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Dichloroacetic Acid in Drinking-water

Background document for development of
WHO *Guidelines for Drinking-water Quality*

Preface

One of the primary goals of WHO and its member states is that "all people, whatever their stage of development and their social and economic conditions, have the right to have access to an adequate supply of safe drinking water." A major WHO function to achieve such goals is the responsibility "to propose ... regulations, and to make recommendations with respect to international health matters ...."

The first WHO document dealing specifically with public drinking-water quality was published in 1958 as *International Standards for Drinking-water*. It was subsequently revised in 1963 and in 1971 under the same title. In 1984–1985, the first edition of the WHO *Guidelines for Drinking-water Quality* (GDWQ) was published in three volumes: Volume 1, Recommendations; Volume 2, Health criteria and other supporting information; and Volume 3, Surveillance and control of community supplies. Second editions of these volumes were published in 1993, 1996 and 1997, respectively. Addenda to Volumes 1 and 2 of the second edition were published on selected chemicals in 1998 and on microbiological aspects in 2002. The third edition of the GDWQ was published in 2004, and the first addendum to the third edition was published in 2005.

The GDWQ are subject to a rolling revision process. Through this process, microbial, chemical and radiological aspects of drinking-water are subject to periodic review, and documentation related to aspects of protection and control of public drinking-water quality is accordingly prepared and updated.

Since the first edition of the GDWQ, WHO has published information on health criteria and other supporting information to the GDWQ, describing the approaches used in deriving guideline values and presenting critical reviews and evaluations of the effects on human health of the substances or contaminants of potential health concern in drinking-water. In the first and second editions, these constituted Volume 2 of the GDWQ. Since publication of the third edition, they comprise a series of free-standing monographs, including this one.

For each chemical contaminant or substance considered, a lead institution prepared a background document evaluating the risks for human health from exposure to the particular chemical in drinking-water. Institutions from Canada, Denmark, Finland, France, Germany, Italy, Japan, Netherlands, Norway, Poland, Sweden, United Kingdom and United States of America prepared the documents for the third edition and addenda.

Under the oversight of a group of coordinators each of whom was responsible for a group of chemicals considered in the GDWQ, the draft health criteria documents were submitted to a number of scientific institutions and selected experts for peer review. Comments were taken into consideration by the coordinators and authors. The draft documents were also released to the public domain for comment and submitted for final evaluation by expert meetings.

During the preparation of background documents and at expert meetings, careful consideration was given to information available in previous risk assessments carried

out by the International Programme on Chemical Safety, in its Environmental Health Criteria monographs and Concise International Chemical Assessment Documents, the International Agency for Research on Cancer, the Joint FAO/WHO Meetings on Pesticide Residues and the Joint FAO/WHO Expert Committee on Food Additives (which evaluates contaminants such as lead, cadmium, nitrate and nitrite, in addition to food additives).

Further up-to-date information on the GDWQ and the process of their development is available on the WHO Internet site and in the current edition of the GDWQ.

Acknowledgements

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The work of the following working group coordinators was crucial in the development of this document and others contributing to the first addendum to the third edition:

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The draft text was discussed at the Working Group Meeting for the first addendum to the third edition of the GDWQ, held on 17–21 May 2004. The final version of the document takes into consideration comments from both peer reviewers and the public. The input of those who provided comments and of participants in the meeting is gratefully acknowledged.

The WHO coordinator was Dr J. Bartram, Coordinator, Water Sanitation and Health Programme, WHO Headquarters. Ms C. Vickers provided a liaison with the International Chemical Safety Programme, WHO Headquarters. Mr Robert Bos, Water Sanitation and Health Programme, WHO Headquarters, provided input on pesticides added to drinking-water for public health purposes.

Ms Penny Ward provided invaluable administrative support at the Working Group Meeting and throughout the review and publication process. Ms Marla Sheffer of Ottawa, Canada, was responsible for the scientific editing of the document.

Many individuals from various countries contributed to the development of the GDWQ. The efforts of all who contributed to the preparation of this document and in particular those who provided peer or public domain review comment are greatly appreciated.

Acronyms and abbreviations used in the text

$BMDL_{10}$	lower-bound confidence limit on the benchmark dose associated with a 10% increase in response over background
CAS	Chemical Abstracts Service
DCA	dichloroacetic acid
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency (USA)
FAO	Food and Agriculture Organization of the United Nations
GDWQ	*Guidelines for Drinking-water Quality*
GSH	glutathione
GST	glutathione-*S*-transferase
ISO	International Organization for Standardization
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
NOAEL	no-observed-adverse-effect level
PPAR	peroxisome proliferator activated receptor
PQL	practical quantification level
THM	trihalomethane
USA	United States of America
WHO	World Health Organization

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1. GENERAL DESCRIPTION

1.1 Identity

CAS No.:	79-43-6
Molecular formula:	$Cl_2CHCOOH$

Dichloroacetic acid is also known as dichloroacetate, dichloroethanoic acid, and DCA.

1.2 Physicochemical properties[1] *(Verschueren, 1977; Weast, 1988; Budavari et al., 1989; HSDB, 2001)*

Property	*Value*
Boiling point (°C)	194
Melting point (°C)	13.5
Density (g/cm^3)	1.56 at 20 °C
Vapour pressure (Pa)	23.9 at 25 °C
Dissociation constant (pK_a) at 25 °C	1.26
Water solubility (g/litre)	86.3
Log octanol–water partition coefficient	0.92

1.3 Organoleptic properties

No information is available on the taste or odour threshold of dichloroacetic acid in water.

1.4 Major uses and sources in drinking-water

Chlorinated acetic acids, including dichloroacetic acid, are formed from organic material during water chlorination (Coleman et al., 1980; IPCS, 2000).

Dichloroacetic acid is used as a chemical intermediate in the synthesis of organic materials, as an ingredient in pharmaceuticals and medicines, as a topical astringent, and as a fungicide (Hawley, 1981; HSDB, 2001). Dichloroacetic acid has been used as a therapeutic agent to treat lactic acidosis, diabetes, and familial hyperlipidaemia in humans (Stacpoole et al., 1998a).

[1] Conversion factor in air: 1 ppm = 5.27 mg/m^3.

2. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

2.1 Air

No information is available on the concentrations of dichloroacetic acid in air. Dichloroacetic acid is not a volatile compound and is not expected to be present in air unless it is dissolved in atmospheric water vapour.

Reimann et al. (1996) reported dichloroacetic acid concentrations of 0.05–4 µg/litre measured in rainwater. Rainwater in Germany contained 1.35 µg of dichloroacetic acid per litre (IARC, 1995).

2.2 Water

IARC (1995) reported that chlorinated drinking-water in Japan contained 4.5 and 7.5 µg of dichloroacetic acid per litre and that a maximum concentration of 200 µg/litre was found for dichloroacetic acid in chlorinated water in Australia.

Data for drinking-water supplies in the USA indicate that dichloroacetic acid was detected in groundwater and surface water distribution systems at mean concentrations of 6.9 and 17 µg/litre, respectively. Concentrations ranged from <1.0 to 99 µg/litre in surface water distribution systems and from <1.0 to 71 µg/litre in groundwater systems (US EPA, 2001).

Dichloroacetic acid has also been detected in swimming pool water. In a German study of 15 indoor and 3 outdoor swimming pools (Clemens & Scholer, 1992), dichloroacetic acid concentrations averaged 5.6 µg/litre and 119.9 µg/litre in indoor and outdoor pools, respectively. The mean concentration of dichloroacetic acid in three indoor pools in the USA was 419 µg/litre (Kim & Weisel, 1998). The difference between the results of these two studies may be due to differences in the amounts of chlorine used to disinfect swimming pools, sample collection time relative to chlorination of the water, or addition or exchanges of water in the pools. The formation of dichloroacetic acid (and other haloacetic acids) in pools is discussed in Volume 2 of the WHO *Guidelines for Safe Recreational Water Environments* (WHO, in revision).

2.3 Food

Chlorine is used in food production and processing, including the following: disinfection of chicken in poultry plants; processing of seafood, poultry, and red meats; and oxidizing and bleaching in the flour industry. It is also used in sanitizing equipment and containers and in cooling heat-sterilized foods (US EPA, 1994). Therefore, dichloroacetic acid is likely to be found as a disinfection by-product in meat and other food products.

Reimann et al. (1996) examined the concentrations of dichloroacetic acid in a limited number of samples of several vegetables, fruits, grain, and beer. Dichloroacetic acid

concentrations ranged from <0.9 to 3.5 µg/kg in vegetables, from <0.6 to 11.1 µg/kg in grains, from 0.8 to 19.8 µg/kg in flours/breads, and from 1.5 to 15.2 µg/litre in beer. It was not detected in fruits or tomatoes. Raymer et al. (2001) found that dichloroacetic acid was stable in water during boiling and was taken up by foods during cooking in water. Carrots, green beans, pinto beans, and chicken were tested; uptake ranged from 11% for chicken to 85% for pinto beans.

2.4 Estimated total exposure and relative contribution of drinking-water

The available data are sufficient to demonstrate that food and water are relevant sources for exposure to dichloroacetic acid. The data are not adequate to quantify the contributions of each source for an overall assessment of exposure.

3. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

Dichloroacetic acid is rapidly absorbed into the bloodstream from the gastrointestinal tract in rats and mice (Stacpoole, 1987; James et al., 1998; Schultz et al., 1999, 2002) and via both the oral and dermal routes in humans (Kim & Weisel, 1998; Stacpoole et al., 1998a). It is initially distributed to liver and muscle and subsequently to other target organs (Evans, 1982; James et al., 1998).

In young adult rats administered a single radiolabelled gavage dose of 50 mg of sodium dichloroacetate per kg of body weight (42.4 mg of radiolabelled dichloroacetate per kg of body weight), the radioactivity present as a percentage of the administered dose was localized in the muscle (11.9%), liver (6.19%), gastrointestinal tract (3.74%), fat (3.87%), and kidney (0.53%). "Other tissues," including plasma, spleen, heart, skin, bone, brain, lung, and testes, accounted for 9.46% of the administered dose (James et al., 1998). In rats, dogs, and humans given single doses of sodium dichloroacetate intravenously, average half-lives of the parent compound in plasma were 2.97, 20.8, and 0.43 h, respectively. The apparent dose dependence in plasma clearance suggested that metabolic transformation becomes the rate-limiting step at high doses (Lukas et al., 1980).

Dichloroacetic acid is dechlorinated to glyoxylate and then oxidized to oxalate, all of which are excreted in the urine. Transamination of glyoxylate forms glycine and can distribute the label from dichloroacetic acid to urinary glycine conjugates, such as hippuric acid (Stacpoole, 1989; James et al., 1998; Stacpoole et al., 1998a). Some dichloroacetic acid is also converted to carbon dioxide and eliminated via expired air (James et al., 1998). Rats administered repeated high doses of dichloroacetic acid also eliminate unmetabolized compound (Gonzalez-Leon et al., 1997; Cornett et al., 1999).

Following a single oral dose of 50 mg/kg of body weight in humans, urinary excretion of unchanged dichloroacetate was negligible after 8 h, and cumulative excretion was less than 1% of the total dose in all subjects (Lukas et al., 1980). However, two human subjects who ingested drinking-water containing 4 or 6.3 µg of dichloroacetic acid per litre excreted 2–5% of the dose as unmodified dichloroacetic acid in the urine

shortly after exposure (Kim et al., 1999). The plasma elimination of dichloroacetic acid by rats was slowed by administration of a single prior dose of the acid, suggesting that dichloroacetic acid inhibits its own metabolism (James et al., 1997). The mean plasma half-life increased from 63.3 to 374 min in human volunteers after intravenous administration of five 50 mg/kg of body weight doses (Curry et al., 1985).

The enzyme that catalyses the GSH-dependent oxygenation of dichloroacetate has been identified as GST-zeta (Tong et al., 1998a, 1998b). GST-zeta is identical to maleylacetoacetate isomerase, an enzyme in the metabolic pathway for tyrosine catabolism (Fernandez-Canon & Penalva, 1998). There are interspecies and intraspecies differences in the activity of GST-zeta with dichloroacetic acid as a substrate (Tong et al., 1998a). Blackburn et al. (2001) identified four variant forms of the enzyme among a group of 128 Caucasian blood donors (GST-zeta 1a-1a, 1b-1b, 1c-1c, and 1d-1d). The most frequent human variant (1c-1c) identified by Blackburn et al. (2001) was found to be the least active in the metabolism of dichloroacetic acid (Tzeng et al., 2000), whereas the most active variant (1a-1a) had a low distribution in the population.

Dichloroacetic acid inhibition of GST-zeta appears to be the result of the formation of a covalent complex between GSH and dichloroacetic acid that can either dissociate, releasing glyoxylate as a product, or covalently bind to a nucleophilic residue in the enzyme, causing irreversible inhibition (Anderson et al., 1999). Using chlorofluoroacetate as a substrate, Lantum et al. (2002) found that, after inhibition, the 1a-1a variant retained 12% of its initial activity, whereas the 1b-1b, 1c-1c, and 1d-1d variants retained only 3–5% of their original activity.

4. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO *TEST SYSTEMS*

4.1 Acute exposure

Oral LD_{50}s of 4480 and 5520 mg of dichloroacetic acid per kg of body weight have been reported in rats and mice, respectively (Woodard et al., 1941). Acute effects included narcosis, with either death or complete recovery within 36 h. In another study on male rats, the LD_{50} was 2820 mg/kg of body weight, and the dermal LD_{50} was 0.1 ml/kg of body weight (about 795 mg/kg of body weight) (Smyth et al., 1951).

Groups of Long-Evans rats (10 per group) administered a single gavage dose of 0, 300, 1000, or 2000 mg of dichloroacetic acid per kg of body weight exhibited neurobehavioural toxicity 4–24 h following dosing, as indicated by decreased hind limb grip strength and decreased motor activity. Based on decreased grip strength, the lowest dose of 300 mg/kg of body weight was a LOAEL (Moser et al., 1999). These effects were reversible, with recovery occurring 7–14 days after dosing.

4.2 Short-term exposure

Increased liver weight and localized areas of liver necrosis were reported in male B6C3F1 and male and female Swiss-Webster mice treated with dichloroacetic acid in drinking-water for 14 days at concentrations of 1 or 2 g/litre (250 or 500 mg/kg of body weight per day); male B6C3F1 mice were also treated with 0.3 g/litre (75 mg/kg of body weight per day), and no effects on the liver were observed at this dose. Increased cell proliferation in the livers of male B6C3F1 mice occurred at 2 g/litre (500 mg/kg of body weight per day) after 5 but not 14 days (Sanchez & Bull, 1990).

Male B6C3F1 mice given dichloroacetic acid in drinking-water at concentrations of 0 or 0.1 to 3 g/litre (0 or 16 to 490 mg/kg of body weight per day) for up to 8 weeks showed significant dose-dependent increases in the glycogen content of the liver at concentrations of 0.5 g/litre (approximately 80 mg/kg of body weight per day) and higher (Kato-Weinstein et al., 1998).

Sprague-Dawley rats (10 per sex per dose) were administered sodium dichloroacetate by gavage at dose levels of 0, 125, 500, or 2000 mg/kg of body weight per day for 3 months. Two rats of each sex in the 2000 mg/kg of body weight per day group died during the study. The major signs of intoxication were hind limb paralysis and frequent urination. Effects included a dose-dependent decrease in body weight and increased relative weights of liver, kidneys, and adrenals at all dose levels. Brain and testes were the principal target organs; brain lesions, characterized by vacuolation of the myelinated white tracts resembling oedema, were observed in the cerebrum and cerebellum of treated rats of both sexes in all dose groups. Based on organ weight effects and brain lesions, the lowest dose tested, 125 mg/kg of body weight per day, was identified as a LOAEL (Katz et al., 1978, 1981).

Beagle dogs were given sodium dichloroacetate by capsule at 50, 75, or 100 mg/kg of body weight per day for 13 weeks. Effects included dose-dependent weight loss, a progressive depression in haematological parameters, and decreased mean blood glucose, lactate, and pyruvate levels at all dose levels. Histopathological effects included slight to moderate vacuolation of white myelinated tracts in the cerebrum and, to a lesser extent, in the cerebellum; an increased incidence of haemosiderin-laden Kupffer's cells in the liver and cystic mucosal hyperplasia in the gall bladder were observed at all dose levels. In this study, the lowest dose tested, 50 mg/kg of body weight per day, was the LOAEL (Katz et al., 1978, 1981).

Beagle dogs (five per sex per dose) received dichloroacetate in capsules at daily doses of 0, 12.5, 39.5, or 72 mg/kg of body weight per day for 90 days. At 72 mg/kg of body weight per day, effects included dyspnoea, partial paralysis of the hind limbs, and decreased erythrocyte count and haemoglobin levels. At 39.5 mg/kg of body weight per day and above, effects included decreased body weight gain in both sexes. At 12.5 mg/kg of body weight per day and above, effects included increased relative liver weights in males, conjunctivitis, and histopathology in the liver, kidney, pancreas, brain, and testes. Lesions included pale and discoloured kidneys; mild vacuolar change, inflammation, and haemosiderosis in the liver; chronic inflammation

and acinar degeneration in the pancreas; mild vacuolization of white myelinated tracts in the cerebrum and/or cerebellum; and testicular abnormalities. The LOAEL for this study was 12.5 mg/kg of body weight per day, the lowest dose tested (Cicmanec et al., 1991).

The neurobehavioural toxicity of dichloroacetic acid was examined in two age groups of rats (young adult and weanling), using two strains of rats (F344 and Long-Evans) and two routes of administration (drinking-water and gavage) for varying lengths of time (8 weeks to 24 months). Daily doses of dichloroacetic acid ranged from 16 to 308 mg/kg of body weight per day for drinking-water exposures and from 30 to 1000 mg/kg of body weight per day for gavage exposures. Gait abnormalities, described as uncoordinated placement of the hind limbs and hunched posture, were observed at daily doses as low as 16 mg/kg of body weight. At higher doses, other effects observed in both F344 and Long-Evans rats included deficits in the righting reflex, decreased hind limb grip strength, and mild tremors; ocular abnormalities and a unique chest-clasping response were observed only in F344 rats. The neurotoxicity was progressive with continued exposure and persisted for up to 2 years following high-dose exposures of 6 months. The neurotoxicity was most severe in F344 rats whose exposure in drinking-water began post-weaning. In general, F344 rats were more sensitive than Long-Evans rats, and weanlings appeared to be somewhat more sensitive than young adults. Histopathology showed that microscopic effects were limited to the central nervous system and were most severe in the spinal cord, which showed degeneration of the posterior columns accompanied by gliosis and loss of myelinated axons. Based on gait abnormalities in drinking-water studies of 12–13 weeks' duration, the LOAEL was 16 mg/kg of body weight per day, the lowest dose tested (Moser et al., 1999).

4.3 Long-term exposure

Male F344 rats (60–78 per group) were given dichloroacetic acid in drinking-water at concentrations of 0, 0.05, 0.5, or 5.0 g/litre for 100 weeks. Time-weighted average daily doses in the control, low, and middle dose groups were 0, 3.6, and 40.2 mg/kg of body weight, respectively, over the course of 100 weeks of treatment. In the high dose group, early signs of peripheral neuropathy resulted in a sequential lowering of the drinking-water concentration to 1.0 g/litre at 52 weeks. The neuropathy did not reverse or diminish, and, as a result, the animals were sacrificed at 60 weeks; the results of this dose group were excluded from the analysis. There was a mild increase in absolute and relative testis weights at 40.2 mg/kg of body weight per day. No liver necrosis was noted in any of the groups (DeAngelo et al., 1996).

In a second study using the same experimental protocol, male F344 rats (78 per group) were given 0 or 2.5 g of dichloroacetic acid per litre in drinking-water. Peripheral neuropathy in the treated group resulted in a sequential lowering of the dichloroacetic acid concentration to 1.0 g/litre at 26 weeks. Treatment at this level was continued to 103 weeks. The time-weighted average daily dose was 139 mg/kg of body weight. Final mean body weights of treated animals were significantly reduced to 73% of control values. Absolute testes weights were significantly decreased, but

there was no change in relative testes weights. Neither changes in other organ weights nor non-neoplastic liver lesions were observed at final sacrifice (DeAngelo et al., 1996).

Male B6C3F1 mice (30–71 per dose group) were administered dichloroacetic acid in drinking-water at concentrations of 0, 0.05, 0.5, 1, 2, or 3.5 g/litre (0, 8, 84, 168, 315, or 429 mg/kg of body weight per day) for 90–100 weeks. A dose-dependent increase in liver weight was seen at 26 and 52 weeks in all treatment groups evaluated for this end-point (84 mg/kg of body weight per day and higher), but only at the two highest dose levels at 100 weeks. At final sacrifice, mean body weights were significantly decreased, and absolute and relative liver weights were significantly increased at 315 mg/kg of body weight per day and higher. A dose-dependent increase in liver toxicity, as indicated by a significant increase in alanine aminotransferase and liver necrosis, was observed at a dose of 168 mg/kg of body weight per day and higher; significantly increased alanine aminotransferase activity was also observed with the 84 mg/kg of body weight per day dose. Hepatic peroxisome proliferation was increased in the highest dose group (DeAngelo et al., 1999).

Male B6C3F1 mice (50 per dose group) received dichloroacetate in their drinking-water at 0, 0.05, 0.5, 3.5, or 5.0 g/litre (0, 7.6, 77, 410, or 486 mg/kg of body weight per day) for 60 weeks. Other groups of mice received dichloroacetate at 7.6 or 77 mg/kg of body weight per day for 75 weeks. In the highest dose group, water consumption was reduced to 60% of that of controls. Body weight was decreased at the two highest dose levels, and relative liver weight was increased at the three highest dose levels. An increase in kidney weight was seen only at 410 mg/kg of body weight per day. No effects were seen on testes or spleen weight. The NOAEL for the 60- and 75-week studies was 7.6 mg/kg of body weight per day (DeAngelo et al., 1991).

4.4 Reproductive and developmental toxicity

Male Sprague-Dawley rats (eight per group) were given dichloroacetic acid by gavage at doses of 0, 18, 54, 160, 480, or 1440 mg/kg of body weight per day for 14 days and evaluated for reproductive tract toxicity. At 480 mg/kg of body weight per day and higher, epididymis weights were decreased. At 160 mg/kg of body weight per day and higher, the percentage of abnormal cauda sperm was significantly increased, and there was a statistically significant decrease in the percentage of motile sperm. At 54 mg/kg of body weight per day and higher, rats exhibited clear histopathological effects on spermiation indicative of spermatotoxicity, which increased in severity with increasing dose. Effects included altered spermiation, including retention of Step 19 spermatids, and atypical formation and resorption of residual bodies. At 18 mg/kg of body weight per day, two animals were judged by the authors to have mild increased retention of Step 19 spermatids; however, the statistical significance of this finding was not reported, and no data on control responses were given. Based on these results, 18 mg/kg of body weight per day was identified as a NOAEL (Linder et al., 1997).

Pregnant Long-Evans rats (20 per group) received dichloroacetic acid by oral gavage on gestation days 6–15 at doses of 0, 900, 1400, 1900, or 2400 mg/kg of body weight per day (first study) or 0, 14, 140, or 400 mg/kg of body weight per day (second study). At 1400 mg/kg of body weight per day and higher, dose-related mortality occurred in treated dams. At 140 mg/kg of body weight per day and higher, maternal body weight gain was significantly reduced. Significant dose-related increases in the relative liver weights of the dams were observed at all dose levels. At 900 mg/kg of body weight per day and above, post-implantation losses were significantly increased, and the number of live fetuses per litter was significantly reduced. No treatment-related effects were observed for pregnancy rates, the total number of implants per litter, or the frequency of pre-implantation losses. Dose-related decreases in fetal growth and increases in total soft tissue malformations occurred at 140 mg/kg of body weight per day and above. In this study, the maternal and developmental NOAELs were both 14 mg/kg of body weight per day. This was based on increased relative maternal liver weight and increased soft tissue abnormalities at 140 mg/kg of body weight per day (Smith et al., 1992).

Pregnant rats were administered gavage doses of dichloroacetate ranging from 1900 to 3500 mg/kg of body weight per day on specific 1- to 3-day periods during gestation in order to examine the effects of treatment during organogenesis. Reduced fetal body weight was observed in the offspring of dams exposed to 1900 mg/kg of body weight per day on gestation days 6–8. Fetal cardiac malformations were reported in the offspring of pregnant dams dosed at 1900 mg/kg of body weight per day on gestation days 9–11 and 12–15, at 2400 mg/kg of body weight per day on gestation days 10 or 12, and at 3500 mg/kg of body weight per day on gestation day 12 (Epstein et al., 1992). Collectively, these studies indicate a developmental LOAEL of 1900 mg/kg of body weight per day.

Saillenfait et al. (1995) studied groups of 10–20 explanted embryos from Sprague-Dawley rats cultured for 46 h in dichloroacetic acid solutions (0–10 mmol/litre). A significant, dose-dependent decrease in crown–rump length was seen at 3.5 mmol/litre and above, while significant, dose-related decreases in yolk sac diameter, head length, somite (embryonic segment) number, protein content, and DNA content were seen at 2.5 mmol/litre and above. In addition, several structural defects not seen in the control or lowest dose group were increased at the higher doses. Data for teratogenicity of dichloroacetic acid were considered to be equivocal in the frog embryo teratogenesis assay – *Xenopus* (Bantle et al., 1999).

4.5 Genotoxicity and related end-points

There have been numerous studies investigating the genotoxicity of dichloroacetic acid (summarized in US EPA, 2003). The results of most *in vitro* tests have been negative or equivocal, with or without metabolic activation. For example, negative or equivocal results were obtained in most reverse mutation tests in *Salmonella typhimurium*, in tests for DNA strand breakage in mammalian cells, and in most forward mutation tests in mouse lymphoma cells. One report indicated that dichloroacetic acid may increase prophage induction in *Escherichia coli* (DeMarini et

al., 1994); this finding has not been confirmed by other laboratories and required extremely high dichloroacetic acid concentrations to achieve significance. The results of *in vivo* studies have been mixed. No consistent pattern of positive or negative results for genotoxicity has been observed in the mouse micronucleus assay, in assays for DNA strand breaks in mouse or rat cells, or in assays for DNA adduct formation (Austin et al., 1996; Parrish et al., 1996; US EPA, 2003). Dichloroacetic acid was reported to induce both gene mutations and gross chromosomal aberrations in L5178Y mouse lymphoma cells *in vitro*, but the concentrations required to induce these effects were in the millimoles per litre range (Harrington-Brock et al., 1998).

Transgenic mice (Big Blue) were exposed to dichloroacetic acid in drinking-water at concentrations of 1 or 3.5 g/litre (approximately 190 or 665 mg/kg of body weight per day) for 60 weeks. After 4 or 10 weeks of treatment, neither concentration induced an increased frequency of mutations in the *lacI* gene; after 60 weeks, both concentrations induced a significantly elevated mutational frequency at this locus. In order to account for possible confounding by clonal expansion, the type of mutation (i.e., base substitutions) was analysed, and duplicate identical mutations in each animal were subtracted from the total number of mutations. Mutational frequencies in the *lacI* gene still differed significantly between treated and control mice after this adjustment (Leavitt et al., 1997).

4.6 Carcinogenicity

Male B6C3F1 mice were given dichloroacetic acid in drinking-water at 0 or 5 g/litre (approximately 0 or 1000 mg/kg of body weight per day) for 61 weeks. An increase in hepatocellular carcinomas was observed in 81% of treated animals (Herren-Freund et al., 1987).

Male B6C3F1 mice receiving dichloroacetic acid in drinking-water at 2 g/litre (300 mg/kg of body weight per day) for 52 weeks developed hepatocellular carcinomas; tumours were not observed in male mice administered 1 g/litre (140 mg/kg of body weight per day) for 52 weeks or 2 g/litre (280 mg/kg of body weight per day) for 37 weeks followed by a 15-week recovery period (Bull et al., 1990).

Male B6C3F1 mice given dichloroacetic acid in drinking-water at a concentration of 0 or 0.5 g/litre (0 or 88 mg/kg of body weight per day) for 104 weeks developed hepatocellular carcinomas in 63% of treated animals, compared with 10% in controls; hepatocellular adenomas in 42% of treated animals, compared with 5% in controls; and hyperplastic nodules in 8% of treated animals, compared with 0% in controls (Daniel et al., 1992).

Male B6C3F1 mice were administered dichloroacetic acid in drinking-water at concentrations of 0, 0.05, 0.5, 3.5, or 5.0 g/litre (0, 7.6, 77, 410, or 486 mg/kg of body weight per day) for 60 weeks. An increase in liver adenomas, carcinomas, and hyperplastic nodules was observed only in the two highest dose groups (DeAngelo et al., 1991).

Male B6C3F1 mice (35–71 per dose) were given dichloroacetic acid in drinking-water at concentrations of 0, 0.05, 0.5, 1.0, 2.0, or 3.5 g/litre (0, 8, 84, 168, 315, or 429 mg/kg of body weight per day, based on measured water consumption) for 90–100 weeks. Interim sacrifices were conducted at 26, 52, and 78 weeks in all dose groups except the lowest one. No hepatocellular tumours were observed in any group after 26 weeks of exposure. At 52 weeks, the incidence of hepatocellular carcinoma was significantly elevated in the two highest dose groups (20% and 50% of animals in the 2.0 and 3.5 g/litre groups, respectively, versus 0% in controls). At 78 weeks, the percentage of animals with this tumour had increased to 50% and 70% in the 2.0 and 3.5 g/litre groups, respectively, compared with a control rate of 10%. At study termination, the incidence of hepatocellular carcinoma was significantly elevated in the three highest dose groups, with 71%, 95%, and 100% of the animals, respectively, developing these tumours, compared with 26% of controls. Hepatic peroxisome proliferation (as measured by cyanide-insensitive palmitoyl coenzyme A oxidase) was significantly elevated only in the highest dose group after 26 weeks of exposure and was not increased at any time point in other dose groups. Hepatocyte proliferation (as measured by incorporation of radiolabelled thymidine) outside of proliferative lesions was not significantly different from control rates at any of the doses that produced tumours. The authors concluded that neither peroxisome proliferation nor hepatocyte proliferation was associated with the induction of liver cancer in these mice (DeAngelo et al., 1999).

Carter et al. (2003) examined the histology slides from the DeAngelo et al. (1999) study. The slides were examined independently by two individuals for the presence of altered hepatic foci, large foci of cellular alteration, adenomas, and carcinomas. The investigators were blind to the dose and time of sacrifice of the animals. Lesions were subcategorized as eosinophilic, dysplastic, and basophilic and/or clear cell. After all of the slides were characterized, they were arrayed by dose and time of sacrifice to determine if there was a pattern in the progression to tumours. Several separate patterns were observed. Eosinophilic cells seemed to progress from altered hepatic foci to eosinophilic adenomas and carcinomas. Basophilic or clear cells progressed either from altered hepatic foci to large foci of cellular alteration to carcinomas or from large foci of cellular alteration to adenomas and carcinomas. Dysplastic cells progressed from altered hepatic foci to carcinomas.

In this same study (Carter et al., 2003), the tissues were also examined for the relationship between necrosis, glycogen accumulation, cytomegaly, accumulation of lipid droplets, atypical nuclei, and enlarged nuclei and tumours. The strongest dose–response correlation was noted for cytomegaly and, to a lesser extent, for atypical nuclei.

Female B6C3F1 mice administered 2.0 g of dichloroacetic acid per litre in drinking-water for 52 weeks did not develop liver tumours (Bull et al., 1990). On the other hand, in female B6C3F1 mice given dichloroacetic acid in drinking-water at concentrations of 0, 0.5, or 3.5 g/litre (0, 77, or 410 mg/kg of body weight per day) for 104 weeks, liver tumours were observed in all animals in the highest dose group (US EPA, 1991).

Female mice (40–90 per dose) were administered dichloroacetic acid in drinking-water at concentrations of 0, 0.26, 0.86, or 2.6 g/litre (reported to be 0, 40, 115, or 330 mg/kg of body weight per day) for 51 or 82 weeks. Increased incidences of adenomas and altered hepatocyte foci were observed in the highest dose group after 51 weeks and in the two highest dose groups after 82 weeks. After 51 weeks at 330 mg/kg of body weight per day, 40% of animals exhibited altered foci and 35% had adenomas. After 82 weeks at 115 mg/kg of body weight per day, 39.3% of animals showed altered foci and 25% had developed liver adenomas; after 82 weeks at 330 mg/kg of body weight per day, 89.5% of animals had altered hepatocyte foci and 84.2% had adenomas. A statistically significant increase in the percentage of animals with liver carcinoma (26.3%) was observed only in the highest dose group after 82 weeks of exposure. The total yield of lesions (altered hepatocyte foci, hepatocellular adenomas, or hepatocellular carcinomas) was statistically increased in the high-dose group at 51 weeks (40% compared with 0% in controls) and in the mid- and high-dose groups at 82 weeks (39.3% in mid-dose group, 89.5% in high-dose group, compared with 11.1% in controls). The dose–response relationship between drinking-water concentrations of dichloroacetic acid and the yield of liver tumours and altered hepatocyte foci was described by the author as being suggestive of non-linearity (Pereira, 1996).

Male Fischer 344 rats were administered time-weighted average concentrations of 0, 0.05, 0.5, or 2.4 g of dichloroacetic acid per litre (0, 4, 40, or 296 mg/kg of body weight per day) in drinking-water, followed by sacrifice at intervals for up to 104 weeks. No hepatoproliferative lesions were seen in the 4 mg/kg of body weight per day group, and the negative control group had only 4% hepatic adenomas. The 40 mg/kg of body weight per day group had 10% hyperplastic nodules, 21% hepatic adenomas, and 10% hepatocarcinomas after 104 weeks. The 296 mg/kg of body weight per day group had 70% hyperplastic nodules, 26% hepatic adenomas, and 4% hepatocarcinomas at terminal sacrifice at 60 weeks (Richmond et al., 1995).

Male F344 rats (60–78 per group) were given dichloroacetic acid in drinking-water at concentrations of 0, 0.05, 0.5, or 5.0 g/litre. Animals in the highest dose group developed early signs of peripheral neuropathy that were not reversed or diminished by a sequential lowering of the drinking-water concentration of dichloroacetic acid; these animals were sacrificed at 60 weeks, and the results of this dose group were excluded from the analysis. Time-weighted average daily doses in the remaining groups were 0, 3.6, or 40.2 mg/kg of body weight over the course of 100 weeks of treatment. At 40.2 mg/kg of body weight per day, the incidence of combined hepatocellular adenomas and carcinomas was 24.1% in treated animals, compared with 4.4% of controls. Total proliferative lesions (combined neoplasms and hyperplastic nodules) were observed in 34.9% of animals in this dose group, compared with 8.7% of controls. No liver histopathology was observed at 3.6 mg/kg of body weight per day (DeAngelo et al., 1996).

In a second study using the same experimental protocol, male F344 rats (78 per group) were given drinking-water containing dichloroacetic acid at concentrations of

0 or 2.5 g/litre. Peripheral neuropathy in the treated group resulted in a sequential lowering of the dichloroacetic acid concentration to 1.0 g/litre at 26 weeks, and treatment was continued to 103 weeks. The time-weighted average doses were 0 or 139 mg/kg of body weight per day. Hepatocellular carcinomas were observed in 21.4% of treated animals, compared with 3% of controls; combined hepatocellular adenomas and carcinomas were found in 28.6% of treated animals, compared with 3.0% of controls. Proliferative lesions were observed in 32.1% of treated animals, compared with 6.1% of controls (DeAngelo et al., 1996).

A number of mechanistic bioassays have shown that altered hepatic foci and hepatocellular tumours initiated or promoted by treatment with dichloroacetic acid are eosinophilic and contain GST-pi (Pereira & Phelps, 1996). Altered hepatic foci and hepatocellular tumours exhibit differences in the mutational spectra of *K*- and *H-ras* proto-oncogenes, compared with spontaneously occurring tumours (Anna et al., 1994; Ferreira-Gonzalez et al., 1995); do not show loss of heterozygosity on chromosome 6 (Tao et al., 1996); and selectively stimulate the replication rate of different populations of immunoreactive cells (Latendresse & Pereira, 1997; Stauber & Bull, 1997).

There are two reports of gene array analysis of liver cells from mice treated with 2 g of dichloroacetic acid per litre for 4 weeks (Thai et al., 2001, 2003). Both reports involve the same tissue samples. The three different gene arrays tested displayed differences between control tissues and those from exposed mice. Not all of the genes affected were identified. Those that were affected fell into three groupings according to the authors: genes involved with tissue remodelling and/or angiogenesis, damage response, and xenobiotic metabolism. In most cases, gene expression was suppressed. The PPAR-alpha gene was present on one of the gene arrays and was not found to be activated by the dose of dichloroacetic acid used.

The information available on the mechanisms of dichloroacetic acid-induced liver tumorigenesis in rodents is not sufficient to identify a single mode of action leading to cancer. It is possible that multiple mechanistic pathways are involved in dichloroacetic acid-induced rodent hepatocarcinogenicity and that these pathways are dose-dependent or species-specific.

5. EFFECTS ON HUMANS

Dichloroacetic acid has been used as a therapeutic agent to treat lactic acidosis, diabetes, and familial hyperlipidaemia in humans; oral or intravenous therapeutic doses are usually in the range of 25–50 mg/kg of body weight per day (Stacpoole et al., 1998a). Biochemical effects of dichloroacetate treatment include significantly reduced fasting blood glucose levels, marked decreases in plasma lactate and alanine, significantly decreased plasma cholesterol levels, decreased triglyceride levels, elevated plasma ketone bodies, and elevated serum uric acid levels (Stacpoole et al., 1978). Approximately 50% of patients receiving 25–50 mg/kg of body weight per day experience anxiolytic or sedative effects following oral, intravenous, or repeated dosing regimens. These effects usually occur within 60 min of dichloroacetic acid

treatment, may last several hours, and appear to be unrelated to gender, age, or route of administration (Stacpoole et al., 1998a).

Several cases of mild peripheral neuropathy following dichloroacetic acid treatment at 50–100 mg/kg of body weight per day for several months to a year have been reported (Stacpoole et al., 1998a; Spruijt et al., 2001). All were completely reversible after cessation of treatment. In one of these cases, dichloroacetic acid was reinstituted at 25 mg/kg of body weight per day following reversal of neurological symptoms, and this dose was maintained for 2 years without further evidence of neuropathy (Stacpoole et al., 1998a). Two children with congenital lactic acidosis were treated orally with 25–75 mg of dichloroacetic acid per kg of body weight per day for several months; they showed a 2-fold increase in serum transaminases, suggesting the possibility of preclinical hepatic toxicity. This increase was reversible after treatment ended (Stacpoole et al., 1998a).

Two young males were administered daily oral doses of 50 mg of dichloroacetate per kg of body weight to treat severe familial hypercholesterolaemia. Total serum cholesterol levels decreased significantly in both patients (Moore et al., 1979). No adverse clinical or laboratory symptoms were detected in one patient, but the second complained of tingling in his fingers and toes after 16 weeks. Physical examination revealed slight decreases in the strength of facial and finger muscles, diminished to absent tendon reflexes, and decreased strength in all muscle groups of the lower extremities. Electromyographic studies showed denervation changes in foot and leg muscles. Mild slowing of conduction velocity was noted in both posterior tibial nerves, and no measurable response was obtained in the peroneal or orbital nerves. Six months after discontinuation of the treatment, the observed peripheral neuropathy had improved, although serum cholesterol returned to high levels (Stacpoole et al., 1979).

To date, there have been no reports of dichloroacetic acid-induced neoplasia in any human tissue and no reports of gonadal toxicity in humans (Stacpoole et al., 1998a). However, dichloroacetic acid is currently being used only in the treatment of lactic acidosis, and mortality among this population is high, at about 20% per year (Stacpoole et al., 1998b), providing a limited opportunity to observe the effects of chronic exposures.

6. PRACTICAL ASPECTS

6.1 Analytical methods and analytical achievability

The chloroacetic acids can be detected in water by EPA Method 552.1 (US EPA, 1992), EPA Method 552.2 (US EPA, 1995), or Standard Method 6251B (APHA et al., 1998). In EPA Method 552.1, the haloacetic acids are extracted on a miniature anion exchange column and converted to methyl esters in the eluant prior to analysis (US EPA, 1992). EPA Method 552.2 involves a liquid–liquid extraction procedure, after which the acetic acids are converted to methyl esters (US EPA, 1995). Both EPA methods use gas chromatography and electron capture detection. Standard Method

6251B uses a micro liquid–liquid extraction procedure combined with gas chromatography and electron capture detection (APHA et al., 1998). Method detection limits range from <0.1 to 0.4 µg/litre. The PQL for dichloroacetic acid is approximately 1 µg/litre (P. Fair, personal communication). There is no ISO method for chloroacetic acids.

6.2 Treatment and control methods and technical achievability

Although it is technically feasible to remove dichloroacetic acid (and other disinfection by-products) prior to distribution, this is the least attractive option for controlling dichloroacetic acid concentrations.

Controlling coagulation to remove organic carbon prior to chlorination can reduce dichloroacetic acid concentrations. Increasing the coagulant dose can give enhanced removal of organic precursors (Hartman et al., 1991). However, the pH needs to be controlled; otherwise, the lower pH associated with higher coagulant doses can lead to increased dichloroacetic acid concentrations (Dixon & Lee, 1991). Some control of dichloroacetic acid concentrations can be achieved by increasing the pH at which chlorination is carried out, but this may lead to increased formation of THMs (Singer et al., 1995; Nikolaou et al., 1999).

Lower dichloroacetic acid concentrations can be achieved by using alternatives to chlorine for disinfection. Plants using ozone followed by chloramine were found to produce lower dichloroacetic acid concentrations than those using free chlorine (Nissinen et al., 2002).

Granular activated carbon can be used to obtain greater than 80% removal of dichloroacetic acid (Lykins et al., 1991); however, removal of dichloroacetic acid, once formed, is unlikely to be the preferred method of control.

7. GUIDELINE VALUE

Dichloroacetic acid exposure is associated with both tumorigenic and non-tumorigenic health effects in humans and laboratory animals. The data from humans are primarily the product of the use of dichloroacetic acid in the treatment of patients suffering from hereditary lactic acidosis (Stacpoole et al., 1998a). They are, accordingly, not suitable for deriving risk-based values for a healthy population. A subchronic study by Cicmanec et al. (1991) found adverse effects on the liver, testes, and nervous system in groups of five male and female dogs at doses as low as 12.5 mg/kg of body weight per day. The hepatic and nervous system effects are consistent with some of the observations from the human clinical reports (Stacpoole et al., 1998a, 1998b) and are supported by data from animal studies (Katz et al., 1978, 1981; Moser et al., 1999). At higher doses (≥140 mg/kg of body weight per day), dose-related decreases in fetal growth and increases in total soft tissue malformations were observed in pregnant Long-Evans rats (Smith et al., 1992). Effects on sperm in rats were reported by Linder et al. (1997).

Dichloroacetic acid has been observed to be tumorigenic in rats and mice by a number of researchers (Herren-Freund et al., 1987; Bull et al., 1990; Daniel et al., 1992; Richmond et al., 1995; DeAngelo et al., 1996, 1999; Pereira, 1996). Genotoxicity data are considered to be inconclusive, particularly at lower doses. Glycogen deposition, peroxisome proliferation, changes in signal transduction pathways, and DNA hypomethylation have all been observed following dichloroacetic acid exposure and have been hypothesized to be involved in its carcinogenicity. However, the available data are not sufficient to establish a cancer mode of action with reasonable certainty, especially at the very low exposure levels expected to apply to humans ingesting chlorinated drinking-water. Recent data from Carter et al. (2003) suggest that there may be more than one mechanism leading to tumours, since altered hepatic foci from treated mice were found to have three different types of cellular characteristics.

IARC (2002) recently reclassified dichloroacetic acid as Group 2B (possibly carcinogenic to humans), based on the absence of data on human carcinogenicity and sufficient evidence of its carcinogenicity in experimental animals. This classification replaced an earlier Group 3 classification (not classifiable as to its carcinogenicity in humans) from 1995.

The tumour prevalence data from male mice (DeAngelo et al., 1999) were used to quantify the cancer risk from dichloroacetic acid. The combined data for carcinomas and adenomas in male B6C3F1 mice exposed to doses of 0, 8, 84, 168, 315, or 429 mg/kg of body weight per day for up to 2 years were plotted using the US EPA's Benchmark Dose software version 1.3.1. The slope factor of 0.0075 (mg/kg of body weight per day)$^{-1}$ was derived from the $BMDL_{10}$ using a linear multistage model of the dose–response data. If it is assumed that a 60-kg person ingests 2 litres of water per day, the concentration of dichloroacetic acid in drinking-water associated with upper-bound excess lifetime cancer risks of 10^{-4}, 10^{-5}, and 10^{-6} are 400, 40, and 4 μg/litre, respectively.

The concentration associated with a 10^{-5} upper-bound excess lifetime cancer risk is usually identified as the health-based guideline for drinking-water when the contaminant is a carcinogen. However, it may not be possible to provide for adequate disinfection treatment of potable water and maintain dichloroacetic acid at levels of 40 μg/litre or less. Accordingly, the guideline value is provisionally established as 50 μg/litre. The guideline value is designated as provisional because the data on treatment are insufficient to ensure that the 40 μg/litre value is technically achievable under a wide range of circumstances. Difficulties in meeting a guideline value must never be a reason to compromise adequate disinfection. It should be possible to achieve a dichloroacetic acid concentration at or below the 50 μg/litre provisional guideline value by appropriate control of the water treatment processes.

8. REFERENCES

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