g/mol)	107.16
Flash point (°C)	85
Octanol/water partition coefficient (unitless)	1.32

[a]Values obtained from IARC (2000).

No RfD, RfC, or cancer assessment for *o*-toluidine is included in the EPA's IRIS database (U.S. EPA, 2012a) or on the Drinking Water Standards and Health Advisories List (U.S. EPA, 2006). No RfD or RfC values have been reported in the Health Effects Assessment Summary Tables (HEAST; U.S. EPA, 2012b). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1994a) includes a listing for a Health and Environmental Effects Profile (HEEP) for *o*-toluidine. The toxicity of *o*-toluidine has not been reviewed by the

ATSDR (2008). The World Health Organization (WHO, 2011) has not produced an Environmental Health Criteria Document for *o*-toluidine but did produce a Concise International Chemical Assessment Document (WHO, 1998). CalEPA (2008) has not derived acute toxicity values for *o*-toluidine. The American Conference of Governmental Industrial Hygienists (ACGIH) has derived an 8-hour time-weighted average (TWA) exposure limit of 2 ppm (8.8 mg/m^3) along with a skin notation for *o*-toluidine (ACGIH, 2008). The National Institute of Occupational Safety and Health (NIOSH, 2010) has not established a Recommended Exposure Limit (REL) but considers *o*-toluidine to be a potential occupational carcinogen that may be absorbed through skin. NIOSH set an immediately dangerous to life or health (IDLH) value for *o*-toluidine at 50 ppm (220 mg/m^3). The Occupational Safety and Health Administration (OSHA, 2010) has derived a TWA Permissible Exposure Level (PEL) of 5 ppm (22 mg/m^3) for *o*-toluidine.

The HEAST (U.S. EPA, 2011b) does not include a cancer assessment for *o*-toluidine, but HEEP (U.S. EPA, 1984) provides a potency factor of 0.24 (mg/kg-day)$^{-1}$ for oral exposure. The International Agency for Research on Cancer (IARC, 2010) has classified *o*-toluidine as a Group 1 agent (*Carcinogenic to Humans*) based on sufficient evidence in humans and animals. *o*-Toluidine is included in the *12th Report on Carcinogens* (NTP, 2011) and is listed as *Reasonably Anticipated to be a Human Carcinogen*. CalEPA (2008) has derived an inhalation slope factor of 1.8×10^{-1} per mg/kg-day, an inhalation unit risk of 5.1×10^{-5} per μg/m^3, and an oral slope factor of 1.8×10^{-1} per mg/kg-day for *o*-toluidine.

Literature searches were conducted on sources published from 1950s through November 2011 for studies relevant to the derivation of provisional toxicity values for *o*-toluidine, CASRN 95-53-4. Searches were conducted using EPA's Health and Environmental Research Online (HERO) database of scientific literature. HERO searches the following databases: AGRICOLA; American Chemical Society; BioOne; Cochrane Library; DOE: Energy Information Administration, Information Bridge, and Energy Citations Database; EBSCO: Academic Search Complete; GeoRef Preview; GPO: Government Printing Office; Informaworld; IngentaConnect; J-STAGE: Japan Science & Technology; JSTOR: Mathematics & Statistics and Life Sciences; NSCEP/NEPIS (EPA publications available through the National Service Center for Environmental Publications [NSCEP] and National Environmental Publications Internet Site [NEPIS] database); PubMed: MEDLINE and CANCERLIT databases; SAGE; Science Direct; Scirus; Scitopia; SpringerLink; TOXNET (Toxicology Data Network): ANEUPL, CCRIS, ChemIDplus, CIS, CRISP, DART, EMIC, EPIDEM, ETICBACK, FEDRIP, GENE-TOX, HAPAB, HEEP, HMTC, HSDB, IRIS, ITER, LactMed, 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 also searched for health-related information: 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 an overview of the database for *o*-toluidine and includes all potentially relevant repeated subchronic- and chronic-duration studies. Entries for the principal studies are bolded. Unless otherwise qualified, the phrase "significant" means "statistical significance" with a *p*-value of less than 0.05).

Table 2. Summary of Potentially Relevant Data for *o*-Toluidine (CASRN 95-53-4)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Human								
			1. Oral (mg/kg-d)[a]					
Subchronic	ND							
Chronic	ND							
Developmental	ND							
Reproductive	ND							
Carcinogenicity	ND							
			2. Inhalation (mg/m^3)[a,d]					
Subchronic	ND							
Chronic	ND							
Developmental	ND							
Reproductive	ND							
Carcinogenicity	1643 male and 106 female (total 1749), retrospective cohort, from <5-yr to over 20-yr exposure to *o*-toluidine with or without aniline	Categories were "definitely exposed," "possibly exposed," and "probably not exposed"	Bladder cancer standardized incidence ratio [SIR] (90% confidence interval [CI]) Definitely exposed: 6.48 (3.04−12.2) Duration of employment <5 yr = 0 5 to <10 yr = 8.8 (0.45−41.7) ≥10 yr = 27.2 (11.8−53.7) $p < 0.001$ for linear trend	NA	Not run	NA	Ward et al. (1991)	

Table 2. Summary of Potentially Relevant Data for *o*-Toluidine (CASRN 95-53-4)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Carcinogenicity	Not reported but apparently the same cohort as listed above was used	Not reported	19 additional cases of bladder cancer in the same cohort from 1973 to 1988	NA	Not run	NA	Markowitz and Levin (2004)	
	1643 male and 106 female (total 1749), retrospective cohort, from <5 yr to over 20 yr; bladder cancer incidence was determined from December 31, 1988 to December 31, 1994 (re-analysis of Ward et al., 1991)	Categories were "definitely exposed," "possibly exposed," and "probably not exposed"	Bladder cancer SIR (95% CI) Definitely exposed: 5.84 (2.91−10.45)* Duration of employment <5 yr = 1.25 (0.03−6.97) 5−<10 yr = 3.67 (0.09−20.44) ≥10 yr = 11.09 (5.07−21.05)* *$p < 0.001$	NA	Not run	NA	Carreón et al. (2010)	
	2160 males, cohort, at least 6 mo of employment from 1955−1984	Not reported	Bladder cancer; for subgroup exposed to *o*-toluidine, standardized mortality ratio [SMR] = 1589 Relative risk (95% CI) for years of employment: 1−4 yr = 6.73 (1.59−28.41)* ≥5 yr = 7.65 (1.03−56.87)* *$p < 0.05$ test for trend $p = 0.002$	NA	Not run	NA	Sorahan et al. (2000)	

Table 2. Summary of Potentially Relevant Data for *o*-Toluidine (CASRN 95-53-4)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Carcinogenicity	2160 males, cohort, at least 6 mo of employment from 1955−1984 (update of study listed above)	Not reported	Bladder cancer; for subgroup exposed to *o*-toluidine, SMR = 1116 (95% CI: 230−3261) Relative risk (95% CI) for years of employment: 1−4 yr = 4.68 (1.66−13.2)* ≥5 yr = 6.99 (1.69−28.9)* *$p < 0.05$ test for trend $p < 0.001$	NA	Not run	NA	Sorahan (2008)	
Animal								
			1. Oral (mg/kg-day)[a]					
Subchronic	10 rat (sex and strain not specified), diet, duration not specified but at least 91 d	Not specified; authors added *o*-toluidine (final concentration of 7.5 to 12 mg per d) in rice flour diet	Three animals exhibited metaplasia and early epithelial proliferation in bladder mucous membrane	ND	Not run	ND	Ekman and Strömbeck (1947)	
	20/0 F344/N rat, diet, 7 d/wk, 13 wk	**0 or 301 (Adjusted)[e,f]**	**Decreased body weight; increased relative weight of the liver, kidney, spleen, and testes; and increased incidence of lesions in the liver, kidneys, bladder, and spleen**	**None**	**Not run**	**301**	**NTP (1996a)**	**PS, PR**
	20/0 F344/N rat, diet, 7 d/wk, 26 wk	0 or 285 (Adjusted)[e,f]	Decreased body weight; increased relative weight of the liver, kidney, spleen, and testes; and increased incidence of lesions in the liver, kidneys, bladder, and spleen	None	Not run	285	NTP (1996b)	

Table 2. Summary of Potentially Relevant Data for *o*-Toluidine (CASRN 95-53-4)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Subchronic	20/0 F344/N rat, diet, 7 d/wk, 13 wk with 13 wk recovery	0 or 304 (Adjusted)[e,f]	Decreased body weight after treatment and recovery; increased relative spleen weight; and increased incidence of lesions in the kidney, liver, and spleen after recovery	None	Not run	304	NTP (1996c)	
Chronic	50/50 F344 rat, diet, 7 d/wk, 101−104 wk	0, 231 and 525 in males; 0, 264, and 600 in females (Adjusted)[e,g]	Decreased survival	None	Not run	None; 231 is a frank effect level (FEL)	NCI (1979a)	
Developmental	ND							
Reproductive	ND							
Carcinogenicity	50/50 F344 rat, diet, 7 d/wk, 101−104 wk	0, 63.0, and 138.8 in males; 0, 63.7 and 140.4 in females[e,h]	Significant increase in several types of cancer, including skin (fibromas), spleen, and bladder cancer; BMDL based on subcutaneous fibroma in males	NA	10.4	NA	NCI (1979a)	
	25/0 CD rat, diet, 7 d/wk, 18 mo followed by 6 mo on the control diet	**0, 72.1, and 144.1[e,h]**	**Significant increase in the incidence of dermal subcutaneous fibroma and fibrosarcoma**	**NA**	**4.5**	**NA**	**Weisburger et al. (1978a)**	**PS, PR**
	30/0 F344 rat, diet, 7 d/wk, 72 wk followed by 21-wk recovery	0 or 44.2[e,h]	Significant increases in fibromas of the skin, spleen, and mammary tissue, and in peritoneal tumors; nonsignificant increases in neoplasms of the bladder, liver, and mammary carcinomas; decreased survival (significance not reported)	NA	Not run	NA	Hecht et al. (1982)	

Table 2. Summary of Potentially Relevant Data for *o*-Toluidine (CASRN 95-53-4)

Category	Number of Male/Female, Strain, Species, Study Type, Study Duration	Dosimetry[a]	Critical Effects	NOAEL[a]	BMDL/ BMCL[a]	LOAEL[a,b]	Reference (Comments)	Notes[c]
Carcinogenicity	50/50 B6C3F$_1$ mouse, diet, 7 d/wk, 102−103 wk	0, 23.0, and 73.8 in males; 0, 26.5, and 91.9 in females[e,h]	Significant increase in hepatocellular carcinomas, adenomas, and hemangiosarcomas; BMDL based on hepatocellular adenomas and carcinomas in females	NA	22.2	NA	NCI (1979b)	
	25/25 CD-1 mouse, diet, 7 d/wk, 18 mo followed by 3 mo on the control diet	0, 210.5, and 420.9 in males; 0, 211.8, and 423.7 in females[e,h]	Significant increase in vascular tumors	NA	23	NA	Weisburger et al. (1978b)	
2. Inhalation (mg/m^3)[a]								
Subchronic	ND							
Chronic	ND							
Developmental	ND							
Reproductive	ND							
Carcinogenicity	ND							

[a]Dosimetry: NOAEL, BMDL/BMCL, and LOAEL values are converted to an adjusted daily dose (ADD in mg/kg-d) for oral noncancer effects and to a human equivalent dose (HED in mg/kg-d) for oral carcinogenic effects. All long-term exposure values (4 wk and longer) are converted from a discontinuous to a continuous (weekly) exposure. Values from animal developmental studies are not adjusted to a continuous exposure.
[b]Not reported by the study author, but determined from data.
[c]PS = Principal study, PR = Peer reviewed, NPR = Not peer reviewed, NA = Not applicable, ND = No data.
[d]These studies were occupational studies where exposure is presumably via inhalation, but dermal and oral exposures are also possible.
[e]Compound administered as toluidine hydrochloride.
[f]Average daily doses were provided in the study report in mg/kg-day presumably based on the concentrations in the diet (also provided in the study report), body weights, and food consumption (both of which were routinely measured).
[g]Doses were converted from ppm (as presented in the study report) by using the following equation ppm × average daily food consumption × (1 ÷ body weight) × (days dosed ÷ total days). Average food consumption was obtained from EPA (1988) and average body weights were obtained from the study report (NCI, 1979).
[h]These doses are HEDs that were calculated as follows: average daily dose in mg/kg-day × (average animal body weight ÷ average human body weight)$^{0.25}$.

HUMAN STUDIES

Oral Exposures

No human oral exposure studies on *o*-toluidine were identified

Inhalation Exposures

The effects of inhalation exposure of humans to *o*-toluidine have been evaluated in five carcinogenicity studies (i.e., Ward et al., 1991; Markowitz and Levin, 2004; Carreón et al., 2010; Sorahan et al., 2000; Sorahan, 2008).

Subchronic-duration Studies

No subchronic-duration studies on *o*-toluidine were identified.

Chronic-duration Studies

No chronic-duration studies on *o*-toluidine were identified.

Developmental Studies

No developmental toxicity studies on *o*-toluidine were identified.

Reproductive Studies

No reproductive toxicity studies on *o*-toluidine were identified.

Carcinogenicity Studies

Human studies have been limited to those examining cancer outcomes, specifically bladder cancer, in subjects presumed to have occupational exposure to azo dyes. Although for the purpose of this document, the exposures are considered via inhalation because there are no specifics in the study reports. Occupational exposures to compounds, such as aromatic amines, might have an appreciable dermal component, likely due to the relatively low vapor pressure of these compounds (Baan et al., 2008). The majority of the studies examined exposures to mixtures of several chemicals, including *o*-toluidine, and, in some cases, other suspected or known carcinogens (i.e., Ward et al., 1991; Markowitz and Levin, 2004; Carreón et al., 2010; Sorahan et al., 2000; Sorahan, 2008).

Ward et al. (1991) used a retrospective cohort design to investigate the incidence of bladder cancer in 1749 workers (1643 males, 106 females) at a New York chemical plant with exposures to *o*-toluidine and aniline. The workers were assigned to three groups: (1) "definitely exposed" (n = 708; those who worked in the *o*-toluidine department), (2) "possibly exposed" (n = 288; maintenance, janitors, yard workers, and shipping), (3) and "probably not exposed" (n = 753; all others). The observed (O) incidence rate of bladder cancer in the chemical plant was compared to that of the expected (E) general population of the State of New York (excluding New York City). Workers in the "definitely exposed" group had a standardized incidence ratio (SIR) for bladder cancer of 6.48 (90% confidence interval [CI]: 3.04−12.2; O/E = 7/1.08). SIRs for <5 years, 5 to <10 years, or 10 years or more of employment were 0 (O/E = 0/0.75), 8.8 (O/E = 1/0.11), and 27.2 (O/E = 6/0.22) respectively. Using <5 years as a reference group, the standard rate ratios for these categories were 1.0, 3.31, and 16.0, which were significant for trend ($p < 0.001$). Ward et al. (1996) reported urinary levels of *o*-toluidine in both exposed (98.7 ± 119.4 µg/L) and unexposed (2.8 ± 1.4 µg/L) workers. They further looked at the

hemoglobin adducts showing higher levels of *o*-toluidine than aniline, thereby suggesting that exposure to *o*-toluidine exceeded that of aniline.

Markowitz and Levin (2004) conducted a follow-up study of the Ward et al. (1991) cohort and found an additional 19 cases of bladder cancer based on data from attorneys who represented civil litigation and from a bladder cancer screening program sponsored by the chemical plant, which employed the workers. Their analysis indicated that further follow-up should be conducted to characterize the occupational cohort.

Another reanalysis by Carreón et al. (2010) changed the group assignments of the workers based on a walk-through and observations of the workers in the chemical plant. Although the categories remained the same, the number of individuals in the categories changed, with more being placed in the definitely exposed category (i.e., definitely exposed, $n = 962$; possibly exposed, $n = 187$; and probably not exposed, $n = 600$). The bladder cancer incidence was determined for the period of December 31, 1988 to December 31, 1994. The calculated SIR for the updated "definitely exposed" cohort was reported as 5.84 (95% CI: 2.91−10.45), which was significantly higher than the "probably not exposed" group. Duration of exposure was also reevaluated with an SIR of 11.09 (95% CI: 5.07−21.05) for those employed for 10 years or more. These results support the initial Ward et al. (1991) study, which also found a significant excess risk of bladder cancer among the cohort of workers in the chemical plant.

Sorahan et al. (2000) examined the cancer mortality and incidence in 2160 males working in a factory manufacturing chemicals for the rubber industry for at least 6 months from 1955 to 1984 in northern Wales. Exposure assessments were done by using job histories for 300 different jobs from 1930 to 1984. However, a specific exposure-versus-time matrix for *o*-toluidine was not possible, and the subjects were evaluated by duration of employment in the *o*-toluidine department. There were 1131 observed deaths between 1955 and 1996, which were quite close to the expected deaths of 1114.5. Of the workers in the *o*-toluidine department ($n = 53$), there were three deaths due to bladder cancer, compared to the expected rate of 0.2, the standard mortality ratio (SMR) was reported to be 1589 (no CI provided). Relative risks (RRs) of bladder cancer mortality due to working in the *o*-toluidine department for 1−4 years or ≥5 years, using unlagged employment history, were both elevated, at 4.44 (95% CI: 0.76−25.79) and 5.48 (95% CI: 0.51−59.14), respectively. However, RRs of total bladder cancer incidence for those working in the *o*-toluidine department for 1−4 years or ≥5 years were 6.73 (95% CI: 1.59−28.41) and 7.65 (95% CI: 1.03−56.87), respectively, which were significantly increased and followed a significant trend.

Sorahan (2008) provided an update, with bladder cancer incidence and deaths examined through December 2005. The SMR for workers in the *o*-toluidine department was 1116 (95% CI: 230−3261). Relative risk of bladder cancer due to working in the *o*-toluidine department for 1−4 years or ≥5 years remained elevated and significant, consistent with the 2000 analysis at 4.68 (95% CI: 1.66−13.2) and 6.99 (95% CI: 1.69−28.9), respectively.

ANIMAL STUDIES

Oral Exposures

In animal studies, *o*-toluidine is generally administered as *o*-toluidine HCl. The effects of oral exposure of animals to *o*-toluidine HCl have been evaluated in subchronic-duration (Ekman

and Strombeck, 1947; NTP, 1996a,b,c), chronic-duration (NCI, 1979a,b), and cancer (NCI, 1979a,b; Weisburger et al., 1978a,b; Hecht et al., 1982) studies. The NTP rat studies examined 13-week (designated as NTP, 1996a), 26-week (designated as NTP, 1996b), and 13-week with a 13-week recovery period (designated as NTP, 1996c) exposures. The NCI (1979) and Weisburger et al. (1978) studies were conducted in rats and mice. Rat studies were designated as NCI (1979a) and Weisburger et al. (1978a), while mouse studies were designated NCI (1979b) and Weisburger et al. (1978b) as indicated in Table 2. When the complete study was discussed throughout the report, the letter designations were not employed. Two subacute (14-day) studies were also conducted and are reported under the section of "Other Data" below.

Subchronic-duration Studies

In a study by Ekman and Strömbeck (1947), 10 rats (sex and strain not specified) were given a diet of rice flour with *o*-toluidine that was supplemented with a slice of carrot every other day (duration not specified). The methods of the study are not well reported, and the dose of the experiment was lowered soon after study initiation. The study authors reported the dose in the latter part of the study as approximately 7.5 to 12 mg of *o*-toluidine per day, with levels about twice as high during the beginning of the study. Study investigators reported the average lifespan of the animals to be 91 days. No other methods or details of the experiment were provided. Three of the rats were stated to have changes in the mucous membrane of the bladder, with metaplasia and early epithelial proliferation.

NTP (1996)

The 13-week component of the peer-reviewed rat study by NTP (1996a) is selected as the principal study for derivation of the screening subchronic p-RfD. NTP (1996a,b,c) conducted a study performed under Good Laboratory Practices (GLP) that compared the toxicity and carcinogenicity of *o*-nitrotoluene and *o*-toluidine HCl administered in feed at one dose level. Sixty male F344/N rats (20/group) were administered 5000 ppm of *o*-toluidine HCl (100% pure as tested by the study laboratory) in the diet and sacrificed after continuous exposure for 13 weeks (Group 1) or 26 weeks (Group 2). A third group was administered 5000-ppm *o*-toluidine HCl for 13 weeks, followed with a 13-week recovery period before sacrifice. The study authors reported the daily doses received by these groups as 301 mg/kg-day for the 13-week continuous exposure, 285 mg/kg-day for the 26-week continuous exposure, and 304 mg/kg-day for the 13-week exposure with 13-week recovery group. Homogeneity and stability tests were conducted. Results demonstrated that 9% of the test compound was lost due to preparation, but the dose formulations were homogeneous and stable for 28 days when stored at −20°C to 5°C. Samples stored at room temperature lost 10% of the test material. There were a total of 40 unexposed control rats. However, 20 were used to examine the effects of altered gastrointestinal flora (which were used as part of the testing for *o*-nitrotoluene). Clinical signs, body weight, and food consumption were recorded throughout the study. At study termination, animals were necropsied, and the following organs were weighed: epididymis, right kidney, liver, spleen, and right testis. Histopathology was only routinely performed on gross lesions, epididymides, liver, kidneys, testes, spleen, and urinary bladder. Continuous data with a normal distribution were analyzed with a Dunnett's test. A Fischer's Exact test was used to evaluate histopathological lesions, and a Wilcoxon rank sum test was used for the placental glutathione *S*-transferase-positive data.

No mortalities were observed among treated or control animals (NTP, 1996a,b,c). Although statistical analyses were not performed, there was a 11% decrease in body-weight, relative to concurrent controls after 13 weeks of treatment. The lowered relative body-weight persisted after the 13-week recovery period (11% lower than the concurrent control). After 26 weeks of treatment, *o*-toluidine-treated animals had a body-weight 18% lower than the concurrent controls. This was accompanied by lower food consumption. There was a significant increase in the relative weight of the right kidney, liver, spleen, and right testis, but not in the epididymides, after both 13 (see Table B.1) and 26 weeks (see Table B.2) of treatment. After 13 weeks of treatment followed by 13 weeks of recovery, only the relative weight of the spleen remained significantly elevated (155% of the control weight) (see Table B.2). However, the spleen weight, after the recovery period, was not as elevated as after 13 weeks (350% of the controls) or 26 weeks (433% of the controls) of continuous exposure. In the liver, accumulation of hemosiderin pigment of the Kupffer cell cytoplasm was significantly increased in all groups (20/20 after 13 weeks and 26 weeks, 11/20 after a 13-week recovery period compared to 0/20 in the controls after 13 weeks and 0/10 after 26 weeks). Placental isozyme of glutathione *S*-transferase (PGST)-positive foci (number and size) were also found to be significantly increased at 26 weeks (not measured in the other two treatment groups). All treated animals experienced a significant increase in hemosiderin pigment accumulation in the tubule epithelium of the kidney. There was a significant increase in transitional cell hyperplasia in the urinary bladder after 13 (10/20) and 26 (17/20) weeks of treatment but not after the 13-week recovery period (0/20). At necropsy, the spleens were stated to be grossly enlarged, with granular plaques on the capsular surface, with some plaques containing fluid-filled cysts at 13 weeks. At 13 and 26 weeks, all the treated animals had the following histopathological lesions in the spleen: congestion, hematopoietic cell proliferation, hemosiderin pigmentation, and fibrosis capsules. These were significantly increased compared with the incidence in the controls, and with the exception of hematopoietic cell proliferation, were still significantly increased after a 13-week recovery period. There was thrombosis in the spleen in a few animals after 13 (3/20) and 26 (2/20) weeks of treatment that were not observed in either the controls or the 13-week recovery animals. The only end points that seemed to progress in severity from 13 weeks to 26 weeks were the increase in spleen weight and the increase in the severity of fibrosis capsules in the spleen. After a 13-week recovery period, effects were still observed in the spleen, with relative weight still increased and numerous lesions still observed. The LOAEL is 5000 ppm (285 to 304 mg/kg-day), the only dose tested, based on the numerous effects observed among the three groups.

Chronic-duration/Carcinogenic Studies

NCI (1979a)

NCI (1979a) sponsored a chronic-duration/carcinogenic feeding study of *o*-toluidine HCl (purity >99% as measured by the study laboratory) to male and female F344 rats (20 controls/sex, 50/dose/sex), obtained from the NCI Frederick Cancer Research Center animal farm. The rats received concentrations of 0, 3000, or 6000 ppm in the diet for a period of 101 to 104 weeks (equivalent to 231 and 525 mg/kg-day in males and 264 and 600 mg/kg-day in females based on average body weight from the study and daily food consumption [U.S. EPA, 1988]). The human equivalent doses (HEDs) for the carcinogenicity study are equivalent to 63.0 and 138.8 mg/kg-day in males and 63.7 and 140.4 mg/kg-day in females. Doses were selected based on a 7-week study that was conducted to determine a maximum tolerated dose. Diets were freshly prepared every 1 to 1.5 weeks and stored at 5°C, but there is no indication that the diets

were tested for concentration, stability, or homogeneity. Animals were checked twice daily for morbidity and mortality. Clinical observations, palpations, and body-weight measurements were conducted once per month. Animals were necropsied and evaluated for gross signs of toxicity, and tissues were examined microscopically. The statistical tests conducted were numerous and typical of those conducted in NCI studies. Animals that died from other than natural causes were statistically censored at the time of death; animals dying from natural causes were not statistically censored. A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors.

There was a significant decrease in survival relative to controls, at both doses, in both male and female rats; there was approximately 90%, 27%, and 0% (males) and 80%, 55%, and 21% (females) survival at the end of the study for control, low, and high doses, respectively (estimated from Figure 2 of NCI, 1979a). The majority of mortalities in the high-dose group occurred late in the exposure, after Week 60. Decreases in mean body weights of surviving rats relative to concurrent controls were also noted beginning at around 40 weeks of treatment for the low-dose group and at 10 weeks of treatment for the high-dose group. Proliferative lesions in the spleen, abdomen, scrotum, urinary bladder epithelium, and renal pelvis of dosed rats were observed. There was an increase in the incidence of liver necrosis in high-dose females and both low- and high-dose male groups and bladder epithelial hyperplasia in both low- and high-dose groups in males and females (see Table B.3). *o*-Toluidine also induced subcutaneous fibromas and mammary fibroadenomas (see Table B.4). Statistical analyses conducted by the authors showed a significant, dose-related increase in subcutaneous fibroma in male (but not female) rats, with the incidence at both dose levels (28/50 low, 27/49 high) significantly higher than matched controls (0/20) in male rats. Sarcomas in multiple organs and fibrosarcomas in multiple organs were found to be significantly elevated in male rats as compared to controls. In female rats, sarcomas (fibrosarcomas, angiosarcomas, and osteosarcomas) of multiple organs were found to be significantly higher than controls. The study authors also reported that *o*-toluidine significantly induced urinary bladder transitional cell carcinomas in female rats, fibroadenomas/adenomas of the mammary gland in female rats, and mesotheliomas in multiple organs or tunica vaginalis in male rats. A NOAEL for noncancer effects was not reported by the study authors. No NOAEL can be derived from the data. There is no LOAEL because the lowest dose (231 mg/kg-day) is a frank effect level (FEL) based on decreased survival.

Weisburger et al. (1978a)

The peer-reviewed rat study by Weisburger et al. (1978a) is selected as the principal study for derivation of the cancer p-OSF. Weisburger et al. (1978a) administered *o*-toluidine HCl (purity 97−99%) to groups of 25 male Sprague-Dawley-derived CD rats from Charles River in a 24-month study beginning at concentrations of 8000 or 16,000 ppm in the diet for 3 months, then reduced to 4000 or 8000 ppm in the diet for 15 additional months, after which they were fed a control diet for 6 additional months before scheduled sacrifice. Several chemicals were tested and reported in this report. For all compounds, the higher dose was selected to be the maximum tolerated dose, but if high mortality occurred or body weight became 10% lower than the concurrent control, the doses were reduced. Body weight was routinely measured to keep track of the differences. Each compound was run in conjunction with a control, which is considered the concurrent control. The pooled control combines the control groups for each compound (start times were stratified over an 8-month period). The time-weighted human equivalent doses are 72.1 and 144.1 mg/kg-day calculated based on the 3-month exposure to 8000 or 16,000 ppm,

the 15-month exposure to 4000 or 8000 ppm, and the 6 months on control diet (using average body weight [U.S. EPA, 1994b] and daily food consumption [U.S. EPA, 1988]), and the ratio of animal-to-human body-weight adjustment raised to the ¼ power. Necropsies were performed only on animals that died or were sacrificed after 6 months of exposure. The following tissues were processed for histopathology: gross lesions and tumor masses, lung, liver, spleen, kidney, adrenals, intestines, stomach, heart, bladder, reproductive organs, and pituitary gland. A Fischer's Exact test was used to determine the differences between treatment groups and the control groups. There was a significant increase in subcutaneous fibromas and fibrosarcomas in both the low- (18/23) and high- (21/24) dose groups compared to the control group (concurrent: 0/16; pooled: 18/111) (see Table B.5). There was a nonsignificant increase in bladder tumors in both the low- (3/23) and high- (4/24) dose groups compared to the control (concurrent: 0/16; pooled: 5/111). Multiple tumors (this is assumed to be more than one tumor per rat, although the study authors did not specify) were significantly increased above the pooled controls in the high-dose group (8/24) but not the low-dose group (6/23) compared to the control groups (concurrent: 3/16; pooled: 14/111).

Hecht et al. (1982)

Hecht et al. (1982) administered *o*-toluidine HCl (purity not reported) to 30 male F344 rats at a concentration of 0.028 mol/kg diet for 72 weeks. Study authors calculated a mean daily dose of *o*-toluidine HCl to be 0.062 g/rat based on measured mean daily food consumption. This is equivalent to 163 mg/kg-day based on a standard average body weight of 0.380 kg in F344 rats during a chronic-duration study (U.S. EPA, 1994b). The human equivalent dose based on an animal-to-human body-weight ratio raised to the ¼ power is 44.2 mg/kg-day. After 72 weeks of dosing, animals were placed on control diet. Animals were sacrificed when found moribund or after 93 weeks and were necropsied. It was stated that all major organs were processed for histopathology, but a detailed list was not provided. There was a decrease in survival of dosed rats beginning in the 18th month. Although the study authors stated that a chi-square test was used, it appears that results were compared to the *o*-nitrosotoluene group and not the control. Therefore, a Fisher's Exact test is used to analyze the data. There was a significant increase in the following tumors in treated rats compared to the concurrent controls: skin fibromas (25/30 versus 1/27), spleen fibromas (10/30 versus 0/27), mammary fibroadenomas (11/30 versus 0/27), and peritoneal tumors (14/30 versus 2/27). Smaller, nonsignificant, increases were observed for liver hepatomas (2/30 treated; 0/27 controls), bladder papilloma (3/30 treated; 0/27 controls), bladder carcinoma (1/30 treated; 0/27 controls), and mammary carcinomas (2/30 treated; 0/27 controls). The use of a single dose level precludes dose-response modeling.

NCI (1979b)

B6C3F$_1$ mice (20 controls/sex, 50/dose/sex) were dosed in the diet at levels of 0, 1000, or 3000 ppm for a period of 102 to 103 weeks (NCI, 1979b). Study details were similar to those discussed above in the rat study (NCI, 1979a). HEDs are 23.0 and 73.8 mg/kg-day in males and 26.5 and 91.9 mg/kg-day in females calculated based on average body weight from the study and daily food consumption (U.S. EPA, 1988), and the ratio of animal-to-human body-weight adjustment raised to the ¼ power. Animals were checked twice daily for morbidity and mortality. Clinical observations, palpations, and body-weight measurement were conducted once per month. Animals were necropsied and evaluated for gross signs of toxicity, and tissues were examined microscopically. There was a decrease in mean body weight in treated male and female mice, but, in contrast to the rats, there was no significant dose-related decrease in

survival. Statistical analyses conducted by the study authors indicate that incidence of hemangiosarcomas of all sites in male mice is significantly increased ($p < 0.05$ trend test; see Table B.6). In female mice, investigators reported a significant, dose-related increase in the number of combined hepatocellular carcinomas and adenomas, which was not observed in male mice.

Weisburger et al. (1978b)

Weisburger et al. (1978b) also administered *o*-toluidine HCl (purity 97−99%) to groups of 25/dose/sex CD-1 mice in a 24-month study, beginning at concentrations of 16,000 or 32,000 ppm in the diet for 3 months, followed by 8000 or 16,000 ppm in the diet for 15 additional months, after which they were fed the control diet for 6 additional months before scheduled sacrifice. Methodology mirrored that in the rat experiment, except the pituitary was not routinely examined histologically. The time-weighted HEDs are 210.5 and 420.9 mg/kg-day in male mice and 211.8 and 423.7 mg/kg-day in female mice. Necropsies were performed only on animals that died after 6 months of exposure. Tissues were processed for histopathology. There was a significant increase in vascular tumors in both the low- and high-dose groups compared to the control in male and female groups (see Table B.7). Incidence of vascular tumors in the simultaneous control, pooled control, low-dose group, and high-dose group were 0/14, 5/99, 5/14, and 9/11, respectively, in male mice and 0/15, 9/102, 5/18, and 9/21, respectively, in female mice.

Developmental and Reproduction Studies

No studies could be located regarding the effects on development and reproduction resulting from oral exposure of animals to *o*-toluidine. Hiles and Abdo (1990) report on two dermal exposure studies that examined the developmental and reproductive effects of *o*-toluidine that indicate chemical-related effects. The original sources of Malysheva and Zaitseva (1982) and Malysheva et al. (1983) were unavailable for review at this time. The original study publications were published in a foreign language. The brief summary of the two studies stated that an unspecified number of animals (species not specified) were treated dermally (doses and purity not reported) for 4 months with *o*-toluidine. The summary also stated treatment resulted in changes in ovarian cycle, ovarian morphostructure, ability to reproduce in females, and stimulation of spermatogenesis in males. It is further stated that progeny were also affected, but no specific effects were reported.

Inhalation Exposures

There is no suitable information to provide in this regard.

OTHER DATA (SHORT-TERM TESTS, OTHER EXAMINATIONS)

Short-term Studies

Haskell Laboratory (1994) conducted a study sponsored by Dupont & Co. that examined urinary bladder toxicity in rats after a 14-day feeding exposure. *o*-Toluidine (purity 99.5%) was added to the diet via an ethanol vehicle and administered to F344 rats (10−15 rats/sex/dose) for 14 days at dose levels of 0, 500, 3000, or 6000 ppm. The mean daily intake was estimated by the study authors to be 40.4, 238, and 449 mg/kg-day in males and 43.5, 251, and 481 mg/kg-day in females. It was reported that the test substance was not stable in the diet (30% decrease in concentration after 7 days), and doses were not adjusted for test substance stability. Cage side observations and checks for moribund animals were made twice daily, body weights were

measured three times weekly, and urine was collected on Days 6−7 and Days 13−14. Average daily food consumption was measured weekly. At sacrifice, blood was collected and analyzed specifically for methemoglobin levels. Necropsies were performed at the end of the study period, and bladder and duodenum were removed and processed for analysis of cell proliferation (5 rats/sex/dose) and unscheduled DNA synthesis (5 rats/sex/dose).

None of the animals died during treatment (Haskell Laboratory, 1994). Clinical signs of toxicity included wet and stained perineums in females of the high-dose group. There was a significant decrease in body weight in high-dose males and females that was likely due to the low weight gain during the first week of exposure. A significant decrease in food consumption was noted in the mid- and high-dose males and females during the first week. Significant increases in bladder epithelial cell proliferation were observed in mid- and high-dose females and high-dose males. Histopathology revealed mild urothelial hyperplasia in females, slight urothelial thickening in males, and unscheduled DNA synthesis (UDS) in both males and females in the high-dose groups. Furthermore, the investigators observed a significant and dose-related increase in methemoglobin levels in both sexes at all dose levels. The study authors established a NOAEL of 500 ppm (equivalent to 40.4 and 43.5 mg/kg-day in males and females, respectively) for urinary bladder toxicity (LOAEL is 238 and 251 mg/kg-day in male and females, respectively). The study authors could not establish a NOAEL for methemoglobinemia; therefore, the LOAEL is 40.4 and 43.5 mg/kg-day in males and females, respectively, based on increased levels of methemoglobin.

Short et al. (1983) conducted a short-term toxicity study where they administered *o*-toludine (purity 99.3%) to male F344 rats (10/time point) via daily gavage at a dose level of 225 mg/kg-day for 5, 10, or 20 days. Body weight, organ weight, and histopathology were evaluated. Clinical signs reported by the study authors included transient cyanosis and rough hair coat. Of the 30 rats treated, 10 of the rats died before the end of the 20 days of treatment. Body weight was significantly lower after 5 and 10 days but was equal to the control by Day 20. Spleen weight was significantly increased at all time points, but liver and kidney weights were not affected. The effects on the spleen were confirmed with significant increases in spleen congestion, hemosiderosis, and hematopoiesis in the survivors at all three time points. Hypercellularity of bone marrow was significantly increased only at the 10-day time point. No changes in histopathology were noted in the liver. The LOAEL is 225 mg/kg-day and was the only dose tested.

Other Studies

Toxicokinetic data demonstrate that *o*-toluidine is readily absorbed via the gastrointestinal tract and excreted within 24 hours in the urine (Cheever et al., 1980; Son et al., 1980). A greater amount of *o*-toluidine is excreted unchanged (21%) compared to either of the other toluidine isomers (2.5%). *o*-Toluidine is not mutagenic in Ames assays (see Table 3). However, in a review or *o*-toluidine genotoxicity, Danford (1991) states that this is only the case in standard Ames assays, and positive mutagenicity results have been obtained at high concentrations using different types of metabolic activation systems. Additionally, Danford (1991) states that most yeast studies have also been negative, but *o*-toluidine has been found to cause deletion mutations in yeast at concentrations that reduced survival (Brennan and Schiestl, 1999). *o*-Toluidine was weakly positive for Trp+ revertants in modified yeast (*Saccaromyces cerevisiae* C658-K42) (Morita et al., 1989). There have been mixed results with cytogenic

assays. Danford (1991) concludes that it is a clastogen but only with prolonged exposure. Morita et al. (1997) summarized results from six independent micronuclei assays in male $B6C3F_1$ mice receiving one to four intraperitoneal (ip) injections at doses up to 1000 mg/kg (the LD_{50} was stated to be 800 mg/kg). There was a small—but significant—increase in micronucleated polychromatic erythrocytes in the bone marrow only with the 800- or 1000-mg/kg dose. This was not considered biologically significant, due to the deaths in these animals, and the tests were considered negative. Chromosomal aberrations have been found in Chinese hamster ovary (CHO) cells in vitro (Gulati et al., 1985) but not after ip injection to $B6C3F_1$ mice in vivo (McFee et al., 1989). *o*-Toluidine has been found to cause sister chromatid exchange (SCE) in both CHO cells in vitro (Gulati et al., 1985) and in $B6C3F_1$ mice in vivo (McFee et al., 1989) and to cause transformations in BALB/c-3T3 and Syrian hamster embryo (SHE) transformation assays (Matthews et al., 1993; Kerckaert et al., 1998) (see Table 3).

Table 3. Other Studies

Tests	Materials and Methods	Results	Conclusions	References
Toxicokinetic	Studied distribution of ^{14}C following single dose of either 50- or 400 mg/kg subcutaneous dose of *o*-[methyl-^{14}C]toluidine to male F344 rats (2/dose) after 24 and 48 h.	Urine was the major excretory pathway at both dose levels, followed by fecal excretion and breath. Tissue concentration of radiolabel after 48 h was highest in liver with approximately 0.12% and 0.325% at the 50- and 400-mg/kg dose, respectively.	NA	Son et al. (1980)
Toxicokinetic	Studied excretion and metabolism of ^{14}C following single oral dose of 50 or 500 mg/kg of *o*-toluidine to male Sprague-Dawley rats; *p*-toluidine and *m*-toluidine were also tested.	92% of *o*-toluidine was excreted via the urine within 24 h of dosing. The amount of parent compound excreted was greater for *o*-toluidine (21%) than either of the other isomers (2.5%). Aminomethylphenols were the metabolite detected in the urine.	NA	Cheever et al. (1980)
Genotoxicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) TA1535, TA1537, TA1538, TA98, and TA100 with and without metabolic activation at a dose level of 1000 µg or 9.33 µmole.	Nonmutagenic in all strains tested.	*o*-Toluidine was not considered to be mutagenic.	Simmon (1979)
Genotoxicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) TA100 with metabolic activation and for DNA damage in Chinese hamster lung fibroblasts (V79) with metabolic activation incubated for 2 h with concentrations ranging from 0.3 to 10.0 mM (32−1070 mg).	Nonmutagenic and did not cause DNA breakage.	*o*-Toluidine was not considered to be mutagenic or cause DNA damage.	Zimmer et al. (1980)
Genotoxicity	Tested for sperm head abnormalities using ip of 0.05, 0.1, 0.2, 0.25, 0.3, 0.4, or 0.5 mg/kg-day for 5 days in male (CBA × BALB/c)F_1 mice.	Negative.	*o*-Toluidine did not cause sperm head abnormalities.	Topham (1980)

Table 3. Other Studies

Tests	Materials and Methods	Results	Conclusions	References
Genotoxicity	Tested for reverse mutation in *Salmonella typhimurium* (Ames assay) TA1535, TA1537, TA1538, TA98, and TA100 with and without metabolic activation at doses of 0.32, 1.0, 3.2, or 10.0 µg/plate.	Nonmutagenic in all test strains.	*o*-Toluidine was not considered to be mutagenic.	Baker and Bonin (1981)
Genotoxicity	Tested in vivo mutagenicity in the bone marrow micronucleus test (injected at 0 and 24 h, samples taken at 48, 72, and 96 h); dose level was at 50, 80, and 100% of LD_{50} in $B6C3F_1$ mice (800 mg/kg).	Nonclastogenic.	*o*-Toluidine is not considered to be clastogenic.	Salamone et al. (1981)
Genotoxicity	Tested for chromosomal aberrations and sister chromatid exchanges (SCEs) in Chinese hamster ovary (CHO) cells with concentrations up to 500 µg/ml in the absence of metabolic activation and 5000 µg/ml in the presence of metabolic activation.	The highest dose was generally cytotoxic. However, in the one assay that was not toxic at 500 µg/plate without metabolic activation, there was nearly twice as many SCEs as in the vehicle control. There was also an increase in chromosomal aberrations, with a concentration of 500 µg/plate or greater depending on the assay conditions.	*o*-Toluidine was considered positive for inducing SCE in the presence and absence of metabolic activation. *o*-Toluidine was considered positive for chromosomal aberration only when fixation was done at 6 h.	Gulati et al. (1985)
Genotoxicity	Tested for chromosomal aberrations and sister chromatid exchanges (SCE) in vivo after a single ip injection at doses of 150, 300, or 600 (maximum tolerated dose) mg/kg to male $B6C3F_1$ mice.	There was not an increase in chromosomal aberrations or micronuclei, but there was an increase in the frequency of SCE in two separate trials.	*o*-Toluidine was positive for inducing SCE but not chromosomal aberrations or micronuclei in vivo.	McFee et al. (1989)
Genotoxicity	Tested for Trp+ reversion in *Saccaromyces cerevisiae* C658-K42 (strain with increased permeability), with concentrations ranging from 0.5 to 3.0 mg/mL.	At 3.0 mg/mL there was an increase in Trp+ revertants (6.9 ± 1.9) in the presence of metabolic activation compared to the control (2.4 ± 1.3).	It was concluded that *o*-toluidine was weakly positive.	Morita et al. (1989)
Genotoxicity	BALB/c-3T3 cell transformation assay.	50% cytotoxicity occurred with a concentration of 4.33 mM. Statistical sensitivities for the three trials were stated to be 87/110, 106/110, and 59/110.	*o*-Toluidine was considered active in the BALB/c-3T3 cell transformation assay.	Matthews et al. (1993)

Table 3. Other Studies

Tests	Materials and Methods	Results	Conclusions	References
Genotoxicity	Tested in vivo mutagenicity in the bone marrow micronucleus test with 1 to 4 ip injections up to 1000 mg/kg (LD_{50} = 800 mg/kg) in male $B6C3F_1$ mice, results were measure 24 h after last dose.	There was a slight increase in micronucleated polychromatic erythrocytes in the bone marrow of dying animals, which was not considered biologically significant.	*o*-Toluidine was not considered to be clastogenic.	Morita et al. (1997)
Genotoxicity	Tested for genotoxicity using the 24-h Syrian hamster embryo (SHE) transformation assay at a pH of 6.7 with concentrations up to 1200 µg/mL (higher concentrations were not soluble).	There was a significant increase in transformations with concentrations ranging from 750 to 1200 µg/mL, but there was only 38% cytotoxicity at the highest dose tested.	*o*-Toluidine was positive in the SHE transformation assay.	Kerckaert et al. (1998)
Genotoxicity	Tested for deletion (DEL) recombination in yeast, with concentrations ranging from 3.0 to 6.0 mg/mL.	An increase in revertants (more than 3-fold) was observed, with concentrations that caused decreased survival (5.0 and 6.0 mg/mL, survival 3.90 and 0.49% compared to 93% in the control). Survival was increased and revertants decreased when the antioxidant *N*-acetyl cysteine was added to the culture.	*o*-Toluidine caused deletion mutations in yeast.	Brennan and Schiestl (1999)

DERIVATION OF PROVISIONAL VALUES

Tables 5 and 6 below present a summary of noncancer and cancer reference values, respectively. IRIS data are indicated in the table if available.

DERIVATION OF ORAL REFERENCE DOSE

Derivation of Subchronic Provisional RfD (Subchronic p-RfD)

There are two subchronic-duration studies available (see Table 4). Ekman and Strömbeck (1947) did not provide sufficient details. There is no information provided on the doses used, and there does not appear to be a control group. While the NTP (1996) study was a peer-reviewed GLP study, only one dose was tested. The NTP (1996) study was conducted to compare the effects of *o*-toluidine HCl to *o*-nitrotoluene, to demonstrate the progression of toxic effects over time (13 weeks on the diet compared to 26 weeks on the diet), and, also, to illustrate the reversibility of effects (13 weeks on the diet followed by 13 weeks on control diet); therefore, doses were selected to ensure a response.

A potential POD, blood methemoglobin, is suggested by a short-term study (Haskell Laboratory, 1994) that observed a dose-dependent increase in methemoglobin levels. However, other studies with toluidine isomers suggest that the methemoglobin response likely decreases with longer durations of exposure, while other organ effects likely related to blood effects such as methemoglobinemia become apparent (Malik-Brys and Senczuk, 1995). For example, some of the splenic and hepatic effects noted in the NTP (1996a) 13-week study are consistent with persistent chronic blood effects. The NTP (1996b) study demonstrates that the splenic effects progress with time on the diet. Therefore, the methemoglobin levels observed in the 14-day study (Haskell Laboratory, 1994) are supportive of some of the effects seen in the NTP (1996) study but are not useful for deriving the subchronic p-RfD.

Table 4 below describes studies that are relevant for deriving the oral provisional RfDs. The NTP (1996a) 13-week study provides the best data to derive a subchronic p-RfD. The NTP (1996b) study, after 26 weeks, reported similar effects to those seen at 13 weeks in the NTP (1996a) study at a slightly lower dose level, and supports the selection of a POD from the NTP (1996a) 13-week study. Although both the NTP (1996a) and NTP (1996b) studies could serve as the principal study for derivation of a subchronic p-RfD, the 13-week study (NTP, 1996a) is selected because the exposure duration is considered to be subchronic for rodent studies. Because there is not enough dose-response information available, the data are not amenable to BMD modeling for derivation of the subchronic p-RfD. Further, the NTP (1996a) study provides only a LOAEL as the POD, thus requiring four full UFs (UF_A, UF_H, UF_D, UF_L) resulting in a composite UF (UF_C) of 10,000. Consequently, the high uncertainty combined with a very limited database precludes derivation of a subchronic p-RfD. However, the NTP (1996a) study does provide sufficient information to derive a screening subchronic p-RfD in Appendix A. A chronic p-RfD is not derived because no acceptable chronic-duration toxicity studies are available and extrapolation from the subchronic study, NTP (1996a) would entail applying a full suite of 10-fold UFs (UF_A, UF_D, UF_H, UF_L, UF_S) resulting in a composite UF_c of 100,000. Additionally, the low survival seen in the chronic-duration NCI (1979a) rat study (approximately 27% at the low dose of 231 mg/kg-day in males and 55% at 264 mg/kg-day in females) suggests that the subchronic-duration LOAEL of 301 mg/kg-day from the principal study (NTP, 1996a) should not be extrapolated to a chronic-duration LOAEL.

Table 4. Summary of Relevant Oral Systemic Toxicity Studies for *o*-Toluidine

References	Species, # /Sex (M/F)	Exposure (ppm unless otherwise noted)	Frequency/ Duration	$NOAEL_{ADJ}$[a] (mg/kg-d)	$LOAEL_{ADJ}$[b] (mg/kg-d)	Critical End point
Ekman and Strömbeck (1947)	Rat, 10 (sex not specified)	Not specified; authors added *o*-toluidine (7.5 to 12 mg/d) in rice flour diet	At least 91 d	ND	ND	Three animals exhibited metaplasia and early epithelial proliferation in bladder mucous membrane
NTP (1996a)	**Rat, 20 M**	**0, 5000**	**13 wk in diet**	**None[c]**	**301[d]**	**Decreased body weight; increased relative weight of the liver, kidney, spleen, and testes; and increased incidence of lesions in the liver, kidneys, bladder, and spleen**
NTP (1996b)	Rat, 20 M	0, 5000	26 wk in diet	None	285[d]	Decreased body weight; increased relative weight of the liver, kidney, spleen, and testes; and increased incidence of lesions in the liver, kidneys, bladder, and spleen
NCI (1979a)	Rat, 50M/50F	0, 3000, 6000	101−104 wk in diet	None	None. 231[d] is an FEL	Decreased survival

[a]$NOAEL_{ADJ}$ = NOAEL (ppm or mg/kg food) × food consumption (kg/day) ÷ body weight (kg).
[b]$LOAEL_{ADJ}$ = LOAEL (ppm or mg/kg food) × food consumption (kg/day) ÷ body weight (kg).
[c]No NOAEL was identified. The NOAEL is considered equal to a LOAEL ÷ 10 for screening purposes.
[d]These values were provided in the study report.

ND = Not determined.

Table 5. Summary of Noncancer Reference Values for *o*-Toluidine

Toxicity Type (Units)	Species/Sex	Critical Effect	p-Reference Value[a]	POD Method	POD	UF_C	Principal Study
Screening subchronic p-RfD	F344 rat/M	Increased spleen weight and incidence of spleen lesions	2×10^{-2}	LOAEL	301	10,000	NTP (1996a)
Chronic p-RfD (mg/kg-day)	None						
Subchronic p-RfC (mg/m^3)	None						
Chronic p-RfC (mg/m^3)	None						

[a]The screening subchronic p-RfD adjusts for the fact that *o*-toluidine HCl was administered instead of *o*-toluidine using the following calculation: p-RfD *o*-toluidine = p-RfD *o*-toluidine HCl × (molecular weight of *o*-toluidine ÷ molecular weight of *o*-toluidine HCl) or 3×10^{-2} mg/kg-day × (107.16 ÷ 143.62) = 2×10^{-2} mg/kg-day.

Table 6. Summary of Cancer Reference Values for *o*-Toluidine

Toxicity Type	Species/Sex	Tumor Type	Cancer Value[a]	Principal Study
p-OSF	F344 rat/M	Subcutaneous fibroma and fibrosarcoma	1.6×10^{-2} $(mg/kg\text{-}day)^{-1}$	Weisburger et al. (1978a)
p-IUR	None			

[a]The p-OSF adjusts for the fact that *o*-toluidine HCl was administered instead of *o*-toluidine using the following calculation: p-OSF *o*-toluidine = p-OSF *o*-toluidine HCl × (molecular weight of *o*-toluidine ÷ molecular weight of *o*-toluidine HCl) or 2.2×10^{-2} $(mg/kg\text{-}day)^{-1}$ × (107.16 ÷ 143.62) = 1.6×10^{-2} $(mg/kg\text{-}day)^{-1}$.

Derivation of Chronic Provisional RfD (Chronic p-RfD)

No chronic p-RfD values can be derived. The Weisburger et al. (1978a,b) study provides only cancer incidence and does not provide information on any possible noncancer effects or on mortality. The only chronic-duration study available that provides any nonneoplastic results (i.e., NCI, 1979a) also had decreased survival (i.e., 27% survival in males and 55% in females at 3000 ppm) in rats relative to controls (i.e., 90% and 80% survival in control males and females, respectively) at the lowest dose tested (see Table B.3). The decreased survival in the NCI (1979a) study occurred at doses (231 mg/kg-day in males and 264 mg/kg-day in females) that were equivalent to the dose that caused reduced survival in the NTP (1996) subchronic-duration rat studies (301 mg/kg-day), which precludes using the subchronic-duration study to extrapolate to a chronic p-RfD. In addition, using a subchronic-duration LOAEL to extrapolate to a chronic-duration NOAEL would entail applying a full suite of 10-fold UFs (UF_A, UF_D, UF_H, UF_L, UF_S) resulting in a UF_C of 100,000. Consequently, derivation of a chronic p-RfD is precluded.

DERIVATION OF INHALATION REFERENCE CONCENTRATIONS

No subchronic or chronic p-RfC values can be derived. There are no animal inhalation studies, and the epidemiology studies in humans do not provide any concentrations for *o*-toluidine, nor can the studies make any definitive relationship between *o*-toluidine exposure and any toxic effect.

CANCER WEIGHT-OF-EVIDENCE (WOE) DESCRIPTOR

Table 7 identifies the cancer weight-of-evidence (WOE) descriptor for *o*-toluidine.

Table 7. Cancer WOE Descriptor for *o*-Toluidine

Possible WOE Descriptor	Designation	Route of Entry (Oral, Inhalation, or Both)	Comments
"Carcinogenic to Humans"	N/A	N/A	Occupational studies, although consistently and positively associated with bladder cancer, cannot establish any definitive link with exposures to *o*-toluidine and are confounded with exposure to other known or suspected carcinogens.
"Likely to Be Carcinogenic to Humans"	**Selected**	**Oral**	**Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), *o*-toluidine is *"Likely to be Carcinogenic to Humans"* based on evidence of carcinogenicity in rats and mice in the NCI (1979a,b) and Weisburger et al. (1978a,b) oral bioassays.**
"Suggestive Evidence of Carcinogenic Potential"	N/A	N/A	
"Inadequate Information to Assess Carcinogenic Potential"	N/A	N/A	
"Not Likely to Be Carcinogenic to Humans"	N/A	N/A	No strong evidence of noncarcinogenicity in humans or animals is available.

MODE-OF-ACTION DISCUSSION

The *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005) define mode of action (MOA) as the following: "…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…There are many examples of possible modes of carcinogenic action, such as mutagenicity, mitogenesis, inhibition of programmed cell death, cytotoxicity with reparative cell proliferation, and immune suppression" (p. 1−10).

Summary of Mutagenicity and Genetic Toxicology Studies

Data on the mutagenicity of *o*-toluidine are equivocal. *o*-Toluidine is not mutagenic in standard Ames assays but has been positive in modified assays including the use of different types of metabolic activation systems at high concentrations. It has also been generally negative in yeast studies, but it has been found to cause deletion mutations in yeast (Brennan and Schiestl, 1999) and was weakly positive for Trp+ revertants in modified yeast (*Saccaromyces cerevisiae* C658-K42) (Morita et al., 1989). There have also been mixed results with cytogenic assays. Danford (1991) concludes that it is a clastogen but only with prolonged exposure. Morita et al. (1997) summarize results from six independent micronuclei assays in male $B6C3F_1$ mice receiving one to four ip injections at doses exceeding the LD_{50} (800 mg/kg). There was a small—but significant—increase in micronucleated polychromatic erythrocytes in the bone marrow only in dying animals. The study author did not consider this to be biologically significant, and the tests were considered negative. Chromosomal aberrations have been found in CHO cells in vitro (Gulati et al., 1985) but not after ip injection to $B6C3F_1$ mice in vivo (Salamone et al., 1981). *o*-Toluidine has been found to cause SCE in both CHO cells in vitro (Gulati et al., 1985) and in $B6C3F_1$ mice in vivo (McFee et al., 1989) and to cause transformations in BALB/c-3T3 and SHE transformation assays (Matthews et al., 1993; Kerckaert et al., 1998). Taken together, there are insufficient data to determine the MOA of *o*-toluidine.

DERIVATION OF PROVISIONAL CANCER POTENCY VALUES

There are insufficient data to determine the carcinogenic mode of action. Therefore, a linear approach is applied.

Derivation of Provisional Oral Slope Factor (p-OSF)

The study by Weisburger et al. (1978a) is selected as the principal study. The cancer end point is the incidence of subcutaneous fibromas and fibrosarcomas in male rats. This study is generally well conducted, and the data from this study are able to support a quantitative cancer dose-response assessment. There is no GLP statement, but the study otherwise meets the standards of study design, performance, and presentation of information to examine carcinogenicity. Details are provided in the "Review of Potentially Relevant Data" section. Among the available, acceptable studies, Weisburger et al. (1978a) represents the highest OSF from relevant studies in the database. A supporting study (i.e., NCI, 1979) is a peer-reviewed technical report from the National Cancer Institute (NCI).

There were two acceptable carcinogenicity studies, each performed in rats and mice, showing significant increases in a number of different tumor end points. All relevant tumor end points in the Weisburger et al. (1978a,b) and NCI (1979a,b) studies were modeled using BMDS software version 2.1.2 (see Table C.1; U.S. EPA, 2010c). Individual tumor types were modeled

and all tumor types that could be combined were also modeled. All the remaining tumor types that were not significantly different from the control animal frequencies were not considered biologically relevant. Weisburger et al. (1978a) was selected as the principal study because the increase in subcutaneous fibromas and fibrosarcomas in male rats provided the highest OSF with adequate model fit. An increase in subcutaneous fibromas and/or fibrosarcomas was also found in the NCI study in rats (1979a) and mice (1979b) and in the Hecht et al. (1982) study in male rats. Although the Hecht (1982) study also found an increase in subcutaneous fibromas, with results at a lower dose (i.e., 44.2 compared to 72.1 mg/kg-day in the Weisburger et al. [1978a] study), the Hecht study only used one dose, and no dose-response can be established. However, the tumor findings of these three chronic-duration studies lend internal consistency to the entire *o*-toluidine database. Because the study administered *o*-toluidine HCl, the p-OSF is converted to reflect the molecular weight difference between *o*-toluidine and the salt form. In addition, because the study lowered the dose after 3 months, additional time-weighted adjustments were made for the two different concentrations.

The following dosimetric adjustments were made for dietary treatment in adjusting doses for oral cancer analysis for the first 3 months (90 days) of treatment:

$(DOSE_{ADJ, HED})_{Weisburger\ et\ al.,\ 1978a}$ = $(concentration)_{Weisburger\ et\ al.,\ 1978a}$ × 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_A = 0.267 kg (average body weight for male rats in subchronic-duration study) (U.S. EPA, 1994b)

BW_H = 70 kg (human reference body weight) (U.S. EPA, 1997)

Body-weight adjustment = $(0.267 \div 70)^{1/4} = 0.2485$

$(DOSE_{ADJ, HED})_{Weisburger\ et\ al.,1978a}$ = 8000 mg/kg × (0.023 kg/day) × (1 ÷ 0.267 kg) × (90 days ÷ 90 days) × 0.2485

= 689.08 mg/kg-day × 1 × 0.2485

$(DOSE_{ADJ, HED})_{Weisburger\ et\ al.,\ 1978a}$ = 171.26 mg/kg-day

The following dosimetric adjustments were made for dietary treatment in adjusting doses for oral cancer analysis for the last 15 months (455 days) of treatment:

$(DOSE_{ADJ, HED})_{Weisburger\ et\ al.,\ 1978a}$ = $(concentration)_{Weisburger\ et\ al.,\ 1978a}$ × 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_A = 0.523 kg (average body weight for male rats in chronic-duration study) (U.S. EPA, 1994b)

BW_H = 70 kg (human reference body) (U.S. EPA, 1997)

Body-weight adjustment = $(0.523 /70)^{1/4}$ = 0.294

$(DOSE_{ADJ, HED})_{Weisburger et al., 1978a}$ = 4000 mg/kg × (0.036 kg/day) × (1 ÷ 0.523 kg) × (455 days ÷ 455 days) × 0.294
= 275.328 mg/kg-day × 1 × 0.294
= 275.328 mg/kg-day × 0.294
$(DOSE_{ADJ, HED})_{Weisburger et al., 1978a}$ = 80.95 mg/kg-day

The time-weighted average HED including the 6 months on the control diet = [$(DOSE_{ADJ, HED}$3 months × 90 days) + ($DOSE_{ADJ, HED}$15 months × 455 days)] ÷ study duration = **[(171.26 × 90) + (80.95 × 455)] ÷ (90 + 455 + 180) = 72.1 mg/kg-day**; similar calculations for the high dose (16,000 ppm reduced to 8000 ppm) derived a value of 144.1 mg/kg-day.

Table 8 presents BMD input data for incidence of subcutaneous fibromas and fibrosarcomas in male F344 rats exposed to *o*-toluidine in the diet for 18 months.

Table 8. BMD Input for Incidence of Subcutaneous Fibromas and Fibrosarcomas in Male F344 Rats Exposed to *o*-Toluidine via the Diet[a]

$(Dose)_n$ (ppm)	$(DOSE_{ADJ,HED})_n$ (mg/kg-d)	Number of Subjects	Response[b]
0	0	16	0 (0)
8000 reduced to 4000	72.1	23	18(78)[c]
16,000 reduced to 8000	144.1	24	21(87.5)[c]

[a]Weisburger et al. (1978a).
[b]Number of rats with tumors, ( ) = percentage of rats with tumors.
[c]Statistically significant in pairwise test versus control.

Table 9 shows the modeling results. Adequate model fit is obtained for the subcutaneous fibroma and fibrosarcoma incidence data using the BMDS version 2.1.2 (U.S. EPA, 2010c) multistage cancer model. The BMD modeling results with 10% extra risk for subcutaneous fibroma and fibrosarcoma yield a BMD_{10} of 6.08 mg/kg-day and a $BMDL_{10}$ of 4.50 mg/kg-day (see Table 13).

Table 9. Model Predictions for Subcutaneous Fibroma and Fibrosarcomas[a]

Model	Goodness of Fit *p*-Value[b]	AIC for Fitted Model	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)	Conclusions
Multistage cancer	0.57	45.25	6.08	4.50	Lowest AIC Lowest BMDL

[a]Weisburger et al. (1987).
[b]Values <0.10 fail to meet conventional goodness-of-fit criteria.

AIC = Akaike's Information Criteria; BMD = benchmark dose; BMD10 = BMD at a response rate of 10% incidence, extra risk; BMDL = lower confidence limit (95%) on the benchmark dose.

The curve and BMD output for the selected model, only, are provided in the BMD supplement to the document (Appendix C).

$$\text{The p-OSF}_{o\text{-toluidine HCl}} = 0.1 \div BMDL_{10}$$
$$= 0.1 \div 4.50 \text{ mg/kg-day}$$
$$= 2.2 \times 10^{-2} \text{ (mg/kg-day)}^{-1}$$

Conversion of the p-OSF based on *o*-toluidine HCl salt (molecular weight of 143.62) to *o*-toluidine (molecular weight of 107.16) is as follows:

$$\textbf{p-OSF}_{o\textbf{-toluidine}} = \text{p-OSF}_{o\text{-toluidineHCl}} \times (\text{MW of } o\text{-toluidine} \div \text{MW of } o\text{-toluidine HCl})$$
$$= 2.2 \times 10^{-2} \text{ (mg/kg-day)}^{-1} \times (107.16 \div 143.62)$$
$$= 2.2 \times 10^{-2} \text{ (mg/kg-day)}^{-1} \times 0.746$$
$$= \mathbf{1.6 \times 10^{-2} \text{ (mg/kg-day)}^{-1}}$$

Derivation of Provisional Inhalation Unit Risk (p-IUR)

No human or animal studies examining the carcinogenicity of o-toluidine following inhalation exposure have been located. Therefore, derivation of an IUR is precluded.

APPENDIX A. PROVISIONAL SCREENING VALUES

For reasons noted in the main PPRTV document, it is not possible to derive a provisional subchronic p-RfD for *o*-toluidine. 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.

DERIVATION OF SCREENING SUBCHRONIC ORAL REFERENCE DOSE

The 13-week component of the NTP (1996a) report is selected as the principal study for the derivation of the screening subchronic p-RfD. The critical end point is spleen toxicity as measured by increased relative spleen weight accompanied by increased incidence of lesions in the spleen in male F344 rats. The effects progressed with longer duration and were not entirely reversible after a 13-week recovery period. This study is peer reviewed and performed according to GLP principles. Details are provided in the "Review of Potentially Relevant Data" section. BMD analysis is not possible with these data because there was only one dose level presented in addition to the control. Among the available, acceptable studies, NTP (1996a) represents the lowest POD for developing a subchronic p-RfD. The POD in this study is a LOAEL of 301 mg/kg-day.

Dosimetric adjustments for daily exposure

No dosimetric adjustments were made for the dose in the principal study for dietary treatment, as the study authors report the average daily dose of 301 mg/kg-day.

The screening subchronic p-RfD for *o*-toluidine, based on the LOAEL of 301 mg/kg-day in male rats exposed for 13 weeks, is derived as follows:

$$\textbf{Screening Subchronic p-RfD}_{\textbf{o-toluidineHCl}} = LOAEL_{ADJ} \div UF$$
$$= 301 \text{ mg/kg-day} \div 10{,}000$$
$$= \mathbf{3 \times 10^{-2} \text{ mg/kg-day}}$$

This value is normalized to account for the actual amount of *o*-toluidine from the compound used, *o*-toluidine HCl:

$$\textbf{Screening Subchronic p-RfD}_{\textbf{o-toluidine}} = \text{p-RfD}_{o\text{-toluidineHCl}} \times (\text{MW of } o\text{-toluidine} \div \text{MW of } o\text{-toluidine HCl})$$
$$= 3 \times 10^{-2} \text{ mg/kg-day} \times (107.16 \div 143.62)$$
$$= 3 \times 10^{-2} \text{ mg/kg-day} \times 0.746$$
$$= \mathbf{2 \times 10^{-2} \text{ mg/kg-day}}$$

Table A.1 summarizes the uncertainty factors for the screening subchronic p-RfD for *o*-toluidine.

DERIVATION OF SCREENING CHRONIC ORAL REFERENCE DOSE

A screening chronic p-RfD is not derived because the only available chronic-duration study that reported noncancer effects (i.e., NCI, 1979) also reported low survival at both tested doses. Further, deriving a screening chronic p-RfD based on the same subchronic study (NTP, 1996) that was used to derive the screening subchronic p-RfD would result in a UF_C of 100,000 based on 10-fold values for each of the following UFs: UF_A, UF_D, UF_H, UF_L, UF_S. Consequently, the derivation of a screening chronic p-RfD is precluded. See the section titled "Derivation of Chronic Provisional RfD (Chronic p-RfD)" for further discussion.

Table A.1. Uncertainty Factors for Screening Subchronic p-RfD for *o*-Toluidine

UF	Value	Justification	Notes
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 liver, spleen, kidney, and bladder toxicity of *o*-toluidine.	
UF_D	10	A UF_D of 10 is applied because there are no acceptable two-generation reproduction studies or developmental studies.	
UF_H	10	A UF_H of 10 is applied for intraspecies differences to account for potentially susceptible individuals in the absence of information on the variability of response to humans.	
UF_L	10	A UF_L of 10 is applied for using a POD based on a LOAEL.	Only one dose was tested.
UF_S	1	A UF_S of 1 is applied because a subchronic-duration study was utilized.	
UF_C ≤3000	10,000		

APPENDIX B. DATA TABLES

Table B.1. Results in Male F344 Rats Exposed to Oral (in Feed) *o*-Toluidine for 13 Weeks[a]

Parameter	Adjusted Daily Dose (mg/kg-d)[b]	
	Control	301
Relative Body-weight (g)	331	293 (11%)[c]
Food consumption (g/d)	14.9	13.5 (91%)
Necropsy body weight (g)	345 ± 5[d]	298 ± 4 (86%)**
Relative right kidney weight (mg/g)	3.27 ± 0.03	3.50 ± 0.05 (107%)**
Relative liver weight (mg/g)	34.91 ± 0.31	42.57 ± 0.36 (122%)**
Relative spleen weight (mg/g)	2.12 ± 0.03	7.43 ± 0.16 (350%)**
Relative right testis weight (mg/g)	4.61 ± 0.03	5.07 ± 0.07 (110%)**
Hemosiderin pigmentation in the liver	0/10[e]	20/20**
Kidney pigmentation	0/10	20/20**
Transitional epithelium hyperplasia in the urinary bladder	0/10	10/20**
Congestion in the spleen	0/10	20/20**
Hematopoietic cell proliferation in the spleen	2/10	20/20**
Hemosiderin pigmentation in the spleen	0/10	20/20**
Thrombosis in the spleen	0/10	3/20
Fibrosis capsule in the spleen	0/10	20/20**

[a]NTP (1996a).
[b]Animals were administered diets with 5000 ppm; adjusted daily doses were provided in the study report.
[c]Number in parentheses is the percent of control.
[d]Mean ± standard error.
[e]Number of animals with lesions/number of animals.
**$p < 0.01$

Table B.2. Results in Male F344 Rats Exposed to Oral (in Feed) *o*-Toluidine for 26 Weeks or for 13 Weeks with a 13-Week Recovery Period[a]

Parameter	Adjusted Daily Dose (mg/kg-d)[b]		
	Control	26 Wk (285)	13 Wk with 13-Wk Recovery (304)
Body-weight (g)	382	314 (18%)[c]	342 (11%)
Food consumption (g/d)	14.7	13.4 (91%)	13.9 (95%)
Necropsy body weight (g)	389 ± 5[d]	316 ± 3 (81%)**	351 ± 9 (90%)**
Relative right kidney weight (mg/g)	3.55 ± 0.08	3.99 ± 0.09 (112%)**	3.56 ± 0.05 (100%)
Relative liver weight (mg/g)	37.09 ± 0.69	45.71 ± 0.88 (123%)**	39.58 ± 1.13 (107%)
Relative spleen weight (mg/g)	2.08 ± 0.05	9.01 ± 0.26 (433%)**	3.23 ± 0.16 (155%)**
Relative right testis weight (mg/g)	4.16 ± 0.06	4.84 ± 0.05 (116%)**	4.28 ± 0.12 (103%)
Hemosiderin pigmentation in the liver	0/10[e]	20/20 **	11/20**
Placental glutathione *S*-transferase-positive foci in the liver	17 ± 2	145 ± 61**	Not conducted
Kidney pigmentation	0/10	20/20**	20/20**
Transitional epithelium hyperplasia in the urinary bladder	0/10	17/20**	0/20
Congestion in the spleen	0/10	20/20**	20/20**
Hematopoietic cell proliferation in the spleen	3/10	20/20**	1/20
Hemosiderin pigmentation in the spleen	3/10	20/20**	18/20**
Thrombosis in the spleen	0/10	2/20	0/20
Fibrosis capsule in the spleen	0/10	20/20**	20/20**
Capsule, lymphatic, angiectasis in the spleen	0/10	6/20	15/20**

[a]NTP (1996b,c).
[b]Animals were administered diets with 5000 ppm; adjusted daily doses were provided in the study report.
[c]Number in parentheses is the percent of control.
[d]Mean ± standard error.
[e]Number of animals with lesions/number of animals.
**$p < 0.01$.

Table B.3. Incidence of Selected Noncancer Parameters in the F344 Rat Exposed to Oral (in Feed) *o*-Toluidine for 101 to 104 Weeks[a]

Parameter	Exposure Group (ppm) (Average Daily Dose, mg/kg-d)		
Males	**0**	**3000 (231)**	**6000 (525)**
Probability of survival[b]	90%	27%	0%
Liver Necrosis	2/20 (10%)[c]	12/50 (24%)	17/49 (35%)*
Bladder epithelial hyperplasia	0/20 (0%)	9/50 (18%)*	7/44 (16%)
Females	**0**	**3000 (264)**	**6000 (600)**
Probability of survival[b]	80%	55%	21%
Liver Necrosis	0/20 (0%)	1/50 (2%)	15/49 (31%)*
Bladder epithelial hyperplasia	0/20 (0%)	21/45 (47%)*	13/47 (28%)*

[a]NCI (1979a).
[b]Actual mortality was not provided in the study report; results were estimated for the study termination from Kaplan and Meier curves provided in the study report. There were significant positive dose-response trends in mortality.
[c]Number of animals with lesions/total number of animals (%).
*$p < 0.05$ Fischer's Exact Test performed for this PPRTV document.

Table B.4. Incidence of Tumors in the F344 Rat Exposed to Oral (in Feed) *o*-Toluidine for 101 to 104 Weeks[a]

Parameter	Exposure Dose (ppm) (Human Equivalent Dose, mg/kg-d)		
Males	**0**	**3000 (63.0)**	**6000 (138.8)**
Subcutaneous fibroma	0/20 (0%)[b]	28/50 (56%)**	27/49 (55%)**
Spleen sarcoma, NOS[c]	0/20	1/49 (2%)	3/42 (7%)
Mesotheliomas of the tunica vaginalis or multiple organs	0/20	17/50 (34%)**	9/49 (18%)*
Multiple organ osteosarcoma	0/20 (0%)	3/50 (6%)	5/49 (10%)
Multiple organ fibrosarcoma	0/20 (0%)	8/50 (16%)	20/49 (41%)**
Multiple organ sarcoma, NOS	0/20 (0%)	3/50 (6%)	11/49 (22%)*
Multiple organ sarcoma, NOS, fibrosarcoma, angiosarcoma, or osteosarcoma	0/20	15/50 (30%)**	37/49 (76%)**
Females	**0**	**3000 (63.7)**	**6000 (140.4)**
Mammary fibroadenoma	6/20 (30%)	20/50 (40%)	35/49 (71%)*
Bladder transitional-cell carcinoma	0/20 (0%)	9/45 (20%)*	22/47 (47%)**
Multiple organ osteosarcoma	0/20 (0%)	0/50 (0%)	18/49 (37%)**
Multiple organ sarcoma, NOS	0/20 (0%)	1/50 (2%)	2/49 (4%)
Multiple organ sarcoma, NOS, fibrosarcoma, angiosarcoma, or osteosarcoma	0/20	3/50 (6%)	21/49 (43%)**
Spleen sarcoma, NOS, angiosarcoma, or osteosarcoma	0/20	9/49 (18%)*	12/49 (24%)*
Spleen fibroma	0/20 (0%)	4/49 (8%)	6/49 (12%)
Spleen angiosarcoma	0/20 (0%)	7/49 (14%)	9/49 (18%)*
Spleen sarcoma, NOS	0/20 (0%)	1/49 (2%)	3/49 (6%)

[a]NCI (1979a).
[b]Number of animals with tumor/number of animals (%).
[c]NOS = Not otherwise stated.
*$p < 0.05$; **$p < 0.01$.

Table B.5. Incidence of Tumors in Male CD Rats Exposed to Oral (in Feed) *o*-Toluidine for 18 Months[a]

Parameter	Human Equivalent Dose (mg/kg-d)[b]			
	Concurrent Control	Pooled Control	72.1	144.1
Subcutaneous fibroma and fibrosarcoma	0/16 (0%)[c]	18/111 (16%)	18/23 (78%)[d]	21/24 (88%)[d]
Bladder tumors	0/16 (0%)	5/111 (5%)	3/23 (13%)	4/24 (17%)
Multiple tumors	3/16 (19%)	14/111 (13%)	6/23 (26%)	8/24 (33%)[e]

[a]Weisburger et al. (1978a).
[b]Animals were administered diets with 8000 or 16,000 ppm for 3 months followed by 4000 or 8,000 ppm for an additional 15 months, then placed on control diet for 6 months.
[c]Number of animals with tumor/number of animals (%).
[d]$p < 0.025$ denotes significant difference from all controls.
[e]$p < 0.025$ denotes significant difference from pooled controls only

Table B.6. Incidence of Tumors in the $B6C3F_1$ Mice Exposed to Oral (in Feed) *o*-Toluidine for 101 to 104 Weeks[a]

Parameter	Exposure Group (ppm) (Human Equivalent Dose, mg/kg-d)		
Males	**0**	**1000 (23.0)**	**3000 (73.8)**
Hepatocellular adenoma	1/20 (5%)[b]	3/50 (6%)	3/50 (6%)
Hepatocellular carcinoma	4/19 (21%)	16/50 (32%)	11/50 (22%)
Hepatocellular adenoma and carcinoma	5/19 (26%)	19/50 (38%)	14/50 (28%)
Hemangiosarcoma (all sites)	1/20 (5%)	1/50 (2%)	10/50 (20%)[c]
Females	**0**	**1000 (26.5)**	**3000 (91.9)**
Hepatocellular adenoma	0/20 (0%)	2/49 (4%)	6/50 (12%)
Hepatocellular carcinoma	0/20 (0%)	2/49 (4%)	7/50 (14%)[c]
Hepatocellular adenoma and carcinoma	0/20 (0%)	4/49 (8%)	13/50 (26%)**
Hemangiosarcoma (all sites)	1/20 (5%)	1/49 (2%)	2/50 (4%)

[a]NCI (1979b).
[b]Number of animals with tumor/number of animals (%).
[c]Significant ($p < 0.05$) trend but incidence not significantly different from the control.
**$p < 0.01$.

Table B.7. Incidence of Vascular Tumors in the CD-1 Mice Exposed to Oral (in Feed) *o*-Toluidine for 18 Months[a]

Parameter	Human Equivalent Dose (mg/kg-d)[b]			
Males	**Concurrent control**	**Pooled control**	**210.5**	**420.9**
Vascular tumors	0/14 (0%)[c]	5/99 (5%)	5/14 (36%)**	9/11 (82%)**
Females	**Concurrent control**	**Pooled control**	**211.8**	**423.7**
Vascular tumors	0/15 (0%)	9/102 (9%)	5/18 (28%)*	9/21 (43%)**
Males and females combined	**Concurrent control**	**Pooled control**	**211.2**	**422.3**
Vascular tumors	0/29 (0%)	14/201 (7%)	10/32 (31%)	18/32 (56%)

[a]Weisburger et al. (1978b).
[b]Animals were administered diets with 16,000 or 32,000 ppm for 3 months followed by 8000 or 16,000 ppm for an additional 15 months, then were on control diet for an additional 3 months.
[c]Number of animals with tumor/number of animals (%).
*$p < 0.05$; **$p < 0.025$.

APPENDIX C. BMD OUTPUTS

Table C.1. Multistage Cancer Model Predictions for Tumor Data for *o*-Toluidine

Tumor Type/Site	Gender/Species	Goodness-of-Fit *p*-Value[a]	AIC[b] for Fitted Model	$BMDL_{10}$[c] (mg/kg-d)	Slope Factor	Reference
Subcutaneous fibroma and fibrosarcoma	**Male rat**	**0.57**	**45.25**	**4.50**	**0.0222316**	**Weisburger et al. (1978a)**
Vascular tumor data	Female mouse	0.96	52.02	50.00	0.00199986	Weisburger et al. (1978b)
Vascular tumor data	Male mouse	0.54	31.99	23.00	0.00434693	Weisburger et al. (1978a)
Bladder tumors	Male rat	0.85	41.74	40.3	0.00248149	Weisburger et al. (1978a)
Multiple tumors	Male rat	0.64	71.32	34.25	0.00292001	Weisburger et al. (1978a)
Subcutaneous fibroma	Male rat	0.01	146.48	10.41	0.00960838	NCI (1979a)
Multiple organ sarcomas, NOS[d], fibrosarcoma, angiosarcoma, or osteosarcoma	Male rat	1.00	119.64	13.77	0.00726365	NCI (1979a)
Multiple organ sarcoma, NOS, fibrosarcoma, angiosarcoma, or osteosarcoma	Female rat	0.59	92.80	47.03	0.00212622	NCI (1979a)
Spleen sarcoma, NOS, angiosarcoma, or osteosarcoma	Female rat	0.55	100.40	32.71	0.00305746	NCI (1979a)
Multiple organ osteosarcoma	Male rat	0.95	57.09	73.66	0.00135766	NCI (1979a)
Multiple organ osteosarcoma	Female rat	0.09	74.80	61.92	0.00161487	NCI (1979a)
Multiple organ sarcoma, NOS	Male rat	0.62	77.90	45.27	0.00220876	NCI (1979a)
Multiple organ sarcoma, NOS	Female rat	1.00	28.52	137.23	0.000728707	NCI (1979a)
Multiple organ fibrosarcoma	Male rat	0.76	112.81	22.89	0.00436794	NCI (1979a)
Spleen fibromas	Male rat	0.01	73.52	Failed	failed	NCI (1979a)
Spleen fibromas	Female rat	0.85	66.45	61.62	0.00162297	NCI (1979a)
Spleen angiosarcomas	Female rat	0.56	86.85	42.73	0.00234007	NCI (1979a)
Spleen sarcomas	Female rat	0.76	25.71	132.25	0.000756154	NCI (1979a)

Table C.1. Multistage Cancer Model Predictions for Tumor Data for *o*-Toluidine

Tumor Type/Site	Gender/Species	Goodness-of-Fit *p*-Value[a]	AIC[b] for Fitted Model	$BMDL_{10}$[c] (mg/kg-d)	Slope Factor	Reference
Spleen sarcoma, NOS	Male rat	0.81	24.93	122.54	0.000816071	NCI (1979a)
Mammary fibroadenoma	Female rat	0.86	154.40	14.99	0.00667223	NCI (1979a)
Rat bladder transitional-cell carcinoma	Female rat	0.82	112.41	19.10	0.0052354	NCI (1979a)
Hepatocellular carcinoma	Female mouse	1.00	59.21	39.22	0.00254972	NCI (1979b)
Hemangio-sarcomas, all sites	Male mouse	0.26	72.92	38.35	0.00260727	NCI (1979b)
Hemangio-sarcomas, all sites	Female mouse	0.21	31.41	100.95	0.000990631	NCI (1979b)
Hepatocellular adenoma and carcinoma	Female mouse	1.00	87.02	22.20	0.00450356	NCI (1979b)
Hepatocellular adenoma	Female mouse	0.99	55.43	43.29	0.00231009	NCI (1979b)

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria.
[b]AIC = Akaike's Information Criteria.
[c]$BMDL_{10}$ = lower confidence limit (95%) on the benchmark dose at 10% incidence, extra risk.
[d]NOS = not otherwise stated.

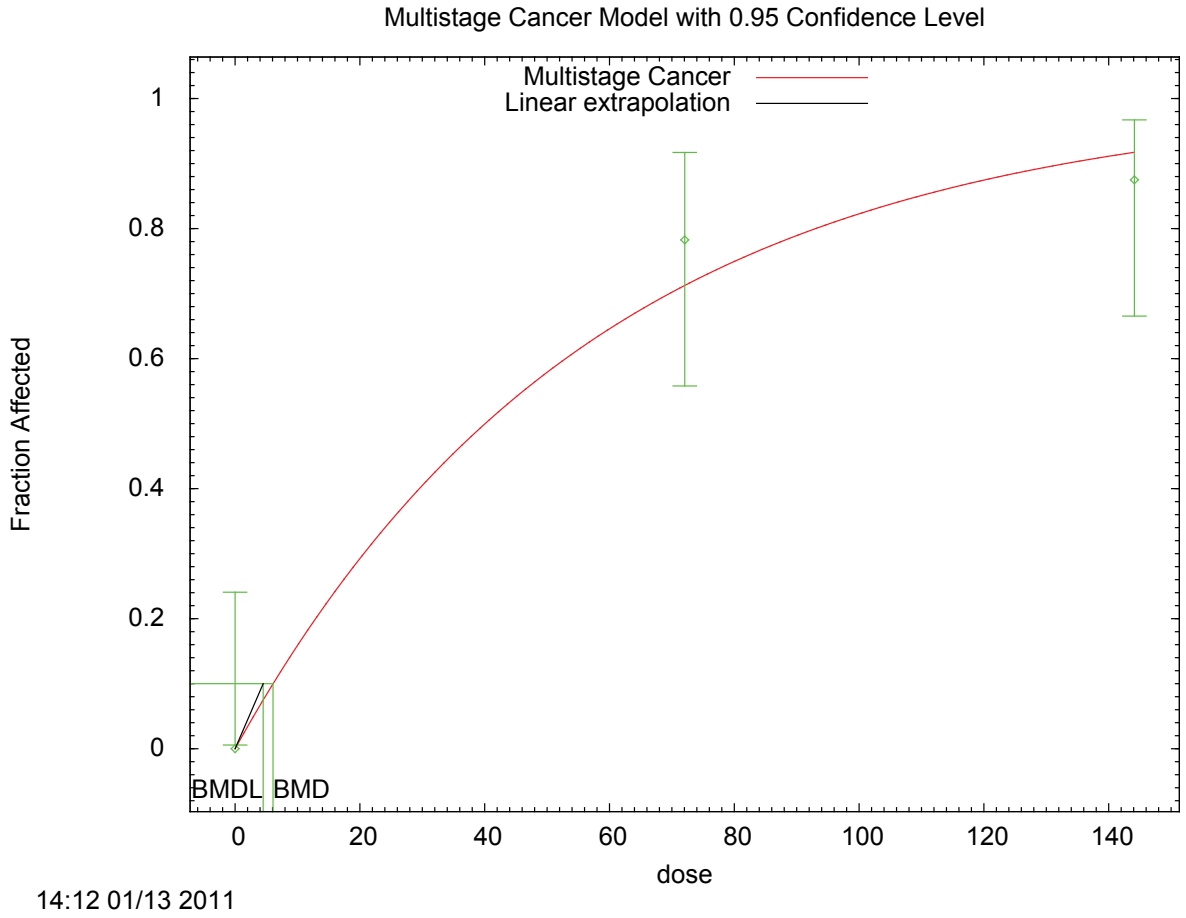

Figure C.1.° Multistage Cancer BMD Model for Subcutaneous Fibroma and Fibrosarcoma in Male F344 Rats Data (Weisburger et al., 1978)

Text Output for 1° Multistage cancer BMD Model for Subcutaneous Fibroma and Fibrosarcoma in Male F344 Rats Data (Weisburger et al., 1978)

```
=============================================================================
        Multistage Cancer Model. (Version: 1.9;  Date: 05/26/2010)
        Input Data File:
C:/18/Jan2011/Weisburger_1978_fibroma_fibrosarcoma_rat_m_MultiCanc1_1.(d)
        Gnuplot Plotting File:
C:/18/Jan2011/Weisburger_1978_fibroma_fibrosarcoma_rat_m_MultiCanc1_1.plt
                                                Thu Jan 13 14:12:20 2011
 =============================================================================

 Male_rat_subcutaneous_fibroma_and_fibrosarcoma
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   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 = DichPerc
  Independent variable = Dose

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


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



                  Default Initial Parameter Values
                     Background =     0.149654
                        Beta(1) =     0.014428


           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)        0.0173338            *                *                  *

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -21.085         3
   Fitted model        -21.6251         1       1.08023      2          0.5827
  Reduced model        -41.8653         1       41.5606      2         <.0001

           AIC:        45.2502


                                Goodness  of  Fit
                                                                      Scaled
     Dose     Est._Prob.    Expected    Observed     Size          Residual
  ----------------------------------------------------------------------
    0.0000     0.0000         0.000     0.000          16          0.000
   72.0626     0.7132        16.405    18.000          23          0.736
```

```
  144.1251     0.9178        22.026    21.000          24       -0.763

 Chi^2 = 1.12      d.f. = 2        P-value = 0.5704


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        6.07834

            BMDL =        4.49811

            BMDU =        8.32855

Taken together, (4.49811, 8.32855) is a 90     % two-sided confidence
interval for the BMD

Multistage Cancer Slope Factor =     0.0222316
```

APPENDIX D. REFERENCES

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