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TRICHLOROETHYLENE, TETRACHLOROETHYLENE, AND SOME OTHER CHLORINATED AGENTS

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IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS

International Agency for Research on Cancer

World Health Organization

DICHLOROACETIC ACID

This substance was considered by previous Working Groups in February 1995 and October 2004 (IARC, 1995, 2004). New data have since become available, and these have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Identification of the agent

1.1.1 Nomenclature

(a) Dichloroacetic acid

Chem. Abstr. Serv. Reg. No.: 79-43-6

Deleted Chem. Abstr. Serv. Reg. No.: 42428-47-7

Chem. Abstr. Serv. Name: Dichloroacetic acid

IUPAC Systematic Name: Dichloroacetic acid

Synonyms: DCA; DCA (acid); dichloracetic acid; dichlorethanoic acid; dichloroethanoic acid; bichloracetic acid

(b) Sodium dichloroacetate

Chem. Abstr. Serv. Reg. No.: 2156-56-1

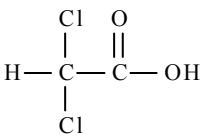
Chem. Abstr. Serv. Name: Sodium dichloroacetate

IUPAC Systematic Name: Sodium 2,2-dichloroacetate

Synonyms: Dichloroacetate, sodium salt; dichloroacetic acid sodium salt; sodium 2,2-dichloroacetate

1.1.2 Structural and molecular formulae and relative molecular mass

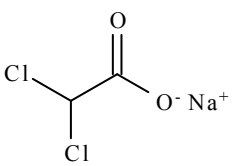
(a) Dichloroacetic acid



$C_2H_2Cl_2O_2$

Relative molecular mass: 128.94

(b) Sodium dichloroacetate



$C_2HCl_2NaO_2$

Relative molecular mass: 150.92

1.1.3 Chemical and physical properties of the pure substance

(a) Dichloroacetic acid

Description: Corrosive liquid; pungent odour (O'Neil *et al.*, 2006)

Table 1.1 Methods for the analysis of dichloroacetic acid in water

Sample preparation	Assay procedure	Limit of detection	Reference
Extract methyl-*t*-butyl ether; derivatize to methyl ester; acidify; extract with methanol	GC/ECD	0.24 µg/L	EPA (2003)
Add ammonium chloride and ^{13}C-labelled internal standards; direct injection	IC-ESI-MS/MS	0.055 µg/L	EPA (2009)

ECD, electron capture detection; ESI-MS/MS, electrospray ionization tandem mass spectrometry; GC, gas chromatography; IC, ion chromatography

Boiling-point: 193–194 °C (O'Neil *et al.*, 2006)

Melting-point: 9.7 °C and −4 °C; apparently occurs in two crystalline forms (O'Neil *et al.*, 2006)

Density: 1.563 at 20 °C/relative to H_2O at 4 °C (O'Neil *et al.*, 2006)

Spectroscopy data: Infrared (prism [2806]), nuclear magnetic resonance [166] and mass spectral data have been reported (Weast & Astle, 1985)

Solubility: Slightly soluble in water; miscible with ethanol, ethyl ether (O'Neil *et al.*, 2006) soluble in acetone; slightly soluble in carbon tetrachloride (Haynes, 2012)

Volatility: Vapour pressure, 0.023 kPa at 25 °C (Haynes, 2012)

Stability: Dissociation constant (K_a), 5.14×10^{-2} (Morris & Bost, 1991)

Octanol/water partition coefficient (P): Log P, 0.92 (Hansch *et al.*, 1995)

Conversion factor: $mg/m^3 = 5.27 \times ppm$ (calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (760 mm Hg)

(b) Sodium dichloroacetate

Description: White powder (Haynes, 2012)

Melting-point: 198 °C (decomposes) (Haynes, 2012)

Solubility: Soluble in cold water (Haynes, 2012)

1.1.4 Technical products and impurities

Dichloroacetic acid is commercially available as a technical-grade liquid with the following typical specifications: purity, 98.0% minimum; monochloroacetic acid, 0.2% maximum; trichloroacetic acid, 1.0% maximum; and water, 0.3% maximum (Clarian GmbH, 2002). Sodium dichloroacetate is available as a powder with a purity of 98%, containing < 2% ethyl alcohol (Sigma-Aldrich, 2012).

Trade names for dichloroacetic acid include Urner's liquid.

1.1.5 Analysis

Methods for the analysis of dichloroacetic acid have been reviewed by Delinsky *et al.* (2005). Selected methods for the analysis of dichloroacetic acid in water, exhaled air, blood and urine are identified in Table 1.1. A headspace gas chromatography–mass spectrometry method has been developed for measuring trichloroacetic acid in urine (Cardador & Gallego, 2010).

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

Dichloroacetic acid was reported to be first synthesized in 1864 by the further chlorination of monochloroacetic acid with chlorine (Beilstein Online, 2002).

The most common production method for dichloroacetic acid is the hydrolysis of dichloroacetyl chloride, which is produced by the oxidation of trichloroethylene. It can also be obtained by hydrolysis of pentachloroethane with 88–99% sulfuric acid or by oxidation of 1,1-dichloroacetone with nitric acid and air. In addition, dichloroacetic acid can be produced by catalytic dechlorination of trichloroacetic acid or ethyl trichloroacetate with hydrogen over a palladium catalyst (Koenig *et al.*, 1986; Morris & Bost, 1991).

Sodium dichloroacetate is readily formed when dichloroacetic acid is dissolved in an aqueous solution. In addition, haloacetic acids may form de novo as disinfection by-products in chlorinated drinking-water (Nissinen *et al.*, 2002).

(b) Production

Dichloroacetic acid was produced by two companies in the USA and one company each in China, Japan and Mexico (Chemical Information Services, 2002; IARC, 2004). It was formulated into pharmaceutical products by one company each in New Zealand and Turkey (Chemical Information Services, 2002).

1.2.2 Use

Dichloroacetic acid and its esters are intermediates in organic synthesis, used in the production of glyoxylic acid, dialkoxy and diaroxy acids, and sulfonamides and in the preparation of iron chelates in the agricultural sector (Koenig *et al.*, 2011). It can also be used as an analytical reagent in fibre manufacture (polyethylene terephthalate).

Dichloroacetic acid is used in medical practice as a cauterizing agent. It rapidly penetrates and cauterizes the skin and keratins. Its cauterizing ability compares with that of electrocautery or freezing. It is used on calluses, hard and soft corns, xanthoma palpebrarum, seborrhoeic keratoses, in-grown nails, cysts and benign erosion of the cervix (Gennaro, 2000). It can also be used as a medicinal disinfectant as a substitute for formalin. Dichloroacetic acid has also been proposed for use in targeted therapy against cancer (Tennant *et al.*, 2010).

Dichloroacetic acid and its salts have been used therapeutically to treat the rare condition of congenital lactic acidosis (Stacpoole *et al.*, 2006, 2008). They have also been tested for effects on diabetes and on tumour growth (Michelakis *et al.*, 2010; Stacpoole & Greene, 1992). Due to side-effects, these substances are not in common use as therapeutic agents.

1.3 Occurrence and exposure

1.3.1 Natural occurrence

Dichloroacetic acid is not known to occur as a natural product.

1.3.2 Environmental occurrence

(a) Air

No data were available to the Working Group.

(b) Water

Dichloroacetic acid is produced as a by-product during the chlorination of water containing humic substances and may occur in drinking-water or swimming pools after chlorine-based disinfection of raw waters that contain natural organic substances (IARC, 2004).

Table 1.2 Concentrations of dichloroacetic acid in water

Country	Location	Concentration (µg/L)		Reference
		Mean	Range	
Drinking-water				
Australia	Seven cities	NR	1–46	Simpson & Hayes, (1998)
China	Eight water supplies	NR	0.3–10.9	Liu *et al.* (2011)
	Beijing	11.1	9.6–12.9	Wang & Wong, (2005)
	Beijing	2.69	13.02[b]	Wei *et al.* (2010)
Greece	Athens	NR	2.3–24.5	Golfinopoulos & Nikolaou (2005)
	Mytilene	NR	2.6–3.5	Leivadara *et al.* (2008)
Spain	Eleven provinces	1.8[a]	0.7–18.0	Villanueva *et al.* (2012)
United Kingdom	England	6.8	3.12–15.0	Zhang *et al.* (2010a)
	Three large regions	9.1	23[b]	Malliarou *et al.* (2005)
		39.9	116[b]	
		23.7	58[b]	
Raw and surface water				
China	Eight water supplies	NR	29.3–155.7	Liu *et al.* (2011)
Republic of Korea	Four regions	50.4	44.2–58.1	Kim (2009)
Swimming-pool water				
Republic of Korea	*Seoul*			Lee *et al.* (2010)
	Pools treated with Chlorine	68.3	14.1–246	
	Pools treated with ozone + chlorine	12.0	ND–31.9	
	Pools treated with EGMO	33.7	1.5–98.5	

[a] Median
[b] Maximum
EGMO, electrically generated mixed oxidants; ND, not detected; NR, not reported
Note: Data from earlier periods can be found in the previous *IARC Monograph* (IARC, 2004)

Table 1.2 summarizes some recent levels of dichloroacetic acid found in surface waters, groundwater and drinking-water worldwide.

(c) Food

No data were available to the Working Group.

(d) Other

No data were available to the Working Group.

1.3.3 Occupational exposure

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 1592 employees in 39 facilities in the USA were potentially exposed to dichloroacetic acid (NIOSH, 1994). The estimate was based on a survey of companies and did not involve measurements of actual exposure.

Recently, occupational exposure of swimming-pool attendants to dichloroacetic acid in indoor and outdoor pools was evaluated by analysis of urine samples. After an exposure of 2 hours, the urine of 24 exposed indoor-pool attendants contained dichloroacetic acid at a concentration of ~300 ng/L (range, 230–448 ng/L). Exposure levels in outdoor pools were much lower at ~50 ng/L (range, < 30–60 ng/L), despite higher concentrations of the chemical in the water of the outdoor pools than in the indoor pools (Cardador & Gallego, 2011). The concentrations in urine of indoor-pool

workers increased by 40% after the length of the shift doubled (4 hours). [The Working Group noted that it was unclear by what route the pool attendants had been exposed.]

1.3.4 Exposure of the general population

Kim & Weisel (1998) measured the amount of dichloroacetic acid excreted by people who had swum in a chlorinated pool; values ranged from 25 to 960 ng per urine void. Mean concentrations at the time of visit were 1.4 ng/mg creatinine in those having swum in water with low concentrations of chlorination-disinfection by-products, and 1.82 ng/mg creatinine in those having swum in water with high concentrations of these by-products (Weisel *et al.*, 1999).

In a study of swimmers who attended swimming pools for two sessions (duration, 1 hour) per week, the average concentrations of dichloroacetic acid in urine were 2294 ng/L in 13 adults and 3102 ng/L in 6 children in an indoor pool and 4979 ng/L in 8 swimmers in an outdoor pool (Cardador & Gallego, 2011).

1.4 Regulations and guidelines

The maximum concentration of haloacetic acids (five) (HAA5) allowable as contaminants in drinking-water is 0.060 mg/L. HAA5 is the sum of the concentrations in milligrams per litre of the haloacetic acid compounds (monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid), rounded to two significant figures after addition (EPA, 2008).

Dichloroacetic acid is not listed as a carcinogen by the United States Environmental Protection Agency (EPA), the National Toxicology Program (NTP), or the European Union. Dichloroacetic acid was classified as a carcinogen in 1996 in California by the Safe Drinking-water and Toxic Enforcement Act. The only countries with established limits for occupational exposure are Belgium and the Republic of Korea, with a limit value of 0.5 ppm [2.5 mg/m^3] at 8 hours (GESTIS, 2013).

2. Cancer in Humans

Dichloroacetic acid is a chemical that occurs in drinking-water and swimming pools as part of a mixture of disinfection by-products. The chemicals in water-disinfection by-products do not occur in an isolated manner and there is no epidemiological evidence on risk of cancer associated specifically with them. A detailed description of water-disinfection by-products and cancer risk is given in *IARC Monograph* Volume 101 (IARC, 2012).

3. Cancer in Experimental Animals

Because of the potential role of dichloroacetic acid in carcinogenicity as a metabolite of trichloroethylene, studies with dichloroacetic acid have focused almost exclusively on the liver.

Therefore assessment of cancer at other sites has been very limited (see Table 3.1 and Table 3.2).

3.1 Mouse

See Table 3.1

3.1.1 Oral administration

As part of an initiation–promotion study, Herren-Freund *et al.* (1987) examined induction of liver cancer in male B6C3F$_1$ mice given drinking-water containing dichloroacetic acid at a concentration of 5 g/L for 61 weeks. Control animals were given drinking-water containing sodium chloride (NaCl) at a concentration of 2 g/L to control for the sodium hydroxide (NaOH) used to neutralize dichloroacetic acid.

Table 3.1 Studies of carcinogenicity in mice exposed to dichloroacetic acid by oral administration or skin application

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mice, B6C3F$_1$ (M) 61 wk Herren-Freund *et al.* (1987)	NaCl, 2 g/L (control), DCA, 5 g/L in drinking-water 27, 26/group	At terminal kill (five mice unaccounted for in the control group): Liver [hepatocellular] adenomas: 2/22, 25/26* Hepatocellular carcinoma: 0/22, 21/26*	*$P < 0.01$, Fisher exact test	Purity, > 99% Small numbers of mice. Short duration of exposure. Based on data from other studies, it is probable that drinking-water consumption was significantly depressed in the treated group, Pathological examination limited to the liver.
Mice, B6C3F$_1$ (M) 52 wk Bull *et al.* (1990)	0 (control), 1, 2 g/L in drinking-water 35, 11, 24/group	Total gross liver lesions: 2/35, 2/11, 23/24* Six hepatocellular carcinomas confirmed in five mice in the group at 2 g/L	Statistical analysis, NR *[$P < 0.0001$]	Analytical grade; purity, NR. Small numbers of mice/group, short duration, and only 45/120 gross liver lesions were examined and characterized. Only the liver was examined for gross pathology or histopathology. Ten females survived to 52 wk with no lesions noted.
Mice, B6C3F$_1$ (M) 60–75 wk DeAngelo *et al.* (1991)	*Experiment 1A (60 wk):* NaCl, 2 g/L (control); DCA, 0.05, 0.5, 5 g/L in drinking-water 9, 9, 9, 30/group *Experiment 1B (75 wk):* NaCl, 2 g/L (control); DCA, 0.05, 0.5 g/L 19, 20, 18/group *Experiment 2 (60 wk):* acetic acid, 2 g/L (control); DCA, 3.5 g/L 10, 12/group	Data from all experiments were combined for reporting prevalence at terminal kill *DCA, 5 g/L* Hepatocellular adenoma: 24/30* Hepatocellular carcinoma: 25/30* *Control, and DCA, 0.05, 0.5, 3.5 g/L* Hepatocellular adenoma: 0/28, 2/29, 1/27, 12/12* Hepatocellular carcinoma: 2/28, 5/29, 2/27, 8/12*	*$P < 0.001$	Purity, > 99% Drinking-water consumption significantly decreased at 5 g/L; only histopathological results from the liver were reported; limited reporting of the study.
Mice, B6C3F$_1$ (M) 104 wk Daniel *et al.* (1992)	0, 0.5 g/L in drinking-water Experiment 1: 10, 16/group Experiment 2: 10, 8/group	Data from both experiments were combined for reporting prevalence at terminal kill Liver adenoma: 1/20, 10/24* Hepatocellular carcinoma: 2/20, 15/24* Hepatic adenoma or carcinoma (combined): 3/20, 18/24*	Fisher exact test; *$P \leq 0.01$	Purity, > 95% Small number of mice/group and single dose limit statistical power. Histopathology nor reported for mice dying during experiment.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mice, B6C3F$_1$ (M) 76 wk Anna *et al.* (1994)	0, 5 g/L drinking-water Start: 24, 110/group	Prevalence at terminal kill: Hepatocellular adenoma: 2/24, 83/89* Hepatocellular carcinoma: 2/24, 66/89*	Fisher exact test *[$P < 0.0001$]	Purity, NR Only the liver was examined grossly or microscopically for pathology. Consumption of drinking-water at this high dose was not discussed by the authors.
Mice, B6C3F$_1$ (F) Up to 586 days Pereira (1996)	NaCl, 1.15 g/L; DCA, 0.259, 0.86, 2.59 g/L in drinking-water, or repeated treatment cycle of 24 days with DCA at 2.59 g/L followed by 48 days without DCA 134, 90, 50, 40, 34/group	Prevalence: *360 days:* Hepatocellular adenoma: 1/40, 0/40, 3/20, 7/20, 0/15 Hepatocellular carcinoma: 0/40, 0/40, 0/20, 1/20, 0/15 *576 days:* Hepatocellular adenoma: 2/90, 3/50, 7/28, 16/19, 3/34 Hepatocellular carcinoma: 2/90, 0/50, 1/28, 7/19, 1/34	Kruskal–Wallis test $P < 0.01$; adenoma at high dose, 360 and 576 days $P < 0.05$; carcinoma at high dose, 576 days	Purity, NR Only cancer bioassay (except initiation-promotion studies) in the female mouse. Drinking-water consumption reduced at the high dose for the first week, but not beyond. The only organ examined was the liver.
Mice, B6C3F$_1$ (F) 104 wk Schroeder *et al.* (1997)	0, 0.5, 3.5 g/L in drinking-water 39, 25, 25/group	Hepatocellular carcinoma: 1/39, 1/25, 23/25*	*[$P < 0.001$]	Purity, NR Study was primarily intended to characterize *ras* mutation spectra in liver tumours. The only organ examined was the liver and reporting of histopathology was limited.
Mice, B6C3F$_1$ (M) Up to 100 wk DeAngelo *et al.* (1999)	0, 0.5, 1, 2, 3.5 g/L in drinking-water 88, 55, 71, 55, 46/group	*Hepatocellular adenoma* At 78 wk ($n = 10$): 10%, 10%, 20%, 50%, 50% At 79–100 wk: 10% ($n = 50$), 20% ($n = 24$), 51.4%* ($n = 32$), 42.9%* ($n = 14$), 45%*($n = 8$) *Hepatocellular carcinoma* At 78 wk ($n = 10$): 10%, 0%, 20%, 50%, 70%* At 79–100 wk: 26% ($n = 50$), 48% ($n = 24$), 71%* ($n = 32$), 95%* ($n = 14$), 100%* ($n = 8$)	Trend (Fisher-Irwin test) *$P < 0.05$	Purity, > 99%; no contaminants detected Early sacrifice of groups of 10–15 mice at wk 26, 52 and 78. Number of mice at terminal kill varied as indicated. Not always apparent what the effective number of mice was at terminal kill. Inconsistent reporting. Limited pathology examination of tumours sites other than the liver.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mice, B6C3F$_1$ (M) Up to 87 wk Bull *et al.* (2002)	52 wk: 0, 0.1, 0.5, 2 g/L in drinking-water; 87 wk: 0, 0.5, 2 g/L Number of mice at start unclear	Combined incidence of liver hyperplastic nodules or hepatocellular adenoma or carcinoma: 52 wk: 1/20, 2/20, 5/20, 12/19* 87 wk: 4/7, 17/19*, 5/5	Fisher exact test *$P < 0.05$	Purity, NR Primarily an interaction study between DCA and TCA as they contribute to carcinogenicity of trichloroethylene. Limited statistical power of overall study, but particularly because of small numbers of mice available at 87 wk. Only liver was examined as a target organ. Limited histopathological diagnosis of lesions. Lesions include grossly observable nodules, hepatocellular adenoma or carcinoma.
Mice, Tg.AC hemizygous (M) 41 wk NTP (2007), Kissling *et al.* (2009)	0, 0.5, 1, 2 g/L in drinking-water 10, 10, 10, 10/group	Bronchioloalveolar adenoma: 1/10, 2/10, 7/10*, 3/10	Fisher exact test *$P < 0.01$	Purity, > 99% Liver tumours were not observed. Small numbers of mice. Short duration of treatment.
Mice, Tg.AC hemizygous (M) 39 wk NTP (2007), Kissling *et al.* (2009)	0, 31.25, 125, 500 mg/kg bw, skin application 10, 10, 10, 10/group	Skin papilloma: 0/10, 0/10, 2/10, 8/10*	Fisher exact test *$P < 0.01$	Small number of mice. Short duration of treatment.
Mice, Tg.AC hemizygous (F) 39 wk NTP (2007), Kissling *et al.* (2009)	0, 31.25, 125, 500 mg/kg bw, skin application 10, 10, 10, 10/group	Skin papilloma: 0/10, 0/10, 0/10, 6/10*	Fisher exact test *$P < 0.01$	Small number of mice. Short duration of treatment.

bw, body weight; DCA, dichloroacetic acid; F, female; M, male; mo, month; NR, not reported; NS; not significant; TCA, trichloroacetic acid; wk, week

Table 3.2 Integration of the studies of carcinogenicity in F344 rats given drinking-water containing dichloroacetic acid reported in Richmond *et al.* (1995) and DeAngelo *et al.* (1996)

Parameter	Study 1[a]				Study 2[b]	
	DCA (g/L)				DCA (g/L)	
	0 (NaCl, 2.0 g/L)	0.05	0.5	2.4[d]	0 (Water)[c]	1.6 [c,e]
Mean daily dose (mg/kg bw per day)	-	3.6	40.2	NR	-	139.1
No. of rats at start	50	60	60	60	78	78
No. of unscheduled deaths	6	12	10	NR	17	23
No. of rats killed at interim (45 and 60 wk)	21	27	27	NR	28	27
No. of rats killed at termination (100–104 wk)	23	21	23	NA	33	28
No. of rats surviving > 78 wk	[23]	26	29	NA	33	28
Prevalence of HN/HA/HC[f] (No.)	4.4%, 4.4%, 0% (23)	0%, 0%, 0%	10.3%, 17.2%, 10.3% (29)	NR	3%, 0%, 3%	3.6%, 10.7%, 21.4%*
Incidence of HN/HA/HC[c,f]	0/7, 0/7, 0/7	0/7, 0/7, 0/7	0/7, 1/7, 0/7	19/27,* 7/27, 1/27		
Prevalence of mononuclear cell leukaemia[b]	24%	20%	43%	NR	9%	11%

* $P < 0.05$

[a] Richmond *et al.* (1995) study, but some data for the control group, and groups receiving DCA at 0.05, 0.5 and 2.4 g/L were taken from DeAngelo *et al.* (1996)

[b] DeAngelo *et al.* (1996) study

[c] Termination for this group was at 60 wk. Data were taken from Richmond *et al.* (1995)

[d] The starting concentration for this group identified in the Richmond *et al.* (1995) study was 2.4 g/L and was maintained throughout the study; the DeAngelo *et al.* (1996) study identifies the starting concentration as 5 g/L in the abstract, and that this was lowered in stages to 1 g/L

[e] This is a time-weighted average dose: 2.5 g/L for 8 wk, 1.5 g/L from 8 to 26 wk, and 1.0 g/L from 26 wk to study termination. There was inconsistency in describing the starting concentration (5.0 g/L is mentioned in the abstract, but 2.5 g/L in the methods section).

[f] Hepatic nodules are lesions distinct from altered foci and that express similar phenotypes as hepatocellular adenoma and hepatocellular carcinoma.

bw, body weight; DCA, dichloroacetic acid; HA, hepatocellular adenoma; HC, hepatocellular carcinoma; HN, hepatic nodules; NA, not applicable; NR, not reported; wk, week

In mice receiving dichloroacetic acid for 61 weeks, 25 out of 26 ($P < 0.01$) had multiple liver [hepatocellular] adenomas (average, 4.58 ± 0.51 per mouse) and 21 out of 26 ($P < 0.01$) had multiple hepatocellular carcinomas (average, 1.69 ± 0.29 per mouse). Incidences of these lesions in mice in the control group were 2 out of 22, and 0 out 22, respectively. [The Working Group noted that this study was limited by examination of the liver only, the short duration of exposure, no reporting of consumption of drinking-water (but that this was likely to be depressed at 5 g/L based upon results of other studies), and the small numbers of mice examined.]

Male B6C3F$_1$ mice were given dichloroacetic acid (neutralized with NaOH) at a concentration of 1 g/L or 2 g/L for 37 or 52 weeks (Bull *et al.*, 1990). Controls received distilled water. There was a clear dose-related increase in the incidence of gross lesions in the liver. Some of these lesions were identified after histopathological examination as hyperplastic nodules, [hepatocellular] adenoma or hepatocellular carcinoma. The incidence of gross lesions after 52 weeks of exposure was 2 out of 35 in the control group, 2 out of 11 in the group receiving dichloroacetic acid at 1 g/L, and 23 out of 24 [$P < 0.0001$] in the group at 2 g/L. Only 45 of a total of 120 gross lesions found in the liver of mice receiving dichloroacetic acid and mice in the control group were examined histologically. In the control group and the group at 1 g/L, a single hyperplastic nodule was confirmed in each group after 52 weeks of treatment. In the group at 2 g/L, 15 hyperplastic nodules were confirmed in 9 mice, 2 liver adenomas in 2 mice, and 6 hepatocellular carcinomas in 5 mice. [The Working Group noted that observations were restricted to the liver, histopathological examination was only carried out on a fraction of the gross lesions observed, and statistical analyses were limited.]

DeAngelo *et al.* (1991) conducted two experiments in male B6C3F$_1$ mice. In the first experiment, mice were given drinking-water containing dichloroacetic acid at a concentration of 0 (control), 0.05, 0.5, or 5 g/L of for 60–75 weeks. The controls received NaCl at 2 g/L. In the second experiment, mice were given drinking-water containing acetic acid at 1.5 g/L (control) or dichloroacetic acid at 3.5 g/L. Dichloroacetic acid in the drinking-water was neutralized with NaOH.

In both experiments, mice treated with dichloroacetic acid at 3.5 or 5 g/L were killed after 60 weeks of treatment; mice treated with lower doses in the first experiment were killed after 60 weeks (nine mice per group) with the remaining mice killed after 75 weeks. The data from all experiments were combined for reporting. Statistically significant increases ($P < 0.001$) in the prevalence and multiplicity of hepatocellular adenoma and carcinoma of the liver were observed in the group at 5 g/L. A statistically significant increase ($P < 0.001$) in prevalence and multiplicity was also observed in the group at 3.5 g/L group. [The Working Group noted that liver, kidney, testes and spleen were examined for gross lesions and histopathology, but results were presented only for the liver. The treatments were of short duration, which may have prevented the expression of carcinogenesis at the lower doses. The Working Group also noted the limited reporting of the study.]

Daniel *et al.* (1992) presented the results of two experiments in which male B6C3F$_1$ mice were given drinking-water containing dichloroacetic acid at a concentration of 0.5 g/L, with mice in the control group being given distilled water. Dichloroacetic acid in drinking-water was neutralized with NaOH. In the first experiment, the initial number of mice in the control group and in the treated group was 23. In the control group, five mice were killed after 30 weeks and 60 weeks, and three died prematurely. Five mice were killed after 5 weeks and two died in the treated group, leaving ten survivors in the control group and sixteen in the treated group at termination of the study (104 weeks). In the second

experiment, there were 10 mice in the control group and in the treatment group. There were no interim kills, and while there were no premature deaths in the control group, there were two in the treated group. The data were combined for reporting. After 104 weeks, the prevalence of hepatocellular adenoma in surviving mice was 1 out of 20 in the control group, and 10 out of 24 ($P \leq 0.01$) in the group receiving dichloroacetic acid. Hepatocellular carcinoma was found in 2 out of 20 mice in the control group, and in 15 out of 24 ($P \leq 0.01$) in the group receiving dichloroacetic acid. [The Working Group noted that complete histopathological examinations were not performed, and that although selected organs (kidney, liver, testes and spleen) of survivors were examined, no data other than for liver were shown or discussed. The Working Group also noted the limited reporting, that no histopathology was reported for mice dying prematurely, and that the study was limited by the single dose and small number of mice.]

A group of 110 male B6C3F$_1$ mice were given drinking-water containing dichloroacetic acid at 5 g/L for 76 weeks, while a control group of 50 male mice were given distilled water (Anna *et al.*, 1994). Dichloroacetic acid in the drinking-water was neutralized with NaOH. In the control group, 24 mice were killed after 76 weeks, while the remaining mice were killed after 96, 103, or 134 weeks. [Only the 24 controls that were killed at the same time as the treated mice were considered by the Working Group.] Only the liver was examined grossly and microscopically for pathology.

Hepatocellular adenoma was detected in 2 out of 24 mice in the control group, and 2 out of these 24 mice were found to have hepatocellular carcinoma. Of the mice receiving dichloroacetic acid, 83 out of 89 [$P < 0.0001$] had hepatocellular adenoma and 66 out of 89 [$P < 0.0001$] had hepatocellular carcinoma. [The Working Group noted that the study was limited to a single high dose in a large group of mice, but only a limited number of mice in the control group were killed at the same time as the treated mice. Consumption of drinking-water at this high dose was not discussed by the authors. Liver was the only tissue for which lesions were characterized histopathologically.]

Groups of female B6C3F$_1$ mice were given drinking-water containing dichloroacetic acid at 0 (control group, $n = 134$), 2.0 mM [0.259 g/L] ($n = 90$), 6.67 mM [0.86 g/L] ($n = 50$), or 20.0 mM [2.59 g/L] ($n = 40$) (Pereira, 1996). Mice were killed after 360 or 576 days of treatment. The drinking-water of mice in the control group was supplemented with NaCl at 20.0 mM [1.15 g/L] to control for the amount of NaOH that was required to neutralize dichloroacetic acid in the drinking-water of treated mice. An additional group of 34 mice underwent repeated dosing with dichloroacetic acid at 2.59 g/L for 24 days, followed by 48 days without treatment [intermittent treatment]. The authors stated that this schedule was designed to provide the same total dose as the group receiving continuous treatment with dichloroacetic acid at 0.86 g/L. At day 360, 40 mice in the control group, 40 mice at 0.259g/L, 20 mice from each of the groups at 0.86 g/L and 2.59 g/L, and 15 mice from the intermittent-treatment group were killed. The remaining mice were killed at day 576 (90 in the control group, 50 in the group at 0.259 g/L, 28 in the group at 0.86 g/L, 19 in the group at 2.59 g/L, and 34 in the intermittent-treatment group). The incidences of hepatocellular adenoma and carcinoma in the treatment groups and by duration of treatment is shown in Table 3.1. Statistically significant increases in the incidence of hepatocellular adenoma were observed in the group at 2.59 g/L at day 360 and at day 576. An increase in the incidence of hepatocellular carcinoma was observed in the group at 2.59 g/L after 576 days. The incidence of liver foci per mouse in the group receiving intermittent treatment was similar to that in the group dosed continuously at 0.86 g/L after 576 days, but the incidence of hepatocellular

adenoma in the intermittent-treatment group was only 3 out of 34 versus 7 out of 28 in mice dosed continuously at 0.86 g/L. One hepatocellular carcinoma was observed in the intermittent-treatment group and in the group dosed continuously at 0.86 g/L (groups receiving equivalent total doses). [The Working Group noted that this study focused on liver; no other tissues were examined histopathologically.]

In an experiment that was designed primarily for the purpose of characterizing Ha-*ras* mutations in tumours induced by dichloroacetic acid, the incidence of hepatocellular carcinoma was 1 out of 39, 1 out of 25, and 23 out of 25 [$P < 0.001$] in female B6C3F$_1$ mice given drinking-water containing dichloroacetic acid at a concentration of 0, 0.5, or 3.5 g/L, respectively, for 104 weeks (Schroeder *et al.*, 1997). Mice in the control group were given 1.5% acetic acid. The incidence of hepatocellular adenoma was not reported. [The Working Group noted the limited reporting of this experiment, and that histopathological examination was restricted to the liver.]

DeAngelo *et al.* (1999) conducted a 2-year study with interim kills in male B6C3F$_1$ mice given drinking-water containing dichloroacetic acid at a concentration of 0 (n = 88), 0.5 (n = 55), 1 (n = 71), 2 (n = 55), or 3.5 g/L (n = 46). Dichloroacetic acid in the drinking-water was neutralized with NaOH. Water consumption was significantly reduced by dichloroacetic acid at the two higher doses over the first year of the study, but increased considerably in these groups during the second year and exceeded that of the other groups. The increase in water consumption during the second year was also noted at the lowest dose (0.5 g/L). A total of 35 mice in the control group and 30 mice from each of the groups receiving dichloroacetic acid were killed after 26, 52, and 78 weeks of treatment. Unscheduled deaths were reported for three mice in the control group, one mouse at 0.5 g/L, nine mice at 1 g/L, eleven mice at 2 g/L, and eight mice at 3.5 g/L. Thus 50, 24, 32, 14, and 8 mice remained at terminal kill (at 100 weeks), respectively. The number of mice per group for which pathological examination of the liver was performed was 85, 55, 65, 51, and 41, respectively. Data were reported as tumour prevalence and also as mean number of tumours per mouse, since multiple tumours are characteristic in mice given dichloroacetic acid at concentrations greater than 2 g/L (mean number of hepatocellular carcinomas at 0, 2, 3.5 and 5 g/L, respectively, was 0.3, 1.3, 2.5, 2.9, after 79–100 weeks of treatment). Hepatocellular carcinomas began to appear after 26 weeks of treatment in mice at 3.5 g/L. The prevalence of hepatocellular carcinoma was statistically significantly increased after 79–100 weeks in mice at 1, 2, or 3.5 g/L. [The Working Group noted that this was a group of studies presented together in one report. It was limited by the number of mice studied per group, inconsistent reporting, and limited pathology examination of tumour sites other than the liver. Data were reported as percentage of mice with tumours, and it was not always apparent what the effective number of mice was at terminal kill.]

A study attempted to determine the extent to which dichloroacetic acid and trichloroacetic acid contributed to liver tumours induced by trichloroethylene (Bull *et al.*, 2002). The only organ examined was the liver. Among other treatments, the study included assessment of the tumorigenic effects of drinking-water containing dichloroacetic acid at three concentrations (0.1, 0.5, and 2 g/L) in male B6C3F$_1$ mice. Dichloroacetic acid in the drinking-water was neutralized with NaOH. Mice were killed after 52 or 87 weeks of treatment and the data reported as combined incidence of liver hyperplastic nodules, hepatocellular adenoma or carcinoma. Increases in the incidence of liver hyperplastic nodules, hepatocellular adenoma, or carcinoma (combined) were observed in some groups of mice treated with dichloroacetic acid. [The Working Group noted that the study was limited by the examination of the liver only, the

short duration of exposure, the small number of mice remaining at 87 weeks, the uncertainty of reporting lesion prevalence (i.e. random selection of gross lesions for histopathology examination), and the issue of lesion grouping.]

Dichloroacetic acid has been tested in genetically modified mouse strains: the Tg.AC hemizygous and p53 haploinsufficient strains (NTP, 2007; Kissling *et al.*, 2009). Drinking-water containing dichloroacetic acid at concentrations of 0.5, 1, and 2 g/L was given to males and females of both strains. The duration of the studies was 41 weeks. While there was no evidence for induction of liver tumours, there was an increase in the incidence of bronchioloalveolar adenoma in male Tg.AC hemizygous mice (control group, 1 out of 10; 0.5 g/L, 2 out of 10; 1 g/L, 7 out of 10 ($P < 0.01$); 2 g/L, 3 out of 10).

3.1.2 Skin application

Male and female Tg.AC mice received dichloroacetic acid at a dose of 0, 31.25, 125, or 500 mg/kg bw applied to the skin (NTP, 2007; Kissling *et al.*, 2009). After 39 weeks, there was a statistically significant increase ($P < 0.01$) in the incidence of skin papilloma at the highest dose in males (8 out of 10 versus 0 out of 10 in the control group) and females (6 out of 10 versus 0 out of 10 in the control group). [The Working Group noted the small number of mice and the short duration of treatment used in this study.]

3.2 Rat

Oral administration

The two publications reporting studies in rats given drinking-water containing dichloroacetic acid (Richmond *et al.*, 1995; DeAngelo *et al.*, 1996) appeared to contain much of the same data. Since there were some inconsistencies in reporting of the two studies, the Working Group prepared a table (Table 3.2) to clarify how the data overlapped.

As the data were more completely reported for some groups in DeAngelo *et al.* (1996), the Working Group preferentially placed these data into the table when there were small discrepancies in reporting. The Working Group recognized these inconsistencies, but did not believe they affected the utility of the data for the evaluation of the carcinogenicity of dichloroacetic acid.

In a study of phenotypical changes in liver lesions according to duration of treatment and lesion type, male F344 rats (age, 28 days) were given drinking-water containing dichloroacetic acid at a concentration of 0 (drinking-water containing NaCl at 2.0 g/L to control for NaOH added to neutralize dichloroacetic acid), 0.05, 0.5, or 2.4 g/L for 45, 60, or 100–104 weeks (Richmond *et al.*, 1995). Results were only reported for the liver. All surviving rats in the group at the highest dose were killed at 60 weeks. After 45 weeks of treatment, a single adenoma was noted in the group at the highest dose. After 60 weeks, no lesions were observed in rats at the two lower doses, but in rats in the group at 2.4 g/L, 19 out of 27 had hyperplastic nodules ($P < 0.05$), 7 out of 27 had hepatocellular adenomas (not statistically significant), and 1 out of 27 (not statistically significant) had hepatocellular carcinomas. At terminal kill, in the control group, 1 out of 23 rats had hepatocellular adenoma; at 0.05 g/L, 0 out of 26 rats had any lesion; and at 0.5 g/L, 3 out of 29 had hyperplastic nodules, 6 out of 29 had hepatocellular adenoma, and 3 out 29 had hepatocellular carcinoma. [The Working Group noted that the limitations of this study were that only the liver was examined by histopathology, rats that died during the course of the experiment were not examined by histopathology, rats at 2.4 g/L were killed at 60 weeks, and the terminal kill of rats in the control group was at 104 weeks while that of rats in groups receiving dichloroacetic acid was at 100 weeks.]

The DeAngelo *et al.* (1996) study repeated part of the data set from Richmond *et al.* (1995), but added a water control and a single dose of dichloroacetic acid (neutralized with NaOH) that was given to male F344 rats in drinking-water at initial concentrations of 0 or 2.5 g/L [Study 2, Table 3.2] beginning at age 28–30 days (DeAngelo *et al.*, 1996). [The Working Group noted that the dose was reduced from 2.5 g/L to 1.5 g/L at 8 weeks, and then to 1 g/L at 26 weeks, resulting in a time-weighted average of 1.6 g/L over the study duration.] Prevalence data were provided for the terminal kill at 103 weeks. In the 33 rats remaining at termination in the control group, prevalences were: hyperplastic nodules, 3%; hepatocellular adenoma, 0%; and hepatocellular carcinoma, 3%. In the group receiving dichloroacetic acid, prevalences at termination were: hyperplastic nodules, 3.6%; hepatocellular adenoma, 10.7%; and hepatocellular carcinoma, 21.4%. [The Working Group noted that rats from interim kills of both studies did not appear to have been examined for neoplastic lesions, but were used to investigate mechanistic questions.] A renal tubular adenoma was found in the group receiving dichloroacetic acid, while none were observed in the control group. [The Working Group noted that the incidence of this tumour in historical controls in F344 rats was 10 out of 1352 (0.7%) (Haseman *et al.*, 1998).]

The DeAngelo *et al.* (1996) study indicated that mononuclear cell leukaemia was observed at a prevalence of 24% in the control group receiving NaCl, 20% in the group receiving dichloroacetic acid at 0.05 g/L, and 43% in the group receiving dichloroacetic acid at 0.5 g/L in the Richmond *et al.* (1995) study. Neither study indicated the prevalence of mononuclear cell leukaemia at 2.4 g/L. In the study by DeAngelo *et al.* (1996), the prevalence of mononuclear cell leukaemia was 9% in the control group receiving water, and 11% in the group receiving dichloroacetic acid at 1.6 g/L. [The Working Group noted that both experiments had limited statistical power. Although the reporting of the study by DeAngelo *et al.* (1996) was limited, with no data for individual animals, it nevertheless contained two separate experiments both reporting a positive response in the liver.]

3.3 Co-administration with known carcinogens or other modifying factors

In the Herren-Freund *et al.* (1987) initiation–promotion study in male B6C3F$_1$ mice, cited above (see Section 3.1.1), mice were initiated with an intraperitonal injection of *N*-ethyl-*N*-nitrosourea (ENU) at 2.5 mg/kg bw on postnatal day 15. From postnatal day 28 and continuing for 61 weeks, the mice were given drinking-water containing dichloroacetic acid at 0 (control; NaCl, 2 g/L), 2 or 5 g/L. The study focused on liver tumorigenesis. In the control group of mice initiated with ENU and maintained on water containing NaCl, 1 out of 22 mice had liver [hepatocellular] adenoma, and 1 out of 22 had hepatocellular carcinoma. In the group of mice initiated with ENU and subsequently treated with dichloroacetic acid at 2 g/L, 22 out of 29 ($P < 0.01$) mice had liver adenoma, and 19 out of 29 ($P < 0.01$) had hepatocellular carcinoma. In mice initiated with ENU, but treated with dichloroacetic acid at 5 g/L, incidences of these lesions were 31 out of 32 ($P < 0.01$) and 25 out of 32 ($P < 0.01$), respectively.

An initiation–promotion study assessed dichloroacetic acid as a promoter in female B6C3F$_1$ mice (Pereira & Phelps, 1996). Mice were initiated with an intraperitoneal injection of *N*-methyl-*N*-nitrosourea (MNU) at 25 mg/kg bw on postnatal day 15. Treatment with dichloroacetic acid began at age 7 weeks and was continued for 52 weeks. Dichloroacetic acid was administered in the drinking-water at concentrations of 2.0 ($n = 9$), 6.67 ($n = 9$), and 20.0 ($n = 24$) mM [i.e. 0.259, 0.86, and 2.59 g/L].

The study focused on liver tumorigenesis and data were expressed as numbers of lesions per mouse and percentage of mice with the indicated lesion. A "recovery" group (n = 12) was given dichloroacetic acid at 20 mM [2.59 g/L] for 31 weeks, after which the treatment was suspended, and the mice were killed at experimental week 52. After 52 weeks, there were increases in the incidences of hepatocellular adenoma and carcinoma in groups of MNU-initiated mice treated with dichloroacetic acid at 2.0, 6.7, or 20 mM relative to MNU-initiated controls (n = 39). The percentages of mice with hepatocellular adenoma were 10% (control), 40%, 20%, and 19.2%; and the percentages of mice with hepatocellular carcinoma were 17.5% (control), 20%, 10%, and 73.1%, for increasing doses. The percentages of mice with hepatocellular adenoma and hepatocellular carcinoma in the recovery group were 46.2% and 15.4%, respectively. [The Working Group noted the limited reporting of the study and the small number of mice used.]

Another study from the same group (Pereira *et al.*, 1997) examined the ability of mixtures of dichloroacetic acid and trichloroacetic acid to promote MNU-initiated liver tumours in female B6C3F$_1$ mice. All mice were initiated with an intraperitonal injection of MNU at 25 mg/kg bw on postnatal day 15. Nine groups were given dichloroacetic acid or trichloroacetic acid alone, or combinations of dichloroacetic acid and trichloroacetic acid. An additional control group was treated with MNU only. Dichloroacetic acid and trichloroacetic acid in the drinking-water were neutralized with NaOH. Treatments with dichloroacetic acid and trichloroacetic acid started at age 6 weeks and continued for 44 weeks. Survival (number surviving out of initial number of animals) was: controls, 29 out of 30; dichloroacetic acid, 1 g/L, 17 out of 20; dichloroacetic acid, 2 g/L, 19 out of 20; dichloroacetic acid, 3.2 g/L, 29 out of 30; trichloroacetic acid, 1 g/L, 20 out of 20; trichloroacetic acid, 4 g/L, 29 out of 30; dichloroacetic acid (3.2 g/L) + trichloroacetic acid (1 g/L), 21 out of 25; dichloroacetic acid (2 g/L) + trichloroacetic acid (1 g/L), 42 out of 45; dichloroacetic acid (1 g/L) + trichloroacetic acid (1 g/L), 22 out of 25; and trichloroacetic acid (4 g/L) + dichloroacetic acid (2 g/L), 19 out of 20. Dichloroacetic acid produced a dose-dependent increase in the incidence of hepatocellular adenoma per mouse relative to the MNU-initiated controls (MNU only, 0.07; MNU + dichloroacetic acid at 1 g/L, 0.06; MNU + dichloroacetic acid at 2 g/L, 0.32; MNU + dichloroacetic acid at 3.2 g/L, 1.8 [$P < 0.05$]). [The conclusions regarding interactions between dichloroacetic acid and trichloroacetic acid were discussed without presenting the detailed data.] A fixed dose of TCA at 1 g/L statistically significantly enhanced the yield of total proliferative lesions (liver foci and hepatocellular adenomas, combined) observed with dichloroacetic acid at 1 g/L, but effects were less than additive with treatments with dichloroacetic acid at 2 or 3.2 g/L. [The Working Group noted that the doses given were somewhat higher than those used in cancer bioassays, and probably affected consumption of drinking-water. As a consequence, the true dose received by the mouse may not have been linearly related to the concentration of dichloroacetic acid in the drinking-water.]

A study examined the interactions of three tumour promoters (dichloroacetic acid, trichloroacetic acid and carbon tetrachloride) in male B6C3F$_1$ mice initiated with vinyl carbamate (Bull *et al.*, 2004). Vinyl carbamate was administered at a dose of 3 mg/kg bw [administration route not reported] at age 2 weeks. Groups of 10 mice were treated with different doses of the individual promoters or mixtures for 18, 24, 30, or 36 weeks (70 different experimental groups in total). Macroscopically observable liver lesions were all sectioned, but only a subsample was randomly examined microscopically for diagnosis of hyperplastic nodules, hepatocellular adenoma or hepatocellular carcinoma. No attempt was made to differentiate between these lesions

in the analysis of the data. In mice receiving dichloroacetic acid at a concentration of 0.1, 0.5 or 2 g/L, the number and the size of liver lesions was increased compared with mice treated with vinyl carbamate only. There were significant interactions between the three agents that both enhanced or inhibited the development of liver lesions. The interactions between lesion size and number were frequently reciprocal in direction. [The Working Group noted the complexity of the data set, that only a representative sampling was submitted for histopathological analysis as a check on the gross observations, and that the size of individual groups was small.]

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

Toxicokinetic studies of dichloroacetic acid have been detailed extensively in Volume 84 of the *IARC Monographs* (IARC, 2004). Therefore, this information is summarized here:

Dichloroacetic acid is readily absorbed from the gut and widely distributed systemically in humans and rodents. Dichloroacetic acid is metabolized to glyoxylate via the enzyme glutathione *S*-transferase zeta 1 (GST-zeta1) in humans and rodents. Glyoxylate is converted via lactate dehydrogenase to oxalate, which is excreted in the urine (Fig. 4.1). Transamination of glyoxylate in peroxisomes can produce glycine, which can be incorporated into protein. In rats and humans, dichloroacetic acid has been shown to inhibit its own metabolism by inhibiting GST-zeta1, the key enzyme responsible for its metabolism (Fig. 4.2).

The inhibitory effect of dichloroacetic acid on its own metabolism has been further explored in more recent studies in humans and in rodents:

In a stable-isotope study by Schultz & Shangraw (2006), the authors tested the effect of pretreatment with dichloroacetic acid on the pharmacokinetics of later doses of dichloroacetic acid in eight male and eight female volunteers. In the absence of pretreatment with dichloroacetic acid at a dose of 0.02 μg/kg bw per day for 14 days, there were no sex differences in the pharmacokinetics of dichloroacetic acid. Only women were affected by pretreatment with dichloroacetic acid, showing an increased area under the curve of concentration–time (AUC) for plasma dichloroacetic acid and a decreased rate of clearance.

Toxicokinetic studies in rodents (Saghir & Schultz, 2002; Schultz *et al.*, 2002, 2004) showed that dichloroacetic acid, even at environmental concentrations (0.2 g/L in drinking-water), inhibits its own metabolism via inhibition of GST-zeta1, slowing down the elimination of dichloroacetic acid and leading to increased potential for carcinogenicity in rodents. In mice, the ability of dichloroacetic acid to inhibit its own metabolism is greatest in the young (Schultz *et al.*, 2002, 2004). In another study by Saghir & Schultz (2005), rats were studied for the effects of depletion of GST-zeta1 on the elimination of mixtures of di- and tri-halogenated acidic acids. Pre-treatment with dichloroacetic acid (to deplete GST-zeta1) increased the elimination of tri-halogenated acetic acids.

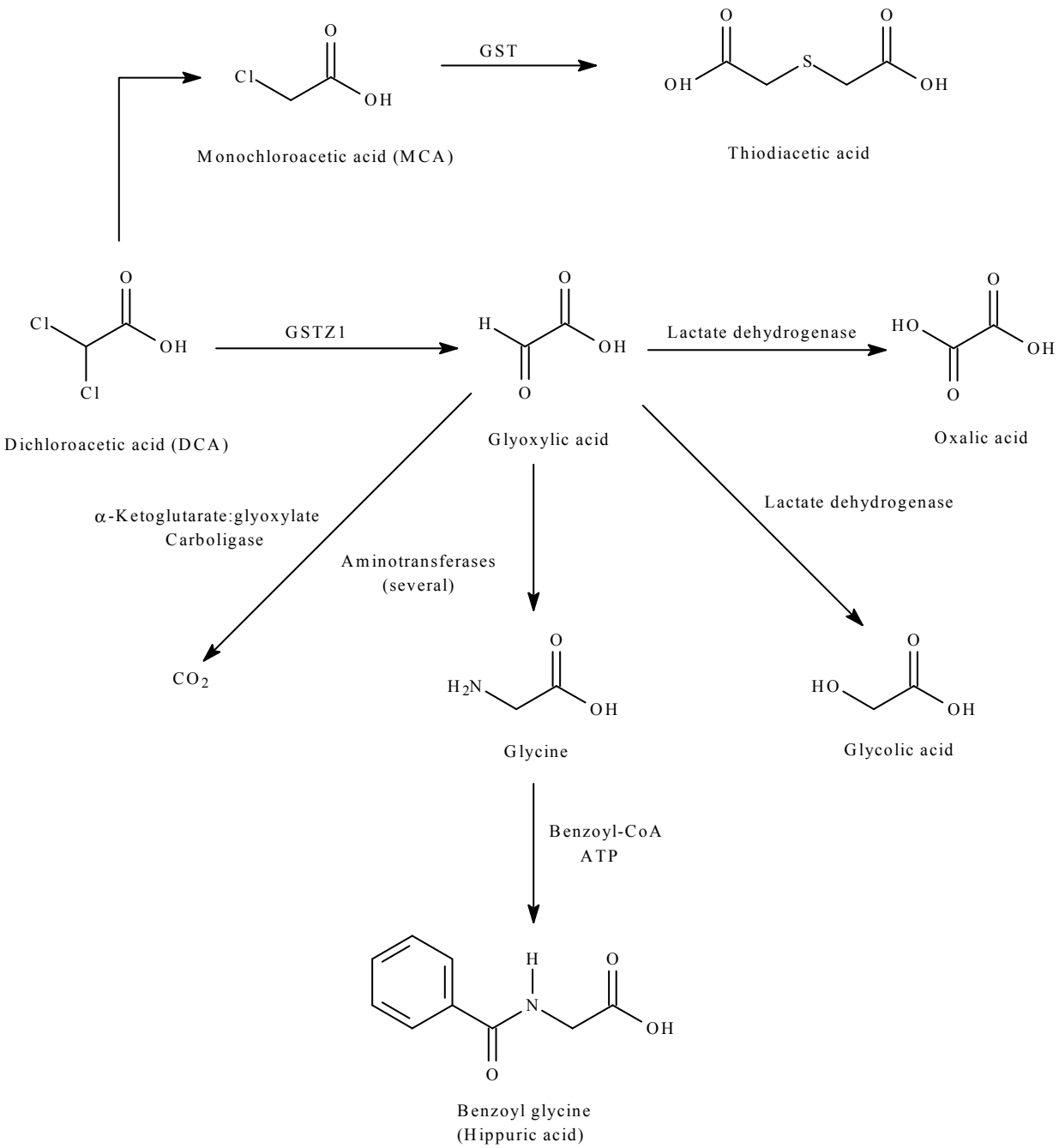
4.2 Genotoxicity and related effects

The results of tests for mutagenicity with dichloroacetic acid in mammalian systems are summarized in Table 4.1.

4.2.1 Humans

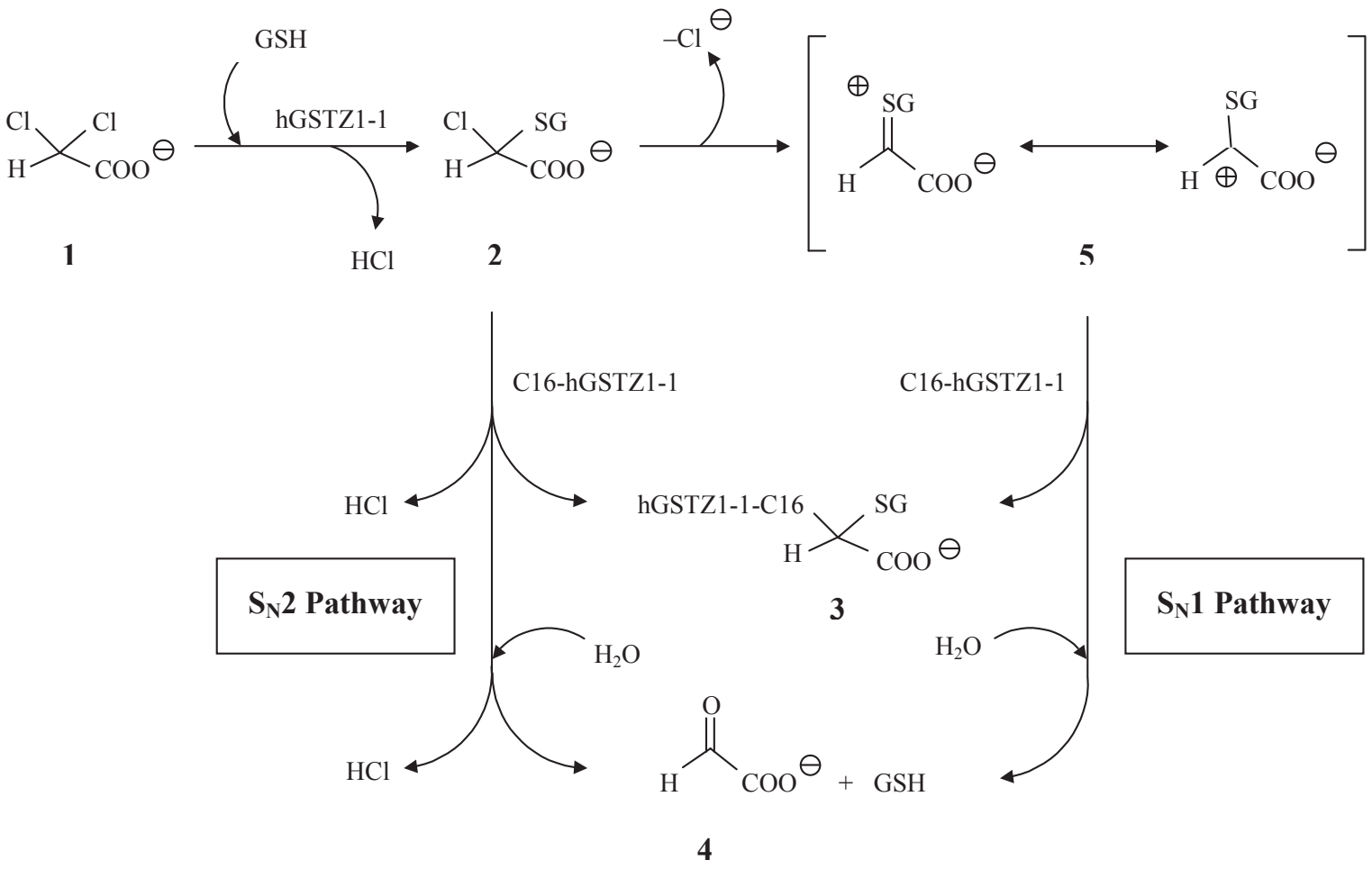
No DNA strand breaks were observed in human CCRF-CEM lymphoblastoid cells exposed to dichloroacetic acid *in vitro* (Chang *et al.*, 1992).

Fig. 4.1 Proposed metabolism of dichloroacetic acid



GST, glutathione-*S*-transferase; GSTZ1, GST-zeta1; P450, cytochrome P450
Prepared by the Working Group

Fig. 4.2 Mechanisms of human GSTZ1-catalysed biotransformation of dichloroacetic acid to glyoxylic acid and inactivation of GSTZ1 by dichloroacetic acid



1, dichloroacetic acid; 2, *S*-(α-chlorocarboxymethyl)glutathione; 3, human GSTZ1 covalently modified at cysteine-16; 4, glyoxylic acid; 5, sulfonium-carbocation intermediate
Adapted with permission from Anderson *et al.* (2002). Copyright (2002) American Chemical Society.

Table 4.1 Studies of genotoxicity with dichloroacetic acid in mammalian systems *in vitro* and *in vivo*

Test system/end-point	Dose[a] (LED or HID)	Results		Reference
		With metabolic activation	Without metabolic activation	
In vitro				
DNA strand breaks and alkali-labile damage, Chinese hamster ovary cells (single-cell gel electrophoresis assay)	3225	NT	–	Plewa *et al.* (2002)
DNA strand breaks, $B6C3F_1$ mouse hepatocytes	2580	NT	–	Chang *et al.* (1992)
DNA strand breaks, F344 rat hepatocytes	1290	NT	–	Chang *et al.* (1992)
Gene mutation, mouse lymphoma cell line L5178Y/$Tk^{+/-}$	5000	–	–	Fox *et al.* (1996a)
Gene mutation, mouse lymphoma cell line L5178Y/$Tk^{+/-}$–3.7.2C	400	NT	+	Harrington-Brock *et al.* (1998)
Gene mutation, Chinese hamster ovary cells, *HGPRT* [*Hprt*] gene mutation assay	129	NT	+	Zhang *et al.* (2010b)
Micronucleus formation, mouse lymphoma L5178Y/$Tk^{+/-}$–3.7.2C cell line	800	NT	–	Harrington-Brock *et al.* (1998)
Chromosomal aberrations, Chinese hamster ovary	5000	–	–	Fox *et al.* (1996a)
Chromosomal aberrations, mouse lymphoma L5178Y/$Tk^{+/-}$–3.7.2C cell line	600	NT	+	Harrington-Brock *et al.* (1998)
Aneuploidy, mouse lymphoma L5178Y/$Tk^{+/-}$–3.7.2C cell line	800	NT	–	Harrington-Brock *et al.* (1998)
DNA strand breaks, human CCRF-CEM lymphoblastoid cells	1290	NT	–	Chang *et al.* (1992)
In vivo				
DNA strand breaks, male $B6C3F_1$ mouse liver	13, oral, × 1	NT	+	Nelson & Bull (1988)
DNA strand breaks, male $B6C3F_1$ mouse liver	10, oral, × 1	NT	+	Nelson *et al.* (1989)
DNA strand breaks, male $B6C3F_1$ mouse liver	1290, oral, × 1	NT	–	Chang *et al.* (1992)
DNA strand breaks, male $B6C3F_1$ mouse splenocytes	1290, oral, × 1	NT	–	Chang *et al.* (1992)
DNA strand breaks, male $B6C3F_1$ mouse epithelial cells from stomach and duodenum	1290, oral, × 1	NT	–	Chang *et al.* (1992)
DNA strand breaks, male $B6C3F_1$ mouse liver	5000, dw, × 7–14 days	NT	–	Chang *et al.* (1992)
DNA strand breaks, alkali-labile sites, cross linking, male $B6C3F_1$ mouse blood leukocytes (single-cell gel electrophoresis assay)	3500, dw, × 28 days	NT	+	Fuscoe *et al.* (1996)
DNA strand breaks, male Sprague-Dawley rat liver	30, oral, × 1	NT	+	Nelson & Bull (1988)
DNA strand breaks, male F344 rat liver	645, oral, × 1	NT	–	Chang *et al.* (1992)

Table 4.1 (continued)

Test system/end-point	Dosea (LED or HID)	Results		Reference
		With metabolic activation	Without metabolic activation	
DNA strand breaks, male F344 rat liver	2000, dw, × 30 wk	NT	–	Chang *et al.* (1992)
Gene mutation, lacI transgenic male B6C3F$_1$ mouse liver assay	1000, dw, × 60 wk	NT	+	Leavitt *et al.* (1997)
Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes	3500, dw, × 9 days	NT	+	Fuscoe *et al.* (1996)
Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes	3500, dw, × 28 days	NT	–	Fuscoe *et al.* (1996)
Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes	3500, dw, × 10 wk	NT	+	Fuscoe *et al.* (1996)
Micronucleus formation, male and female Crl:CD (Sprague-Dawley) BR rat bone-marrow erythrocytes	1100, i.v., × 3	NT	–	Fox *et al.* (1996a)
Micronucleus formation, *Pleurodeles waltl* newt larvae peripheral erythrocytes	80 days	NT	–	Giller *et al.* (1997)

[a] Doses are in µg/mL for tests *in vitro*; mg/kg bw for tests *in vivo*, unless specified.

+, positive; –, negative; dw, drinking-water (in mg/L); HID, highest ineffective dose; i.v., intravenous injection; LED, lowest effective dose; NT, not tested; wk, week.

4.2.2 Experimental systems

(a) Mammalian systems

(i) Gene mutation

Mutation frequencies were studied in male transgenic B6C3F$_1$ mice harbouring the bacterial *lacI* gene and given drinking-water containing dichloroacetic acid at 1.0 g/L or 3.5 g/L (Leavitt *et al.*, 1997). No statistically significant differences in mutation frequency were observed after 4 or 10 weeks of treatment at either dose when compared with controls. However, at 60 weeks, mice treated with dichloroacetic acid at 1.0 g/L showed a slight increase (1.3-fold) in mutation frequency compared with controls, and mice treated with dichloroacetic acid at 3.5 g/L showed an increase of 2.3-fold. Mutational spectrum analysis revealed that ~33% had G:C to A:T transitions and 21% had G:C to T:A transversions; this mutation spectrum was different to that seen in the untreated mice, indicating that the mutations were probably induced by treatment with dichloroacetic acid.

Harrington-Brock *et al.* (1998) evaluated dichloroacetic acid for mutagenic activity in L5178Y/*Tk*$^{+/-}$–3.7.2C mouse lymphoma cells. A dose-related increase in mutation frequency (and cytotoxicity) was observed at concentrations of 400–800 µg/mL. Most mutagenic activity of dichloroacetic acid at the *Tk* locus was due to the production of small-colony *Tk* mutants (indicating chromosomal mutations). There was no effect of pH on the induction of mutants.

Zhang *et al.* (2010a) tested the cytotoxic and genotoxic effects of dichloroacetic acid at 0, 200, 1000, 5000 or 10 000 µM [0, 129, 645 and 1290 µg/mL]) in a microplate-based test for cytotoxicity and an assay for HGPRT [*Hprt*] gene mutation with Chinese hamster ovary K1 cells, respectively. Two parameters were used to indicate long-term cytotoxicity: the lowest concentration at which cytotoxicity was apparent, and the percentage C1/2 value (the concentration at which cell density was reduced to 50% of values for negative controls). The lowest concentration at which dichloroacetic acid caused cytotoxicity was 2.87×10^{-3} M [370 µg/mL]. A statistically significant increase in the frequency of HGPRT mutation was observed at a concentration of 1000 µM [129 µg/mL].

(ii) Chromosomal aberration

Harrington-Brock *et al.* (1998) evaluated dichloroacetic acid for its potential to induce chromosomal aberration in mouse lymphoma cells treated with dichloroacetic acid at 0, 600, or 800 µg/mL). Results were clearly positive at both concentrations tested. However, no chromosomal aberrations were found in Chinese hamster ovary cells exposed to dichloroacetic acid (Fox *et al.*, 1996a).

(iii) Micronucleus formation

Fuscoe *et al.* (1996) investigated genotoxic potential *in vivo* in male B6C3F$_1$ mice given drinking-water containing dichloroacetic acid (pH-adjusted exposures, 0.5, 1, 2 and 3.5 g/L; available *ad libitum*, for up to 31 weeks). At the highest exposure tested, a statistically significant increase in the frequency of micronucleated erythrocytes was observed after exposure to dichloroacetic acid for 9 days, but not against a higher background at 28 days. A small but statistically significant increase was also observed after exposure for 10 weeks at the highest dose of dichloroacetic acid tested (3.5 g/L). The results of the alkaline single-cell gel electrophoresis (comet) assay are discussed below.

No statistically significant increase in micronucleus formation was observed in mouse lymphoma cells treated with dichloroacetic acid at 0, 600, or 800 µg/mL (Harrington-Brock *et al.*, 1998).

(iv) DNA damage

Fuscoe *et al.* (1996) also investigated genotoxic potential *in vivo* in bone marrow and blood leukocytes of male B6C3F$_1$ mice given drinking-water containing dichloroacetic acid for up

to 31 weeks. DNA crosslinking was observed in blood leukocytes of mice exposed to dichloroacetic acid at 3.5 g/L for 28 days.

Nelson & Bull (1988) and Nelson *et al.* (1989) reported positive results for DNA unwinding with dichloroacetic acid, with Nelson *et al.* (1989) reporting the same response with dichloroacetic acid at 10 and 500 mg/kg bw in mice. Chang *et al.* (1992) conducted studies of DNA damage *in vitro* and *in vivo*, finding that primary rat (F344) hepatocytes and primary mouse hepatocytes treated with dichloroacetic acid for 4 hours did not exhibit DNA single-strand breaks as detected by the alkaline DNA unwinding assay. Similarly, analysis of DNA single-strand breaks in mice killed 1 hour after a single dose of dichloroacetic acid at 1, 5, or 10 mM/kg bw [129, 645, 1290 mg/kg bw] suggested that dichloroacetic acid did not cause DNA damage. There was no detectable DNA damage in F344 rats killed 4 hours after a single gavage dose of dichloroacetic acid (1–10 mM/kg bw [129–1290 mg/kg bw]).

(v) Mutational analyses of tumours

Anna *et al.* (1994) exposed male B6C3F$_1$ mice to drinking-water containing dichloroacetic acid at a concentration of 0 (50 animals) or 5 g/L (110 animals; about 900 mg/kg bw per day), 5 days per week, for 76 weeks. Dichloroacetic acid increased the incidence of hepatic adenoma (93% of exposed mice versus 8% of control mice had at least one adenoma), and hepatocarcinoma (74% of exposed mice versus 8% of control mice had at least one carcinoma). The frequency of mutation at H-*ras* codon 61 did not differ among dichloroacetic acid-induced and spontaneous hepatocellular tumours. However, significant changes were seen in the mutation spectra of H-*ras* [*Hras*] codon 61 after exposure to dichloroacetic acid. In the spontaneous tumours from the controls (study controls plus historical controls), the CAA of codon 61 became AAA in 58% of the tumours, CGA in 27% and CTA in 14%. In the dichloroacetic acid-exposed mice, H-*ras* codon 61 changes were AAA in 28%, CGA in 35% and CTA in 38%.

In a study by Ferreira-Gonzalez *et al.* (1995), male B6C3F$_1$ mice were given drinking-water containing dichloroacetic acid at a concentration of 1.0 or 3.5 g/L (180 or 630 mg/kg bw per day) for 104 weeks. The incidence of liver carcinoma was 19%, 70.6% and 100% in the control group, and in the groups at 180 mg/kg bw per day and 630 mg/kg bw per day, respectively. DNA samples were examined from 32 spontaneous liver tumours from the control group, 13 tumours from the group at 180 mg/kg bw per day, and 33 tumours from the group at 630 mg/kg bw per day. Similar frequencies of mutation at H-*ras* proto-oncogene exon 2 were found in all three groups (spontaneous tumours, 58%; 180 mg/kg bw per day, 48%; and 630 mg/kg bw per day, 50%). Mutation frequencies in exons 1 and 3 were minimal. Comparative sequence analysis of exon 2 mutations in spontaneous and dichloroacetic acid-induced tumours revealed a substantial shift in the spectrum of base changes in codon 61. In spontaneous tumours, changes in codon 61 from CAA to AAA in 80% and CAA to CGA in 20% of the examined tumours were revealed, while no conversion of CAA to CTA was observed. In contrast, the frequency of conversion of CAA to AAA was 16% and 21% at doses of 180 and 630 mg/kg bw per day, respectively. Conversion of CAA to CGA was noted in 50% of the tumours from mice treated with dichloroacetic acid at 180 or 630 mg/kg bw per day, and conversion of CAA to CTA was observed in 34% and 29% in these two groups, respectively. Thus, although dichloroacetic acid-induced and spontaneous tumours involved similar levels of H-*ras* mutation, the mechanisms of tumour induction may be different. Differences in codon 61 mutation spectra between spontaneous and dichloroacetic acid-induced tumours in this study are similar to those reported in the study by Anna *et al.* (1994), in which there was also a lower number of CAA

Table 4.2 Studies of genotoxicity with dichloroacetic acid in bacterial systems

Test system/end-point	Dose[a] (LED or HID)	Results		Reference
		With metabolic activation	Without metabolic activation	
λ Prophage induction, *Escherichia coli* WP2s	2500	+	–	DeMarini *et al.* (1994)
SOS chromotest, *E. coli* PQ37	500	–	(+)	Giller *et al.* (1997)
Salmonella typhimurium, DNA repair-deficient strains TS24, TA2322, TA1950	31 000	–	–	Waskell (1978)
S. typhimurium TA100, TA1535, TA1537, TA1538, reverse mutation	NR	–	–	Herbert *et al.* (1980)
S. typhimurium TA100, reverse mutation	50	+	+	DeMarini *et al.* (1994)
S. typhimurium TA100, TA1535, TA1537, TA98, reverse mutation	5000	–	–	Fox *et al.* (1996a)
S. typhimurium TA100, reverse mutation, liquid medium	100	+	+	Giller *et al.* (1997)
S. typhimurium RSJ100, reverse mutation	1935	–	+	Kargalioglu *et al.* (2002)
S. typhimurium TA104, reverse mutation, microsuspension	150 µg/plate	–	–	Nelson *et al.* (2001)
S. typhimurium TA98, reverse mutation	10 µg/plate	(+)	–	Herbert *et al.* (1980)
S. typhimurium TA98, reverse mutation	5160	–	+	Kargalioglu *et al.* (2002)
S. typhimurium TA100, reverse mutation	1935	+	+	Kargalioglu *et al.* (2002)
E. coli WP2*uvrA*, reverse mutation	5000	–	–	Fox *et al.* (1996a)

[a] Doses are in µg/mL for tests *in vitro*, unless specified.
+, positive; (+), weakly positive; –, negative; HID, highest ineffective dose; LED, lowest effective dose; NR, not reported

to AAA conversions and a higher number of CAA to CTA conversions in the dichloroacetic acid-induced tumours than in the spontaneous tumours.

Schroeder *et al.* (1997) examined dichloroacetic acid-induced tumours in female B6C3F$_1$ mice for mutations in H-*ras* codon 61. There was an H-*ras* mutation in only one of 22 tumours, revealing a CAA to CTA conversion.

(b) Bacterial and fungal systems: gene mutation

Studies to evaluate the mutagenicity of dichloroacetic acid in various strains of *S. typhimurium* and *E. coli* (Waskell, 1978; Herbert *et al.*, 1980; DeMarini *et al.*, 1994; Fox *et al.*, 1996a; Giller *et al.*, 1997; Nelson *et al.*, 2001; Kargalioglu *et al.*, 2002) are summarized in Table 4.2. Dichloroacetic acid was mutagenic in three strains of *S. typhimurium*: strain TA100 in three out of five studies, strain RSJ100 in a single study, and strain TA98 in two out of three studies. Dichloroacetic acid failed to induce point mutations in other strains of *S. typhimurium* (TA104, TA1535, TA1537, and TA1538) or in *E. coli* strain WP2*uvrA*. In one study, dichloroacetic acid caused a weak induction of SOS repair in *E. coli* strain PQ37 (Giller *et al.*, 1997).

4.3 Non-genotoxic mechanisms of carcinogenesis

4.3.1 Liver

The available evidence for non-genotoxic mechanisms for the induction by dichloroacetic acid of liver tumours in rodents (mouse) comprises the following: (a) epigenetic effects (especially DNA hypomethylation); (b) cytotoxicity and oxidative stress; (c) alteration of

proliferation and apoptosis, and clonal expansion; (d) PPARα activation; and (e) disruption of gap-junctional communication. Evidence supporting each of these non-genotoxic mechanisms from studies in humans and experimental animals is presented below.

(a) Epigenetic effects

Epigenetic events that have been studied primarily include studies of changes in DNA methylation, both of total DNA and of particular genes. Expression of the affected genes, and activity of DNA methyltransferases, has also been investigated.

(i) Humans

No dichloroacetic acid-specific data regarding alteration in DNA methylation from studies in humans were available to the Working Group.

(ii) Experimental systems

Hypomethylation of DNA may be related to the carcinogenicity of trichloroacetic acid and dichloroacetic acid in mice.

In female B6C3F_1 mice that received an intraperitoneal injection of MNU and were then given drinking-water containing trichloroacetic acid or dichloroacetic acid, DNA methylation in the resulting hepatocellular adenomas and carcinomas was about half that observed in non-tumour tissue from the same animal or from animals given only MNU (Tao *et al.*, 1998). Exposure of female B6C3F_1 mice to drinking-water containing trichloroacetic acid or dichloroacetic acid for 11 days also decreased total liver DNA methylation by 60% (Tao *et al.*, 1998). The same investigators (Tao *et al.*, 2004) also demonstrated hypomethylation of a region of the *Igf2* gene in liver and tumours from mice initiated with MNU and subsequently exposed to trichloroacetic acid or dichloroacetic acid. An association between hypomethylation and cell proliferation in liver of mice exposed to trichloroacetic acid or dichloroacetic acid was demonstrated by Ge *et al.* (2001). Hypomethylation of the internal cytosine of CCGG sites in the promoter region of the *Myc* gene began between 48 and 72 hours from the initiation of trichloroacetic acid or dichloroacetic acid exposure and continued to 96 hours. Pereira *et al.* (2001) investigated the effect of dichloroacetic acid treatment on hypomethylation and expression of the *Myc* gene and the promotion of liver tumours, in combination with chloroform. In a study by Pereira *et al.* (2001), female B6C3F_1 mice (age, 7–8 weeks) were given drinking-water containing chloroform at a concentration of 400, 800, or 1600 mg/L for 17 days. On the last 5 days of treatment, the mice were also given dichloroacetic acid at a dose of 500 mg/kg bw per day by gavage. Dichloroacetic acid decreased methylation and increased gene expression of *Myc* to a greater degree than did chloroform. Chloroform at doses greater than 800 mg/kg bw per day, co-administered with dichloroacetic acid, significantly reduced the ability of dichloroacetic acid to increase gene expression.

In a separate study, Pereira *et al.* (2004) gave female B6C3F_1 mice drinking-water containing dichloroacetic acid at a concentration of 3.2 g/L for 8 or 44 weeks. Dietary exposure to methionine (4 or 8 g/kg bw) abrogated DNA hypomethylation, reduced glycogen accumulation by 25% and was without effect on the increased liver/body weight ratio or peroxisome proliferation. Tumour multiplicity was decreased by methionine. The multiplicity of foci of altered hepatocytes was increased by methionine at the lower dose, and decreased by methionine at the higher dose, consistent with a slowing of progression of foci to tumours.

(b) Cytotoxicity and oxidative stress

(i) Humans

No studies on liver toxicity or oxidative stress in humans exposed to dichloroacetic acid were available to the Working Group.

(ii) Experimental systems

Histological examination of liver in most studies found little or no evidence of damage or of overt cytotoxicity.

Austin *et al.* (1996) investigated the potential for dichloroacetic acid to increase intercellular lipid peroxidation and the oxidation of DNA. Male B6C3F$_1$ mice were treated with a single oral dose of dichloroacetic acid (0, 30, 100, or 300 mg/kg bw). Nuclear DNA was extracted at various times to assess increases in relative guanosine hydroxylation. A statistically significant increase was seen in the group dosed at 300 mg/kg bw from 4 to 6 hours after dosing, but returned to near control levels at 8 hours after dosing. The level of hydroxylation appeared to be related to the ability to induce thiobarbituric acid-relative substances (TBARS), which is an indicator of lipid peroxidation. Statistically significant increases in lipid peroxidation have also been shown in cultured primary rat and mouse hepatocytes following exposure to dichloroacetic acid at concentrations as low as 0.5 mM [64.5 µg/mL] (in mice) and 1.0 mM [129 µg/mL] (in rats) (Everhart *et al.*, 1998).

(c) Alteration of cell proliferation and apoptosis, and clonal expansion

(i) Humans

No studies providing evidence of alteration of cell proliferation and apoptosis, or clonal expansion, after exposure to dichloroacetic acid in humans were available to the Working Group.

(ii) Experimental systems

Carter *et al.* (1995) gave male B6C3F$_1$ mice drinking-water containing dichloroacetic acid at 0, 0.5, or 5 g/L (0, 95, or 440 mg/kg bw per day, respectively) for up to 30 days. Significant, dose-related increases in absolute and relative (to total body weight) liver weights were seen at each 5-day interval. These trends increased with the length of exposure. Reduced thymidine incorporation (labelling index) and inhibition of mitosis was seen. Differences from the control group were statistically significant at 20 and 25 days, but not at 30 days. In mice in both treatment groups, hepatocytes had enlarged nuclei, consistent with polyploidy, and exhibited glycogen accumulation.

Tsai & DeAngelo (1996) examined responsiveness to growth factors in hepatocytes isolated from male B6C3F$_1$ mice given dichloroacetic acid. Inhibition of basal DNA synthesis was noted in cells isolated from mice exposed to dichloroacetic acid for 30, 60, or 90 days. However, this inhibition was reversed when cells from dichloroacetic acid-treated mice were treated in culture with growth factors.

Stauber *et al.* (1998) demonstrated that dichloroacetic acid increases cell proliferation of c-Jun-positive hepatocytes *in vitro*. Statistically significantly increased colony formation (no cytotoxicity) was seen in hepatocytes isolated from neonatal mice exposed to drinking-water containing dichloroacetic acid at 0.5 g/L. Colonies induced by dichloroacetic acid were positive for c-Jun, as were liver tumours induced in mice exposed *in vivo* (Stauber & Bull, 1997).

Male and female B6C3F$_1$ mice (age, 5 weeks) were given drinking-water containing dichloroacetic acid at 3.2 g/L, either alone, or together with chloroform at a concentration of 800 or 1600 mg/L (Pereira *et al.*, 2001). Before exposure to dichloroacetic acid, the mice were initiated with a single intraperitoneal dose of MNU at 300 mg/kg bw at age 15 days. The mice were killed at age 36 weeks. Greater numbers of hepatic foci were observed in dichloroacetic acid-treated animals (females more than in males). The tumour response was greater in males than in females. Chloroform in conjunction with dichloroacetic acid at both doses drastically reduced the incidence of adenoma and adenocarcinoma.

Snyder *et al.* (1995) examined the role of apoptosis (programmed cell death) suppression as a contributing factor to hepatocarcinogenicity induced by dichloroacetic acid. Regression

analysis revealed a statistically significant trend towards decreased apoptosis as the dose and duration 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 days 15, 25, and 30. For the group at the highest dose, apoptosis was statistically significantly depressed when compared with controls for all time-points.

Walgren *et al.* (2005) demonstrated that in cultured hepatocytes from male Long-Evans rats, treatment with dichloroacetic acid at 0.01–1.0 mM [1.3–129 μg/mL] for 10–40 hours did not alter the incorporation of [^{3}H]thymidine. However, dichloroacetic acid synergistically enhanced proliferation induced by epidermal growth factor. Additionally, dichloroacetic acid significantly reduced the normal background cell loss, suggesting an inhibition of apoptosis.

In the study by Ge *et al.* (2001) discussed above, an increase in DNA replication (evidenced by increased proliferating cell nuclear antigen labelling index and mitotic labelling index) was observed 72 hours and 96 hours after the first daily gavage dose of either trichloroacetic acid or dichloroacetic acid.

A small initial increase in cell division has been reported in normal liver after treatment with dichloroacetic acid. In all cases, however, cell replication rates in normal liver decreased with long-term treatment (Stauber & Bull, 1997; Bull, 2000). Decreased rates of cell replication were paralleled by decreased rates of spontaneous apoptosis (Snyder *et al.*, 1995).

However, dichloroacetic acid increased cell replication rates in a dose-dependent statistically significant manner in altered hepatic foci and small tumours when long-term treatment was followed by continued administration of dichloroacetic acid at different doses (Stauber & Bull, 1997). These studies indicate that dichloroacetic acid has selective effects on cell replication. Another experiment, conducted *in vivo*, demonstrated that the growth of tumours, as measured by magnetic resonance imaging, slowed when treatment with dichloroacetic acid was suspended (Miller *et al.*, 2000). This effect was also demonstrated as increased growth of colonies when isolated anchorage-independent hepatocytes from B6C3F$_1$ mice were treated with dichloroacetic acid (Stauber *et al.*, 1998).

(d) Activation of peroxisome proliferator-activated receptor-α

The sections below review the evidence that dichloroacetic acid induces activation of peroxisome proliferator-activated receptor-α (PPARα).

(i) Humans

No studies were identified that addressed the dichloroacetic acid-induced activation of a PPARα mechanism in human liver. However, studies of transactivation *in vitro* have shown that human (and murine) versions of PPARα are activated by dichloroacetic acid (and trichloroacetic acid), while trichloroethylene is relatively inactive (Zhou & Waxman, 1998; Maloney & Waxman, 1999). Walgren *et al.* (2000a) showed that dichloroacetic acid did not increase oxidation of palmitoyl-coenzyme A in primary human hepatocyte cultures; the effects of dichloroacetic acid on cell proliferation in this study are addressed below.

(ii) Experimental systems

Direct evidence for activation of PPARα come from several studies of transactivation *in vitro*, which have shown that murine versions of PPARα are activated by both trichloroacetic acid and dichloroacetic acid, while tetrachloroethylene is relatively inactive. Activation of murine PPARα by chlorinated hydrocarbons in COS1 cells containing a murine PPARα reporter plasmid was tested (Zhou & Waxman, 1998; Maloney & Waxman, 1999). Treatment with trichloroacetic acid and dichloroacetic acid for 24 hours resulted in activation of the reporter plasmid at concentrations of 1 mM [129 μg/mL]

and 5 mM [645 µg/mL] with a statistically significant concentration–response relationship. Walgren *et al.* (2000b) tested transactivation of murine PPARα using a reporter plasmid in HL8.5 cells cotransfected with mouse retinoic acid receptor α. Dichloroacetic acid caused an increase in activity (4 mM [516 µg/mL]), although the effect was not statistically significant.

Several studies have shown indirect evidence for PPARα activation by demonstrating that dichloroacetic acid is a peroxisome proliferator in mice and rats (Mather *et al.*, 1990; DeAngelo *et al.*, 1999). Induction of peroxisome proliferation has been associated repeatedly with long-term toxicity and carcinogenicity of dichloroacetic acid in the liver (DeAngelo *et al.*, 1989). Dichloroacetic acid induces peroxisome proliferation in the livers of both mice and rats, as indicated by increased activities of palmitoyl-coenzyme A oxidase and carnitine acetyl transferase, the appearance of a peroxisome proliferation-associated protein and increased volume density of peroxisomes after exposure to dichloroacetic acid for 14 days. With further treatment, peroxisome markers returned to control levels after 45–60 weeks (DeAngelo *et al.*, 1999).

Two reports suggest that the concentrations of dichloroacetic acid and trichloroacetic acid that result in peroxisome proliferation or PPARα activation are much higher than those that induce liver tumours (Bull, 2004; Bull *et al.*, 2004).

Indirect evidence for activation of PPARα comes from studies using enzyme markers. Laughter *et al.* (2004) reported that the induction of acyl-coenzyme A oxidase, palmitoyl-coenzyme A oxidase, and CYP4A by trichloroacetic acid and dichloroacetic acid was substantially diminished in PPARα-null mice.

Walgren *et al.* (2000a) found that both trichloroacetic acid and dichloroacetic acid (2 mM [258 µg/mL]), a concentration that was not cytotoxic) activated palmitoyl-coenzyme A oxidation in rat (LEH) and mouse ($B6C3F_1$) primary hepatocytes, and dichloroacetic acid was shown to be about twice as potent as trichloroacetic acid.

(e) Inhibition of intracellular communication

(i) Humans

No dichloroacetic acid-specific data on inhibition of gap-junctional communication in studies in humans were available to the Working Group.

(ii) Experimental systems

Benane *et al.* (1996) demonstrated an effect of dichloroacetic acid on gap-junctional communication in clone 9 cell cultures (normal rat hepatocytes). The shortest and lowest exposure to statistically significantly reduce dye transfer was 10 mM [1290 µg/mL] for 6 hours. The ability of dichloroacetic acid to disrupt communication was weaker (~5.8-fold) than other chlorinated compounds tested, including tetrachloroethylene, trichloroacetic acid, trichloroethanol, and chloral hydrate.

(f) Comparative analyses of liver tumours induced by dichloroacetic acid or trichloroacetic acid

Biomarkers of cell growth, differentiation, and metabolism in proliferative hepatocellular lesions promoted by dichloroacetic acid were investigated by Latendresse & Pereira (1997) to further determine differences between dichloroacetic acid and trichloroacetic acid in terms of mechanisms of carcinogenesis. Female $B6C3F_1$ mice were initiated with an intraperitoneal injection of MNU at age 15 days and treated with drinking-water containing dichloroacetic acid. More than half of tumours from dichloroacetic acid-treated mice expressed transforming growth factor-α, c-myc, CYP2E1, CYP4A1, and GST-π in more than 50% of cells. A different profile of histochemical markers was induced by trichloroacetic acid, supporting different mechanisms for these two haloacetic acids. Bull *et al.*

(2002) similarly observed that dichloroacetic acid-induced tumours often expressed c-jun, while trichloroacetic acid-induced tumours were uniformly lacking in c-jun expression.

Pereira (1996) studied the characteristics of lesions in female B6C3F$_1$ mice to evaluate differences between dichloroacetic acid and trichloroacetic acid. Foci of altered hepatocytes and tumours induced by dichloroacetic acid were reported to be predominantly eosinophilic. Foci induced by trichloroacetic acid were equally distributed between basophilic and eosinophilic, while hepatic tumours induced by trichloro-acetic acid were predominantly basophilic, including all observed hepatocellular carcinomas (n = 11), and lacked GST-π expression. These characteristics for trichloroacetic acid-induced tumours were also reported by Pereira *et al.* (1997). Tumours in control mice were also mostly basophilic, or mixed basophilic and eosinophilic. Since comparable numbers of the foci of trichloroacetic acid-treated mice were basophilic and eosinophilic, it suggested that the basophilic foci induced by treatment with trichloroacetic acid may be more likely to progress to tumours. Based on differences in the shape of the dose–response curves and staining characteristics of tumours, Pereira (1996) concluded that dichloroacetic acid and trichloroacetic acid act through different mechanisms. The characteristics of the foci and tumours induced by trichloroacetic acid were described as being consistent with the predominant basophilic staining observed in tumours induced by peroxisome proliferators, suggesting that this pathway might be involved in the observed hepatocarcinogenicity of trichloroacetic acid.

Similarly, Bull *et al.* (1990) also presented evidence that the mechanisms of carcinogenesis for trichloroacetic acid and dichloroacetic acid are different. In this study, dichloroacetic acid-treated mice showed marked cytomegaly, substantial glycogen accumulation, and necrosis of the liver. The dose–response relationship between proliferative liver lesions and dichloroacetic acid treatment followed a "hockey stick" pattern. In contrast, these effects were either minimal or absent in trichloroacetic acid-treated mice, and accumulation of lipofuscin (an indication of lipid peroxidation) was observed only in trichloroacetic acid-treated mice. In contrast to the dose–response relationship for dichloroacetic acid, the dose–response curve for trichloroacetic acid and proliferative lesions was linear.

4.3.2 Kidney

(a) Humans

No dichloroacetic acid-specific data from studies in humans were available to the Working Group.

(b) Experimental animals

Few studies have examined any effects, or potential mechanisms, of dichloroacetic acid in the kidney.

Mather *et al.* (1990) evaluated toxicological effects in groups of 10 male Sprague-Dawley rats given drinking-water containing dichloroacetic acid at concentrations of 0, 50, 500, or 5000 ppm [5000 µg/mL] for 90 days. At 500 and 5000 ppm [500 and 5000 µg/mL], relative kidney weights were statistically significantly ($P \leq 0.05$) increased when compared with controls. Changes in kidney histopathology (diffuse degeneration of the tubular epithelium and cells of the glomeruli) were observed in the group at 5000 ppm [5000 µg/mL].

In a follow-up study, Tao *et al.* (2005) treated B6C3F$_1$ mice with drinking-water containing dichloroacetic acid (3.2 g/L) for 7 days concurrently. In male, but not female mouse kidney, dichloroacetic acid decreased the methylation of DNA and the *c-myc* gene. To determine whether methionine co-administration would also prevent hypomethylation in the kidneys, male mice were fed diet containing methionine concurrently with drinking-water containing

dichloroacetic acid. Methionine prevented dichloroacetic acid-induced hypomethylation of the *c-myc* gene.

4.3.3 Other target tissues

Few studies have examined the effects of dichloroacetic acid in other target tissues, or their possible mechanisms. Madhok *et al.* (2010) demonstrated that dichloroacetic acid (20 mM [2580 µg/mL]) induces apoptosis and cell-cycle arrest in cancerous and non-cancerous cells of colorectal origin. Cancerous cells were more sensitive than non-cancerous cells to the growth-inhibitory effects of dichloroacetic acid.

4.4 Susceptibility data

4.4.1 Inter-individual variability

There were no data demonstrating that any particular human subpopulation is especially susceptible to the toxic effects of dichloroacetic acid. It has been suggested, however, that potential susceptibility may be related to polymorphisms in enzymes that are key to the metabolism of dichloroacetic acid.

For instance, the enzyme GST-zeta1 (*GSTZ1*) (Board *et al.*, 2001) is critical for dichloroacetic acid metabolism; it has been demonstrated that *Gstz1*-null mice fail to metabolize [^{13}C]-labelled dichloroacetic acid to [^{13}C]glyoxylate (Ammini *et al.*, 2003). In studies by Fang *et al.* (2006), a total of 10 single-nucleotide polymorphisms (SNPs) were identified in African, and Australian European subjects in a region 1.5 kb upstream of the *GSTZ1* start of transcription. Most recent studies suggest that there are four common polymorphic alleles of *GSTZ1*: 1a, 1b, 1c, and 1d (Board & Anders, 2011). *GSTZ1c* is the most common and is designated as the wild-type gene.

Dichloroacetic acid is an inactivator of *GSTZ1* in humans, rats, and mice. However, human *GSTZ1* is more resistant to inactivation than mouse or rat *Gstz1* (Tzeng *et al.*, 2000). The polymorphic variants of human *GSTZ1* differ in their susceptibility to inactivation, with 1a-1a being more resistant to inactivation than the other variants (Blackburn *et al.*, 2000; Blackburn *et al.*, 2001). A pharmacokinetic study (Li *et al.*, 2008) concluded that apparent inhibition of GSTZ-mediated metabolism of dichloroacetic acid is minimal at low doses (µg/kg bw per day), but may be significant for therapeutic doses of dichloroacetic acid and that polymorphisms of *GSTZ1* may help explain inter-individual variability in the plasma kinetics of dichloroacetic acid.

Short-term treatment of B6C3F$_1$ mice with dichloroacetic acid was shown to lead to an increase in activity of hepatic superoxide dismutase and catalase (Hassoun & Cearfoss, 2011). Because oxidative stress in the liver was suggested as one of the mechanisms of carcinogenesis by dichloroacetic acid (Austin *et al.*, 1995), polymorphisms in these protective enzymes may be of potential importance in protection against oxidative stress induced by dichloroacetic acid.

Individuals with glycogen storage disease (an inherited deficiency or alteration in any one of the enzymes involved in glycogen degradation) represent another group that may be more susceptible to toxicity caused by dichloroacetic acid. There is some evidence that alterations in glycogenolysis precede the development of many types of tumour (Bannasch, 1986; Bannasch *et al.*, 1986). The dose–response relationship for dichloroacetic acid-induced effects on hepatic glycogen is in the same range as that required for inducing liver tumours (Bull, 2000).

In addition, individuals with hyperoxaluria type 1, a rare genetic disorder, may be susceptible to elevated levels of glyoxylate originating from dichloroacetic acid metabolism. In this condition, the inability to convert glyoxylate to glycine leads to the formation and excretion of oxalate (Ribaya & Gershoff, 1982).

4.4.2 Life-stage susceptibility

The effect of dichloroacetic acid on its own metabolism is age-dependent in humans (Shroads *et al.*, 2008). Two randomized, double-blind, placebo-controlled clinical trials have been reported in which subjects received dichloroacetic acid at a dose of 12.5 mg/kg bw, twice per day for 6 months. In 43 children being treated for congenital lactic acid acidosis, no neurotoxicity was observed (Stacpoole *et al.*, 2006). In 30 adults, the trial had to be terminated prematurely because of the high incidence of symptomatic peripheral neuropathy. In studies by Shroads *et al.* (2008), nine patients were treated for 6 months with dichloroacetic acid at 25 mg/kg bw per day, and rats of varying ages were treated for 5 days with dichloroacetic acid at 50 mg/kg bw per day. Long-term administration of dichloroacetic acid showed a striking age-dependent decrease in plasma clearance in rats and humans. Monochloroacetate, a known neurotoxin, increased as a function of age in the urine of rats. This neurotoxin was detectable only in the plasma of older rats.

In female rats, exposure to dichloroacetic acid during gestation has been shown to result in the impairment of fetal maturation and soft-tissue anomalies (primarily of cardiac origin) indicating that the developing fetus may be uniquely susceptible to dichloroacetic acid-induced toxicity (Smith *et al.*, 1992). The study of Moser *et al.* (1999) provided additional limited evidence for increased susceptibility of rats to dichloroacetic acid-induced neurotoxicity when exposures begin shortly after weaning.

4.4.3 Sex differences

In a stable-isotope study by Schultz & Shangraw (2006), the effect of pretreatment with dichloroacetic acid on the pharmacokinetics of later doses of dichloroacetic acid was tested in eight male and eight female volunteers. In the absence of pretreatment with dichloroacetic acid (0.02 µg/kg bw per day for 14 days), there were no sex differences in the pharmacokinetics of dichloroacetic acid. Only women were affected by pretreatment, showing an increased AUC for plasma dichloroacetic acid and a decreased rate of clearance.

In a 26- and 39-week studies of carcinogenesis in Tg.AC hemizygous mice given dichloroacetic acid by dermal application (NTP, 2007), kidney nephropathy (observed in males) was the only non-cancer pathology to occur differently in males and females. This pathology was not observed in male or female mice of the same strain when dichloroacetic acid was given in the drinking-water, or in 26- and 41-week studies of carcinogenesis in p53 haplo-insufficient mice treated with dichloroacetic acid in drinking-water (NTP, 2007).

4.4.4 Effect of co-morbidities

The pharmacokinetics of dichloroacetic acid was evaluated in several small cohorts of humans with disease conditions. Most of the studies examined parameters of distribution and excretion.

In children (four boys and four girls, aged 1.5–10 years) with lactic acidosis caused by severe malaria, who were given dichloroacetic acid intravenously at a dose of 50 mg/kg bw, the average plasma half-life of dichloroacetic acid was 1.8 ± 0.4 hours, volume of distribution was 0.32 ± 0.09 L/kg, and the average AUC was 378 ± 65 mg/L per hour (Krishna *et al.*, 1995).

Two studies were conducted on the pharmacokinetics of dichloroacetic acid in patients with severe malaria. In one study that included 13 adults ([sex not reported]; average age, 27 ± 8 years) who were given dichloroacetic acid intravenously at a dose of 46 mg/kg bw over 30 minutes, the elimination half-life was 2.3 ± 1.8 hours, the clearance was 0.32 ± 0.16 L/h per kg and the volume of distribution was 0.75 ± 0.35 L/kg (Krishna *et al.*,

1994). In a second study, 11 adults (eight men and three women; average age, 32 ± 10 years) were given dichloroacetic acid intravenously at a dose of 46 mg/kg bw and a second dose (46 mg/kg bw) was given 12 hours later. The mean plasma half-life was 3.4 ± 2 hours after the first dose and 4.4 ± 2 hours after the second dose, the volume of distribution was 0.44 ± 0.2 L/kg and the plasma clearance was 0.13 ± 0.03 L/h per kg (Krishna *et al.*, 1996).

The effect of end-stage liver disease and liver transplantation on the pharmacokinetics of dichloroacetic acid was studied in 33 subjects [sex and age not reported] who were given dichloroacetic acid at a dose of 40 mg/kg bw by a 60-minute intravenous perfusion, then a second dose (40 mg/kg bw) by intravenous perfusion 4 hours later, before and during the anhepatic stage. The clearance of dichloroacetic acid during the paleohepatic, anhepatic and neohepatic stages was 1.0, 0.0 and 1.7 mL/kg per minute, respectively, indicating a major role of the liver in the metabolism of dichloroacetic acid (Shangraw & Fisher, 1996). The effect of cirrhosis on the pharmacokinetics of dichloroacetic acid was reported in six healthy volunteers (five men and one woman; age, 30 ± 3 years) and seven subjects with end-stage cirrhosis (five men and two women; age, 47 ± 3 years) who were given dichloroacetic acid at a dose of 35 mg/kg bw by intravenous perfusion over 30 minutes. The clearance of dichloroacetic acid was 2.14 mL/kg per minute in control subjects and 0.78 mL/kg per minute in patients with cirrhosis (Shangraw & Fisher, 1999).

The pharmacokinetics of dichloroacetic acid was studied in 111 patients with lactic acidosis (66 men; age, 56.0 ± 18.4 years), who received dichloroacetic acid (50 mg/kg bw) by intravenous perfusion over 30 minutes, then a second perfusion of 50 mg/kg bw, 2 hours after the beginning of the first. The pharmacokinetics were complex in the acutely ill patients studied and differed markedly from those observed in healthy volunteers. In healthy volunteers, the pharmacokinetics fitted a one-compartment model, while in the patients the data fitted one-, two- and three-compartment models. In the two-compartment model, the plasma half-life and plasma clearance were 18.15 ± 3.12 hours (mean ± standard error [SE]) and 0.041 L/kg per hour, respectively, after the first treatment, while the two values were 68.30 ± 14.50 hours (mean ± SE) and 0.017 L/kg per hour, respectively, after the second treatment. Plasma clearance of dichloroacetic acid tended to decrease as either the number of compartments or the number of treatments increased. The prolonged half-life and decreased plasma clearance indicate that repeated administration of dichloroacetic acid impairs its metabolism (Henderson *et al.*, 1997).

The pharmacokinetics of dichloroacetic acid was compared in healthy volunteers (27 subjects) and in patients with traumatic brain injury (25 subjects; average age, 52.8 ± 18.1 years). The healthy volunteers were given cumulative intravenous doses (two doses, 8 hours apart) of dichloroacetic acid at 45, 90 or 150 mg/kg bw; 16 patients with acute traumatic brain injury were given a single intravenous dose of dichloroacetic acid at 60, 100 or 200 mg/kg bw; six other patients were given three intravenous doses [dose not stated] of dichloroacetic acid at 24-hour intervals; and three patients were given six intravenous doses [dose not stated] at 12-hour intervals. The initial clearance of dichloroacetic acid (4.82 L/h) declined (1.07 L/h) after repeated doses in patients with traumatic brain injury.

4.5 Mechanistic considerations

Weak to moderate evidence suggested that dichloroacetic acid may be genotoxic. No induction of DNA strand breaks was observed in the only available study in a human lymphoblast cell line *in vitro*.

In mammalian systems, gene mutations were reported in experiments *in vivo* and limited

evidence existed for increased frequency of mutation after treatment with dichloroacetic acid *in vivo* and *in vitro*. Dichloroacetic acid clearly induced chromosomal aberrations in mouse lymphoma cells, but not in Chinese hamster ovary cells. With regard to micronucleus formation, results were conflicting *in vivo* and negative *in vitro* in mouse lymphoma cells. Inconsistent evidence existed to suggest that dichloroacetic acid could cause DNA damage (DNA unwinding) in studies *in vivo* in bone marrow and blood leukocytes in animals. In addition, several studies have found specific mutations in H-*ras* codon 61 in liver tumours after dichloroacetic acid administration, distinct from those in spontaneous tumours. In tests for genotoxicity in bacterial and fungal systems, only positive results were observed in assays for base substitution mutations in strains TA100 (three out of five tests), RSJ 100, and TA98.

Overall, the strength of evidence for the liver as a target organ is strong. Available mechanistic data come almost exclusively from studies in animals. Multiple mechanisms have been identified including epigenetic effects (global DNA hypomethylation and hypomethylation of the *Myc* gene promoter), oxidative stress (oxidative DNA damage and lipid peroxidation), effects on cell proliferation/apoptosis (a decrease in both cell proliferation and apoptosis, but selective enhancement of Jun-positive cells), induction of the peroxisome proliferation response (strong direct and indirect evidence for activation of PPARα in rodents, limited evidence for dichloroacetic acid as a ligand of human PPARα), disruption of gap-junctional intercellular communications (limited evidence from one study in a rat hepatocyte cell line *in vitro*). Because dichloroacetic acid is a metabolite of other chlorinated solvents, several studies have compared mutational and phenotypic profiles of liver tumours induced by various chlorinated solvents and concluded that little similarity exists.

Overall, the strength of evidence for the kidney as a target organ is weak. Some evidence of kidney toxicity has been reported in studies in animals. Several studies evaluated the effects of dichloroacetic acid in rodents and demonstrated increased relative kidney weight and effects on kidney histopathology in male rats exposed to high doses of dichloroacetic acid in drinking-water for 90 days. However, no similar effect was observed in mice. Hypomethylation of global DNA and of the *Myc* gene has been observed in kidney of male but not female mice.

Dichloroacetic acid is a sedative in animals and humans, and high doses have been shown to cause adverse effects on the central nervous system. In addition, peripheral neuropathy has been observed in humans (at therapeutic concentrations), and in rodents and dogs. There were no studies available that suggested a mechanism for these effects.

There is the potential for inter-individual variability in the adverse effects of dichloroacetic acid. GST-zeta1 is an important enzyme in the metabolism of dichloroacetic acid and common polymorphisms that result in differences in activation have been reported in humans. With respect to life-stage susceptibilities, neurotoxicity has been observed in adults, but not in children.

Dichloroacetic acid has been used in therapeutic studies for a variety of conditions related to impaired metabolism. Dichloroacetic acid activates pyruvate dehydrogenase. This effect has been suggested to be beneficial for human conditions associated with lactic acidosis, hypercholesterolaemia and hyperglycaemia. A suggestion of anti-cancer effects of dichloroacetic acid is based on its anti-proliferative effects and activation of pyruvate dehydrogenase which may in turn affect glycolysis, the major oxidative metabolic pathway in tumours.

5. Summary of Data Reported

5.1 Exposure data

Dichloroacetic acid is used as an intermediate in the production of glyoxylic acid, dialkoxy and diaroxy acids, sulfonamides and iron chelates. It is used to a lesser extent as a cauterizing agent and as a therapeutic agent for metabolic diseases. Dichloroacetic acid is readily transformed into dichloroacetate salts in aqueous solutions. Data on occupational exposure were only available for a small group of swimming-pool attendants who had very low levels in urine. Exposure of the general population to dichloroacetic acid occurs at the level of micrograms per litre in drinking-water (range, 10–40 μg/L) and from swimming pools (range, 10–100 μg/L) as a result of chlorine-based disinfection of water.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Dichloroacetic acid has been evaluated for its carcinogenicity in seven studies with drinking-water (some involving more than one experiment) in male mice and two studies with drinking-water in female mice. Two studies with drinking-water (involving more than one experiment) were conducted in male rats. These studies varied significantly in quality and statistical power.

In all studies in male and female mice, there was an increase in the incidence of hepatocellular adenoma and/or hepatocellular carcinoma. In all studies in male rats, an increased incidence of hepatocellular adenoma and hepatocellular carcinoma was observed. The main deficiency of all these studies was that they uniformly focused on the development of liver tumours. As a result, they did not provide a basis for considering whether tumours in other organs might have been induced.

Dichloroacetic acid increased the incidence of bronchioloalveolar adenoma in female Tg.AC hemizygous mice after administration in drinking-water, and of skin papilloma in both males and females of the same strain after skin application.

The four initiation–promotion studies with dichloroacetic acid in mice provided positive results. Dichloroacetic acid was found to be an efficient promoter of *N*-ethyl-*N*-nitrosourea- and vinyl carbamate-initiated hepatocellular tumours.

5.4 Mechanistic and other relevant data

Major similarities exist between humans and laboratory animals with regard to the absorption, distribution, metabolism and excretion of dichloroacetic acid. Dichloroacetic acid has a very similar plasma half-life in humans and laboratory animals. Dichloroacetic acid is primarily metabolized through glutathione-*S*-transferase zeta 1 (GST-zeta1) to glyoxylic acid and then to oxalic and glycolic acids, glycine and CO_2. The minor metabolic pathway of dichloroacetic acid is to monochloroacetic acid with further processing to thiodiacetic acid. Dichloroacetic acid acts as an inhibitor of its own metabolism by inactivating GST-zeta1. Such inhibition has a major impact on plasma half-life depending on the duration of exposure. Repeated administration of dichloroacetic acid has been shown to increase plasma half-life in both humans and laboratory animals by about 10 times.

Weak to moderate experimental evidence was available to suggest that dichloroacetic acid is a genotoxic agent. Target organs for adverse health outcomes of dichloroacetic acid are liver, nervous system, and kidney. Cancer findings in

animals and toxicity findings in humans and laboratory animals designated liver as a major target organ for dichloroacetic acid. Available data suggested that dichloroacetic acid may also act through multiple non-genotoxic mechanisms in liver carcinogenesis. There is a potential for inter-individual variability in the adverse effects of dichloroacetic acid, because dichloroacetic acid is primarily metabolized through GST-zeta1; this enzyme is polymorphic, and such polymorphisms have been shown to have an impact ont the function of GST-zeta1.

6. Evaluation

6.1 Cancer in humans

There is *inadequate evidence* in humans for the carcinogenicity of dichloroacetic acid.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of dichloroacetic acid.

6.3 Overall evaluation

Dichloroacetic acid is *possibly carcinogenic to humans (Group 2B).*

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