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TRICHLOROETHYLENE, TETRACHLOROETHYLENE, AND SOME OTHER CHLORINATED AGENTS

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International Agency for Research on Cancer

World Health Organization

TRICHLOROACETIC ACID

Trichloroacetic acid was considered by previous IARC Working Groups in 1995 and 2004 (IARC, 1995, 2004). New data have since become available, and, together with information about sodium trichloroacetate (trichloroacetic acid, sodium salt), have been taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 Nomenclature

(a) Trichloroacetic acid

Chem. Abstr. Serv. Reg. No.: 76-03-9

Chem. Abstr. Serv. Name: Trichloroacetic acid

IUPAC Systematic Name: 2,2,2-Trichloroacetic acid

Synonyms: TCA; TCA (acid); trichloracetic acid; trichloroethanoic acid; trichloromethane carboxylic acid

(b) Sodium trichloroacetate

Chem. Abstr. Serv. Reg. No.: 650-51-1

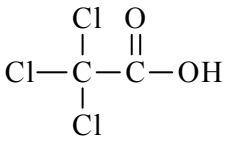
Chem. Abstr. Serv. Name: Sodium trichloroacetate

IUPAC Systematic Name: Sodium 2,2,2-trichloroacetate

Synonyms: TCA-sodium; TCA sodium salt; trichloroacetic acid, sodium salt

1.1.2 Structural and molecular formulae, and relative molecular mass

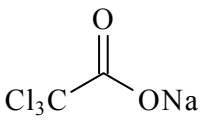
(a) Trichloroacetic acid



$C_2HCl_3O_2$

Relative molecular mass: 163.39

(b) Sodium trichloroacetate



$C_2Cl_3NaO_2$

Relative molecular mass: 185.37

1.1.3 Chemical and physical properties of the pure substance

(a) Trichloroacetic acid

Description: Very deliquescent crystals; slight, characteristic odour (O'Neil *et al.*, 2006)

Boiling-point: 196–197 °C (O'Neil *et al.*, 2006)

Melting-point: 57–58 °C (O'Neil *et al.*, 2006)

Density: 1.629 at 61 °C/4 °C (O'Neil *et al.*, 2006)

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)

Solubility: Very soluble in water, ethanol, ethyl ether (O'Neil *et al.*, 2006) soluble in most organic solvents, including acetone, benzene, methanol and *o*-xylene (Morris & Bost, 1991)

Volatility: Vapour pressure, 1 kPa at 83.8 °C (Haynes, 2012)

Stability: Decomposes by heating with caustic alkalis into chloroform and alkali carbonate. Corrosive. Decomposition products are chloroform, hydrochloric acid, carbon dioxide and carbon monoxide (O'Neil *et al.*, 2006)

Octanol/water partition coefficient (P): log P, 1.33 (Hansch *et al.*, 1995)

Conversion factor: mg/m^3 = 6.68 × ppm, calculated from: mg/m^3 = (relative molecular mass/24.45) × ppm, assuming normal temperature (25 °C) and pressure (101 kPa)

(b) Sodium trichloroacetate

Description: Yellow powder (HSDB, 2012)

Melting-point: Decomposes at 165–200 °C (HSDB, 2012)

Density: 1.808 g/cm^3 (Guidechem, 2012)

Solubility: Soluble in water at 1.2 kg/L at 25 °C; soluble in ethanol; soluble in methanol at 232 g/L, acetone at 7.6 g/L, diethyl ether at 0.2 g/L, benzene at 0.07 g/L, carbon tetrachloride at 0.04 g/L, heptane at 0.02 g/L (all at 25 °C) (HSDB, 2012)

1.1.4 Technical products and impurities

Trichloroacetic acid and sodium trichloroacetate are marketed at various degrees of purity. Trichloroacetic acid is available as aqueous solutions, with concentrations ranging from 3% to 100% (w/v) (Spectrum Chemical, 2012). Sodium trichloroacetate is available as a granular powder at a purity of > 97% (Acros Organics, 2012).

Trade names for trichloroacetic acid include Aceto-Caustin and Amchem Grass Killer.

Trade names for sodium trichloroacetate include: Eribitox T95G, acp grasskiller, Antiperz; Antyperz, varitox weedmaster grass killer and Remazol SaltFD.

1.1.5 Analysis

Methods for the analysis of trichloroacetic acid have been reviewed by Delinsky *et al.* (2005). Selected methods for the analysis of trichloroacetic acid in water 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) and headspace gas chromatography methods have been developed for measuring trichloroacetic acid in blood and urine (Monster & Boersma,1975; Skender *et al.*, 1993).

1.2 Production and use

1.2.1 Production process

(a) Manufacturing processes

Trichloroacetic acid was reported to have been first synthesized in 1840 by chlorination of acetic acid in sunlight (Beilstein Online, 2002). It 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 (such as iron or copper compounds) have been used in some cases. Trichloroacetic acid is isolated from

Table 1.1 Methods for the analysis of trichloroacetic 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	0.02 µg/L	EPA (2003) 552.3
Add ammonium chloride and ^{13}C-labelled internal standards; direct injection	IC-ESI-MS/MS	0.09 µg/L	EPA (2009) 557

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

the crude product by crystallization (Koenig *et al.*, 2011).

Sodium trichloroacetate is produced industrially by neutralizing trichloroacetic acid with sodium hydroxide solution or sodium carbonate (Ullmans, cited in HSDB, 2012).

(b) Production volume

Trichloroacetic acid was produced by nine companies in India, two companies each in China, Germany and Mexico, and one company each in France, Israel, Italy, Japan, the Russian Federation, and Spain (Chemical Information Services, 2002a). It was 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).

Between the late 1940s and 1990, about 30 000 tonnes of trichloroacetate were applied as herbicide in Germany (Schöler *et al.*, 2003). [This amount may not all have been produced in Germany.] By 1993, only about 1000 tonnes per annum of trichloroacetic acid were produced in Germany (OECD–SIDS, 2000). HSDB (2012) reported production volumes of 5–230 tonnes per annum in the USA.

1.2.2 Use

The main application of trichloroacetic acid, usually as its sodium salt, has been as a selective herbicide. It was formulated as a water-soluble liquid or powder (Koenig *et al.*, 2011). Its main use was as grass killer for perennial grasses such as common Bermuda grass, quack grass and Johnson grass (Monaco *et al.*, 2002). Common crops on which it was used include sugar beet, sugar cane and canola.

Historically, trichloroacetate has been combined with 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) to sterilize soil (Crafts, 1975) or for control of weeds. In Germany and Switzerland, the sale and import of sodium trichloroacetate as herbicide has been prohibited since 1989 (HSDB, 2012). In the USA, registrations for herbicidal products containing trichloroacetic acid were voluntarily cancelled by 1992 (California EPA, 1999), although existing stocks may have been used for some time after this date.

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 can be used as a caustic agent on the skin or mucous membranes to treat local lesions and for the treatment of various dermatological diseases. There are reports of its use in removing tattoos, treating genital warts and in dermal peeling. It is also used 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 Environmental occurrence

(a) Air

No data were available to the Working Group.

(b) Water

Trichloroacetic 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 summarizes some recent levels of trichloroacetic acid found in surface waters, groundwater and drinking-water worldwide.

1.3.3 Occupational exposure

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 35 124 employees in seven industries in the USA had potentially experienced occupational exposure to trichloroacetic acid (NIOSH, 1994). The estimate was based on a survey of companies and did not involve measurement of actual exposure.

Recently, occupational exposure of swimming-pool attendants to trichloroacetic acid in indoor and outdoor pools was evaluated by analysis of urine samples. After an exposure of 2 hours, the urine of 24 pool attendants contained trichloroacetic acid at a concentration of ~120 ng/L (range, < 60–163 ng/L). No trichloroacetic acid was detected in the urine of attendants at outdoor pools. Concentrations of trichloroacetic acid in the urine of pool attendants working indoors increased by 30% after the length of the shift doubled (4 hours) (Cardador & Gallego, 2011). [The Working Group noted that it was unclear by what route the pool attendants had been exposed.]

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 these compounds. It is also a metabolite of 1,1,1-trichloroethane (see IARC, 1979, 1987, 1999), and chloral hydrate (see *Monograph* on Chloral and Chloral Hydrate in this Volume; and IARC, 2004) 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 1.3.

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 after a request from the local labour-inspection agency or medical officer. The data showed that: (a) concentrations of trichloroacetic acid decreased by four times between 1947 and 1985; (b) the highest concentrations were observed in the iron and metal, chemical and dry-cleaning industries; (c) concentrations of trichloroacetic acid were twice as high in men than in women in the iron and metal, and dry-cleaning industries; (d) concentrations of trichloroacetic acid were higher in younger than in older workers; and (e) people working in an area in which trichloroethylene was used, but not working with trichloroethylene themselves, also showed urinary concentrations of trichloroacetic acid indicative of exposure.

Urinary concentrations of trichloroacetic acid in a large database of more than 3000 workers exposed to 1,1,1-trichloroethane, tetrachloroethylene and trichloroethylene in Finland showed a rapid 10-times decline in men between 1985 and 1992; the decline observed among

Table 1.2 Concentrations of trichloroacetic acid in water

Country	Location	Concentration (µg/L)		Reference
		Mean or median[a]	Range	
Drinking-water				
Australia	Seven cities	NR	< 0.02–14	Simpson & Hayes (1998)
China	Eight typical water supplies	NR	8.4–30.9	Liu *et al.* (2011)
	Beijing	1.7	1.5–2.2	Wang & Wong (2005)
	Beijing	3.38	NR–20.10	Wei *et al.* (2010)
Greece	Athens	NR	3.5–17.9	Golfinopoulos & Nikolaou (2005)
	Mytilene (bottled water)	NR	NR–1.5	Leivadara *et al.* (2008)
Spain	Eleven provinces	3.1[a]	1.5–5.0	Villanueva *et al.* (2012)
United Kingdom	England	9.8	4.1–18.5	Zhang *et al.* (2010a)
	Three large regions served by three water companies	12.7	NR–34	Malliarou *et al.* (2005)
		29.3	NR–95	
		21.1	NR–51	
	Scotland	20[a] [HAA]	11–134 [HAA]	Goslan *et al.* (2009)
Raw and surface water				
China	Eight typical water supplies	NR	33.6–488.5	Liu *et al.* (2011)
Republic of Korea	Four regions	46.6	43.5–55.8	Kim (2009)
Turkey	Country-wide (29 regions)	NR	18–149 [HAA]	Ates *et al.* (2007)
Swimming-pool water				
Republic of Korea	Seoul	156.4	19.7–636	Lee *et al.* (2010)
		17.4	1.3–85.8	
		97.2	1–413	

[a] Median
EGMO, electrochemically generated mixed oxidants; HAA, total halogenated acetic acids; NR, not reported

women was less steep, such that the median exposure of women was higher than that of men (Anttila *et al.*, 1995).

Recent studies have shown that urinary concentrations of trichloroacetic acid in workers in electronic and related industries in China (Green *et al.*, 2004) are above those in dry cleaners in the USA (McKernan *et al.*, 2008).

1.3.4 Exposure of the general population

Concentrations of trichloroacetic acid in urine and blood in the general population are presented in Table 1.4.

Concentrations of trichloroacetic acid as a metabolite of trichloroethylene and tetrachloroethylene were determined in the urine of people living in the vicinity of dry-cleaning shops in Germany and the USA where tetrachloroethylene was used (Popp *et al.*, 1992; Schreiber *et al.*, 2002). Trichloroacetic acid has also been measured in adults with no known exposure to these chemicals (Ikeda & Ohtsuji, 1969; Hajimiragha *et al.*, 1986; Skender *et al.*, 1993; Calafat *et al.* 2003; Zhou *et al.*, 2012).

In China, trichloroacetic acid was measured in the urine of 418 male partners in couples seeking medical treatment for infertility (Xie *et al.*, 2011). Urinary creatinine-adjusted concentrations of trichloroacetic acid ranged from 0.4 to 43.1 mg/g creatinine, with a mean of 6.4 mg/g creatinine.

In a study of exposures at swimming pools, the average urinary concentrations of trichloroacetic

Table 1.3 Concentrations of trichloroacetic acid in blood and urine measured by biological monitoring in occupational settings with exposure to various chlorinated solvents

Country	Job/task	No. of subjects	Agent to which exposure occurred	Air levels Range [mg/m³][a]	Matrix	Concentrations in blood or urine (range)	Reference
China	Electronic and related industries	70	Trichloroethylene	32 ppm (range, 0.5–252 ppm) (estimated based on concentrations of TCA in urine)	Urine	1–386 mg/g creatinine	Green *et al.* (2004)
Denmark	Various industries	2397 measurements	Trichloroethylene	NR	Urine	78 mg/L 1947–53; *n* = 396) 57 mg/L (1954–59; *n* = 214) 58 mg/L (1960–64; *n* = 290) 49 mg/L (1965–69; *n* = 413) 49 mg/L (1970–74; *n* = 499) 28 mg/L (1975–79; *n* = 459) 14 mg/L (1980–85; *n* = 126)	Raaschou-Nielsen *et al.* (2001)
Finland	Solvent exposure	10 783 measurements from 3976	1,1,1-Trichloroethane, tetrachloroethylene, trichloroethylene	NR	Urine	Median: Women, 63 µmol/L Men, 48 µmol/L	Anttila *et al.* (1995)
The former state union of Serbia and Montenegro	Dry cleaning, degreasing	32	Trichloroethylene	NR	Blood Urine	0.43–154.92 µmol/L [0.07–25.3 mg/L] 0.58–42.44 mmol/mol creatinine [0.84–61 mg/g]	Skender *et al.* (1988)
The former state union of Serbia and Montenegro	Dry cleaning	10	Trichloroethylene	25–40 ppm [134–215 mg/m³]	Blood Urine	13.47–393.56 µmol/L [2.2–64 mg/L] 1.92–77.35 mmol/mol creatinine [2.8–112 mg/g]	Skender *et al.* (1991)
		18	Tetrachloroethylene	33–53 ppm [224–359 mg/m³]	Blood Urine	1.71–20.93 µmol/L [0.3–3.4 mg/L] 0.81–15.76 mmol/mol creatinine [1.2–23 mg/g]	
Germany	Printing and ceramics workshop	31	Trichloroethylene	5–70 ppm [26.9–376 mg/m³]	Urine	2.0–201.0 mg/g creatinine	Triebig *et al.* (1982)

Table 1.3 (continued)

Country	Job/task	No. of subjects	Agent to which exposure occurred	Air levels Range [mg/m³][a]	Matrix	Concentrations in blood or urine (range)	Reference
Germany	Dry cleaning (nine shops)	12	Tetrachloroethylene	NR	Urine	Mean, 682 µg/L; maximum, 1720 µg/L	Popp *et al.* (1992)
Japan	Workshop	85	Trichloroethylene	3–175 ppm [16.1–940 mg/m³]	Urine	9–297 mg/L	Ikeda *et al.* (1972)
Japan	Printing factory	46	1,1,1-Trichloroethane	4.3–53.5 ppm [23–289 mg/m³]	Urine	0.5–5.5 mg/L	Seki *et al.* (1975)
Japan	Automobile workshop	25	Trichloroethylene	1–50 ppm [5–269 mg/m³]	Urine	Average, 136 mg/g creatinine	Ogata *et al.* (1987)
Japan	Workshop	3	Trichloroethylene	NR	Urine	Range of means, 108–133 mg/L (trichloroacetate)	Itoh (1989)
Japan	Printing workshop	48	1,1,1-Trichloroethane	5–65 ppm [27–351 mg/m³]	Urine	2–5 mg/L	Kawai *et al.* (1991)
Republic of Korea	Solvent exposure	13	Tetrachloroethylene	0–61 ppm [0–414 mg/m³]	Urine	0.6–3.5 mg/L	Jang *et al.* (1993)
Spain	Swimming pool	24	Trichloroacetic acid	NR	Urine	< 60–163 ng/L	Cardador & Gallego (2011)
Sweden	Degreasing	31	Trichloroethylene	3–114 mg/m³	Urine	2–260 µmol/L [0.3–42.5 mg/L]	Ulander *et al.* (1992)
Sweden	Various industries	1670	Trichloroethylene	81% < 20 ppm (estimated from concentrations of TCA in urine)	Urine	81% < 50 mg/L	Axelson *et al.* (1994)
Switzerland	Metal degreasing	26	Trichloroethylene	10–300 ppm [54–1611 mg/m³]	Urine	57–980 mg/L	Boillat (1970)
USA	Metal degreasing	19	Trichloroethylene	170–420 mg/m³	Urine	3–116 mg/g creatinine	Lowry *et al.* (1974)
USA	Dry cleaning	18	Tetrachloroethylene	GM, 1.64 ppm; GSD, 3.26	Urine	Pre-shift: GM, 0.29 mg/g creatinine; GSD, 7.23 Post-shift: GM, 0.51 mg/g creatinine; GSD, 3.89	McKernan *et al.* (2008)

GM, geometric mean; GSD, geometric standard deviation; NR, not reported; TCA, trichloroacetic acid

[a] Converted by the Working Group, except where stated in mg/m³ in the article

Table 1.4 Concentrations of trichloroacetic acid in urine and blood of the general population with known or unknown exposures

Country Year of study	Subjects	No. of subjects	Concentration in urine		Concentration in blood		Reference
			Mean	Range	Mean	Range	
China	Male partners of subfertile couples	418	6.4	0.4–43.1 mg/g creatinine	NR	NR	Xie *et al.* (2011)
China	Pregnant women	398	13.4 µg/g creatinine	1–123 µg/g creatinine	NR	NR	Zhou *et al.* (2012)
Croatia 1993	No known exposure to solvents	39	NR	NR	45 µg/L	14–160 µg/L	Skender *et al.* (1993)
Germany	Unexposed	43	NR	NR	24 µg/L	5–221 µg/L	Hajimiragha *et al.* (1986)
Germany 1992	Living near dry-cleaning shops	29	105 µg/L	NR	NR	NR	Popp *et al.* (1992)
Japan	Students	66	ND–930 µg/g creatinine	NR	NR	NR	Ikeda & Ohtsuji (1969)
Spain	Swimmers, indoor pool	13	4390 ng/L	NR	NR	NR	Cardador & Gallego (2011)
	Child swimmers, indoor pool	6	6092 ng/L	NR	NR	NR	
	Swimmers, outdoor pool	8	4757 ng/L	NR	NR	NR	
USA	Living near dry cleaner	10	6.6 µg/g creatinine	NR	< 0.83–21	NR	Schreiber *et al.* (2002)
USA	NHANES	402	2.9 µg/L	< 0.5–25 µg/L	NR	NR	Calafat *et al.* (2003)

ND, not detected; NHANES, National Health and Nutrition Examination Survey; NR, not reported

Table 1.5 National regulations and guidelines for trichloroacetic acid

Country or region	Concentration (mg/m³)	Unit
Australia	6.7	TWA
Austria	5	TWA
Belgium	6.8	TWA
Canada, Quebec	6.7	TWA
Denmark	1	TWA
France	5	TWA
New Zealand	6.7	TWA
Singapore	6.7	TWA
Spain	6.8	TWA
Switzerland	7	TWA
USA, NIOSH	7	TWA

NIOSH, National Institute for Occupational Safety and Health; TWA, 8-hour time-weighted average
From GESTIS – database on hazardous substances (Information system on hazardous substances of the German Social Accident Insurance) (IFA, 2012)

acid were 4390 ng/L in 13 adults and 6092 ng/L in 6 children swimming in an indoor pool and 4757 ng/L in 8 adults using an outdoor pool (Cardador & Gallego, 2011). Exposure levels in the swimmers were one order of magnitude higher than those in the pool attendants (313 ng/L and 51 ng/L, respectively, for workers at indoor and outdoor pools after a 2-hour shift). [Exposures may be higher for swimmers than for pool attendants because the main route of exposure is through ingestion.]

1.4 Regulations and guidelines

1.4.1 Comparison of exposure limits worldwide

Trichloroacetic acid is classified by the United States Environmental Protection Agency as a possible human carcinogen based on evidence of carcinogenicity in experimental animals. Trichloroacetic acid is considered to be a confirmed carcinogen in experimental animals, with unknown relevance to humans by the American Conference of Industrial Hygienists (HSDB, 2012).

Table 1.5 summarizes current limits for occupational exposure.

2. Cancer in Humans

Trichloroacetic acid is a chemical that occurs in drinking-water and in swimming pools as part of a mixture of by-products resulting from water disinfection. 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, 2013).

3. Cancer in Experimental Animals

Studies in experimental animals exposed to trichloroacetic acid were limited by incomplete pathology examination, short durations of exposure, and small numbers of animals. Studies with such a design cannot show the full expression of carcinogenic response. For any study in which trichloroacetic acid is administered in the drinking-water, palatability is an issue; decreases in drinking-water consumption have been observed at high doses. Such decreased drinking-water consumption not only affects the dose

administered but also may affect the health of the animals.

See Table 3.1

3.1 Mouse

As part of an initiation–promotion study in male B6C3F$_1$ mice (age, 4 weeks), trichloroacetic acid was administered in a buffered solution of drinking-water at a concentration of 5 g/L (Herren-Freund *et al.*, 1987). Controls were given drinking-water containing sodium chloride at 2 g/L. The experiment was terminated at 61 weeks because of tumour induction. Histopathological examination was restricted to the liver. At termination of the experiment, mice treated with trichloroacetic acid were found to have an increased incidence of hepatocellular adenoma (2 out of 22 in controls versus 8 out of 22 in treated mice, [$P < 0.05$]) and hepatocellular carcinoma (0 out of 22 in controls versus 7 out of 22 in treated mice, $P < 0.01$). [The Working Group noted that the study was limited by observations only for the liver, the low statistical power due to the small numbers of mice studied, the short duration of exposure, and the use of a single dose.]

Three groups of male B6C3F$_1$ mice (age, 5 weeks) were given drinking-water containing trichloroacetic acid at a concentration of 0, 1, or 2 g/L for up to 52 weeks (Bull *et al.*, 1990). Solutions of trichloroacetic acid were neutralized with sodium hydroxide (NaOH). Five mice in the groups at 0 and 2 g/L were killed at 15, 24, 37 weeks. Eleven animals from each group had treatment suspended at 37 weeks and were killed at 52 weeks. Thirty-five mice in the control group, 11 mice treated with trichloroacetic acid at 1 g/L, and 24 mice treated with trichloroacetic acid at 2 g/L were killed after 52 weeks of continuous treatment. Only the liver was examined for macroscopic lesions, and histopathology was not conducted on all lesions. One mouse out of 35 in the control group was found to have a hyperplastic nodule at 52 weeks. Mice treated with trichloroacetic acid at 1 g/L for 52 weeks were found to have three hyperplastic nodules, two liver [hepatocellular] adenomas, and two hepatocellular carcinomas distributed among five of the eleven mice treated [$P < 0.05$ versus controls]. At 2 g/L, 10 hyperplastic nodules, 1 liver [hepatocellular] adenoma and 4 hepatocellular carcinomas were found among 19 of the 24 mice treated [$P < 0.05$ versus controls]. Mice for which treatment at 2 g/L was terminated at 37 weeks and that were killed at 52 weeks were found to have two hyperplastic nodules, no adenomas, and three carcinomas distributed among four of the eleven mice treated. [The Working Group noted that this study was limited by observations being restricted to the liver, limited histopathological confirmation of diagnoses, varying numbers of animals in control and treatment groups, and the short duration of the study.]

In another study, groups of female B6C3F$_1$ mice (age, 7 weeks) were given drinking-water containing trichloroacetic acid at 0 (controls; drinking-water contained sodium chloride at 20 mM to control for the neutralization of trichloroacetic acid with NaOH in treated groups; $n = 134$), 2.0 ($n = 93$), 6.67 ($n = 46$), or 20 mM ($n = 38$) for 360 or 576 weeks (Pereira, 1996). The concentrations of trichloroacetic acid in drinking-water corresponded to 0.327, 1.09, and 3.27 g/L. Histopathology examination was restricted to the liver. The incidence of tumours was reported as the number of tumour-bearing mice compared with the total number of mice examined at termination of the experiments. The number of mice examined varied greatly between treatment groups ($n = 18$–90). The results for the incidence of hepatocellular adenoma and of hepatocellular carcinoma are provided in Table 3.1. A statistically significant treatment-related increase in the incidence of hepatocellular adenoma and of hepatocellular carcinoma was observed. A greater response was reported at 567 days than at 360 days. [The Working Group

Table 3.1 Studies of carcinogenicity in experimental animals given trichloroacetic acid by oral administration

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 61 wk Herren-Freund *et al.* (1987)	NaCl, 2 g/L; TCA, 5 g/L, in drinking-water 22/group	Hepatocellular adenoma: 2/22, 8/22* Hepatocellular carcinoma: 0/22, 7/22**	*[$P < 0.05$] **$P < 0.01$	Purity, > 99% Small number of mice. Histopathology restricted to the liver. Decreases in drinking-water consumption likely at this high dose. Short duration of exposure.
Mouse, B6C3F$_1$ (M) 52 wk Bull *et al.* (1990)	0, 1, 2 g/L, in drinking-water 35, 11, 24/group	Gross lesions: liver hyperplastic nodules, liver adenoma or hepatocellular carcinoma (combined): 2/35 [6%], 5/11* [45%], 19/24* [79%] Mice with confirmed hepatocellular carcinoma: 0, 2, 4	*[$P < 0.05$]	Analytical grade; purity, NR Histopathology restricted to the liver and not conducted on all gross lesions. Short exposure duration. Two groups of 10 females treated at 0 or 2 g/L were maintained until wk 52; no lesions were noted by the authors.
Mouse, B6C3F$_1$ (F) Up to 576 days Pereira (1996)	NaCl, 20 mM; TCA, 2.0, 6.67, 20.0 mM in drinking-water [0, 0.327, 1.09, and 3.27 g/L] 134, 93, 46, 38/group	*360 days* Hepatocellular adenoma: 1/40, 3/40, 3/19, 2/20 Hepatocellular carcinoma: 0/40, 0/40, 0/19, 5/20* *576 days* Hepatocellular adenoma: 2/90, 4/53, 3/27, 7/18* Hepatocellular carcinoma: 2/90, 0/53, 5/27*, 5/18*	*$P < 0.05$	Purity, NR Histopathology restricted to the liver.
Mouse, B6C3F$_1$ (M) 52 wk Bull *et al.* (2002)	0, 0.5, 2 g/L, in drinking-water 32, 20, 40/group	Liver adenoma: 0/20, 5/20*, 6/20* Hepatocellular carcinoma: 0/20, 3/20, 3/20 Hepatocellular adenoma or carcinoma (combined): 0/20, 6/20**, 8/20***	*[$P < 0.05$] ** [$P < 0.05$] *** [$P < 0.05$]	Purity, NR Study was focused on tumour phenotype and did not provide histopathology for all lesions.

Table 3.1 (continued)

Species, strain (sex) Duration Reference	Dosing regimen Animals/group at start	Incidence of tumours	Significance	Comments
Mouse, B6C3F$_1$ (M) 60 wk or 104 wk DeAngelo *et al.* (2008)	*Experiment 1 (60 wk):* NaCl, 2 g/L; TCA, 0.05, 0.5, 5 g/L in drinking-water 50/group *Experiment 2 (104 wk):* NaCl, 2 g/L; TCA, 4.5 g/L, in drinking-water 57, 58/group *Experiment 3 (104 wk):* acetic acid, 1.5 g/L; TCA, 0.05, 0.5 g/L, in drinking-water 72/group [In all experiments, all groups included interim kills]	*Experiment 1* Hepatocellular adenoma: 7% (n = 30), 15% (n = 27), 21% (n = 29), 38% (n = 29)* Hepatocellular carcinoma: 7% (n = 30), 4% (n = 27), 21% (n = 29), 38% (n = 29)* Hepatocellular adenoma or carcinoma (combined): 13% (n = 30), 15% (n = 27), 38% (n = 29)**, 55% (n = 29)*** *Experiment 2* Hepatocellular adenoma: 0% (n = 25), 59% (n = 36)* Hepatocellular carcinoma: 12% (n = 25), 78% (n = 36)* Hepatocellular adenoma or carcinoma (combined): 12% (n = 25), 89% (n = 36)* *Experiment 3* Hepatocellular adenoma: 21% (n = 42), 23% (n = 35), 51% (n = 37)* Hepatocellular carcinoma: 55% (n = 42), 40% (n = 35), 78% (n = 37)* Hepatocellular adenoma or carcinoma (combined): 64% (n = 42), 57% (n = 35), 87% (n = 37)*	* $P \leq 0.03$	The data set was reported as one study, but was actually three separate experiments, each with a different background tumour rate. The controls received different dosing solutions. There was limited reporting of lesions except for liver tumours. Some tumour data appears to have been mislabelled. The difference in data for 60-week and 104-week exposure could not be easily interpreted due to differences between the paradigms and in background tumour rate.
Rat, F344/N (M) 104 wk DeAngelo *et al.* (1997)	NaCl, 2 g/L; TCA, 0.05, 0.5, 5 g/L in drinking-water 50/group [All groups included interim kills]	Hepatocellular adenoma: 4.4% (n = 23), 4.2% (n = 24), 15% (n = 20), 4.6% (n = 22) Hepatocellular carcinoma: 0% (n = 23), 0% (n = 24), 0% (n = 20), 4.6% (n = 22)	NS	Purity, > 99% Small number of animals/group. Study focused on liver tumours, with limited histopathological examination and reporting of results. Historical background incidences of hepatocellular adenoma and carcinoma are low for this species and strain; the incidences of adenoma at the intermediate dose and carcinoma at highest dose are higher than in concurrent and historical controls.

F, female; M, male; NaCl, sodium chloride; NR, not reported; NS, not significant; TCA, trichloroacetic acid; wk, week

noted that this study was limited by examination being restricted to the liver.]

A similar paradigm was reported as part of an initiation–promotion study in female B6C3F$_1$ mice (age, 6 weeks) exposed for a shorter period (Pereira & Phelps, 1996). Groups of 10–15 mice were exposed for 31 weeks and groups of 19–40 mice were exposed for 52 weeks to trichloroacetic acid at the same concentrations used in Pereira (1996). The study gave negative results after 31 weeks of exposure to trichloroacetic acid alone. After 52 weeks, 4 out of 20 [$P < 0.05$] mice at 20.0 mM developed hepatocellular carcinoma (0.50 ± 0.18 tumour per mouse; $P < 0.05$, Mann–Whitney test) versus 0 out of 40 controls. [The Working Group noted that the study was limited by examination being restricted to the liver, and that the small number of animals studied and brief exposure period limited the power of the study to detect a carcinogenic response.]

A study was conducted to determine the extent to which trichloroacetic acid and dichloroacetic acid contribute to the carcinogenicity of trichloroethylene in B6C3F$_1$ mice. The study included groups that received treatment with trichloroacetic acid alone (Bull *et al.*, 2002). Histopathology was restricted to the liver. In these groups, male mice were treated with drinking-water containing trichloroacetic acid (neutralized with NaOH) at 0, 0.5 or 2 g/L for 52 weeks. Dose-dependent increases in the incidences of liver [hepatocellular] adenoma, and liver adenoma or hepatocellular carcinoma (combined) were observed in mice treated with trichloroacetic acid (Table 3.1). [The Working Group noted that the study was limited by examination being restricted to the liver, the short duration of exposure, the uncertainty of reporting lesion prevalence (i.e. random selection of gross lesions for histopathological examination), issues of lesion grouping, and the limited statistical power.]

The carcinogenicity of trichloroacetic acid and of several other chemicals was assessed in groups of 23–24 male and groups of 23–24 female neonatal B6C3F$_1$ mice (Von Tungeln *et al.*, 2002). In a first experiment, three sevenths of the total dose of trichloroacetic acid (2000 nmol per mouse, i.e. 327 µg per mouse) was administered by intraperitoneal injection on postnatal day 8 and four sevenths of the total dose on postnatal day 15. The mice were observed for 12 months. In a 20-month experiment, one third of the total dose (1000 nmol per mouse, i.e. 164 µg per mouse) was administered on postnatal day 8 and the remaining two thirds on postnatal day 15. [The dose in mg/kg bw was not reported and trichloroacetic acid was apparently not neutralized.] Histopathology was restricted to the liver. The incidences of hepatocellular adenoma, carcinoma and adenoma or carcinoma (combined) were not increased in either experiment. [The Working Group noted that the study was limited by histopathological examination being restricted to the liver, the small numbers of animals studied, the nonphysiological route of exposure, and the use of a low dose.]

In a study in male B6C3F$_1$ mice given drinking-water containing trichloroacetic acid (DeAngelo *et al.*, 2008), the three experiments reported were conducted in separate laboratories, as indicated by the very different background rates of tumour incidence. In these experiments, different doses were used with different control solutions. The first experiment involved mice treated with drinking-water containing trichloroacetic acid at concentrations of 0 (control), 0.05, 0.5 or 5 g/L for 60 weeks. The control group was given drinking-water containing sodium chloride at 2 g/L. In the second experiment, mice were treated with trichloroacetic acid at a concentration of 0 (control) or 4.5 g/L for 104 weeks. In this case, the control group received acetic acid at 1.5 g/L. In the third experiment, mice were treated with trichloroacetic acid at 0 (control), 0.05, or 0.5 g/L for 104 weeks. The control group for this experiment was given sodium chloride at 2 g/L. There was limited

reporting of lesions, except for liver tumours. The incidence of hepatocellular adenoma and of hepatocellular carcinoma in each experiment is presented in Table 3.1. In the first experiment, the incidences of hepatocellular adenoma and hepatocellular carcinoma were increased (relative to controls) in mice treated at 5 g/L for 60 weeks. The incidence of hepatocellular adenoma or carcinoma (combined) was increased in mice at 0.5 g/L. In the second experiment, substantial increases in the incidences of both tumour types were observed in mice treated with trichloroacetic acid at 4.5 g/L at 104 weeks relative to controls. In the third experiment, the incidences of both tumour types were also increased in mice treated with trichloroacetic acid at 0.5 g/L at 104 weeks relative to controls. [The Working Group noted that there was limited reporting of lesions except for liver tumours, that there was also no allowance of full expression of tumour response within 60 weeks, and that there was a very high background rate of incidence of liver tumours in control animals for the third experiment.]

3.2 Rat

Only one study in rats treated with trichloroacetic acid was available (DeAngelo *et al.*, 1997). Groups of 50 male F344/N rats (age, 28–30 days) were given drinking-water containing trichloroacetic acid at a concentration of 0, 0.05, 0.5, or 5 g/L. Trichloroacetic acid was neutralized with NaOH; to compensate for the resultant salt formation, control rats were given drinking-water containing sodium chloride at 2 g/L. Results were reported for 23, 24, 19, and 22 rats in the control group and groups at 0.05, 0.5, and 5.0 g/L, respectively, that were examined at terminal kill at 104 weeks, with one extra rat (from the group at the intermediate dose) examined for the liver tumour analysis. Six rats in the control group, and 8, 13, and 7 rats in the groups at the lowest, intermediate, and highest dose died during the course of the study. Interim kills of six rats per group were also carried out at 15, 30, 45, and 60 weeks for separate enzyme analysis. Complete gross pathological examinations were performed for rats in the group at the highest dose at 104 weeks; light microscopic examination was performed for kidney, liver, spleen, and testis only at the highest dose. [No indication was given as to whether a complete necropsy and pathological examination was performed for rats in the control group at terminal kill.] It was reported that trichloroacetic acid slightly decreased water consumption at all doses. Only data on liver tumours were presented, but not evaluated microscopically for rats that died early. The data were reported as the percentage of rats examined with hepatocellular adenoma (4.4%, 4.2%, 15%, and 4.6% for each group, respectively) and hepatocellular carcinoma (i.e. 0%, 0%, 0%, and 4.6%) as shown in Table 3.1. Although there were increases in the incidence of adenoma in the group receiving the intermediate dose, and in the incidence of carcinoma in the group receiving the highest dose, these increases were not statistically significant. [The Working Group noted that the historical control values for hepatocellular tumours, adenoma and especially carcinoma, are low in this species and strain, and that the percentage of rats with tumours exceeding the value for concurrent controls in this study also exceeds historical background levels (Haseman *et al.*, 1998). The Working Group also noted that this study was limited by the relatively small numbers of animals examined at 104 weeks and the limited histopathological examination and reporting of results.]

3.3 Administration with known carcinogens or other modifying factors

There have been several initiation–promotion studies with trichloroacetic acid, as well as studies of interactions with other carcinogenic

chemicals in which trichloroacetic acid consistently displayed hepatocellular tumour-promoting activity (Herren-Freund *et al.*, 1987; Pereira & Phelps, 1996; Pereira *et al.*, 1997, 2001; Bull *et al.*, 2004).

Two of these studies, which showed the most significant results in terms of promotion of hepatocellular tumours, are described here in more detail.

The Herren-Freund *et al.* (1987) study cited in Section 3.1 was an initiation–promotion study in male B6C3F$_1$ mice. Histopathological examination was restricted to the liver. Mice were initiated by intraperitoneal injection with *N*-ethyl-*N*-nitrosourea (ENU) at a dose of 2.5 or 10 mg/kg bw on postnatal day 15. Drinking-water containing trichloroacetic acid at 2 or 5 g/L was given from postnatal day 28 for 61 weeks. Controls treated with ENU only were given drinking-water containing sodium chloride at 2.5 g/L. The incidences of hepatocellular adenoma and carcinoma in mice treated only with ENU at 2.5 mg/kg bw were 1 out of 22, and 1 out of 22, respectively, and the incidences in mice treated only with ENU at 10 mg/kg bw were 9 out of 23, and 9 out of 23, respectively. The incidences in mice initiated with ENU at 2.5 mg/kg bw and subsequently treated with trichloroacetic acid at 2 g/L were 11 out of 33 ($P < 0.01$), and 16 out of 33 ($P < 0.01$), respectively. When mice were initiated with ENU at the same dose, but subsequently treated with trichloroacetic acid at 5 g/L, the incidences of these tumours were 6 out of 23 ($P < 0.01$), and 11 out of 23 ($P < 0.01$), respectively. The incidences in mice initiated with ENU at 10 mg/kg bw and treated with trichloroacetic acid at 5 g/L were 11 out of 28, and 15 out of 28, respectively.

In an initiation–promotion study with trichloroacetic acid in female B6C3F$_1$ mice (Pereira & Phelps, 1996), groups of mice were initiated with *N*-methyl-*N*-nitrosourea (MNU) as an intraperitoneal dose at 25 mg/kg bw on postnatal day 15. Treatment with drinking-water containing trichloroacetic acid began at age 7 weeks and was continued for 31 or 52 weeks. Histopathological examination was restricted to the liver. The data were expressed as numbers of hepatocellular tumours per mouse and percentage of mice with the indicated tumour were provided in parentheses. Concentrations of trichloroacetic acid were 2, 6.67, and 20.0 mM [i.e. 0.327, 1.09, and 3.27 g/L]. A "recovery" group was given trichloroacetic acid at 20 mM for 31 weeks, at which point treatment was suspended, and the mice were killed at week 52. Mice in the control group were given drinking-water containing sodium chloride at 2 mM. In MNU-initiated mice treated with trichloroacetic acid for 52 weeks, there were increases in the number of hepatocellular adenomas ($P < 0.05$) and hepatocellular carcinomas ($P < 0.05$) in the groups at 6.7 and 20 mM relative to controls treated with MNU only, i.e. hepatocellular adenoma: 0.28 ± 0.11 (17.5%), 2.00 ± 0.82 (83.3%), and 1.29 ± 0.24 (66.7%); hepatocellular carcinoma: 0.10 ± 0.05 (10%), 1.33 ± 0.42 (83.3%), and 2.79 ± 0.48 (83.3%); for the control group, and groups at 6.7 and 20 mM, respectively. The incidences of hepatocellular adenoma and hepatocellular carcinoma were also increased ($P < 0.05$) in the "recovery group": 0.91 ± 0.28 (63.6%), and 0.73 ± 0.33 (36.4%), respectively.

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, and excretion

In humans and animals, trichloroacetic acid is rapidly absorbed dermally or orally, but is only slowly metabolized, accumulating to a steady-state after successive exposures. Most of the absorbed dose is excreted in the urine as the parent compound. Metabolism that does occur

is mainly oxidative through cytochrome P450 to dichloroacetic acid via a dichloroacetic acid radical (shown in Fig. 4.1). The results of several studies indicate that the urinary elimination or plasma clearance of trichloroacetic acid is slower in humans than in rodents. Also, protein binding in the plasma is greater in humans than in rodents.

4.1.1 Absorption

(a) Humans

Trichloroacetic acid is rapidly absorbed by the oral and dermal routes of exposure, based on the results of studies with drinking-water and in swimming pools treated with disinfectants, including trichloroacetic acid. In studies by Kim & Weisel (1998), subjects stood in swimming pools for 30 minutes and the content of trichloroacetic acid of the pool water was compared with that of urine samples taken before and after exposure. Urinary excretion of trichloroacetic acid, an indirect biomarker of exposure, increased with increasing exposure. No studies were available on the absorption of inhaled trichloroacetic acid.

(b) Experimental systems

Studies in animals also indicated that trichloroacetic acid is rapidly absorbed in the gut and is slowly metabolized (Larson & Bull, 1992a; Xu *et al.*, 1995). In studies by Larson & Bull (1992a), male F344 rats and B6C3F$_1$ mice were given single oral doses of ^{14}C-labelled trichloroacetic acid of between 5 and 100 mg/kg bw. Radiolabel recovered in the urine over 48 hours ranged from 57% to 72% of the administered dose, with 81–90% being parent compound. In expired air, radiolabelled carbon dioxide ($^{14}CO_2$) represented 4–8% of the administered dose. The plasma half-life of trichloroacetic acid was about 6 hours, and the volume of distribution was 365–485 mL/kg in rats and 335–555 mL/kg in mice. These data indicate the ready absorption of trichloroacetic acid.

4.1.2 Distribution

(a) Humans

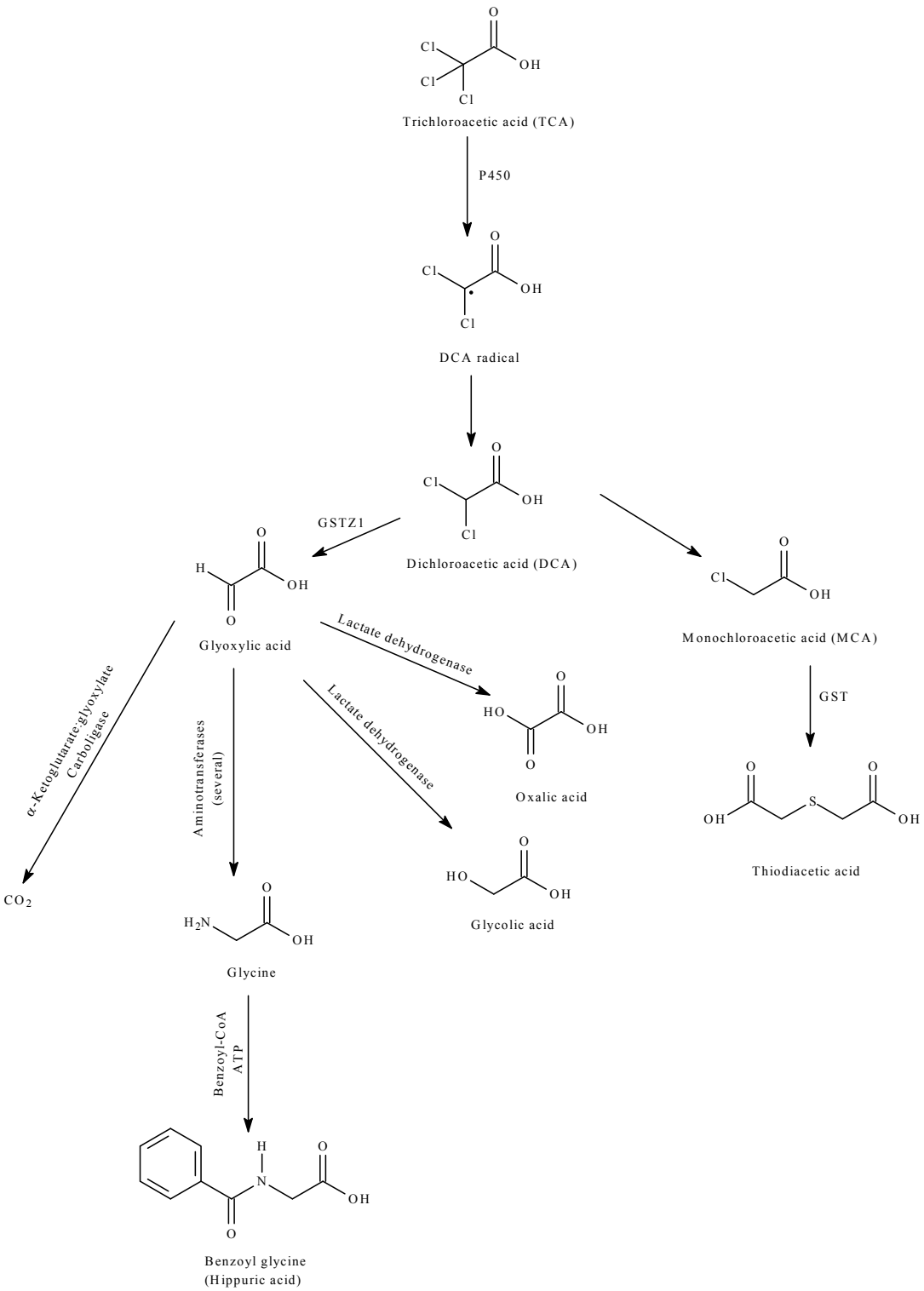
In a study by Müller *et al.* (1974), the half-life of trichloroacetic acid in plasma was approximately 50 hours and the volume of distribution was 115 mL/kg after a single oral dose of 3 mg/kg bw in healthy volunteers. The long half-life of plasma trichloroacetic acid is consistent with extensive binding to plasma proteins (Sellers & Koch-Weser, 1971; Stenner *et al.*, 1997, 1998). Templin *et al.* (1995) found that approximately 80% of ^{14}C-labelled trichloroacetic acid (dose, 6–61 nmol/mL [0.98–10 mg/mL]) in human plasma was bound to protein, a percentage that is higher than that observed in animals (see below).

A study by Lumpkin *et al.* (2003) used equilibrium dialysis to measure the binding of trichloroacetic acid at a range of concentrations to plasma of humans, rats and mice. Dissociation values did not vary between species, but the number of binding sites in plasma was higher in humans (2.97) than in rats (1.49) or mice (0.17). The greater plasma-protein binding in human plasma would be expected to increase the residence time for trichloroacetic acid in plasma and also reduce the amount of trichloroacetic acid available in other tissues.

(b) Experimental systems

The tissue distribution of absorbed trichloroacetic acid was reported by Yu *et al.* (2000). Male F344 rats were injected intravenously with ^{14}C-labelled trichloroacetic acid at doses of 0, 1, 10 or 50 mg/kg bw. Concentrations of trichloroacetic acid equivalents, based on detected radioactivity, were measured in plasma and in tissues at various times up to 24 hours after exposure. At early time-points, the highest concentrations of trichloroacetic acid equivalents were found in plasma, followed by kidney, erythrocytes, liver, skin, small intestine, muscle and fat. However, by 24 hours, the highest concentrations were found in the liver. Yu *et al.* (2000) hypothesized that

Fig. 4.1 Proposed metabolism of trichloroacetic acid



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

trichloroacetic acid was cleared from the liver more slowly than from the plasma because of a concentration-transport process in hepatocyte plasma membranes.

Templin *et al.* (1995) measured the binding of ^{14}C-labelled trichloroacetic acid to protein in the plasma of the mouse, rat, and dog. At concentrations of 6–61 nmol/mL [0.98–10 mg/mL], the percentage bound was approximately 60% (dog) or 50% (mouse and rat).

4.1.3 Metabolism

According to data from studies in humans and animals, trichloroacetic acid is poorly metabolized, with most of the absorbed dose being excreted as the parent compound in the urine.

(a) Humans

In studies reported by Paykoc & Powell (1945), six patients received trichloroacetic acid in aqueous solution at a dose of between 1.5 and 3 g via an intravenous drip for 1 hour. By 10 days after dosing, approximately 75% of the dose had been excreted unchanged in the urine, indicating little metabolism. No metabolites were measured. The evidence for metabolites of trichloroacetic acid comes from studies in experimental animals, described below.

(b) Experimental systems

Fig. 4.1 shows a proposed scheme for metabolism based on the results of studies in experimental animals. In the study by Xu *et al.* (1995), male B6C3F$_1$ mice were given uniformly labelled ^{14}C-labelled trichloroacetic acid at a dose of 100 mg/kg bw. After 24 hours, the distribution of radioactivity, as a percentage of the administered dose, was 55% in urine, 5% in exhaled CO_2 and 5% in faeces. Small amounts of urinary radioactivity in both studies were found in the form of metabolites, dichloroacetate, monochloracetate, glyoxylate, glycolate, and oxalate. In the studies by Larson & Bull, the metabolism of trichloroacetic acid was studied in male Fischer 344 rats and male B6C3F$_1$ mice (Larson & Bull, 1992a). The animals were given ^{14}C-labelled trichloroacetic acid orally at a dose of 5, 20 or 100 mg/kg bw. Approximately 50% of any dose of trichloroacetic acid was excreted unchanged in the urine of rats and mice. The half-life of trichloroacetic acid in rats and mice given a dose of 20 or 100 mg/kg bw ranged from 4.2 to 7.0 hours, and the clearance ranged from 36 to 66 mL/kg per hour. The combined excretion of glyoxylic acid, oxalic acid and glycolic acid (known metabolites of dichloroacetic acid) in urine amounted to 4.9–10.8% of the administered dose. Dichloroacetic acid was detected in the urine of rats and mice, indicating the reduction of trichloroacetic acid. Trichloroacetic acid thus undergoes reduction to the dichloroacetyl radical (•CCl_2COOH), which may abstract a hydrogen atom to form dichloroacetic acid, or may react with oxygen to form a hydroperoxyl radical (•$OOCCl_2COOH$) that may yield oxalic acid (Larson & Bull, 1992a).

The metabolic fate of trichloroacetic acid has been investigated in male B6C3F$_1$ mice given [1,2-^{14}C]-labelled trichloroacetic acid at a dose of 100 mg/kg bw by gavage. About 5%, 55% and < 10% of the administered dose was eliminated in exhaled air as CO_2, in the urine, and in the faeces, respectively; about 25% was found in the carcass. Trichloroacetic acid, dichloroacetic acid, monochloroacetic 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 treated male B6C3F$_1$ mice given trichloroacetic acid intravenously at a dose of 100 mg/kg bw (Merdink *et al.*, 1998). In contrast with other reports (Larson & Bull, 1992a; Xu *et al.*, 1995), quantifiable concentrations of dichloroacetic acid were not detected in the blood of mice given

trichloroacetic acid. 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 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 ^{14}C-labelled trichloroacetic acid was studied in male Fischer 344 rats given trichloroacetic acid intravenously at a dose of 6.1, 61 or 306 µmol/kg bw [1, 10, or 50 mg/kg bw] (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 CO_2 decreased from 12% to 8% of the total dose. 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).

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-phenyl-nitrone [the concentration of oxygen (O_2) 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). 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 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. The same product was formed when trichloroacetic acid was incubated with phenyl-*tert*-butyl nitroxide, ferrous sulfate and hydrogen peroxide (Fenton reaction system) (Merdink *et al.*, 2000).

Overall, the identified metabolites of trichloroacetic acid include dichloroacetic acid and its metabolites (CO_2, glyoxylic acid, oxalic acid, and glycolic acid). The extent of metabolism is not large, with identified metabolites accounting for less than 20% of the administered dose. Recovery of trichloroacetic acid in urine is around 50–75%, and the remaining fraction unidentified.

4.1.4 Excretion

(a) Humans

The major excretory route for trichloroacetic acid is the urine (see Section 4.1.3a).

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 chloral hydrate at 15 mg/kg bw (Breimer *et al.*, 1974) and was 50.6 hours after oral administration of trichloroacetic acid at 3 mg/kg bw (Müller *et al.*, 1972, 1974). Paykoc & Powell (1945) reported a plasma half-life of 82 hours for trichloroacetic acid administered intravenously in six patients.

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.

In the swimming pool studies of Kim & Weisel (1998) urinary excretion of trichloroacetic acid, in terms of μg per m^2 of surface area, increased with increasing exposures. Peak urinary trichloroacetic acid excretion rates occurred within 5 to 10 minutes after exposure and TCA was no longer detected after 3 hours. The data were consistent with rapid dermal absorption and subsequent urinary excretion of trichloroacetic acid.

Studies by the same group (Kim *et al.*, 1999), in which the content of trichloroacetic acid of chlorinated drinking-water ingested by subjects was compared to urinary trichloroacetic acid output, also indicated that trichloroacetic acid was rapidly absorbed from the gastrointestinal tract and was only slowly metabolized, accumulating to a steady-state after successive exposures. In additional studies of swimming pools, Cardador & Gallego (2011) analysed the urine of 49 swimmers (adults and children, male and female) after 1 hour of swimming and found trichloroacetic acid at an average concentration of 4400 ng/L. The major route of exposure was determined to be oral, with 5% exposure by inhalation and about 1% exposure via the dermal route.

Overall, available studies in humans indicated that trichloroacetic acid is slowly metabolized and excreted unchanged in urine, with a half-life estimated to be between 2 and 4 days (Paykoc & Powell, 1945; Müller *et al.*, 1972, 1974; Froese *et al.*, 2002).

(b) Experimental systems

In a study by Xu *et al.* (1995), male $B6C3F_1$ mice were given uniformly labelled ^{14}C-labelled trichloroacetic acid at a dose of 100 mg/kg bw. After 24 hours, the distribution of radioactivity, as a percentage of the administered dose, was 55% in urine, 5% in exhaled CO_2 and 5% in faeces.

Larson & Bull (1992a) studied excretory routes for trichloroacetic acid in male Fischer 344 rats and male $B6C3F_1$ mice. Results are detailed above in Section 4.1.3.

The kinetics of elimination were studied in male $B6C3F_1$ mice given trichloroacetic acid by gavage at a dose of 0.03, 0.12, or 0.61 mmol/kg bw [5, 20 and 100 mg/kg bw] (Templin *et al.*, 1993). The half-life ranged from 5.4 to 6.4 hours. A comparison of the area under the concentration–time curve for distribution of trichloroacetic acid to the blood and liver after exposure to trichloroethylene showed that distribution favoured the blood over the liver.

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

Stenner *et al.* (1997) reported similar concentrations of trichloroacetic acid in blood and bile of rats given trichloroacetic acid intravenously at a dose of 100 mg/kg bw, suggesting that enterohepatic recirculation may be occurring.

The kinetics of the elimination of trichloroacetic acid as a metabolite of inhaled trichloroethylene or after intravenous administration has been reported in pregnant rats and in lactating rats and nursing pups (Fisher *et al.*, 1989, 1990). In pregnant rats given trichloroacetic acid intravenously at a dose of 4 mg/kg bw 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 constant in lactating dams given trichloroacetic acid intravenously at 4.4 mg/kg bw was 0.086/hour.

4.2 Genotoxicity and related effects

Trichloroacetic acid has been studied in a variety of assays for genotoxic potential. Table 4.1, Table 4.2, and Table 4.3 summarize the studies carried out *in vivo*, *in vitro*, and in bacterial systems, respectively. [The Working Group noted that the evaluation of the studies of genotoxic potential *in vitro* must consider cytotoxicity and acidification of medium resulting in precipitation of proteins, as trichloroacetic acid is commonly used as a reagent to precipitate proteins.]

4.2.1 Human cell lines

Mackay *et al.* (1995) investigated trichloroacetic acid (as free acid) in an assay for chromosomal aberration *in vitro* using cultured human lymphocytes, both in the presence and absence of metabolic activation. Trichloroacetic acid induced chromosomal damage at concentrations (2000 and 3500 µg/mL) that significantly reduced the pH of the medium. However, neutralized trichloroacetic acid was without effect, even at a cytotoxic concentration of 5000 µg/mL. To further evaluate the role of pH changes in the induction of chromosomal damage, isolated liver-cell nuclei from $B6C3F_1$ mice were suspended in a buffer at various pH levels. A decrease in chromatin staining intensity was observed as pH decreased, suggesting that pH changes, independent of trichloroacetic acid exposure, can alter chromatin conformation. [The Working Group noted that it was possible that the reduced pH was responsible for clastogenicity induced by trichloroacetic acid in this study].

Chang *et al.* (1992) used the human lymphoblastic cell line, CCRF-CEM, to study the effect of trichloroacetic acid on DNA single-strand breaks. No effect was observed.

4.2.2 Experimental systems

(a) Mammalian systems

(i) Gene mutation

Harrington-Brock *et al.* (1998) examined the potential of trichloroacetic acid at concentrations up to 2150 µg/mL without metabolic activation, and up to 3400 µg/mL with metabolic activation from S9 (9000 × *g* supernatant) to induce mutations in L5178Y/*Tk*+/−–3.7.2C mouse lymphoma cells. The mutation frequency doubled at concentrations of ≥ 2250 µg/mL with metabolic activation, including at several concentrations at which survival was > 10%. In the absence of metabolic activation, trichloroacetic acid increased the mutation frequency by twofold or greater only at concentrations of 2000 µg/mL with 11% survival rates. Both large- and small-colony mutants were observed, with small-colony mutants indicative of chromosomal damage. [The Working Group noted that no rigorous statistical analysis was conducted in this study.]

Trichloroacetic acid was tested in a microplate-based test for cytotoxicity, and an assay for HGPRT [*Hprt*] gene mutation in Chinese hamster ovary K1 cells (Zhang *et al.*, 2010b). Trichloroacetic acid was the least cytotoxic of seven haloacetic acids tested. Trichloroacetic acid, at concentrations of 0, 200, 1000, 5000 and 10 000 µM [33, 163, 817 and 1630 µg/mL], visibly increased mutation frequency, but did not show any statistically significant increase at any of the doses tested.

(ii) Chromosomal aberration

Chromosomal aberrations in bone marrow were observed *in vivo* after intraperitoneal or oral administration of trichloroacetic acid in mice (Bhunya & Behera, 1987), and after intraperitoneal administration of trichloroacetic acid in chickens (Bhunya & Jena, 1996).

Table 4.1 Studies of genotoxicity with trichloracetic acid *in vivo*

Test system/end-point	Doses[a] (LED or HID)	Results	Reference
DNA strand breaks, B6C3F$_1$ mouse liver	1630, oral, × 1	+	Nelson & Bull (1988)
DNA strand breaks, B6C3F$_1$ mouse liver	500, oral, × 1	+	Nelson *et al.* (1989)
DNA strand breaks, B6C3F$_1$ mouse liver	500, oral, 10 repeats	–	Nelson *et al.* (1989)
DNA strand breaks, B6C3F$_1$ mouse liver and epithelial cells from stomach and duodenum	1630, oral, × 1	–	Chang *et al.* (1992)
DNA strand breaks, male B6C3F$_1$ mice	500 (neutralized) oral, × 1	–	Styles *et al.* (1991)
DNA strand breaks, male B6C3F$_1$ mouse liver	77, oral, 1/day, 13 wk	+	Hassoun *et al.* (2010a)
Micronucleus formation, Swiss mice	125, i.p., × 2	+	Bhunya & Behera (1987)
Micronucleus formation, female C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes	1300, i.p., × 2	–	Mackay *et al.* (1995)
Micronucleus formation, male C57BL/6JfBL10/Alpk mouse bone-marrow erythrocytes	1080, i.p., × 2	–	Mackay *et al.* (1995)
Micronucleus formation, *Pleurodeles waltl* newt larvae peripheral erythrocytes	80 µg/mL	+	Giller *et al.* (1997)
Chromosomal aberrations, Swiss mouse bone-marrow cells	125, i.p., × 1	+	Bhunya & Behera (1987)
	100, i.p., × 5	+	Bhunya & Behera (1987)
	500, oral, × 1	+	Bhunya & Behera (1987)
Chromosomal aberrations, chicken *Gallus domesticus* bone marrow	200, i.p., × 1	+	Bhunya & Jena (1996)
Sperm morphology, Swiss mice, *in vivo*	125, i.p., × 5	+	Bhunya & Behera (1987)

[a] Doses are in mg/kg bw unless otherwise specified.

+, positive; –, negative; HID, highest ineffective dose; i.p., intraperitoneal; LED, lowest effective dose; wk, week

(iii) Micronucleus formation

Trichloroacetic acid caused micronucleus formation in the bone marrow of mice (Bhunya & Behera, 1987) and chickens (Bhunya & Jena, 1996) exposed *in vivo via* intraperitoneal injection. A small increase in the frequency of micronucleated erythrocytes at 80 µg/mL in a test for micronucleus formation in the newt (*Pleurodeles waltl* larvae) was observed after exposure to trichloroacetic acid (Giller *et al.*, 1997). However, Mackay *et al.* (1995) found that trichloroacetic acid did not induce formation of micronuclei in bone marrow of C57BL mice given trichloroacetic acid intraperitoneally at doses of up to 1080 mg/kg bw per day for males and 1300 mg/kg bw per day for females for two consecutive days.

(iv) Other studies of DNA damage

The ability of trichloroacetic acid to induce single-strand DNA breaks (SSBs) has been examined in several studies, both *in vivo* and *in vitro* (see Table 4.1; Table 4.2; Nelson & Bull, 1988; Nelson *et al.*, 1989; Styles *et al.*, 1991; Chang *et al.*, 1992).

Nelson & Bull (1988) evaluated the ability of single oral doses of trichloroacetic acid to induce SSBs in Sprague-Dawley rats and B6C3F$_1$ mice *in vivo*. Dose-dependent increases in the frequency of SSBs were induced in both rats and mice, with mice being more susceptible than rats. The lowest dose of trichloroacetic acid to produce significant SSBs was 0.6 mmol/kg (98 mg/kg bw) in rats, but 0.006 mmol/kg (0.98 mg/kg bw) in mice.

A single oral dose of trichloroacetic acid at 500 mg/kg bw rapidly induced SSBs in the liver of male B6C3F$_1$ mice, but repair of SSBs also

Table 4.2 Studies of genotoxicity with trichloroacetic acid in mammalian systems *in vitro*

Test system/end-point	Doses[a] (LED or HID)	Results		Reference
		With metabolic activation	Without metabolic activation	
DNA strand breaks, B6C3F$_1$ mouse and F344 rat hepatocytes	1630	NT	–	Chang *et al.* (1992)
DNA damage, Chinese hamster ovary cells, comet assay	490	NT	–	Plewa *et al.* (2002)
DNA strand breaks, human CCRF-CEM lymphoblastic cells	1630	NT	–	Chang *et al.* (1992)
Gene mutation, mouse lymphoma L5178Y/*Tk*+/- cells	2250	(+)	?	Harrington-Brock *et al.* (1998)
Gene mutation, *HGPRT*, Chinese hamster ovary cells	1630 µM	NT	–	Zhang *et al.* (2010b)
Chromosomal aberration, human lymphocytes	5000 (neutralized)	NT	–	Mackay *et al.* (1995)

[a] Doses are in µg/mL, unless otherwise stated

+, positive; (+), weakly positive; –, negative; ?, inconclusive; HID, highest ineffective dose; LED, lowest effective dose; NT, not tested

proceeded rapidly, with a return to control levels within 8 hours after dosing (Nelson *et al.*, 1989). However, in the follow-up study in male B6C3F$_1$ mice given 10 repeated doses of trichloroacetic acid at 500 mg/kg bw by oral gavage, there was no effect on SSBs in whole-liver homogenate (Nelson *et al.*, 1989).

In a follow-up experiment with a similar experimental design, Styles *et al.* (1991) tested trichloroacetic acid for its ability to induce strand breaks in male B6C3F$_1$ mice in the presence and absence of liver growth induction. The test animals were given one, two, or three daily doses of neutralized trichloroacetic acid (500 mg/kg bw) by gavage and killed 1 hour after the final dose. Additional mice were given a single dose of 500 mg/kg bw by gavage and killed 24 hours after treatment. No induction of SSBs was observed in DNA from the liver under the conditions of this assay.

Chang *et al.* (1992) gave B6C3F$_1$ mice single oral doses of trichloroacetic acid (1–10 mmol/kg [163–1630 mg/kg]) and reported no dose-related effect on DNA strand breaks as determined by the alkaline unwinding assay. No genotoxic activity (evidence for strand breakage) was detected in F344 rats given trichloroacetic acid by gavage at up to 5 mmol/kg (817 mg/kg bw).

DNA SSBs were increased a dose-dependent manner in male B6C3F$_1$ mice treated with trichloroacetic acid by gavage for 4 weeks or 13 weeks. SSBs were increased in the liver by 75%, 125%, and 300% at doses of 77, 154, and 410 mg/kg bw per day at 4 weeks; and by 125%, 200%, and 310% at doses of 77, 154, and 410 mg/kg bw per day at 13 weeks.

Plewa *et al.* (2002) evaluated the induction of DNA strand breaks induced by trichloroacetic acid (1–3 mM [163–490 µg/mL]) in Chinese hamster ovary cells *in vitro* and did not observe genotoxicity.

Chang *et al.* (1992) used primary hepatocytes from mouse or rat to investigate the potential of trichloroacetic acid to induce DNA strand breaks. No effect of trichloroacetic acid was observed.

(b) Bacterial systems: gene mutation

Trichloroacetic acid has been evaluated in several test systems *in vitro*, including bacterial assays (Ames) using different strains of *S. typhimurium*, such as TA98, TA100, TA104, TA1535, and RSJ100 (see Table 4.3). The majority of

Table 4.3 Studies of genotoxicity with trichloroacetic acid in bacterial systems

Test system/end-point	Doses[a] (LED or HID)	Results		Reference
		With metabolic activation	Without metabolic activation	
λ Prophage induction, *E. coli* WP2s	10 000	–	–	DeMarini *et al.* (1994)
SOS chromotest, *E. coli* PQ37	10 000	–	–	Giller *et al.* (1997)
S. typhimurium TA1535, 1536, 1537, 1538, reverse mutation	20 µg/plate	NT	–	Shirasu *et al.* (1976)
S. typhimurium TA100, 98, reverse mutation	450 µg/plate	–	–	Waskell (1978)
S. typhimurium TA100, 1535, reverse mutation	4000 µg/plate	–	–	Nestmann *et al.* (1980)
S. typhimurium TA1537, 1538, 98, reverse mutation	2000 µg/plate	–	–	Nestmann *et al.* (1980)
S. typhimurium TA100, reverse mutation	520 µg/plate	NT	–	Rapson *et al.* (1980)
S. typhimurium TA100, 98, reverse mutation	5000 µg/plate	–	–	Moriya *et al.* (1983)
S. typhimurium TA100, reverse mutation	600	–	–	DeMarini *et al.* (1994)
S. typhimurium TA100, reverse mutation, liquid medium	1750	–	+	Giller *et al.* (1997)
S. typhimurium TA100, reverse mutation, liquid medium	3000	+	NT	Giller *et al.* (1997)
S. typhimurium TA104, reverse mutation, microsuspension	250 µg/plate	–	–	Nelson *et al.* (2001)
S. typhimurium TA100, RSJ100, reverse mutation	16 300	–	–	Kargalioglu *et al.* (2002)
S. typhimurium TA98, reverse mutation	13 100	–	–	Kargalioglu *et al.* (2002)
S. typhimurium TA1535, SOS DNA repair	NR	+	–	Ono *et al.* (1991)

[a] Doses are in µg/mL unless otherwise specified.
+, positive; –, negative; HID, highest ineffective dose; LED, lowest effective dose; NR, not reported; NT, not tested

these studies did not report positive findings for genotoxicity.

However, trichloroacetic acid induced a small increase in SOS DNA repair (an inducible error-prone repair system) in *S. typhimurium* strain TA1535 in the presence of metabolic activation from S9 (Ono *et al.*, 1991). Furthermore, Giller *et al.* (1997) reported genotoxic activity of trichloroacetic acid at noncytotoxic concentrations of 1750 to 2250 µg/mL in an Ames fluctuation test in *S. typhimurium* TA100 in the absence of S9. The addition of S9 decreased the genotoxic response, with effects observed at 3000–7500 µg/mL. Cytotoxic concentrations in the Ames fluctuation assay were 2500 and 10 000 µg/mL without and with microsomal activation, respectively.

4.3 Nongenotoxic mechanisms of carcinogenesis

4.3.1 Liver

The available evidence for nongenotoxic mechanisms for the rodent (mouse) liver tumours induced by trichloroacetic acid comprises the following: (i) epigenetic effects (especially DNA hypomethylation); (ii) cytotoxicity and oxidative stress; (iii) alteration of proliferation and apoptosis, and clonal expansion; (iv) PPARα activation; and (v) disruption of gap-junctional communication. Evidence from humans and from experimental animals supporting each of these nongenotoxic mechanisms of tumour

induction by trichloroacetic acid is presented below.

(a) Epigenetic effects

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

(i) Humans

No data on alterations in DNA methylation specific to trichloroacetic acid are available from studies in humans.

(ii) Experimental systems

The hypomethylation of DNA in response to exposure to trichloroacetic acid was investigated by Tao *et al.* (1998). Female B6C3F$_1$ mice (age, 15 days) received intraperitoneal injections of *N*-methyl-*N*-nitrosourea (MNU) at a dose of 25 mg/kg bw, and were subsequently given drinking-water containing trichloroacetic acid (neutralized to a concentration of 25 mmol/L [4085 mg/L]) for 44 weeks. After 11 days of exposure to trichloroacetic acid without pretreatment with MNU, the levels of 5-methylcytosine (5MeC) in total liver DNA was decreased (by about 60%) relative to untreated controls. After 44 weeks of exposure to trichloroacetic acid, levels of 5MeC were not different from those in controls that had received MNU only. No difference in DNA methylation was observed between the control groups in the short term (drinking-water control) and long-term (MNU only control) experiments. In hepatocellular adenomas and carcinomas promoted by trichloroacetic acid, the level of 5MeC in DNA was decreased by 40% and 51% when compared with non-tumour tissue from the same animal, or with liver tissue from control animals given only MNU, respectively. Cessation of treatment with trichloroacetic acid at 1 week before termination did not change the levels of 5MeC in either adenomas or carcinomas; however, levels remained lower than in non-tumour tissue. Levels of 5MeC in DNA from carcinomas were lower than in DNA from adenomas, suggesting that DNA methylation decreases with tumour progression.

Tao *et al.* (2000a) evaluated the methylation and expression of *c-jun* and *c-myc* proto-oncogenes in mouse liver after short-term exposure to trichloroacetic acid. Female B6C3F$_1$ mice were given water containing trichloroacetic acid (neutralized to pH 6.5–7.5 with sodium hydroxide) at a dose of 500 mg/kg bw per day by gavage for 5 days. Mice received methionine at a dose of 0, 30, 100, 300, or 450 mg/kg bw by intraperitonal injection, 30 minutes after the last exposure to trichloroacetic acid and were killed 70 minutes after dosing. Decreased methylation in the promoter regions of the *c-jun* and *c-myc* genes and increased levels of corresponding mRNA and proteins were found in the liver of mice exposed to trichloroacetic acid. Methionine prevented decreases in methylation of the two genes in a dose-dependent manner, with an effective dose of 100 mg/kg bw. At 450 mg/kg bw, methionine also prevented increases in the levels of mRNA and proteins from the two genes.

In another study, Tao *et al.* (2000b) examined the methylation of *c-jun* and *c-myc* genes, expression of both genes, and activity of DNA methyltransferase in liver tumours initiated by MNU and promoted by trichloroacetic acid in female B6C3F$_1$ mice. Mice aged 15 days were given either MNU at a dose of 25 mg/kg bw, or the saline vehicle control. Starting at age 6 weeks, the mice were given drinking-water containing neutralized trichloroacetic acid at a concentration of 20 mmol/L (3268 mg/L) continuously until age 52 weeks. The promoter regions of *c-jun* and *c-myc* in tumours were found to be hypomethylated relative to the promoter regions in non-tumour liver tissue from mice treated with trichloroacetic acid. The expression of mRNA and protein for each gene was also increased in trichloroacetic acid-promoted tumours relative

to non-tumour liver tissue. DNA methyltransferase activity was significantly increased in liver tumours from trichloroacetic acid-promoted mice when compared with non-tumour liver tissue from the same mice.

In a related study, Tao *et al.* (2004) reported hypomethylation of DNA and increased expression of the insulin-like growth factor II (*Igf2*) gene in liver tumours promoted by trichloroacetic acid. Specifically, the level of 5MeC in DNA from non-tumour liver tissue in mice exposed to trichloroacetic acid was decreased relative to that in DNA from mice initiated with MNU but not exposed to trichloroacetic acid. The level of 5MeC in trichloroacetic acid-promoted tumours was also decreased relative to the non-tumour liver tissue, indicating hypomethylation. Sequencing of the differentially methylated region-2 of the *Igf2* gene promoter revealed that 21–24 cytosine-guanine dinucleotide sites were methylated in initiated liver, compared with 15–17 sites in non-tumour liver tissue from mice with tumours promoted by trichloroacetic acid. Thus, exposure to trichloroacetic acid reduced the percentage of cytosine-guanine dinucleotide sites that were methylated from approximately 79% to 58%. The number of methylated cytosine-guanine dinucleotide sites was further reduced to approximately 11% in liver tumours promoted by trichloroacetic acid. Expression of mRNA was significantly increased (5.1 times) in liver tumours relative to non-tumour liver tissue from mice treated with trichloroacetic acid, but was not increased in non-tumour liver tissue from trichloroacetic acid-promoted mice when compared with the level of expression in MNU-initiated mice in the control group.

The temporal association of DNA methylation and cell proliferation (the latter discussed below) in mice exposed to trichloroacetic acid has been investigated by Ge *et al.* (2001). Female B6C3F_1 mice were given neutralized trichloroacetic acid by gavage at a dose of 500 mg/kg bw per day. Southern blot analysis indicated that the tumour promoter region of the *c-myc* proto-oncogene in the liver was hypomethylated at 72 hours and 96 hours.

Pereira *et al.* (2001) examined the effect of chloroform (a disinfection by-product present as a co-contaminant with trichloroacetic acid in drinking-water) on dichloroacetic acid- or trichloroacetic acid-induced hypomethylation and expression of the c-*myc* proto-oncogene in female B6C3F_1 mice. Both dichloroacetic acid and trichloroacetic acid decreased methylation in the promoter region of the *c-myc* gene and increased expression of *c-myc* mRNA. Coadministration of chloroform did not affect the extent of trichloroacetic acid-induced hypomethylation or mRNA expression, or the incidence or multiplicity of liver tumours promoted by trichloroacetic acid. By contrast, coadministration of chloroform prevented the hypomethylation and mRNA expression of the *c-myc* gene and the promotion of liver tumours by dichloroacetic acid.

(b) Cytotoxicity and oxidative stress

The available evidence for this mechanism includes both cytotoxicity and oxidative stress after exposure to trichloroacetic acid.

(i) Humans

No studies on liver toxicity or oxidative stress in humans exposed to trichloroacetic acid were identified.

(ii) Experimental systems

Cytotoxicity

Mather *et al.* (1990) evaluated toxicological effects in male Sprague-Dawley rats given drinking-water containing neutralized trichloroacetic acid at concentrations of 0, 50, 500, or 5000 ppm (approximately 0, 4.1, 36.5, or 355 mg/kg bw per day) for 90 days. At 355 mg/kg bw per day, relative liver weights were statistically significantly ($P < 0.05$) increased (7%) compared with controls. Although hepatomegaly was observed in the group at the highest dose, no microscopic

lesions were observed. No consistent treatment-related effects were seen on clinical chemistry or immune-function parameters.

In a study by Bhat *et al.* (1991), groups of five male Sprague-Dawley rats were given one quarter of a median lethal dose (LD_{50}) of trichloroacetic acid in drinking-water for 90 days. One quarter of the reported LD_{50} for trichloroacetic acid, 3300 mg/kg bw, corresponds to an administered dose of approximately 825 mg/kg bw per day. Trichloroacetic acid induced minimal to moderate collagen deposition (an indication of liver injury) in portal triads and large central veins in four out of five rats (minimal collagen deposition was observed in one out of five controls). Morphological changes in the liver included portal vein dilation/extension of minimal to moderate severity in five out of five rats treated with trichloroacetic acid.

Acharya *et al.* (1995) evaluated liver toxicity caused by trichloroacetic acid as part of a study on the interactive toxicity of tertiary butyl alcohol and trichloroacetic acid. Groups of five to six young male Wistar rats (age, 50 days) were exposed to water containing trichloroacetic acid at 0 or 25 ppm (approximately 0 or 3.8 mg/kg bw per day, assuming water intake of 0.15 L/kg bw per day) for 10 weeks. Little, if any, trichloroacetic acid-induced liver toxicity was observed; relative liver weight was unaffected, and no significant changes were detected in the indicators of liver injury: aspartate and alanine aminotransferases, or alkaline and acid phosphatases. In contrast, indicators of lipid and carbohydrate homeostasis were affected by trichloroacetic acid. The activity of succinate dehydrogenase was increased by approximately 30% compared with controls. In the liver, levels of triglyceride and cholesterol were significantly decreased, while glycogen levels increased approximately eight times. Levels of serum cholesterol were also increased approximately twofold. There was little evidence for induction of oxidative stress in the liver. No increase in lipid peroxidation was observed in the liver.

In a follow-up study using the same exposure protocol (Acharya *et al.*, 1997), minimal hepatic alterations were observed in the group treated with trichloroacetic acid. Histopathological changes noted in the liver included centrilobular necrosis, hepatocyte vacuolation, loss of hepatic architecture, and hypertrophy of the periportal region.

Oxidative stress

The ability of trichloroacetic acid to induce oxidative-stress responses, such as lipid peroxidation and oxidative DNA damage, have been tested in a series of short-term or single-dose studies in mice (Larson & Bull, 1992a; Austin *et al.*, 1995, 1996; Parrish *et al.*, 1996). Trichloroacetic acid induced lipid peroxidation, as measured by induction of thiobarbituric acid-reactive substances (TBARS), and oxidative DNA damage, as measured by detection of 8-hydrodeoxyguanosine adducts (8-OHdG) after administration of single oral doses.

In a study by Austin *et al.* (1996), groups of six male $B6C3F_1$ mice were given a single oral dose of neutralized trichloroacetic acid at 0, 30, 100, or 300 mg/kg bw in water. Mice were deprived of food for 3 hours before dosing. A significant increase in 8-OHdG in nuclear DNA in the liver was observed in the group at 300 mg/kg bw at 8–10 hours after dosing. The maximum level of 8-OHdG was observed at 8 hours. Levels of 8-OHdG in groups at 30 or 100 mg/kg bw were not reported.

Austin *et al.* (1996) reported that the maximum concentration of trichloroacetic acid-induced TBARS in the liver occurred 9 hours after dosing in in mice. In an earlier study, Larson & Bull (1992b) also reported that the maximum concentration of TBARS in the liver occurred 9 hours after dosing in mice given trichloroacetic acid at a dose of 2000 mg/kg bw. In the study by Larson & Bull (1992b), it was reported that 9 hours after

a single oral dose of trichloroacetic acid at 100, 300, 1000, and 2000 mg/kg bw, levels of TBARS were 1.15, 1.7, 2, and 2.7 times greater than those of controls, respectively.

Parrish *et al.* (1996) evaluated the ability of haloacetic acids to induce oxidative DNA damage in mouse liver. Groups of six male B6C3F$_1$ mice were given drinking-water containing trichloroacetic acid at 0, 100, 500, or 2000 mg/L for either 3 or 10 weeks. Oxidative damage to DNA, as measured by 8-OHdG adducts, did not occur with prolonged treatment with trichloroacetic acid, although peroxisome proliferation was induced, as indicated by increased activity of palmitoyl-coenzyme A oxidase (palmitoyl-coA oxidase) and 12-hydroxylation of lauric acid.

Austin *et al.* (1995) tested the ability of trichloroacetic acid to induce markers of CYP450 after short-term treatments (the latter effects are discussed below). Male B6C3F$_1$ mice were given trichloroacetic acid at 0 or 1000 mg/L for 14 days. The following parameters were evaluated: (i) changes in microsomal 12-(ω) hydroxylation of lauric acid (an indicator for the activity of CYP4A); (ii) hydroxylation of *p*-nitrophenol (as an index of CYP2E1 activity); and (iii) protein levels for a panel of CYP450s. CYP4A activities doubled in mice treated with trichloroacetic acid, while no increase in CYP2E1 activity and no change in the overall amount of total liver microsomal P450 were found.

Hassoun & Ray (2003) investigated activation of cultured macrophages (J744A.1 cell line) *in vitro* by neutralized trichloroacetic acid (8–32 mM [1.3–5 mg/kg bw] for 24–60 hours). Reduced cell viability was observed at all concentrations and correlated well with increased activity of lactate dehydrogenase in media. After incubation for 24 hours, trichloroacetic acid caused increases in the levels of superoxide anion; however, incubations of 36 and 60 hours statistically significantly increased superoxide anion levels at 16, 24, and 32 mM [2.6; 4; 5 mg/mL] ($P < 0.05$). Superoxide dismutase activity was also affected by treatment with trichloroacetic acid. Significant increases in superoxide dismutase activity occurred at lower trichloroacetic acid concentrations (8–24 mM [1.3–4 mg/mL]) than in controls, but activity at the highest concentration (32 mM [5 mg/mL]) for 24–36 hours was similar to that of controls. Incubation of cells with trichloroacetic acid at 32 mM [5 mg/mL] for 60 hours resulted in 100% cell death. These results indicated that incubation with trichloroacetic acid at 8–32 mM [1.3–5 mg/mL] for 24–60 hours induces macrophage activation, which resulted in cytotoxicity due to oxidative stress.

The activation of phagocytic cells was supported by studies *in vivo* (Hassoun & Dey, 2008). Male B6C3F$_1$ mice were given trichloroacetic acid at 300 mg/kg bw by gavage and killed after 6 or 12 hours. At 12 hours, the superoxide anion increased by 62.5% in cells obtained by peritoneal lavage and 17.6% in hepatic tissue.

In further 4-week and 13-week studies, groups of male B6C3F$_1$ mice were given trichloroacetic acid at a dose of 7.7, 77, 154, or 410 mg/kg bw day by gavage for 4 and 13 weeks (Hassoun *et al.*, 2010a, b). These doses were comparable to those inducing hepatocarcinogenicity.

In the liver, dose- and time-dependent increases were seen in the production of superoxide anion (increases of up to 167% at 4 weeks, and up to 200% at 13 weeks), and lipid peroxidation (increases of up to 567% at 4 weeks, and up to 733% at 13 weeks). Trichloroacetic acid also induced dose-dependent increases in biomarkers of phagocytic activation in cells obtained by peritoneal lavage after 4, but not 13, weeks of exposure. The production of superoxide anion at 4 weeks increased up to 175% at doses of 77, 154, and 410 mg/kg bw per day, whereas at 13 weeks, the production increased significantly (60%) in the group treated at 77 mg/kg bw per day only. Similarly, the increase in myeloperoxidase activity was robust at 4 weeks, and modest at 13 weeks. TNF-α, released by peritoneal-lavage cells, increased dose-dependently up to ninefold

at 4 weeks, whereas at 13 weeks the increase (1.8-fold) was only found at 77 mg/kg bw per day.

In a 50-day study with trichloroacetic acid in drinking-water (Celik, 2007), female Sprague-Dawley rats (age, 4 months; numbers not reported) were given trichloroacetic acid at 2000 ppm (300 mg/kg bw per day, assuming a default water intake of 0.15 L/kg bw per day), while the control group received natural spring water. Trichloroacetic acid significantly increased the activity of serum aspartate aminotransferases, alanine aminotransferases, creatine phosphokinase, and acid phosphatase ($P < 0.05$) in treated rats. A slight but statistically insignificant increase in malondialdehyde was found in the erythrocytes and liver. The antioxidant enzymes, superoxide dismutase and catalase, were significantly increased. However, no changes in the activities of glutathione, glutathione reductase, or glutathione-*S*-transferase were found in any tissue.

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

(i) Humans

No studies of altered cell proliferation and apoptosis, or clonal expansion, in humans exposed to trichloroacetic acid were identified.

(ii) Experimental systems

Several studies have observed hepatocyte proliferation in response to trichloroacetic acid in mice (Sanchez & Bull, 1990; Dees & Travis, 1994; Pereira, 1996; Stauber & Bull, 1997; DeAngelo *et al.*, 2008). For instance, Dees & Travis (1994) observed relatively small (two- to threefold), but statistically significant, increases in [^{3}H]thymidine incorporation in hepatic DNA in mice exposed for 11 days to trichloroacetic acid at doses (1 g/kg bw) that increased relative liver weight. Increased labelling of hepatic DNA was observed at doses lower than those associated with evidence of necrosis, suggesting that cell proliferation induced by trichloroacetic acid is not due to regenerative hyperplasia.

Miyagawa *et al.* (1995) examined the effect of trichloroacetic acid on replicative DNA synthesis in the liver of male B6C3F$_1$ mice. Mice were given a single dose by gavage of one-half of the maximum tolerated dose (250 mg/kg bw, as estimated from data provided by the authors), or the maximum tolerated dose (500 mg/kg bw), and incorporation of [^{3}H]thymidine in harvested hepatocytes was measured 24, 39, or 48 hours after dosing. For trichloroacetic acid, positive responses were observed at 250 mg/kg bw at 24 and 39 hours (6.5 and 4.9 times above controls) and at 500 mg/kg bw (9.8 times above controls).

Pereira (1996) evaluated cell proliferation in the liver of female B6C3F$_1$ mice treated with drinking-water containing trichloroacetic acid at 0, 2, 6.67, or 20 mM [327, 1090, or 3200 mg/L] for 5, 12, or 33 days by estimating the bromodeoxyuridine labelling index in hepatocytes. Cell proliferation was enhanced by 5 days exposure to trichloroacetic acid, but not for longer exposures of 12 or more days.

In a study reported by Stauber & Bull (1997), male B6C3F$_1$ mice were pretreated with drinking-water containing trichloroacetic acid at 2000 mg/L [480 mg/kg bw per day] for 50 weeks. The mice were then given drinking-water containing trichloroacetic acid at 0, 20, 100, 500, 1000, or 2000 mg/L [estimated doses of 0, 5, 23, 115, 230, or 460 mg/kg bw per day] for two additional weeks. Cell division rates in trichloroacetic acid-induced altered hepatic foci and tumours were high at all doses. Rates of cell division in altered hepatic foci and tumours remained high in mice for which exposure was terminated during the last 2 weeks of the study, indicating that these rates were independent of continued treatment with trichloroacetic acid.

Ge *et al.* (2001) exposed female B6C3F$_1$ mice to neutralized trichloroacetic acid at a dose of 500 mg/kg bw per day by gavage. Relative liver weights were significantly increased after

36, 72, and 96 hours. The proliferating cell nuclear antigen labelling index was significantly increased in liver cells at 72 and 96 hours, relative to controls. The mitotic index was significantly elevated in liver cells at 96 hours after the first dose.

A study *in vitro* by Channel & Hancock (1993) reported that exposure to medium containing trichloroacetic acid at 100 µg/mL decreased the rate of progression through S-phase of the cell cycle in WB344 cells (a nontumorigenic, epithelial, rat hepatocyte cell line).

(d) Activation of PPARα

The sections below review the evidence that trichloroacetic acid induces activation of PPARα.

(i) Humans

No studies were identified that addressed trichloroacetic acid-induced peroxisome proliferation or activation of PPARα in human liver. However, studies of transactivation *in vitro* have shown that human PPARα is activated by trichloroacetic acid and dichloroacetic acid. Maloney & Waxman (1999) demonstrated comparable transactivation potency against human and mouse PPARα with trichloroacetic acid at concentrations higher than 1 mM [163.39 µg/mL], and activation was dose-dependent up to 5 mM [817 µg/mL], tested. Walgren *et al.* (2000a) showed that neither trichloroacetic acid nor dichloroacetic acid had an effect on oxidation of palmitoyl-coenzyme A (palmitoyl-coA) in human hepatocyte cultures; however, no palmitoyl-coA oxidation activity was detected in control human hepatocytes in these experiments.

(ii) Experimental systems

Direct evidence for activation of PPARα

Several studies of transactivation *in vitro* have shown that murine versions of PPARα are activated by both trichloroacetic acid and dichloroacetic acid, while trichloroethylene itself is relatively inactive. Issemann & Green (1990) demonstrated transactivation by trichloroacetic acid of a mouse PPARα construct in mouse kidney COS1 cells, albeit with less potency than other known PPARα ligands.

Zhou & Waxman (1998) tested activation of mouse PPARα by chlorinated hydrocarbons using mouse kidney COS1 cells containing the murine PPARα reporter plasmid. Exposure to trichloroacetic acid for 24 hours resulted in activation of the reporter plasmid at concentrations higher than 1 mM [163.39 µg/mL]. In these experiments, trichloroacetic acid was about twice as potent as dichloroacetic acid. Trichloroethylene at concentrations of up to 5 mM had no effect.

In a similar study of transactivation with murine PPARα, a significant concentration–response relationship was obtained with trichloroacetic acid (Maloney & Waxman, 1999).

Walgren *et al.* (2000b) tested transactivation of murine PPARα using a reporter plasmid in HL8.5 cells co-transfected with mouse retinoic acid receptor α (mRXR). Trichloroacetic acid (4 mM [653 µg/mL]) showed a significant effect.

Indirect evidence for activation of PPARα: peroxisome proliferation

Elcombe (1985) examined peroxisome proliferation in male Wistar rats and male Swiss mice given corn oil containing trichloroacetic acid at a dose of 10–200 mg/kg bw per day by gavage for 10 consecutive days. Peroxisome volume densities were increased. Effects on other markers of peroxisome proliferation, presented below, demonstrated concomitant increases in β-oxidation activity.

DeAngelo *et al.* (1989) assessed relative species and strain sensitivities to the induction of hepatic peroxisome proliferation by chloroacetic acids. In the study in rats, male Sprague-Dawley, F344, and Osborne-Mendel rats received drinking-water supplemented with trichloroacetic acid at 0, 6, 12, or 31 mM (approximately 0, 212, 327, or 719 mg/kg bw per day) for 14 days. The volume fraction of cytoplasm from hepatic tissue

occupied by peroxisomes was decreased to less than half that seen in controls in this strain. [The Working Group noted that the reason for this paradoxical effect was not addressed.]

DeAngelo *et al.* (1989) also studied groups of six male mice of each of four strains ($B6C3F_1$, C3H, Swiss-Webster, and C57BL/6) that were exposed to drinking-water containing trichloroacetic acid at 0, 12, or 31 mM (approximately 0, 261, or 442 mg/kg bw per day) for 14 days. No effects were seen on body weight, but liver-to-body weight ratios were significantly increased at both dosages in all four strains as well as increases in the number and size of peroxisomes in the liver cytoplasm.

Indirect evidence for activation of PPARα: enzyme markers

Mather *et al.* (1990) evaluated toxicological effects in male Sprague-Dawley rats dosed with drinking-water containing neutralized trichloroacetic acid at concentrations of 0, 50, 500, or 5000 ppm (approximately 0, 4.1, 36.5, or 355 mg/kg bw per day) for 90 days. At the highest dose, hepatic peroxisomal enzyme activity was statistically significantly increased (15%, $P < 0.05$) as measured by the activity of palmitoyl-coA oxidase.

Effects consistent with peroxisome proliferation (e.g. induction of lipid-metabolism enzymes, such as acyl-coA oxidase and palmitoyl-coA oxidase, increased liver weight) have been observed in male F344 rats exposed to trichloroacetic acid by gavage for 14 days (Goldsworthy & Popp, 1987), in male F344 rats exposed to drinking-water containing trichloroacetic acid for 14 days (DeAngelo *et al.*, 1989), or 104 weeks (DeAngelo *et al.*, 1997), in male Osborne-Mendel rats exposed via drinking-water for 14 days (DeAngelo *et al.*, 1989), and in male Sprague-Dawley rats exposed via drinking-water for 90 days (Mather *et al.*, 1990). In mice, peroxisome proliferation or changes consistent with peroxisome proliferation have been reported in male $B6C3F_1$mice exposed via drinking-water for 2–10 weeks (DeAngelo *et al.*, 1989; Sanchez & Bull, 1990; Austin *et al.*, 1995; Parrish *et al.*, 1996), in male $B6C3F_1$ mice exposed by gavage for 10 days (Goldsworthy & Popp, 1987), and in male C57BL/6 and Swiss-Webster mice exposed via drinking-water for 14 days (DeAngelo *et al.*, 1989). Furthermore, PPARα-null mice exposed to drinking-water containing trichloroacetic acid at 2 g/L for 7 days did not show the characteristic responses of acyl-coA oxidase, palmitoyl-coA oxidase, and CYP4A induction associated with PPARα activation and peroxisome proliferation in wild-type mice (Laughter *et al.*, 2004). In addition, the livers from wild-type mice, but not PPARα-null mice, exposed to trichloroacetic acid developed centrilobular hepatocyte hypertrophy, although no significant increase in relative liver weight was observed.

As discussed above, Elcombe (1985) demonstrated species differences in peroxisome proliferation in male Wistar rats and male Swiss mice given corn oil containing trichloroacetic acid at 10–200 mg/kg bw per day by gavage for 10 consecutive days. Dose-related increases in cyanide-insensitive palmitoyl-coA oxidation were observed in rats and mice after treatment with trichloroacetic acid. At doses of 200 mg/kg bw per day for 10 days, peroxisomal β-oxidation increased 6.5 times in Wistar rats and 4.8 times in Swiss mice. On the other hand, trichloroacetic acid had no effect on hepatic catalase activity. Dose-related increases in cyanide-insensitive palmitoyl-coA oxidation were also observed in cultured rat and mouse hepatocytes exposed to trichloroacetic acid; however, no stimulation of peroxisomal β-oxidation was observed in cultured human hepatocytes prepared from two samples of human liver and treated with trichloroacetic acid.

Goldsworthy & Popp (1987) assessed cyanide-insensitive activity of palmitoyl-coA oxidase in adult male F344 rats given corn oil containing trichloroacetic acid at 0 or 500 mg/kg bw per day by gavage for 10 consecutive days.

Hepatic peroxisomal enzyme activity increased statistically significantly ($P < 0.05$) in rats receiving trichloroacetic acid, resulting in levels of enzyme activity approximately 2.8 times greater than in controls. Liver-to-body weight ratios were also statistically significantly increased (41%, $P < 0.05$) relative to those in controls. Body weight gain was not changed.

DeAngelo *et al.* (1989) assessed relative species and strain sensitivities to the induction of hepatic peroxisome proliferation markers by chloroacetic acids. In the study in rats, male Sprague-Dawley, F344, and Osborne-Mendel rats received drinking-water supplemented with 0, 6, 12, or 31 mM [0, 212, 327, or 719 mg/kg bw per day] for 14 days. Activity of palmitoyl-coA oxidase was elevated in Osborne-Mendel rats by 2.4 times and in F344 rats by 1.6 times over control values at the highest dose. Although palmitoyl-coA oxidase activity was not affected in treated Sprague-Dawley rats at any dose, carnitine acetyl-coA transferase activity was increased by 321% above control values in Sprague-Dawley rats at the highest dose (significant increases were not observed at lower doses). The volume fraction of cytoplasm from hepatic tissue occupied by peroxisomes was decreased to less than half that seen in controls in this strain.

In the study of DeAngelo *et al.* (1989) in mice, groups of six male mice per each of four strains of mice ($B6C3F_1$, C3H, Swiss-Webster, and C57BL/6) were exposed drinking-water that contained trichloroacetic acid at 0, 12, or 31 mM [0, 261, or 442 mg/kg bw per day] for 14 days. No effects were seen on body weight, but liver-to-body weight ratios were significantly increased at both dosages in all strains. The activity of palmitoyl-coA oxidase was elevated in all four strains for all dose groups. Palmitoyl-coA oxidase levels were 276%, 325%, and 456% above controls at 12 mM and 648%, 644%, and 678% above controls at 31 mM for Swiss-Webster, C3H, and $B6C3F_1$ mice, respectively. Palmitoyl-coA oxidase activity in C57BL/6 mice was increased by 2100% and 2500% above control levels at the highest and lowest doses of trichloroacetic acid, respectively, indicating high strain sensitivity. DeAngelo *et al.* (1989) also reported that catalase activity was increased by 461% above controls in $B6C3F_1$ mice at the highest dose, with accompanying increases in the level of peroxisome proliferation-associated protein and the number and size of peroxisomes in liver cytoplasm described above. To summarize, the results of DeAngelo *et al.* (1989) indicated that mice, in general, are more sensitive than rats to the effects of trichloroacetic acid on peroxisome proliferation, as indicated by palmitoyl-coA oxidase activity.

Goldsworthy & Popp (1987) investigated induction of hepatic and renal peroxisome proliferation markers in adult male $B6C3F_1$ mice given trichloroacetic acid at a dose of 0 or 500 mg/kg bw per day in corn oil for 10 days via gavage. Hepatic peroxisomal-enzyme activity increased statistically significantly ($P < 0.05$) in mice receiving trichloroacetic acid, resulting in levels of enzyme activity that were 280% those of the controls. Liver-to-body weight ratios were also statistically significantly increased (40%; $P < 0.05$) relative to controls.

Austin *et al.* (1995) explored the relationship between trichloroacetic acid-induced lipid peroxidation and the ability of trichloroacetic acid to induce markers of peroxisome proliferation. Groups of 18 male $B6C3F_1$ mice were exposed to trichloroacetic acid at 0 or 1000 mg/L for 14 days. Mice pretreated with water or trichloroacetic acid were divided into groups of six and given a single dose of trichloroacetic acid of 300 mg/kg bw or an equivalent volume of distilled water by gavage (control). The mice were killed 9 hours after the single dose. The following end-points were evaluated: (1) lipid-peroxidation response, as measured by the production of TBARS; (2) indicators of peroxisome proliferation, as measured by increased activity of palmitoyl-coA oxidase and increased catalase activity. TBARS were measured after 14 days of pretreatment and after the

single dose. Mice treated with trichloroacetic acid had a lower mean concentration of TBARS compared with controls, but the difference was not statistically significant. In the single-dose experiment, mice pretreated with trichloroacetic acid exhibited a significant decrement in TBARS in liver homogenates after a single dose of trichloroacetic acid, when compared with mice that received the same single dose, but that had not been pretreated. In contrast, pretreatment with trichloroacetic acid caused increases in the activities of palmitoyl-coA oxidase and catalase by 4.5- and 1.7-fold, respectively.

Walgren *et al.* (2000b) assessed the effects of trichloroacetic acid on palmitoyl-CoA oxidation in rat (LEH) and mouse ($B6C3F_1$) primary hepatocytes and found that trichloroacetic acid (2 mM [327 µg/mL], a concentration that was not cytotoxic) activated palmitoyl-CoA oxidation in mouse and rat cells.

(e) Inhibition of intracellular communication

(i) Humans

No trichloroacetic acid-specific data on inhibition of gap-junctional communication were available from studies in humans.

(ii) Experimental systems

Experimental evidence that trichloroacetic acid inhibits gap-junctional communication was available from studies *in vitro*. Benane *et al.* (1996) assessed the effects of trichloroacetic acid on gap-junctional intercellular communication in clone 9 (ATCC CRL 1439), a normal liver epithelial cell line from a male Sprague-Dawley rat aged 4 weeks. The cells were grown in a nutrient mixture, plated, and exposed to trichloroacetic acid at concentrations of 0, 0.5, 1.0, 2.5, or 5 mM for varying times (1, 4, 6, 24, 48, or 168 hours). Lucifer yellow scrape-load dye transfer was used as a measure of gap-junctional intercellular communication. The lowest concentration and shortest time to reduce dye transfer was 1 mM over 1 hour. The reduction in dye transfer increased with higher concentrations and longer treatment time. Klaunig *et al.* (1989) performed a series of experiments to determine the effects of trichloroacetic acid on gap-junctional intercellular communication in primary cultured hepatocytes from male $B6C3F_1$ mice and F344 rats aged 6–8 weeks. Cells were exposed to trichloroacetic acid (dissolved in dimethyl sulfoxide) at 0, 0.1, 0.5, or 1 mM [0, 16.3, 82, 163 µg/mL] for up to 24 hours. Trichloroacetic acid inhibited Lucifer yellow dye transfer in mouse hepatocytes, either freshly plated or after 24 hours. Dye coupling was significantly reduced at all tested concentrations after 4 hours of treatment, but not after 8 or 24 hours. The inhibitory effect on dye transfer in mouse cells was unaffected by treatment with SKF-525A, an inhibitor of CYP450. In rat hepatocytes, dye transfer was unaffected by treatment with trichloroacetic acid at concentrations up to 1 mM [163 µg/mL] for as long as 24 hours or 6 hours in freshly plated cells. The results obtained in primary F344 rat hepatocytes by Klaunig *et al.* (1989) differed from those reported by Benane *et al.* (1996), who observed inhibition of dye transfer in cells from a Sprague-Dawley rat epithelial cell line treated with 1 mM [163 µg/mL] for durations of 1–168 hours. [The Working Group noted that the reason for the differential response in rat liver cells was unknown, but may be related to differences in the originating strain or in the type of cell tested (primary cultured hepatocytes versus established cell line)].

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

See Section 4 of the *Monograph* on Dichloroacetic Acid in this Volume.

4.3.2 Kidney

Few studies had examined the effects of trichloroacetic acid on the kidney, or possible mechanisms.

Acharya *et al.* (1995) evaluated kidney toxicity in young male Wistar rats (age, 50 days) exposed to water containing trichloroacetic acid at 0 or 25 ppm [≈3.8 mg/kg bw per day, assuming water intake of 0.15 L/kg bw per day] for 10 weeks. While serum enzyme levels were unaffected by trichloroacetic acid, kidney, but not liver, levels of glutathione-*S*-transferase were decreased to approximately 66% of control values. No examination of kidney histology was performed in this study.

In a follow-up study using the same exposure protocol (Acharya *et al.*, 1997), histopathological changes were noted in the kidneys of animals treated with trichloroacetic acid, and included degeneration of renal tubules.

Goldsworthy & Popp (1987) assessed cyanide-insensitive activity of palmitoyl-coA oxidase in adult male F344 rats given trichloroacetic acid in corn oil at a dose of 0 or 500 mg/kg bw per day by gavage for 10 consecutive days. Activity of renal peroxisomal enzymes was statistically significantly ($P < 0.05$) increased by approximately 1.8 times over that in controls in rats. Kidney weights were not affected by treatment.

Mather *et al.* (1990) evaluated toxicological effects in male Sprague-Dawley rats dosed with drinking-water containing neutralized trichloroacetic acid at concentrations up to 5000 ppm [355 mg/kg bw per day] for 90 days. At 355 mg/kg bw per day, relative kidney weights were statistically significantly ($P < 0.05$) increased (11%) compared with controls. There were no changes in kidney histopathology.

Pereira *et al.* (2001) examined kidney weight in B6C3F$_1$ mice injected with MNU at age 15 days and then exposed to drinking-water containing trichloroacetic acid (4.0 g/L) from age 4 weeks to age 36 weeks. No effect of trichloroacetic acid on kidney weight was found.

The temporal association of DNA methylation and cell proliferation (the latter discussed below) in mice exposed to trichloroacetic acid was investigated by Ge *et al.* (2001). Female B6C3F$_1$ mice were given neutralized trichloroacetic acid at a dose of 500 mg/kg bw per day by gavage. Trichloroacetic acid decreased methylation in the promoter region of the *c-myc* gene in the kidney and urinary bladder after 72 and 96 hours of treatment, but the response was less pronounced than in liver. Trichloroacetic acid had no effect on relative kidney weights.

In a follow-up study, Tao *et al.* (2005) treated B6C3F$_1$ mice with drinking-water containing trichloroacetic acid (4 g/L) for 7 days concurrently. In male, but not female mouse kidney, trichloroacetic acid decreased the methylation of DNA and of the *c-myc* gene. Coadministration of methionine in the diet prevented hypomethylation in the kidneys of male mice.

4.4 Susceptibility data

4.4.1 Inter-individual variability

It is not clear whether trichloroacetic acid is metabolized to dichloroacetic acid in any significant quantities (Bull, 2000; Lash *et al.*, 2000; Kim *et al.*, 2009). Genetic polymorphisms have been identified in glutathione *S*-transferase zeta 1 (*GSTZ1*), a key enzyme involved in the metabolism of dichloroacetic acid (Board *et al.*, 2001). *Gstz1*-null mice fail to metabolize ^{13}C-labelled dichloroacetic acid to [^{13}C]glyoxylate (Ammini *et al.*, 2003). Polymorphisms in *GSTZ1* would be relevant to susceptibility to trichloroacetic acid only if dichloroacetic acid is a metabolite of trichloroacetic acid, which is as yet unclear. Four polymorphic alleles of *GSTZ1* have been identified: 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 human, rat, and mouse GSTZ1. 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.

Short-term treatment of $B6C3F_1$ mice with trichloroacetic acid was shown to induce hepatic activity of superoxide dismutase and catalase (Hassoun & Cearfoss, 2011). Because oxidative stress in the liver was suggested as one of the mechanisms of carcinogenesis by which trichloroacetic acid acts (Austin *et al.*, 1995), polymorphisms in these enzymes may be of importance in protection against trichloroacetic acid-induced oxidative stress.

Trichloroacetic acid also induces glycogen accumulation; prolonged glycogen accumulation can become irreversible (Kato-Weinstein *et al.*, 1998). Thus, individuals with glycogen storage disease, in the form of an inherited or spontaneous deficiency or alteration in any one of the enzymes involved in glycogen metabolism, may constitute another group that may be more susceptible than the general population to the toxicity of trichloroacetic acid.

4.4.2 Life-stage susceptibility

There is evidence that the developing fetus is highly susceptible to maternal exposure to trichloroacetic acid. Smith *et al.* (1989) examined developmental effects in pregnant Long-Evans rats given trichloroacetic acid at doses of up to 1800 mg/kg bw per day by gavage on days 6–15 of gestation. Maternal toxicity (reduced body-weight gain, increases in spleen and kidney weight) was evident in groups at doses exceeding 800 mg/kg bw per day. However, much more pronounced effects were observed in the fetuses. Dose-dependent increases in the frequency of fetus resorption, decreases in weight and length, and malformations in soft tissue, and skeletal and cardiac muscle were observed.

Singh (2006) examined the effect of trichloroacetic acid on the developing brain in Charles Foster rats given doses up to 1800 mg/kg bw per day by gavage on days 6–15 of gestation. A significant dose-dependent decrease in fetal brain weight was found at doses of 1200 mg/kg bw per day and higher.

Von Tungeln *et al.* (2002) investigated the potency of trichloroacetic acid in an assay for cancer in neonatal mice. In this study, male and female neonatal $B6C3F_1$ mice were given two intraperitoneal injections of trichloroacetic acid, with a total dose of 1000 or 2000 nmol [≈16 or 32 mg/kg bw], at age 8 and 15 days. The mice were killed and evaluated for liver tumours at age 12 months (higher dose) or 20 months (lower dose). The incidence of hepatic tumours in treated mice did not differ significantly from incidence in mice receiving the solvent only.

4.4.3 Sex differences

In one cancer bioassay, male and female mice were concurrently exposed to drinking-water containing trichloroacetic acid for 52 weeks (Bull *et al.*, 1990). A clear dose-related increase in the incidence of proliferative lesions (hyperplastic nodules, adenoma, or carcinoma) was observed in male $B6C3F_1$ mice, but not in females. Other available cancer bioassays in either males or females, conducted in separate laboratories, also suggested that males may be more sensitive than females to carcinogenicity induced by trichloroacetic acid. For example, Pereira *et al.* (2001) observed a tumour incidence of 25% in female $B6C3F_1$ mice exposed to drinking-water containing trichloroacetic acid at a dose of 784 mg/kg bw per day for 51 weeks. In studies by Bull and co-workers (Bull *et al.*, 1990; Bull, 2000), tumour incidences ranging from 55% to 83% have been reported in male mice exposed to drinking-water containing trichloroacetic acid at lower doses (309–480 mg/kg bw per day) for a comparable duration.

4.5 Mechanistic considerations

Evidence suggests that trichloroacetic acid is not genotoxic. In one study in human lymphocytes *in vitro*, no genotoxicity (chromosomal aberrations or DNA strand breaks) was observed with neutralized trichloroacetic acid. In mammalian systems *in vivo*, inconsistent evidence was available that trichloroacetic acid affects the induction of DNA strand breaks, micronucleus formation or chromosomal aberration. In mammalian studies *in vitro*, trichloroacetic acid had no genotoxic effects. Likewise, overwhelmingly negative results were obtained in bacterial and fungal test systems after exposure to trichloroacetic acid.

Major target organs for the adverse health outcomes associated with trichloroacetic acid are the liver and kidney.

Overall, the strength of evidence for liver as a target organ is strong. Multiple mechanisms are probably operative with regard to liver carcinogenesis in rodents. The following mechanisms have been identified, almost exclusively from studies in rodents or rodent test systems *in vitro*: epigenetic effects (global DNA hypomethylation and hypomethylation of the *c-myc* gene promoter), oxidative stress (oxidative DNA damage and lipid peroxidation, activation of phagocytic cells that may lead to generation of oxidants), increase in cell proliferation (an effect not observed in PPARα-null mice in a 7-day study), induction of the peroxisome proliferation response (strong direct and indirect evidence for activation of PPARα in rodents, limited evidence for trichloroacetic acid as a ligand of human PPARα), disruption of gap-junctional intercellular communications (limited evidence from several studies in rat cells *in vitro*). While there were consistent results in several rodent species and the overall body of evidence was coherent, no studies had been conducted to demonstrate that suppression of mechanistic processes leads to tumour suppression. Because trichloroacetic acid is a metabolite of other chlorinated solvents, several studies compared mutational and phenotypic profiles of liver tumours induced by various chlorinated solvents or their metabolites and concluded that little similarity exists. The strength of evidence for nongenotoxic mechanisms of liver cancer with trichloroacetic acid is moderate.

Some evidence suggests that kidney may also be a target organ for trichloroacetic acid. An increase in relative kidney weight has been reported in rats, but not mice. However, hypomethylation of global DNA and the *c-myc* gene has been observed in male but not female mouse kidney. The relevance of these observations to potential cancer hazard in kidney is unknown, given the absence of renal tumours.

There is weak evidence for potential inter-individual variability in the adverse effects of trichloroacetic acid. Glutathione *S*-transferase zeta 1 is an enzyme that has a major role in the metabolism of dichloroacetic acid, and common polymorphisms in the corresponding gene (*GSTZ1*) that result in differences in activation have been reported in humans (see the *Monograph* on Dichloracetic Acid in this Volume). Because dichloroacetic acid is a metabolite of trichloroacetic acid, it is not clear what relevance these polymorphisms may have to humans. It has been shown that trichloroacetic acid may cause oxidative stress in the liver in rodents, and that superoxide dismutase and catalase are induced; it is thus plausible that polymorphisms in these genes may have an impact on inter-individual variation in susceptibility. However, since this mechanism has not been confirmed in studies in humans, the relevance of such susceptibility is uncertain. With respect to life-stage susceptibilities, the developing fetus was suggested to be highly susceptible to trichloroacetic acid-induced toxicity. Males have been shown to be more prone to hepatocarcinogenicity after exposure to trichloroacetic acid in studies in rodents.

5. Summary of Data Reported

5.1 Exposure data

Trichloroacetic acid has been mainly used as the sodium salt as a selective herbicide. It is also used 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. The highest exposures to trichloroacetic acid result from it being a major end metabolite of several chlorinated organic solvents, in particular, trichloroethylene and tetrachloroethylene, and trichloroacetic acid in urine has therefore been widely used as a biological marker of exposure to these solvents. Widespread exposure also occurs at much lower levels in drinking-water and swimming pools as a by-product of chlorine-based water disinfection.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

Several long-term bioassays (some including more than one experiment) have primarily focused on induction of liver tumours by trichloroacetic acid, with only limited pathology analyses of other tissues. Four drinking-water studies in male mice and two studies in female mice showed an increased incidence of hepatocellular adenoma and/or hepatocellular carcinoma. The only available study in rats given trichloroacetic acid in drinking-water had limited capacity to detect a carcinogenic response. Two initiation–promotion studies in mice showed that trichloroacetic acid is an efficient promoter of hepatocellular tumours initiated by *N*-ethyl-*N*-nitrosourea and *N*-methyl-*N*-nitrosourea.

5.4 Mechanistic and other relevant data

Similarities exist between humans and laboratory animals with regard to the absorption, distribution and metabolism of trichloroacetic acid. Trichloroacetic acid has a much longer plasma half-life in humans (2–4 days) than in rodents (5–6 hours), which is indicative of much slower excretion and metabolism in humans. The metabolism of trichloroacetic acid is rather slow, with the parent compound being the main urinary excretion product, and dichloroacetic acid being the main proximate metabolic product in all species studied. Dichloroacetic acid is further metabolized through GST-zeta1 to glyoxylic acid and then to oxalic and glycolic acids, glycine and carbon dioxide. The available evidence suggests that trichloroacetic acid is not a genotoxic agent. The available data in animals designate the liver as a major target organ for trichloroacetic acid. There is moderate evidence suggesting that trichloroacetic acid may act through multiple nongenotoxic mechanisms, leading to liver carcinogenesis.

Some data from studies in animals suggest that kidney may also be a target organ for trichloroacetic acid. The relevance of the apparent effects in the kidney to the cancer hazard potential of trichloroacetic acid in the kidney is unknown. There is a potential for inter-individual variability in adverse effects of trichloroacetic acid, because its major metabolite, dichloroacetic acid, is further metabolized by GST-zeta1. This enzyme is polymorphic, and such polymorphisms have been shown to have an impact on its function.

6. Evaluation

6.1 Cancer in humans

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

6.2 Cancer in experimental animals

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

6.3 Overall evaluation

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

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