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Provisional Peer-Reviewed Toxicity Values for

2,4,6-Trichloroaniline
(CASRN 634-93-5)

and

2,4,6-Trichloroaniline Hydrochloride
(CASRN 33663-50-2)

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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 2,4,6-TRICHLOROANILINE (CASRN 634-93-5) AND 2,4,6-TRICHLOROANILINE HYDROCHLORIDE (CASRN 33663-50-2)

BACKGROUND

HISTORY

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

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

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

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

DISCLAIMERS

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

in question. PPRTVs are periodically updated; therefore, users should ensure that the values contained in the PPRTV are current at the time of use.

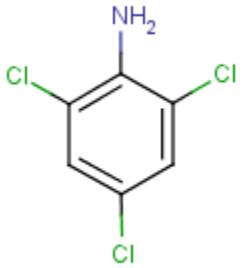
It is important to remember that a provisional value alone tells very little about the adverse effects of a chemical or the quality of evidence on which the value is based. Therefore, users are strongly encouraged to read the entire PPRTV document and understand the strengths and limitations of the derived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

QUESTIONS REGARDING PPRTVS

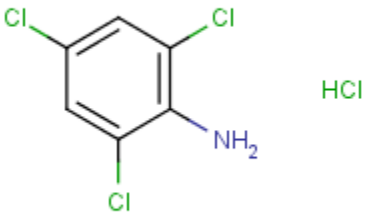
Questions regarding the contents of the PPRTVs and their appropriate use (e.g., on chemicals not covered, or whether chemicals have pending IRIS toxicity values) may be directed to the EPA Office of Research and Development's National Center for Environmental Assessment, Superfund Health Risk Technical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

INTRODUCTION

No RfDs, RfCs, or cancer assessments for 2,4,6-trichloroaniline or 2,4,6-trichloroaniline hydrochloride (see Figure 1 for chemical structures) are included on the IRIS database (U.S. EPA, 2009) or the Drinking Water Standards and Health Advisories List (U.S. EPA, 2006). No RfD or RfC values were reported in the HEAST (U.S. EPA, 1997). The Chemical Assessments and Related Activities (CARA) list (U.S. EPA, 1994, 1991) included a Health and Environmental Effects Document (HEED) for trichloroanilines (U.S. EPA, 1987) that did not derive noncancer toxicity values for 2,4,6-trichloroaniline or 2,4,6-trichloroaniline hydrochloride due to inadequate noncancer data and potential carcinogenicity of the chemicals (see below). The toxicity of 2,4,6-trichloroaniline and 2,4,6-trichloroaniline hydrochloride has not been reviewed by ATSDR (2009) or the World Health Organization (WHO, 2009). CalEPA (2009a,b) has not derived toxicity values for exposure to 2,4,6-trichloroaniline or 2,4,6-trichloroaniline hydrochloride. No occupational exposure limits for 2,4,6-trichloroaniline or 2,4,6-trichloroaniline hydrochloride have been derived by the American Conference of Governmental Industrial Hygienists (ACGIH, 2009), the National Institute of Occupational Safety and Health (NIOSH, 2009), or the Occupational Safety and Health Administration (OSHA, 2009).



2,4,6-Trichloroaniline



2,4,6-Trichloroaniline Hydrochloride

Figure 1. Chemical Structures of 2,4,6-Trichloroaniline and 2,4,6-Trichloroaniline Hydrochloride

The HEAST reported an EPA (1986) cancer weight-of-evidence classification of Group C (*Possible Human Carcinogen*) and an oral slope factor (OSF) of 2.9×10^{-2} $(mg/kg/day)^{-1}$ for 2,4,6-trichloroaniline hydrochloride based on an increased incidence of vascular tumors in male mice in an 18-month study (Weisburger et al., 1978). The HEAST cited the HEED (U.S. EPA, 1987) as the source of the OSF. The HEAST and HEED also reported a Group C classification (*Possible Human Carcinogen*) and an OSF of 3.4×10^{-2} $(mg/kg/day)^{-1}$ for 2,4,6-trichloroaniline based on the same study and calculated by multiplying the OSF for 2,4,6-trichloroaniline hydrochloride by the ratio of molecular weights (232.92 for 2,4,6-trichloroaniline hydrochloride and 196.46 for 2,4,6-trichloroaniline). Neither compound has been evaluated under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005). The International Agency for Research on Cancer (IARC, 2009) has not reviewed the carcinogenic potential for 2,4,6-trichloroaniline or 2,4,6-trichloroaniline hydrochloride. 2,4,6-Trichloroaniline and 2,4,6-trichloroaniline hydrochloride have not been evaluated for potential carcinogenicity by the National Toxicology Program (NTP, 2009) subsequent to the study by Weisburger et al. (1978), and neither compound is included in the *11th Report on Carcinogens* (NTP, 2005). CalEPA (2009b) has not prepared a cancer assessment for 2,4,6-trichloroaniline or 2,4,6-trichloroaniline hydrochloride.

Literature searches were conducted from the 1960s through March 2010 for studies relevant to the derivation of provisional toxicity values for 2,4,6-trichloroaniline and 2,4,6-trichloroaniline hydrochloride. Databases searched included MEDLINE, TOXLINE (with NTIS), BIOSIS, TSCATS/TSCATS2, CCRIS, DART, GENETOX, HSDB, RTECS, Chemical Abstracts, and Current Contents (last 6 months). The HEED (U.S. EPA, 1987) was also reviewed for pertinent studies.

REVIEW OF PERTINENT DATA

Literature searches for toxicological data on 2,4,6-trichloroaniline hydrochloride did not identify any information (although the chemical tested by Weisburger et al. [1978] was reported as 2,4,6-trichloroaniline hydrochloride in the HEED, it is likely that the test material was actually the free base). In fact, there are data suggesting that 2,4,6-trichloroaniline hydrochloride is not likely to be stable in the environment, with rapid conversion to the free base under most conditions (see discussion of ***Acid-Base Interactions*** under **Other Studies**, below). Due to the lack of data on the toxicity of 2,4,6-trichloroaniline hydrochloride and the high likelihood that any exposure to this chemical in the environment will be to the free base variant, the following review is limited to information on 2,4,6-trichloroaniline (with the exceptions noted above), and toxicity values are derived only for this form of the chemical.

HUMAN STUDIES

No data on the effects of 2,4,6-trichloroaniline in humans following inhalation or oral exposure were located in the literature searches.

ANIMAL STUDIES

Oral Exposure

Subchronic Studies—Groups of white rats (128 rats of both sexes; strain and number of rats per group not specified) were administered 2,4,6-trichloroaniline (purity not specified) via gavage in an 8% oil solution (type not specified) at 0, 80, 160, or 800 mg/kg-day for 45 days (Sapegin et al., 1985). Animals were monitored for mortality and clinical signs. Changes in body weight, hematology (including the concentration of formed elements and serum hemoglobin), clinical chemistry (residual nitrogen, pyruvic acid, catalase, alanine aminotransferase [ALT], and aspartate aminotransferase [AST] levels in serum), and other parameters (EKG at lead II and oxygen consumption) were recorded before the start of the experiment and on Treatment Days 10, 20, 30, and 45. Absolute and relative organ weights (not specified) were measured at study termination; lactate dehydrogenase (LDH) and succinic dehydrogenase (SDH) activities in the liver and kidney were determined. Histological analyses were performed, but the organs examined were not specified.

No mortality was reported (Sapegin et al., 1985). Rats administered the high dose exhibited clinical signs of toxicity including depression, cyanosis, hair loss, and hematuria; a lag in body-weight gain compared to controls was also noted (data not shown). The concentration of hemoglobin in the blood (Day 45) and the total number of red blood cells (RBCs) were statistically significantly ($p < 0.05$) reduced in the high-dose group (approximately 25 and 27% less than controls, respectively; see Table 1). Data were presented for the control and high-dose groups at one time point only. Polychromaphilic and hypochromic RBCs, signs of anisocytosis, poikilocytosis, and a tendency toward leucopenia were noted in the high-dose group (data not shown). The activities of ALT and AST in the serum were increased approximately by about 45% and 20%, respectively, and the ALT/AST ratio was decreased in high-dose rats compared to controls. Levels of residual serum nitrogen and serum pyruvic acid were statistically significantly ($p < 0.05$) increased, and rates of oxygen consumption and serum

catalase activity were statistically significantly ($p < 0.02$) decreased in the high-dose group (see Table 1). Inhibition of SDH and LDH activities in the liver and the kidneys of high-dose rats was reported (data not shown).

Table 1. Significant Changes in White Rats Treated with 2,4,6-Trichloroaniline via Gavage for 45 Days

Parameter	Dose in mg/kg-day	
	Control	800[a]
Hematology		
Concentration of hemoglobin on Day 45 (g%)	15.88 ± 0.82[b]	12.02 ± 2.08[c]
Number of RBCs (millions)	6.38 ± 0.25	4.63 ± 0.79[d]
Clinical chemistry		
ALT (mmole)	2.57 ± 0.37	4.69 ± 0.5[d]
AST (mmole)	2.95 ± 0.27	3.74 ± 0.45[c]
Residual serum nitrogen (mg%)	34 ± 2.4	45.5 ± 6[d]
Serum pyruvic acid (mg%)	1.67 ± 0.1	2.36 ± 0.32[d]
Catalase activity (index)	1.04 ± 0.11	0.18 ± 0.13[d]
Oxygen consumption at 15 minutes (mL/100 g)	56.4 ± 7.2	42.2 ± 6.3[e]

[a]Data for other dose groups were not reported
[b]Values are presented as means ± standard deviation (SD)
[c]Significantly different from control at $p < 0.02$
[d]Significantly different from control at $p < 0.001$
[e]Significantly different from control at $p < 0.01$

Source: Sapegin et al. (1985)

Relative weights of the heart, liver, kidneys, and spleen were increased in high-dose rats compared to controls (data not shown) (Sapegin et al., 1985). Degenerative changes, including evidence of hemorrhage in the myocardium, kidneys, liver, spleen, and brain were observed in rats administered the high dose (incidence data not shown). Decreased weight and volume of the testicles were noted in high-dose animals. Histological alterations were noted in the testicles (increased incidence of tubules with desquamated spermatogenic epithelium), but not the ovaries, of high-dose rats (data not shown). The researchers reported that similar, but less pronounced, evidence of toxicity was apparent in mid-dose rats, and that insignificant changes in some of the parameters occurred at the low dose (data not shown). This study is limited by inadequate data reporting. Strain, size, and sex distribution of the control and treatment groups and the statistical methods utilized are not given. In addition, the data presented by the authors are limited to only a few endpoints for the control and high-dose groups at a single time point. Complete histopathology examinations were not performed. Though with severe uncertainties in reporting, the available data provide limited evidence for a NOAEL and a LOAEL of 80 mg/kg-day and 160 mg/kg-day, respectively.

Chronic Studies—In a chronic study conducted by the same researchers, 180 white rats (120 females and 60 males; strain, size, and sex distribution/group not specified) were administered 2,4,6-trichloroaniline (purity not specified) via gavage as 0.04, 0.4, or 4% oil solutions (type not specified) at doses of 0.4, 4, or 40 mg/kg-day (0.3, 3.0, and 29 mg/kg-day, adjusted by multiplying 5/7), respectively, 5 days/week, for 6 months (Sapegin et al., 1985). Although a control group was reportedly used, neither the size nor sex distribution of this group

was reported. The condition of the animals was monitored every 30 days throughout the treatment period. In addition to the toxicological parameters assessed in the subchronic study, conditioned reflexes and methemoglobin concentration in the blood were evaluated in the chronic study (time points not specified). Relative organ weights and LDH and SDH activities in the liver and kidneys were measured at terminal sacrifice. Organs (not specified) were examined for gross pathology; histology was apparently limited to the reproductive organs.

Mortality was not reported by the researchers (Sapegin et al., 1985). As with the subchronic study, data were presented for the control and high-dose groups at a single time point only. Decreased weight gain was noted in high-dose rats when compared with controls (data not shown). Rats administered the high dose had increased numbers of hypochromic RBCs (data not shown) and doubled levels of methemoglobin in the blood in the 6^{th} month of treatment ($p < 0.02$ when compared to controls). Other hematological alterations, including anisocytosis, poikilocytosis, reticulocytosis, hypochromia, and the presence of Heinz bodies in the RBCs were noted at the high dose (incidence data not shown). Oxygen consumption at 15 minutes was statistically significantly ($p < 0.05$) decreased in high-dose rats with respect to controls (see Table 2). High-dose rats required statistically significantly ($p < 0.001$) higher numbers of associations for conditioned reflexes compared to controls (see Table 2). Changes in the relative weights of the brain (increased) and liver (decreased) were reported at the high dose (data not shown). Levels of SDH activity in the liver and LDH activity in the liver and kidneys were reportedly reduced in high-dose rats compared to controls (data not shown). Degenerative changes (not specified) were noted in the blood vessels of the brain, liver, and kidneys of high-dose rats (data not shown). The researchers reported that similar—but less pronounced—evidence of toxicity was apparent in mid-dose rats; only insignificant changes in some of the parameters occurred at the low dose (data not shown). This study is limited by inadequate data reporting including the strain, size, and sex distribution of the control and treatment groups, and the statistical methods utilized. In addition, the data presented by the authors were limited to only a few endpoints for the control and high-dose groups at one (unspecified) time point. Complete histopathology examinations were not performed. These limitations preclude the identification of NOAEL and LOAEL values for this study. From information available qualitatively, a NOAEL at 0.3 mg/kg-day and a LOAEL at 3 mg/kg-day can be identified for hematologic and degenerative changes in brain, liver, and kidneys.

Table 2. Significant Changes in White Rats Treated with 2,4,6-Trichloroaniline via Gavage for 6 Months

Parameter	Dose in mg/kg-day	
	Control	29[a]
Number of associations required for conditioned reflexes	8.3 ±1.6[b]	23.2 ± 2.2[c]
Concentration of methemoglobin: Month 6 (%)	4.04 ± 1.2	8.07 ± 0.89[d]
Oxygen consumption at 15 minutes (mL/100 g)	57.0 ± 3.8	46.4 ± 2.8[e]
Chromosomal aberrations: bone marrow cells (%)	0.4	1.6[e]

[a]Data for other dose groups were not reported
[b]Values are presented as means ± SD
[c]Significantly different from control at $p < 0.001$
[d]Significantly different from control at $p < 0.02$
[e]Significantly different from control at $p < 0.05$

Source: Sapegin et al. (1985)

In a chronic carcinogenicity study of 21 aromatic amines, Charles River CD rats (25 males/group) were administered 2,4,6-trichloroaniline (97–99% pure; purity of individual test compounds not specified) at concentrations of 0, 3,000, or 6,000 ppm in the diet for 5 months, followed by 0, 1,500 or 3,000 ppm, respectively, in the diet for 13 months (Weisburger et al., 1978). Doses of 0, 79, or 303 mg/kg-day (based on time-weighted average concentrations of 0, 1,917, or 3,833 ppm) were calculated for this review.[1] Rats were observed for up to 6 months after the end of the treatment period. Animals were monitored daily for mortality and clinical signs of toxicity. Body weights were recorded periodically. Complete necropsies were conducted on all animals that died after ≥6 months of treatment or at study termination. Histological examinations of grossly abnormal organs, tumor masses, the lung, liver, kidneys, spleen, adrenal, heart, bladder, stomach, intestines, reproductive organs, and pituitaries were performed.

Doses were lowered after 5 months of treatment; according to the study protocol, this action was taken either when there were treatment-related deaths or when body-weight gains in exposed animals were lower than corresponding controls by at least 10% (Weisburger et al., 1978). The study authors did not specify which effect led to the decrease in doses of 2,4,6-trichloroaniline. The results reported in the study were limited to neoplastic changes; no data on mortality, clinical signs of toxicity, body weights, or nonneoplastic findings were given. No significant increase in tumor incidence was observed in any group of rats (data not shown).

In a companion mouse study, albino CD-1 mice (25/sex/group) were treated with concentrations of 0, 6,000, or 12,000 ppm in the diet for 18 months and observed for an additional 3 months following treatment (Weisburger et al., 1978). Doses of 0, 1,040, or 2,070 mg/kg-day for female mice and 0, 1,030, or 2,060 mg/kg-day for male mice were estimated for this review.[2] The same toxicological parameters that were evaluated in rats were

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

also evaluated in mice, and the same tissues were subjected to histological examination—except that pituitaries were not examined. No data regarding mortality, clinical signs of toxicity, or body weights were reported; however, there was no dose modification during the study, suggesting that body-weight gains remained within 10% of corresponding controls (Weisburger et al., 1978).

No significant increase in tumor incidence was observed in either exposed group of female mice (data not shown; Weisburger et al., 1978). However, a dose-related, statistically significant ($p < 0.025$) increase in the incidence of vascular tumors (not further characterized) was observed in dosed male mice (56 and 75% for the low- and high-dose groups, respectively) compared to concurrent controls (13%) and compared to pooled controls from similarly designed experiments (5%; see Table A-1). The incidence of hepatocellular carcinomas in male mice was statistically significantly ($p < 0.025$) increased in the low-dose group—but not the high-dose group—compared to the incidence in pooled, but not concurrent, controls (incidences in the pooled control, concurrent control, low-dose, and high-dose groups were 7/99, 1/16, 5/18, and 1/16, respectively). The lack of a dose-response relationship suggests that the effect was not treatment related. This carcinogenicity study is limited in that small sample sizes were used, only two positive doses were tested, and data reporting was incomplete (growth and survival data were not reported).

Reproductive Studies—The chronic toxicity study conducted by Sapegin et al. (1985) included a reproductive toxicity component. White rats (120 males and 60 females; strain, size, and sex distribution/group not specified) were administered 2,4,6-trichloroaniline via gavage as oil solutions (type not specified) at doses of 0.4, 4, or 40 mg/kg-day, 5 days/week, for 6 months (adjusted to 0.3, 3, or 29 mg/kg-day). Although a control group was reportedly used, neither the size nor sex distribution of this group was reported. The animals were mated at the end of the 6-month treatment period. At study termination, microscopic examination of the reproductive organs was performed. Effects on spermatogenesis and ovogenesis, embryotoxicity, and teratogenicity were assessed (specific endpoints evaluated and methods utilized were not further specified). The authors indicated that there were no significant variations in the "morphofunctional" indices (endpoints not specified) for the male and female reproductive organs (data not shown). Increased incidences of pre- and postimplantation fetal mortality and decreased numbers of fetuses/dam were reported at the mid-dose (data not shown; statistical analyses not reported). Massive hematomas were observed in the abdominal cavities of mid-dose adult rats. The researchers did not indicate whether the effects reported for mid-dose rats also occurred in high-dose rats. This study is limited by inadequate data reporting, including the strain, size, and sex distribution of the control and treatment groups, the methods utilized, and the endpoints evaluated. Based on available information, although limited, a reproductive NOAEL at 0.3 mg/kg-day and a LOAEL at 3 mg/kg-day can be identified for critical endpoints, such as implantation losses and decreased number of fetuses per dams.

Inhalation Exposure

No data on the effects of 2,4,6-trichloroaniline in animals following subchronic or chronic inhalation exposure to 2,4,6-trichloroaniline were located in the literature searches.

OTHER STUDIES

Acute or Short-term Studies

2,4,6-Trichloroaniline (purity not specified) was administered to 80 white rats and 95 white mice (size and sex not specified) via gavage as an oil solution (type not specified) at unspecified doses in an acute lethality study (Sapegin et al., 1985). LD_{50} values were calculated as 3850–4228 mg/kg in rats and 5681–5800 mg/kg in mice. Clinical signs of toxicity (including signs of CNS depression, hypoxia, dyspnea, cyanosis, and weak reactions to external stimuli) were noted in treated animals. Focal hemorrhages, vascular thrombosis, and unspecified degenerative changes to the myocardium, brain, liver, and kidneys were reported (data not shown).

Genotoxicity

Limited information is available regarding the potential genotoxicity of 2,4,6-trichloroaniline. 2,4,6-Trichloroaniline did not induce mutations in *Salmonella typhimurium* strains TA98, TA100, or TA1537 in the presence or absence of metabolic activation in plate-incorporation assays; microsomal-suspension assays using the same strains and metabolic-activation preparations were also negative (Zimmer et al., 1980). However, using the preincubation method, 2,4,6-trichloroaniline tested positive for mutagenicity in *Salmonella* and *Escherichia coli* (strains not specified) (Shimuzu and Takemura, 1984). 2,4,6-Trichloroaniline tested negative in the *Salmonella* umu (SOS response) assay (Ono et al., 1992) and failed to induce DNA repair in rat hepatocytes (Yoshimi et al., 1988). In vivo, 2,4,6-trichloroaniline was mutagenic in the wing spot test in *Drosophila* (Kugler-Steigmeier et al., 1989). Rats treated orally with 2,4,6-trichloroaniline at 40 mg/kg-day (but not 0.4 or 4 mg/kg-day) for 6 months showed a small but statistically significant increase ($p < 0.05$) in the number of bone marrow cells containing chromosomal aberrations when compared with controls (1.6% vs. 0.4%, respectively); however, this study did not provide any study design details (Sapegin et al., 1985).

Acid-Base Interactions

2,4,6-Trichloroaniline is a weak base. Like other bases, 2,4,6-trichloroaniline may be protonated under acidic conditions to form salts (e.g., 2,4,6-trichloroaniline hydrochloride). The property that determines this behavior is the acid dissociation constant, or pK_a (Lyman et al., 1990). When the pH equals the pK_a, the protonated and free-base forms of the chemical are in equilibrium. The higher the pH relative to the pK_a, the greater the proportion of the chemical found as the free base (increase of an order of magnitude for each unit of pH above pK_a). The SPARC on-line calculator (SPARC, 2009) was used to estimate pK_a for 2,4,6-trichloroaniline based on the chemical's structure. The pK_a for 2,4,6-trichloroaniline was estimated as -0.25, which is very low. The associated speciation plot indicated that there would be essentially no protonated 2,4,6-trichloroaniline at pH ≥2. Therefore, any 2,4,6-trichloroaniline hydrochloride in the environment (pH 5–9) is expected to dissolve in moisture and immediately convert to the free base, 2,4,6-trichloroaniline.

DERIVATION OF PROVISIONAL SUBCHRONIC AND CHRONIC ORAL RFD VALUES FOR 2,4,6-TRICHLOROANILINE

Oral data are limited to poorly reported subchronic and chronic toxicity studies (including a reproductive toxicity component) in rats (Sapegin et al., 1985) and chronic cancer bioassays in rats and mice (Weisburger et al., 1978). The cancer bioassays (Weisburger et al., 1978) were not designed to assess noncancer endpoints and provided no information relevant to noncancer toxicity assessment. Clinical signs of toxicity, decreased body-weight gain, serum chemistry changes, hematological effects, organ weight changes, degenerative changes, and reproductive effects (including increased pre- and postimplantation losses, decreased numbers of fetuses/dam, and hematomas in the abdominal cavity) were reported in rats by Sapegin et al. (1985). Although sufficient dose-response information for all exposed doses pertinent to critical effects are unavailable, the chronic studies (Sapegin et al., 1985) clearly identified a point of departure (POD) at 0.3 mg/kg-day. Lack of quantitative data precluded BMD analysis. Based on this information, the NOAEL (0.3 mg/kg-day) can be used to derive p-RfD values by applying a composite UF of 300 and 3000 for subchronic and chronic RfDs, respectively.

The subchronic p-RfD for 2,4,6-trichloroaniline based on the NOAEL of 0.4 (adjusted to 0.3 mg/kg-day in rats [Sapegin et al., 1985]) is derived as follows:

Subchronic p-RfD = $NOAEL_{ADJ} \div UF$
= 0.3 mg/kg-day ÷ 1000
= **0.0003 mg/kg-day or 3×10^{-4} mg/kg-day**

The composite UF of 300 is composed of the following UFs:

- UF_H: A factor of 10 is applied for extrapolation to a potentially susceptible human subpopulation in the absence of data on variability of response in humans.
- UF_A: A factor of 10 is applied for animal-to-human extrapolation.
- UF_D: A factor of 10 is applied due to lack of developmental and multigenerational studies.

Derivation of the chronic p-RfD requires an additional uncertainty factor of 10 for extrapolation to chronic values. Since the composite uncertainty factor is 10,000, a provisional screening chronic p-RfD is presented in Appendix A of this document.

FEASIBILITY OF DERIVING PROVISIONAL SUBCHRONIC AND CHRONIC INHALATION RFC VALUES FOR 2,4,6-TRICHLOROANILINE

No data on the effects of 2,4,6-trichloroaniline in humans or animals following inhalation exposure were located in the literature searches. Derivation of p-RfC values for 2,4,6-trichloroaniline is precluded by the absence of data.

PROVISIONAL CARCINOGENICITY ASSESSMENT FOR 2,4,6-TRICHLOROANILINE

WEIGHT-OF-EVIDENCE DESCRIPTOR

Under the 2005 *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), there is "*Suggestive Evidence of [the] Carcinogenic Potential"* of 2,4,6-trichloroaniline. No information on the carcinogenicity of 2,4,6-trichloroaniline in humans was located. Weisburger et al. (1978) observed an increased incidence of tumors in male CD-1 mice administered 2,4,6-trichloroaniline in the diet for 18 months. Although the Weisburger et al. (1978) report was ambiguous about the exact identity of the test material, and a previous EPA assessment (U.S. EPA, 1987) considered it to be 2,4,6-trichloroaniline hydrochloride, it is concluded here that the test material was the free base, 2,4,6-trichloroaniline. This study reported a statistically significant ($p < 0.025$), dose-related increase in the incidence of vascular tumors in treated male mice (see Table A-1). A statistically significant ($p < 0.025$) increase in the incidence of hepatocellular carcinomas was observed in low-dose, but not high-dose, male mice. This study was limited by incomplete data reporting, small numbers of animals/group, and lack of details regarding the nature and sites of the observed vascular tumors. Although growth and survival data were not presented, the authors reported using doses intended to correspond to the maximum tolerated dose (MTD) and ½ the MTD (Weisburger et al., 1978).

Although in vitro studies suggest that 2,4,6-trichloroaniline is predominantly nonmutagenic, 2,4,6-trichloroaniline has given positive results in vivo. 2,4,6-Trichloroaniline tested positive for mutation in one study in bacteria (Shimuzu and Takemura, 1984) but did not induce mutations in other bacterial assays (Zimmer et al., 1980); this compound also tested negative in the *Salmonella* umu (SOS response) assay (Ono et al., 1992) and failed to induce DNA repair in rat hepatocytes (Yoshimi et al., 1988). However, 2,4,6-trichloroaniline was mutagenic in the wing spot test in *Drosophila* in vivo (Kugler-Steigmeier et al., 1989). Rats treated orally with 2,4,6-trichloroaniline at 40 mg/kg-day (but not 0.4 or 4 mg/kg-day) for 6 months showed a statistically significant increase in the number of bone marrow cells containing chromosomal aberrations; however, few study details were presented (Sapegin et al., 1985).

No other information is available on the mode of action by which 2,4,6-trichloroaniline acts in the development of tumors. In summary, the data are insufficient to postulate a mode of action for 2,4,6-trichloroaniline-induced vascular tumors in mice.

QUANTITATIVE ESTIMATES OF CARCINOGENIC RISK

Since the cancer descriptor is "*Suggestive Evidence of [the] Carcinogenic Potential,"* quantitative treatment is provided in Appendix A as a screening p-OSF.

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APPENDIX A. PROVISIONAL SCREENING VALUES

For reasons noted in the main PPRTV document, it is inappropriate to derive provisional toxicity values for 2,4,6-trichloroaniline. 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 PROVISIONAL ORAL REFERENCE DOSES

Derivation of Screening Chronic Provisional RfD (Chronic p-RfD)

A screening chronic p-RfD based on hematological and reproductive effects reported above (Sapegin et al., 1985) can be derived by dividing the $NOAEL_{ADJ}$ of 0.3 mg/kg-day by a composite UF of 3000, as shown below:

Screening Chronic p-RfD = $NOAEL_{ADJ} \div UF$
= 0.3 mg/kg-day ÷ 10,000
= **0.00003 mg/kg-day or 3×10^{-5} mg/kg-day**

The composite UFs for the screening chronic p-RfD are similar to the subchronic p-RfD. A UF_S of 10, however, is applied for duration of exposure (6 months).

Confidence in the principal study is low. The subchronic and reproductive study evaluated multiple dose levels administered by gavage using adequate endpoints. However, results were poorly reported, making evaluation difficult. There are no developmental and multigenerational studies available. Overall confidence in the data and subchronic and screening chronic p-RfD values is low.

DERIVATION OF SCREENING PROVISIONAL CANCER POTENCY VALUES

Derivation of Screening Provisional Oral Slope Factor (p-OSF)

Weisburger et al. (1978) reported an increased incidence of vascular tumors in male CD-1 mice administered 2,4,6-trichloroanline in the diet for 18 months and observed for 3 additional months. As shown in Table A-1, a statistically significant ($p < 0.025$) increase in tumor incidence was observed in both low-dose and high-dose male mice. The dose-response data for vascular tumors shown in Table A-1 can be used to derive an OSF for 2,4,6-trichloroaniline. In order to determine a POD for OSF derivation, animal doses in the Weisburger et al. (1978) study were first adjusted for lifetime exposure as follows:

$$Dose_{ADJ} = \text{dose (mg/kg-day)} \times (\text{months of treatment} \div [\text{months of treatment} + \text{months of observation period}])$$
$$= \text{dose (mg/kg-day)} \times (18 \div 21)$$

Table A-1. Incidences of Tumors in Male CD-1 Mice Treated with 2,4,6-Trichloroaniline for 18 Months

Dose (mg/kg-day)	Incidence of Vascular Tumors	Incidence of Hepatocellular Carcinomas
0 (concurrent)	2/16	1/16
0 (pooled)	5/99	7/99
1030	10/18[a]	5/18[b]
2060	12/16[a]	1/16

[a]Significantly different from incidence in concurrent and pooled controls at $p < 0.025$
[b]Significantly different from incidence in pooled controls at $p < 0.025$

Source: Weisburger et al. (1978)

The dose-adjusted values, shown in Table A-2, were then converted to human equivalent doses (HEDs) by adjusting for differences in body weight between humans and 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 of 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. Since Weisburger et al. (1978) did not report body weights of mice used in the principal study, a default body-weight value for chronic exposure of 0.0373 kg for male $B6C3F_1$ mice (U.S. EPA, 1988) was used to calculate the animal:human body-weight ratios. The equation used to calculate the HED values is shown below; the HED values are presented in Table A-2.

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

where

$Dose_{ADJ}$ = average daily dose adjusted for lifetime exposure (mg/kg-day)
animal BW = average male mouse body weight (0.0373 kg; default value from U.S. EPA, 1988)
human BW = reference human body weight (70 kg; U.S. EPA, 1988)

Table A-2. Dose-response Data for Incidence of Vascular Tumors in Male CD-1 Mice Treated with 2,4,6-Trichloroaniline for 18 Months

Animal Dose (mg/kg-day)	$Dose_{ADJ}$[a] (mg/kg-day)	HED[b] (mg/kg-day)	Incidence of Vascular Tumors
0	0	0	2/16
1030	883	134	10/18
2060	1766	268	12/16

[a]$Dose_{ADJ}$ = Dose (mg/kg-day) × (months of treatment ÷ [months of treatment + months of observation period]), where (months of treatment ÷ [months of treatment + months of observation period]) = (18 ÷ 21)
[b]HED = $Dose_{ADJ}$ × (animal BW ÷ human BW)$^{1/4}$, where animal body weight = 0.0373 kg (default value from U.S. EPA, 1988 for male mice) and human body weight = 70 kg

Source: Weisburger et al. (1978)

The tumor data shown in Table A-2 were modeled as described in Appendix A using a benchmark response (BMR) of 10% extra risk (U.S. EPA, 2000). The BMD_{10HED} and $BMDL_{10HED}$ values predicted by the multistage cancer model for the data on vascular tumors in male mice were 22 and 14 mg/kg-day, respectively. The $BMDL_{10HED}$ of 14 mg/kg-day was selected as the POD for the screening p-OSF derivation. In the absence of information on the cancer mode of action of 2,4,6-trichloroaniline, a linear extrapolation to the origin was conducted. A screening p-OSF 2,4,6-trichloroanline was calculated as follows:

$$\begin{aligned} \textbf{Screening p-OSF} &= \text{BMR} \div BMDL_{10HED} \\ &= 0.1 \div 14 \text{ mg/kg-day} \\ &= \mathbf{0.007 \text{ or } 7 \times 10^{-3} \text{ (mg/kg-day)}^{-1}} \end{aligned}$$

The screening p-OSF for 2,4,6-trichloroaniline should not be used with exposures exceeding the POD ($BMDL_{10HED}$ = 14 mg/kg-day) because, at exposures above these levels, the fitted dose-response model better characterizes what is known about the carcinogenicity of 2,4,6-trichloroaniline. Table A-3 shows the doses associated with specific levels of cancer risk based on the p-OSF for 2,4,6-trichloroaniline.

Table A-3. Doses of 2,4,6-Trichloroaniline Associated with Specific Levels of Cancer Risk

Risk Level	Dose (mg/kg-day)
10^{-4}	0.014
10^{-5}	0.0014
10^{-6}	0.00014

APPENDIX B. DETAILS OF BENCHMARK DOSE MODELING FOR ORAL SLOPE FACTOR (OSF)

MODEL-FITTING PROCEDURE FOR CANCER INCIDENCE DATA

The multistage-cancer model in the EPA benchmark dose software (BMDS) is fit to the incidence data using the extra risk option and 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 of the models providing adequate fit to the data, the lowest BMDL is selected as the point of departure when the difference between the Benchmark Dose Lower bound 95% confidence intervals (BMDLs) estimated from these models is more than 3-fold (unless it is an outlier); otherwise, the BMDL from the model with the lowest Akaike Information Criterion (AIC) is chosen. In accordance with EPA (2000) guidance, benchmark doses (BMDs) and BMDLs associated with a BMR of 10% extra risk are calculated.

MODEL-FITTING RESULTS FOR THE INCIDENCE OF VASCULAR TUMORS IN MALE CD-1 MICE (WEISBURGER ET AL., 1978)

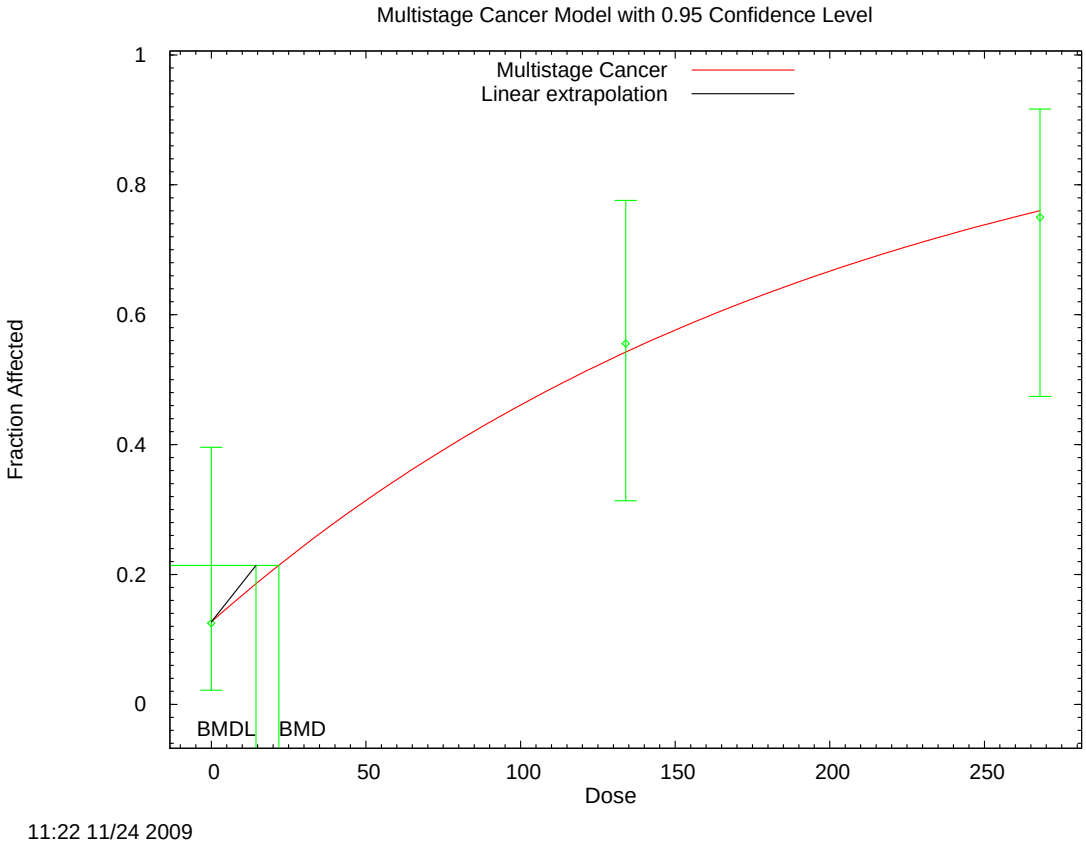
Applying the procedure outlined above to the human equivalent doses (HEDs) and incidences of vascular tumors in male CD-1 mice (see Table A-2), both the 1- and 2-degree multistage-cancer models provided adequate fit to the data and gave the same results (see Table B-1). The benchmark dose (BMD_{10HED}) and associated 95% lower confidence limit ($BMDL_{10HED}$) were 21.84 and 14.44 mg/kg-day, respectively (see Table B-1).

Table B-1. Model Predictions for Vascular Tumors in Male CD-1 Mice Treated with 2,4,6-Trichloroaniline for 18 Months

Model	Degrees of Freedom	χ^2	χ^2 Goodness-of-Fit *p*-Value[a]	AIC	BMD_{10HED} (mg/kg-day)	$BMDL_{10HED}$ (mg/kg-day)
Multistage-cancer (degree = 1)[b]	1	0.02	0.8817	58.80	21.84	14.44
Multistage-cancer (degree = 2)[b]	1	0.02	0.8817	58.80	21.84	14.44

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

AIC = Akaike Information Criterion



BMD and BMDLs indicated are associated with an extra risk of 10% and are human equivalent doses (HEDs) in units of mg/kg-day.

Source: Weisburger et al. (1978).

Figure B-1. Fit of Multistage-Cancer (1-Degree) Model to Incidence Data for Vascular Tumors in Male CD-1 Mice Administered 2,4,6-Trichloroaniline in the Diet for 18 Months