# DICHLOROACETIC ACID

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

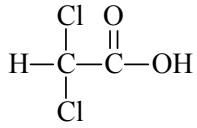
## 1. Exposure Data

### 1.1 Chemical and physical data

1.1.1 *Nomenclature*

*Chem. Abstr. Serv. Reg. No.*: 79-43-6
*Deleted CAS Reg. No.*: 42428-47-7
*Chem. Abstr. Name*: Dichloroacetic acid
*IUPAC Systematic Name*: Dichloroacetic acid
*Synonyms*: DCA; DCA (acid); dichloracetic acid; dichlorethanoic acid; dichloroethanoic acid

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_2H_2Cl_2O_2$ Relative molecular mass: 128.94

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: Colourless, highly corrosive liquid (Koenig *et al*., 1986)
(*b*) *Boiling-point*: 192 °C (Koenig *et al*., 1986)
(*c*) *Melting-point*: 13.5 °C (freezing-point) (Koenig *et al*., 1986)
(*d*) *Density*: 1.5634 at 20 °C/4 °C (Morris & Bost, 1991)

(*e*) *Spectroscopy data*: Infrared (prism [2806]), nuclear magnetic resonance [166] and mass spectral data have been reported (Weast & Astle, 1985)
(*f*) *Solubility*: Miscible with water; soluble in organic solvents such as alcohols, ketones, hydrocarbons and chlorinated hydrocarbons (Koenig *et al*., 1986)
(*g*) *Volatility*: Vapour pressure, 0.19 kPa at 20 °C (Koenig *et al*., 1986)
(*h*) *Stability*: Dissociation constant ($K_a$), $5.14 \times 10^{-2}$ (Morris & Bost, 1991)
(*i*) *Octanol/water partition coefficient (P)*: log P, 0.92 (Hansch *et al*., 1995)
*(j) Conversion factor*: $mg/m^3 = 5.27 \times ppm$[a]

### 1.1.4 *Technical products and impurities*

Dichloroacetic acid is commercially available as a technical-grade liquid with the following typical specifications: purity, 98.0% min.; monochloroacetic acid, 0.2% max.; trichloroacetic acid, 1.0% max.; and water, 0.3% max. (Clariant Corp., 2001; Clariant GmbH, 2002).

### 1.1.5 *Analysis*

Dichloroacetic acid has been determined in water using liquid–liquid extraction, conversion to its methyl ester and gas chromatography (GC) with electron capture detection. This method has been applied to drinking-water, groundwater, water at intermediate stages of treatment and raw source water, with a detection limit of 0.24 µg/L (Environmental Protection Agency, 1995; American Public Health Association/American Water Works Association/Water Environment Federation, 1999).

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

These methods were modified for an analytical survey of 16 drinking-water sources in Australia (Simpson & Hayes, 1998) and in a survey of treated water from 35 Finnish waterworks during different seasons (Nissinen *et al*., 2002).

## 1.2 Production and use

Dichloroacetic acid was reported to be first synthesized in 1864 by the further chlorination of monochloroacetic acid with chlorine (Beilstein Online, 2002). Haloacetic acids were first detected in 1983 as disinfection by-products in chlorinated drinking-waters, 9 years after the discovery of trihalomethanes in chlorinated waters (Nissinen *et al*., 2002).

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

### 1.2.1 *Production*

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

Available information indicates that dichloroacetic acid is produced by two companies in the USA and one company each in China, Japan and Mexico (Chemical Information Services, 2002).

Available information indicates that dichloroacetic acid is formulated into pharmaceutical products by one company each in New Zealand and Turkey (Chemical Information Services, 2002).

### 1.2.2 *Use*

Dichloroacetic acid, particularly in the form of its esters, is an intermediate in organic synthesis, used in the production of glyoxylic acid, dialkoxy and diaroxy acids, and sulfonamides and in the preparation of iron chelates in the agricultural sector. It is also used as an analytical reagent in fibre manufacture (polyethylene terephthalate) and as a medicinal disinfectant (substitute for formalin) (Koenig *et al*., 1986; Morris & Bost, 1991; Clariant GmbH, 2002).

Dichloroacetic acid is used in medical practice as a cauterizing agent. It rapidly penetrates and cauterizes the skin and keratins. Its cauterizing ability compares with that of electrocautery or freezing. It is used on calluses, hard and soft corns, xanthoma palpebrarum, seborrhoeic keratoses, in-grown nails, cysts and benign erosion of the cervix (Gennaro, 2000).

## 1.3 Occurrence

### 1.3.1 *Natural occurrence*

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

### 1.3.2 *Occupational exposure*

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

### 1.3.3 *Air*

No data were available to the Working Group.

### 1.3.4 *Water*

Dichloroacetic acid is produced as a by-product during chlorination of water containing humic substances (Christman *et al*., 1983; Miller & Uden, 1983; Legube *et al*., 1985; Reckhow & Singer, 1990; Reckhow *et al*., 1990). Consequently, it may occur in drinking-water after chlorine-based disinfection of raw waters containing natural organic substances (Hargesheimer & Satchwill, 1989; see IARC, 1991a) and in swimming pools (Stottmeister & Naglitsch, 1996; Kim & Weisel, 1998). The concentrations of dichloroacetic acid measured in various water sources are summarized in Table 1.

Geist *et al.* (1991) measured concentrations of dichloroacetic acid ranging from < 3 to 522 μg/L in surface water downstream from a paper mill in Austria, and Mohamed *et al.* (1989) measured concentrations ranging from 14 to 18 μg/L in effluent from a kraft pulp mill in Malaysia.

Levels of dichloroacetic acid tend to decline with length of time in the distribution system (Chen & Weisel, 1998), and concentrations tend to be higher in warmer seasons (LeBel *et al*., 1997; Chen & Weisel, 1998). It has been identified as a major chlorinated by-product of the photocatalytic degradation of tetrachloroethylene in water and as a minor by-product of the degradation of trichloroethylene (Glaze *et al*., 1993).

Dichloroacetic acid has also been detected in the Great Lakes, Canada (Scott *et al*., 2002) and in fog samples (0.12–5.0 μg/L) at ecological research sites in north-eastern Bavaria, Germany (Römpp *et al*., 2001). Clemens and Schöler (1992a) measured 1.35 μg/L dichloroacetic acid in rainwater in Germany. Precipitation samples in Canada contained dichloroacetic acid concentrations ranging from < 0.0004 to 2.4 μg/L and concentrations in Canadian lakes varied from < 0.0001 to 4.7 μg/L (Scott *et al.*, 2000). Dichloroacetic acid has a relatively short persistence (~ 4 days) in pond waters (Ellis *et al*., 2001)

### 1.3.5 *Other*

Dichloroacetic acid has been reported as a biotransformation product of methoxyflurane (Mazze & Cousins, 1974) and dichlorvos (see WHO, 1989; IARC, 1991b), and it may occur in the tissues and fluids of animals treated with dichlorvos for helminthic infections (Schultz *et al*., 1971).

Kim and Weisel (1998) measured the amount of dichloroacetic acid excreted by humans after having swum in a chlorinated pool, and this ranged from 25 to 960 ng per one urine void. The background excretion rate varied from 109 to 253 ng per one urine void. Weisel *et al.* (1999) measured an average excretion rate of 1.04 ng/min dichloroacetic acid in subjects exposed to low levels (1.76 μg/L) and 1.47 ng/min dichloroacetic acid in subjects exposed to high levels (32.7 μg/L) of dichloroacetic acid in water.

**Table 1. Concentrations of dichloracetic acid in water**

| Water type (location) | Concentration range (μg/L) | Reference |
|---|---|---|
| Treatment plant and distribution system (Canada) | 1.8–53.2 | LeBel *et al.* (1997) |
| Treatment plant (Canada) | 0.3–163.3 | Williams *et al.* (1997) |
| Treatment plant and distribution system (Poland) | 13.34–25.55 | Dojlido *et al.* (1999) |
| Treatment plant and distribution system (South Korea) | 0.8–5.2[a] | Shin *et al.* (1999) |
| Drinking-water (USA)[b] | 63.1–133 | Uden & Miller (1983) |
| Drinking-water (USA)[b] | 5.0–7.3 | Krasner *et al*. (1989) |
| Drinking-water (USA)[b] | 4.7–23 | Jacangelo *et al.* (1989) |
| Drinking-water (USA)[b] | 8–79 | Reckhow & Singer (1990) |
| Drinking-water (Spain) | ND–2.0[b] | Cancho *et al*. (1999) |
| Drinking-water and distribution system (USA)[b] | 1–100 | Obolensky *et al.* (2003) |
| Distribution system (Canada) | 0.2–120.1 | Williams *et al.* (1997) |
| Distribution system (USA) | 1.7–14 | Chen & Weisel (1998) |
| Distribution system (Australia) | 1–46 | Simpson & Hayes (1998) |
| Drinking-water (USA) | 7.5–21 | Lopez-Avila *et al*. (1999) |
| Drinking-water (USA) | 0.33–110 | Weisel *et al.* (1999) |
| Drinking-water (Canada) | 8.1–12.7 | Scott *et al.* (2000) |
| Drinking-water (Finland) | < 2.2–42 | Nissinen *et al.* (2002) |
| Drinking-water (Spain) | 0.2–21.5 | Villanueva *et al*. (2003) |
| Swimming pool (Germany) | indoors: 0.2–10.6 open air: 83.5–181.0[c] | Clemens & Schöler (1992b) |
| Swimming pools (Germany) | | Stottmeister & Naglitsch (1996) |
| Indoor | 1.5–192 | |
| Hydrotherapy | 1.8–27 | |
| Outdoor | 6.2–562 | |
| Swimming pool (USA) | 52–647 | Kim & Weisel (1998) |

[a] Based on the assumption that dichloroacetic acid makes up approximately 40% of haloacetic acids
[b] Samples taken from water leaving the treatment plant
[c] The authors suggest that the higher levels found in open-air swimming pools may be due to greater input of organic matter.
ND, not detected

Dichloroacetic acid has been detected in spruce needles from the Black Forest in Germany and the Montafon region in Austria, both of which are considered to be relatively unpolluted areas, in the range of 10 to several hundred micrograms per kilogram (Frank *et al*., 1989).

### 1.4 Regulations and guidelines

The WHO (1998) has established a provisional guideline of 50 μg/L for dichloroacetic acid in drinking-water. A provisional guideline is established when there is some evidence of a potential health hazard but where available data on health effects are limited, or where an uncertainty factor greater than 1000 has been used in the derivation of the tolerable daily intake.

In Australia and New Zealand, the drinking-water guideline for dichloroacetic acid is 100 μg/L (National Health and Medical Research Council and Agriculture and Resource Management Council of Australia and New Zealand, 1996). This guideline also notes that minimizing the concentration of all chlorination by-products is encouraged by reducing naturally occurring organic material from the source water, reducing the amount of chlorine added, or using an alternative disinfectant, while not compromising disinfection.

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

The European Union (European Commission, 1998) and Canada (Health Canada, 2001) have not set guideline values, but encourage the reduction of total disinfection by-product concentrations.

## 2. Studies of Cancer in Humans

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

## 3. Studies of Cancer in Experimental Animals

*Previous evaluation*

In four studies evaluated previously, dichloroacetic acid induced hepatocellular adenomas and carcinomas in male B6C3$F_1$ mice, and studies in experimental animals have since focused on its activity in the liver. The previous evaluation of dichloroacetic acid concluded that there was limited evidence in experimental animals for its carcinogenicity (IARC, 1995).

*New studies*

## 3.1 Oral administration

### 3.1.1 *Mouse*

Groups of 90 female B6C3$F_1$ mice, 7–8 weeks of age, were administered dichloroacetic acid continuously in the drinking-water at concentrations of 2.0, 6.67 and 20.0 mmol/L [236, 854 or 2350 mg/L], adjusted to pH 6.5–7.5 with sodium hydroxide; a control group of 134 animals received 20.0 mmol/L sodium chloride. Mice were killed after 360 or 576 days of exposure. The livers were weighed and evaluated for foci of altered hepatocytes (eosinophilic and basophilic), adenomas and carcinomas. After 360 or 576 days of exposure, dichloroacetic acid increased the ratio of liver-to-body weight dose-dependently. Data from mice administered dichloroacetic acid were compared with those from control mice using Fisher's exact test with a $p$-value $< 0.05$. At 360 days, only the high concentration of dichloroacetic acid (20.0 mmol/L) induced a significant increase in the incidence of altered hepatocyte foci (8/20; 40%) and hepatocellular adenomas (7/20; 35%) compared with control values of 0/40 and 1/40 (2.5%), respectively. The multiplicity of hepatocyte foci and adenomas was increased from 0 and $0.03 \pm 0.03$ to $0.60 \pm 0.22$ and $0.45 \pm 0.17$, respectively. After 576 days of exposure, 6.67 mmol/L dichloroacetic acid increased the incidence of foci (11/28; 39.3%) and adenomas (7/28; 25%), while 20.0 mmol/L increased the incidence of foci (17/19; 89.5%), adenomas (16/19; 84.2%) and carcinomas (5/19; 26.3%). In control mice, the incidence of lesions was 1/40 adenomas at 360 days and 10/90 (11.1%) foci, 2/90 adenomas (2.2%) and 2/20 carcinomas (2.2%) at 576 days. An intermittent exposure group [number unspecified] was treated during 72-day cycles of 24 days of exposure to 20.0 mmol/L dichloroacetic acid followed by 48 days without exposure so that the time-weighted average concentration was equivalent to that of the 6.67-mmol/L group. Intermittent exposure to 20.0 mmol/L dichloroacetic acid resulted in a much lower incidence and multiplicity of hepatocellular adenomas than those in mice that received the same time-weighted average concentration of 6.67 mmol/L continuously (3/34 [8.8%] versus 7/28 [25%] and $0.09 \pm 0.05$ versus $0.32 \pm 0.13$). The dose–response relationship for total liver lesions per mouse did not appear to be linear for dichloroacetic acid (Pereira, 1996).

Groups of 25 female B6C3$F_1$ mice, 28 days of age, were given 0.5 or 3.5 g/L dichloroacetic acid in the drinking-water adjusted to 6.9–7.1 with sodium hydroxide for 104 weeks; a group of 39 controls received 1.5% acetic acid. A complete necropsy was performed. The high dose of dichloroacetic acid decreased body weights of the mice and increased the liver-to-body weight ratio. The high dose, but not the low dose, of dichloroacetic acid increased the incidence and multiplicity of liver carcinomas. The incidence of hepatocellular carcinomas was 2.6 (1/39), 4.0 (1/25) and 92% (23/25) and the multiplicity was $0.05 \pm 0.32$, $0.04 \pm 0.20$ and $2.96 \pm 1.67$ in control, low-dose and high-dose mice, respectively (Schroeder *et al*., 1997).

Groups of male B6C3$F_1$ mice, 28–30 days of age, were given 0 (50 animals), 0.5 (50 animals), 1 (71 animals), 2 (55 animals) or 3.5 (46 animals) g/L dichloroacetic acid in the

drinking-water adjusted to pH 6.9–7.1 with sodium hydroxide. Starting at a different time, other mice were exposed to 0 (33 animals) or 0.05 (50 animals) g/L dichloroacetic acid. The mice were killed between 90 and 100 weeks. Body, liver, kidney, testis and spleen weights were measured and livers, kidneys, spleens and testes were examined microscopically. No statistical differences with respect to tumour incidence or multiplicity were found between the control groups placed on study at different times or the two groups combined. Five animals from the high-dose group had a complete pathological examination. Animals that received the two higher doses of dichloroacetic acid (2 and 3.5 g/L) had significantly decreased body weights (18% versus control animals). At 1, 2 and 3.5 g/L, dichloroacetic acid increased the incidence and multiplicity of hepatocellular adenomas and carcinomas, while 0.5 g/L increased the multiplicity, but not the incidence of both lesions. After 90–100 weeks of treatment, the percentage of animals with hepatocellular adenomas was 10, 20, 51.4, 42.9 and 45% [$p$-value $\leq 0.05$ for the three highest percentages] and that of animals with carcinomas was 26, 48, 71, 95 and 100% [$p$-value $\leq 0.05$ for the three highest percentages] following administration of 0, 0.5, 1, 2 and 3.5 g/L dichloroacetic acid, respectively. [The numbers of animals that survived to 79–100 weeks and underwent histopathological examination were 50 (controls), 33 (0.05 g/L), 35 (0.5 g/L), 35 (1 g/L), 21 (2 g/L) and 11 (3 g/L) (personal communication from the author)] (DeAngelo *et al.*, 1999).

The ability of mixtures of dichloroacetic acid and trichloroacetic acid to induce liver tumours was studied in 6-week-old male B6C3$F_1$ mice. Treatments administered included 0.1, 0.5 and 2.0 g/L dichloroacetic acid and 0.5 and 2.0 g/L trichloroacetic acid, and selected combinations of these treatments. Twenty animals were assigned to each of 10 groups that received the above concentrations in the drinking-water for 52 weeks. Dose-related increases in liver tumour incidence (adenomas and carcinomas combined) were observed with the individual compounds and, when the animals were exposed to mixtures of dichloroacetic acid and trichloroacetic acid, it appeared that there was an additive effect in terms of tumour incidence (see Table 2) (Bull *et al.*, 2002).

### 3.1.2 *Rat*

Groups of 60 male Fischer 344/N rats, 28–30 days of age, received 0.05, 0.5 or 5 g/L dichloroacetic acid daily in the drinking-water for 100 weeks; a control group of 50 mice received 2 g/L sodium chloride for 104 weeks. In another experiment, a group of 78 males received a dose of 2.5 g/L dichloroacetic acid (neutralized) daily, which, due to toxicity, was lowered to 1.5 g/L at 8 weeks and 1.0 g/L at 26 weeks (time-weighted average daily concentration, 1.6 g/L) and a control group of 78 animals received deionized water. Rats were killed after 103 weeks of exposure. The highest concentration of dichloroacetic acid (5 g/L) caused severe, irreversible peripheral neuropathy and rats were killed at 60 weeks and excluded from the analysis. The neurotoxicity observed at 2.5 g/L abated when the dose was lowered to 1.0 g/L. The liver, kidneys, testes, thyroid, stomach, rectum, duodenum, ileum, jejunum, colon, urinary bladder and spleen were examined for gross lesions. Body, liver, kidney, testes and spleen weights were measured and the organs were exa-

**Table 2. Effect on liver tumour incidence of administration of dichloroacetic acid (DCA) and trichloroacetic acid (TCA) to B6C3F$_1$ male mice in the drinking-water for 52 weeks**

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

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

mined microscopically. Exposure to 1.6 g/L dichloroacetic acid reduced the terminal body weight to 73% that of the control value (308 ± 9 g versus 424 ± 4 g; $p < 0.05$) and reduced the relative liver and kidney weights. No other effect on body and organ weight was reported in this or the other treatment groups. A significant increase in the incidence of hepatocellular carcinomas was noted in animals administered 1.6 g/L dichloroacetic acid (21.4% [6/28] versus 3.0% [1.33]; p-value ≤ 0.05). Animals treated with 0.5 g/L dichloroacetic acid did not show a significant increase in the incidences of adenomas (5/29; 17.2%) or carcinomas (3/29; 10.3%), although the combined incidence of adenomas and carcinomas (7/19; 24.1%) was greater than that in controls (1/23; 4.4%) [$p$-value ≤ 0.05]. No liver lesions were found in rats exposed to 0.05 g/L dichloroacetic acid (DeAngelo *et al*., 1996). [The data for the treatments with 2 g/L sodium chloride and 0.05 and 0.5 g/L dichloroacetic acid were published previously, but the incidences of adenoma and proliferative lesions were reported to be slightly higher (Richmond *et al.*, 1995).]

### 3.2 Administration with known carcinogens or modifying factors

*Tumour-promotion studies*

Groups of 10–40 female B6C3F$_1$ mice, 15 days of age, were initiated with an intraperitoneal injection of 25 mg/kg bw *N*-methyl-*N*-nitrosourea (MNU). At 49 days of age, the animals received 2.0, 6.67 or 20.0 mmol/L [256, 854 or 2560 mg/L] dichloroacetic acid adjusted to pH 6.5–7.5 with sodium hydroxide or 20.0 mmol/L sodium chloride as a control

for the sodium salt in the drinking-water. At 31 weeks, administration of 20.0 mmol/L dichloroacetic acid in the drinking-water was stopped for 12 mice that were held untreated until 52 weeks. Some mice were killed after 31 weeks of exposure and the remainder after 52 weeks. Dichloroacetic acid did not significantly increase the incidence or multiplicity of hepatocellular adenocarcinomas. The high dose of dichloroacetic acid increased the incidence of hepatocellular adenomas in MNU-initiated mice from 0/10 to 5/10 (50%) at 31 weeks and from 7/40 (17.5%) to 19/26 (73.1%) at 52 weeks. The multiplicity of hepatocellular adenomas was increased ($p < 0.01$) from 0.00 to 1.80 ± 0.83 and from 0.28 ± 0.11 to 3.62 ± 0.70 at 31 and 52 weeks, respectively. The two lower doses of dichloroacetic acid did not significantly increase tumour incidence or multiplicity. The high dose of dichloroacetic acid also promoted MNU-initiated altered hepatocyte foci. The foci and tumours promoted by dichloroacetic acid were eosinophilic and contained glutathione *S*-transferase-π. When exposure to dichloroacetic acid was stopped after 31 weeks, the tumours that it had promoted appeared to regress (1.80 ± 0.83 at week 31 and 0.69 ± 0.26 at week 52) (Pereira & Phelps, 1996).

Combinations of dichloroacetic acid and trichloroacetic acid have been evaluated for tumour-promoting activity (Pereira *et al*., 1997). Groups of 20–45 female B6C3$F_1$ mice, 15 days of age, were initiated with an intraperitoneal injection of 25 mg/kg bw MNU on day 15 of age. At 4 weeks of age, they received 0, 7.8, 15.6 or 25 mmol/L dichloroacetic acid in the drinking-water with or without 6.0 mmol/L trichloroacetic acid or 25 mmol/L trichloroacetic acid with or without 15.6 mmol/L dichloroacetic acid. The pH of the dose solutions was adjusted to 6.5–7.5 with sodium hydroxide. Treatment was continued up to 48 weeks of age, at which time the mice were killed. The high dose of dichloroacetic acid (25 mmol/L) or trichloroacetic acid (25 mmol/L) significantly ($p < 0.05$) increased the multiplicity of hepatocellular adenomas from 0.07 ± 0.05 (no dichloroacetic acid or trichloroacetic acid) to 1.79 ± 0.29 and 0.52 ± 0.11, respectively. The lower doses of dichloroacetic acid and trichloroacetic acid did not significantly increase the incidence or multiplicity of adenomas. Although the combination containing 25 mmol/L dichloroacetic acid and 6.0 mmol/L trichloroacetic acid produced a less than synergistic increase in the liver-to-body weight ratio, it produced an additive increase in the multiplicity of hepatocellular adenomas. The multiplicity of adenomas in mice treated with the combination was 2.33 ± 0.42 compared with 1.79 ± 0.29 and 0.15 ± 0.08 for dichloroacetic acid and trichloroacetic acid alone. The results following the combination containing 15.6 mmol/L dichloroacetic acid and 25 mmol/L trichloroacetic acid were also consistent with an additive increase in the multiplicity of adenomas: 0.32 ± 0.11 (15.6 mmol/L dichloroacetic acid), 0.53 ± 0.20 (25.0 mmol/L trichloroacetic acid) and 0.52 ± 0.11 (combination of dichloroacetic acid and trichloroacetic acid).

The effect of chloroform on the promotion of liver and kidney tumours by dichloroacetic acid and trichloroacetic acid was evaluated in groups of 6–29 male and female B6C3$F_1$ mice initiated at 15 days of age with an intraperitoneal injection of 30 mg/kg bw MNU. At 5 weeks of age, the mice received 0 or 3.2 g/L dichloroacetic acid with 0, 800 or 1600 mg/L chloroform in the drinking-water. The pH of the dose solutions was neutra-

lized with sodium hydroxide. The concentrations of chloroform were chosen because they prevented dichloroacetic acid-induced DNA hypomethylation and increased mRNA expression of the c-*myc* gene. The mice were killed at 36 weeks of age. The results were analysed for statistical significance by a one-way ANOVA followed by the Tukey test with a $p$-value < 0.05. In MNU-initiated mice that did not receive dichloroacetic acid, liver adenomas were found in 2/29 (6.9%) females and 2/8 males (25%). No hepatocellular adenocarcinomas were found. Dichloroacetic acid increased the incidence of liver adenomas (17/24 [70.8%] females, 21/25 [84.0%] males). The multiplicity of liver tumours (adenomas plus adenocarcinomas) was increased by dichloroacetic acid from 0.25 ± 0.16 to 3.17 ± 0.76 tumours per mouse (females) and from 0.07 ± 0.04 to 3.92 ± 0.54 (males). In mice administered dichloroacetic acid, co-administration of chloroform decreased the incidence of adenomas from 17/24 (70.8%) to 2/8 (25%; 800 mg/L) and 0/6 (0%; 1600 mg/L) in females and from 21/25 (84.0%) to 7/12 (58.3%; 1600 mg/L) in males. The multiplicity of tumours was also decreased by chloroform to 0.8 and 0 for doses of 800 and 1600 mg/L in females and to 1.1 for the dose of 1600 mg/L in males. No altered hepatocyte foci, adenomas or adenocarcinomas were found in six MNU-initiated male mice that were administered 1600 mg/L chloroform. Renal tumours of tubular origin were found in male mice. The majority (> 70%) were papillary cystic adenomas and the rest were cystic adenomas and, to a lesser extent, adenocarcinomas (~5%). No kidney tumours were found in the eight MNU-initiated mice. In MNU-initiated mice that received dichloroacetic acid, a significant increase in the incidence or multiplicity of kidney tumours was observed: 24% (6/25) with tumours and 0.28 ± 0.11 tumours per mouse. However, co-administration of 1600 mg/L chloroform with dichloroacetic acid significantly increased the incidence of kidney tumours in male mice to 100% (12/12) and the multiplicity to 1.75 ± 0.39 tumours per mouse ($p$-value < 0.01). No kidney tumours were found in the six MNU-initiated male mice administered 1600 mg/L chloroform alone. In female mice, the incidence of kidney tumours in all treatment groups was not significantly increased and ranged from 0 to 28.6% and the multiplicity ranged from 0 to 0.29 tumours per mouse. Hence, dichloroacetic acid promoted kidney tumours only in mice that were also administered chloroform (Pereira *et al*., 2001).

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

## 4.1 Absorption, distribution, metabolism and excretion

### 4.1.1 *Humans*

The disposition of dichloroacetic acid (10 or 20 mg/kg bw given intravenously) was studied in four human volunteers (26, 38, 42 and 52 years old [sex not stated]). In subjects given 10 mg/kg bw dichloroacetic acid, the average plasma half-life was 0.34 h (range,

0.33–0.36 h), the average volume-of-distribution was 337 mL/kg (range, 308–366 mL/kg) and the average plasma clearance was 11.31 mL/min/kg (range, 10.86–11.76 mL/min/kg); in subjects given 20 mg/kg bw dichloroacetic acid, the average plasma half-life was 0.51 h (range, 0.41–0.61 h), the average volume-of-distribution was 190 mL/kg (range, 186–195 mL/kg) and the average plasma clearance was 4.55 mL/min/kg (range, 3.53–5.58 mL/min/kg) (Lukas *et al*., 1980).

Dichloroacetic acid was given intravenously (50 mg/kg bw) or orally (50 mg/kg bw or 50 mg/kg bw plus 50 mg thiamine [vitamin $B_1$]) to healthy human volunteers (eight men and four women, aged 18–45 years), and a range of pharmacokinetic parameters were measured (Curry *et al*., 1991). On average, there was no evidence of an effect of vitamin $B_1$ on the kinetics of dichloroacetic acetic. The average plasma half-life was 2.7 ± 0.4 h, the average volume of distribution was 19.9 ± 1.7 L, the average area-under-the-curve (AUC) was 608.9 ± 61.0 μg/h/mL and the average renal clearance was 53.0 ± 15.9 mL/h. There was no difference in the AUC or elimination half-life between men and women. Only 0.7 ± 0.5% dichloroacetic acid was excreted unchanged in the urine. Urinary excretion of oxalic acid was similar after oral or intravenous administration of dichloroacetic acid (2.1 ± 0.8 mg versus 2.3 ± 0.5 mg). However, the elimination half-life was markedly prolonged after a second dose of dichloroacetic acid (Curry *et al*., 1991).

The pharmacokinetics of dichloroacetic acid has been studied in humans with a range of diseases. In children (four boys and four girls, aged 1.5–10 years) with lactic acidosis due to severe malaria, dichloroacetic acid given intravenously at a dose of 50 mg/kg bw showed an average plasma half-life of 1.8 ± 0.4 h, a volume of distribution of 0.32 ± 0.09 L/kg and an average AUC of 378 ± 65 mg/L/h (Krishna *et al*., 1995).

Two studies were conducted on the pharmacokinetics of dichloroacetic acid in patients with severe falciparum malaria. In one study that included 13 adults ([sex not stated]; average age, 27 ± 8 years) who were given 46 mg/kg bw dichloroacetic acid intravenously over 30 min, the elimination half-life was 2.3 ± 1.8 h, the clearance was 0.32 ± 0.16 L/h/kg and the volume of distribution was 0.75 ± 0.35 L/kg (Krishna *et al*., 1994). In a second study, 11 adults (eight men and three women; average age, 32 ± 10 years) were given 46 mg/kg bw dichloroacetic acid intravenously and a second dose (46 mg/kg bw) was given 12 h later. The mean plasma half-life was 3.4 ± 2 h after the first dose and 4.4 ± 2 h after the second dose, the volume of distribution was 0.44 ± 0.2 L/kg and the plasma clearance was 0.13 ± 0.03 L/h/kg (Krishna *et al*., 1996).

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

women, 47 ± 3 years old) who were given 35 mg/kg bw dichloroacetic acid by intravenous perfusion over 30 min. The clearance of dichloroacetic acid was 2.14 mL/kg/min in control subjects and 0.78 mL/kg/min in patients with cirrhosis (Shangraw & Fisher, 1999).

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

The pharmacokinetics of dichloroacetic acid was compared in healthy volunteers (27 subjects) and in patients with traumatic brain injury (25 subjects; average age, 52.8 ± 18.1 years). The healthy volunteers were given cumulative intravenous doses (two doses 8 h apart) of 45, 90 or 150 mg/kg bw dichloroacetic acid; 16 patients with acute traumatic brain injury were given 60, 100 or 200 mg/kg bw dichloroacetic acid as a single intravenous dose; six other patients were given three intravenous doses [dose not stated] of dichloroacetic acid at 24-h intervals; and three patients were given six intravenous doses [dose not stated] at 12-h intervals. The initial clearance of dichloroacetic acid (4.82 L/h) declined (1.07 L/h) after repeated doses in patients with traumatic brain injury. Although the authors suggested several mechanisms by which the clearance of dichloroacetic acid might be decreased after repeated doses, they proposed that the enzyme responsible for the metabolism of dichloroacetic acid might be destroyed after repeated treatment with the compound (Williams *et al*., 2001).

### 4.1.2 *Experimental systems*

The pharmacokinetics of dichloroacetic acid was studied in rats and dogs. Three male Sprague-Dawley rats and two male beagle dogs were given 100 mg/kg bw dichloroacetic acid intravenously; the average plasma elimination half-life was 2.97 h (range, 2.1–4.4 h) in rats and 20.8 h (range, 17.1–24.6 h) in dogs; the average volume of distribution was 932 mL/kg (range, 701–1080 mL/kg) in rats and 256 mL/kg (range, 249–262 mL/kg) in dogs; and the average plasma clearance was 4.22 mL/min/kg (range, 1.84–5.94 mL/min/kg) in rats and 0.146 mL/min/kg (range, 0.123–0.168 mL/min/kg) in dogs (Lukas *et al*., 1980).

The disposition and elimination kinetics of [$^{14}$C]dichloroacetic acid were studied in male Fischer 344 rats and B6C3F$_1$ mice. In rats given 5, 20 or 100 mg/kg bw [$^{14}$C]dichloro-

acetic acid by gavage, 23.9–29.3% of the dose was eliminated as carbon dioxide and 19.6–24.4% was excreted in the urine. Only 1.0–2.2% of the dose was excreted unchanged in the urine. The major urinary metabolites in rats were glyoxylic, oxalic and glycolic acids, which amounted to 10.5–15.0% of the dose, and thiodiacetic acid amounted to 6.3–6.8% of the dose. In mice given 20 and 100 mg/kg bw dichloroacetic acid by gavage, 2.2 and 2.4% of the dose was eliminated as carbon dioxide and 2.2–2.3% was excreted as unchanged dichloroacetic acid. The major urinary metabolites of dichloroacetic acid in mice were glyoxylic, oxalic and glycolic acids and thiodiacetic acid, which amounted to 13.4–18.4% and 7.9–12.3% of the dose, respectively (Larson & Bull, 1992).

The adduction of haemoglobin and albumin by metabolites of dichloroacetic acid was investigated in male Fischer 344 rats and male $B6C3F_1$ mice given 5 mg/kg bw [1,2-$^{14}$C]-dichloroacetic acid orally. Adduct levels were determined by measuring the difference between total level incorporated and the fraction of the label present in serine and glycine. In rats, haemoglobin and albumin adducts amounted to 3–9 and 177–228 pmol equivalents/mg protein, respectively, whereas in mice, these adducts ranged from undetectable to < 1 and 65–152 pmol equivalents/mg protein, respectively (Stevens *et al*., 1992).

The effect of prior treatment with dichloroacetic acid on its pharmacokinetics was studied in male Fischer 344 rats. Animals were given unlabelled dichloroacetic acid (0.2 or 2.0 g/L) in the drinking-water for 14 days before receiving [1,2-$^{14}$C]dichloroacetic acid intravenously (bolus dose of 5, 20 or 100 mg/kg bw) or orally by gavage (100 mg/kg bw). In pretreated rats, the half-life of dichloroacetic acid after intravenous administration was significantly increased (10.8 versus 2.4 h) and the total body clearance was decreased (42.7 versus 267.4 mL/h/kg) compared with controls. The fraction of [1,2-$^{14}$C]dichloroacetic acid (100 mg/kg bw given orally or intravenously) excreted as carbon dioxide appeared to be decreased in pretreated rats. The urinary excretion of dichloroacetic acid, glycolic acid, glyoxylic acid, chloroacetic acid and acetic acid was significantly increased in rats pretreated with 2.0 g/L dichloroacetic acid for 14 days before receiving the labelled compound (the excretion of oxalic acid remained unchanged). The in-vitro biotransformation of [1,2-$^{14}$C]dichloroacetic acid to glyoxylic, oxalic and glycolic acids required glutathione for maximal activity and was decreased in rat liver cytosol isolated from rats pretreated with dichloroacetic acid (Gonzalez-Leon *et al*., 1997).

These data were reanalysed and extended in another study. Rats were administered dichloroacetic acid (65 mg/kg bw) via jugular vein cannula and whole blood and plasma concentrations of the parent compound were determined. The unbound fraction of dichloroacetic acid in plasma amounted to 0.94% and its renal clearance (corrected for plasma protein binding) was 3.1 mL/h/kg (Schultz *et al*., 1999).

The pharmacokinetics and oral bioavailability of dichloroacetic acid were studied in control naive male Fischer 344 rats administered 1–20 mg/kg bw and in glutathione *S*-transferase (GST)-zeta (GSTZ1-1)-depleted male Fischer 344 rats administered 0.05–20 mg/kg bw intravenously or by gavage (Saghir & Schultz, 2002). GSTZ1-1 activity was depleted by exposing rats to 0.2 g/L dichloroacetic acid in drinking-water for 7 days before pharmacokinetic studies (dichloroacetic acid being an inhibitor of the GSTZ1-1 enzyme). The authors

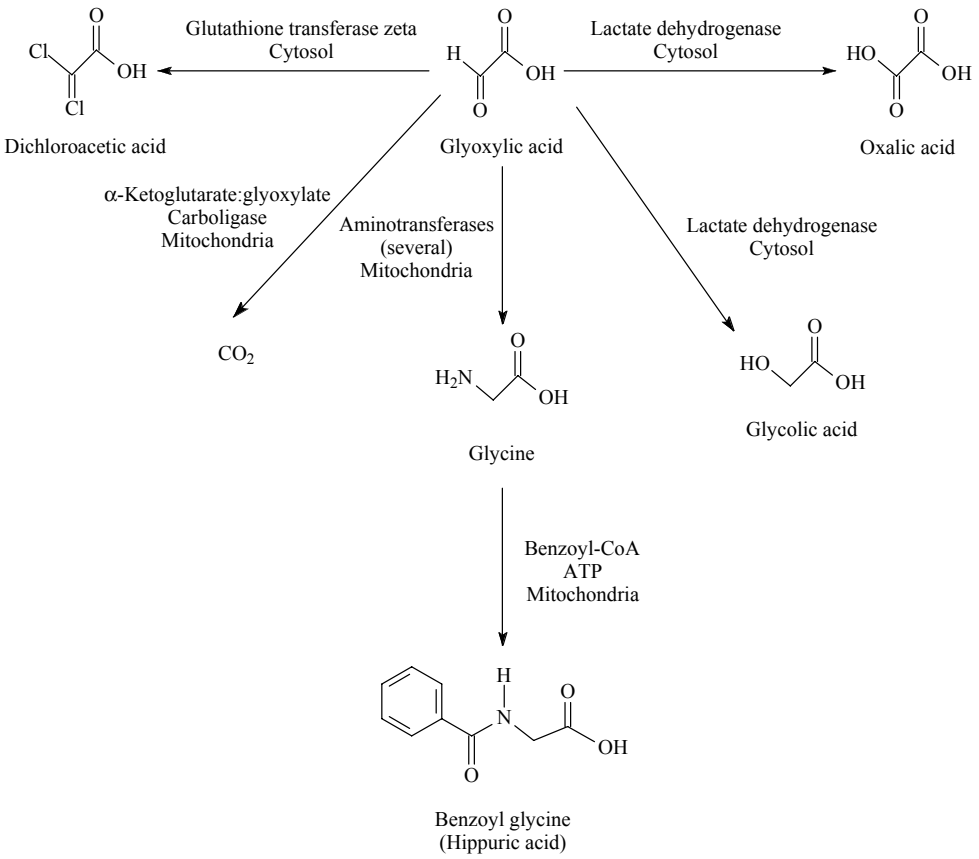
also compared the in-vitro metabolism of dichloroacetic acid in human liver cytosol with that in cytosol obtained from naive and GSTZ1-1-depleted rats. The half-life for the elimination of dichloroacetic acid from the plasma was dose-dependent and increased with increasing dose of dichloroacetic acid; it was also increased in GSTZ1-1-depleted rats given dichloroacetic acid in the drinking-water. The oral bioavailability of dichloroacetic acid was 0–13% in control naive rats and 14–75% in GSTZ1-1-depleted rats. The authors predicted that the human oral bioavailability of dichloroacetic acid in drinking-water would be low (< 1%). The intrinsic metabolic clearance ($Cl_{int}$) from control naive rat liver cytosol was 3.86 mL/h/mg protein compared with 0.25 mL/h/mg protein in GSTZ1-1-depleted rats; the $Cl_{int}$ from human liver cytosol was not significantly different from that observed in the GSTZ1-1-depleted rats. The authors concluded that exposure of rodents to a high level of dichloroacetic acid depletes GSTZ1-1 activity, causing the pharmacokinetics in rats to become comparable to that in humans (Saghir & Schultz, 2002).

The metabolic fate of dichloroacetic acid was investigated in male Fischer 344 rats given either 282 mg/kg bw [1-$^{14}$C]dichloroacetic acid or 282 mg/kg bw [2-$^{14}$C]dichloroacetic acid or 28.2 mg/kg [2-$^{14}$C]dichloroacetic acid by gavage. The disposition of [1-$^{14}$C]- or [2-$^{14}$C]dichloroacetic acid at 282 mg/kg bw was similar, except that the fraction of the dose eliminated through expiration as carbon dioxide decreased (25.0 versus 34.4%) whereas the fraction of the administered radioactivity excreted in the urine increased (35.2 versus 12.7%) as the dose of [2-$^{14}$C]dichloroacetic acid was increased from 28.2 to 282 mg/kg bw. Most of the increase in excreted radioactivity was attributable to unchanged dichloroacetic acid. The major urinary metabolites identified were glycolic acid, glyoxylic acid and oxalic acid. The fraction of administered radioactivity measured in tissues 48 h after treatment ranged from 20.8% to 36.4%; liver and muscle contained the most radioactivity (Lin *et al*., 1993).

The pharmacokinetics and metabolic fate of dichloroacetic acid were studied in male Sprague-Dawley rats given 50 mg/kg bw dichloroacetic acid containing 280–400 μCi/kg bw [1-$^{14}$C]dichloroacetic acid, 50 mg/kg bw [1,2-$^{13}$C]dichloroacetic acid or a mixture of [1-$^{14}$C]- and [1,2-$^{13}$C]dichloroacetic acid (1:99) by gavage as sodium dichloroacetate in water. The plasma elimination half-life of dichloroacetic acid was 0.11 ± 0.02 or 5.38 ± 0.76 h in rats given one or two doses of dichloroacetic acid, respectively. The fraction of the dose eliminated as carbon dioxide ranged from 17 to 46% of the dose. Oxalic acid, glyoxylic acid and the glycine conjugates hippuric acid and phenylacetylglycine were identified as metabolites; the glycine conjugates apparently arise from the transamination of glyoxylic acid to give glycine followed by conjugation with benzoic acid or phenylacetic acid. The fraction of the dose excreted in urine as unchanged dichloroacetic acid ranged from 0.36 to 20.2%, depending on the size of the rats and on whether they were fed or fasted (James *et al.*, 1998). The metabolic fate of dichloroacetic acid in rats is shown in Figure 1.

The kinetics of the biotransformation of dichloroacetic acid was studied in livers isolated from male Fischer 344 rats and perfused with Krebs-Ringer buffer containing bovine serum albumin, glucose and taurocholate and an initial concentration of 25 or 250 μM (3.2

**Figure 1. Metabolic fate of dichloroacetic acid in rats**




Modified from James *et al.* (1998)

or 32 µg/mL). In livers perfused with 250 µM (32 µg/mL), the free dichloroacetic acid concentration after 5 min of perfusion was 112 µM (14.5 µg/mL) or 47% of the total concentration, indicating binding to bovine serum albumin, whereas at the end of the 120-min perfusion, the concentration of free dichloroacetic acid was 7 µM (0.9 µg/mL) or 41% of the total concentration present at the end of the perfusion. A total of 0.2% of the total concentration of dichloroacetic acid was excreted in the bile during the 120-min perfusion. The half-life and the elimination rate constant for dichloroacetic acid were 32 min and 0.022/min, respectively (Toxopeus & Frazier, 1998).

The in-vitro degradation of dichloroacetic acid followed the order: liver >> lung > kidney > intestine ≈ muscle in mouse 700 × *g* supernatant fractions (cytosol, microsomes and mitochondria). The highest rate of degradation of dichloroacetic acid was found in liver cytosol, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen

were not required for maximal activity in microsomes. The degradation of dichloroacetic acid was decreased when diethyl maleate or chlorodinitrobenzene, which deplete glutathione (GSH) concentrations, was included in the reaction mixture and increased when GSH was added. Because the enzyme that catalysed the degradation of dichloroacetic acid was not retained on a GSH–sepharose column, it was concluded that a GST was not involved; carbon monoxide and SKF525-A, inhibitors of cytochrome P450 activity, did not inhibit the degradation of dichloroacetic acid, indicating that cytochromes P450 played no role in this degradation (Lipscomb *et al*., 1995).

The proposed formation of glyoxylic acid as an intermediate in the metabolism of dichloroacetic acid to oxalic acid, carbon dioxide and glycine was confirmed by the identification of [1-$^{14}$C]- and [1,2-$^{13}$C]glyoxylic acid as metabolites of [1-$^{14}$C]- and [1,2-$^{13}$C]dichloroacetic acid. The enzymes that catalysed this biotransformation were located in hepatic cytosolic fractions isolated from male Sprague-Dawley rats and from humans and required GSH, but not NADPH or NADH, for maximal activity. The rate of biotransformation to glyoxylic acid was decreased in hepatic cytosolic fractions from rats pretreated with 50 mg/kg bw dichloroacetic acid for 2 days compared with rats given water (James *et al*., 1997).

A GSH-dependent enzyme that catalysed the oxygenation of dichloroacetic acid to glyoxylic acid as the sole product was purified to homogeneity from male Fischer 344 rat liver (Tong *et al*., 1998a). Antibodies to human GSTZ cross-reacted with the rat liver enzyme, thereby identifying it as the rat orthologue of human GSTZ1-1 (hGSTZ1-1), which was identified by interrogating expressed sequence tag databases (Board *et al*., 1997). hGSTZ1-1 is identical to maleylacetoacetate isomerase, which catalyses the penultimate step in the degradation of phenylalanine and tyrosine (Fernández-Cañón & Peñalva, 1998). Rat liver GSTZ1-1 catalysed the biotransformation of [2-$^{13}$C]dichloroacetic acid to [2-$^{13}$C]glyoxylic acid, which was identified by $^{13}$C nuclear magnetic resonance spectroscopy. GSH was required for the GSTZ-catalysed oxygenation of dichloroacetic acid metabolism, but it was neither consumed nor oxidized; it was completely recovered from the incubation mixtures, and no formation of GSH disulfide was associated with oxygenation of dichloroacetic acid, as compared with controls. Immunoblotting with anti-hGSTZ1-1 antibodies demonstrated the presence of immunoreactive GSTZ in rat, mouse and human hepatic cytosolic fractions. Kinetic studies with human, rat (male Fischer 344) and mouse (male B6C3F$_1$) liver cytosolic fractions with dichloroacetic acid as the variable substrate showed that the $V_{max}/K_m$ followed the order human < rat < mouse. These data identified GSTZ1-1 as the cytosolic enzyme that catalyses the biotransformation of dichloroacetic acid to glyoxylic acid (Tong *et al.*, 1998b).

Immunohistochemical studies on the localization of GSTZ1-1 in male Fischer 344 rat tissues reported intense staining in the liver, testis and prostate, and moderate-to-sparse staining in a range of tissues. Hepatic GSTZ1-1 activities with maleylacetone or chlorofluoroacetic acid as substrates were markedly decreased after administration of 1.2 mmol/kg (154.8 mg/kg bw) dichloroacetic acid intraperitoneally for 5 days, but no change in residual GSTZ1-1 activities in the testis and other tissues was observed. The

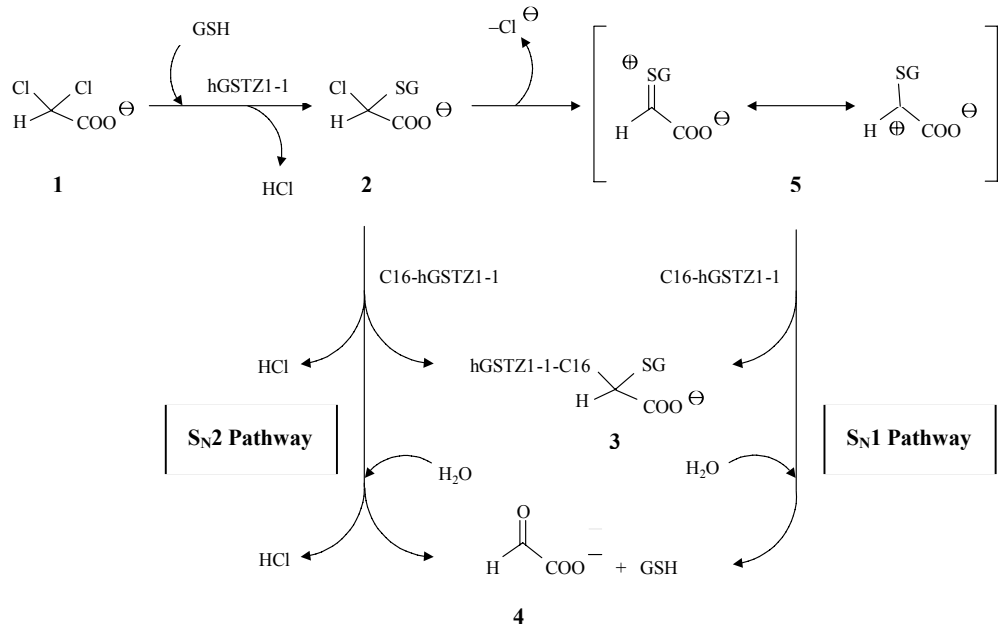
tissue-dependent differences in activities with both substrates reflected the pattern of expression of GSTZ1-1 observed by immunohistochemistry (Lantum *et al*. 2002).

The biotransformation of dichloroacetic acid to glyoxylic acid in rat liver cytosol (male Fischer 344 rats) was not linear with time and reached a plateau after 20–30 min of incubation; the reaction proceeded at the initial rate when a second dose of rat liver cytosol was added, whereas the addition of a second dose of dichloroacetic acid did not result in further glyoxylic acid formation, indicating that dichloroacetic acid inactivated GSTZ1-1. In rats given 0.3 mmol/kg (38.7 mg/kg) bw dichloroacetic acid for 12 days, GSTZ1-1 activity with dichloroacetic acid as the substrate reached a nadir 12 h after the first dose and did not return to control values until 10–12 days after treatment. The loss of GSTZ1-1 activity was paralleled by the loss of immunoreactive GSTZ1-1 protein. Data on dichloroacetic acid-induced inactivation of GSTZ1-1 were used to determine the rate of turnover of GSTZ *in vivo*. GSTZ1-1 was degraded in rat liver at the rate of –0.21/day, which corresponded to a half-life of 3.3 days (Anderson *et al*., 1999).

The kinetics of in-vitro inactivation of mouse (male $B6C3F_1$), rat (male Fischer 344) and human (male) GSTZ1-1 and of recombinant human GSTZ1-1 (hGSTZ1-1) by dichloroacetic acid was studied. The half-life for dichloroacetic acid-induced inactivation of GSTZ1-1 in mouse, rat and human liver cytosol was 6.61, 5.44 and 22 min, respectively (Tzeng *et al*., 2000). Four polymorphic variants of hGSTZ1-1 have been identified (Blackburn *et al*., 2000, 2001). The half-lives for dichloroacetic acid-induced inactivation of hGSTZ1a-1a, -1b-1b, -1c-1c and -1d-1d were 23, 9.6, 10.1 and 9.5 min, respectively (Tzeng *et al*., 2000). The inactivation of hGSTZ1c-1c was accompanied by a covalent modification of the enzyme: when [1-$^{14}$C]dichloroacetic acid was incubated with hGSTZ1c-1c in the presence of GSH, [1-$^{14}$C]dichloroacetic acid-derived radioactivity was irreversibly bound to the protein; similarly, when [$^{35}$S]GSH was incubated with hGSTZ1c-1c in the presence of dichloroacetic acid, [$^{35}$S]GSH-derived radioactivity was irreversibly bound to the protein. These data show that dichloroacetic acid-induced inactivation of hGSTZ1c-1c is accompanied by the covalent modification of the protein by both dichloroacetic acid and GSH (Tzeng *et al*., 2000). Additional studies showed that the partition ratio (turnover number) for dichloroacetic acid-induced, mechanism-based inactivation of hGSTZ1c-1c was $5.7 \pm 0.5 \times 10^2$ (Anderson *et al*., 2002). hGSTZ1c-1c, which is modified covalently by both dichloroacetic acid and GSH, has been characterized by matrix-assisted laser-desorption-ionization time-of-flight mass spectrometry (MALDI–TOF–MS) and by liquid chromatography–MS (LC-MS) (Anderson *et al*., 2002). The stoichiometry of dichloroacetic acid binding to hGSTZ1c-1c was ~0.5 mol dichloroacetic acid/mol enzyme monomer. A single dichloroacetic acid-derived adduct was observed and was assigned to cysteine-16 by a combination of MALDI–TOF–MS and electrospray ionization quadrupole ion-trap LC–MS analyses, and by analysis of [1-$^{14}$C]dichloroacetic acid binding to hGSTZS1c-1c. The dichloroacetic acid-derived adduct with hGSTZ1c-1c contained both GSH and the carbon skeleton of dichloroacetic acid, presumably in a dithioacetal linkage. Hence, the mechanism of biotransformation of dichloroacetic acid to glyoxylic acid and of inactivation of GSTZ1-1 involves the displacement of chloride from dichloroacetic acid to

give *S*-(α-chlorocarboxymethyl)GSH, which may react with cysteine-16 of hGSTZ1c-1c to give the covalently modified protein, or with water to give glyoxylic acid and GSH (Figure 2) (Anderson *et al.*, 2002).

**Figure 2. Mechanisms of the hGSTZ1-1-catalysed biotransformation of dichloroacetic acid to glyoxylic acid and of the mechanism-based inactivation of hGSTZ1-1 by dichloroacetic acid**




Modified from Anderson *et al.* (2002)
1, dichloroacetic acid; 2, *S*-(α-chlorocarboxymethyl)glutathione; 3, hGSTZ1-1 covalently modified at cysteine-16; 4, glyoxylic acid; 5, sulfonium-carbocation intermediate

### 4.1.3 *Comparison of humans and animals*

The kinetics of elimination of dichloroacetic acid in humans and rats is quantitatively similar: the half-lives range from 0.3 to 3.5 h. The half-life of dichloroacetic acid in dogs is 20.8 h (Lukas *et al.*, 1980). In both humans and rats, the half-life of dichloroacetic acid is prolonged by prior treatment with the compound. Studies with expressed recombinant hGSTZ1-1 showed that dichloroacetic acid is a mechanism-based inactivator of the enzyme, which explains the observed change in half-life in rodents and humans caused by prior treatment with the compound (Tzeng *et al.*, 2000; Anderson *et al.*, 2002).

## 4.2 Toxic effects

### 4.2.1 *Humans*

The pharmacological and toxic effects of dichloroacetic acid in humans have been studied extensively because of its potential use in the treatment of various disorders (see Section 1.2.2). Dichloroacetic acid lowers blood sugar levels in animals and humans with diabetes mellitus by stimulating peripheral use of glucose and inhibiting gluconeogenesis. In addition, long-term administration of dichloroacetic acid reduces plasma triglyceride and cholesterol levels (a particularly important effect in patients with congenital hypercholesterolaemia, who have no cholesterol receptors), and it facilitates oxidation of lactate by activating pyruvate dehydrogenase in patients with acquired and congenital forms of lactic acidosis (Stacpoole, 1989).

Drowsiness is a fairly frequent side-effect of dichloroacetic acid and has been observed in healthy volunteers, adults with type II diabetes and patients with lactic acidosis (Stacpoole *et al.*, 1978). A patient with homozygous familial hypercholesterolaemia who received single oral doses of 3 g [concentration not specified] dichloroacetic acid daily for 4 months developed reversible peripheral neuropathy characterized by loss of reflexes and muscle weakness; the effect subsided several weeks after cessation of treatment (Moore *et al.*, 1979). A second case of peripheral neuropathy in the lower extremities was reported in a 13-year-old girl treated with dichloroacetate for 1 year (Saitoh *et al*., 1998). A more systematic study of nerve conduction velocities was carried out in 27 patients with congenital lactic acidaemia who were treated with dichloroacetate for 1 year (Spruijt *et al*., 2001). For 25 cases, electrophysiological results were available before administration of dichloroacetate. Ten male and four female patients developed abnormal nerve conduction velocities and amplitudes within 3–6 months of the start of treatment. Motor neurons were more greatly than sensory neurons. The patients were all co-medicated with thiamine to reduce the incidence of polyneuropathy due to oxalate, a metabolite of dichloroacetic acid (Bilbao *et al.*, 1976; Stacpoole *et al.*, 1984).

### 4.2.2 *Experimental systems*

Exposure of male and female Sprague-Dawley rats to dichloroacetic acid at target doses of 10–600 mg/kg bw per day in the drinking-water for 14 days resulted in reduced weight gain only in the group given the highest dose. Treatment also increased urinary excretion of ammonia and changed the activities of ammoniagenesis enzymes (phosphate-dependent and -independent glutaminase), indicating renal compensation for an acid load (Davis, 1986).

Male Sprague-Dawley rats administered dichloroacetic acid in the drinking-water for 90 days at concentrations providing daily doses of about 3.9, 25.5 or 345.0 mg/kg bw had decreased body weights. Animals given the high dose also showed histological and biochemical signs of liver and kidney damage and increased hepatic peroxisomal β-oxidation activity (Mather *et al.*, 1990).

Ocular toxicity was observed in beagle dogs (which are susceptible to drug-induced cataract formation) that were treated for 13 weeks with approximate doses of 50, 75 and 100 mg/kg bw dichloroacetic acid in the drinking-water. No similar organ-specific effect has been seen in other studies or in other species (rats or humans) (Katz *et al.*, 1981).

Administration of the sodium salt of dichloroacetic acid at a target dose of 50 or 1100 mg/kg bw to male Sprague-Dawley rats in the drinking-water for 7 weeks resulted in severe hindlimb weakness, vacuolation and demyelinization of cerebral and cerebellar parenchyma and thiamine depletion in the high-dose group. The neurotoxic effects were partially prevented by providing thiamine supplementation during the treatment period (Stacpoole *et al.*, 1990). These results confirmed the observed association between neurotoxicity induced by dichloroacetic acid and the histopathological changes in the brain seen with thiamine deficiency (McCandless *et al.*, 1968). The underlying mechanism may involve stimulation of the thiamine-dependent enzymes pyruvate dehydrogenase and α-ketoacid dehydrogenase by dichloroacetic acid, resulting in increased turnover of this vitamin (Stacpoole *et al.*, 1990). Oxalate, a metabolite of dichloroacetic acid in humans and rodents, has been shown to cause both peripheral neuropathy and cataracts (Bilbao *et al.*, 1976; Fielder *et al.*, 1980); however, the renal and testicular oxalate crystals commonly seen in such cases have not been observed after administration of high doses of dichloroacetic acid (Katz *et al.*, 1981; Yount *et al.*, 1982; Stacpoole *et al.*, 1990).

A behavioural study documented effects of dichloroacetate on gait and grip strength in Fischer 344 and Long-Evans rats. Gait was affected at doses as low as 16 mg/kg bw per day within 9 weeks. Hindlimb grip strength was affected within 8 weeks after doses in excess of 90 mg/kg bw per day. Other stereotypical behaviours were also observed. Effects at high doses were irreversible, but there was partial recovery from the effects of a 13-week intake of 172 mg/kg bw, 14 weeks after treatment ended (Moser *et al.*, 1999).

Exposure of male and female $B6C3F_1$ mice to dichloroacetic acid at 1000 and 2000 mg/L in drinking-water for up to 52 weeks induced severe cytomegaly associated with extensive accumulation of glycogen, the effects progressing to multiple focal areas of necrosis, regenerative cell division and hepatomegaly (Bull *et al.*, 1990; Sanchez & Bull, 1990; Bull *et al.*, 1993).

The occurrence of infrequent and scattered acinar necrosis and a small initial increase in cell division have been reported in normal liver after treatment with dichloroacetic acid, but studies are not consistent. Histological examination of liver in other studies found little or no evidence of such damage or of overt cytotoxicity. In all cases, however, cell replication rates in normal liver decreased with chronic treatment (Stauber & Bull, 1997; Bull, 2000). Decreased cell replication rates were paralleled by decreased rates of spontaneous apoptosis (Snyder *et al.*, 1995). However, dichloroacetate significantly increased cell replication rates in a dose-dependent manner in altered hepatic foci and small tumours when chronic treatment was followed by continued administration of dichloroacetic acid at different doses (Stauber & Bull, 1997). These studies indicate that dichloroacetate has selective effects on cell replication. Another experiment, conducted *in vivo*, demonstrated that the growth of tumours, as measured by magnetic resonance imaging, slowed to a rate not

statistically different from zero when treatment with dichloroacetate was suspended (Miller *et al*., 2000). This effect was also demonstrated as increased growth of colonies when isolated anchorage-independent hepatocytes from $B6C3F_1$ mice were treated with dichloroacetate (Stauber *et al*., 1998).

Induction of peroxisome proliferation has been repeatedly associated with the chronic toxicity and carcinogenicity of dichloroacetic acid in the liver (DeAngelo *et al.*, 1989). It induced peroxisome proliferation in the livers of both mice and rats, as indicated by increased activities of palmitoyl-coenzyme A oxidase and carnitine acetyl transferase, the appearance of a peroxisome proliferation-associated protein and increased volume density of peroxisomes after exposure to dichloroacetic acid for 14 days. With further treatment, peroxisome markers returned to control levels after 45–60 weeks (DeAngelo *et al*., 1999). Dichloroacetate concentrations in the range of 0.5–2 mM (64.5–258 μg/mL) increased peroxisome proliferation in cultured hepatocytes derived from $B6C3F_1$ mice and Long-Evans rats (Everhart *et al*., 1998). However, systemic concentrations of dichloroacetate with minimally carcinogenic effects were found to be in the range of 1g/L (8 mM) in mice (Daniel *et al.*, 1992), while a concentration of 3.5 g/L (27 mM) dichloroacetate produced a transitory increase in liver palmitoyl-coenzyme A oxidase activity, suggesting that peroxisome proliferation does not contribute significantly to the development of tumours induced by lower doses of dichloroacetate (DeAngelo *et al.*, 1999).

As described below, dichloroacetate induced changes in carbohydrate metabolism at all doses that gave rise to tumours. The most notable change was an increase in hepatic glycogen (Bull *et al.*, 1990; Sanchez & Bull, 1990; Carter *et al*., 1995; Kato-Weinstein *et al*., 1998). In-vitro studies in isolated mouse hepatocytes demonstrated that this effect was mediated through phosphatidylinositol 3-kinase, but was independent of insulin (Lingohr *et al*., 2002). In intact $B6C3F_1$ mice, treatment with dichloroacetic acid resulted in a substantial and dose-related decrease in serum insulin concentrations and in the levels of insulin receptor expression in the liver (Kato-Weinstein *et al*., 2001a; Lingohr *et al*., 2001). Down-regulation of the insulin receptor was not observed in hepatic tumours induced by dichloroacetate, since the amount of insulin receptor protein was higher in liver tumours than in normal liver tissue taken from the same animal and in the livers of mice that received no dichloroacetate treatment (Lingohr *et al*., 2001).

Short-term treatment (11 days) of mice with dichloroacetate produced hypomethylation of DNA in liver, but this effect disappeared with longer-term treatment (44 weeks) (Tao *et al*., 1998). However, the extent of methylation at 5-methylcytosine sites in DNA of liver tumours was reduced by chronic treatment (44 weeks) with dichloroacetic acid, but returned to normal if treatment was suspended 1 week prior to sacrifice. Hypomethylation of DNA and of the specific proto-oncogenes c-*jun* and c-*myc* was associated with an increase in their expression in dichloroacetate-promoted tumours when compared with surrounding non-tumorous liver (Tao *et al*., 2000a). The hypomethylation was reversed by prior administration of methionine, suggesting that dichloroacetate acts by depleting the availability of *S*-adenosyl-methionine for methylation (Tao *et al*., 2000b). It appears that hypomethylation of promoter region for the c-myc gene occurs in several tissues (liver, kidney and bladder)

in mice and precedes cell replication within these tissues; dichloroacetate prevents the methylation of hemimethylated sites in newly synthesized strands of DNA (Ge *et al*., 2001).

As discussed in Section 4.1.1, dichloroacetate inhibits its own metabolism. The enzyme involved in its metabolism, GSTZ1-1, is also known as maleylacetoacetate isomerase (MAAI) and converts maleylacetoacetate to fumarylacetoacetate and maleylacetone to fumarylacetone. The enzyme is part of the tyrosine degradation pathway and interference with tyrosine is associated with human disease, including the development of hepatocellular carcinomas (Tanguay *et al*., 1996). Cornett *et al*. (1999) demonstrated that as little as 4 mg/kg bw per day dichloroacetate given to rats for 5 days significantly inhibited this enzyme. A dose rate of 200 mg/kg bw per day for the same period inhibited it by > 90%. This latter dose was shown to increase significantly excretion of maleylacetone in the urine. The authors found that the human enzyme was insensitive to inhibition by dichloroacetate *in vitro* while the rat enzyme was inhibited; they suggested that accumulation of toxic metabolites of tyrosine may be responsible for some toxicities seen in animals. While this hypothesis remains viable for some of the toxic effects of dichloroacetate, it does not appear to account for the carcinogenic effects of dichloroacetate in mouse liver. Schultz *et al*. (2002) found that activity of MAAI was only affected by dichloroacetate in young mice (10 weeks old) and not in 60-week-old animals. These observations suggest that, if MAAI plays a role, it would only be in the early stages of treatment and that animals may be able to adapt to inhibition of this enzyme. This is consistent with the observation that *MAAI*/*GSTZ1-1* knockout mice do not develop hepatocellular carcinomas spontaneously (Fernández-Cañón *et al.*, 2002).

## 4.3 Reproductive and prenatal effects

### 4.3.1 *Humans*

No data were available to the Working Group.

### 4.3.2 *Experimental systems*

The developmental toxicity of dichloroacetic acid has been reviewed previously (IARC, 1995).

To identify the most sensitive period of organogenesis, single doses of dichloroacetic acid were administered to pregnant Long-Evans rats. Anomalies (particularly defects in the audiovascular system) were produced with treatment on gestational days 9–12 (Epstein *et al.*, 1992). When 300 mg/kg bw dichloroacetate was administered to Sprague-Dawley rats on days 6–15 of gestation, a decrease of 5% in fetal body weight was noted, but no increase in fetal heart malformations (Fisher *et al.*, 2001).

The developmental toxicity of dichloroacetate has also been evaluated in whole-embryo cultures. In rat embryos explanted on day 10 of gestation and cultured for 46 h, malformations were not observed until concentrations of dichloroacetate of 2.5 mM [322.5 mg/L] and above were used in the incubation medium (Saillenfait *et al*., 1995). In a mouse embryo

explanted on day 9 of gestation and cultured for 24–26 h, dichloroacetate produced an increased incidence of neural tube defects only when concentrations in the medium exceeded 5.8 mM [748.2 mg/L]. In comparison, acetic acid induced significant increases in neural tube defects at a lower concentration (4 mM) [516 mg/L] (Hunter *et al.*, 1996).

A subsequent evaluation in Sprague-Dawley rats showed delayed spermiation and formation of atypical residual bodies at doses as low as 54 mg/kg bw per day for 14 days, and sperm fusion and other abnormalities at doses of 160 mg/kg bw or more (Linder *et al.*, 1997).

## 4.4 Genetic and related effects

### 4.4.1 *Humans*

No data were available to the Working Group.

### 4.4.2 *Experimental systems*

#### (*a*) *DNA adduct formation*

Dichloroacetic acid given by gavage caused a slight increase in 8-hydroxydeoxyguanosine DNA adduct formation in B6C3F$_1$ mouse hepatocytes *in vivo* (Austin *et al.*, 1996), but it had no effect in this assay when given in drinking-water (Parrish *et al.*, 1996).

#### (*b*) *Mutagenic and allied effects* (see Table 3 for details and reference)

Table 3 is an attempt to provide a comprehensive review of the literature on the genotoxic effects of dichloroacetic acid to date, and the text below reviews primarily the data not previously reviewed in IARC (1995).

In a single study, dichloroacetic acid caused a weak induction of SOS repair in *E. coli* strain PQ37. Dichloroacetic acid did not induce differential toxicity in DNA repair-deficient strains of *Salmonella typhimurium* but did induce prophage in *Escherichia coli* in one study. It was mutagenic in three strains of *S. typhimurium*: strain TA100 in three of five studies, strain RSJ100 in a single study, and strain TA98 in two of three studies. Most of the mutations in 400 revertants of dichloroacetic acid-treated *S. typhimurium* TA100 cultures were GC→AT transitions (DeMarini *et al.*, 1994). Dichloroacetic acid failed to induce point mutations in other strains of *S. typhimurium* (TA104, TA1535, TA1537, TA1538) or in *E. coli* strain WP2*uvrA*.

DNA strand breaks were not induced in mammalian cells *in vitro* in the absence of an exogenous metabolic activation system. Dichloroacetic acid failed to induce DNA damage as detected by the single-cell gel electrophoresis assay in Chinese hamster ovary cells *in vitro*.

In the gene mutation assay in mouse lymphoma cells *in vitro*, dichloroacetic acid did not show an effect in one study, but gave a positive response at 10-fold lower doses in a test in sealed tubes in another. The compound failed to induce micronuclei in a single study in the

**Table 3. Genetic and related effects of dichloroacetic acid**

| Test system | Result[a] | | Dose[b] (LED or HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic activation | With exogenous metabolic activation | | |
| λ Prophage induction, *Escherichia coli* WP2s | – | + | 2500 | DeMarini *et al.* (1994) |
| SOS chromotest, *Escherichia coli* PQ37 | (+) | – | 500 | Giller *et al.* (1997) |
| *Salmonella typhimurium*, DNA repair-deficient strains TS24, TA2322, TA1950 | – | – | 31 000 | Waskell (1978) |
| *Salmonella typhimurium* TA100, TA1535, TA1537, TA1538, reverse mutation | – | – | NR | Herbert *et al*. (1980) |
| *Salmonella typhimurium* TA100, reverse mutation | + | + | 50 | DeMarini *et al.* (1994) |
| *Salmonella typhimurium* TA100,TA1535, TA1537, TA98, reverse mutation | – | – | 5000 | Fox *et al*. (1996) |
| *Salmonella typhimurium* TA100, reverse mutation, liquid medium | + | + | 100 | Giller *et al.* (1997) |
| *Salmonella typhimurium* RSJ100, reverse mutation | + | – | 1935 | Kargalioglu *et al*. (2002) |
| *Salmonella typhimurium* TA104, reverse mutation, microsuspension | – | – | 150 µg/plate | Nelson *et al.* (2001) |
| *Salmonella typhimurium* TA98, reverse mutation | – | (+) | 10 µg/plate | Herbert *et al*. (1980) |
| *Salmonella typhimurium* TA98, reverse mutation | + | – | 5160 | Kargalioglu *et al*. (2002) |
| *Salmonella typhimurium* TA100, reverse mutation | + | + | 1935 | Kargalioglu *et al*. (2002) |
| *Escherichia coli* WP2*uvr*A, reverse mutation | – | – | 5000 | Fox *et al*. (1996) |
| DNA strand breaks and alkali-labile damage, Chinese hamster ovary cells *in vitro* (single-cell gel electrophoresis assay) | – | NT | 3225 µg/mL | Plewa *et al*. (2002) |
| DNA strand breaks, $B6C3F_1$ mouse hepatocytes *in vitro* | – | NT | 2580 | Chang *et al*. (1992) |
| DNA strand breaks, Fischer 344 rat hepatocytes *in vitro* | – | NT | 1290 | Chang *et al*. (1992) |
| Gene mutation, mouse lymphoma cell line L5178Y/$TK^{+/-}$ *in vitro* | – | – | 5000 | Fox *et al*. (1996) |
| Gene mutation, mouse lymphoma cell line L5178Y/$TK^{+/-}$-3.7.2C *in vitro* | + | NT | 400 | Harrington-Brock *et al.* (1998) |
| Micronucleus formation, mouse lymphoma L5178Y/$TK^{+/-}$-3.7.2C cell line *in vitro* | – | NT | 800 | Harrington-Brock *et al.* (1998) |
| Chromosomal aberrations, Chinese hamster ovary *in vitro* | – | – | 5000 | Fox *et al*. (1996) |

**Table 3 (contd)**

| Test system | Result[a] | | Dose[b] (LED or HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic activation | With exogenous metabolic activation | | |
| Chromosomal aberrations, mouse lymphoma L5178Y/Tk$^{+/-}$ -3.7.2C cell line *in vitro* | + | NT | 600 | Harrington-Brock *et al.* (1998) |
| Aneuploidy, mouse lymphoma L5178Y/Tk$^{+/-}$-3.7.2C cell line *in vitro* | - | NT | 800 | Harrington-Brock *et al.* (1998) |
| DNA strand breaks, human CCRF-CEM lymphoblastoid cells *in vitro* | – | NT | 1290 | Chang *et al*. (1992) |
| DNA strand breaks, male B6C3F$_1$ mouse liver *in vivo* | + | | 13 po × 1 | Nelson & Bull (1988) |
| DNA strand breaks, male B6C3F$_1$ mouse liver *in vivo* | + | | 10 po × 1 | Nelson *et al*. (1989) |
| DNA strand breaks, male B6C3F$_1$ mouse liver *in vivo* | – | | 1290 po × 1 | Chang *et al.* (1992) |
| DNA strand breaks, male B6C3F$_1$ mouse splenocytes *in vivo* | – | | 1290 po × 1 | Chang *et al.* (1992) |
| DNA strand breaks, male B6C3F$_1$ mouse epithelial cells from stomach and duodenum *in vivo* | – | | 1290 po × 1 | Chang *et al*. (1992) |
| DNA strand breaks, male B6C3F$_1$ mouse liver *in vivo* | – | | 5000 dw × 7–14 d | Chang *et al.* (1992) |
| DNA strand breaks, alkali-labile sites, cross linking, male B6C3F$_1$ mouse blood leukocytes *in vivo* (single-cell gel electrophoresis assay) | + | | 3500 dw × 28 d | Fuscoe *et al.* (1996) |
| DNA strand breaks, male Sprague-Dawley rat liver *in vivo* | + | | 30 po × 1 | Nelson & Bull (1988) |
| DNA strand breaks, male Fischer 344 rat liver *in vivo* | – | | 645 po × 1 | Chang *et al.* (1992) |
| DNA strand breaks, male Fischer 344 rat liver *in vivo* | – | | 2000 dw × 30 w | Chang *et al*. (1992) |
| Gene mutation, *lacI* transgenic male B6C3F$_1$ mouse liver assay *in vivo* | + | | 1000 dw × 60 w | Leavitt *et al*. (1997) |
| Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes *in vivo* | + | | 3500 dw × 9 d | Fuscoe *et al.* (1996) |
| Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes *in vivo* | – | | 3500 dw × 28 d | Fuscoe *et al.* (1996) |
| Micronucleus formation, male B6C3F$_1$ mouse peripheral erythrocytes *in vivo* | + | | 3500 dw × 10 w +21[c] | Fuscoe *et al.* (1996) |

**Table 3 (contd)**

| Test system | Result[a] | | Dose[b] (LED or HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic activation | With exogenous metabolic activation | | |
| Micronucleus formation, male and female Crl:CD (SD) BR rat bone-marrow erythrocytes *in vivo* | – | | 1100 iv × 3 | Fox *et al.* (1996) |
| Micronucleus formation, *Pleurodeles waltl* newt larvae peripheral erythrocytes *in vivo* | – | | 80[d] | Giller *et al.* (1997) |
| Inhibition of intercellular communication, male Sprague-Dawley rat liver clone 9 cells *in vitro* | + | NT | 1290 | Benane *et al*. (1996) |

[a] +, positive; (+), weakly positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, µg/mL; in-vivo tests, mg/kg bw; NR, dose not reported; po, orally; dw, drinking-water (in mg/L); d, day; w, week; iv, intravenous
[c] 10 weeks of dichloroacetic acid-containing drinking-water, followed by 21 weeks on dichloroacetic acid-free drinking-water
[d] Larvae reared in dichloroacetic acid-containing water

same system. Dichloroacetic acid increased the frequency of chromosomal aberrations in mouse lymphoma cell cultures but not in Chinese hamster ovary cells. In a single study, dichloroacetic acid did not induce aneuploidy in mouse lymphoma cells *in vitro*.

Contradictory results were obtained for the induction of DNA strand breaks in mammals *in vivo*. No effects were seen in either mouse or rat hepatic cells after single or repeated dosing, and no effects were observed in epithelial cells from spleen, stomach or duodenum after a single dose. In one study *in vivo*, dichloroacetic acid induced a significant decrease in DNA migration, consistent with the presence of DNA cross-linking in leukocytes *in vivo*, as detected by the single cell gel electrophoresis assay. Dichloroacetic acid caused mutations in male transgenic B6C3F$_1$ mice harbouring the bacterial *lacI* gene. In the latter study, in which mice received dichloroacetic acid in the drinking-water for 60 weeks, the mutation spectrum recovered from treated mice showed a significant decrease in GC→AT transitions (32.8% versus 53.2% for controls) and an increase in mutations at TA sites (32.79% versus 19.15% for controls) (Leavitt *et al.*, 1997). [The Working Group noted that the doses used (3.5 or 1.0 g/L) were high and tumorigenic.]

In one study in male B6C3F$_1$ mice polychromatic erythrocytes *in vivo*, dichloroacetic acid induced the formation of micronuclei in two of three treatments: a dose-related increase after a 9-day exposure; no significant increase after a 28-day exposure [the value for control in that experiment was rather high (higher than after 9 days of exposure)]; and a small but significant increase in the frequency of micronucleated normochromatic erythrocytes following exposure for more than 10 weeks. Coadministration of the antioxidant vitamin E did not affect the ability of dichloroacetic acid to induce this damage, indicating that the small induction of micronuclei by dichloroacetic acid was probably not caused by oxidative damage. Based on the lack of any difference observed in the proportion of kinetochore-positive micronuclei between the treated and control animals, micronuclei were assumed to arise from clastogenic events (Fuscoe *et al.*, 1996). After intravenous administration to male and female Crl:CD (SD) BR rats *in vivo*, dichloroacetic acid failed to induce micronuclei in bone-marrow erythrocytes in a single study. Dichloroacetic acid did not induce micronuclei in erythrocytes of new larvae *in vivo*.

Dichloroacetic acid given in drinking-water (0.5 and 5 g/L) to male B6C3F$_1$ mice significantly reduced apoptosis in hepatocytes in a dose-dependent manner compared to untreated controls (Snyder *et al*., 1995).

In the livers of female B6C3F$_1$ mice given dichloroacetic acid in drinking-water for 11 days, the level of 5-methylcytosine in DNA was decreased, it was also decreased in liver tumours. In the livers of female B6C3F$_1$ mice initiated with *N*-methyl-*N*-nitrosourea before receiving dichloroacetic acid in drinking-water for 44 weeks, termination of exposure to dichloroacetic acid 1 week prior to sacrifice resulted in an increase in the level of 5-methylcytosine in adenomas to the level found in non-involved livers (Tao *et al.*, 1998). These authors noted that the restauration of DNA methylation in adenomas upon removal of dichloroacetic acid corresponded to the regression of adenomas upon removal of dichloroacetic acid (Bull *et al.*, 1990; Pereira & Phelps, 1996). Gap-junctional intercellular communication was inhibited in rat hepatocytes *in vitro* (Benane *et al.*, 1996).

*Mutations of proto-oncogenes in tumours induced by dichloroacetic acid*

In a tumour assay by Anna *et al*. (1994), numerous foci of cellular alteration (presumed preneoplastic lesions) were noted in the livers of mice treated with dichloroacetic acid for 76 weeks, but only rare foci were found in the livers of controls. No neoplasms related to treatment were found at other sites. The frequency of mutations in codon 61 of H-*ras* (CAA) was not significantly different in the hepatocellular tumours from 64 treated mice (62%) and in those from 74 combined historical and concurrent controls (69%); however, the spectrum of these mutations showed a significant decrease in AAA and an increase in CTA in the treated mice in comparison with the controls. No other H-*ras* mutations were found, and only one K-*ras* mutation was detected in tumours from the treated and concurrent control groups. The authors interpreted these findings as suggesting that exposure to dichloroacetic acid provides the environment for a selective growth advantage for spontaneous CTA mutations in codon 61 of H-*ras* (Anna *et al.*, 1994).

Point mutations in exons 1, 2 and 3 of K- and H-*ras* proto-oncogenes were studied in dichloroacetic acid-induced liver tumours of male $B6C3F_1$ mice (104-week treatment). Dichloroacetic acid did not modify the incidence of mutations for exon 2 of *H-ras* in carcinomas (50% versus 58% for control). Only three liver carcinomas from dichloroacetate-treated mice showed mutations in the other exons of *H-ras* or in *K-ras*. In tumours with mutation in exon 2 of *H-ras*, treatment with dichloroacetic acid induced a decrease in the frequency of mutations CAA→AAA (21% versus 80% for control), an increase in the frequency of mutations CAA→CGA (50% versus 20% for control) and the appearance of a new mutation CAA→CTA (29% versus 0% for control, about one third of the mutations) (Ferreira-Gonzalez *et al.*, 1995).

H-*ras* codon 61 mutations were studied in female $B6C3F_1$ mouse liver tumours given 3.5 g/L dichloroacetic acid in the drinking-water over a period of 104 weeks. Only one mutation in exon 2 was found among the 22 tumours analysed (4.5%), and it was a CAA→CTA transversion in H-*ras* codon 61 (Schroeder *et al*., 1997).

In *N*-methyl-*N*-nitrosourea-initiated and dichloroacetic acid-promoted female $B6C3F_1$ mice, no loss of heterozygosity on chromosome 6 was observed in the 24 liver tumours promoted by dichloroacetic acid (Tao *et al.*, 1996).

## 4.5 Mechanistic considerations

Section 4.4 provides a consistent data set to indicate that dichloroacetic acid induces mutations and chromosomal effects. However, the concentrations of dichloroacetic acid that are required to produce these effects raise serious questions as to whether damage to DNA is involved in carcinogenic responses at low doses (Moore & Harrington-Brock, 2000). This conclusion is reinforced by observations suggesting that alternative mechanisms may adequately account for the carcinogenic responses that have been observed in rodents. These data are discussed in some detail in the following paragraphs.

A series of studies examined the extent to which the mutation frequency and spectra in *ras* genes might provide evidence of a genotoxic mechanism. The first of these studies examined liver tumours that were induced by administering 5 g/L dichloroacetic acid in drinking-water to male B6C3F$_1$ mice for 76 weeks (Anna *et al*., 1994). Tumours induced by dichloroacetic acid did not have mutations in codons 12 or 13 of the H-*ras* oncogene, whereas genotoxic carcinogens generally induce mutations at these two codons. The incidence of mutations at codon 61 of the H-*ras* oncogene in tumours from dichloroacetic acid-treated mice was not different from that of spontaneous tumours in control mice: 62 and 69% of the animals, respectively. However, the types of mutation were differed: dichloroacetic acid-induced tumours had 27% CAA→AAA and 37% CAA→CTA transversions of codon 61. Of the spontaneous tumours, 58% had a CAA→AAA transversion at this locus and only 13% had CAA→CTA transversions. These differences in the mutation spectrum were statistically significant. Thus, the mutation analysis of the H-*ras* oncogene in dichloroacetic acid-induced tumours is consistent with a non-genotoxic mechanism for carcinogenesis, with dichloroacetic acid promoting the development of a subclass of spontaneous tumours with CAA→CTA transversions.

In a subsequent study in male B6C3F$_1$ mice, dichloroacetic adid did not induce mutations in codons 12 or 13 of H-*ras* but induced liver tumours with mutations at codon 61 (Ferreira-Gonzalez *et al.*, 1995). Similar to the results of Anna *et al*. (1994), the incidence of tumours with mutations in codon 61 was not different from that of spontaneous liver tumours. In addition, dichloroacetic acid-induced tumours had a decreased incidence of CAA→AAA transversions.

In a more recent study, tumours induced in male B6C3F$_1$ mice by dichloroacetic acid at a concentration of 2 g/L were examined at 52 weeks of treatment, whereas those induced by 0.5 g/L were examined at 87 weeks of treatment to ensure a sufficient number of tumours. Concomitant control animals were used in these studies, but the spontaneous rate of tumour production was too small to provide adequate measurements of mutation frequency and spectra. A total of 64 dichloroacetic acid-induced tumours were evaluated: mutation frequencies in these tumours were significantly lower than those in historical controls (33% versus 56%). There was an excess of CTA mutations relative to control, consistent with earlier studies. However, most tumours contained less than 50% H-*ras* mutant sequences and only one was completely without a wild-type sequence, and the frequency of mutations appeared to increase with duration of treatment (or age) (Bull *et al.*, 2002). These data indicated that H-*ras* mutations were most probably a late event rather than an initiating event in dichloroacetic acid-induced liver carcinogenesis. Despite the lack of mutations, however, it was found that expression of H-*ras* was substantially and uniformly elevated in both spontaneous and dichloroacetic acid-induced tumours (Bull *et al.*, 2002). In addition, the lower mutation frequency observed in dichloroacetic acid-induced tumours was found to be consistent with tumours induced by other non-genotoxic carcinogens, which also have lower H-*ras* mutation frequency than spontaneous tumours (Fox *et al*., 1990; Hegi *et al*., 1993; Stanley *et al*., 1994).

The mutation spectrum in the H-*ras* oncogene in dichloroacetic acid-induced liver tumours in female B6C3F$_1$ mice has also been reported (Schroeder *et al*., 1997). Female B6C3F$_1$ mice were exposed to 1.5% acetic acid (control) or 3.5 g/L dichloroacetic acid in the drinking-water for 104 weeks. Only 1/22 (45%) tumours in the dichloroacetic acid-treated female mice had a mutation at codon 61 (Schroeder *et al.*, 1997), in contrast to the 62% in dichloroacetic acid-treated male mice (Anna *et al.*, 1994). The incidence of H-*ras* mutations in spontaneous tumours in control female mice was not determined (only one spontaneous tumour was available). Nevertheless, these data are consistent with the finding that female mice have a lower incidence of spontaneous liver tumours than male mice. Moreover, dichloroacetic acid-treated male mice have a higher percentage of carcinomas relative to adenomas and higher frequency of H-*ras* mutations in carcinomas compared with adenomas, suggesting that codon 61 mutations in H-*ras* may be late events in male mice (Anna *et al.*, 1994; Bull *et al.*, 2002).

Studies vary in their ability to detect a small initial increase in cell division within the liver after the beginning of treatment with dichloroacetic acid. In all cases, however, cell replication rates within the non-neoplastic portions of the liver were seen to decrease with chronic treatment (Carter *et al.*, 1995; Stauber & Bull, 1997; Bull, 2000). Decreased cell replication rates were paralleled by decreased rates of spontaneous apoptosis (Snyder *et al*., 1995). However, dichloroacetic acid significantly increased cell replication rates in a dose-dependent manner within foci and small tumours when the treatment used to induce the tumours (2 g/L) was followed by continued administration of doses ranging from 0.1 to 2 g/L for 2 weeks (Stauber & Bull, 1997). A parallel experiment demonstrated that the growth of tumours induced by a dose of 2 g/L, as measured *in situ* by magnetic resonance imaging, slowed to a rate not statistically different from zero when treatment with dichloroacetic acid was suspended (Miller *et al*., 2000). Dichloroacetic acid duplicated these effects by stimulating the growth of anchorage-independent colonies with the same phenotype from hepatocytes isolated from B6C3F$_1$ mice (Stauber *et al*., 1998; Kato-Weinstein *et al*., 2001a,b).

Several studies have documented changes in the expression of different genes following treatment with dichloroacetic acid. Lingohr *et al*. (2001) found that expression of insulin receptor was decreased in the liver of male B6C3F$_1$ mice following administration of dichloroacetic acid in the drinking-water at concentrations of 0.5 g/L and above for 2 and 10 weeks of treatment. Depressed expression of insulin receptor was also observed in hepatocytes derived from B6C3F$_1$ mice treated with dichloroacetic acid in culture (Lingohr *et al*., 2002). These changes were accompanied by substantial decreases in concentrations of serum insulin in the animal. Because the latent period for the development of the changes in serum insulin coincided with accumulation of glycogen in the liver, it was suggested that they may be indirectly mediated by changes in the regulation of carbohydrate metabolism. As insulin receptor concentrations in tumours induced by dichloroacetic acid remained elevated, this could provide these cells with a selective advantage over normal hepatocytes (Bull *et al.*, 2002; Lingohr *et al.*, 2002).

Increased expression of stearoyl-coenzyme A desaturase and depressed expression of α-1 protease inhibitor, cytochrome b5 and carboxylesterase were observed by an RNA differential display technique in the liver of mice treated with dichloroacetic acid at 2 g/L in drinking-water for 4 weeks (Thai *et al*., 2001). Increased expression of c-*jun* and c-*myc* in liver has also been observed in female mice treated with 500 mg/kg bw dichloroacetic acid by gavage (Tao *et al*., 2000b). The increases in c-*jun* and c-*myc* expression were associated with changes in the methylation status of the promoter regions of the respective genes in liver DNA (Tao *et al*., 2000a). Treatment with dichloroacetic acid at a concentration of 25 mmol/L (3.2 g/L) in drinking-water for 11 days was shown to produce hypomethylation of DNA in liver, but this effect disappeared with longer-term treatment (44 weeks) (Tao *et al*., 1998). However, the extent of methylation at 5-methylcytosine sites in DNA of liver tumours was reduced after chronic treatment with dichloroacetic acid, but returned to normal when treatment was suspended. Hypomethylation of DNA was associated with increased expression of c-*jun* and c-*myc* proto-oncogenes in dichloroacetate-promoted tumours when compared with surrounding non-tumorous liver (Tao *et al*., 2000a). The hypomethylation could be reversed by prior administration of methionine, suggesting that depletion of *S*-adenosyl-methionine may be responsible for the effect (Tao *et al*., 2000b). It appeared that hypomethylation of promotor region for the c-*myc* gene occurred in several tissues (liver, kidney and bladder) of mice and preceded cell replication within these tissues (Ge *et al*., 2001).

# 5. Summary of Data Reported and Evaluation

## 5.1 Exposure data

Dichloroacetic acid is used as an intermediate in the production of glyoxylic acid, dialkoxy and diaroxy acids, sulfonamides and iron chelates. It is used to a lesser extent as a cauterizing agent in dermatology. Wider exposure to dichloroacetic acid occurs at microgram-per-litre levels in drinking-water and swimming pools as a result of chlorination and chloramination.

## 5.2 Human carcinogenicity data

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

### 5.3 Animal carcinogenicity data

In eight studies, neutralized dichloroacetic acid administered in the drinking-water to male and/or female mice increased the incidences of hepatocellular adenomas and/or carcinomas. Following oral administration of dichloroacetic acid in the drinking-water to male rats, an increased incidence of hepatocellular carcinomas was found at a dose that decreased body weight and an increase in the combined incidence of adenomas and carcinomas was found at a lower dose. When administered in the drinking-water, dichloroacetic acid promoted hepatocellular carcinomas in carcinogen-initiated male and female mice in three studies.

### 5.4 Other relevant data

Dichloroacetic acid is metabolized to glyoxylic acid, which may be oxidized to oxalic acid, reduced to glycolic acid and transaminated to glycine. The metabolism of dichloroacetic acid to glyoxylic acid is catalysed by glutathione *S*-transferase zeta-1. Dichloroacetic acid is a mechanism-based inactivator of this enzyme, which decreases its own metabolism in humans or rats treated with the compound.

Clinically, administration of dichloroacetic acid for the treatment of congenital lactic acidosis has been associated with nervous system toxicity, which has also been observed in experimental animals. Animal studies have demonstrated toxic effects in the liver and testis. Treatment of rats with dichloroacetic acid has also given rise to developmental effects, primarily in the cardiovascular system. However, these have not been observed consistently.

Dichloroacetic acid produces a variety of effects on intermediary metabolism, including increases in hepatic glycogen at low concentrations ($\leq 1$ μM) in the blood and inhibition of pyruvate dehydrogenase kinase at higher concentrations ($\geq 100$ μM). Dichloroacetic acid also affects gene expression, including various proto-oncogenes and enzymes involved in lipid metabolism. In some cases, changes in gene expression have been associated with decreased methylation of DNA in the promoter region of the gene.

Studies on proto-oncogenes in mice have compared mutation induction in codon 61 of H-*ras* proto-oncogenes in hepatic tumours from dichloroacetic acid-treated mice and untreated mice. The spectrum of mutations showed a decrease in the frequency of CAA→AAA mutations and an increase in the frequency of mutations from CAA→CGA and from CAA→CTA in treated mice compared with controls. No loss of heterozygosity on chromosome 6 was observed in liver tumours promoted by dichloroacetic acid in female mice.

The evidence for induction of DNA strand breaks in liver cells of rodents exposed to dichloroacetic acid *in vivo* was inconclusive, as were the results of measurements of dichloroacetic acid-induced 8-hydroxydeoxyguanosine DNA adducts in mouse liver. Dichloroacetic acid caused a decrease in the level of 5-methylcytosine in DNA of liver cells and liver tumours of female mice. In peripheral blood cells of mice *in vivo*, dichloroacetic acid induced DNA damage in the single-cell gel electrophoresis assay. It caused mutations

(decrease in G:C→A:T and increase in mutations at T:A sites) in male transgenic mice harbouring the bacterial *lacI* gene. Dichloroacetic acid induced the formation of micronuclei *in vivo* in mouse polychromatic erythrocytes but not in rat bone-marrow cells.

DNA strand breaks were not induced in human or rodent cells *in vitro*. The results of assays for mutagenesis in bacteria and in mouse lymphoma cells were inconsistent. Dichloroacetic acid did not induce micronuclei in a mouse lymphoma cell line *in vitro* or in erythrocytes of newt larvae *in vivo*. It caused chromosomal aberrations in one of two studies *in vitro*. Dichloroacetic acid induced no aneuploidy in mouse lymphoma cells *in vitro*.

Dichloroacetic acid affects cell proliferation and cell death both in normal livers and tumours throughout the dose range that induces liver tumours in mice. These changes are associated with differential effects on intermediary metabolism in preneoplastic lesions versus normal liver and by changes in gene expression and DNA hypomethylation.

Dichloroacetic acid is genotoxic *in vivo* and *in vitro*. It also causes DNA hypomethylation *in vivo*. Thus, a genotoxic effect, possibly involving an indirect, epigenetic mechanism, may contribute to the carcinogenic mode of action of dichloroacetic acid.

### 5.5 Evaluation

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

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

### Overall evaluation

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

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