TRICHLOROACETIC ACID

This substance was considered by a previous Working Group, in February 1995 (IARC, 1995). Since that time, new data have become available and these have been incorporated into the monograph and taken into consideration in the present evaluation.

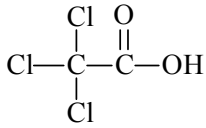
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 76-03-9
Chem. Abstr. Name: Trichloroacetic acid
IUPAC Systematic Name: Trichloroacetic acid
Synonyms: TCA; TCA (acid); trichloracetic acid; trichloroethanoic acid; trichloromethane carboxylic acid

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2HCl_3O_2$ Relative molecular mass: 163.39

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless to white deliquescent crystals with characteristic odour (Morris & Bost, 1991; Gennaro, 2000)
(*b*) *Boiling-point*: 197.5 °C (Morris & Bost, 1991)
(*c*) *Melting-point*: 59 °C (Morris & Bost, 1991)
(*d*) *Density*: 1.6218 at 64 °C/4 °C (Morris & Bost, 1991)

(*e*) *Spectroscopy data*: Infrared [2376], ultraviolet [1-6], nuclear magnetic resonance [6] and mass [1026] spectral data have been reported (Weast & Astle, 1985; Sadtler Research Laboratories, 1991)
(*f*) *Solubility*: Very soluble in water (1306 g/100 g at 25 °C) and most organic solvents, including acetone, benzene, ethyl ether, methanol and *ortho*-xylene (Morris & Bost, 1991)
(*g*) *Volatility*: Vapour pressure, 1 mm/Hg at 51 °C (Verschueren, 2001)
(*h*) *Stability*: Dissociation constant (K_a), 0.2159; undergoes decarboxylation when heated with caustics or amines to yield chloroform (Morris & Bost, 1991)
(*i*) *Octanol/water partition coefficient (P)*: log P, 1.33 (Hansch *et al*., 1995)
(*j*) *Conversion factor*: $mg/m^3 = 6.68 \times ppm$[a]

1.1.4 *Technical products and impurities*

Trade names for trichloroacetic acid include Aceto-Caustin and Amchem Grass Killer. Trichloroacetic acid is marketed at various degrees of purity. Typical specifications for commercially available trichloroacetic acid are presented in Table 1. Trichloroacetic acid is also available as aqueous solutions with concentrations ranging from 3 to 100% (w/v) (Spectrum Chemical, 2002).

1.1.5 *Analysis*

Trichloroacetic acid has been determined in water using liquid–liquid extraction, conversion to its methyl ester and gas chromatography with electron capture detection. This method has been applied to drinking-water, groundwater, water at intermediate stages of treatment and raw source water, with a limit of detection of 0.08 µg/L (Environmental Protection Agency, 1995; American Public Health Association/American Water Works Association/Water Environment Federation, 1999).

A similar method was used in a 1993 national survey of chlorinated disinfection by-products in Canadian drinking-water. Methyl esters were analysed by gas chromatography–mass spectrometry with selected ion monitoring. The minimum quantifiable limit for this method was 0.01 µg/L (Health Canada, 1995; Williams *et al.*, 1997).

Modifications of these methods have been used in an analytical survey of 16 drinking-water sources in Australia (Simpson & Hayes, 1998) and in a survey of treated water from 35 Finnish waterworks during different seasons (Nissinen *et al*., 2002).

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[a] Calculated from: mg/m^3 = (molecular weight/24.45) × ppm, assuming normal temperature (25 °C) and pressure (760 mm Hg)

Table 1. Typical quality specifications for trichloroacetic acid[a]

Property	Grade					
	Crude[a]		Technical[b]		Ph. Eur.[c]	ACS[b]
Trichloroacetic acid, % min.	96.5	86.0[d]	98	87.6[d]	98	99.0
Dichloroacetic acid, % max.	2.5	2.5	1.2	1.1	–	0.5
Sulfuric acid, % max.	0.5	0.5	0.3	0.3	–	0.02
Sulfated ash, % max.	–	–	–	–	0.1	0.03
Water, % max.	0.5	–	0.2	–	–	0.5
Heavy metals in the form of:						
Lead, ppm max.	10	–	–	–	–	20
Iron, ppm (mg/kg) max.	20	20	10	10	–	10
Halogenides, mg/kg max.	–	–	–	–	–	10
Sulfates, mg/kg max.	–	–	–	–	–	200
Phosphate, ppm (mg/kg) max.	–	–	–	–	–	5
Chloride, ppm (mg/kg) max.	–	–	–	–	100	10
Nitrate, ppm (mg/kg) max.	–	–	–	–	–	20

[a] From Koenig *et al*. (1986); Clariant GmbH (2002a,c)
[b] From Clariant Corp. (2001, 2002); Clariant GmbH (2002a,b,c); cfm Oskar Tropitzsch (2002)
[c] From Council of Europe (2002)
[d] 90% trichloroacetic acid in water (Clariant GmbH, 2002c)

1.2 Production and use

Trichloroacetic acid was reported to have been first synthesized in 1840 by chlorination of acetic acid in sunlight (Beilstein Online, 2002). Haloacetic acids were first detected in 1983 as disinfection by-products in chlorinated drinking-waters, 9 years after the discovery of trihalomethanes in chlorinated waters (Nissinen *et al.*, 2002).

1.2.1 *Production*

Trichloroacetic acid is produced on an industrial scale by chlorination of acetic acid or chloroacetic acid at 140–160 °C. Calcium hypochlorite may be added as a chlorination accelerator, and metal catalysts have been used in some cases. Trichloroacetic acid is isolated from the crude product by crystallization (Koenig *et al*., 1986; Morris & Bost, 1991).

Available information indicates that trichloroacetic acid is produced by nine companies in India, two companies each in China, Germany and Mexico and one company each in France, Israel, Italy, Japan, Russia and Spain (Chemical Information Services, 2002a).

Available information indicates that trichloroacetic acid is formulated into pharmaceutical products by five companies in Italy, three companies in France, two companies in Poland and one company each in Argentina, Spain and Turkey (Chemical Information Services, 2002b).

1.2.2 *Use*

The main application of trichloroacetic acid, usually as its sodium salt, is as a selective herbicide and, historically, in herbicidal formulations with 2,4-D and 2,4,5-T (IARC, 1977, 1987). Trichloroacetic acid is also used as an etching or pickling agent in the surface treatment of metals, as a swelling agent and solvent in the plastics industry, as an auxiliary in textile finishing, as an additive to improve high-pressure properties in mineral lubricating oils and as an analytical reagent. Trichloroacetic acid, and particularly its esters, are important starting materials in organic syntheses (Koenig *et al*., 1986; Morris & Bost, 1991; Clariant GmbH, 2002a,b,c).

Trichloroacetic acid is used as a caustic on the skin or mucous membranes to treat local lesions and for the treatment of various dermatological diseases. Its chief medicinal use is in the treatment of ordinary warts and juvenile flat warts (escharotic), although there are reports of its use in removing tattoos, treating genital warts and in dermal peeling. It is also used extensively as a precipitant of protein in the chemical analysis of body fluids and tissue extracts, and as a decalcifier and fixative in microscopy (Gennaro, 2000; Royal Pharmaceutical Society of Great Britain, 2002).

1.3 Occurrence

1.3.1 *Natural occurrence*

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

1.3.2 *Occupational exposure*

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 35 124 employees in the USA had potential occupational exposure to trichloroacetic acid in seven industries and 1562 plants (National Institute for Occupational Safety and Health, 1994). The estimate is based on a survey of companies and did not involve measurement of actual exposures.

Because trichloroacetic acid is a major end-metabolite of trichloroethylene (IARC, 1976, 1979, 1987, 1995) and tetrachloroethylene (IARC, 1979, 1987, 1995) in humans, it has been used for many years as a biological marker of exposure to those compounds. It is also a metabolite of 1,1,1-trichloroethane (see IARC, 1979, 1987, 1999), and chloral hydrate (see the monograph in this volume) is rapidly oxidized to trichloroacetic acid in humans. The levels of trichloroacetic acid reported in human blood and urine after occupational and environmental exposure to trichloroacetic acid, trichloroethylene, tetrachloroethylene or 1,1,1-trichloroethane are summarized in Table 2.

The average concentration of trichloroacetic acid in 177 urinary measurements made in various industries in 1986–88 at the Finnish Institute of Occupational Health was 77.1 μmol/L [12.6 mg/L], with a range of < 50–860 μmol/L [< 8.2–140.5 mg/L]. The

Table 2. Concentrations of trichloroacetic acid in human blood and urine following exposure to chlorinated solvents

Job description (country)	Exposure	Concentration of trichloroacetic acid	Reference
Occupational			
Metal degreasing (Switzerland)	Trichloroethylene, 10–300 ppm [54–1611 mg/m^3]	57–980 mg/L (urine)	Boillat (1970)
Metal degreasing (USA)	Trichloroethylene, 170–420 mg/m^3	3–116 mg/g creatinine (urine)	Lowry *et al*. (1974)
Workshop (Japan)	Trichloroethylene, 3–175 ppm [16.1–940 mg/m^3]	9–297 mg/L (urine)	Ikeda *et al*. (1972)
Printing factory (Japan)	1,1,1-Trichloroethane, 4.3–53.5 ppm [23–289 mg/m^3]	0.5–5.5 mg/L	Seki *et al*. (1975)
Workshop (Japan)	Trichloroethylene	Range of means, 108–133 mg/L (urine) (trichloroacetate)	Itoh (1989)
Automobile workshop (Japan)	Trichloroethylene, 1–50 ppm [5–269 mg/m^3]	Average, 136 mg/g creatinine (urine)	Ogata *et al*. (1987)
Dry cleaning, degreasing (former Yugoslavia)	Trichloroethylene	0.43–154.92 µmol/L (blood) [0.07–25.3 mg/L] 0.58–42.44 mmol/mol creatinine (urine) [0.84–61 mg/g]	Skender *et al*. (1988)
Solvent exposure (Republic of Korea)	Tetrachloroethylene, 0–61 ppm [0–414 mg/m^3]	0.6–3.5 mg/L (urine)	Jang *et al*. (1993)
Degreasing (Sweden)	Trichloroethylene, 3–114 mg/m^3	2–260 µmol/L (urine) [0.3–42.5 mg/L]	Ulander *et al*. (1992)

Table 2 (contd)

Job description (country)	Exposure	Concentration of trichloroacetic acid	Reference
Dry cleaning (former Yugoslavia)	Trichloroethylene, 25–40 ppm [134–215 mg/m^3] Tetrachloroethylene, 33–53 ppm [224–359 mg/m^3]	13.47–393.56 µmol/L (blood) [2.2–64 mg/L] 1.92–77.35 mmol/mol creatinine (urine) [2.8–112 mg/g] 1.71–20.93 µmol/L (blood) [0.3–3.4 mg/L] 0.81–15.76 mmol/mol creatinine (urine) [1.2–23 mg/g]	Skender *et al*. (1991)
Printing workshop (Japan)	1,1,1-Trichloroethane, 5–65 ppm [27–351 mg/m^3]	2–5 mg/L (urine)	Kawai *et al*. (1991)
Printing and ceramics workshop (Germany)	Trichloroethylene, 5–70 ppm [26.9–376 mg/m^3]	2.0–201.0 mg/g creatinine (urine)	Triebig *et al*. (1982)
Environment			
Environmental levels (Italy)	Trichloroethylene: air: 1.7–26.9 µg/m^3 water: 12–123 µg/L Tetrachloroethylene: air: 2.9–40 µg/m^3 water: 2–68 µg/L	8.1–60.0 µg/L (plasma) 6.2–72.0 µg/g creatinine (urine)	Ziglio *et al*. (1985)
Environmental levels (Australia)	Trichloroacetic acid: drinking-water: 1.1–52 µg/L	0.8–38 µg/day urine	Froese *et al.* (2002)
Environmental levels (USA)	Trichloroacetic acid: drinking-water: 0.25–120 µg/L	1– ~42 µg/day urine	Weisel *et al*. (1999); Kim *et al.* (1999)
	Trichloroacetic acid: swimming pools: 57–871 µg/L	Background, 0.15–1.18 µg/one urine void After exposure, 0.29–1.59 µg/one urine void	Kim & Weisel (1998)

samples were usually taken upon request, and seven exceeded the Finnish biological action level of 360 μmol/L [59 mg/L] (Rantala *et al*., 1992).

Raaschou-Nielsen *et al.* (2001) examined 2397 measurements of trichloroacetic acid in urine collected between 1947 and 1985 from workers in various industries in Denmark. The urine samples were usually taken following a request from the local labour inspection agency or medical officer and the concentration of trichloroacetic acid ranged from 0.5 to 150 mg/L. The data showed that (*a*) a fourfold decrease in concentrations of trichloroacetic acid occurred from 1947 to 1985; (*b*) the highest concentrations were observed in the iron and metal, chemical and dry cleaning industries; (*c*) levels of trichloroacetic acid were twice as high among men than among women in the iron and metal and dry cleaning industries; (*d*) concentrations of trichloroacetic acid concentrations were higher among younger than among older workers; and (*e*) persons working in an area in which trichloroethylene was used, but not working with trichloroethylene themselves, also showed urinary levels of trichloroacetic acid indicative of exposure.

1.3.3 *Air*

No data were available to the Working Group.

1.3.4 *Water*

Trichloroacetic acid is produced as a by-product during chlorination of water containing humic substances (Christman *et al*., 1983; Miller & Uden, 1983; Legube *et al*., 1985; Reckhow *et al*., 1990). Consequently, it may occur in drinking-water after chlorine-based disinfection of raw waters containing natural organic substances (Hargesheimer & Satchwill, 1989; see IARC, 1991) and in swimming pools (Kim & Weisel, 1998; Stottmeister & Naglitsch, 1996). The concentrations of trichloroacetic acid measured in various water sources are summarized in Table 3.

Geist *et al.* (1991) measured concentrations of trichloroacetic acid ranging from < 3 to 558 μg/L in surface water downstream from a paper mill in Austria, and Mohamed *et al.* (1989) measured concentrations ranging from 838 to 994 μg/L in effluent from a kraft pulp mill in Malaysia.

Clemens and Schöler (1992a) measured concentrations of trichloroacetic acid of 0.9 μg/L in rainwater and 0.05 μg/L in groundwater in Germany. Plümacher and Renner (1993) measured concentrations ranging from 0.1–20 μg/L in rainwater.

Levels of trichloroacetic acid tend to decline with length of residence in the distribution system (Chen & Weisel, 1998), and tend to be higher in warmer seasons (LeBel *et al*., 1997; Chen & Weisel, 1998). Trichloroacetic acid has been identified as a major chlorinated by-product of the photocatalytic degradation of tetrachloroethylene (IARC, 1979, 1987, 1995) in water but a minor by-product of that of trichloroethylene (IARC, 1976, 1979, 1987, 1995) in water (Glaze *et al*., 1993).

Table 3. Concentrations of trichloroacetic acid in water

Water type (location)	Concentration range (μg/L)	Reference
Treatment plant and distribution system (Canada)	0.2–36.8	LeBel *et al.* (1997)
Treatment plant and distribution system (Poland)	8.18–15.20	Dojlido *et al.* (1999)
Treatment plant and distribution system (Republic of Korea)	0.6–4.2[a]	Shin *et al.* (1999)
Treatment plant (Canada)	0.1–273.2	Williams *et al.* (1997)
Drinking-water (USA)[b]	33.6–161	Uden & Miller (1983)
Drinking-water (USA)[b]	3.2–67	Norwood *et al*. (1986)
Drinking-water (USA)[b]	4.0–6.0	Krasner *et al*. (1989)
Drinking-water (USA)[b]	1.3–22	Jacangelo *et al.* (1989)
Drinking-water (USA)[b]	15–64	Reckhow & Singer (1990)
Drinking-water (Switzerland)[b]	3.0	Artho *et al*. (1991)
Drinking-water (Spain)[b]	0.3–2.5	Cancho *et al*. (1999)
Drinking-water and distribution system (USA)[b]	1–170	Obolensky *et al.* (2003)
Drinking-water (Germany)	Not detected–3	Lahl *et al*. (1984)
Distribution system (Canada)	0.1–473.1	Williams *et al.* (1997)
Distribution system (USA)	5.5–7.5	Chen & Weisel (1998)
Distribution system (Australia)	0.2–14	Simpson & Hayes (1998)
Drinking-water (USA)	6.4–14	Lopez-Avila *et al*. (1999)
Drinking-water (USA)	0.25–120	Weisel *et al.* (1999)
Drinking-water (Canada)	3.0–8.4	Scott *et al.* (2000)
Drinking-water (Australia)	1.1–52	Froese *et al.* (2002)
Drinking-water (Finland)	< 2.5–210	Nissinen *et al.* (2002)
Drinking-water (Spain)	0.1–25.5	Villanueva *et al*. (2003)
Irrigation water (USA)[c]	0–297	Comes *et al*. (1975)
Swimming pools (Germany)	18–136	Lahl *et al.* (1984)
Swimming pools (Germany)	Indoor: 3.3–9.1 Open-air: 46.5–100.6	Clemens & Schöler (1992b)
Swimming pools (Germany)	1.5–64.2	Mannschott *et al.* (1995)
Swimming pools (Germany)		Stottmeister & Naglitsch (1996)
Indoor	3.5–199	
Hydrotherapy	1.1–45	
Outdoor	8.2–887	
Swimming pools (USA)	57–871	Kim & Weisel (1998)

[a] Based on the assumption that trichloroacetic acid makes up approximately 32% of haloacetic acids

[b] Samples taken in water leaving the treatment plant

[c] Trichloroacetic acid was present in the irrigation water from its use as a herbicide.

Trichloroacetic acid has also been detected in the Great Lakes, Canada (Scott *et al*., 2002) and in fog samples (0.02–2.0 µg/L) at ecological research sites in north-eastern Bavaria, Germany (Römpp *et al.*, 2001). Precipitation samples in Canada contained trichloroacetic acid concentrations ranging from < 0.0006 to 0.87 µg/L and concentrations in the Canadian lakes varied from < 0.0001 to 0.037 µg/L (Scott *et al*., 2000). Trichloroacetic acid has a residence time of approximately 40 days in pond waters (Ellis *et al*., 2001).

1.3.5 *Food*

Residues of trichloroacetic acid have been found in the seed of wheat, barley and oats after its use as a postemergence herbicide (Kadis *et al*., 1972). Trace concentrations (0.01–0.20 ppm [0.01–0.20 mg/kg) have been detected in vegetables and fruits from fields irrigated with water containing trichloroacetic acid; slightly higher levels (0.13–0.43 mg/kg) were detected in field bean pods and seeds (Demint *et al*., 1975).

1.3.6 *Other*

Concentrations of trichloroacetic acid were determined in the urine of people living in the vicinity of dry cleaning shops in Germany where tetrachloroethylene was used. The mean values were 105 µg/L for 29 neighbours and 682 µg/L for 12 workers (maximum, 1720 µg/L) (Popp *et al*., 1992). In Zagreb, Croatia, the levels of trichloroacetic acid in fluids from 39 people with no known exposure to solvents were 14–160 µg/L in plasma and 2–292 µg/24 h in urine (Skender *et al*., 1993). In 66 students in Japan, the levels ranged from not detected to 930 µg/g creatinine (urine) (Ikeda & Ohtsuji, 1969); those in 94 unexposed subjects in Germany were 5–221 µg/L in serum and 0.6–261 µg/24 h in urine (Hajimiragha *et al*., 1986).

Trichloroacetic acid was detected at levels of 10–150 µg/kg in spruce needles from the Black Forest in Germany and the Montafon region in Austria, both considered to be relatively unpolluted areas (Frank *et al*., 1989; Frank, 1991). The concentrations of trichloroacetic acid in pine needles from an urban area in Germany were 0.7–175 µg/kg fresh wt (Plümacher & Renner, 1993); those in conifer needles in Finland were 3–126 µg/kg fresh wt (Frank *et al*., 1992). In Sitka spruce needles from Scotland, concentrations of up to approximately 22 µg/kg fresh needles were measured (Reeves *et al.,* 2000). In the vicinity of a pulp mill in Finland, concentrations of 2–135 µg/kg were found in pine needles (Juuti *et al*., 1993). Trichloroacetic acid was also detected in earthworms (at 150–400 µg/kg wet wt) from a contaminated forest site (Back & Süsser, 1992).

1.4 Regulations and guidelines

The WHO (1998) has established a provisional guideline of 100 µg/L for trichloroacetic acid in drinking-water. A provisional guideline is established when there is some evidence of a potential health hazard but where available data on health effects are limited, or

where an uncertainty factor greater than 1000 has been used in the derivation of the tolerable daily intake.

In Australia and New Zealand, the guideline for trichloroacetic acid in drinking-water is 100 μg/L (National Health and Medical Research Council and Agriculture and Resource Management Council of Australia and New Zealand, 1996). This guideline also notes that the minimization of the concentration of all chlorination by-products is encouraged by reducing the quantity of naturally occurring organic material in the source water, reducing the amount of chlorine added or using an alternative disinfectant, without compromising disinfection.

In the USA, the Environmental Protection Agency (1998) regulates trichloroacetic acid as one of a combination of five haloacetic acids, which also include monochloracetic acid, dichloroacetic acid and mono- and dibromoacetic acids. The maximum contaminant level for the sum of these five haloacetic acids is 60 μg/L.

The European Union (European Commission, 1998) and Canada (Health Canada, 2003) have not set guideline values but encourage the reduction of concentrations of total disinfection by-product.

2. Studies of Cancer in Humans

See Introduction to the monographs on chloramine, chloral and chloral hydrate, dichloroacetic acid, trichloroacetic acid and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5*H*)-furanone.

3. Studies of Cancer in Experimental Animals

Previous evaluation

Trichloroacetic acid has been evaluated previously (IARC, 1995) and was found to induce hepatocellular adenomas and carcinomas in male B6C3F_1 mice and to possess promoter activity. The previous evaluation of trichloroacetic acid indicated that there was limited evidence in experimental animals for its carcinogenicity (IARC, 1995).

New studies

3.1 Oral administration

3.1.1 *Mouse*

Groups of 93, 46 and 38 female B6C3F_1 mice, 7–8 weeks of age, were administered trichloroacetic acid in the drinking-water at concentrations of 2.0, 6.67 and 20.0 mmol/L [324, 1080 or 3240 mg/L], adjusted to pH 6.5–7.5 with sodium hydroxide; a control group of 134

animals received 20.0 mmol/L sodium chloride. Mice were killed after 360 or 576 days (when high-dose mice became moribund) of exposure. The livers were weighed and evaluated for foci of altered hepatocytes (basophilic and eosinophilic foci), adenomas and carcinomas. After 360 or 576 days of exposure, the liver-to-body weight ratio was increased dose-dependently following treatment with trichloroacetic acid. Data from mice administered trichloroacetic acid were compared with those from control mice using Fisher's exact test with a p-value < 0.05. The high dose of trichloroacetic acid (20.0 mmol/L) increased, in comparison with controls, the incidence of foci (11/18 versus 10/90 at 576 days), adenomas (7/18 versus 2/90 at 576 days) and carcinomas (5/20 versus 0/40 at 360 days and 5/18 versus 2/90 at 576 days). The mid dose of trichloroacetic acid (6.67 mmol/L) increased the incidence of foci (9/27) and hepatocellular carcinomas (5/27) at 576 days, while the low dose of 2.0 mmol/L did not alter the incidence of any liver lesion. In control mice, the incidence of lesions was 1/40 adenoma (2.5%) at 360 days and 10/90 foci (11.1%), 2/90 adenomas (2.2%) and 2/90 carcinomas (2.2%) at 576 days (Pereira, 1996).

The ability of mixtures of di- and trichloroacetic acid to induce liver tumours was studied in 6-week-old B6C3F_1 male mice. Treatments administered included 0.1, 0.5 and 2.0 g/L dichloroacetic acid, and 0.5 and 2.0 g/L trichloroacetic acid, and selected combinations of these treatments. Twenty animals were assigned to each of 10 groups that received the above concentrations in their drinking-water for 52 weeks. Dose-related increases in the incidence of liver tumours (adenomas and carcinomas combined) were observed with the individual compounds and, when the animals were exposed to mixtures of di- and trichloroacetic acid, it appeared that there was an approximately additive effect in terms of tumour incidences (see Table 4) (Bull *et al.*, 2002).

3.1.2 *Rat*

Groups of 50 male Fischer 344/N rats, 28–30 days of age, received 0.05, 0.5 and 5 g/L neutralized trichloroacetic acid, adjusted to pH 6.9–7.1 with sodium hydroxide, or 2 g/L sodium chloride in the drinking-water for a total 104 weeks. Interim sacrifices were made at 15, 30, 45 and 60 weeks. A complete necropsy of the animals was performed. The liver, kidney, spleen, testes and gross lesions were examined microscopically. A complete pathological examination was carried out on all tissues from all animals in the high-dose group. The high dose of trichloroacetic acid but not the low or mid dose decreased body weight (~11%). Trichloroacetic acid did not affect the absolute or relative (to body weight) weights of the liver, kidneys, spleen or testes except for a decrease in the absolute liver weight in rats administered 5.0 g/L ($p \leq 0.05$). At 104 weeks, the number of animals per treatment group ranged from 20 to 24 including one rat that died after 76 weeks. The number of rats with hepatocellular adenomas varied between one and three among the treatment groups (4.2–15.0%). A single hepatocellular carcinoma (1/22, 4.6%) was found in the high-dose trichloroacetic acid-treated group). None of the treatment groups had a significant increase in the incidence of any tumour in other organs (DeAngelo *et al.*, 1997).

Table 4. Effect on liver tumour incidence of dichloroacetic acid (DCA) and trichloroacetic acid (TCA) administered in the drinking-water for 52 weeks to male B6C3F$_1$ mice

Treatment	Tumour incidence (adenomas and carcinomas)	Tumour multiplicity[a]
Control (drinking-water)	1/20	0.05 ± 0.0
0.1 g/L DCA	2/20	0.10 ± 0.07
0.5 g/L DCA	5/20[b]	0.35 ± 0.15[b]
2 g/L DCA	12/19[b]	1.7 ± 0.5[b]
0.5 g/L TCA	11/20[b]	0.70 ± 0.16[b]
2 g/L TCA	9/20[b]	0.60 ± 0.18[b]
0.1 DCA + 0.5 TCA g/L	9/20[b]	0.65 ± 0.22[b]
0.1 DCA + 2 TCA g/L	15/20[b]	1.3 ± 0.2[b]
0.5 DCA + 0.5 TCA g/L	13/19[b]	1.4 ± 0.3[b]
0.5 DCA + 2 TCA g/L	13/20[b]	1.5 ± 0.3[b]

From Bull *et al.* (2002)
[a] Total number of tumours divided by total number of animals
[b] Significantly different from control at $p < 0.05$

3.2 Administration with known carcinogens or modifying factors

Tumour-promotion studies

Groups of 6–40 female B6C3F$_1$ mice, 15 days of age, were initiated with an intraperitoneal injection of 25 mg/kg *N*-methyl-*N*-nitrosourea (MNU). At 49 days of age, the animals received 2.0, 6.67 or 20.0 mmol/L [324, 1080 or 3240 mg/L] trichloroacetic acid, adjusted to pH 6.5–7.5 with sodium hydroxide, or 20.0 mmol/L sodium chloride as a control for the sodium salt in the drinking-water. Mice were killed after 31 or 52 weeks of exposure. At 52 weeks, the mid (6.67 mmol/L) and high (20.0 mmol/L) doses of trichloroacetic acid significantly ($p < 0.01$) increased the incidence of carcinomas in MNU-initiated mice from 4/40 (10.0%) to 5/6 (83.3%) and 20/24 (83.3%), and multiplicity from 0.10 ± 0.05 to 1.33 ± 0.42 and 2.79 ± 0.48, respectively. At 31 weeks, the high dose of trichloroacetic acid increased the incidence of hepatocellular adenomas in MNU-initiated mice from 0/10 to 6/10 (60%) and multiplicity from 0.00 to 1.30 ± 0.045. At 52 weeks, the mid and high doses of trichloroacetic acid increased the incidence of adenomas from 7/40 (17.5%) to 16/24 (66.7%) and 5/6 (83.3%), respectively, and the multiplicity from 0.28 ± 0.11 to 2.00 ± 0.82 and 1.29 ± 0.24. In mice that were not administered MNU, the high dose of trichloroacetic acid significantly increased the incidence of carcinomas from 0/40 to 5/20 (25.0%) (Pereira & Phelps, 1996).

Combinations of dichloroacetic acid and trichloroacetic acid have been evaluated for tumour-promoting activity. Female $B6C3F_1$ mice, 15 days of age, were initiated with MNU (25 mg/kg bw) followed by exposure to 0, 7.8, 15.6 and 25 mmol/L dichloroacetic acid with or without 6.0 mmol/L trichloroacetic acid or 0, 6.0 and 25 mmol/L trichloroacetic acid with or without 15.6 mmol/L dichloroacetic acid. The pH of the dose solutions was adjusted to 6.5–7.5 with sodium hydroxide. Exposure was from week 4 to 48 of age, at which time the mice were killed. The high dose of dichloroacetic acid (25 mmol/L) and trichloroacetic acid (25 mmol/L) significantly increased ($p < 0.05$) the multiplicity of hepatocellular adenomas from 0.07 ± 0.05 (no dichloroacetic acid or trichloroacetic acid) to 1.79 ± 0.29 and 0.52 ± 0.11, respectively. The lower doses of dichloroacetic acid and trichloroacetic acid did not significantly increase the incidence or multiplicity of adenomas (Pereira *et al*., 1997).

The effect of chloroform on liver and kidney tumour promotion by trichloroacetic acid as well as dichloroacetic acid has been investigated. The concentrations of chloroform were chosen because they prevented dichloroacetic acid-induced DNA hypomethylation and increased mRNA expression of the c-*myc* gene. However, chloroform did not alter trichloroacetic acid-induced DNA hypomethylation and expression of the c-*myc* gene. Groups of male and female $B6C3F_1$ mice, 15 days of age, were initiated with 30 mg/kg MNU. At 5 weeks of age, the mice started to receive in the drinking-water 4.0 g/L trichloroacetic acid or 3.2 g/L dichloroacetic acid neutralized with sodium hydroxide with 0, 800 or 1600 mg/L chloroform and were killed at 36 weeks of age. The results were analysed for statistical significance by a one-way ANOVA followed by the Tukey test with a p-value < 0.05. In MNU-initiated mice that did not receive trichloroacetic acid, hepatocellular adenomas were found in 2/29 (6.9%) females and 2/8 (25%) males, while no hepatocellular carcinomas were found. Trichloroacetic acid increased the incidence of liver adenocarcinomas (10/16 [62.5%]) and adenomas (12/16 [75%]) in male mice. In female mice administered trichloroacetic acid, the incidence of mice with hepatocellular adenocarcinomas and adenomas was not significantly altered: 4/14 (28.6%) and 2/14 (14.3%). In male mice administered trichloroacetic acid plus 0, 800 and 1600 mg/L chloroform, the incidence of hepatocellular adenocarcinomas was 10/16 (62.5%), 7/9 (87.5%) and 6/8 (75%) and that of hepatocellular adenomas was 12/16 (75%), 6/9 (75%) and 1/8 (12.5%), respectively. The incidence of mice with hepatocellular adenomas was significantly lower in mice administered trichloroacetic acid plus 1600 mg/L chloroform than in mice administered trichloroacetic acid ($p < 0.05$). No altered hepatocyte foci, adenomas or adenocarcinomas were found in six MNU-initiated male mice that were administered 1600 mg/L chloroform. Multiplicity of tumours (adenomas plus adenocarcinomas) was increased in male mice from 0.25 ± 0.16 to 3.81 ± 0.82 ($p < 0.001$), but not in female mice, with 0.07 ± 0.04 and 0.64 ± 0.22 for control and trichloroacetic acid-exposed mice, respectively. Sixty per cent of the tumours were adenocarcinomas, indicating that the multiplicity of adenocarcinomas was significantly increased in male mice exposed to trichloroacetic acid. Renal tumours of tubular origin were found only in male mice. The majority (more than 70%) were papillary cystic adenomas with the rest were cystic adenomas and to a lesser extent adenocarcinomas (~5%). Trichloroacetic

acid increased the incidence and the multiplicity of MNU-initiated kidney tumours from 0 (0/8) to 87.5% (14/16) and 1.68 tumours per mouse. Chloroform did not alter the promotion of kidney tumours by trichloroacetic acid; the incidence of mice with tumours was 71.4–87.5% and multiplicity was 1.00–1.68 kidney tumours per mouse for mice administered trichloroacetic acid with 0, 800 or 1600 mg/L chloroform. No kidney tumours were found in the six MNU-initiated male mice administered 1600 mg/L chloroform only. In female mice, the incidence of kidney tumours among all the treatment groups ranged from 0 to 28.6% and the multiplicity ranged from 0 to 0.29 tumours per mouse (Pereira *et al*., 2001).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

A physiologically based pharmacokinetic model for trichloroacetic acid in humans has been developed on the basis of data from studies on the kinetics of trichloroacetic acid in rodents (Allen & Fisher, 1993). The rate of systemic clearance of trichloroacetic acid in humans was slower than that in rodents (0.045–0.1/h/kg in rats and mice). Trichloroacetic acid is eliminated largely (93%) unchanged (Allen & Fisher, 1993).

A second-generation physiologically based pharmacokinetic model for trichloroethylene that includes trichloroacetic acid as a metabolite has been developed (Fisher *et al.*, 1998). Nine male (20–36 years old) and eight female (20–30 years old) volunteers were exposed by inhalation to 50 or 100 ppm [269 or 538 μg/L] trichloroethylene for 4 h. The first-order rate constants for the excretion of trichloroacetic acid in urine averaged 1.54/h (range, 0.7–3.0/h) and 1.1/h (range, 0.13–2.0/h) in women and men, respectively. The pharmacokinetic model successfully simulated the cumulative excretion of trichloroacetic acid in the urine of both men and women (Fisher *et al.*, 1998).

Several studies have examined the elimination half-life of trichloroacetic acid in humans. The plasma half-life of trichloroacetic acid ranged from 4 to 5 days after oral ingestion of 15 mg/kg bw chloral hydrate (Breimer *et al*., 1974) and was 50.6 h after oral administration of 3 mg/kg bw trichloroacetic acid (Müller *et al*., 1972, 1974).

Froese *et al*. (2002) estimated the half-life of trichloroacetic acid in humans after drinking tap-water containing a range of disinfection products, including trichloroacetic acid. The intake of trichloroacetic acid was 20–82 μg/day during the 12-day study period. Although 10 volunteers (eight men, two women) were enrolled in the study, useful elimination data were obtained for only three, in whom the elimination half-lives ranged from 2.3 to 3.67 days.

The fraction of trichloroacetic acid bound to human plasma proteins ranged from 74.8 to 84.3% at concentrations of 6, 61 and 612 nmol/mL trichloroacetic acid; the binding of trichloroacetic acid to human plasma proteins is greater than that to rat (38.3–53.5%) or dog (54.2–64.8%) plasma protein (Templin *et al*., 1995).

4.1.2 *Experimental systems*

The metabolism of trichloroacetic acid was studied in male Fischer 344 rats and male B6C3F$_1$ mice (Larson & Bull, 1992). The animals were given 5, 20 or 100 mg/kg bw [^{14}C]trichloroacetic acid orally, and the ^{14}C content of the urine, faeces, exhaled air and carcass was measured. Approximately 50% (47.8–64.6%) of any dose of trichloroacetic acid was excreted unchanged in the urine of both rats and mice. The half-life of trichloroacetic acid in rats and mice given 20 or 100 mg/kg bw ranged from 4.2 to 7.0 h, and the clearance ranged from 36 to 66 mL/kg/h. The combined excretion of glyoxylic acid, oxalic acid and glycolic acid in urine amounted to 4.9–10.8% of the administered dose. Although not stated by the authors, glyoxylic acid, oxalic acid and glycolic acid are known metabolites of dichloroacetic acid and may have been formed from trichloroacetic acid-derived dichloroacetic acid. Dichloroacetic acid was detected in the urine of rats and mice, indicating the reduction of trichloroacetic acid. The authors proposed that trichloroacetic acid undergoes reduction to the dichloroacetyl radical ($\bullet CCl_2COOH$), which may abstract a hydrogen atom to form dichloroacetic acid or may react with oxygen to form a hydroperoxyl radical ($\bullet OOCCl_2COOH$) that may yield oxalic acid (Larson & Bull, 1992).

The kinetics of the elimination of trichloroacetic acid as a metabolite of inhaled trichloroethylene or following intravenous administration has been reported in pregnant rats and in lactating rats and nursing pups (Fisher *et al*., 1989, 1990). In pregnant rats exposed by inhalation to 618 ppm [3.3 mg/L] trichloroethylene for 4 h on day 12 of gestation or given 4 mg/kg bw trichloroacetic acid intravenously on days 14–15 of pregnancy, the elimination rate constant was 0.045/h. Fetal exposure to trichloroacetic acid was estimated at 63–64% of the maternal dose (Fisher *et al.*, 1989). The elimination rate constants in lactating dams exposed by inhalation to 600.4 ppm [3.2 mg/L] trichloroethylene for 4 h or given 4.4 mg/kg bw trichloroacetic acid intravenously were 0.063/h and 0.086/h, respectively, whereas that in nursing pups whose dams had been exposed to trichloroethylene by inhalation was 0.014/h. It was estimated that 4.2–6.8% of the trichloroacetic acid formed as a metabolite of trichloroethylene was eliminated by lactational transfer (Fisher *et al.*, 1989).

The kinetics of the elimination was studied in male B6C3F$_1$ mice given 0.03, 0.12 and 0.61 mmol/kg bw [5, 20 and 100 mg/kg bw] trichloroacetic acid by gavage (Templin *et al.*, 1993). The half-life ranged from 5.4 to 6.4 h. A comparison of the area-under-the-curve for distribution of trichloroacetic acid to the blood and liver following exposure to trichloroethylene showed that distribution favoured the blood over the liver. Analysis of the binding of trichloroacetic acid to plasma proteins gave a K_D and B_{max} of 248 and 310 µM [40.5 and 50.6 mg/L], respectively.

The half-lives for the elimination of trichloroacetic acid in male Fischer 344 rats given 0.15 and 0.76 mmol/kg bw [24.5 and 124 mg/kg bw] orally were 7.9 and 13 h, respectively, whereas those in male beagle dogs given 0.15, 0.38 and 0.76 mmol/kg bw [24.5, 62 and 124 mg/kg bw] orally were 200, 175 and 238 h, respectively (Templin *et al*., 1995).

The half-life of trichloroethanol-derived trichloroacetic acid in male Fischer 344 rats that had no enterohepatic circulation because of the placement of a bile shunt and were given 5, 20 or 100 mg/kg bw trichloroethanol intravenously ranged from 7.7 to 11.6 h (Stenner *et al*., 1997). The areas-under-the-curve for trichloroacetic acid in the blood and bile of rats without enterohepatic circulation that were given 5, 20 or 100 mg/kg bw trichloroethanol were 1.6, 12.1 or 38.3 and 5.4, 12.8 or 28.3 mg/h/mL, respectively. In rats with intact enterohepatic circulation given 100 mg/kg bw trichloroacetic acid intravenously, the half-life for the elimination of trichloroacetic acid from the blood was 11.6 h. These data were incorporated into a physiologically based pharmacokinetic model for trichloroethylene and its metabolites that included enterohepatic circulation of metabolites of trichloroethylene (Stenner *et al*., 1998). The authors concluded that the elimination of trichloroacetic acid is best described by a multi-exponential decay in which the long half-life of trichloroacetic acid is associated with its renal re-absorption.

Second-generation physiologically based pharmacokinetic models for trichloroethylene that include the kinetics of trichloroacetic acid formation and excretion in B6C3F_1 mice have been developed (for review, see Fisher, 2000). These models differ from first-generation models in that a proportionality constant was not used to describe the stoichiometry of the metabolism of trichloroethylene to trichloroacetic acid. A physiologically based pharmacokinetic model for trichloroethylene that includes kinetic constants for the urinary excretion of trichloroacetic acid has been reported (Abbas & Fisher, 1997). In male B6C3F_1 mice given 1200 mg/kg bw trichloroethylene orally, the first-order rate constant values for the metabolism of trichloroacetic acid to dichloroacetic acid and for the urinary excretion of trichloroacetic acid were 0.35 and 1.55/h/kg, respectively. The authors noted that 66% of the trichloroethylene-derived trichloroacetic acid was excreted in the urine and the remaining 34% was presumed to be metabolized to dichloroacetic acid. Because dichloroacetic acid was not detected in the urine of mice given trichloroethylene orally, it was speculated that dichloroacetic acid must be metabolized extensively (Abbas & Fisher, 1997). In a study of the rates of elimination of trichloroacetic acid and its metabolism to dichloroacetic acid in male B6C3F_1 mice exposed by inhalation to 100 or 600 ppm trichloroethylene [0.538 or 3.2 mg/L], the first-order rate constant values for the conversion of trichloroacetic acid to dichloroacetic acid and for the urinary excretion of trichloroacetic acid were 0.004 and 2.50/h/kg, respectively (Greenberg *et al*., 1999).

The metabolic fate of trichloroacetic acid has been investigated in male B6C3F_1 mice given 100 mg/kg bw trichloroacetic acid containing [1,2-^{14}C]trichloroacetic acid by gavage; ~5, ~55 and < 10% of the dose was eliminated as carbon dioxide and in the urine and faeces, respectively, and ~25% was found in the carcass. Trichloroacetic acid, dichloroacetic acid, chloroacetic acid, glyoxylic acid, glycolic acid, oxalic acid and unidentified metabolites

accounted for 44.5, 0.2, 0.03, 0.06, 0.11, 1.5 and 10.2% of the urinary metabolites (Xu *et al*., 1995).

The metabolism of trichloroacetic acid to dichloroacetic acid was studied in control and dichloroacetic acid-treated male B6C3F$_1$ mice given 100 mg/kg bw trichloroacetic acid intravenously (Merdink *et al*., 1998). Prolonged treatment with dichloroacetic acid depletes GSTZ1-1, which catalyses its metabolism which is thereby reduced (Saghir & Schultz, 2002). In contrast with other reports (Larson & Bull, 1992; Xu *et al*., 1995), quantifiable concentrations of dichloroacetic acid were not detected in the blood of mice given trichloroacetic acid. The authors concluded that, although there is uncertainty about the metabolism of trichloroacetic acid to dichloroacetic acid, pharmacokinetic simulations indicate that dichloroacetic acid is probably formed as a short-lived metabolite of trichloroacetic acid and that its rapid elimination compared with its relatively slow formation prevents its ready detection (Merdink *et al*., 1998). The artefactual formation of dichloroacetic acid from trichloroacetic acid has also been noted: for example, trichloroacetic acid was converted to dichloroacetic acid in freshly drawn blood samples (Ketcha *et al*., 1996).

The tissue disposition and elimination of [1-^{14}C]trichloroacetic acid was studied in male Fischer 344 rats given 6.1, 61 or 306 μmol/kg bw [1, 10 and 50 mg/kg bw] trichloroacetic acid intravenously (Yu *et al*., 2000). The fraction of the initial dose excreted in the urine increased from 67 to 84% as the dose increased and faecal excretion decreased from 7 to 4%. The elimination of trichloroacetic acid as carbon dioxide decreased from 12 to 8% of the total dose. The authors noted that the hepatic intracellular concentrations of trichloroacetic acid were significantly greater than the free plasma concentrations, indicating concentrative uptake by hepatocytes, and that trichloroacetic acid filtered at the glomerulus appears to be reabsorbed from either the renal tubular urine or the bladder (Yu *et al.*, 2000).

Gonthier and Barret (1989) reported that trichloroacetic acid (60 μL/mL neat compound) did not yield a spin adduct when incubated with liver or brain microsomes from male Sprague-Dawley rats in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) and the spin trap α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone. The absence of a hydroxyl radical signal during microsomal metabolism of trichloroacetic acid remains a controversial point (Gonthier & Barret, 1989). [The Working Group noted that trichloroacetic acid may have inactivated enzymes responsible for its reduction. Moreover, the reaction mixtures were apparently incubated in the presence of air, which may also have prevented its reduction.]

The biotransformation of trichloroacetic acid was studied in hepatic microsomal fractions isolated from control and pyrazole-treated male B6C3F$_1$ mice (Ni *et al.*, 1996). When trichloroacetic acid (5 mM [817 μg/mL]) was incubated with a microsomal fraction and a NADPH-generating system in the presence of the spin trap *N*-*tert*-butyl-α-phenylnitrone [the concentration of oxygen in the closed reaction flasks was not stated], analysis by electron spin resonance spectroscopy indicated the presence of a carbon-centred radical, which was not characterized (Ni *et al*., 1996).

The mechanism of the reduction of trichloroacetic acid to dichloroacetic acid has been investigated (Merdink *et al*., 2000). Microsomal fractions from male B6C3F$_1$ mice or

Fischer 344 rats were incubated with a NADPH-generating system, trichloroacetic acid (1 mM) [163.4 μg/mL] and the spin trap phenyl-*tert*-butyl nitroxide (PBN) in an argon (anaerobic) atmosphere. Gas chromatographic–mass spectrometric analysis of methylated extracts of the reaction mixture revealed the formation of 2-*tert*-butyl-4,4-dichloro-3-phenylisoxazolidin-5-one derived from the dichloroacetate radical, whose mass spectrum was interpreted to be a cyclized form of the expected PBN/dichloroacetyl radical. The same product was formed when trichloroacetic acid was incubated with PBN, ferrous sulfate and hydrogen peroxide (Fenton reaction system) (Merdink *et al*., 2000).

4.1.3 *Comparison of humans and animals*

The results of several studies indicate that the urinary elimination or plasma clearance of trichloroacetic acid is slower in humans than in rodents.

4.2 Toxic effects

This section reviews the literature on the toxic effects of trichloroacetic acid not cited in the previous monograph (IARC, 1995).

4.2.1 *Humans*

No new data were available to the Working Group.

4.2.2 *Experimental systems*

As reported in the previous monograph on trichloroacetic acid (IARC, 1995), short-term treatment (≤ 14 days) resulted in increases in cell replication rates in the liver of mice. The elevated rates of replication were not sustained and became substantially reduced compared with controls with and without chronic pretreatment (Pereira, 1996; Stauber & Bull, 1997). In an experiment in which treatment of male $B6C3F_1$ mice with 2 g/L trichloroacetic acid was terminated after 1 year (50 weeks), cell replication rates within tumours were not dependent upon continued treatment (for an additional 2 weeks). Trichloroacetic acid did not stimulate replication of initiated cells. As only one time-point was measured, the possibility that trichloroacetic acid affected replication rates of preneoplastic lesions cannot be ruled out (Stauber & Bull, 1997).

The previous review showed that trichloroacetic acid induced peroxisome proliferation in rodents (IARC, 1995). A recent in-vitro study of COS-1 cells transiently co-transfected with a peroxisome proliferator-activated receptor (PPAR) expression plasmid, pCMV-mPPARα, together with a reporter plasmid containing a peroxisome proliferator response element, Pluc4A6-880, clearly demonstrated that trichloroacetate directly activates the PPARα (Zhou & Waxman, 1998). In other studies, trichloroacetic acid induced peroxisome proliferation in primary cultures of hepatocytes from rats and mice but not in those from humans (Elcombe, 1985; Walgren *et al*., 2000a). In addition, human hepatocytes that

expressed endogenous human PPARα did not respond to trichloroacetic acid, whereas human cells co-transfected with mouse PPARα and mouse retinoid X receptor plasmids displayed increased activity of the peroxisome proliferator response element reporter after treatment with trichloroacetic acid and other peroxisome proliferators. Retinoid X receptor that forms a heterodimer with PPAR enhanced PPAR–DNA binding and transcriptional activation (retinoid X receptor is a common partner for many steroid receptors) (Walgren *et al*., 2000b). The phenotype of trichloroacetic acid-induced liver tumours in mice is also consistent with a mechanism of action that is parallel to or dependent upon activation of peroxisome synthesis (Nakano *et al*., 1994; Latendresse & Pereira, 1997; Stauber *et al*., 1998; Bull *et al*., 2002).

Chronic treatment with trichloroacetic acid produced relatively mild changes in carbohydrate metabolism compared with dichloroacetic acid. In male mice, it reduced liver glycogen content (Kato-Weinstein *et al*., 2001), whereas dichloroacetate increased it. It had no measurable effects on serum insulin concentrations, but produced an elevation in serum glucose at high doses (3 g/L). These findings also contrast with dichloroacetate, which has been shown to decrease serum insulin levels substantially with no effect on serum glucose concentrations (Kato-Weinstein *et al*. 2001; Lingohr *et al*., 2001).

Short-term oral treatment (11 days) of mice with trichloroacetate (25 mmol/L) inhibited methylation of DNA in liver, an effect that was not observed with long-term treatment (44 weeks) (Tao *et al*., 1998). However, methylation of DNA was depressed in trichloroacetate-promoted liver tumours at 44 weeks and suspension of treatment 1 week prior to sacrifice did not reverse this effect. An increased expression of *c-jun* and *c-myc* proto-oncogenes was observed when the 5-methylcytosine levels in their respective promoter regions decreased (Tao *et al*., 2000a) and administration of methionine 30 min after trichloroacetate inhibited expression of both proto-oncogenes (Tao *et al*., 2000b). Increased cell replication rates and decreased methylation of the c-*myc* gene were first observed simultaneously in mice 72 h after the start of exposure to trichloroacetic acid. Trichloroacetic acid induced DNA hypomethylation by inducing DNA replication and preventing the methylation of the newly synthesized strands of DNA (Ge *et al.*, 2001). The authors speculated that trichloroacetate depleted *S*-adenosylmethionine levels. Depressed levels of 5-methylcytosine were observed in the kidney and bladder as well as the liver.

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Sprague-Dawley rats administered 2730 ppm trichloroacetic acid in the drinking-water (mean daily dose, 291 mg/kg) throughout pregnancy had significant increases in the number of fetal resorptions (2.7 per litter compared with 0.70 per litter for controls) (Johnson *et al*.,

1998a,b). These were accompanied by a significant increase in the incidence of heart anomalies (from 13/605 to 12/114 fetuses) that were described somewhat differently from those reported by Smith *et al*. (1989) because of the more detailed dissection technique used.

A subsequent study in which trichloroacetate was administered by gavage at a daily dose of 300 mg/kg on days 6–15 of gestation during organogenesis did not produce clear evidence of a teratogenic effect (Fisher *et al*., 2001). The dissections were the same as those used by Johnson *et al*. (1998a,b). However, the study by Fisher *et al*. (2001) was complicated by widely divergent incidences of anomalies in the two different vehicle control groups (soya bean oil versus water). Consequently, the in-vivo information on the teratogenic effects of trichloroacetate appears to be inconclusive.

Trichloroacetate was evaluated for effects on the growth and development of Sprague-Dawley rat embryos in whole-embryo culture (Saillenfait *et al*., 1995). Malformed embryos were observed when the concentration of trichloroacetate reached 2.5 mM (no effect at 1 mM). There appeared to be some effects on growth at 1 mM, as total DNA and protein content per embryo was reduced (but not significantly) at this concentration. Hunter *et al*. (1996) reported a significant dose-dependent increase in the incidence of malformations, including neural tube defects, rotational defects, eye defects, pharyngeal arch defects and heart defects, in mouse whole-embryo cultures treated with 1–5 mM trichloroacetate.

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems*

(*a*) *DNA binding*

The level of 8-hydroxydeoxyguanosine–DNA adducts in the liver of $B6C3F_1$ mice was not modified after administration of trichloroacetic acid through drinking-water (Parrish *et al.*, 1996), was slightly increased after administration by gavage (Austin *et al.*, 1996) and was clearly increased after intraperitoneal injection (Von Tungeln *et al.*, 2002). After treatment with trichloroacetic acid, the level of malondialdehyde-derived adducts was increased *in vivo* (Von Tungeln *et al*., 2002) and *in vitro* (Beland, 1999).

(*b*) *Mutagenic and allied effects* (see Table 5 for details and references)

Trichloroacetic acid did not induce λ prophage or SOS repair in *Escherichia coli*. It was not mutagenic to *Salmonella typhimurium* strains in the presence or absence of metabolic activation, except in a single study on strain TA100 using a modified protocol in liquid medium.

The frequency of chlorophyll mutations was increased in *Arabidopsis* after treatment of seeds with trichloroacetic acid.

Table 5. Genetic and related effects of trichloroacetic acid

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic activation		
λ Prophage induction, *Escherichia coli* WP2s	–	–	10 000	DeMarini *et al.* (1994)
SOS chromotest, *Escherichia coli* PQ37	–	–	10 000	Giller *et al.* (1997)
Bacillus subtilis H17 *rec*$^+$ and M45 *rec*$^-$	–	NT	20 µg/plate	Shirasu *et al*. (1976)
Escherichia coli, B/r *try* WP2, reverse mutation	–	NT	20 µg/plate	Shirasu *et al*. (1976)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	450 µg/plate	Waskell (1978)
Salmonella typhimurium TA100, TA1535, reverse mutation	–	–	4000 µg/plate	Nestmann *et al*. (1980)
Salmonella typhimurium TA100, reverse mutation	–	NT	520 µg/plate	Rapson *et al*. (1980)
Salmonella typhimurium TA100, TA98, reverse mutation	–	–	5000 µg/plate	Moriya *et al*. (1983)
Salmonella typhimurium TA100, reverse mutation	–	–	600 ppm [600]	DeMarini *et al.* (1994)
Salmonella typhimurium TA100, reverse mutation, liquid medium	+	+	1750	Giller *et al.* (1997)
Salmonella typhimurium TA100, RSJ100, reverse mutation	–	–	16 300	Kargalioglu *et al*. (2002)
Salmonella typhimurium TA104, reverse mutation, microsuspension	–	–	250 µg/plate	Nelson *et al.* (2001)
Salmonella typhimurium TA1535, TA1536, TA1537, TA1538, reverse mutation	–	NT	20 µg/plate	Shirasu *et al*. (1976)
Salmonella typhimurium TA1537, TA1538, TA98, reverse mutation	–	–	2000 µg/plate	Nestmann *et al*. (1980)
Salmonella typhimurium TA98, reverse mutation	–	–	13 100	Kargalioglu *et al*. (2002)
Arabidopsis species, mutation	+	NT	1000	Plotnikov & Petrov (1976)
Micronucleus formation, *Pleurodeles waltl* newt larvae peripheral erythrocytes *in vivo*	+		80[d]	Giller *et al.* (1997)
DNA strand breaks, B6C3F$_1$ mouse and Fischer 344 rat hepatocytes *in vitro*	–	NT	1630	Chang *et al.* (1992)
DNA damage, Chinese hamster ovary cells *in vitro*, single-cell gel electrophoresis assay	–	NT	3 mM [490]	Plewa *et al*. (2002)

Table 5 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic activation		
Gene mutation, mouse lymphoma L5178Y/TK$^{+/-}$-3.7.2.C cells *in vitro*	?	(+)	3000	Harrington-Brock *et al*. (1998)
Formation of anchorage-independent colonies, B6C3F1 mouse hepatocytes *in vitro*	+	NT	82	Stauber *et al*. (1998)
DNA strand breaks, human CCRF-CEM lymphoblastic cells *in vitro*	–	NT	1630	Chang *et al.* (1992)
Chromosomal aberrations, human lymphocytes *in vitro*	–	–[c]	5000	Mackay *et al*. (1995)
DNA strand breaks, B6C3F$_1$ mouse liver *in vivo*	+		1.0 po × 1	Nelson & Bull (1988)
DNA strand breaks, B6C3F$_1$ mouse liver *in vivo*	+		500 po × 1	Nelson *et al.* (1989)
DNA strand breaks, B6C3F$_1$ mouse liver *in vivo*	–		500 po × 10	Nelson *et al*. (1989)
DNA strand breaks, B6C3F$_1$ mouse liver and epithelial cells from stomach and duodenum *in vivo*	–		1630 po × 1	Chang *et al.* (1992)
DNA strand breaks, Sprague-Dawley rat liver *in vivo*	+		100 po × 1	Nelson & Bull (1988)
DNA strand breaks, Fischer 344 rat liver *in vivo*	–		1630 po × 1	Chang *et al.* (1992)
Micronucleus formation, Swiss mice *in vivo*	+		125 ip × 2	Bhunya & Behera (1987)
Micronucleus formation, female C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes *in vivo*	–		1300 ip × 2	Mackay *et al.* (1995)
Micronucleus formation, male C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes *in vivo*	–		1080 ip × 2	Mackay *et al.* (1995)
Chromosomal aberrations, Swiss mouse bone-marrow cells *in vivo*	+		125 ip × 1	Bhunya & Behera (1987)
Chromosomal aberrations, Swiss mouse bone-marrow cells *in vivo*	+		100 ip × 5	Bhunya & Behera (1987)
Chromosomal aberrations, Swiss mouse bone-marrow cells *in vivo*	+		500 po × 1	Bhunya & Behera (1987)
Chromosomal aberrations, chicken *Gallus domesticus* bone marrow *in vivo*	+		200 ip × 1	Bhunya & Jena (1996)

Table 5 (contd)

Test system	Result[a]		Dose[b] (LED or HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic activation		
Inhibition of intercellular communication, B6C3F$_1$ mouse hepatocytes *in vitro*	+	NT	16.3	Klaunig *et al.* (1990)
Inhibition of intercellular communication, Sprague-Dawley rat liver clone 9 cells *in vitro*	+	NT	163	Benane *et al*. (1996)
Sperm morphology, Swiss mice *in vivo*	+		125 ip × 5	Bhunya & Behera (1987)

[a] +, positive; (+), weakly positive; –, negative; ?, inconclusive; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw; po, orally; ip, intraperitoneally
[c] Neutralized trichloroacetic acid
[d] Larvae reared in water containing trichloroacetic acid

Trichloroacetic acid did not induce DNA strand breaks or DNA damage in mouse, rat or hamster cells *in vitro* but was weakly mutagenic in mouse lymphoma cells. DNA strand breaks were not induced by trichloroacetic acid *in vitro* in human cells nor were chromosomal aberrations in human lymphocytes exposed *in vitro* to trichloroacetic acid neutralized to avoid the effects of low pH seen in cultured mammalian cells.

In one study, trichloroacetic acid induced micronuclei and chromosomal aberrations in bone-marrow cells and abnormal sperm morphology after injection into Swiss mice *in vivo*. In another study, in which a 10-fold higher dose was injected into C57BL/6JfBL10/Alpk mice, no micronucleus formation was observed. Trichloroacetic acid induced the formation of micronuclei in erythrocytes of newt larvae *in vivo*. It also induced chromosomal aberrations *in vivo* in the bone marrow of the chicken *Gallus domesticus*. Gap-junctional intercellular communication was inhibited in mouse and rat hepatocytes *in vitro*.

Mutation of proto-oncogenes in tumours induced by trichloroacetic acid

Point mutations in exons 1, 2 and 3 of K- and H-*ras* proto-oncogenes were studied in trichloroacetic acid-induced liver tumours of male B6C3F_1 mice (104-week treatment with 4.5 g/L in drinking-water). Trichloroacetic acid did not modify the incidence of mutations in exon 2 of H-*ras* in carcinomas (45% versus 58% for control). Only four carcinomas showed mutations in the other exons of H-*ras* or in K-*ras*. In tumours with mutation in exon 2 of H-*ras*, treatment with trichloroacetic acid did not modify the mutational spectrum compared with that of spontaneous liver tumours, that is to say 80% of the mutations in codon 61 were CAA→AAA, and 20% were CAA→CGA (Ferreira-Gonzalez *et al.*, 1995).

In *N*-methyl-*N*-nitrosourea (MNU) initiated female B6C3F_1 mice, 27% of liver tumours promoted by trichloroacetic acid exhibited loss of heterozygosity for at least two loci on chromosome 6 (Tao *et al.*, 1996).

4.5 Mechanistic considerations

There is little evidence that trichloroacetic acid induces mutation (see Section 4.4). Therefore, it is improbable that it acts through genotoxic mechanisms.

High doses of trichloroacetic acid were required to induce proliferation of peroxisomes in B6C3F_1 mice (palmitoyl-coenzyme A oxidase activity increased eightfold), but the compound was less effective in Fischer 344 rats (approximately twofold induction of palmitoyl-coenzyme A oxidase) (DeAngelo *et al*., 1989). Maloney and Waxman (1999) demonstrated that trichloroacetic acid directly activated hPPAR-α and mPPAR-α, but only weakly activated the mPPAR-γ receptor. The external doses administered as well as the systemic concentrations that resulted in a carcinogenic response in the liver of B6C3F_1 mice were generally consistent with those required to activate PPAR receptors and induce peroxisome proliferation [interaction was not examined].

Tumours from trichloroacetic acid-treated male B6C3F_1 mice did not have mutations in codons 12 or 13 of the H-*ras* oncogene, which are frequently observed with genotoxic

carcinogens. Liver tumours from these mice were found to have mutations at codon 61 of the H-*ras* gene (Ferreira-Gonzalez *et al*., 1995), the incidence of which was not different from that in spontaneous liver tumours (see also Bull *et al*., 2002). The frequency of codon 61 mutations in the H-*ras* gene was higher than that expected of other activators of the PPAR-α receptor such as ciprofibrate (Fox *et al.*, 1990; Hegi *et al*. 1993) but was consistent with that of peroxisome proliferators that activate PPAR-γ, such as LY-171883 (Helvering *et al.*, 1994; Kliewer *et al*., 1994). In contrast to mice, rats did not develop liver tumours when treated with trichloroacetic acid at concentrations of up to 5 g/L in the drinking-water, which is consistent with the weak peroxisome proliferative activity of this compound in rats (DeAngelo *et al*., 1997).

Treatment for up to 14 days with trichloroacetic acid enhanced cell proliferation in the liver of $B6C3F_1$ mice, but this increase was not apparent after longer exposures when chronic treatment even depressed cell division rates relative to controls (Pereira, 1996; Stauber & Bull, 1997). The growth of anchorage-independent colonies with the same phenotype as that observed *in vivo* were stimulated by appropriate concentrations of trichloroacetic acid (Stauber *et al*., 1998; Kato-Weinstein *et al.*, 2001). These data suggest that trichloroacetic acid promotes the clonal expansion of a subset of abnormal cells that arise spontaneously *in vivo*.

Expression of the mRNA for the c-*myc* gene was increased by trichloroacetic acid in both tumorous and non-tumorous liver in $B6C3F_1$ mice (Tao *et al*., 2000a,b). The c-*myc* proto-oncogene is a cellular transcription factor that plays a pivotal role in apoptosis, and cell replication and differentiation (Holden *et al*., 1998; Christensen *et al*., 1999). In female $B6C3F_1$ mice administered daily doses of 500 mg/kg trichloroacetic acid by gavage, enhancement of liver cell proliferation and decreased methylation of the *c-myc* gene were observed simultaneously 72 h after the start of exposure (Ge *et al.*, 2001). Methylation of CpG sites in the promoter region of a gene regulates in part the expression of its mRNA (Jones & Buckley, 1990; Wainfan & Poirier, 1992; Razin & Kafri, 1994). Trichloroacetic acid has been shown to decrease the methylation of DNA and of CCGG sites in the promoter region of the c-*myc* and c-*jun* genes and to induce their overexpression (Tao *et al.*, 1998, 2000a). A c-jun phenotype is typical of tumours induced in male B6C3F1 mice by peroxisome proliferators (Nakano *et al*. 1994). In the liver of female $B6C3F_1$ mice given trichloroacetic acid in drinking-water for 11 days, the level of 5-methylcytosine in DNA was decreased. When female $B6C3F_1$ mice were initiated with a single dose of MNU before receiving trichloroacetic acid in the drinking-water for 44 weeks, the amount of 5-methylcytosine in adenomas 1 week after termination of the treatment did not recover to the level observed in non-cancerous liver (Tao *et al.*, 1998), which is consistent with an aggressive rate of cell replication within trichloroacetic acid-induced tumours after cessation of treatment (Stauber & Bull, 1997).

In summary, studies suggest that the carcinogenic activity of trichloroacetic acid in $B6C3F_1$ mice is consistent with its activity as a peroxisome proliferator. This is further supported by a lack of a carcinogenic response in rats, in which the extent of peroxisome proliferation after treatment with trichloroacetic acid is much lower than in mice. In

addition, various other effects on cell replication rates, decreased DNA methylation and increased proto-oncogene expression are consistent with an epigenetic mechanism of carcinogenesis in mice.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Trichloroacetic acid is mainly used as a selective herbicide. It also finds use in the metal, plastics and textile industries and as an analytical reagent. It is used in the topical treatment of warts, cervical lesions and other dermatological conditions. Trichloroacetic acid is a major end metabolite of trichloroethylene and tetrachloroethylene. Wider exposure to trichloroacetic acid occurs at microgram-per-litre levels in drinking-water and swimming pools as a result of chlorination or chloramination.

5.2 Human carcinogenicity data

Several studies analysed risk with respect to one or more measures of exposure to complex mixtures of disinfection by-products that are found in most chlorinated and chloraminated drinking-water. No data specifically on trichloroacetic acid were available to the Working Group.

5.3 Animal carcinogenicity data

In four studies, neutralized trichloroacetic acid, when administered in the drinking-water to female and/or male mice, increased the incidences of hepatocellular adenomas and carcinomas. In a study in male rats, trichloroacetic acid did not increase the incidence of liver tumours or tumours at any other site. When administered in the drinking-water, trichloroacetic acid promoted the induction of hepatocellular adenomas and/or carcinomas in carcinogen-initiated male and female mice and of kidney tumours in male mice.

5.4 Other relevant data

The half-life of trichloroacetic acid, given orally or formed as a metabolite of trichloroethylene or trichloroethanol, is longer in humans than in rodents. Trichloroacetic acid may be reduced *in vivo* to dichloroacetic acid, but the artefactual conversion of trichloroacetic acid to dichloroacetic acid hinders any clear conclusions. A fraction of trichloroacetic acid is metabolized to carbon dioxide.

Trichloroacetic acid induces peroxisome proliferation in the livers of mice at doses within the same range as those that induce hepatic tumours. A brief stimulation of cell division is observed in the liver during the first days of treatment, but depressed cell replication

results from chronic treatment. The initial increase in cell proliferation was correlated with decreased methylation of the promoter regions of the c-*jun* and c-*myc* proto-oncogenes and increased expression of these genes.

Effects of trichloroacetic acid on reproduction and development in rats have been reported, but were not confirmed in a subsequent study. In-vitro results suggest that trichloroacetic acid can produce teratogenic effects at high doses.

In male mice, trichloroacetic acid modified neither the incidence of mutations in exon 2 of H-*ras* in carcinomas, nor the mutational spectrum observed in tumours that bore a mutation in exon 2. In female mice, 27% of tumours promoted by trichloroacetic acid exhibited loss of heterozygosity at a minimum of two loci on chromosome 6.

In mouse liver *in vivo*, measurements of trichloroacetic acid-induced 8-hydroxydeoxyguanosine DNA adducts gave different results depending on the route of administration. Trichloroacetic acid induced abnormal sperm in mice *in vivo* in one study and chromosomal aberrations in mouse and chicken bone marrow *in vivo*. The results of in-vivo studies in rodents on the induction of DNA strand breaks and micronuclei were inconsistent. It induced the formation of micronuclei in newt larvae *in vivo*.

In human cells *in vitro*, trichloroacetic acid did not induce chromosomal aberrations or DNA strand breaks in single studies. In single studies on cultured rodent cells, trichloroacetic acid was weakly mutagenic; no effect was observed in a DNA strand-break assay or a single-cell gel assay. It also inhibited intercellular communication in cultured rodent cells. Trichloroacetic acid caused neither mutation in bacteria nor SOS repair.

5.5 Evaluation

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

There is *limited evidence* in experimental animals for the carcinogenicity of trichloroacetic acid.

Overall evaluation

Trichloroacetic acid is *not classifiable as to its carcinogenicity to humans (Group 3)*.

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