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Trichloroacetic acid (TCA); CASRN 76-03-9; 00/00/0000

Human health assessment information on a chemical substance is included in IRIS only after a comprehensive review of toxicity data by U.S. EPA health scientists from several program offices, regional offices, and the Office of Research and Development. Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the positions that were reached during the review process. Supporting information and explanations of the methods used to derive the values given in IRIS are provided in the guidance documents located on the IRIS website at http://www.epa.gov/iris/backgrd.html.

STATUS OF DATA FOR TRICHLOROACETIC ACID (TCA)

File First On-Line 10/01/1992

Category (section)	Status	Last Revised
Chronic Oral RfD Assessment (I.A.)	on-line	00/00/0000
Chronic Inhalation RfC Assessment (I.B.)	discussion	00/00/0000
Carcinogenicity Assessment (II.)	on-line	00/00/0000

_I. HEALTH HAZARD ASSESSMENTS FOR NONCARCINOGENIC EFFECTS

__I.A. REFERENCE DOSE (RfD) FOR CHRONIC ORAL EXPOSURE

Substance Name -- Trichloroacetic acid (TCA)
CASRN -- 76-03-9
Section I.A. Last Revised -- 00/00/0000

The RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD is intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear

(possibly threshold) mode of action. It is expressed in units of mg/kg-day. Please refer to the guidance documents at http://www.epa.gov/iris/backgrd.html for an elaboration of these concepts. Because RfDs can be derived for the noncarcinogenic health effects of substances that are also carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

An RfD for TCA was not previously available on the IRIS database.

___I.A.1. CHRONIC ORAL RfD SUMMARY

Critical Effect	Point of Departure*	UF	Chronic RfD
Hepatocellular necrosis Male B6C3F$_1$ mice 60-Week drinking water exposure study DeAngelo et al., 2008	BMDL$_{10}$ = 18 mg/kg-day	1000	0.02 mg/kg-day

*Conversion Factors and Assumptions -- The rats in the principal study were exposed continuously via drinking water; therefore, no adjustment for intermittent dosing was required. Doses were estimated from drinking water concentrations using measured values for body weight and drinking water consumption.

___I.A.2. PRINCIPAL AND SUPPORTING STUDIES

DeAngelo et al. (2008) exposed male B6C3F$_1$ mice to nominal concentrations of 0.05, 0.5, or 5 g/L TCA in the drinking water (50/dose at study initiation) for 60 weeks (Study 1); 0 or 4.5 g/L TCA (58 animals/group) for 104 weeks (Study 2); or 0, 0.05, or 0.5 g/L TCA (72/group) for 104 weeks (Study 3). The pH of the dosing solutions was adjusted to 6.0–7.1 by the addition of 10 N sodium hydroxide. Mice in the control group in Study 1 received 2 g/L sodium chloride in the drinking water, while those in Study 2 received 1.5 g/L neutralized acetic acid to account for any taste aversion of TCA in dosing solutions. In Study 3, deionized water served as the control. Body weights and water consumption were measured twice monthly for the first 2 months and then monthly afterwards. In Study 1, groups of five animals from each dose group were examined at necropsy at 4, 15, 31, and 45 weeks. In Study 2, serial necropsies were conducted at 15, 30, 45, and 60 weeks. In Study 3, serial necropsies were conducted at 26, 52, and 78 weeks.

At interim and terminal necropsies, gross lesions, livers, kidneys, spleens, and testes were examined by a board-certified veterinary pathologist. For all other tissues, a complete pathological examination was performed on five mice from the high-dose and control groups. If the number of any histopathologic lesions in a tissue was significantly increased above that in

the control animals, then that tissue was examined in all TCA dose groups. To determine long-term hepatocellular damage during TCA treatment, arterial blood was collected at 30 and 60 weeks (Study 1) and 4, 30, and 104 weeks (Study 2), and serum lactate dehydrogenase (LDH) activity was measured. Portions of liver tissue from the interim-sacrifice animals (5/group/duration) were frozen and analyzed for palmitoyl-CoA oxidase (PCO) activity, a marker of peroxisome proliferation. Five days prior to each scheduled necropsy, osmotic pumps containing 200 µL [^{3}H]thymidine (62–64 Ci/mmol) or 20 mg/mL bromodeoxyuridine (BrdU) (Study 3) were implanted subcutaneously. Autoradiography using paraffin-embedded sections of liver was performed to evaluate hepatocyte proliferation, as measured by the incorporation of ^{3}H-labeled thymidine or BrdU into nuclear DNA. The labeling index was calculated by dividing the number of labeled hepatocyte nuclei (S-phase) by the total number of hepatocyte nuclei scored.

For Study 1, time-weighted mean doses of 8, 68, and 602 mg/kg-day were calculated by the study authors from nominal TCA concentrations (0.05, 0.5, and 5 g/L, respectively) and drinking water consumption data for the low-, mid-, and high-dose groups. Animals in the mid- and high-dose groups consumed significantly less water than the controls. No significant differences in animal survival were noted for any treatment group. The study authors estimated the mean doses to be 572 mg/kg-day for a nominal drinking water concentration of 4.5 g/L TCA (Study 2), and 6 and 58 mg/kg-day for nominal concentrations of 0.05 and 0.5 mg/kg-day (Study 3). DeAngelo et al. (2008) also reported measured TCA concentrations in drinking water. Doses calculated by EPA based on those concentrations and reported drinking water consumption are as follows: Study 1: 7.7, 68.2, and 602.1 mg/kg-day for measured TCA concentrations of 0.05, 0.48, and 5.06 g/L, respectively; Study 2: 571.5 mg/kg-day for a measured TCA concentration of 4.43 g/L; and Study 3: 6.7 and 81.2 mg/kg-day for measured TCA concentrations of 0.06 and 0.70 g/L, respectively. With the exception of liver neoplasia, all data presented by DeAngelo et al. (2008) were from the 60-week study (Study 1).

No decrease in animal survival was found at any TCA dose in any of the three studies. Exposure to TCA in the drinking water decreased body weight by 15% in the high-dose group relative to the control. Significant, dose-related increases in absolute and relative liver weights were observed in the 0.5 and 5 g/L treatment groups at all scheduled sacrifices, with the exception of the 0.5 g/L dose group at 30 days.

Nonneoplastic alterations in the liver and testes were seen at study termination at 60 weeks and appeared to be dose related. The nonneoplastic alterations observed in the liver included hepatocellular cytoplasmic alteration, necrosis, and inflammation. Cytoplasmic alterations were observed in all treatment groups; however, the incidence did not increase monotonically with dose. These lesions were most prominent in the 5 g/L TCA group throughout the study and were most severe after 60 weeks of treatment. The alterations were characterized by an intense eosinophilic cytoplasm with deep basophilic granularity and slight cytomegaly. The distribution ranged from centrilobular to diffuse. Hepatic necrosis was observed in the middle- and high-dose group at all time points and was reported to be most severe at 30–45 weeks. A significant increase in the severity of inflammation was seen in the high-dose group at 60 weeks. A dose-related increase in serum lactate dehydrogenase (LDH) activity (a measure of liver damage) was observed at 30 weeks, and significant increases were

measured in the 0.5 and 5.0 g/L dose groups. No change in LDH activity was found in any treatment groups at 60 weeks. No other hepatic changes showed statistically significant increases in incidence or severity level. An increased incidence of testicular tubular degeneration was seen in the 0.5 and 5 g/L treatment groups. No treatment-related changes were observed in the spleen or kidney.

Incidence and severity of nonneoplastic lesions in male B6C3F$_1$ mice exposed to TCA in drinking water for 60 weeks

Lesion	Treatment	Control	0.05 g/L TCA	0.5 g/L TCA	5 g/L TCA
	Dose	0	8	68	602
	Number[a]	30	27	29	29
Hepatocellular cytoplasmic alteration	Incidence[b]	7%	48%[d]	20.6%[d]	93%[d]
	Severity[c]	0.10 ± 0.40	0.70 ± 0.82	0.34 ± 0.72	1.60 ± 0.62[d]
Hepatocellular inflammation	Incidence[b]	10%	0	7%	24%[d]
	Severity[c]	0.13 ± 0.40	0	0.07 ± 0.03	0.24 ± 0.44
Testicular tubular degeneration	Incidence[b]	7%	0	14%[d]	21%[d]
	Severity[c]	0.10 ± 0.40	0	0.17 ± 0.47	0.21 ± 0.41

[a]Number of animals examined.
[b]Percentage of animals with alteration.
[c]Severity: 0 = no lesion, 1 = minimal, 2 = mild, 3 = moderate, 4 = severe (reported as the average severity of all animals in the dose group).
[d]Statistically significant from the control group, $p \leq 0.05$.

Source: DeAngelo et al. (2008).

Areas of inflammation (at high dose only) and necrosis (at mid- and high dose) were present during the early course of TCA administration, but abated after week 60 in all studies. Similarly, LDH activity was elevated in the mid- and high-dose groups at week 30 but not at week 60. Cytoplasmic alterations occurred as early as week 4 and persisted throughout the three studies at all doses, indicating that this effect did not correlate with other nonneoplastic changes in the liver.

For the 60-week study, EPA determined the lowest-observed-adverse-effect level (LOAEL) for effects on the liver (increased liver weight, hepatic necrosis, and serum LDH activity at 30 weeks) and testes (testicular tubular degeneration) to be 0.5 g/L (68 mg/kg-day) and the no-observed-adverse-effect level (NOAEL) to be 0.05 g/L (8 mg/kg-day).

Methods of Analysis. Hepatocellular necrosis in male B6C3F$_1$ mice exposed to TCA in drinking water for 30–45 weeks as reported in the DeAngelo et al. (2008) 60-week study was identified as the critical effect. All of the available dichotomous models in U.S. EPA's Benchmark Dose Software (BMDS, version 1.4.1) were fit to the incidence data for hepatocellular necrosis. Doses (i.e., benchmark dose [BMD$_{10}$] and 95% lower confidence limit on the benchmark dose [BMDL$_{10}$]) associated with a benchmark response (BMR) of 10% extra

risk were calculated. A BMR of 10% is generally used in the absence of information regarding what level of change is considered biologically significant, and also to facilitate a consistent basis of comparison across assessments (U.S. EPA, 2000). The log-logistic model, which provided the best fit of the hepatocellular necrosis data, yielded a BMD_{10} of 40.7 mg/kg-day and a $BMDL_{10}$ of 17.9 mg/kg-day. The $BMDL_{10}$ or 17.9 mg/kg-day was selected as the point of departure (POD) for the RfD.

___I.A.3. UNCERTAINTY FACTORS

UF = 1000

An uncertainty factor (UF) of 10 was selected for interindividual variability to account for human-to-human variability in susceptibility in the absence of quantitative information to assess the toxicokinetics and toxicodynamics of TCA in humans.

An UF of 10 was selected for interspecies extrapolation to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability) because information was unavailable to quantitatively assess toxicokinetic or toxicodynamic differences between animals and humans for TCA.

An UF of 10 was used to account for database deficiencies. There are no TCA-specific systemic toxicity data in humans. Although subchronic and chronic animal studies of TCA have been conducted in rats and mice, most studies have focused primarily or exclusively on liver lesions and have not examined other organs for microscopic lesions. DeAngelo et al. (2008) is the only study in mice that included histopathological examination of organs other than the liver; however, complete histopathologic examinations were performed on only five mice from the high-dose and control groups. Other data gaps include lack of a multigeneration reproductive toxicity study. Available developmental studies were conducted at high doses, and did not allow identification of a NOAEL.

An UF for study duration was not required in this assessment because the principal study was of chronic duration.

An UF for LOAEL-to-NOAEL extrapolation was not applied because the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, a BMR of 10% increase in the incidence of hepatocellular necrosis was selected under an assumption that it represents a minimally biologically significant change.

___I.A.4. ADDITIONAL STUDIES/COMMENTS

No human epidemiology studies of TCA were located. Case reports and accounts of the medical use of TCA for skin treatment demonstrate its potential for skin corrosion and eye irritation.

In animals, TCA induces systemic, noncancer effects that can be grouped into three general categories: liver toxicity, metabolic alterations, and developmental toxicity. Studies in rats and mice indicate that TCA primarily affects the liver, although effects on the lungs and kidneys have also been noted in rats. Observed hepatic effects in rodents include increased size and weight, collagen deposition, indications of altered lipid and carbohydrate metabolism, histopathologic changes, peroxisome proliferation, evidence of lipid peroxidation, and oxidative damage to hepatic DNA. TCA may influence intermediary carbohydrate metabolism, as shown by altered glycogen content in the livers of mice treated with TCA. Administration of TCA to female rats during pregnancy induced developmental effects in six studies at doses that also resulted in maternal toxicity. Two of these studies are single-dose studies. The observed effects include fetal cardiac malformations, decreased crown-rump length, and reduced fetal body weight. The pattern of observed fetal cardiac malformation effects is not consistent across the available studies. The reason for this inconsistency is unknown, but may be related to factors such as the differences in susceptibility of the test animal strain and/or the poor definition of the cardiac malformations.

___I.A.5. CONFIDENCE IN THE CHRONIC ORAL RfD

Study -- Medium
Database -- Medium
RfD -- Medium

The overall confidence in this RfD assessment is medium. Confidence in the principal study (DeAngelo et al., 2008) is medium. The study was well designed, with a study duration of up to 104 weeks, and well conducted. Quantitative data for the incidence and severity of the various endpoints were included in the published paper. Complete histopathologic examination was conducted for control and high-dose groups. Confidence in the database is medium. Human data are limited primarily to case reports of skin or eye effects associated with medical treatments, and information on systemic toxicity is lacking. Significant gaps in the animal database include the absence of a multigeneration reproductive toxicity study.

___I.A.6. EPA DOCUMENTATION AND REVIEW OF THE CHRONIC ORAL RfD

Source Document -- *Toxicological Review of Trichloroacetic Acid* (U.S. EPA, 2011).

This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of Trichloroacetic Acid* (U.S. EPA, 2011).

Agency Completion Date -- __/__/__

___I.A.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

__I.B. REFERENCE CONCENTRATION (RfC) FOR CHRONIC INHALATION EXPOSURE

Substance Name -- Trichloroacetic acid (TCA)
CASRN -- 76-03-9
Section I.B. Last Revised -- 00/00/0000

The RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory effects). The inhalation RfC (generally expressed in units of mg/m^3) is analogous to the oral RfD and is similarly intended for use in risk assessments for health effects known or assumed to be produced through a nonlinear (possibly threshold) mode of action.

Inhalation RfCs are derived according to *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994). Because RfCs can also be derived for the noncarcinogenic health effects of substances that are carcinogens, it is essential to refer to other sources of information concerning the carcinogenicity of this chemical substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

An inhalation RfC for TCA was not previously available on the IRIS database.

___I.B.1. CHRONIC INHALATION RfC SUMMARY

No inhalation studies adequate for the derivation of an RfC were located. The respiratory tract has not been examined in oral studies of TCA. Because the liver is the critical target organ for oral toxicity and a first-pass effect by the liver is expected following oral administration, the route of exposure may influence the hepatic response to TCA. Physiologically based pharmacokinetic (PBPK) models that would support route-to-route extrapolation for TCA have not been published. Thus, the available information is inadequate for extrapolation of oral toxicity data to the inhalation pathway. For these reasons, an RfC for TCA was not derived.

___I.B.2. PRINCIPAL AND SUPPORTING STUDIES

Not applicable.

___I.B.3. UNCERTAINTY FACTORS

Not applicable.

___I.B.4. ADDITIONAL STUDIES/COMMENTS

Not applicable.

___I.B.5. CONFIDENCE IN THE CHRONIC INHALATION RfC

Not applicable.

___I.B.6. EPA DOCUMENTATION AND REVIEW OF THE CHRONIC INHALATION RfC

Source Document -- *Toxicological Review of Trichloroacetic Acid* (U.S. EPA, 2011).

This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of Trichloroacetic Acid* (U.S. EPA, 2011).

Agency Completion Date -- __/__/__

___I.B.7. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

_II. CARCINOGENICITY ASSESSMENT FOR LIFETIME EXPOSURE

Substance Name -- Trichloroacetic acid (TCA)
CASRN -- 76-03-9
Section II. Last Revised -- 00/00/0000

This section provides information on three aspects of the carcinogenic assessment for the substance in question: the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral and inhalation exposure. Users are referred to Section I of this file for information on long-term toxic effects other than carcinogenicity.

The rationale and methods used to develop the carcinogenicity information in IRIS are described in the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a) and the

Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens (U.S. EPA, 2005b). The quantitative risk estimates are derived from the application of a low-dose extrapolation procedure, and are presented in two ways to better facilitate their use. First, route-specific risk values are presented. The "oral slope factor" is a plausible upper bound on the estimate of risk per mg/kg-day of oral exposure. Similarly, a "unit risk" is a plausible upper bound on the estimate of risk per unit of concentration, either per μg/L drinking water (see Section II.B.1.) or per $\mu g/m^3$ air breathed (see Section II.C.1.). Second, the estimated concentration of the chemical substance in drinking water or air when associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000 is also provided.

In the previous IRIS assessment (posted in 1996), TCA had a classification of C (possible human carcinogen). The previous IRIS assessment did not provide quantitative estimates of carcinogenic risk from oral or inhalation exposure.

__II.A. EVIDENCE FOR HUMAN CARCINOGENICITY

___II.A.1. WEIGHT-OF-EVIDENCE CHARACTERIZATION

Under the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005a), TCA is "likely to be carcinogenic to humans" based on statistically significantly increased incidences of hepatocellular adenomas and carcinomas in male and female $B6C3F_1$ mice in multiple studies following lifetime and less-than-lifetime oral exposures in drinking water (DeAngelo et al., 2008; Bull et al., 2002, 1990; Pereira, 1996; Pereira and Phelps, 1996; Herren-Freund et al., 1987). Additionally, liver tumors were observed in mouse tumor promotion assays with and without initiation (Pereira et al., 2001, 1997; Pereira and Phelps, 1996; Herren-Freund et al., 1987). Treatment-related tumors were not observed in a study of male F344/N rats following lifetime exposure in drinking water (DeAngelo et al., 1997). No information is available on the carcinogenicity of TCA in humans.

The Cancer Guidelines emphasize the importance of weighing all of the evidence in reaching conclusions about the human carcinogenic potential of agents. Choosing a descriptor is a matter of judgment and cannot be reduced to a formula. Each descriptor may be applicable to a wide variety of potential data sets and weights of evidence. The "likely to be carcinogenic to humans" descriptor is appropriate when the weight of the evidence is adequate to demonstrate carcinogenic potential to humans but does not reach the weight of evidence for the descriptor "carcinogenic to humans." Examples provided in the Cancer Guidelines (U.S. EPA, 2005a) include "an agent that has tested positive in animal experiments in more than one species, sex, strain, site, or exposure route, with or without evidence of carcinogenicity in humans" and "a positive tumor study that raises additional biological concerns beyond that of a statistically significant result, for example, a high degree of malignancy, or an early age at onset."

TCA induced liver adenomas and carcinomas in male and female $B6C3F_1$ mice in multiple drinking water bioassays. EPA acknowledges that the mouse, and in particular the $B6C3F_1$ mouse, is relatively susceptible to liver tumors, thus background incidence of this tumor is generally high. For these reasons, use of mouse liver tumor data in risk assessment has been a subject of controversy (King-Herbert and Thayer, 2006). The majority of TCA drinking water bioassays in the $B6C3F_1$ mouse (DeAngelo et al., 2008; Bull et al., 2002, 1990; Pereira, 1996) reported relatively low incidences of liver adenomas and carcinomas in control animals (ranging

from 0 to 13%), thereby minimizing the possible confounding of compound-related liver tumors. Furthermore, it is noteworthy that statistically significant increases in tumor incidence were induced by TCA following drinking water exposures of only 51–82 weeks in these studies. In tumor promotion assays, TCA induce liver tumors in mice with and without pre-treatment with an initiator (Herren-Freund et al., 1987; Pereira and Phelps, 1996; Pereira et al., 2001).

Although there is evidence from multiple drinking water bioassays that TCA can cause cancer in male and female mice, the characterization of the carcinogenic potential to humans is complicated by the limited scope of testing of TCA for carcinogenicity (i.e., all but one assay were conducted in a single mouse strain by a single route of exposure).

As emphasized by EPA in Section 2.5 of the Cancer Guidelines (U.S. EPA, 2005a), cancer descriptors represent points along a continuum of evidence; consequently, there are gradations and borderline cases. Therefore, although the tumor data for TCA can be considered consistent with the descriptor of "likely to be carcinogenic to humans," the evidence supporting the descriptor of "suggestive evidence of carcinogenic potential" was also considered. This descriptor is appropriate when a concern for potential carcinogenic effects in humans is raised, but the data are judged not sufficient for a stronger conclusion or when there are few pertinent data to form a conclusion about the agent's carcinogenic potential (U.S. EPA, 2005a).

EPA identified several limitations associated with the carcinogenic database for TCA and with some of the studies in particular. The only lifetime TCA cancer bioassay (104-week study by DeAngelo et al., 2008) was conducted in male mice only. The interpretation of the positive liver tumor findings (increases of 44 and 71% at the low and high dose, respectively) in this study is complicated by a high rate of background tumors (i.e., 55% in the controls). The remaining mouse cancer bioassays for TCA are limited to the $B6C3F_1$ strain only; other mouse strains have not been tested for TCA carcinogenicity. Additionally, other than Bull et al. (1990), the studies only evaluated a single sex and none of the studies performed a comprehensive histologic evaluation in all of the mice tested.

Cancer bioassay information in other species is limited to a lifetime study in male F344 rats (104-week study by DeAngelo et al., 1997) in which TCA did not induce tumors. GGT-positive foci (closely linked to the subsequent development of tumors) were observed in DEN-initiated male Sprague-Dawley rats following promotion with TCA; one rat developed a liver carcinoma (Parnell et al., 1988). However, the attribution of this effect to TCA is confounded by the fact that the treated rats also received a partial hepatectomy, which can itself act as a promoter. Because female rats were not included in the DeAngelo et al. (1997) bioassay and because other species have not been tested for TCA carcinogenic potential, limited conclusions can be drawn about the carcinogenic properties of TCA in experimental animals other than the $B6C3F_1$ mouse.

Based on an in-depth review of the available information related to TCA tumor induction and after consideration of all pertinent issues, EPA has concluded that despite the limited scope of testing, the positive findings observed in male and female $B6C3F_1$ mice and the early age of onset of tumor development in multiple studies is most consistent with a characterization that TCA is "likely to be carcinogenic to humans."

EPA's Cancer Guidelines (U.S. EPA, 2005a) indicate that for tumors occurring at a site other than the initial point of contact, the weight of evidence for carcinogenic potential may apply to all routes of exposure that have not been adequately tested at sufficient doses. An exception occurs when there is convincing toxicokinetic data that absorption does not occur by other routes. For TCA, systemic tumors were observed in mice following oral exposure.

Information on carcinogenic effects via the inhalation or dermal routes in humans or animals is absent. Data evaluating absorption by the inhalation route are unavailable and limited data are reported for dermal absorption (Kim and Weisel, 1998). However, TCA is highly soluble in water. Thus, it is reasonable to assume that TCA can be absorbed and taken up into the blood via the inhalation route. Moreover, based on the observance of systemic tumors following oral exposure, and in the absence of information to indicate otherwise, it is assumed that an internal dose will be achieved regardless of the route of exposure. Therefore, TCA is "likely to be carcinogenic to humans" by all routes of exposure.

The MOA for TCA-induced liver carcinogenesis has not been established. The available data collectively provide limited evidence regarding the genotoxicity of TCA. Tumor induction appears to include perturbation of cell growth, both through growth inhibition of normal cells and proliferation of selected cell populations. Specific mechanisms of altered growth control that have been investigated for TCA include activation of the PPARα pathway, global DNA hypomethylation, reduced intercellular communication, and oxidative stress. Of these, PPARα agonism has been advanced as the most likely MOA contributing to the development of liver tumors. However, significant gaps in knowledge exist in the hypothesized PPARα MOA (Ito et al., 2007; Yang et al., 2007), such that the formation of liver tumors cannot be sufficiently accounted for by this proposed MOA and the existence of other contributing MOA(s) is assumed.

___II.A.2. HUMAN CARCINOGENICITY DATA

None. There are no epidemiological studies of TCA carcinogenicity in humans. Most of the human health data for chlorinated acetic acids concern components of complex mixtures of water disinfectant byproducts. These complex mixtures of disinfectant byproducts have been associated with increased potential for bladder, rectal, and colon cancer in humans (reviewed by Boorman et al. [1999]; Mills et al. [1998]).

___II.A.3. ANIMAL CARCINOGENICITY DATA

The experimental database for carcinogenicity of TCA consists of studies in rats and mice. Studies in mice indicate that TCA is a complete carcinogen that significantly increased the incidence of liver tumors in male and female $B6C3F_1$ mice exposed via drinking water for 52–104 weeks (DeAngelo et al., 2008; Bull et al., 2004, 2002, 1990; Pereira et al., 2001, 1997; Pereira, 1996; Pereira and Phelps, 1996; Herren-Freund et al., 1987). Incidence of tumors increased with increasing TCA concentrations (DeAngelo et al., 2008; Bull et al., 2002, 1990; Pereira, 1996). These results were obtained under conditions where the background incidence of tumors in control animals was generally low. The development of tumors in animals exposed to TCA progressed rapidly, as evident from the observation of significant numbers of tumors in less-than-lifetime studies of ≤82 weeks. Positive evidence for tumor promotion by TCA (following exposure to known tumor initiators) has been reported for liver tumors in $B6C3F_1$ mice (Pereira et al., 2001, 1997) and for gamma-glutamyl transferase (GGT)-positive foci in livers of partially hepatectomized Sprague-Dawley rats (Parnell et al., 1988).

In contrast to the results observed for mice, treatment-related tumors were not observed in a study of male F344/N rats exposed to TCA via drinking water for 104 weeks (DeAngelo et

al., 1997). The carcinogenicity of TCA has not been evaluated in female rats or in other species of experimental animals. However, treatment of primary cultures of male Long-Evans rat hepatocytes with 0.01–1.0 mM TCA for 10–40 hours did not induce proliferation of the cultured hepatocytes (Walgren et al., 2005).

___II.A.4. SUPPORTING DATA FOR CARCINOGENICITY

Evidence for genotoxic activity of TCA is inconclusive. No mutagenicity was reported in *Salmonella typhimurium* strain TA100 in the absence of metabolic activation (Rapson et al., 1980) or in an alternative protocol using a closed system (DeMarini et al., 1994), but a mutagenic response was induced in this same strain in the Ames fluctuation test reported by Giller et al. (1997). Mutagenicity in mouse lymphoma cells was only induced at cytotoxic concentrations (Harrington-Brock et al., 1998). Measures of DNA-repair responses in bacterial systems are similarly inconclusive, with induction of DNA repair reported in *S. typhimurium* (Ono et al., 1991) but not in *Escherichia coli* (Giller et al., 1997). Although positive results were reported for unneutralized TCA in three in vivo cytogenetic assays by Bhunya and Behera (1987), later in vitro studies by Mackay et al. (1995), using neutralized TCA, reported negative results, suggesting that TCA-induced clastogenicity may occur secondary to pH changes. Some evidence for TCA-induction of hepatic DNA strand breaks and chromosome damage has been reported (Harrington-Brock et al., 1998; Giller et al., 1997; Nelson and Bull, 1988); however, these effects have not been uniformly reported (Chang et al., 1992; Styles et al., 1991) and may be related to low pH when TCA was not neutralized. TCA induced oxidative DNA damage in the livers of mice following a single dose (Austin et al., 1996), but not following repeated dosing over 3 or 10 weeks (Parrish et al., 1996).

__II.B. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM ORAL EXPOSURE

___II.B.1. SUMMARY OF RISK ESTIMATES

____II.B.1.1. Oral Slope Factor -- 7×10^{-2} (mg/kg-day)$^{-1}$

The oral slope factor is derived from the LED_{10}, the 95% lower bound on the exposure associated with an 10% extra cancer risk, by dividing the risk (as a fraction) by the LED_{10}, and represents an upper bound, continuous lifetime exposure risk estimate:

LED_{10}, lower 95% bound on exposure at 10% extra risk – 1.5 mg/kg-day
ED_{10}, central estimate of exposure at 10% extra risk – 5.7 mg/kg-day

The slope of the linear extrapolation from the central estimate ED_{10} is $0.1/(5.7 \text{ mg/kg-day}) = 1.8 \times 10^{-2}$ per mg/kg-day.

The slope factor for TCA should not be used with exposures exceeding the POD (1.5 mg/kg-day), because above this level the fitted dose-response model better characterizes what is known about the carcinogenicity of TCA.

____II.B.1.2. Drinking Water Unit Risk* -- 2×10^{-6} per µg/L

Drinking water concentrations at specified risk levels

Risk level	Lower bound on concentration estimate*
E-4 (1 in 10,000)	50 µg/L
E-5 (1 in 100,000)	5 µg/L
E-6 (1 in 1,000,000)	0.5 µg/L

* The unit risk and concentration estimates assume a water consumption of 2 L/day by a 70-kg human.

____II.B.1.3. Extrapolation Method

Multistage model with linear extrapolation from the POD (LED_{10}).

___II.B.2. DOSE-RESPONSE DATA

Tumor type − Hepatocellular adenomas or carcinomas
Test species – Male $B6C3F_1$ mice
Route – Oral (drinking water)
Reference – DeAngelo et al. (2008)

Incidence of hepatocellular adenomas, carcinomas, or adenomas and carcinomas combined in male $B6C3F_1$ mice exposed to TCA in drinking water for 104 weeks (DeAngelo et al., 2008)

TCA concentration (g/L)	Estimated intake[a] (mg/kg-day)	Human lifetime equivalent dose[b] (mg/kg-day)	Incidence of adenomas[c]	Incidence of carcinomas[c]	Incidence of adenomas or carcinomas[c]
0	0	0	10/56	26/56	31/56
0.05	6.7	1	10/48	15/48	21/48
0.5	81.2	12.8	20/51	32/51	36/51

[a]Estimated daily intakes were calculated with the mean measured TCA concentrations reported by DeAngelo et al. (2008) where available; if not, the nominal concentration for the dose group was used. See Appendix D, Table D-1 of the *Toxicological Review of Trichloroacetic Acid* (U.S. EPA, 2011) for details.
[b]Estimated daily intakes of TCA from the mouse study were converted to human equivalent doses for continuous lifetime exposure using an interspecies body weight scaling factor (body weight to the ¾ power) and exposure time adjustment factors.

[c]Individual animal data were obtained through the study author (email dated February 1, 2010, from Anthony DeAngelo, National Health and Environmental Effects Research Laboratory (NHEERL), Office of Research and Development (ORD), U.S. EPA, to Diana Wong, National Center for Environmental Assessment (NCEA), ORD, U.S. EPA). Because the first liver tumor occurred at 52 weeks or after, adenoma or carcinoma data for all mice examined histopathologically between weeks 52 and 104 were included.

___II.B.3. ADDITIONAL COMMENTS

In addition to the 104-week study of TCA in male $B6C3F_1$ mice that served as the basis for the TCA cancer slope factor, four other bioassays in $B6C3F_1$ mice exposed to TCA in drinking water were selected for analysis and derivation of candidate oral slope factor for TCA. These four bioassays consisted of two 52-week studies in male mice (Bull et al., 2002, 1990), a 60-week study in male mice (DeAngelo et al., 2008), and an 82-week study in female mice (Pereira, 1996). The candidate oral cancer slope factors derived from these four bioassays in mice ranged from 2.1×10^{-2} to 1.1×10^{-1} $(mg/kg\text{-}day)^{-1}$.

Consideration was also given to whether the liver tumor incidence data from the three bioassays conducted by DeAngelo et al. (2008) could be combined to derive an oral cancer slope factor. Statistical analysis revealed that two liver tumor data sets from DeAngelo et al. (2008), i.e., the 60-week study and the multi-dose 104-week study, were statistically compatible to be combined for multistage Weibull (MSW) time-to-tumor modeling. The cancer slope factor derived from the combined data set was 7.2×10^{-2} $(mg/kg\text{-}day)^{-1}$, and was similar to the cancer slope factor of 7×10^{-2} $(mg/kg\text{-}day)^{-1}$ derived from male mouse liver tumor data from the 104-week DeAngelo et al. (2008) study using the multistage model in BMDS.

___II.B.4. DISCUSSION OF CONFIDENCE

Confidence in the oral slope factor and extrapolation of cancer risks to low doses would be increased with the identification of precursor events for TCA-induced liver tumors and additional information concerning tumor responses in mice to drinking water concentrations <0.05 g/L TCA (the lowest tested concentration in the mouse bioassays).

__II.C. QUANTITATIVE ESTIMATE OF CARCINOGENIC RISK FROM INHALATION EXPOSURE

No inhalation unit risk (IUR) for TCA was derived. Cancer bioassays involving inhalation exposure to TCA are not currently available, and PBPK models that could be used to support route-to-route extrapolation for TCA have not been published. In the absence of a PBPK model, route-to-route extrapolation (from oral to inhalation) is not recommended because the liver is the critical target organ for oral toxicity, and first-pass effect by the liver is expected following oral administration. TCA is a strong acid, and it is possible that inhalation exposure may cause pathway-specific toxicity in the respiratory tract. Furthermore, the respiratory tract has not been evaluated in oral exposure studies. Therefore, an IUR for TCA was not derived.

___II.C.1. SUMMARY OF RISK ESTIMATES

Not applicable.

___II.C.2. DOSE-RESPONSE DATA

Not applicable.

___II.C.3. ADDITIONAL COMMENTS

Not applicable.

___II.C.4. DISCUSSION OF CONFIDENCE

Not applicable.

__II.D. EPA DOCUMENTATION, REVIEW, AND CONTACTS (CARCINOGENICITY ASSESSMENT)

___II.D.1. EPA DOCUMENTATION

Source Document -- *Toxicological Review of Trichloroacetic Acid* (U.S. EPA, 2011).

This document has been provided for review to EPA scientists, interagency reviewers from other federal agencies and White House offices, and the public, and peer reviewed by independent scientists external to EPA. A summary and EPA's disposition of the comments received from the independent external peer reviewers and from the public is included in Appendix A of the *Toxicological Review of Trichloroacetic Acid* (U.S. EPA, 2011).

___II.D.2. EPA REVIEW

Agency Completion Date -- __/__/__

___II.D.3. EPA CONTACTS

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202) 566-1676 (phone), (202) 566-1749 (fax), or hotline.iris@epa.gov (email address).

_III. [reserved]
_IV. [reserved]
_V. [reserved]

__VI. BIBLIOGRAPHY

Substance Name -- Trichloroacetic acid (TCA)
CASRN -- 76-03-9
Section VI. Last Revised -- 00/00/0000

__VI.A. ORAL RfD REFERENCES

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__VI.B. INHALATION RfC REFERENCES

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_VII. REVISION HISTORY

Substance Name -- Trichloroacetic acid (TCA)
CASRN -- 76-03-9
File First On-Line 10/01/1992

Date	Section	Description
00/00/0000	I., II.	RfD and cancer assessment sections updated. RfC discussion added.

_VIII. SYNONYMS

Substance Name -- Trichloroacetic acid (TCA)
CASRN -- 76-03-9
Section VIII. Last Revised -- 00/00/0000

76-03-9
Acetic acid, trichloro-
TCA
Aceto-Caustin
Acide trichloracetique [French]
Acido tricloroacetico [Italian]
Acido tricloroacetico [Spanish]

AI3-24157
Amchem Grass Killer
Caswell No. 870
EPA Pesticide Chemical Code 081002
HSDB 1779
Kyselina trichloroctova [Czech]
Trichloorazijnzuur [Dutch]
Trichloressigsäure [German]
Trichloroethanoic acid