

Dichloroacetic acid; CASRN 79-43-6

Human health assessment information on a chemical substance is included in the IRIS database only after a comprehensive review of toxicity data, as outlined in the [IRIS assessment development process](#). Sections I (Health Hazard Assessments for Noncarcinogenic Effects) and II (Carcinogenicity Assessment for Lifetime Exposure) present the conclusions that were reached during the assessment development 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](#).

STATUS OF DATA FOR Dichloroacetic acid

File First On-Line 02/01/1996

Category (section)	Assessment Available?	Last Revised
Oral RfD (I.A.)	yes	09/11/2003
Inhalation RfC (I.B.)	qualitative discussion	09/11/2003
Carcinogenicity Assessment (II.)	yes	09/11/2003

I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

I.A. Reference Dose for Chronic Oral Exposure (RfD)

Substance Name — Dichloroacetic acid
CASRN — 79-43-6
Last Revised — 09/11/2003

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is

essential to refer to other sources of information concerning the carcinogenicity of this 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.

I.A.1. Oral RfD Summary

Critical Effect	Experimental Doses*	UF	MF	RfD
	NOAEL: None			
Lesions observed in the testes, cerebrum, cerebellum, and liver.	LOAEL: 12.5 mg/kg-day	3,000	1	4E-03 mg/kg-day
Dog, Subchronic Oral Cicmanec et al. (1991)				

* Conversion Factors and Assumptions: While a benchmark dose analysis was performed on the data set from this study and others (see U.S. EPA 2003a), the results were considered less reliable than those from the NOAEL/LOAEL approach.

I.A.2. Principal and Supporting Studies (Oral RfD)

A study by Cicmanec et al. (1991) identified the lowest LOAEL (12.5 mg/kg-day) that has been established to date. In this study, groups of 5 male and 5 female juvenile beagle dogs were administered oral doses (12.5 to 72 mg/kg-day) of DCA in capsules for 90 days. Adverse effects noted in the low-dose group (12.5 mg/kg-day) included testicular degeneration, mild to moderate hepatic vacuolization in males and females, and mild vacuolization of the myelinated white tracts of the cerebrum and cerebellum in males.

Overt clinical signs were evident in the high-dose animals throughout the duration of the experiment. Dyspnea (shortness of breath or difficulty in breathing) was observed in high-dose animals starting at day 45, and worsened with time. Partial paralysis of the hind limbs was observed in three animals in the high-dose group during the latter half of the exposure period. Conjunctivitis was observed in 24/30 treated animals and a few controls during the first month, and became more severe later in the study. The occurrence of ocular effects appeared to be dose-related, with 8 of 10 high-dose dogs affected. Reduced food and water intake was noted in DCA-treated dogs, although the effect did not appear to be dose-related. High-dose

males exhibited a 16% reduction in body weight, while high-dose females and mid-dose males experienced a 9% reduction in weight gain over the duration of the study. Mid-dose females exhibited an 11% reduction in weight gain. Dogs in the mid- and high-dose groups experienced sporadic diarrhea. The most severely affected dogs required fluid therapy to prevent severe dehydration. One female and two males treated at 72 mg/kg-day died during the study. The deaths were attributed to pneumonia and dehydration.

Statistically significant decreases in erythrocyte count and hemoglobin levels were observed in high-dose dogs at day 30. Trend analysis of serum biochemistry data indicated apparent increases in lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase activity in the high-dose groups at some time points. The findings were consistent with microscopic tissue observations.

At study termination, organ weights were determined and tissues were examined microscopically. Relative liver weights significantly increased in all dose groups, and absolute liver weight increased in all but high-dose males. Relative kidney weights increased in mid- and high-dose males and females, and relative lung weights increased in high-dose males and females. Pathological examination revealed multiple changes in the organs of animals treated with DCA, including: pale and discolored kidney lesions; mild vacuolar changes (most prevalent at the low dose); inflammation and hemosiderosis in the liver; and chronic inflammation and acinar degeneration in the pancreas. Liver, brain, and pancreas lesions were considered primary, whereas those in kidneys and lungs were considered secondary. The severity of the lesions was ranked as mild or moderate. Microscopic examination of the brain revealed mild vacuolization of white myelinated tracts in the cerebrum and/or cerebellum of some animals in the low-, mid-, and high-dose groups. Brain or spinal cord lesions were not observed in low-dose females, however. Mild vacuolar changes were noted in the medulla and spinal cord of some mid- and high-dose males, while mild meningoencephalitis was present in one high-dose female. Microscopic testicular lesions, which included syncytial giant cell formation and degeneration of testicular germinal epithelium, were also noted in treated males at all doses. Lesion severity increased with dose. The testicular changes were apparent in 4/5 males at the low dose and in all animals in the mid- and high-dose groups; these lesions were also considered to be primary. A LOAEL of 12.5 mg/kg-day can be identified based on visual organ effects (neurological changes, hepatic vacuolization, and testicular effects) and increases in liver weight.

I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF = 3,000

The uncertainty factor includes a factor of 10 to account for potential interhuman variability in susceptibility to DCA, a factor of 3 to account for extrapolation from animal data to humans, a factor of 10 to account for the use of a LOAEL, a factor of 3 to account for the use of a less-than-lifetime study in which frank effects were noted, and a factor of 3 to account for deficiencies in the database.

MF = 1

I.A.4. Additional Studies/Comments (Oral RfD)

Katz et al. (1981) studied the subchronic administration of sodium dichloroacetate (0, 50, 75, or 100 mg/kg-day by capsule for 13 weeks) to four beagle dogs/sex in the control and high-dose groups and three dogs/sex in the other groups. Female dogs at all doses showed markedly reduced appetites and both sexes exhibited dose-dependent weight losses, which were reversed after the treatment ended. One female at 75 mg/kg-day died on day 40, and one at 100 mg/kg-day died on day 88. The animals exhibited anorexia, ataxia, hind limb weakness and reduced activity. Bloody stools, vomiting, and paralysis were also observed at 100 mg/kg-day. Dose-related decreases in erythrocyte counts, hematocrits, and hemoglobin levels were reported. Mean blood glucose, lactate and pyruvate levels significantly decreased in all treated animals. The parameters returned to normal following treatment. Treated dogs also exhibited lung consolidation. Histopathology showed neurological effects (slight to moderate vacuolization of white myelinated tracts in the cerebrum and cerebellum) and liver and gallbladder (an increased incidence of hemosiderin-laden Kupffer cells in the liver and cystic mucosal hyperplasia in the gallbladder); these effects were persistent through the 5-week recovery period. Indirect effects, including increased incidence and/or severity of pulmonary inflammatory lesions were also attributed to DCA treatment. Marked testicular degeneration was noted in male dogs administered doses ≥ 50 mg/kg-day. Based on the study results, the lowest dose of 50 mg/kg-day was identified as a LOAEL.

Although DCA-induced testicular toxicity has not been investigated in the human, it has been reported in rodent models. Bhat et al. (1991) reported significantly ($p < 0.01$) decreased testes weight and signs of tissue atrophy with no mature spermatozoa and few spermatocytes in seminiferous tubules of male Sprague-Dawley rats exposed to 1,100 mg/kg-day DCA (in drinking water) for 90 days. Katz et al. (1981) administered doses of 0, 125, 500, or 2000 mg/kg-day DCA via gavage to adult rats (10-15/sex/dose) daily for 3 months. All males at 2,000 mg/kg-day and 40% of males at 500 mg/kg-day exhibited testicular germinal epithelial degeneration. Further, all males at 2,000 mg/kg-day had aspermatogenic testes with syncytial giant cells in the germinal epithelium and epididymis ducts that were devoid of spermatozoa. Twenty percent of the 500 mg/kg-day males also had syncytial giant cells. No other effects were noted at 500 or 125 mg/kg-day; no reproductive tissue effects were noted in females at

any dose. Some evidence of regeneration of germinal epithelium with spermatogenesis was noted in some high-dose males maintained for 5 weeks on a normal control diet postexposure. Based on these results, a NOAEL of 125 mg/kg-day was identified.

Toth et al. (1992) dosed male Long-Evans rats by oral gavage with 0, 31.25, 62.5, or 125 mg/kg-day dichloroacetate for 10 weeks and evaluated the reproductive effects. Reduced final animal weights were reported in the mid- and high-dose groups. Relative liver weights increased at all doses, while relative kidney and spleen weights increased only at the mid- and high-dose. Significant ($p \leq 0.05$) reductions in the absolute weight of the preputial gland and epididymis were noted at all dose levels, but the absolute weight of the testis was not affected at any dose. At the two higher doses (62.5 and 125 mg/kg-day), there were significant ($p \leq 0.05$) reductions in the percentage of motile sperm, effects on sperm motion (i.e., velocity, linearity, amplitude of lateral head displacement) and reduced epididymis sperm head counts. At 125 mg/kg-day, animals also had reduced accessory organ (prostate and seminal vesicle) weights and increased relative testis weights. Histological examination of testis cross sections did not reveal any gross lesions at any dose, and cellular structures in the epididymis epithelium appeared normal. Impaired spermiation was noted in 4/10 mid-dose animals and 9/10 high-dose animals, and was attributed to the retention of late-step spermatids in the seminiferous tubules.

In addition to the neurotoxicity discussed above in the studies of Cicmanec et al. (1991) and Katz et al. (1981), neurotoxic effects have also been reported following subchronic or chronic exposure of humans and rats to DCA. Stacpoole et al. (1998) reviewed observations in humans that have accrued from nearly 25 years of experimental DCA clinical use, primarily in the treatment of congenital lactic acidosis. Therapeutic doses of DCA are usually in the range of 25-50 mg/kg-day (either oral or intravenous). In a few cases, treatments at 25 mg/kg-day have occurred for as long as 5 years. Evidence of clinically significant DCA toxicity in humans is primarily limited to the central and peripheral nervous system. Approximately 50% of patients receiving 25-50 mg/kg-day experience sedative effects. This effect is observed following oral, intravenous, or repeated dosing regimens. At the time the Stacpoole et al. (1998) study was published, there were three reported cases of peripheral neuropathy following DCA treatment; all were completely reversible within 6 months of cessation of treatment. In one of these cases, following reversal of the neurological symptoms, DCA treatment at 10 to 25 mg/kg-day was resumed and maintained for 2 years without further evidence of neuropathy. Two children that were treated for congenital lactic acidosis with 25-75 mg/kg-day DCA orally for several months had a twofold increase in serum transaminases, suggesting preclinical hepatic toxicity. This increase was also reversible after treatment ended.

In a more recent human study, nerve conduction velocities and amplitudes were studied for one year in 27 patients with congenital lactic acidosis who received sodium dichloroacetate

treatment (Spruijt et al., 2001). The patients (16 males and 11 females whose ages ranged from 9 months to 37.4 years [mean 9.8 ± 9.4 years]), were started on 50 mg/kg-day DCA and were coadministered 100 mg/day thiamine. Lactate and plasma DCA concentrations were measured at 3, 6, and 12 months, and the pharmacokinetics of DCA were measured at 3 and 12 months. All but two of the patients had normal baseline nerve conduction tests prior to DCA administration. Twelve of the patients (9 male, 3 female) who had prior normal baseline electrophysiology showed evidence of neuropathy (decreased nerve conduction velocity and response amplitude) by the end of treatment. Three patients showed neuropathy within 3 months of treatment. Neuropathy increased during treatment in the two patients who exhibited neuropathy prior to the start of therapy. Patients with neuropathy were notably older than those with normal electrophysiology. While age was significantly correlated with the deterioration in conduction of some nerves at some time points, there were insufficient individuals in the study to provide statistical power to test age and the deterioration of most nerves.

Central nervous system effects and peripheral neuropathy have also been reported in male and female rats via oral gavage or drinking water exposures at doses as low as 125 mg/kg-day for 10 weeks or longer (Katz et al., 1981; Moser et al., 1999). Katz et al. (1981) administered sodium dichloroacetate at dose levels of 0, 125, 500, or 2,000 mg/kg-day to Sprague-Dawley rats (10 to 15/sex/group) by gavage for 3 months. Five more rats per sex were added to the control group and to the high-dose group and monitored for an additional 4 weeks after the 3-month feeding was discontinued. The major signs of intoxication were hind limb paralysis and frequent urination. Two rats (one of each sex) exhibiting these signs appeared to recover completely during the 4-week recovery period. Brain lesions (characterized by vacuolization of the myelinated white tracts) were observed in the cerebrum and cerebellum of treated rats of both sexes in all dose groups (combined incidence rates of 60% at 125 mg/kg-day and 100% at 500 and 2,000 mg/kg-day). In three rats from the recovery group, the lesions persisted after cessation of treatment.

Moser et al. (1999) extended the evaluation of the neurotoxic effects of DCA exposure in a series of experiments in weanling and adult rats from the F344 and Long-Evans (LE) strains. The study used a neurobehavioral screening battery under varying exposure durations (acute, subchronic, and chronic) and routes of administration (oral gavage and drinking water). In one experiment, LE and F344 rats (68-69 day old) were administered DCA (via drinking water) at doses of 23, 122, or 220 mg/kg-day (LE rats) and 18, 91, or 167 mg/kg-day (F344 rats) for 8 weeks (plus 2-week recovery period). Some of the F344 rats in the low-dose group showed gait abnormalities. Gait abnormalities and decreased forelimb and hind limb grip strength were noted in the mid- and high-dose LE and F344 rats. With the exception of gait deficit and decreased hind limb grip strength, both strains showed recovery 2 weeks after the exposure

was discontinued. In F344 rats, the low dose of 18 mg/kg-day was a LOAEL for gait abnormalities. In LE rats, 23 mg/kg-day was a NOAEL and the LOAEL was 122 mg/kg-day.

A second experiment by the same authors (Moser et al., 1999) involved the differential susceptibility of weanling LE rats or weanling F344 rats to DCA in drinking water at dose levels of 17, 88 or 192 mg/kg-day (LE) and 16, 89, 173 mg/kg-day (F344) for 13 weeks. Changes were assessed using a functional observation test battery and monitoring of motor activity. The results indicated that both rat strains showed progressive changes in gait in all treated groups (LOAEL, 17 mg/kg-day in LE rats and 16 mg/kg-day in F344 rats). The effect was most pronounced in the high-dose F344 rats. Hind limb grip strength decreased throughout exposure in the mid- and high-dose LE rats (no dose-response relationship was apparent) and in the high-dose F344 rats. The effect was more pronounced in the high-dose F344 rats. Other effects at the high-dose in both strains included tremor, hypotonia and inhibition of pupil reflex. The study authors reported that F344 rats, but not the LE strain, showed a progressive decrease in motor activity, righting deficits, and forelimb grip strength, and an increase in foot splay. Data were presented only for forelimb grip strength which was slightly decreased (<5%) at the mid-dose; the decrease was more pronounced (approximately 20%) at the high dose level.

The results of the Moser et al. (1999) study indicated that DCA is a more potent neurotoxicant when administered to adult rats via drinking water than by gavage (results for gavage studies are not discussed here). The results also revealed that gait abnormality is a critical effect for DCA; this effect was observed at doses as low as 16 mg/kg-day (in the absence of other neuromuscular changes) and was persistent in adult rats of both strains at doses ≥ 91 mg/kg-day (F344) even following a 2-week recovery period. Hind limbs may be preferentially affected by DCA. F344 rats appeared to be more sensitive than the Long-Evans strain. Limited results show that the severity of neuromuscular toxicity was somewhat greater in rats when exposures began shortly after weaning.

The primary metabolic pathway for DCA involves oxidative dechlorination to form glyoxylate (Larson and Bull, 1992). This reaction is NADPH- and GSH-dependent and occurs predominantly in the cytosol (Board et al., 1997; Cornett et al., 1997; Lipscomb et al., 1995; Stacpoole et al., 1998). The rat ortholog of human GST Zeta has been identified (Tong et al., 1998a, 1998b). Rodent and human orthologs of GST Zeta have been identified (Blackburn et al., 2000, 2001; Tong et al., 1998a, b).

Data on DCA metabolism in humans are available because, as mentioned above, DCA has been used experimentally in the therapeutic treatment of several metabolic disorders. The data obtained support the hypothesis that DCA metabolism is similar in both humans and rodents (Stacpoole et al., 1998). Limited data suggest that, in at least some individuals, a reductive

dechlorination pathway can occur initially after DCA administration, but continued DCA metabolism occurs through the oxidative dechlorination pathway (Stacpoole et al., 1998).

GST Zeta appears to be identical to maleylacetoacetate isomerase (MAAI), the enzyme in the pathway for tyrosine catabolism that converts the *cis* double bond in maleylacetoacetate (MAA) to the *trans* double bond in fumarylacetoacetate, using GSH as a cofactor (Fernandez-Canon and Penalva, 1998). In humans, the enzyme is expressed mostly in the liver followed by kidney, skeletal muscle, and brain. It is also expressed in the placenta, mammary tissues, and fetal liver and heart (Polekhina et al., 2000). It is also expressed in the placenta, heart, pancreas, mammary tissues, seminal glands and fetal liver (Fernandez-Canon et al., 1998; Polekhina et al., 2000).

There are species- and age-related differences in the activity of GST Zeta. For example, the relative rate of DCA transformation in mouse hepatic cytosol was greater in rat cytosol which was greater in human hepatic cytosol (Tong et al., 1998a). The K_m and V_{max}/K_m values for DCA in mice were $81.9 \pm 5.6 \mu\text{M}$ and $52.9 \pm 2.46 (x10^{-3})$ respectively, those in rats were $70.1 \pm 5.3 \mu\text{M}$ and $32.4 \pm 4.87 (x10^{-3})$, and those in humans were $47.3 \pm 6.7 \mu\text{M}$ and $8.25 \pm 1.37(x10^{-3})$ (Tong et al., 1998a). Schultz et al. (2002) reported that the K_m values for DCA transformation in young mice exceed those of adult mice.

There are five known polymorphisms of GST Zeta in humans that may account for differences in the ability to metabolize DCA and other halogenated compounds (Blackburn et al., 2000, 2001; Sheehan et al., 2001). Variants are designated as GSTZ1a-1a, GSTZ1b-1b, GSTZ1c-1c, GSTZ1d-1d and GSTZ1e-1e (Blackburn et al., 2000, 2001; Tzeng et al., 2000). Analysis of a one Caucasian population (128 subjects) showed that the allele variants were present with frequencies of 0.086, 0.285, and 0.473, 0.156 and 0, respectively (Blackburn et al., 2001). These results were similar to the analysis of three of the variants in a second group of Caucasian subjects (Blackburn et al., 2000). Although GSTZ1a-1a has a 4- to 5-fold higher activity toward DCA, it appeared with the lowest frequency in the populations studied by Blackburn et al. (2000, 2001).

Prior exposure to DCA also results in significant inhibition of its metabolism (Gonzalez-Leon et al., 1997a, b; Curry et al., 1991; Lukas et al., 1980; Schultz et al., 2002). Studying the plasma half-life of DCA in human volunteers, Curry et al. (1985) found that the mean half-life of DCA increased from 63.3 minutes to an average of 374 minutes following the fifth in a series of 50 mg/kg doses administered intravenously at 2-hour intervals. In another study of healthy adults in which 25 mg/kg DCA was administered daily for five days, the half-life was increased about eightfold on the fifth day as compared to the first ($1.09 \pm 0.45 \text{ hr}$ vs. $8.03 \pm 5.62 \text{ hr}$; Stacpoole et al., 1998). The most likely basis for the decrease in DCA clearance that was observed with repeated or high dose DCA exposure is the inactivation of one or more of

the enzymes involved in its metabolism. Tong et al. (1998a) demonstrated that prior DCA exposure in rats substantially reduces the cytosolic conversion of DCA to glyoxylate, and further demonstrated that this suppression was due to the inhibition of GST Zeta. Tzeng et al. (2000) reported that the most frequent human GST variant (GSTZ1c-1c) observed by Blackburn et al. (2000) is one that has a low activity toward DCA and is also impacted by DCA inhibition to a greater extent than the most active enzyme variant (GSTZ1a-1a). Accordingly, one might expect poor clearance of DCA from human plasma via oxidative dechlorination when exposure is continuous.

For more detail on Susceptible Populations, exit to [the toxicological review, Section 4.7 \(PDF\)](#).

I.A.5. Confidence in the Oral RfD

Study — High

Database — Medium

RfD — Medium

Available data suggest that all of the characteristic noncancer effects in humans and animals occur with similar dose-response patterns, with effect levels of about 25 mg/kg-day or higher. However, the dog appears to be more susceptible, at least with respect to testicular effects in the male. In this case, clear effects have been noted at a dose of 12.5 mg/kg-day (Cicmanec et al., 1991) while a NOAEL has not been established. Besides testicular toxicity, neurological changes, hepatic vacuolization, and increased liver weight were observed in males and females at 12.5 mg/kg-day. The basis for the increased sensitivity of the dog is not certain, but may be due to a relatively low capacity to metabolize DCA and clear it from the plasma. However, the only metabolic data come from one dog and are inadequate to support any firm conclusion on canine metabolism of DCA (Lukas et al., 1980). In addition, there are no data on the correlation of dose-to-GST Zeta inhibition in dogs. Using the LOAEL of 12.5 mg/kg-day identified in the dog, and applying an uncertainty factor of 3000 to account for use of a LOAEL, extrapolation from animals to humans, potential interhuman variability in sensitivity, and database limitations, an oral RfD of 4E-03 mg/kg-day has been derived. Data from Cicmanec et al. (1991) could not be reliably evaluated using the BMD approach.

The overall confidence in the RfD is medium. Confidence in the principal study is high because the study was well-designed and evaluated appropriate endpoints. Although the reproductive toxicity (and systemic toxicity in other organs) has been evaluated in additional species, there currently exists no multigenerational reproductive toxicity study. While no adverse effects have been reported in humans at doses lower than 25 mg/kg-day, systematic investigations of potential hepatic or reproductive effects in therapeutically-treated humans

have not been performed. Metabolic effects have been observed in humans (both those requiring therapeutic treatment and normal controls) at doses ≤ 25 mg/kg-day demonstrate that DCA influences several human enzyme systems. However, the observed metabolic effects (decreases in fasting glucose, plasma lactate, and alanine) cannot be classified as adverse. Data on comparative metabolism in humans and dogs are lacking and it has yet to be established whether the various aspects of DCA toxicity are due to the parent compound or one or more metabolites. The confidence in the database is medium based on an overall confidence in the quality of the studies, but not in the comprehensiveness of them. Therefore, overall confidence in the RfD is medium.

For more detail on Characterization of Hazard and Dose Response, exit to [the toxicological review, Section 6 \(PDF\)](#).

I.A.6. EPA Documentation and Review of the Oral RfD

Source Document — U.S. EPA, 2003a

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS Summary. A record of these comments is included as an appendix to U.S. EPA, 2003a. [To review this appendix, exit to the toxicological review, Appendix A, External Peer Review-Summary of Comments and Disposition \(PDF\)](#).

Date of Agency Consensus — 08/20/2003

I.A.7. EPA Contacts (Oral RfD)

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 for Chronic Inhalation Exposure (RfC)

Substance Name — Dichloroacetic acid

CASRN — 79-43-6

Last Revised — 09/11/2003

The inhalation Reference Concentration (RfC) is analogous to the oral Reference Dose (RfD) and is likewise based on the assumption that thresholds exist for certain toxic effects such as

cellular necrosis. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory effects). It is generally expressed in units of mg/m^3 . In general, the RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily inhalation exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Inhalation RfCs are derived according to the Interim Methods for Development of Inhalation Reference Doses (EPA/600/8-88/066F, August 1989) and subsequently, according to Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry (EPA/600/8-90/066F, October 1994). RfCs can be derived for the noncarcinogenic health effects of substances that are carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this 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.

I.B.1. Inhalation RfC Summary

There are no studies examining inhalation exposure to DCA. Therefore, it is not possible to develop an inhalation RfC for this compound. DCA has low volatility, and inhalation exposure to DCA is not considered to be of great concern.

I.B.2. Principal and Supporting Studies (Inhalation RfC)

Not applicable.

I.B.3. Uncertainty and Modifying Factors (Inhalation RfC)

Not applicable.

I.B.4. Additional Studies/Comments (Inhalation RfC)

Not applicable.

For more detail on Susceptible Populations, exit to [the toxicological review, Section 4.7 \(PDF\)](#).

I.B.5. Confidence in the Inhalation RfC

Not applicable.

For more detail on Characterization of Hazard and Dose Response, exit to [the toxicological review, Section 6 \(PDF\)](#)

I.B.6. EPA Documentation and Review of the Inhalation RfC

Source Document — U.S. EPA, 2003a

[To review this appendix, exit to the toxicological review, Appendix A, External Peer Review-Summary of Comments and Disposition \(PDF\).](#)

Date of Agency Consensus — 08/20/2003

I.B.7. EPA Contacts (Inhalation RfC)

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 — Dichloroacetic acid

CASRN — 79-43-6

Last Revised — 09/11/2003

Section II 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 exposure and inhalation exposure. Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

The rationale and methods used to develop the carcinogenicity information in IRIS is described in the Draft Revised Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999. Guidelines for carcinogen risk assessment. Review Draft, NCEA-F-0644, July. Risk Assessment Forum <http://www.epa.gov/cancerguidelines/draft-guidelines-carcinogen-ra-1999.htm>). The quantitative risk estimates result from application of a low-dose extrapolation procedure, and both the central estimate and upper bound estimate of risk per unit of exposure are presented. The quantitative risk estimates are presented in three ways to facilitate their use. The oral slope factor is the 95% upper bound on the estimate of risk per (mg/kg)/day of oral

exposure. The unit risk is the 95% upper bound on the estimate of risk, either per $\mu\text{g/L}$ drinking water or per $\mu\text{g}/\text{cu.m}$ air breathed. The third form in which risk is presented is the 95% lower bound on the estimated concentration of the chemical in drinking water or air associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000.

II.A. Evidence for Human Carcinogenicity

II.A.1. Weight-of-Evidence Characterization

EPA finds there are no data on humans indicating that DCA is a carcinogen. However, there is sufficient evidence to conclude that DCA is carcinogenic in at least two species of experimental animals. A statistically significant and dose-related incidence of hepatocellular adenomas and carcinomas occur in male and female mice, and male rats. Large foci of cellular alteration (LFCAs, formerly called hyperplastic nodules), which are expected to progress into hepatocellular adenomas and carcinomas, increased in rats and mice. Additional support is provided by: (1) the number of independent studies reporting consistently positive results and at roughly comparable doses, (2) site concordance for tumor formation between two species, (3) clear evidence of a dose-response relationship for tumor incidence and multiplicity, and (4) apparent development of tumors from more than one hepatic cell line and no clear data supporting a cohesive mode of action. Therefore, EPA believes that DCA is *likely to be a carcinogen* in humans.

EPA published a cancer weight-of-evidence review for DCA in IRIS in 1996 (U.S. EPA, 2003c). This review classified DCA as a Group B2 (probable human carcinogen) compound in accordance with EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996). In 1995, IARC concluded that, based on the data available at that time, DCA is not classifiable as to its carcinogenicity to humans and placed DCA in the IARC Group 3 category (not classifiable as to carcinogenicity in humans). However, in 2002, IARC (IARC, 2002) reviewed the expanded DCA database and changed the DCA cancer classification to Group 2B (possibly carcinogenic to humans) based on sufficient evidence of carcinogenicity in experimental animals (two species) and inadequate evidence of carcinogenicity in humans.

For more detail on Characterization of Hazard and Dose Response, exit to [the toxicological review, Section 6 \(PDF\)](#).

For more detail on Susceptible Populations, exit to [the toxicological review, Section 4.7 \(PDF\)](#).

II.A.2. Human Carcinogenicity Data

Inadequate.

Human data on the carcinogenic potential of DCA are not available. The primary source of exposure to dichloroacetic acid in humans is as a byproduct of chlorination of drinking water. Since the early 1970s, numerous epidemiological studies have attempted to assess the relationship between the chlorination byproducts in drinking water and several different human cancers. None of these studies has shown an association of exposure to DCA with an increased incidence of site-specific cancer (U.S. EPA, 1998). Further, even if the data established an increase in cancer risk that is attributable to ingestion of chlorinated water, it cannot be concluded from these studies that DCA per se is carcinogenic in humans, since chlorinated water contains a wide spectrum of potentially carcinogenic disinfection by-products.

II.A.3. Animal Carcinogenicity Data

Sufficient.

DeAngelo et al. (1999) exposed male B6C3F₁ mice to 0, 0.05, 0.5, 1, 2, or 3.5 g/L of DCA in drinking water for 90-100 weeks. This corresponded to mean daily doses of 0, 8, 84, 168, 315, or 429 mg/kg-day, respectively. The cumulative incidence of hepatocellular carcinomas significantly increased in animals exposed to 1 g/L (71%), 2 g/L (95%), and 3.5 g/L (100%) when compared to controls (26%). Hepatocellular carcinoma multiplicity (tumor/animal) significantly increased in all treatment groups as follows: 0.05 g/L (0.58), 0.5 g/L (0.68), 1 g/L (1.29), 2 g/L (2.47) and 3.5 g/L (2.90) compared to the control group (0.28). The cumulative incidence of hepatocellular adenomas was also significantly increased in animals exposed to 1 g/L (51.4%), 2 g/L (42.9%), and 3.5 g/L (45%) as compared to controls (10%) and the 0.5 g/L group (20%). Hepatocellular adenoma multiplicity (tumor/animal) significantly increased in the following dose groups: 0.5 g/L (0.32), 1 g/L (0.80), 2 g/L (0.57), and 3.5 g/L (0.64) as compared to controls (0.12). Hepatic peroxisome proliferation increased in the high-dose group, but did not correlate with liver tumor response. The severity of hepatotoxicity increased with DCA concentration. Below 1 g/L, hepatotoxicity was mild and transitory (as evidenced by histopathological examination and serum enzyme levels) and there was no significant increase in labeling index outside of proliferative lesions. Thus, peroxisome induction or chemically-sustained cell proliferation were not necessary for the development of tumors.

Supporting data for the carcinogenicity of DCA in male mice comes from an older study by DeAngelo et al. (1991) in which groups of 50 male B6C3F₁ mice received drinking water containing neutralized DCA at levels of 0, 0.05, 0.5, 3.5, and 5 g/L (corresponding to time-

weighted mean intakes of 7.6, 77, 410, and 486 mg/kg-day). Interim sacrifices of 5/group were scheduled at 4, 15, 30 and 45 weeks. Controls received 2 g/L sodium chloride (NaCl) or 1.5 g/L acetic acid; an additional group of 12 mice received 3.5 g/L DCA (410 mg/kg-day). At 60 weeks, all mice receiving 3.5 and 5 g/L were sacrificed, as well as 9-10 mice in each of the other groups. The remaining mice in the NaCl control, 0.05 and 0.5 g/L groups (18-19/group) were sacrificed at 75 weeks. Final mean body weights were significantly lower in the 3.5 and 5 g/L groups (87 and 83% of control, respectively). Statistically significant increases in relative liver weights were reported in the 0.5, 3.5, and 5 g/L groups (36, 132, and 250% greater than control) at 60 weeks. Histopathological examination was performed on the liver, kidney, testes and spleen. Hepatocellular adenomas in the 0.5 and 3.5 g/L groups were first observed (in sacrificed animals) at 45 and 30 weeks, respectively, while hepatocellular carcinomas in the 0.05 g/L group were first observed at 60 weeks. In the high-dose group, hepatocellular adenomas and carcinomas were first observed at 45 weeks. At the 60-week sacrifice, hepatocellular adenomas were present in 12/12 (100%) and 24/30 (80%) mice receiving 3.5 and 5 g/L, respectively, and carcinomas were found in 8/12 (67%) and 25/30 (83%) mice, respectively. The incidences of combined hepatocellular tumors showed a statistically significant increase, 100% and 90% for the 3.5 and 5 g/L groups, respectively, and the multiplicity of liver tumors was 4 and 4.5/mouse, respectively. Hyperplastic liver nodules were noted in 7/12 (58%) and 25/30 (83%) of the 3.5 and 5 g/L groups, respectively (incidence not significantly elevated).

Male B6C3F₁ mice (33/group) were treated with 0 or 0.5 g/L DCA (0 or 88 mg/kg-day) in drinking water for 104 weeks (Daniel et al., 1992). Interim sacrifices were performed on five animals/group at 30 weeks and five control animals at 60 weeks. All organs and tissues were examined histopathologically. Survival was not affected in the study. Absolute and relative liver weights were significantly increased in the treated group (52 and 57%, respectively, of control). Nonneoplastic changes in the liver of the 0.5 g/L animals included hepatocellular necrosis (33% incidence), hyperplasia (33%), and cytomegaly (92%), compared with controls (5%). Hepatocellular carcinomas developed in 15/24 (63%) treated mice compared with 2/20 (10%) controls, and hepatocellular adenomas occurred in 10/24 (42%) treated mice compared with 1/20 (5%) controls. The incidence of combined hepatocellular tumors was 18/24 (75%) in 0.5 g/L mice compared with 3/20 (15%) in controls. Individual type and combined tumor incidences showed a statistically-significant increase in the treated group compared with controls. Hyperplastic liver nodules in treated males were not significantly increased over controls.

Herren-Freund et al. (1987) investigated the initiation/promotion potential of DCA in male B6C3F₁ mice (26-32/group). Mice were either given 5 g/L DCA or were pretreated with a single intraperitoneal dose of 2.5 mg/kg ethylnitrosourea (ENU) at 15 days of age and then given 2 or 5 g/L DCA in the drinking water (400 or 1000 mg/kg-day as calculated by the

authors) during the period from 4 to 65 weeks of age. The negative control group (66 animals) received 2 g/L NaCl in drinking water. Animals were sacrificed after 61 weeks of exposure. Survival data were not provided. A significant decrease in the final mean body weight was observed in the 5 g/L groups (uninitiated and initiated) (both 92% of control), while a significant increase in the relative liver weight was found in all treatment groups (140-190% of control). In the uninitiated group receiving 5 g/L DCA, the incidences were 25/26 (96%) for hepatocellular adenomas and 21/26 (81%) for hepatocellular carcinomas compared with 2/22 and 0/22, respectively, for the controls. For the initiated groups, the incidence was 22/29 (76%) and 31/32 (97%) for hepatocellular adenoma in the 2 and 5 g/L DCA groups, respectively, compared with 2/22 (9%) in the controls. The incidence was 19/29 (66%) and 25/32 (72%) for hepatocellular carcinomas in the initiated 2 and 5 g/L DCA groups, respectively, compared with 1/22 (4.5%) in the controls. A statistically-significant increase of all tumor incidences in the treated groups was observed. Hyperplastic nodules were not reported in the treated animals, and no other tumor sites were found.

Bull et al. (1990) also demonstrated that DCA was carcinogenic in male B6C3F₁ mice. In the study, males received 1 g/L DCA (170 mg/kg-day) in drinking water for 52 weeks (11 animals) or 2 g/L (340 mg/kg-day; 11-24 animals) for 37 or 52 weeks. Ten females received 2 g/L DCA for 52 weeks. The control group consisted of 61 males (sacrificed at 15, 24, 37, or 52 weeks) and 10 females (sacrificed at 52 weeks). No effects of treatment on survival or body weight were observed. Food and water consumption data were not reported. A significant increase in the relative liver weight was seen in 1 g/L males (41% increase from control), 2 g/L males (128% increase), and 2 g/L females (88% increase) compared with controls at 52 weeks. Nonneoplastic lesions included degenerative changes (multifocal areas of necrosis with frequent lymphocyte infiltration), cytomegaly and glycogen accumulation in the liver of all treated animals (incidence data were not provided). The high-dose mice also exhibited basophilic foci of cellular alteration in the livers at the 24- and 37-week sacrifice. Hepatocellular adenomas and carcinomas both developed only in the 2 g/L males; in 2/11 and 2/24 males treated for 37 and 52 weeks, respectively. Hepatocellular carcinomas alone developed in 5/24 males treated for 52 weeks. Tumor incidences in all treated groups were statistically significantly elevated. Hyperplastic nodules were observed in 6/11 and 9/24 males treated with 2 g/L DCA for 37 and 52 weeks, respectively, and in 3/10 females treated with 2 g/L for 52 weeks. The appearance of hyperplastic nodules in the treated females suggests that tumors might develop in female mice if DCA exposure continued beyond 52 weeks. Tumors were not found in any other organs. Since the total exposure duration in this study did not exceed 52 weeks, this study may not have evaluated animals for an adequate length of time to observe the full carcinogenic potential of DCA in mice. In addition, the numbers of animals tested are inadequate.

U.S. EPA (1991) reported on the carcinogenicity of DCA in female B6C3F₁ mice. Following exposure to 0, 0.5 or 3.5 g/L DCA in drinking water for 104 weeks, the high-dose group had a 100% hepatocellular tumor incidence and a tumor multiplicity of 8.36 tumors/animal. Females receiving 0.5 g/L DCA had a tumor incidence of 20% and a tumor multiplicity of 0.2 tumors/animal. These values were compared with an incidence of 7.7% and a multiplicity of 0.1 tumors/animal in the control group. It was concluded that, although statistical analysis was not performed for the study, it appeared that female mice may be less sensitive than male mice.

Two more recent studies evaluated the carcinogenic response of DCA exposure in female mice. In the first study, female B6C3F₁ mice were administered 2.0, 6.67, or 20.0 mmol/L DCA in drinking water (40, 115, or 330 mg/kg-day) from 7 to 8 weeks of age to sacrifice at 360 or 576 days (~ 51 or 82 weeks) of exposure (Pereira, 1996). Significant increases in the percentage of animals with altered hepatocyte foci and liver adenomas were seen in the 115 and 330 mg/kg-day groups as follows: after 51 weeks, 40.0% with foci and 35% with adenomas at 330 mg/kg-day; after 82 weeks, 39.3% with foci and 25% with adenomas at 115 mg/kg-day, and 89.5% with foci and 84.2% with adenomas at 330 mg/kg-day. A significant increase in the percentage (26.3%) of animals with liver carcinomas was only seen in the 330 mg/kg-day group after 82 weeks of exposure. The author concluded that the relationships of altered hepatocyte foci frequency, hepatocellular adenoma occurrence, and hepatocellular carcinoma occurrence to DCA concentration were best described by second-order regression.

In the second study, liver tumors were initiated in female B6C3F₁ mice with 25 mg/kg methylnitrosourea (MNU); the mice were then administered 2.0, 6.67, or 20.0 mmol/L DCA in their drinking water (50, 167, or 468 mg/kg-day) from age 7 weeks to sacrifice at 31 or 52 weeks later to characterize tumor promotion by DCA (Pereira and Phelps, 1996). A 4 mL/kg sterile saline vehicle control was included in the study. Significant increases in the percentage of animals with liver adenomas were seen in the 468 mg/kg-day group after 31 weeks of exposure (50.0% versus 0% in control) and 52 weeks of exposure (73.1% versus 17% in control). A significant increase in the percentage of animals with altered hepatocyte foci was also seen after 31 weeks of exposure (80.0% versus 20.0% in control) and 52 weeks of exposure (50.0% versus 10.0% in control). When exposure to 468 mg/kg-day DCA was terminated after 31 weeks, followed by a 21-week recovery period, the yield of foci of altered hepatocytes and tumors decreased, indicating that continued existence of these lesions was dependent on continuous exposure to DCA. The tumor-promoting activity of DCA exhibited a second-order relationship to drinking water concentration, so that a sharp rise in potency was seen between 167 and 468 mg/kg-day.

The tumorigenic response of DCA has also been evaluated in the rat model. In the first study, 28-day-old male F344 rats were given drinking water containing DCA at concentrations of 0

(78/group), 0.05 (60/group), 0.5 (60/group) or 5.0 g/L (78/group) (DeAngelo et al., 1996). Another group was provided water containing 2.0 g/L NaCl (50/group). Body weights and water consumption were measured throughout the study. All animals were treated for 100 weeks, except for the 5.0 g/L group, which began to exhibit signs of peripheral neuropathy. In response to this overt toxic effect, the concentration was sequentially lowered to 2.5 g/L at 9 weeks, then 2.0 g/L at 23 weeks and finally to 1.0 g/L at 52 weeks. When the neuropathy did not reverse or diminish, the animals were sacrificed at 60 weeks and excluded from the report. Based on measured water intake in the 0, 0.05 and 0.5 g/L groups, the time-weighted average doses were 0, 3.6, and 40.2 mg/kg-day respectively. Interim sacrifices for each dose group were performed at 15, 30, 45, 60, and 100 weeks; the NaCl control group was sacrificed at 104 weeks. No differences were observed in water consumption, final body weight, absolute or relative liver, kidneys or spleen weight, at doses of 3.6 or 40.2 mg/kg-day at any time point. However, absolute and relative testicular weights were mildly but significantly increased at the 40.2 mg/kg-day dose at final sacrifice. Increased hepatocellular vacuolization was detected, but there was no increase in hepatocyte proliferation at any dose group. There was also a noted lack of necrosis observed in doses carried out to final sacrifice at 100 weeks. At a dose of 40.2 mg/kg-day DCA, there was a statistically-significant increase in the cumulative incidence of combined hepatocellular neoplasia (21.4% versus 4.4%; $p < 0.05$) and total proliferative lesions in the liver (34.9% versus 8.7%; $p < 0.05$) compared to controls (measured at final sacrifice). This was not observed at the lower dose of 3.6 mg/kg-day. Tumor multiplicity was significantly increased in the 40.2 mg/kg-day group as compared to controls. There was also a significant increase in combined hepatocellular neoplasia (0.04 versus 0.3) and total proliferative lesions (0.41 versus 0.09). Other tumors were not increased over control values.

In the second study, male F344 rats were exposed to DCA concentrations of 2.5 g/L DCA in their drinking water (78/group) or to deionized water (78/group) (DeAngelo et al., 1996). The concentration of DCA was lowered to 2 g/L at 5 weeks, to 1.5 g/L at eight weeks, and to 1.0 g/L at 26 weeks. This corresponded to a time-weighted average concentration of 1.6 g/L and a time-weighted average dose of 139 mg/kg-day over the 103 week exposure period. Interim sacrifices for each dose group were performed at 14, 26, 52, 78 and 103 weeks. In this study the mean final body weight of DCA-exposed animals was significantly reduced to 73% of the deionized water control. Although the absolute testes weight decreased, the relative testes weight was not significantly lower than the control. Signs of liver pathology were also minimal in this study and the DCA dosage suppressed hepatocyte proliferation. Consistent with the first study, there was a lack of liver necrosis observed at final sacrifice. Hepatic tumor incidence significantly increased in exposed animals compared to controls, as follows: carcinoma (21.4% versus 3.0%, $p < 0.05$), combined hepatocellular neoplasia (28.6% versus 3.0%; $p < 0.01$) and total proliferative lesions (32.1% versus 6.1%; $p < 0.01$; DeAngelo et al., 1996). Tumor multiplicity was also significantly increased in this group compared to controls; combined hepatocellular neoplasia (0.36 versus 0.03), total proliferative lesions in the liver

(0.39 versus 0.06), and carcinomas (0.25 versus 0.03). Other tumors examined were not increased over control values.

Male Fischer 344 rats were administered time-weighted average concentrations of 0, 0.05, 0.5, or 2.4 g/L (0, 4, 40, or 296 mg/kg-day) DCA in drinking water, followed by sacrifice at intervals for up to 104 weeks by Richmond et al. (1995). No hepatoproliferative lesions were seen in the 4 mg/kg-day group, and the negative control group had only 4% hepatic adenomas. The 40 mg/kg-day group had 10% hyperplastic nodules, 21% hepatic adenomas, and 10% hepatocarcinomas after 104 weeks, while the 296 mg/kg-day group had 70% hyperplastic nodules, 26% hepatic adenomas, and 4% hepatocarcinomas after terminal sacrifice at 60 weeks. Increased numbers of altered hepatocyte foci were also seen in the 4 and 40 mg/kg-day groups, but the differences were significant only in animals from the 45-week sacrifice.

II.A.4. Supporting Data for Carcinogenicity

One hypothesized mechanism of action of DCA tumorigenesis is via a mutagenic and/or genotoxic pathway. However, the majority of evidence indicates that DCA is a weak mutagen, inducing mutations and chromosome damage in *in vitro* and *in vivo* assays predominantly at high concentrations.

Concentrations of 31,000 µg/mL DCA did not induce mutations in either the absence or presence of metabolic activation (S9) in bacterial strains TS24, TA2322 or TA1950 (Waskell, 1978). Additional negative results were obtained at doses up to 5,000 µg/plate (±S9) in strains TA98, TA100, TA1535, TA1537, TA1538, and *E. coli* WP2uvrA (DeMarini et al., 1994; Fox et al., 1996; Herbert et al., 1980). Although positive responses were obtained, ±S9, in strains TA98 and TA1538 at a dose range of 1-10 µg/plate. the positive response in TA1538 was not completely related to dose (Herbert et al., 1980). A dose of 58.5 µg/mL DCA increased DNA repair in *S. typhimurium* TA1535 when rat liver homogenates were induced with phenobarbital/5,6-benzoflavone, but the response was negative in the absence of exogenous metabolic activation (Ono et al., 1991). Waskell (1978) also reported the absence of DNA repair in repair-deficient bacterial strains, -S9, at 31 mg/plate.

Fox et al. (1996) found no increase in chromosome aberrations in Chinese hamster ovary cells incubated with up to 5,000 µg/mL either in the presence or absence of Aroclor 1254- induced rat S9. In contrast, Harrington-Brock et al. (1998) reported an increase in mutations and chromosome aberrations in mouse lymphoma cells at a dose range of 600-800 µg/mL in the absence of exogenous metabolic activation. The study authors did not observe an increase in micronuclei in the same cell line.

The ability of DCA to induce micronuclei following *in vivo* exposure has been investigated in two studies. Fox et al. (1996) dosed Sprague-Dawley rats via i.v. injection with doses of 275, 550, and 1,100 mg/kg and did not observe an increase in micronuclei. Transient increases in micronucleated polychromatic erythrocytes (PCEs) of male B6C3F₁ mice were observed after 9 days of exposure to drinking water at 665 mg/kg-day, but not during the rest of the 28-day exposure period (Fusco et al., 1996). Lower doses (95, 190, and 380 mg/kg-day) did not have an effect.

In an alkaline unwinding assay, administration of oral doses of 1-10 mmol/kg DCA to B6C3F₁ mice and Fischer 344 rats did not induce DNA strand breaks in a dose-related manner (Chang et al., 1992). This negative finding contrasts with earlier results by Nelson and Bull (1988) who demonstrated that DCA induced a steep log-dose-response curve for single-strand DNA breaks *in vivo* in Sprague-Dawley rats beginning at 0.1 mmol/kg DCA. DCA also induced single-strand breaks in B6C3F₁ mice at similar dose levels, with a less steep dose-response curve (Nelson and Bull, 1988). These positive results were noted at doses much lower than those used in the Chang et al. (1992) study and may reflect differences in experimental methodology. Fuscoe et al. (1996) reported no significant increase in the migration of DNA (taken as evidence of DNA strand breaks) isolated from mice exposed via drinking water to DCA concentrations up to 3.5 g/L. A reduction in migration was observed at the highest dose and was interpreted as evidence of DNA cross-linking. This result conflicts with negative data from other assays that can measure DNA cross-linking (Chang et al., 1992; Fox et al., 1996).

DCA has also been found to induce mutations in the lac I gene of transgenic mice (Big Blue) when administered in drinking water at approximate doses of 190 mg/kg-day and 665 mg/kg-day (Leavitt et al., 1997). The mutation frequency was increased compared to controls only at the 60-week time point, and not after 4 or 10 weeks of exposure. These data suggest that a large cumulative dose is required to induce mutagenesis in this test system and that the mutational event might be secondary to toxic effects in the liver.

As discussed previously, the primary metabolic pathway for DCA is enzymatic oxidative dechlorination of DCA via GST Zeta yielding glyoxylate that is oxidized to oxalate or transaminated to form glycine. An alternate pathway, originally proposed by Stacpoole et al. (1998a), involves reductive dechlorination of the chlorinated acetates to form monochloroacetate (MCA) and eventually thiodiacetic acid through glutathione conjugation. The formation of free radicals in this latter pathway suggests that lipid peroxidation may be involved in DCA toxicity. These free radical species might be expected to initialize DNA damage such as strand breaks and cross-links, which might trigger ameliorative cellular repair processes leading, in turn, to gene mutation and chromosomal aberrations (Chang et al., 1992). Larson and Bull (1992) also suggested that lipid peroxidation plays a role in the induction of focal necrosis as observed in mice by Bull et al. (1990) and Daniel et al. (1992).

DCA has been shown to be a weak peroxisome proliferator in mice and rats (DeAngelo et al., 1989; Daniel et al., 1992; Mather et al., 1990; DeAngelo et al., 1999). Transient transfection studies demonstrated that DCA activates PPARs (Zhou and Waxman, 1998). DCA has also been shown to activate mouse and human PPARs with similar receptor sensitivity (Maloney and Waxman, 1999). The relevance of this finding to DCA tumorigenesis is not fully understood. One of the effects attributed to PPARs is the suppression of *c-Jun* activity and expression (Sakai et al., 1995), yet several recent studies have demonstrated that DCA-induced tumors are *c-Jun* positive (Stauber and Bull, 1997; Stauber et al., 1998). This finding is inconsistent with a role for PPARs in DCA-induced tumorigenesis. Further, DCA induces hepatic tumors in rodents at doses that are significantly below those required to induce significant peroxisome proliferation (DeAngelo et al., 1999). Collectively, these observations suggest that peroxisome proliferation is not likely to be important in the tumorigenicity of DCA.

Necrosis and reparative hyperplasia are additional hypothesized modes of action for DCA to induce tumors. There is some evidence in mice of liver toxicity at doses that induce tumors. Frank necrosis has not been observed in the rat cancer studies, however DeAngelo et al. (1996) and later studies in mice that have used lower doses have not reported evidence of cytotoxicity and reparative hyperplasia (DeAngelo et al., 1999). Further, data from a Carter et al. (1995) study that involved exposing male B6C3F₁ mice with 0, 0.5, or 5 g/L (0, 95, or 440 mg/kg-day, respectively) of DCA in drinking water for up to 30 days indicate that DCA exposure initially inhibits rather than stimulates cell proliferation (hyperplasia), and that the increased liver weight observed in the mice is due to hepatocyte enlargement rather than regenerative hyperplasia following cell death. Both treatment groups had enlarged nuclei, which may suggest polyploidy. The hepatocytes also exhibited glycogen accumulation, suggesting alterations in cellular metabolism. The available data indicate that necrosis, followed by reparative hyperplasia is unlikely to be responsible for the tumorigenesis occurring in rats and in mice at lower doses.

Other potential mechanisms of action are tumor promotion and alterations of cellular replication and death. DCA has been shown to act as a tumor promoter in animal studies (Herren-Freund et al., 1987; Pereira and Phelps, 1996). Nevertheless, DCA also acts as a complete carcinogen. Cellular replication may be affected by DCA treatment. For example, Stauber and Bull (1997) reported that DCA-induced altered hepatic foci (AHF) and tumors in male B6C3F₁ mice were largely basophilic and reacted uniformly to antibodies against *c-Jun* and *c-Fos* (nuclear transcription factors). The *c-Jun* protein was localized in the cytoplasm and the *c-Fos* was found in the nucleus. The AHF and tumors that were *c-Jun* positive displayed a dose-dependent increase in cell replication during a labeling period. The cell replication rate in DCA-induced AHF and tumors were dependent on treatment but this effect was observed only

in the regions of the lesions that were *c-Jun* positive. Stauber et al. (1998) also demonstrated that DCA increases cell proliferation of *c-Jun* positive hepatocytes *in vitro*.

The role of mutations in the *H-ras* gene in tumor tissue from DCA-treated B6C3F₁ mice was investigated by Anna et al. (1994). *Ras* proteins are GTPases that are involved in the activation of a series of protein kinases that control cell growth and differentiation. *Ras* is activated by binding of a ligand to a cell surface receptor. No differences were reported in mutation frequency for the *Ras* gene between control mice and those who had been exposed to drinking water containing 5 g/L DCA (about 900 mg/kg-day) for 5 days/week for 76 weeks. However, significant changes were seen in the mutation spectra of *H-ras* codon 61 in the DCA-treated mice as compared to the control animals. In the spontaneous tumors from the controls, the CAA of codon 61 became AAA in 59% of the tumors, CGA in 28% and CTA in 14%. In the DCA-treated mice, the *H-ras* codon 61 changes were 28% AAA, 35% CGA and 38% CTA. The authors suggest that these differences were due to nonspecific secondary DNA damage by DCA. The data are supported by limited data by Velazquez (1995), Ferreira-Gonzalez et al. (1995) and Schroeder et al. (1997). Hypomethylation of some nuclear protein genes involved in transcriptional response and proliferation of cells (e.g., *c-myc* gene) after DCA exposure, has also been investigated (Pereira et al., 2001). Hypomethylation of the gene seems to enhance its expression and thus cell division. DCA decreased *c-myc* methylation and increased expression of the gene in exposed male mice.

Snyder et al. (1995) examined the role of apoptosis (programmed cell death) suppression as a contributing factor to DCA-induced hepatocarcinogenicity. Apoptotic cells were visualized by *in situ* nick-end labeling of DNA from the livers of animals administered DCA in drinking water at varying concentrations for up to 30 days and sacrificed at 5-day intervals. Regression analysis revealed a significant trend toward decreased apoptosis as dose and length of exposure increased. The lowest dose, 0.5 g/L, significantly ($p < 0.05$) decreased apoptosis at the earliest time point (5 days) and also at 15, 25, and 30 days. For the high-dose group (5 g/L), apoptosis was significantly depressed as compared to controls for all time points except the 20-day point. The authors suggested that DCA may suppress the apoptotic mechanism by which initiated tumor cells would otherwise be removed.

In an effort to shed light on the cellular events preceding the development of malignant liver tumors in male B6C3F₁ mice, Carter et al. (2003) examined 1,355 slides from liver samples from 327 animals used by DeAngelo et al. (1999). Tissues collected from mice sacrificed throughout the study were used to evaluate the effects of dose (0, 0.05, 0.5, 1.0, 2.0 and 3.5 g/L DCA), and time (26, 52, 78 and 100 weeks) on liver lesions. Slides were processed for standard histological examination. They were evaluated for the occurrence of altered hepatic foci (AHF), large foci of cellular alteration (LFCA, formally called hyperplastic nodules), adenomas (AD) and carcinomas (CA). To minimize interhuman variability during the

classification of tissue abnormalities, all of the slides were read by two observers who were blinded to treatment group and time of sacrifice.

In addition to the four main categories described above, lesions were subcharacterized into the following three groupings: eosinophilic, dysplastic, and basophilic and/or clear cell.

Eosinophilic cells showed increases in smooth endoplasmic reticulum and mitochondria. The basophilic cells had increased rough endoplasmic reticulum and/or ribosomes. The clear cells had accumulation of glycogen and/or lipids (steatosis). The dysplastic cells displayed atypical or enlarged nuclei. Tissue lesions from all four major categories and all three subcategories were identified in liver tissues from control and exposed animals.

Following histological examination, the data were arrayed by dose and time-to-sacrifice and examined to determine if there is a pattern of lesion progression with either dose or duration of exposure. The observed patterns of lesion frequency and their progression across the time- and dose-range gave rise to the hypothesis that there were three routes to the development of malignant tumors. In one case, eosinophilic cells seemed to progress from eosinophilic AHF to eosinophilic AD and CA. The basophilic cells and clear cells showed two patterns of progression. They either progressed from AHF to LFCA and then to CA or from LFCA to AD and then to CA. The dysplastic cells seemed to progress directly from AHF to CA. All three patterns of lesion progression were observed in the livers of mice treated with DCA and were significantly different from controls at some time or dose points. The majority of cancers arose from basophilic/clear cell progression. The data suggest that tumors can originate from several different cell lines and through more than one pathway.

The researchers also examined the relationship of necrosis, glycogen accumulation, cytomegaly, accumulation of lipid droplets, atypical nuclei and enlarged nuclei to malignancies. The strongest correlation was observed for cytomegaly. A correlation with glycogen accumulation and necrosis was also observed for some doses but there was no consistent dose-response pattern. The lack of dose-response at the high doses might be the result of a decrease in the amount of liver tissue (non-involved liver) that had not been impacted by the tumors (AD or CA). Clear cells (lipid containing) were negatively correlated to length of DCA exposure. However, this is consistent with the hypolipidemic effects of DCA.

**Biomarkers of Tissue DCA Exposure
Incidence in Percent of Animals Affected**

Dose/Tissue Abnormality	Control	0.05 g/L	0.5 g/L	1.0 g/L	2.0 g/L	3.5 g/L
Cytomegaly	1.2	0	0	30.8	41.2	34.9
Glycogen	3.8	0	20.0	10.8	11.8	27.9
Steatosis	26.3	66.7	34.5	21.5	0	7.0
Necrosis	2.5	6.1	1.8	20.0	11.8	30.2
Atypical nuclei	22.5	33.3	32.7	55.4	58.8	46.5
Enlarged nuclei	41.2	39.4	54.5	55.4	45.1	41.9
CA	7.5	15.2	10.9	20.0	39.2	37.2

Source: Adapted from Carter et al. (2003); CA=carcinomas

II.B. Quantitative Estimate of Carcinogenic Risk from Oral Exposure

II.B.1. Summary of Risk Estimates

II.B.1.1. Oral Slope Factor, 95% upper bound - 5E-02 per mg/kg-day

II.B.1.2. Drinking Water Unit Risk, 95% upper bound - 1.4E-06 per µg/L
(This value is based on an ingestion rate of 2L/day and a body weight of 70 kg)

II.B.1.3. Extrapolation Method - Multistage model with Benchmark Dose Modeling

Drinking Water Concentrations at Specified Risk Levels:

Upper Bound Risk Values:

Risk Level	Concentration
E-4 (1 in 10,000)	70 µg/L
E-5 (1 in 100,000)	7.0 µg/L
E-6 (1 in 1,000,000)	0.7 µg/L

Central Tendency Risk Values:

Risk Level	Concentration
E-4 (1 in 10,000)	230 µg/L
E-5 (1 in 100,000)	23 µg/L
E-6 (1 in 1,000,000)	2.3 µg/L

II.B.2. Dose-Response Data (Carcinogenicity, Oral Exposure)

Tumor Type - hepatadenoma and hepatocarcinoma

Test Species - male B6C3F₁ mice

Route - ingestion, drinking water

Reference - DeAngelo et al., 1999

Cancer dose-response data evaluated using BMD modeling: male mice^a

Conc. in water (g/L)	No. of mice entering study	Mean BW (g) at 100 weeks	Dose (mg/kg-day)		Animals with hepatocarcinomas at 100 weeks		Animals with hepatadenomas at 100 weeks		Animals with either hepatocarcinomas or adenomas at 100 weeks	
			Mouse	HED ^b	%	N	%	N	%	N
0	50	43.9	0	0	26%	13	10%	5	36	18
0.05	33	43.3	8.0	1.3	33%	11	3%	1	33	11
0.5	25	42.1	84	13.2	48%	12	20%	5	56	14
1	35	43.6	168	26.5	71%	25	51%	18	86	30
2	21	36.1	315	47.5	95%	20	43%	9	100	21
3.5	11	36.0	429	64.6	100%	11	45%	5	100	11

^a High-dose group excluded from benchmark modeling (see text)

^b HED calculated using a dose scaling factor of $BW^{0.75}$

Source: adapted from DeAngelo et al. (1999)

Summary of cancer BMD modeling results^{a,b}

Model	BMD^c	BMDL^c	p-value	AIC
Multi-Stage(2)	6.86	2.05	0.981	174.62
Quantal-quadr.	6.86	5.69	0.981	174.62
Probit	3.16	2.54	0.816	175.54
Logistic	3.10	2.43	0.728	176.11
Weibull	7.53	2.50	0.916	176.59
Gamma	8.45	2.55	0.858	176.81
Log-Probit	9.36	4.27	0.779	177.10
Log-Logistic	9.17	4.07	0.703	177.45
Quantal-linear	1.88	1.37	0.370	178.41

^a Data = DeAngelo et al. (1999), animals with hepatocarcinoma or adenoma, excluding high-dose group

^b BMD estimated using BMR = 0.10, BMDL estimated as 95% LCL

^c Units are (mg/kg-day)⁻¹

II.B.3. Additional Comments (Carcinogenicity, Oral Exposure)

Multiple studies in B6C3F₁ mice were performed which investigated the carcinogenic response to oral exposures of DCA and established that increased incidence of hepatic tumors resulted. Of these studies, the best one for cancer dose-response was deemed to be that of DeAngelo et al. (1999), because the study was specifically designed to establish a multipoint dose-response curve and data were available for five dose groups plus a control. The duration

of the study spanned the expected lifetime of a mouse. The administered doses in the mouse study were converted to human equivalent doses using a dose scaling factor of $BW^{0.75}$.

The dose-response curve was modeled using the Benchmark Dose-Response (BMD) method. The high-dose was excluded from the modeling because the dose approached the MTD of the study, as evidenced by the significantly increased hepatic necrosis indices throughout the study and the decreases in body weight gain from 52 weeks on (a sensitivity analysis on the BMD results indicated that no significant changes resulted from exclusion of the highest dose). Nine different models were used to develop BMD values corresponding to a 10% benchmark response (BMR). The 95% lower confidence limit on the central tendency estimate was designated the Benchmark Dose Low (BMDL₁₀). This value was used to develop the Cancer Slope Factor (CSF).

The multistage and quantal-quadratic models provide essentially identical fits to the data. The multistage model estimate was selected for dose-response extrapolation because the quantal-quadratic model has no first-order term and therefore may predict zero slope at zero dose. Given the uncertainty surrounding the carcinogenic mechanism of DCA, it was decided that the zero slope assumption was not justified. The multistage model gives a BMDL₁₀ estimate of 2.1 mg/kg-day (2.05, rounded to two significant figures). This is defined as the point of departure (POD). Consistent with EPA's Proposed and Draft Final Cancer Assessment Guidelines (U.S. EPA, 1999, 2003b), extrapolation from the POD to low dose is performed by assuming a linear dose-response curve between the POD and the origin. Based on this, the slope factor for DCA is calculated as follows:

$$\text{Slope factor} = \text{BMR} / \text{POD (BMDL}_{10}) = 0.1 / (2.1 \text{ mg/kg-day}) = 0.048 \text{ (mg/kg-day)}^{-1}$$

The slope factor derived from the central tendency estimate of the cancer response is simply the BMR divided by the BMD.

$$\text{Slope Factor} = \text{BMR} / \text{BMD} = 0.1 / (6.9 \text{ mg/kg-day}) = 0.015 \text{ (mg/kg-day)}^{-1}$$

II.B.4. Discussion of Confidence (Carcinogenicity, Oral Exposure)

The CSF for DCA was determined from the dose-response data on adenomas and carcinomas from a study in male B6C3F₁ mice by DeAngelo et al. (1999). The study is representative of a larger cancer database in mice and provides data for five dose groups in addition to the control. It is accordingly well suited to modeling using the BMD approach. There is uncertainty regarding the suitability of mice as a model for human carcinogenesis because of the high spontaneous liver tumor response in this species.

DCA has been used therapeutically in humans at doses as low as 25 mg/kg-day (Stacpoole et al., 1998; Spruijt et al., 2001). However, carcinogenic endpoints following these exposures have not been evaluated. To extrapolate mouse tumor data for DCA to the human situation, it is assumed that humans will respond similarly to the mouse. It is not clear that this is the case. Nevertheless, the robust tumor response in male rats, following ingestion exposures to DCA, increases the confidence that DCA can elicit liver tumors across different species. In the absence of human data, the choice for using the mouse data from DeAngelo et al. (1999) is a reasonable assumption and is protective of human health.

Overall, there are inadequate data to support any conclusive mode of carcinogenic action. Some studies indicate that DCA is genotoxic (Fusco et al., 1996; Harrington-Brock et al., 1998; Leavitt et al., 1997) but the doses required to induce these effects are very high and far exceed exposure concentrations that are relevant to humans. Studies are conflicting regarding lipid peroxidation and the production of oxygenated radicals; further, cancer is induced at doses lower than those required to induce lipid peroxidation in the rat. Data suggest that tumors can originate from several different cell lines and through more than one pathway. Findings related to glycogen accumulation and necrosis as they apply to the tumorigenic response are not clear. Given the uncertainty regarding the mode of action, the dose-response was modeled using a BMD approach which assumed linearity at low doses. This decision is protective of public health. The cancer risk estimation presented for DCA is considered to be protective of susceptible groups, including children.

II.C. Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

Lack of data regarding the inhalation route of exposure for DCA precludes the development of a quantitative estimate of carcinogenic risk from inhalation exposure. Nevertheless, due to the low volatility of the compound, it is not believed that inhalation exposure would be significant.

II.C.1. Summary of Risk Estimates

Not applicable.

II.C.2. Dose-Response Data for Carcinogenicity, Inhalation Exposure

Not applicable.

II.C.3. Additional Comments (Carcinogenicity, Inhalation Exposure)

Not applicable.

II.C.4. Discussion of Confidence (Carcinogenicity, Inhalation Exposure)

Not applicable.

II.D. EPA Documentation, Review, and Contacts (Carcinogenicity Assessment)

II.D.1. EPA Documentation

Source Documents -- U.S. EPA, 2003a, 1994, 1991

The Toxicological Review of Dichloroacetic Acid (U.S. EPA, 2003a) was externally peer reviewed in the fall of 2001 by three noted scientists familiar with the toxicology of this compound. The 1991 Drinking Water Criteria Document was externally peer reviewed by the Drinking Water Subcommittee of the Science Advisory Board from April 4-5, 1991. In addition, a review of the Criteria Document was completed by the American Water Works Association in August 1992. Comments from all reviews have been carefully evaluated and considered in the revision and finalization of this IRIS Summary. A record of the comments are included in the IRIS documentation files. A record of the comments specific to the Toxicological Review (U.S. EPA, 2003a) is included as an appendix to the Toxicological Review. [*To review this appendix, exit to the toxicological review, Appendix A, External Peer Review-Summary of Comments and Disposition \(PDF\).*](#)

II.D.2. EPA Review (Carcinogenicity Assessment)

Agency Consensus Date — 08/20/2003

II.D.3. EPA Contacts (Carcinogenicity Assessment)

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 — Dichloroacetic acid
CASRN — 79-43-6

VI.A. Oral RfD References

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VII. Revision History

Substance Name — Dichloroacetic acid
CASRN — 79-43-6

Date	Section	Description
02/01/1996	II.	Carcinogenicity assessment on-line
09/11/2003	I., II., VI.	Added an RfD, added a qualitative discussion for the RfC, revised the cancer slope factor

VIII. Synonyms

Substance Name — Dichloroacetic acid

CASRN — 79-43-6

Last Revised — 09/11/2003

- acetic acid, dichloro-
- acide dichloracetique [French]
- acido dicloroacetico [Spanish]
- AI3-18370
- dichlorethanoic acid
- kyselina dichloroctova [Czech]