United States Environmental Protection Agency

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Provisional Peer-Reviewed Toxicity Values for

Toluene-2,5-diamine
(CASRN 95-70-5)
and Compounds

Toluene-2,5-diamine sulfate (6369-59-1)
[also known as 1,4-Benzenediamine-2-methyl sulfate or
2-Methylbenzene-1,4-diamine sulfate (615-50-9)],
Toluene-2,5-diamine dihydrochloride (615-45-2), and
Toluene-2,5-diamine monohydrochloride (74612-12-7)

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COMMONLY USED ABBREVIATIONS

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

PROVISIONAL PEER-REVIEWED TOXICITY VALUES FOR TOLUENE-2,5-DIAMINE (CASRN 95-70-5) AND COMPOUNDS; TOLUENE-2,5-DIAMINE SULFATE (6369-59-1) [ALSO KNOWN AS 1,4-BENZENEDIAMINE-2-METHYL SULFATE or 2-METHYLBENZENE-1,4-DIAMINE SULFATE(615-50-9)], TOLUENE-2,5-DIAMINE DIHYDROCHLORIDE (615-45-2), AND TOLUENE-2,5-DIAMINE MONOHYDROCHLORIDE (74612-12-7)

BACKGROUND

HISTORY

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

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

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

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

DISCLAIMERS

Users of this document should first check to see if any IRIS values exist for the chemical of concern before proceeding to use a PPRTV. If no IRIS value is available, staff in the regional Superfund and Resource Conservation and Recovery Act (RCRA) program offices are advised to

carefully review the information provided in this document to ensure that the PPRTVs used are appropriate for the types of exposures and circumstances at the Superfund site or RCRA facility in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived provisional values. PPRTVs are developed by the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical Support Center for OSRTI. Other EPA programs or external parties who may choose of their own initiative to use these PPRTVs are advised that Superfund resources will not generally be used to respond to challenges of PPRTVs used in a context outside of the Superfund Program.

QUESTIONS REGARDING PPRTVS

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

INTRODUCTION

Toxicity values for toluene-2,5-diamine (2,5-diaminotoluene) (see Figure 1 for structure of toluene-2,5-diamine) or toluene-2,5-diamine compounds (toluene-2,5-diamine sulfate, toluene-2,5-diamine dihydrochloride, and 2-toluene-2,5-diamine monohydrochloride) are not available on IRIS (U.S. EPA, 2009) or the Drinking Water Standards and Health Advisories list (U.S. EPA, 2006). The HEAST (U.S. EPA, 1997) reported subchronic and chronic oral RfDs for toluene-2,5-diamine of 0.6 mg/kg-day, based on a NOAEL of 56 mg/kg-day in rats fed diets containing toluene-2,5-diamine sulfate for 78 weeks (NCI, 1978) and an uncertainty factor (UF) of 100. The HEAST cites a Health and Environmental Effects Profile (HEEP) for selected toluenediamines (U.S. EPA, 1984) as the source for the RfD values. The HEEP (U.S. EPA, 1984) reported a NOAEL of 2,000 ppm toluene-2,5-diamine sulfate from the National Cancer Institute (NCI) study and converted this to an equivalent dose of 56 mg/kg-day toluene-2,5-diamine based on estimated food consumption in rats and molecular weight adjustment. Other than the HEEP (U.S. EPA, 1984), the Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1994, 1991) did not include any relevant documents. ATSDR (2009) and the World Health Organization (WHO, 2009) have not reviewed the toxicity of toluene-2,5-diamine. CalEPA (2009a,b) has not derived toxicity values for exposure to toluene-2,5-diamine. The American Conference of Governmental Industrial Hygienists (ACGIH, 2009), the National Institute of Occupational Safety and Health (NIOSH, 2009), and the Occupational Safety and Health Administration (OSHA, 2009) have not established exposure limits for toluene-2,5-diamine.

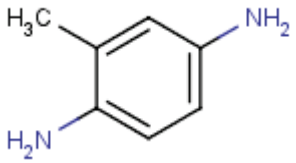

Figure 1. Chemical Structure of Toluene-2,5-Diamine

The HEEP (U.S. EPA, 1984) did not include a cancer assessment for toluene-2,5-diamine due to the absence of the evidence of carcinogenic effects. NCI (1978) concluded that sufficient evidence was not obtained to demonstrate the carcinogenicity of toluene-2,5-diamine sulfate in their study. National Toxicology Program (NTP, 2005) did not include the chemical in the *11th Report on Carcinogens*. The International Agency for Research on Cancer (IARC, 2009) classified toluene-2,5-diamine as Group 3 (*Not Classifiable as to Human Carcinogenicity*) based on inadequate evidence in animals and no data on carcinogenicity in humans.

Literature searches were conducted from the 1960s through May 2010 for studies relevant to the derivation of provisional toxicity values for toluene-2,5-diamine and compounds. Databases searched include MEDLINE, TOXLINE (with NTIS), BIOSIS, TSCATS/TSCATS2, CCRIS, DART, GENETOX, HSDB, RTECS, Chemical Abstracts, and Current Contents (last 6 months). Assessments by the Scientific Committee on Consumer Products of the European Commission (SCCP; 2007) and Pang (1992) were reviewed as well.

REVIEW OF PERTINENT DATA

HUMAN STUDIES

No data were located regarding the effects of toluene-2,5-diamine and compounds in humans following oral or inhalation exposure.

ANIMAL STUDIES

Oral Exposure

Subchronic Studies—NCI (1978) administered toluene-2,5-diamine sulfate (>99% pure) to groups of five female and five male Fischer F344 rats and C57BL/6 mice at reported concentrations of 0, 0.02, 0.05, 0.08, or 0.11% (0, 15.8, 39.5, 63.2, or 79.0 mg-kg/day) in the diet for 28 days in a briefly summarized range-finding study. No mortality occurred. Mean body-weight depression relative to controls was reported in all rats and in female mice but did not appear to correlate with dose (data not shown). No other endpoints were evaluated. The limited scope of this study precludes use of these data for toxicity assessment.

Hill (1997, as cited in SCCP, 2007) administered toluene-2,5-diamine sulfate (99.7% pure) via gavage in deionized water to Sprague-Dawley rats (15/sex/dose) at 0, 2.5, 5, 10, or 20 mg/kg-day for 13 weeks. The original report for this study is not available; SCCP briefly described the study. Animals were observed daily for mortality and clinical signs. Body weights and food intake were recorded weekly. Ophthalmoscopic examinations were performed on all animals before the initiation of treatment and during Week 13. Blood and urine samples

were collected during Week 4 and during Week 12 or 13. Following treatment, all animals were sacrificed and necropsied. Organ weights were recorded, and tissues were subjected to microscopic examination. No dose-related changes in mortality, clinical signs, body weights, body-weight gains, or food consumption were reported (data not shown). The researchers did not consider hematological variations (not further described) to be treatment-related. Aspartate aminotransferase (AST) levels were statistically significantly ($p < 0.05$) increased in females at doses of ≥5 mg/kg-day (data not shown). Increased urine levels, associated with a statistically ($p < 0.05$) significant decrease in specific gravity, were observed at ≥10 mg/kg-day (females) or 20 mg/kg-day (males) (data not shown). Although retinopathy was observed in some animals, a pathology peer review concluded that the incidence of these effects in the treatment groups was similar to the spontaneous incidence for Sprague-Dawley rats. At 20 mg/kg-day, an increased incidence of abnormally shaped pituitary glands was reported. The SCCP (2007) identified a NOAEL of 2.5 mg/kg-day for toluene-2,5-diamine sulfate in this study based on significantly elevated AST levels at 5 mg/kg-day. However, experimental data were not presented in the summary, and the adversity of the reported effects has not been demonstrated (there was no mention of the magnitude or dose response of the observed change in AST, or corresponding changes in other serum enzymes or liver pathology). The available description of this study lacked information to support independent evaluation of the study.

Chronic Studies—NCI (1978) administered toluene 2,5-diamine sulfate (>99% purity) to Fischer 344 rats (50/sex/dose) at time-weighted average concentrations of 0.06 or 0.2% in the diet for 78 weeks. Average daily doses of 47 or 158 mg/kg-day for males and 55 or 183 mg/kg-day for females were estimated for this review[1]. Separate control groups were included for each dose group because the two dose groups were not run simultaneously. Control groups consisted of 25 rats/sex for the high-dose group and 50 rats/sex for the low-dose group. Control groups were started the same week as the corresponding dosed groups. Survival and clinical signs were monitored twice daily; animals were examined monthly for the presence of lesions or tissue masses. Body weights were measured at the start of the experiment, twice weekly for the first 12 weeks, and monthly thereafter. Food consumption was measured using 10 rats/dose on 7 consecutive days/month for the first 9 months, and on 3 consecutive days/month for the rest of the treatment period. No hematology or clinical chemistry endpoints were evaluated. Other than 10 rats/sex sacrificed from the low-dose control group at Week 29 and 5 rats/sex sacrificed from the low-dose and low- and high-dose control groups at Week 78, remaining animals were observed up to 109 weeks before sacrifice. At necropsy, all animals were examined for grossly visible lesions. Comprehensive histological analyses (of 33 tissues) were performed.

Survival was similar in control and treated rats, and no clinical findings were attributed to exposure (NCI, 1978). Body weights of treated rats remained within 10% of their respective control groups except for high-dose female rats, which exhibited a mean body-weight depression of >10% with respect to high-dose female control rats (based on graphical presentation of the data; statistical analyses were not performed). However, mean body weights of high-dose female controls were consistently higher than body weights of low-dose female controls throughout the treatment and observation period; mean body weights of high-dose females remained within 10% of low-dose controls. Feed consumption in treated groups was similar to controls (data not shown). No treatment-related changes in gross or microscopic pathology

[1]Based on chronic reference values for food consumption and body weight in F344 rats (U.S. EPA, 1988).

related to noncancer effects were observed. Limited histopathological findings were considered by the researchers to be age-related and did not correlate with dose. The design of the study is of limited value as an assay for noncancer effects. Animals were examined for pathology only at spontaneous death or 28–31 weeks beyond the 78-week treatment period, allowing time for reversible effects to heal and age-related effects to mask treatment-related effects. Further, high-dose rats and their controls were received in separate shipments, and treatments for low- and high-dose rats were initiated at different times (11 months apart). Despite these limitations, the high dose of 158 mg/kg-day is identified as a NOAEL for chronic toxicity in rats in this study.

In a companion mouse study, NCI (1978) administered toluene-2,5-diamine sulfate (>99% pure) to B6C3F1 mice (50/sex/dose) at time-weighted average concentrations of 0.06 or 0.1% in the diet for 78 weeks. Average daily doses of 103 or 172 mg/kg-day for males and 104 or 173 mg/kg-day for females were estimated[2]. As for rats, separate low- and high-dose control groups (50/sex/dose) were used because the dose groups were started at different times (6 months apart). While low-dose controls were started 2 weeks after the low-dose group, high-dose controls were started 2 months before the high-dose group. Five mice/sex were sacrificed from the high-dose and each control group at Week 78; remaining mice were observed up to 107 weeks preceding sacrifice. The same toxicological parameters that were assessed in rats were also assessed in mice. Comprehensive histological analyses (of 34 tissues) were performed.

Survival rates were not different for treated mice and control mice, and no clinical abnormalities were reported at any dose (NCI, 1978). Body weights of treated mice remained within 10% of their respective control groups except for high-dose female mice, which exhibited a mean body-weight depression of >10% with respect to high-dose female control mice (based on graphical presentation of the data; statistical analyses were not performed). However, mean body weights of high-dose females remained within 10% of low-dose controls throughout most of the treatment period, and the researchers noted that the growth pattern of high-dose female controls was unusual. Feed consumption in treated groups was similar to controls (data not shown). No dose-related increases in the incidence of nonneoplastic lesions were observed. The design of the study is of limited value as an assay for noncancer effects. Animals were examined for pathology only at spontaneous death or 16–19 weeks beyond the 78-week treatment period, allowing time for reversible effects to heal and age-related effects to mask treatment-related effects. Further, dosed mice were received in separate shipments from their respective controls, and treatments were initiated at different times (6 months apart). Despite these limitations, the high dose of 172 mg/kg-day is identified as a NOAEL for chronic toxicity in mice in this study.

All rats and mice were examined for the presence of neoplastic lesions. In rats, the only significant finding was an increase in the incidence of interstitial-cell tumors in the testis of dosed males compared to their respective control groups (see Table 1; $p = 0.039$ and 0.014 using the Fischer's exact test for low- and high-dose groups, respectively). This result was not considered significant by the researchers because the spontaneous incidence of these tumors in F344 rats is traditionally high and variable; regardless, statistical significance compared to controls was achieved for both dose groups. In mice, a significant ($p < 0.05$) increase in the

[2]Based on chronic reference values for food consumption and body weight in $B6C3F_1$ rats (U.S. EPA, 1988).

combined incidence of alveolar/bronchiolar adenomas and carcinomas in high-dose female mice was reported (see Table 2; $p = 0.016$ using Fischer's exact test). However, in the absence of other significant findings, the researchers did not consider this sufficient evidence of compound-related carcinogenicity because high-dose mice were received and housed separately from high-dose control mice. Further, an external review of both studies by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens concluded that deficiencies in the study design warranted further investigation into the carcinogenic potential of toluene-2,5-diamine (NCI, 1978).

Table 1. Incidence of Interstitial-Cell Tumors in the Testis of Male F344 Rats Exposed to Toluene-2,5-Diamine Sulfate for 78 Weeks

Dose (mg/kg-day)	Incidence of Interstitial-Cell Testicular Tumors
0 (low-dose control)	33/45
47	43/48[a]
0 (high-dose control)	19/24
158	47/48[b]

[a]$p = 0.039$ with respect to low-dose control using Fischer's exact test
[b]$p = 0.014$ with respect to high-dose control using Fischer's exact test

Source: NCI (1978)

Table 2. Incidence of Alveolar/Bronchiolar Adenomas and Carcinomas in Female B6C3F1 Mice Exposed to Toluene-2,5-Diamine Sulfate for 78 Weeks

Dose (mg/kg-day)	Incidence of Alveolar/Bronchiolar Adenomas and Carcinomas
0 (low-dose control)	4/46
104	6/42
0 (high-dose control)	1/45
173	8/45[a]

[a]$p = 0.016$ with respect to high-dose control using Fischer's exact test

Source: NCI (1978)

Reproductive/developmental Studies—In an unpublished two-generation reproductive toxicity study available only as a brief description, Bornatowicz (1986, as cited in SCCP, 2007) administered toluene-2,5-diamine sulfate (98.2% pure) via gavage in distilled water to Sprague-Dawley rats (24/sex/dose) at 0, 5, 15, or 45 mg/kg-day for 70 days prior to mating (males) or 14 days prior to mating and throughout mating and lactation (females). The F1 generation was dosed starting at birth for approximately 80 days; the F2 generation was maintained until weaned. Endpoints evaluated included mortality, clinical signs, body-weight gain, food consumption, and reproductive parameters (female sexual cycle, mating,

insemination, gravidity, birth and litter data, postnatal weights, and physiological development). Histopathological analyses were performed for organs with visible abnormalities, for parents with no surviving offspring, and for all parents of the control and high-dose groups. The reproductive organs (including the pituitary gland, mamma, vulva, vagina, cervix, uterus, tubes, ovaries, penis, testes, epididymides, ducti referentes, coagulation gland, prostate gland, and vesicular gland) were examined microscopically. Four mortalities were reported (1 P- and 3 F1-generation, dose groups not specified), all attributed to gavage error. No treatment-related changes in clinical signs, body-weight gain, food consumption, male or female fertility, or pup survival and growth (both generations) were observed (data not shown). Histopathological findings were not reported. SCCP (2007) identified the high dose of 45 mg/kg-day as a NOAEL. The available description of this study is inadequate to support independent evaluation of the study.

In another unpublished study, Osterburg, (1982a, as cited in SCCP, 2007) administered toluene-2,5-diamine sulfate (purity not specified) via gavage in distilled water to groups of 23 pregnant Sprague-Dawley rats at 0, 10, 50, or 80 mg/kg-day on Gestation Days (GDs) 6–15. Rats administered 15 mg/kg-day vitamin A served as positive controls. Animals were observed daily for mortality and clinical signs. Body weights were recorded on GDs 0, 6, 15, and 19. Rats were sacrificed on GD 19. Half of the fetuses from each litter were processed and examined for skeletal abnormalities, while the other half were processed and examined for visceral abnormalities. Two mortalities (one rat dosed at 10 mg/kg-day and one rat dosed 80 mg/kg-day) were reported but were likely due to gavage error. No clinical signs of toxicity were observed. Maternal body weights were slightly reduced in rats administered 50 mg/kg-day and significantly ($p < 0.05$) reduced in rats administered 80 mg/kg-day during the treatment period (data not shown). Resorption was also significantly ($p < 0.05$) increased relative to controls at 80 mg/kg-day (data not shown). Exposure to toluene-2,5-diamine sulfate did not reportedly affect the number of fetuses, sex distribution of the fetuses, or fetal weights. No visceral or skeletal malformations were reported. Based on this study, SCCP (2007) identified a NOAEL of 50 mg/kg-day for maternal toxicity and a NOAEL of 80 mg/kg-day for developmental toxicity (despite the increased resorptions at this dose level) in rats. The available description of this study is inadequate to support independent evaluation of the study.

In a companion unpublished study, Osterburg (1982b, as cited in SCCP, 2007) administered toluene-2,5-diamine sulfate (purity not specified) via gavage in distilled water to groups of 16 pregnant New Zealand white rabbits at 0, 10, 25, or 50 mg/kg-day on GDs 6–18. Rabbits administered 6 mg/kg-day vitamin A served as positive controls. Animals were examined daily for mortality and clinical signs. Body weights were recorded on GDs 0, 6, 18, and 28. On Day 28, rabbits were sacrificed; fetuses were processed and examined for congenital abnormalities and gross macroscopic changes. Half of the fetuses from each litter were processed and examined for skeletal abnormalities, while the other half were processed and examined for visceral abnormalities. Five mortalities (one rabbit dosed at 10 mg/kg-day, one rabbit dosed at 25 mg/kg-day, and three rabbits dosed at 50 mg/kg-day) were reported but were due to gavage error by SCCP (2007). No clinical signs of toxicity were observed. Body weights of treated rabbits did not differ significantly from controls, and incidences of intrauterine deaths did not correlate with dose (data not shown). Exposure to toluene-2,5-diamine did not affect the number or sex of fetuses or fetal weights (data not shown). No dose-related visceral or skeletal abnormalities were apparent. Based on this study, SCCP (2007) identified a NOAEL of

50 mg/kg-day for developmental toxicity in rabbits. The available description of this study is inadequate to support independent evaluation of the study.

In a screening level study, Seidenberg et al. (1986) administered toluene-2,5-diamine sulfate (purity not specified) via gavage in corn oil to 30 pregnant ICR/SIM mice at 160 mg/kg-day on GDs 8–12. Twenty-nine mice served as vehicle-only controls. Maternal body weights were recorded on GDs 7 and 13 and Day 1 postpartum. Mice were allowed to deliver; neonates were examined, counted, and weighed at birth and at age 3 days. Stillborns were recovered and examined for gross external abnormalities. Dams that failed to deliver by GD 21 or 22 were sacrificed and necropsied; uterine contents were examined. There were two maternal deaths in the treated group, which the researchers attributed to toluene-2,5-diamine sulfate exposure (see Table 3). There were no effects on maternal weight gain, number of litters born, or number of litters resorbed. The average number of dead neonates per litter on Day 1 was significantly increased versus controls (see Table 3; $p < 0.05$ using Fischer's exact test); but, survival of neonates from Day 1 to Day 3 was not affected, and birth weight and growth of neonates did not differ from controls. No external abnormalities were reported for dead neonates. Based on the increase in dead neonates on Day 1, the researchers categorized toluene-2,5-diamine sulfate as an embryotoxin. A LOAEL of 160 mg/kg-day is identified for both maternal toxicity and developmental effects in mice.

Table 3. Significant Maternal and Developmental Effects from Exposure to Toluene-2,5-Diamine Sulfate on GDs 8–12

Parameter	Dose (mg/kg-day)	
	0	**160**
Maternal deaths	0/29	2/30
Average number of live neonates/litter on Day 1	12.2 $\pm$ 4.5	11.4 $\pm$ 3.2[a]
Average number of dead neonates/litter on Day 1	0	0.43 $\pm$ 0.8[b]

[a]Mean ± standard deviation
[b]$p < 0.05$ with respect to control using Fischer's exact test

Source: Seidenberg et al. (1986)

A similar screening study performed at a lower dose level found no maternal or developmental effects. Kavlock et al. (1987) administered toluene-2,5-diamine sulfate (purity not reported) via gavage to thirty pregnant CD-1 mice at 80 mg/kg-day on GDs 8–12. Forty mice served as vehicle-only controls. Endpoints evaluated included maternal weight gain; percentages of maternal deaths, pregnancies, and resorptions; pup survival; and pup body weights (expressed as mean values per litter at birth and age 3 days). The data indicate that exposure to toluene-2,5-diamine sulfate did not elicit maternal toxicity, and no significant effects on pup survival or growth were observed. A NOAEL of 80 mg/kg-day is identified for maternal and developmental toxicity in mice.

Inhalation Exposure

Pertinent data regarding the inhalation toxicity of toluene-2,5-diamine and compounds were not located in the available literature.

OTHER STUDIES

Acute or Short-term Studies

In an unpublished range-finding study, Hill (1994, as cited in SCCP, 2007) administered toluene-2,5-diamine sulfate via gavage in distilled water to Sprague-Dawley rats (10/sex/dose) at 0, 7.5, 15, 30, or 60 mg/kg-day for 14 days. Animals were monitored twice daily for mortality and clinical signs. Body weights and food intake were recorded weekly. Blood samples were collected at the end of the treatment period to evaluate hematology and clinical chemistry endpoints. Animals were sacrificed and necropsied; organ weights were recorded, and tissues (unspecified) were examined microscopically. No treatment-related changes in mortality, clinical signs, body weights, food intake, or hematological parameters were reported (data not shown). Clinical chemistry endpoints (including AST, creatinine phosphokinase [CPK], lactate dehydrogenase [LDH], and alanine aminotransferase [ALT; at 60 mg/kg-day only] levels) were altered in rats dosed at ≥30 mg/kg-day (data not shown). Mean absolute and relative liver weights were increased in males at ≥30 mg/kg-day, and in females at 60 mg/kg-day (data not shown). No macroscopic abnormalities were reported; however, myocyte degeneration was noted in the heart, skeletal muscle, tongue, and diaphragm of all dosed rats.

Other Routes

Marks et al. (1981) administered toluene-2,5-diamine sulfate (purity not specified) via subcutaneous injection in sterilized distilled water to groups of pregnant albino CD-1 mice (ranging from 11 to 31/dose) at 0, 16, 32, 48, or 64 mg/kg-day on GDs 6–15. The number of pregnant dams and weight gain during pregnancy (Days 6–17) were recorded. Dams were sacrificed on GD 18; uterine contents were examined. The number of implants and resorptions was noted. Live fetuses were counted, sexed, weighed individually, and examined for external malformations. Stunted fetuses, fetuses with external malformations, and at least one-third of the fetuses from each litter were examined for visceral abnormalities. All processed fetuses were subjected to skeletal examinations.

Maternal mortality was 13% (incidence 4/31) and 82% (incidence 9/11) in the 48 and 64 mg/kg-day groups, respectively (Marks et al., 1981). No maternal mortality occurred in the control group. Although average weight gain during pregnancy was not significantly reduced at any dose, a significant trend for weight gain reduction with increasing dose was reported (Jonckheere's test; $p < 0.05$). No dose-related effects were reported for the average number of implants per pregnant dam, percent resorptions or fetal deaths per total number of implants, number of stunted fetuses, or average number of live fetuses per dam; however, a significant decline in average fetal weights per litter was observed at doses ≥32 mg/kg-day (two-sided Mann-Whitney U test; $p < 0.05$). The average percentage of malformed fetuses was similar for control and treated rats.

In another developmental study, Inouye and Murakami (1977) administered toluene-2,5-diamine dihydrochloride (99.9% pure) via subcutaneous injection in distilled water to groups of pregnant JCL:ddN mice (10–11/group) at 50 mg/kg-day on 1 day during GDs 7–14. An untreated group of 13 females served as controls. All mice were sacrificed on GD 18; uteri were examined for resorptions. Live fetuses were counted, weighed, and examined for external

and skeletal malformations. No mortality was reported. Exposure to toluene-2,5-diamine dihydrochloride did not significantly affect the total number of implantations or live fetuses, the percentage of dead fetuses or resorptions per total number of implantations, or mean fetal weights (statistical analyses not described). A high incidence of malformations was found in fetuses of dams treated on Day 8 of pregnancy (overall incidence of 18% compared to 0% for controls, litter incidence not reported); few fetuses with malformations were found in groups treated on Days 7 or 9. Of the 20 malformed fetuses of dams treated on GD 8, 5 had craniofacial malformations (namely exencephaly or prosoposchisis) and 15 had skeletal malformations (typically fused or distorted thoracic vertebrae associated with fused or absent ribs).

Based on these results, and as an extension of this experiment, Inouye and Murakami, (1977) administered toluene-2,5-diamine dihydrochloride (99.9% pure) to two additional groups of JCL:ddN mice on Day 8 of pregnancy. One group (n = 12) was treated via intraperitoneal injection (i.p.) at 50 mg/kg-day; a second group (n = 10) was treated subcutaneously (s.c.) at 75 mg/kg-day. No additional control group was used. The same toxicological parameters were evaluated. Exposure to toluene-2,5-diamine dihydrochloride at 50 i.p. and 75 mg/kg-day s.c. caused 33 and 60% maternal mortality, respectively. The percentage of dead fetuses or resorptions per total number of implantations (51% for dams treated at 50 mg/kg-day i.p. and 41% for dams treated at 75 mg/kg-day s.c.) was significantly increased in both treatment groups relative to controls (statistical analyses not described). Mean fetal weights were similar for treated and control animals. A high incidence of malformations was found in fetuses of dams treated at both 50 mg/kg-day i.p. (45%) and 75 mg/kg-day s.c. (35%); most malformed fetuses had skeletal (i.e., vertebral and rib) anomalies, and few had craniofacial defects.

Additional reproductive/developmental (Burnett and Goldenthal, 1988; Burnett et al., 1976) and chronic studies (Burnett and Goldenthal 1988; Giles et al., 1976; Burnett et al., 1976, 1975; Kinkel and Holzman, 1973) that examined effects associated with dermal exposure to mixtures containing toluene-2,5-diamine sulfate were not evaluated for this review.

Genotoxicity

A number of studies on the genotoxicity of toluene-2,5-diamine have been published. In the presence of metabolic activation, toluene-2,5-diamine induced mutations in *Salmonella typhimurium* strains TA98, TA100, and TA1538 (Chung et al., 1995; Ames et al., 1975) and tested positive in the *Salmonella* umu (SOS response) assay (Yasunaga et al., 2006). Toluene-2,5-diamine induced rapid lysis mutants in bacteriophage *T4D* (Kvelland, 1985) and chromosomal aberrations in Chinese hamster ovary (CHO) cells in the absence of metabolic activation (Chung et al., 1995) and tested positive in DNA repair assays in rat and hamster hepatocytes (Kornbrust and Barfknecht, 1984). Toluene-2,5-diamine tested positive for the ability to both enhance transformation of primary hamster embryo cells by simian adenovirus and to transform secondary hamster embryo cells (Greene and Friedman, 1980). In vivo, toluene-2,5-diamine was not mutagenic in a recessive spot mutation assay in mice (Soares and Lock, 1980) and did not increase the incidence of dominant lethal effects in rats (Burnett et al., 1977). Oral exposure to toluene-2,5-diamine sulfate did not induce micronuclei in rats (Hossack and Richardson, 1977) and did not cause DNA damage to mouse tissues in two assays (Sekihashi et al., 2002; Sasaki et al., 1999). Rats administered toluene-2,5-diamine sulfate via the oral route tested positive for DNA damage in the stomach but not other tissues (Sekihashi et al., 2002). When administered via i.p., toluene-2,5-diamine sulfate tested positive for the inhibition of testicular DNA synthesis in mice (Greene et al., 1981).

Additional genotoxicity studies, reported as summaries by SCCP (2007), are not available in the open literature. Table 4 summarizes the results from these studies, as reported by SCCP (2007). The results from these studies are generally consistent with the published studies described above in that toluene-2,5-diamine compounds tested positive for bacterial mutagenicity (with activation) and chromosomal aberrations in mammalian cells in vitro but negative in mouse spot test and micronucleus assays in vivo.

Table 4. Summary of Unpublished Genotoxicity Data[a]

Study Type	Test System	Test Substance	Result	Reference[b]
In vitro assays				
Bacterial reverse mutation test	*S. typhimurium*	Toluene-2,5-diamine sulfate	Positive in the presence of metabolic activation	Sokolowski, 2003
Mammalian gene mutation assay (*tk* locus)	L5178Y mouse lymphoma cells	Toluene-2,5-diamine sulfate	Negative	Wollny, 1995
Chromosome aberration test	Chinese hamster V79 cells	Toluene-2,5-diamine sulfate	Positive in the presence or absence of metabolic activation	Schulz, 2002
In vivo assays				
Mouse bone marrow micronucleus test	Crl:NMRI BR mice	Toluene-2,5-diamine sulfate	Negative	Bornatowicz, 1995
Mouse bone marrow micronucleus test	NMRI mice	Toluene-2,5-diamine sulfate	Negative	Völkner, 1995
Unscheduled DNA synthesis in mammalian liver cells	Sprague-Dawley rats	Toluene-2,5-diamine sulfate	Negative	Cinelli, 2004; Getuli, 2002
Mouse spot test	Male T stock and female C57BL/6 mice	Toluene-2,5-diamine dihydrochloride	Negative	Matheson, 1978

[a]All studies conducted in compliance with OECD guidelines
[b]As cited in SCCP, 2007

Source: SCCP, 2007

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RFD VALUES FOR TOLUENE-2,5-DIAMINE AND COMPOUNDS

Table 5 summarizes the database for oral toxicity of toluene-2,5-diamine and compounds which includes short-term, subchronic, chronic, and reproductive and developmental studies. Despite the number of studies that have been conducted, the database in support of an RfD derivation for toluene-2,5-diamine is weak, and all NOAEL and LOAEL values in this table are confounded by limitations of study design, or are available only as brief descriptions in SCCP (2007). Insufficient information is available to support independent evaluations of the studies.

Chronic toxicity studies in rats and mice identified NOAELs of 158 and 172 mg/kg-day, respectively (NCI, 1978). However, these studies were of limited value for deriving an RfD because animals were sacrificed and examined for nonneoplastic lesions only after a lengthy recovery period. Further, animals from different groups within the same study were received in separate shipments and were started on treatment at different times. As described in NCI (1978), an external reviewer of the studies recognized these deficiencies in study design. Further, use of the NOAELs identified from these studies may not be appropriate for derivation of an RfD given that results from subchronic and reproductive and developmental toxicity studies suggest that effects may occur at doses lower than these NOAELs.

The one available subchronic study identified an apparent NOAEL of 2.5 mg/kg-day and a LOAEL of 5 mg/kg-day based on increased AST levels. However, the study was presented in SCCP (2007) as a brief summary, and no experimental data were shown. The absence of additional data (such as the magnitude or dose response of the change in AST levels or changes in other serum enzymes, liver weight, or liver pathology) precludes its use for the derivation of the RfD. In a short-term (14-day) study also described in SCCP (2007), AST levels were reportedly altered at 30 and 60 mg/kg-day and accompanied in this case by changes in other serum enzymes and liver weight. However, no details pertinent to the magnitude in dose response and direction of the observed changes in AST and other serum enzymes were reported. These limitations preclude the use of these studies for health assessment.

Developmental/reproductive toxicity studies identified LOAEL values in the range of 80–160 mg/kg-day (Osterburg, 1982a, Seidenburg et al., 1986 as cited in SCCP, 2007). In a developmental study in rats, adverse effects (including a reduction in maternal body weight and an increased incidence of resorptions) occurred at the high dose of 80 mg/kg-day (Osterburg, 1982a, as cited in SCCP, 2007). In a screening developmental toxicity study in mice (Seidenburg et al., 1986), maternal mortality (2/30 vs. 0/29 in controls) and embryotoxicity (increased average number of dead neonates/litter on Day 1) were observed at 160 mg/kg-day (the only dose tested).

The subchronic and developmental toxicity data suggest that effects may occur at doses lower than the NOAELs identified in the chronic toxicity studies. For all of the available studies, specific study information was lacking such that confidence in the use of the data for the development of an RfD was low. However, the appendix of this document contains screening subchronic and chronic p-RfD values that may be useful in certain instances. Please see the attached appendix for details.

Table 5. Summary of Oral Noncancer Dose-Response Information for Toluene-2,5-Diamine and Compounds

Species and Study Type (n/sex/group)	Exposure	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Responses at the LOAEL	Comments	Reference
Short-term toxicity						
Sprague-Dawley rat 15/sex/group	Administered toluene-2,5-diamine sulfate via gavage in deionized water at 0, 7.5, 15, 30, or 60 mg/kg-d for 14 d	15	30	Clinical chemistry (AST, CPK, LDH) variations; increased absolute and relative liver weights in males	Reported changes not further described and data not shown. NOAEL/LOAEL values based on description provided in SCCP (2007).	Hill, 1994 (as cited in SCCP, 2007)
Subchronic toxicity						
Sprague-Dawley rat 15/sex/group	Administered toluene-2,5-diamine sulfate via gavage in deionized water at 0, 2.5, 5, 10, or 20 mg/kg-d for 13 wks	2.5	5	Increased AST levels in females	Reported changes not further described and data not shown. NOAEL/LOAEL values based on assignment provided in SCCP (2007).	Hill, 1997 (as cited in SCCP, 2007)
Chronic toxicity						
F344 rat 50/sex/group	Administered toluene-2,5-diamine sulfate in the diet at time-weighted average concentrations of 0, 0.6, or 0.2% (0, 47, or 158 mg/kg-d for males or 0, 55, or 183 mg/kg-d for females) daily for 78 wks	158	ND	NA	Study designed as cancer bioassay. Endpoints limited to clinical signs, survival, growth, and pathology. Terminal sacrifices performed after a recovery period of 28–31 wks. NOAEL/LOAEL values due to study limitations for noncancer assessment.	NCI, 1978

Table 5. Summary of Oral Noncancer Dose-Response Information for Toluene-2,5-Diamine and Compounds

Species and Study Type (n/sex/group)	Exposure	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Responses at the LOAEL	Comments	Reference
B6C3F1 mouse 50/sex/group	Administered toluene-2,5-diamine sulfate in the diet at time-weighted average concentrations of 0, 0.06, or 0.1% (0, 103, or 172 mg/kg-d for males and 0, 104, or 173 mg/kg-d for females) daily for 78 wks	172	ND	NA	Study designed as cancer bioassay. Endpoints limited to clinical signs, survival, growth, and pathology. Terminal sacrifices performed after a recovery period of 16–19 wks. NOAEL/LOAEL values due to study limitations for noncancer assessment.	NCI, 1978
Reproductive/developmental toxicity						
Sprague-Dawley rat 24 sex/group	Administered toluene-2,5-diamine via gavage in distilled water at 0, 5, 15, or 45 mg/kg-d for 70 d prior to mating (males) or for 14 d prior to mating and throughout mating and lactation (females). The F1 generation was dosed starting from birth for approximately 80 d	45	ND	NA	No effects reported. Data not shown. NOAEL/LOAEL values based on assignment provided in SCCP (2007).	Bornatowicz, 1986, (as cited in SCCP, 2007)
Sprague-Dawley rat 23 females/group	Administered toluene-2,5-diamine sulfate via gavage in distilled water at 0, 10, 50, or 80 mg/kg-d on GDs 6–15	Maternal: 50 Fetal: 80	Maternal: 80 Fetal: ND	Decreased maternal body weight during dosing period. Increased postimplantation loss	Reported changes not further described and data not shown. NOAEL/LOAEL values based on assignment provided in SCCP (2007).	Osterburg, 1982a (as cited in SCCP, 2007)

Table 5. Summary of Oral Noncancer Dose-Response Information for Toluene-2,5-Diamine and Compounds

Species and Study Type (n/sex/group)	Exposure	NOAEL (mg/kg-day)	LOAEL (mg/kg-day)	Responses at the LOAEL	Comments	Reference
New Zealand white rabbit 16 females/group	Administered toluene-2,5-diamine sulfate via gavage in distilled water at 0, 10, 25, or 50 mg/kg-d on GDs 6–18	Maternal and fetal: 50	Maternal and fetal: ND	NA	No effects reported. Data not shown. NOAEL/LOAEL values based on assignment provided in SCCP (2007).	Osterburg, 1982b (as cited in SCCP, 2007)
ICR/SIM mouse 30 females/group; 29 controls	Administered toluene-2,5-diamine sulfate via gavage in corn oil at 0 or 160 mg/kg-d on GDs 8–12	Maternal and fetal: ND	Maternal and fetal: 160	Maternal mortality (2/30 versus 0/29 in controls). Increased average number of dead neonates per litter on Day 1	Screening level study with limited endpoints conducted at a single dose level. Researchers considered both the maternal mortality and the neonatal effect to be treatment-related. NOAEL/LOAEL values due to study limitations.	Seidenburg et al., 1986
Mouse 30 females/group; 40 controls	Administered toluene-2,5-diamine sulfate via gavage at 0 or 80 mg/kg-d on GDs 8–12	Maternal and fetal: 80	Maternal and fetal: ND	NA	Screening level study with limited endpoints conducted at a single dose level. No effects found. NOAEL/LOAEL values due to study limitations.	Kavlock et al., 1987

NA = not applicable; ND = not determined

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RfC VALUES FOR TOLUENE-2,5-DIAMINE AND COMPOUNDS

No data are available on the effects of toluene-2,5-diamine or compounds in humans or animals exposed via inhalation. Derivation of p-RfC values for toluene-2,5-diamine and compounds is precluded by the absence of data.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR TOLUENE-2,5-DIAMINE AND COMPOUNDS

WEIGHT-OF-EVIDENCE DESCRIPTOR

Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is "***Suggestive Evidence of Carcinogenic Potential***" of toluene-2,5-diamine and compounds. No information was located regarding carcinogenicity in humans following oral or inhalation exposure to toluene-2,5-diamine or compounds. The available animal studies conducted via the oral route of exposure found significant increases in the incidence of interstitial-cell tumors of the testis in male rats and alveolar/bronchiolar adenomas and carcinomas in female mice following chronic exposure to toluene-2,5-diamine sulfate (NCI, 1978). Neither of these findings was considered significant by the researchers because the spontaneous incidence of interstitial-cell tumors is traditionally high and variable in the rat strain used in the bioassay and because dosed mice were received and housed separately from their respective controls. Regardless, statistical significance compared to controls was achieved in male rats for both dose groups, and in high dose female mice. An external reviewer of these assays concluded that deficiencies in study design warranted further investigation into the carcinogenic potential of toluene-2,5-diamine (NCI, 1978). No studies that assessed cancer effects related to chronic inhalation exposure to toluene-2,5-diamine and compounds were available. Genotoxicity data for toluene-2,5-diamine and compounds were mixed; most positive tests were conducted in vitro, while the majority of in vivo tests were negative.

QUANTITATIVE ESTIMATES OF CARCINOGENIC RISK

A provisional oral slope factor and inhalation unit risk for toluene-2,5-diamine and compounds has not been derived. However, Appendix A of this document contains a screening oral slope factor that may be useful in certain instances. Please see Appendix A for details.

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APPENDIX A. DERIVATION OF A SCREENING VALUE FOR TOLUENE-2,5-DIAMINE (CASRN 95-70-5) AND COMPOUNDS; TOLUENE-2,5-DIAMINE SULFATE (6369-59-1) [ALSO KNOWN AS 1,4-BENZENEDIAMINE-2-METHYL SULFATE OR 2-METHYLBENZENE-1,4-DIAMINE SULFATE (615-50-9)], TOLUENE-2,5-DIAMINE DIHYDROCHLORIDE (615-45-2), AND TOLUENE-2,5-DIAMINE MONOHYDROCHLORIDE (74612-12-7)

For reasons noted in the main PPRTV document, it is inappropriate to derive provisional toxicity values for toluene-2,5-diamine and compounds (toluene-2,5-diamine sulfate, toluene-2,5-diamine dihydrochloride, and toluene-2,5-diamine monochloride). However, information is available for this chemical, which although insufficient to support derivation of a provisional toxicity value, under current guidelines, may be of limited use to risk assessors. In such cases, the Superfund Health Risk Technical Support Center summarizes available information in an appendix and develops a "screening value." Appendices receive the same level of internal and external scientific peer review as the PPRTV documents to ensure their appropriateness within the limitations detailed in the document. Users of screening toxicity values in an appendix to a PPRTV assessment should understand that there is considerably more uncertainty associated with the derivation of an appendix screening toxicity value than for a value presented in the body of the assessment. Questions or concerns about the appropriate use of screening values should be directed to the Superfund Health Risk Technical Support Center.

The reported NOAEL of 2.5 mg/kg-day in the study by Hill (1997, briefly described in SCCP, 2007) could serve as a basis for development of screening subchronic and chronic p-RfD values. Similarly, two data sets including (1) the incidence of interstitial-cell tumors of the testis in male F344 rats (NCI, 1978) and (2) the incidence of alveolar/bronchiolar adenomas and carcinomas in female B6C3F1 mice (NCI, 1978) could serve as a basis for the development of a screening p-OSF.

ORAL TOXICITY VALUES

Screening Subchronic p-RfD

The apparent NOAEL of 2.5 mg/kg-day and LOAEL of 5 mg/kg-day for increased serum AST levels in rats treated with toluene-2,5-diamine sulfate by gavage in water for 13 weeks (Hill, 1997, as cited in SCCP, 2007) can be used as the basis for derivation of screening provisional toxicity values for toluene-2,5-diamine sulfate and toluene-2,5-diamine. Based on available information, this appeared to be the most sensitive endpoint identified in the available studies. The choice of endpoint was supported by the results of the 14-day range-finding study, which reported changes in AST and other clinical chemistry measures at 30 mg/kg-day (Hill, 1994, as cited in SCCP, 2007). Reproductive and developmental toxicity studies reported effects only at higher doses (80–160 mg/kg-day) (Kavlock et al., 1987; Seidenburg et al., 1986; Osterberg, 1982a,b, as cited in SCCP, 2007 and reviewed in Pang, 1992).

Using the NOAEL of 2.5 mg/kg-day toluene-2,5-diamine sulfate from the subchronic study in rats (Hill, 1997 as cited in SCCP, 2007) as the POD, **a screening subchronic p-RfD** is derived for **toluene-2,5-diamine sulfate** as follows:

Screening Subchronic p-RfD	=	NOAEL ÷ UF
(Toluene-2,5-Diamine Sulfate)	=	2.5 mg/kg-day ÷ 1,000
	=	**3×10^{-3} mg/kg-day**

The composite uncertainty factor (UF) of 1,000 is composed of the following UFs:

- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation because data for evaluating susceptible human responses are insufficient.
- UF_A: A factor of 10 is applied for animal-to-human extrapolation because data for evaluating relative interspecies sensitivity are insufficient.
- UF_D: The database for oral exposure to toluene-2,5-diamine and compounds consists of short-term and subchronic toxicity studies in rats, chronic toxicity studies in rats and mice, and reproductive and developmental toxicity studies in rats, mice, and rabbits. However, due to limitations of the existing studies and the available reports, a factor of 10 is applied for database inadequacies; the data are insufficient for independently evaluating toxicity.
- UF_L: A factor or 1 is applied for extrapolation from a LOAEL to a NOAEL because the POD was developed using a NOAEL.

The data for toluene-2,5-diamine sulfate can be used to derive an assessment for the free base (toluene-2,5-diamine). In order to calculate the RfD for the free base, the screening subchronic p-RfD for the sulfate is adjusted to compensate for differences in molecular weight between toluene-2,5-diamine sulfate (220.25) and toluene-2,5-diamine (122.17). The **screening subchronic p-RfD** for **toluene-2,5-diamine (free base)** is calculated as follows:

Screening Subchronic p-RfD	=	p-RfD for sulfate × (MW base ÷ MW sulfate)
(Toluene-2,5-Diamine; free base)	=	3×10^{-3} mg/kg-day × (122.17 ÷ 220.25)
	=	3×10^{-3} mg/kg-day × (0.55)
	=	**2×10^{-3} mg/kg-day**

Confidence in the principal study (Hill, 1997, as cited in SCCP, 2007) is low because reporting of results was incomplete, particularly with regard to the magnitude and dose-response of the change in AST levels, changes in other serum enzymes, and liver weight or pathology. Confidence in the database is low. Although the database consists of short-term, subchronic, chronic, and reproductive and developmental toxicity studies, several of the studies had either severe design limitations or were available only as brief summaries with few details and no data shown. Confidence in the screening subchronic p-RfD is accordingly low.

Screening Chronic p-RfD

Oral data for toluene-2,5-diamine and compounds consist of a subchronic toxicity study in rats, chronic toxicity studies in rats and mice, and reproductive and developmental toxicity studies in rats, mice, and rabbits. Because chronic toxicity studies in rats and mice (NCI, 1978) had study limitations that precluded the use of these studies as the basis for the development of a chronic p-RfD value, the POD used to derive the screening subchronic p-RfD was also used to

derive a screening chronic p-RfD. A **screening chronic p-RfD** for toluene-2,5-diamine sulfate is derived as follows:

Screening Chronic p-RfD	=	NOAEL ÷ UF
(Toluene-2,5-Diamine Sulfate)	=	2.5 mg/kg-day ÷ 10,000
	=	**3×10^{-4} mg/kg-day**

The composite UF of 10,000 is composed of the following UFs:

- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation because data for evaluating susceptible human response are insufficient.
- UF_A: A factor of 10 is applied for animal-to-human extrapolation because data for evaluating relative interspecies sensitivity are insufficient.
- UF_D: The database for oral exposure to toluene-2,5-diamine and compounds consists of short-term and subchronic toxicity studies in rats, chronic toxicity studies in rats and mice, and reproductive and developmental toxicity studies in rats, mice, and rabbits. However, due to limitations of the existing studies and the available reports, a factor of 10 is applied for database inadequacies; the data are insufficient for independently evaluating toxicity.
- UF_S: A factor of 10 is applied for using data from a subchronic study to assess potential effects from chronic exposure because data for evaluating the response after chronic exposure are inadequate.
- UF_L: A factor of 1 is applied for extrapolation from a LOAEL to a NOAEL because the POD was developed using a NOAEL.

The **screening chronic p-RfD** for **toluene-2,5-diamine (free base)** is calculated using the ratio of molecular weights, as follows:

Screening Chronic p-RfD	=	p-RfD for sulfate × (MW base ÷ MW sulfate)
(Toluene-2,5-Diamine; Free Base)	=	3×10^{-4} mg/kg-day × (122.17 ÷ 220.25)
	=	3×10^{-4} mg/kg-day × (0.55)
	=	**2×10^{-4} mg/kg-day**

Using molecular weights for the other isomers (toluene-2,5-diamine sulfate, toluene-2,5-diamine dihydrochloride, and toluene-2,5-diamine monohydrochloride) screening subchronic and chronic p-RfDs are presented in Table A-1.

Table A-1. Screening Subchronic and Chronic p-RfDs for Toluene-2,5-diamine Compounds[a]

Compounds	Subchronic p-RfD	Chronic p-RfD
Toluene-2,5-diamine sulfate (also known as 1,4-benzenediamine-2-methyl sulfate or 2-methylbenzene-1,4-diamine sulfate)	3×10^{-3}	3×10^{-4}
Toluene-2,5-diamine dihydrochloride	3×10^{-3}[b]	3×10^{-4}[c]
Toluene-2,5-diamine monohydrochloride	2×10^{-3}[d]	2×10^{-4}[e]

[a]Based on toluene-2,5-diamine sulfate screening subchronic and chronic p-RfDs: p-RfDs of compounds = p-RfDs of toluene-2,5-diamine sulfate × (Molecular weight of salt ÷ Molecular weight of toluene-2,5-diamine sulfate).
[b]$3 \times 10^{-3} \times (195.09 \div 220.25) = 0.0027 = 3 \times 10^{-3}$
[c]$3 \times 10^{-4} \times (195.09 \div 220.25) = 0.00027 = 3 \times 10^{-4}$
[d]$3 \times 10^{-3} \times (158.63 \div 220.25) = 0.0022 = 2 \times 10^{-3}$
[e]$3 \times 10^{-4} \times (158.63 \div 220.25) = 0.00022 = 2 \times 10^{-4}$

As discussed for the screening subchronic p-RfD, confidence is low in the principal study (Hill, 1997, as cited in SCCP, 2007), the database, and the overall assessment. Confidence in the database and the overall assessment for the screening chronic p-RfD is further reduced relative to the screening subchronic p-RfD due to the absence of adequate chronic data.

CARCINOGENICITY ASSESSMENT

Quantitative Estimates of Carcinogenic Risk—Oral Exposure

The two data sets that were considered to derive a screening oral slope factor for toluene-2,5-diamine and compounds are (1) the incidence of interstitial-cell tumors in the testis of male F344 rats administered toluene-2,5-diamine sulfate in the diet for 78 weeks (NCI, 1978); and (2) the incidence of alveolar/bronchiolar adenomas and carcinomas in female B6C3F1 mice administered toluene-2,5-diamine sulfate in the diet for 78 weeks (NCI, 1978). Tables 1 and 2 summarize these data. Appendix B describes the incidence data that were modeled. Table A-2 shows the BMD_{10} and $BMDL_{10}$ values predicted by the multistage model for both tumor types.

Table A-2. Summary of Benchmark Values for Toluene-2,5-Diamine Sulfate Based on Incidence of Interstitial-Cell Tumors in the Testis of Male F344 Rats and Alveolar/Bronchiolar Adenomas and Carcinomas in Female B6C3F1 Mice

Benchmark Value[a]	Form of Toluene-2,5-Diamine	Male F344 Rats (mg/kg-day)	Female B6C3F1 Mice (mg/kg-day)
BMD_{10}	Sulfate	6.4	125
$BMDL_{10}$	Sulfate	3.8	69
BMD_{10HED}	Sulfate	1.7	19
$BMDL_{10HED}$	Sulfate	1.0	10

[a]Human equivalent dose (HED) calculated as described in the text

Source: NCI (1978)

The BMD_{10} and $BMDL_{10}$ values were converted to human equivalent doses (HEDs) of toluene-2,5-diamine sulfate by adjusting for differences in body weight between humans and rats or mice. In accordance with EPA (2005) *Guidelines for Carcinogen Risk Assessment*, a factor of $BW^{3/4}$ was used for cross-species scaling. Using this scaling factor, the dose in humans (mg) is obtained by multiplying the animal dose (mg) by the ratio of human:animal body weight raised to the ¾ power. For doses expressed per unit body weight (mg/kg or mg/kg-day), the relationship is reciprocal, and the human dose is obtained by multiplying the animal dose (mg/kg) by the ratio of animal:human body weight raised to the ¼ power. Because NCI (1978) did not report body weights of the rats or mice used in the principal studies (data were presented graphically), default body-weight values for chronic exposure of 0.38 kg for male F344 rats and 0.0353 kg for female B6C3F1 mice (U.S. EPA, 1988) were used to calculate the animal:human body-weight ratios. The equation used to calculate the BMD_{10HED} and $BMDL_{10HED}$ values is shown below, and Table A-2 presents the BMD_{10HED} and $BMDL_{10HED}$ values.

$$BMD_{10HED} = BMD_{10HED} \times (\text{animal BW} \div \text{human BW})^{1/4}$$

where:

animal BW = body weight of male rats or female mice (kg), based on default values (U.S. EPA, 1988)
human BW = reference human body weight, 70 kg (U.S. EPA, 1988)

The modeling results based on testicular tumors in rats were approximately an order of magnitude more sensitive than the results based on lung tumors in mice, when expressed as HED. Therefore, the rat tumors were selected as the source of the POD for derivation of the screening p-OSF. In the absence of a defined mode of action for toluene-2,5-diamine and compounds, the default linear quantitative methodology was applied. Using the $BMDL_{10HED}$ of 1 mg/kg-day for the incidence of interstitial-cell tumors of the testis in male F344 rats (NCI, 1978) as the POD, **a screening p-OSF** for **toluene-2,5-diamine sulfate** is calculated as follows:

Screening p-OSF = BMR ÷ $BMDL_{10HED}$ for sulfate
(Toluene-2,5-Diamine Sulfate) = 0.1 ÷ 1.0 mg/kg-day
= **0.1 or 1×10^{-1} $(mg/kg\text{-}day)^{-1}$**

The screening p-OSF for toluene-2,5-diamine sulfate should not be used with exposures exceeding the POD ($BMDL_{10HED}$ = 1.0 mg/kg-day), because at exposures above this level, the fitted dose-response model better characterizes what is known about the carcinogenicity of toluene-2,5-diamine sulfate.

The data for toluene-2,5-diamine sulfate can be used to derive an assessment for the free base (toluene-2,5-diamine). In order to calculate the p-OSF for the free base, the $BMDL_{10HED}$ for the sulfate is first adjusted to compensate for differences in molecular weight (MW) between toluene-2,5-diamine sulfate (220.25) and toluene-2,5-diamine (122.17), as follows:

$BMDL_{10HED}$ = $BMDL_{10HED}$ for sulfate × (MW base ÷ MW sulfate)
(Toluene-2,5-Diamine; Free Base) = 1.0 mg/kg-day × (122.17 ÷ 220.25)
= 1.0 mg/kg-day × (0.55)
= **0.55 mg/kg-day**

Then, the **screening p-OSF** for **toluene-2,5-diamine (free base)** is calculated from the BMD_{10HED} for the free base, as follows:

Screening p-OSF	=	BMR ÷ $BMDL_{10HED}$ for free base
(Toluene-2,5-Diamine; Free Base)	=	0.1 ÷ 0.55 mg/kg-day
	=	**0.18 or 1.8 × 10^{-1} (mg/kg-day)$^{-1}$**

APPENDIX B. DETAILS OF BENCHMARK DOSE MODELING FOR SCREENING PROVISIONAL ORAL SLOPE FACTOR

MODEL FITTING PROCEDURE FOR CANCER INCIDENCE DATA

The model fitting procedure for cancer incidence data is as follows. The multistage-cancer model in the EPA Benchmark Dose Software (BMDS) is fit to the incidence data using the extra risk option. The multistage-cancer model is run for all polynomial degrees up to n - 1 (where n is the number of dose groups including control). Adequate model fit is judged by three criteria: goodness-of-fit p-value ($p > 0.1$), visual inspection of the dose-response curve, and scaled residual at the data point (except the control) closest to the predefined benchmark response (BMR). Among all the models providing adequate fit to the data, the lowest BMDL is selected as the POD based on the lowest AIC value. In accordance with EPA (2000) guidance, benchmark doses (BMDs) and lower bounds on the BMD (BMDLs) associated with a BMR of 10% extra risk are calculated.

Model Predictions for Interstitial-Cell Tumors of the Testis in Male F344 Rats (NCI, 1978)

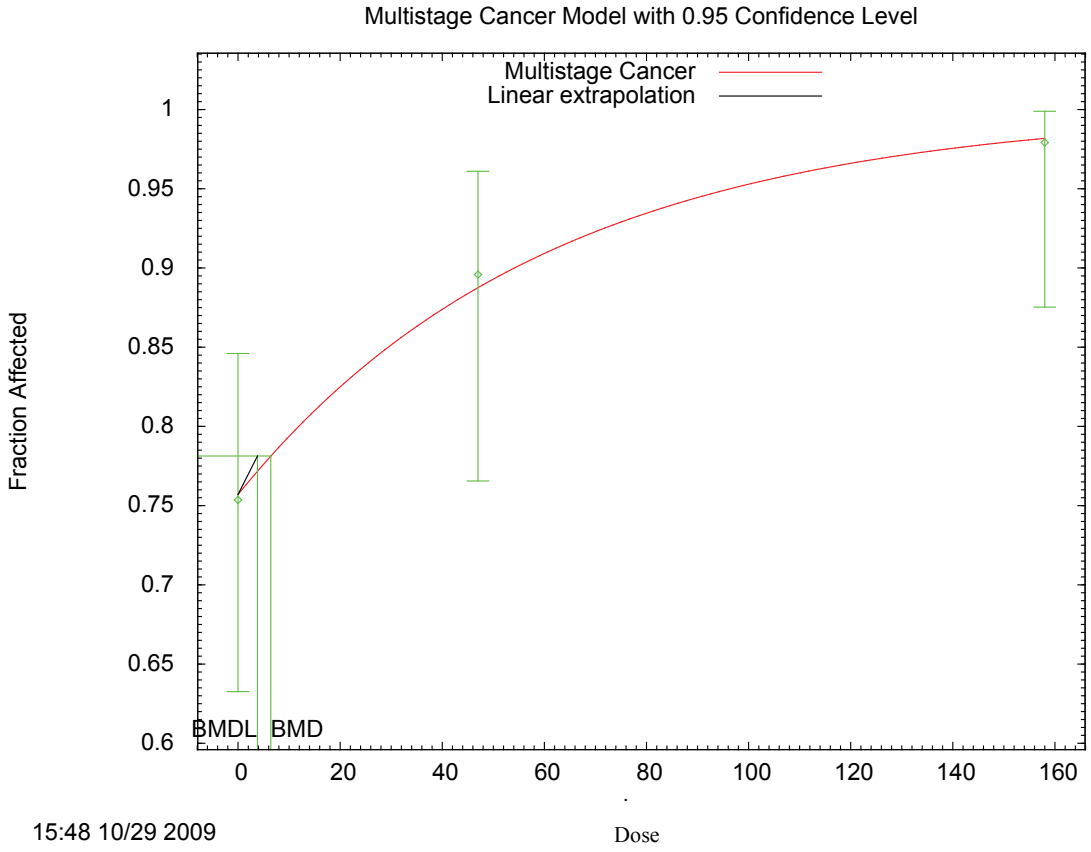
Table B-1 shows the dose-response data on interstitial-cell tumors in the testis of male F344 rats administered toluene-2,5-diamine sulfate for 78 weeks (NCI, 1978). Modeling was performed according to the procedure outlined above using BMDS version 2.1. The low- and high-dose controls were combined for modeling. Table B-1 shows model predictions. The multistage cancer model provided adequate fit (goodness-of-fit p-value >0.1) yielding a BMD_{10} value of 6.4 mg/kg-day with an associated 95% lower confidence limit ($BMDL_{10}$) of 3.8 mg/kg-day. The 2-degree polynomial model converged to the 1-degree model. Figure B-1 shows the fit of the 1-degree multistage cancer model to the incidence data.

Table B-1. Model Predictions for Interstitial-Cell Tumors in the Testis of Male F344 Rats Administered Toluene-2,5-Diamine Sulfate in the Diet for 78 Weeks

Model	Degrees of Freedom	χ^2	χ^2 Goodness-of- Fit p-Value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Multistage (polydegree = 1)[b]	1	0.06	0.8135	122.903	6.41707	3.80415
Multistage (polydegree = 2)[b]	1	0.06	0.8135	122.903	6.41707	3.80415

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria
[b]Betas restricted to ≥0

Source: NCI (1978)



BMDs and BMDLs indicated are associated with an extra risk of 10% and are expressed in units of mg/kg-day.

Figure B-1. Fit of the 1-Degree Multistage Cancer Model to Data on the Incidence of Interstitial-Cell Tumors in the Testis of Male F344 Rats (NCI, 1978)

Model Predictions for Combined Alveolar/Bronchiolar Adenomas and Carcinomas in Female B6C3F1 Mice (NCI, 1978)

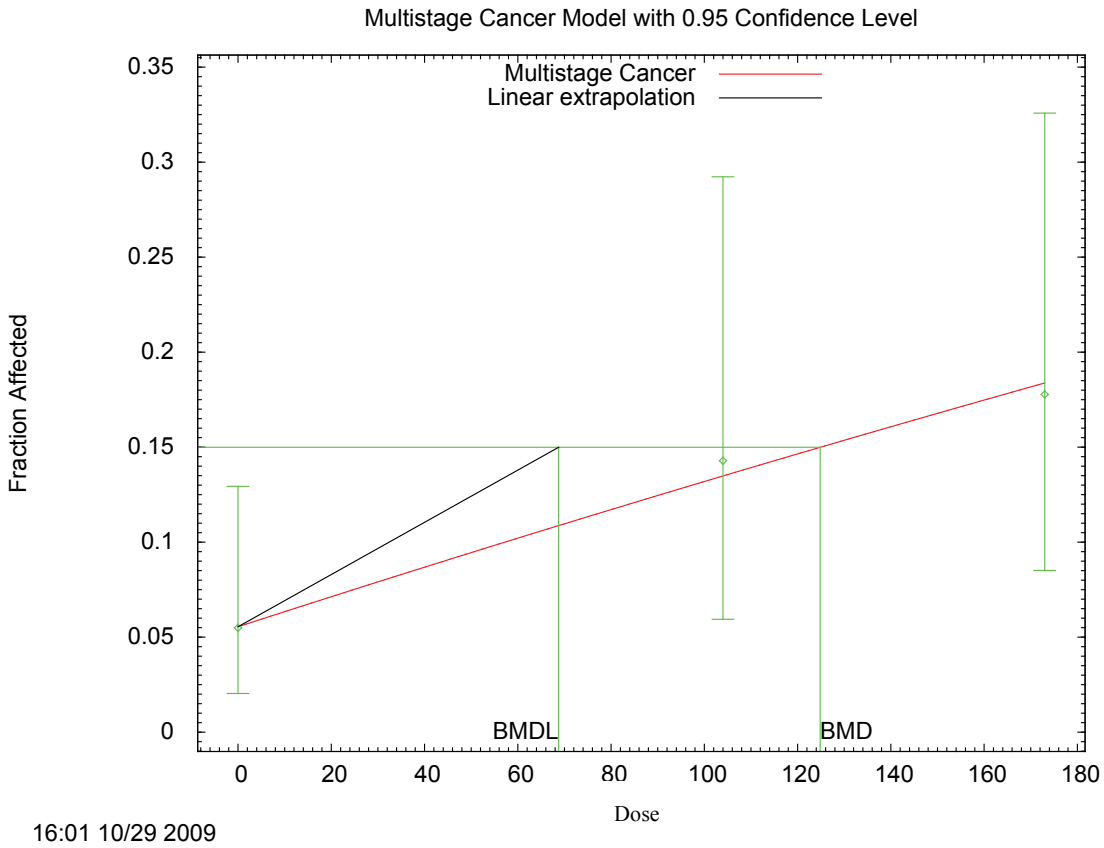
Table B-2 shows the dose-response data on alveolar/bronchiolar adenomas and carcinomas in female B6C3F1 mice administered toluene-2,5-diamine sulfate for 78 weeks (NCI, 1978). Modeling was performed according to the procedure outlined above using BMDS version 2.1. The low- and high-dose controls were combined for modeling. Table B-2 shows model predictions. The multistage cancer model provided adequate fit (goodness-of-fit *p*-value >0.1), yielding a BMD_{10} value of 125 mg/kg-day with an associated 95% lower confidence limit ($BMDL_{10}$) of 69 mg/kg-day. The 2-degree polynomial model converged to the 1-degree model. Figure B-2 shows the fit of the 1-degree multistage cancer model to the incidence data.

Table B-2. Model Predictions for Alveolar/Bronchiolar Adenomas and Carcinomas in B6C3F1 Mice Administered Toluene-2,5-Diamine Sulfate in the Diet for 78 Weeks

Model	Degrees of Freedom	χ^2	χ^2 Goodness-of-Fit *p*-Value[a]	AIC	BMD_{10} (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
Multistage (polydegree = 1)[b]	1	0.03	0.8528	119.339	124.836	68.7632
Multistage (polydegree = 2)[b]	1	0.03	0.8528	119.339	124.836	68.7632

[a]Values <0.10 fail to meet conventional goodness-of-fit criteria
[b]Betas restricted to ≥0

Source: NCI (1978)



BMDs and BMDLs indicated are associated with an extra risk of 10% and are expressed in units of mg/kg-day.

Figure B-2. Fit of the 1-Degree Multistage Cancer Model to Data on the Incidence of Alveolar/Bronchiolar Adenomas and Carcinomas in Female B6C3F1 Mice (NCI, 1978)