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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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Dichloro-acetic acid and sodium dichloro-acetate

No. 188 b

CAS No. 79-43-6
CAS-No. 2156-56-1

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Dichloroacetic acid and sodium dichloroacetate

In addition to the present Toxicological Evaluation on dichloroacetic acid (No. 188 b), a Toxicological Evaluation on dichloroacetyl chloride (No. 188 a) is also available and can be consulted for comparison. Dichloroacetyl chloride hydrolyses in aqueous media to yield dichloroacetic acid and therefore the absorptive effects of the chloride are essentially attributable to dichloroacetic acid. The available body of data on dichloroacetyl chloride is considerably less extensive.

1 Summary and assessment

Dichloroacetic acid is absorbed from the gastrointestinal tract following oral administration. The maximum blood concentrations and the areas under the concentration-time curves (AUC values) are markedly higher in rats than in comparably dosed mice, whereas the half-lives (approx. 2 to 3 hours after single-dose administration) do not differ significantly and the values for clearance and volume of distribution are markedly higher in mice than in rats. Remarkably, repeated administration, when compared with single-dose administration, is associated with the same volume of distribution but a marked increase in the AUC and half-life values in conjunction with lower systemic and metabolic clearance rates, resulting in 8 to 10-fold higher blood levels than after single-dose administration. This effect is observed even when only very small amounts are administered prior to bolus administration. This cumulative property of dichloroacetic acid is reflected in the results from the toxicity studies of the chemical discussed below, which showed a steep dose-response relationship and cumulative effects in the target tissues after longer-term administration. It has been suggested that dichloroacetic acid inhibits metabolic enzymes and in doing so exerts an autoinhibitory effect on its own metabolism. Cytochrome P450-dependent metabolism of dichloroacetic acid leads to the formation of glycolic acid, glyoxylic acid, oxalic acid, acetic acid, thiodiacetic acid, thiodiglycolic acid, traces of monochloroacetic acid and carbon dioxide. Unchanged dichloroacetic acid appears in the urine only in small amounts after single administration. Part of the glycolic acid is channelled into the anabolic metabolism and undergoes transamination to the amino acid glycine, which in

turn is metabolised to another amino acid, serine. Excretion occurs predominantly via the exhaled air and the urine. Only small amounts are excreted in the faeces. Following repeated administration, excretion as carbon dioxide in the exhaled air and renal excretion of oxalic acid both decrease, whilst renal excretion of the unmetabolised compound and its metabolites glycolic acid, glyoxylic acid, monochloroacetic acid and acetic acid increases.

The acute toxicity of dichloroacetic acid after oral administration, inhalation and also intravenous injection is low (LD_{50} rat and mouse oral and intravenous > 2000 mg/kg body weight; no increased mortality in the 8-hour inhalation hazard test in the rat). The only acute dermal test in the rabbit yielded an LD_{50} of approx. 798 mg (0.51 ml)/kg body weight. Administration of dichloroacetic acid to rats in their drinking water for 14 days at dose levels of up to 600 mg/kg body weight (dichloroacetic acid levels of up to 1.875 g/l) causes an acid load which the kidneys attempt to compensate for by increasing ammonia production and the activity of the enzymes involved in the process. In rats and mice, acute and subacute oral administration results in hepatic peroxisome proliferation and lipid peroxidation, and liver weights are increased.

There are no skin or eye irritation studies on dichloroacetic acid that comply with the current guidelines for testing. Dichloroacetic acid was corrosive to both the skin and eyes in older exploratory studies.

In subchronic studies in the rat and dog, oral administration of sodium dichloroacetate or NaOH-neutralised dichloroacetic acid by gavage or in food, drinking water or gelatine capsules (dogs) caused extensive damage to various organ systems. Even the lowest dose levels tested, 12.5 mg/kg body weight/day in the dog (administered in gelatine capsules) and approx. 16 mg/kg body weight/day in the rat (administered in drinking water in a specific 12-week neurotoxicity study), caused nonreversible degenerative CNS lesions. The dose-related lesions in the CNS manifested clinically as hindlimb paralysis and histopathologically as vacuolisation of the white myelinated tracts of the cerebrum, cerebellum and/or spinal cord. The peripheral nervous system was free of histopathological changes, as was the optical nerve. Dose-related testicular damage, as characterised by small testes, degeneration of the germinal epithelium and syncytial giant cell formation, enlarged Sertoli cells, atrophy of the seminiferous tubules and

absence of spermatogonia and spermatozoa in the testes and epididymides, also occurred in the dog, where prostate glandular atrophy was additionally noted, after subchronic administration of as little as 12.5 mg/kg body weight/day and in the rat at higher dose levels of 500 mg/kg body weight and above (by gavage). Kidney weights were increased at dose levels from 35.5 mg/kg body weight/day in the rat and 39.5 mg/kg body weight/day in the dog (administered in drinking water and gelatine capsules, respectively). Diffuse degenerative lesions of the renal tubular epithelium and the glomeruli were noted in the rat after administration of 345 mg/kg body weight/day (in drinking water). In the liver of the rat, mouse and dog, dichloroacetic acid dose-dependently also induced lesions other than those already mentioned (increased organ weights, lipid peroxidation and peroxisomal proliferation). These included marked hepatocellular cytomegaly with glycogen accumulations, haemosiderin-laden Kupffer cells, cytoplasmic vacuolation, bile duct dilation and, in the mouse only, focal necrosis. Serum liver enzymes were elevated as a consequence of hepatotoxicity. Dichloroacetic acid-induced lesions seen only in one of two subchronic studies in the dog, but even in the 50 mg/kg/day dose group, included lenticular opacities with conjunctival hyperaemia and superficial corneal vascularisation as well as signs of keratoconjunctivitis sicca. Another finding also observed only in the dog, and again only in one of the two available subchronic studies, was that males exhibited thymic atrophy, characterised by a depletion of lymphoid tissue, after administration of 72 mg/kg body weight/day. In addition, some studies in rats have also reported increased organ weights for the adrenal glands and spleen in the absence of a histopathological correlate. Changes in clinical chemistry and haematology parameters in the rat and dog (*inter alia* decreases in glucose and lactate levels, red blood cell counts and/or haemoglobin and haematocrit values, increased activity levels of lactate dehydrogenase, alkaline phosphatase and alanine and aspartate aminotransferase, depressed protein levels), some of which were found to occur even at the lowest dose levels tested, proved to be reversible in the recovery groups. A level of 50 ppm (equivalent to 3.9 mg/kg body weight/day), the lowest concentration tested in a subchronic drinking water study, can be considered the *low observed effect level* (LOEL) in the rat, with depressed total serum protein being the only finding observed in that study. It is not possible on the basis of the available subchronic studies to determine a *no observed effect level* (NOEL) or a *no observed adverse effect level* (NOAEL) for the dog, because even the

lowest dose administered, 12.5 mg/kg body weight/day, was noted to cause degenerative changes in brain and testes in males.

The mutagenic activity of dichloroacetic acid and sodium dichloroacetate has been investigated in vitro in Salmonella/microsome assays using *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535, TA 1537, TA 1538 and/or TA 2638, in tests on Escherichia coli WP2 strains, in the HPRT test on Chinese hamster lung fibroblasts (V79) and in the L5178Y/TK test on mouse lymphoma cells (L5178Y/TK$^{+/-}$-3.7.2C). The majority of studies gave no indication of mutagenic activity in the absence or presence of metabolic activation. Strain TA 100, however, gave positive results in the Salmonella/microsome test, performed as a preincubation test and as a test modified for volatile substances. No consistent activity was noted in the L5178Y/TK test. Whereas a test that was carried out with neutralised dichloroacetic acid of ≥ 99.5% purity according to a standardised and validated protocol produced a negative result, another test performed with non-neutralised dichloroacetic acid of unknown purity in the absence of metabolic activation gave a positive result. In-vitro chromosome aberration studies of sodium dichloroacetate (≥ 99.5% pure) in Chinese hamster CHO cells and of non-neutralised dichloroacetic acid (99.5% pure) in Chinese hamster V79 cells gave no indication of any chromosome-damaging potential of the compounds either in the absence or in the presence of metabolic activation. In contrast, chromosome aberration tests performed on Chinese hamster CHL cells and mouse lymphoma cells (L5178Y/TK$^{+/-}$-3.7.2C) with non-neutralised dichloroacetic acid of unknown purity were positive in the absence of metabolic activation. Both the in-vitro micronucleus test in mouse lymphoma cells (L5178Y/TK$^{+/-}$-3.7.2C) and the in-vivo micronucleus tests in CR:CD rats given three intravenous doses of up to 1100 mg/kg body weight and Swiss-Webster mice given two oral administrations of up to 4500 mg/kg body weight showed no indication of chromosome-damaging activity of dichloroacetic acid or sodium dichloroacetate. Inconstant findings were obtained in the male B6C3F1 mouse in a series of micronucleus tests with examination of erythrocytes from the peripheral blood. The test series was conducted with drinking water (adjusted to pH 6.8 to 7.4 with NaOH) containing dichloroacetic acid (> 99% pure) at 0.5, 1, 2 or 3.5 g/l for up to 31 weeks. The treatment induced increases in micronucleated polychromatic erythrocytes in the top dose group to maximum levels of 200% of the relevant controls at several scheduled assessments. The in-

vestigators conducting the test series suggested that the slight increase in frequency of micronuclei could also have been due to systemic toxicity of the administered doses. Dichloroacetic acid did not induce DNA damage in an in-vitro DNA repair test performed only in the absence of metabolic activation on the DNA repair-proficient *Salmonella typhimurium* strain hisGr and the repair-deficient strains TS24recA, TA 2322 polA and TA 1950 uvrB. In-vitro test systems utilising the induction of an SOS response as a parameter for a DNA-damaging effect (the umu test on *Salmonella typhimurium* TA 1535/pSK 1002, the SOS chromotest on *Escherichia coli* PQ37 and the prophage lambda induction test on *Escherichia coli* WP2 lambda) yielded weakly positive results with or without metabolic activation. Dichloroacetic acid was also evaluated as weakly positive in an in-vitro SCE test on Chinese hamster ovary (CHO) cells in which there was a slight but concentration-dependent, significant increase in the number of SCEs/cell at the very high concentration of 2000 µg/ml of NaOH-neutralised dichloroacetic acid in the absence of metabolic activation. According to a report which gives no further details, an in-vivo SCE test performed by the same investigators produced a negative result in the mouse. Neutralised dichloroacetic acid induced no DNA single-strand breaks in primary rat and mouse hepatocytes or in human lymphoblasts (CCRF-CEM cells) in the alkaline elution test (“alkaline unwinding assay”). The positive results that one research team obtained in the in-vivo alkaline elution test in the rat and mouse were not reproducible in the hands of another group, although the latter treated both species at even higher dose levels. In the modified alkaline elution test (“alkaline single cell gel electrophoresis assay”) with subacute oral administration, analysis of leucocyte DNA yielded no indication of DNA strand break induction or alkali-labile lesions or of incomplete DNA repair. As DNA migration in the electric field was decreased in this test, the investigators suspected the formation of cross-links. The sperm-head abnormalities and the decreases in epididymal sperm count observed after subacute administration to mice were more likely, in the opinion of the authors of the study, to be attributable to the compound having a nonspecific toxic effect on the testes rather than a genotoxic effect. A study in transgenic male Big Blue B6C3F1 mice harbouring the *Escherichia coli* lacI gene showed slight increases in mutation frequency. Overall, dichloroacetic acid and sodium dichloroacetate appear to have at most a weak genotoxic potential.

Dichloroacetic acid is a hepatocarcinogen. The hepatocarcinogenic activity of dichloroacetic acid has been investigated in numerous individual studies conducted in the male and female B6C3F1 mouse and the male F344 rat. Dichloroacetic acid, which was always administered in the NaOH-neutralised form – i.e. as the sodium salt – in drinking water, induced dose- and duration of treatment-dependent foci of altered hepatocytes, hyperplastic nodules, adenomas and carcinomas. Dichloroacetic acid acted as a tumour promoter in an initiation-promotion study in female B6C3F1 mice, as evidenced by lower effective dose levels and higher incidences of tumours and/or higher malignancy of the tumours at the same administered dose when compared with studies without prior initiation. When the carcinoma-inducing dose of dichloroacetic acid was discontinued at a stage where female B6C3F1 mice developed only hyperplastic nodules and adenomas but no carcinomas (after 31 weeks), the hyperplastic nodules and adenomas regressed (21-week recovery period). The *no observed effect level* (NOEL) for the hepatocarcinogenicity of dichloroacetic acid administered in drinking water is 0.05 g/l (approx. 3.6 mg/kg body weight/day) in the male F344 rat treated for 100 or 103 weeks while it is 0.5 g/l (approx. 94 mg/kg body weight/day) in the female B6C3F1 mouse treated for 104 weeks. For the male B6C3F1 mouse, the NOEL is below 0.05 g/l (approx. 8 mg/kg body weight/day) and 0.5 g/l (approx. 77 mg/kg body weight/day) for treatment periods of 100 weeks and 75 weeks, respectively. Although frequently investigated, the mechanism by which dichloroacetic acid elicits its hepatocarcinogenic effect has not been fully elucidated so far. Peroxidative processes – measured as lipid peroxidation and peroxisome proliferation –, which can cause oxidative cellular damage with subsequent reparative hyperplasia followed by the clonal expansion of initiated cells, were found only in dose ranges considerably higher than those associated with hepatocarcinogenicity and did not correlate with the neoplastic hepatic lesions. Dichloroacetic acid is even capable of inducing very marked hepatocyte mitoinhibition. The findings regarding the extent to which genotoxic processes are involved in its hepatocarcinogenicity are equivocal. The majority of mechanistic studies, however, assume that dichloroacetic exerts only a very weak genotoxic effect, if any. It appears from the available sequence data for polymerase chain reaction-amplified DNA and RNA that neither the activation of K-ras or H-ras proto-oncogenes nor the inhibition of tumour suppressor genes are necessary prerequisites for hepatocarcinogenicity. Very recent studies suggest that cytotoxic effects of

dichloroacetic acid provide a growth advantage to spontaneously initiated cells, thus enhancing their clonal expansion and ultimately the formation of adenomas and carcinomas. Inhibition of intercellular communication (gap junctions), apoptosis and mitosis have been detected following the administration of dichloroacetic acid at tumorigenic and subtumorigenic dose levels. Most carcinogenicity studies of dichloroacetic acid have been limited to the liver. The studies that included complete examinations provide no indications of other tumour locations. In those studies, male F344 rats were given drinking water containing dichloroacetic acid at levels of up to 1.6 g/l (ca. 139 mg/kg body weight/day) for 100 or 103 weeks, and male B6C3F1 mice were exposed to water containing 0.5 g/l (approx. 88 mg/kg body weight/day, only dose tested) or levels of up to 3.5 g/l (approx. 429 mg/kg body weight/day) for 104 or 100 weeks.

Dichloroacetic acid is embryotoxic and teratogenic at maternally toxic dose levels in the Long-Evans rat. Dose-related malformations of the cardiovascular system, primarily in the form of intraventricular septum defects, have been reported to occur from 140 mg/kg body weight and dose-related malformations of the urogenital system (hydronephrosis) from 1400 mg/kg body weight. The *no observed adverse effect level* (NOAEL) for teratogenicity after oral administration from gestation days 6 to 15 was 14 mg/kg body weight/day for Long-Evans rats in that study. Oral administration of sodium dichloroacetate to male Long-Evans rats for 10 weeks resulted in decreased preputial gland and epididymis weights even at the lowest dose level, 31.25 mg/kg body weight/day, accompanied by depressed body weight gain from that dose level onwards. From 62.5 mg/kg body weight, reductions in sperm count, sperm motility and the percentage of intact sperm and delayed spermiogenesis were additionally noted. A mating study with untreated females given 125 mg/kg body weight (the highest dose level tested) resulted in impaired fertility, observed as reductions in the numbers of pregnant females and implants/dam. Subchronic studies in rats and dogs given higher doses or dosed for prolonged periods revealed degenerative changes in the testes together with reductions in sperm motility and sperm count to the degree of aspermia. Dose-related testicular damage, as characterised by small testes, degeneration of the germinal epithelium and syncytial giant cell formation, enlarged Sertoli cells, atrophy of the seminiferous tubules and absence of spermatogonia and spermatozoa in the testes and epididymides, also occurred in the dog, where pros-

tate glandular atrophy was additionally noted, after subchronic administration of as little as 12.5 mg/kg body weight/day and in the rat at higher doses of and above 500 mg/kg body weight (by gavage).

Immunological parameters (antibody production, delayed hypersensitivity, cytotoxicity of natural killer cells, production of prostaglandin PGE_2 and interleukin IL_2) investigated in the context of a subchronic drinking water study in male Sprague-Dawley rats gave no indication of a harmful effect of dichloroacetic acid on the immune system.

As described above, subchronic oral exposure of rats and dogs to dichloroacetic acid lead to degenerative changes in the CNS, accompanied by corresponding neurological deficits. Specific neurotoxicity studies in rats showed that even acute administration of 300 mg/kg body weight by oral gavage resulted in reversible decrease in hindlimb grip strength, a characteristic sign of dichloroacetic acid neurotoxicity. Subchronic and chronic administration of dichloroacetic acid in the drinking water in these neurotoxicity studies resulted in neurological changes which manifested, in particular, as gait impairment and weakness of the hindlimbs down to the lowest dose range tested (approx. 16 mg/kg body weight/day over a period of 12 weeks and 137 mg/kg body weight/day over a period of 2 years). Further findings included decreased fore- and hindlimb grip strength, abnormal body position, increased landing foot splay when dropped from a height of 30 cm, impairment or loss of righting reflex and pupil response and mild tremor. The incidence and intensity of the individual changes correlated with dose level and duration of treatment. Neurohistopathological examinations revealed pathological changes in a very limited area of the spinal cord. The fasciculus gracilis of the posterior column in the grey spinal cord exhibited marked gliosis with loss of myelinated axons and large granular structures. The peripheral nervous system showed no pathological alterations and also there was no vacuolation in the brain. The neurological changes occurred slowly and were not completely reversible, as seen in animals observed for 14 weeks after administration of 172 mg/kg body weight/day for 3 months and animals observed for 18 months after administration of 235 mg/kg body weight/day for 6 months. A *no observed effect level* (NOEL) for neurological changes in rats can not be established on the basis of these studies, because even the lowest test dose of 16 mg/kg body weight/day, which was administered in drinking water for 12 weeks, produced pathological changes.

Dichloroacetic acid inhibits pyruvate dehydrogenase kinase, thus interfering with a central mechanism of metabolism, the oxidative decarboxylation of pyruvate to acetyl coenzyme A. Both pyruvate and acetyl coenzyme A are central metabolic products and substrates of carbohydrate, lipid and protein metabolism. Therefore, by inhibiting pyruvate decarboxylase kinase, dichloroacetic acid affects numerous intermediary metabolic processes, something that likely explains both the toxicodynamic and the pharmacodynamic effects of the compound, e.g. in hyperglycaemia and hypercholesterolaemia but also in congenital, acquired, general or local metabolic acidosis. However, dichloroacetic acid is not suitable for long-term therapy. A study investigating the use of sodium dichloroacetate for long-term treatment of hypercholesterolaemia, in which a 21-year-old patient with severe familial hypercholesterolaemia received a 50 mg/kg daily oral dose of sodium dichloroacetate, was discontinued after 16 weeks due to the emergence of severe adverse neurological effects. Under sodium dichloroacetate treatment (50 mg/kg body weight/day), the plasma lipoprotein concentrations fell markedly, but on the other hand a polyneuropathy, characterised by weakness of the facial, finger and lower-extremity muscles, developed in parallel after 16 weeks, changes which were also noted in subchronic studies in the dog and rat. In addition, the deep tendon reflexes were diminished and the nerve conduction velocity was slowed. The human study was discontinued owing to these severe adverse effects, and dichloroacetic acid was evaluated as unsuitable for long-term therapy. The adverse effects of sodium dichloroacetate treatment were largely reversible within 6 months after the volunteer stopped taking the compound. A 13-year-old female patient with lactic acidosis and ensuing metabolic encephalopathy, who was treated with an average dichloroacetic acid dose of 55.8 mg/kg body weight three times a week for 24 weeks, also developed comparable adverse neurological effects despite coadministration of 100 mg thiamine per day. The noted gait abnormalities and the impairment of nerve conduction velocity were reversible by 6 months after the end of treatment. Dichloroacetic acid has been patented for use as an acute therapeutic agent to compensate short-term acid-base imbalances due to an acute local underoxygenation and continues to be the subject of clinical investigation.

In the Federal Republic of Germany, the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (“MAK-Kommission”) of the Deutsche Forschungsgemeinschaft has listed di-

chloroacetic acid and its sodium salt in the “Yellow Pages” (“Substances being Examined for the Establishment of MAK Values and BAT Values”) of the 2005 List of MAK and BAT Values on the suggestion of BG Chemie in order that the carcinogenic potential be examined.

2 Name of substance

2.1	Usual name	Dichloroacetic acid Sodium dichloroacetate
2.2	IUPAC name	Dichloroacetic acid Sodium dichloroacetate
2.3	CAS No.	79-43-6 (dichloroacetic acid) 2156-56-1 (sodium dichloroacetate)
2.4	EINECS No.	201-207-0 (dichloroacetic acid) 218-461-3 (sodium dichloroacetate)

3 Synonyms, common and trade names

Acetic acid, dichloro-
Dichloressigsaures Natrium
Dichloressigsäure
2,2-Dichloressigsäure (Natriumsalz)
α,α-Dichloressigsäure (Natriumsalz)
Dichlorethansäure (Natriumsalz)
Dichloroacetic acid (Sodium salt)
2,2-Dichloroacetic acid (Sodium salt)
α,α-Dichloroacetic acid (Sodium salt)
Dichloroethanoic acid (Sodium salt)
Natriumdichloracetat

4 Structural and molecular formulae

4.1	Structural formula	Cl_2CH-COOH (dichloroacetic acid) Cl_2CH-COONa (sodium dichloroacetate)
4.2	Molecular formula	$C_2H_2Cl_2O_2$ (dichloroacetic acid) $C_2HCl_2O_2Na$ (sodium dichloroacetate)

5 Physical and chemical properties

5.1	Molecular mass, g/mol	128.94 (dichloroacetic acid) 150.92 (sodium dichloroacetate)
5.2	Melting point, °C	13.5 (dichloroacetic acid) (Koenig et al., 2001; Lide and Frederikse, 1996)
5.3	Boiling point, °C	192 (at 1013 hPa; dichloroacetic acid) (Clariant, 2003; Koenig et al., 2001) 194 (dichloroacetic acid) (Lide and Frederikse, 1996)
5.4	Vapour pressure, hPa	0.19 (at 20 °C; dichloroacetic acid) (Clariant, 2003) 1.9 (at 20 °C; dichloroacetic acid) (Koenig et al., 2001)
5.5	Density, g/cm³	1.5634 (at 20 °C; dichloroacetic acid) (Lide and Frederikse, 1996) 1.564 (at 20 °C; dichloroacetic acid) (Koenig et al., 2001) 1.56–1.573 (at 20 °C; dichloroacetic acid) (Clariant, 2003)
5.6	Solubility in water	Dichloroacetic acid and sodium dichloroacetate are miscible with water in all proportions (Clariant, 2003; Koenig et al., 2001; Lide and Frederikse, 1996)
5.7	Solubility in organic solvents	Dichloroacetic acid is soluble in all usual solvents (Koenig et al., 2001) Dichloroacetic acid is miscible with ethanol and diethyl ether and soluble in acetone (Lide and Frederikse, 1996)
5.8	Solubility in fat	Partition coefficient log P_{ow}: 0.942 (calculated) (Clariant, 2003)
5.9	pH value	0.64 (as a 10% solution) (Gordon et al., 1998) 1.2 (129 g dichloroacetic acid/l) (Clariant, 2003)

5.10	Conversion factor	1 ml/m³ (ppm) ≙ 5.26 mg/m³ 1 mg/m³ ≙ ml/m³ 0.19 (ppm) (Dichloroacetic acid) 1 ml/m³ (ppm) ≙ 6.16 mg/m³ 1 mg/m³ ≙ ml/m³ 0.16 (ppm) (Sodium dichloroacetate) (at 1013 hPa and 25 °C)

6 Uses

Dichloroacetic acid is a versatile intermediate, *inter alia* in the production of glyoxylic acid. It is used as a medicinal disinfectant and as a test reagent for analyses during the manufacture of polyethylene terephthalate fibres (Koenig et al., 2001). The compound has been used clinically in the treatment of various metabolic imbalances (Stacpoole, 1989; Stacpoole et al., 1998 a, b).

7 Experimental results

7.1 Toxicokinetics and metabolism

The toxicokinetics and metabolism of sodium dichloroacetate were investigated in a study in F344 rats and B6C3F1 mice. Groups of 2 to 4 males/dose, species and time point of assessment received a single dose of sodium dichloroacetate at 5, 20 or 100 mg/kg body weight as an aqueous solution by oral gavage. Irrespective of the dose, each animal was administered 5 to 20 µCi as a mixture of sodium [1,2-^{14}C]-dichloroacetate (> 99% pure, specific activity: 55 mCi/mmol) and unlabelled sodium dichloroacetate (99.0% pure dichloroacetic acid adjusted to pH 7.0 with NaOH). Levels of radioactivity were determined in erythrocytes, whole plasma and protein-poor plasma at intervals over a period of 48 hours. The excretion of radioactivity in the urine, faeces and expired air was monitored for 48 hours and 24 hours in rats and mice, respectively. Determination of residual activity in the body at the end of the study yielded highly variable data as a result of inadequate sample preparation and hence those data were not further analysed. The toxicokinetic parameters found are summarised in Table 1 below.

Table 1. Toxicokinetic parameters for sodium dichloroacetate in the F344 rat and the B6C3F1 mouse following single oral administration (Larson and Bull, 1992 a)			
Parameter	Species	Sodium dichloroacetate, 20 mg/kg body weight	Sodium dichloroacetate, 100 mg/kg body weight
C_{max} (nmol/ml)	Mouse	4 ± 1	20 ± 0
	Rat	15 ± 8	380 ± 2
AUC (nmol/ml/hour)	Mouse	8 ± 2	30 ± 0
	Rat	13 ± 4	750 ± 40
$t_{½}$ (hours)	Mouse	1.5	1.6
	Rat	0.9	0.9
V_d (ml/kg)	Mouse	34800	32500
	Rat	2400	1000
Clearance (ml/kg/hour)	Mouse	16000	14300
	Rat	2900	820
Excretion within 24 hours (mouse) and 48 hours (rat; expressed as percentage of the administered dose):			
In the expired air ($^{14}CO_2$)	Mouse	2.2 ± 0.2	2.4 ± 0.1
	Rat	23.9 ± 1.5	29.3 ± 3.6
In the urine	Mouse	28.2 ± 1.0	27.2 ± 2.0
	Rat	23.4 ± 2.0	24.4 ± 0.4
In the faeces	Mouse	1.0 ± 0.1	1.1 ± 0.1
	Rat	1.4 ± 0.2	1.2 ± 0.2
C_{max} maximum plasma concentration AUC area under the concentration-time curve $t_{½}$ plasma elimination half-life V_d volume of distribution			

As is evident from Table 1, the blood concentrations and AUC values were markedly higher in rats than in mice at the same dose level. Rats showed disproportionate increases in those values whilst mice exhibited a dose-proportionate increase. As regards the half-lives of elimination, the differences between rats and mice were not significant. Volume of distribution and clearance were very much higher in mice than in rats and, in contrast to the rat data, gave no indication of metabolic saturation in the concentration range tested. The amounts of metabolites (not further characterised nonchlorinated acids) detected in plasma were approximately dose-proportionate in both species and generally lower in rats than in mice. Excretion of the administered radioactivity occurred via the expired air, the urine and the faeces. Rats dose-independently exhaled 23.9 to 29.3% of the administered radioactivity as $^{14}CO_2$, whereas mice exhaled only 2.2 to 2.4%. Both species excreted most of the $^{14}CO_2$ within the first two hours after admini-

stration. No other metabolites appeared in the expired air. The fractions of administered radioactivity detected in the urine were 27.2 to 28.2% and 23.4 to 24.4% in mice and rats, respectively. Amounts excreted in the faeces were as small as 1.0 to 1.4% of the administered activity in both species. There were no significant differences between the metabolite patterns observed in rats and those seen in mice. In both species, analysis revealed that the urine contained small amounts of unchanged compound (1.0 to 2.3%), relatively large quantities of the nonchlorinated acids, glycolic acid, glyoxylic acid and oxalic acid (totalling 10.5 to 18.4%) and thiodiacetic acid (6.3 to 12.3%), traces of monochloroacetic acid (0.3% limit of detection) and other, unidentified metabolites (0.1 to 1.0%). The investigators suggested that dichloroacetic acid is metabolised via at least two cytochrome P450-dependent metabolic pathways, one involving hydroxylation and the other reductive dechlorination. Enzymatic hydroxylation, followed by spontaneous dehydrochlorination and subsequent hydrolysis of the thus formed acid chloride intermediate, leads to oxalic acid. Enzymatic reductive dechlorination on the one hand results in the formation of monochloroacetic acid, which undergoes glutathione conjugation and further conversion to thiodiacetic acid, and on the other, via several intermediate steps, in the formation of glycolic acid, which in turn is metabolised to glyoxylic acid and then to oxalic acid, CO_2 or glycine. The observation that 50% and 90% of the radioactivity detected in the blood one hour and 3 hours after administration, respectively, was associated with protein, and further studies by the same team (see Stevens et al., 1992, below), led the investigators to attribute that finding to the formation of the metabolite glyoxylate, which undergoes transamination to the amino acid glycine, which in turn is metabolised to the amino acid serine. Twenty-four hours after administration, the protein-free plasma no longer contained any detectable radioactivity (Bull et al., 1993; Larson and Bull, 1992 a, b).

A later study by the same research group was conducted in male B6C3F1 mice administered [1,2-^{14}C]-dichloroacetic acid in a mixture of unlabelled dichloroacetic acid (both > 99% pure, specific activity 55 mCi/mmol, no information whether or not the compounds were administered after neutralisation with NaOH) to reinvestigate the compound's excretion over a 24-hour period and to analyse its urinary metabolite pattern. Based on the graphic representation of the data, the animals that were treated with 100 mg/kg body weight or traces (no further details) by oral gavage excreted

respective fractions of approx. 45 and 46% of the administered radioactivity as CO_2 in the exhaled air, 20 and 17% in the urine and 3% in the faeces. The carcasses still contained approx. 22 and 28% of the administered radioactivity 24 hours after administration. Urine analyses for both groups detected unmetabolised dichloroacetic acid (0.3 and 0.18%), monochloroacetic acid (0.15 and 0.16%), glyoxylic acid (0.14 and 0.11%), glycolic acid (5.3 and 1.5%), oxalic acid (6.8 and 5.63%), acetic acid (0.07 and 0.23%) and unidentified compounds (7.36 and 8.98%). It was additionally reported for the 100 mg/kg group that the urine contained 0.56% of the administered radioactivity as thiodiglycolate, a metabolite that was not mentioned in the publications from the previous study (see above; Bull et al., 1993; Larson and Bull, 1992 a, b). The fact that, in the presence of otherwise comparable findings, the dose fraction excreted as CO_2 was significantly higher than in the team's earlier study (45 and 46% as opposed to 2.2 and 2.4%) was not commented upon by the investigators (Xu et al., 1995).

Analysis of 24-hour urine from mice given 2 g/l dichloroacetic acid in their drinking water over a period of 26 days detected 0.74 mg dichloroacetic acid, 3.56 mg glycolic acid, 0.46 mg glyoxylic acid and 1.5 mg oxalic acid per ml (Narayanan et al., 1999).

In a subsequent repeated-dose study, the research group of Xu et al. (1995; see above) investigated the toxicokinetics and metabolism of sodium dichloroacetate in male F344 rats given drinking water containing dichloroacetic acid (99% pure, neutralised with NaOH) at 0.2 or 2 g/l (approx. 20 or 114 mg/kg body weight/day) over a period of 14 days. The animals were then offered drinking water without additive for a period of 16 hours. They subsequently received a single intravenous dose of 5, 20 or 100 mg/kg body weight or, by gavage, a single oral dose of 100 mg/kg body weight (mixtures of unlabelled and [1,2-^{14}C]-labelled dichloroacetic acid, 8 and 12 µCi/ml, also neutralised with NaOH) and were placed under observation for 24 hours after dosing. The controls received a single treatment without pretreatment. The toxicokinetic parameters determined for the groups treated intravenously at 100 mg/kg body weight are summarised in Table 2 below.

Table 2. Toxicokinetic parameters for dichloroacetic acid in the F344 rat after single and repeated administration (Gonzalez-Leon et al., 1997 b)		
Parameter	100 mg/kg body weight; intravenous injection, no pretreatment	100 mg/kg body weight; intravenous injection after 14-day administration of 2 g/l in drinking water
AUC (µg/ml/hour)	433.3 ± 233.2	2406.0 ± 405.5
V_{ss} (ml/kg)	618.1 ± 318.7	581.5 ± 146.4
$t_{½}$ (hours)	2.4 ± 0.15	10.8 ± 2.0
CL_b (ml/kg/hour)	267.4 ± 104.8	42.7 ± 8.2
CL_r (ml/kg/hour)	2.9 ± 0.5	8.9 ± 3.3
CL_m (ml/kg/hour)	264.5 ± 103.3	33.8 ± 4.9
Excretion (expressed as % of the administered dose within 24 hours)		
In the expired air ($^{14}CO_2$)	57.4 ± 10.3	19.9 ± 3.7
In the urine	12.4 ± 7.3	38.0 ± 13.2
In the faeces	0.7 ± 0.2	2.2 ± 2.2

AUC area under the concentration-time curve
$t_{½}$ blood elimination half-life
V_{ss} volume of distribution at steady state
CL_b total body clearance
CL_r renal clearance
CL_m metabolic clearance

Compared with single-dose administration, 14-day pretreatment with 2 g/l in drinking water resulted in significant increases in blood concentration levels, area under the concentration-time curve, half-life of elimination from blood and renal clearance. The volume of distribution remained almost unchanged. The values for whole-body clearance and metabolic clearance and the amounts of metabolites detected in the blood were greatly decreased. The metabolite pattern was markedly changed. Compared with single-dose administration, excretion of $^{14}CO_2$ in the exhaled air and renal excretion of oxalic acid both decreased, whilst renal excretion of the unmetabolised compound, in particular, but also the excretion of its metabolites glycolic acid, glyoxylic acid, monochloroacetic acid and acetic acid in the urine increased. As far as reported, similar changes in the metabolism of dichloroacetic acid following repeated administration were observed in all dose groups, including those pretreated with only 0.2 g/l in drinking water. The investigators suggested that dichloroacetic acid inhibits the metabolic enzymes involved and in doing so has an autoinhibitory effect on their excretion (Gonzalez-Leon and Bull, 1996; Gonzalez-Leon et al., 1997 a, b).

Preliminary findings from repeated-dose studies in B6C3F1 mice meanwhile published by the research team have also suggested a decreased rate of elimination. Blood concentration levels were markedly increased following pretreatment. In the mouse, however, excretion in the form of CO_2 was unchanged after repeated-dose administration when compared with single-dose administration (Gonzalez-Leon and Bull, 1996; Gonzalez-Leon et al., 1997 a; Schultz et al., 1999). Human trials investigating the compound as a therapeutic drug also revealed delayed excretion of the compound following repeated administration (see also Section 8; Curry et al., 1991).

Dichloroacetic acid also inhibited its own metabolism in the male B6C3F1 mouse. Mice treated for 2 weeks with drinking water containing dichloroacetic acid at 2 g/l exhibited a markedly lower rate of elimination from the blood after intravenous or oral bolus administration of 20 or 100 mg dichloroacetic acid/kg body weight than did animals treated exclusively with bolus doses. The investigators developed toxicokinetic parameters which were used in one toxicokinetic model to estimate the AUCL values (area under the concentration-time curve in the liver) in the carcinogenesis study conducted by DeAngelo et al. (1999; see also Section 7.7 – Long-term studies) in male mice treated with 0 (controls), 0.05, 0.5, 1, 2, or 3.5 g/l. Using metabolic rates (V_{maxc}) for single-dose and repeated-dose administration of 40 mg/kg body weight/hour (control value) and 8 mg/kg body weight/hour, respectively, a Michaelis constant (K_m) of 0.5 mg/l, a rate of absorption (K_a) of 0.25/hour and a rate of elimination (K_e) of 0.003/hour, the model calculated AUCL values of 0 (control), 0.041, 0.72, 15.8, 417 and 1064 mg x hour/l for the carcinogenesis study mice treated for 100 weeks, of which 0 (controls), 33, 48, 71, 95 and 100% developed liver carcinomas, respectively (Barton et al., 1999).

The excretion and metabolism of [1-^{14}C]-dichloroacetic acid (9.3 mCi/mmol) and [2-^{14}C]-dichloroacetic acid (56 mCi/mmol) after a single oral administration was also investigated in the Fischer-344 rat by another research team, who arrived at comparable results independently of the study discussed above (Bull et al., 1993; Larson and Bull, 1992 a, b). Groups of 4 to 6 males received, by gavage, oral doses of the two compounds (radiochemical purity ≥ 97%) as a mixture with unlabelled dichloroacetic acid, adjusted to pH 6.6 with NaOH, at dose levels of 282 mg/kg body weight (20 µCi/animal). [2-^{14}C]-Dichloroacetic acid was additionally administered at a

dose level of 28.2 mg/kg body weight. The excretion of radioactivity was monitored in the exhaled air, urine and faeces for 48 hours and the residual activity subsequently determined in the tissues and carcass. The excretion of [1-^{14}C]- and [2-^{14}C]-labelled compound showed no significant differences. Within 48 hours, 28.8 and 25.0% of activity, respectively, was excreted as $^{14}CO_2$ in the expired air, 33.3 and 35.2% in the urine and 2.1 and 2.0% in the faeces, the predominant portions of which were excreted within the first 8 hours after administration. Analysis revealed that residual activities in the body were 20.8 and 26.2% of the administered activity. Following administration of the 10-fold lower dose, the residual activity in the body (36.4%) and elimination via the respiratory tract (34.4%) were higher and excretion in the urine (12.7%) and faeces (0.8%) was lower. Excretory products detected in urine after administration of [1-^{14}C]- and [2-^{14}C]-dichloroacetic acid at 282 mg/kg body weight included unchanged dichloroacetic acid (19.8 and 21.1%), glyoxylic acid (1.36 and 1.12%), glycolic acid (4.65 and 6.60%) and oxalic acid (1.86 and 2.34%). After administration of [2-^{14}C]-dichloroacetic acid at 28.2 mg/kg body weight, the fraction of unmetabolised dichloroacetic acid excreted in the urine, 0.62%, was markedly smaller. The relative excretion of the other metabolites differed only slightly from the values found after administration of the high dose (1.34% glyoxylic acid, 1.67% glycolic acid and 3.01% oxalic acid). Of the 20.8 to 36.4% of the administered activity retained in the body after 48 hours, the largest fractions were accounted for by liver (4.89 to 7.87%), muscle (4.52 to 9.86%), skin (3.25 to 4.46%), blood (1.38 to 2.59%), intestines (0.96 to 1.69%), kidneys (0.29 to 0.60%) and adipose tissue (0.25 to 0.7%; Lin et al., 1993).

Published only as an abstract, a study in male Sprague-Dawley rats treated with a single oral dose of 50 mg sodium dichloroacetate + 360 µCi/kg 1-^{14}C-sodium dichloroacetate showed that within 24 hours 36.8% of the administered radioactivity was excreted in the expired air as CO_2 and 9.3% was excreted in urine as metabolites and unchanged compound (0.7% as unchanged compound). When the dose was administered twice at an interval of 24 hours, the expired air and urine contained 43.6% and 15.2% (3.3% unchanged compound) of the administered radioactivity, respectively (no further details; James et al., 1995).

According to a later publication by the same investigators, the toxicokinetics and metabolism of dichloroacetic acid in the rat changed not only with

dosing frequency, but also depended on animals' feeding status and age. Groups of 3 or 4 young Sprague-Dawley rats (aged 3 to 4 months, weighing 180 to 265 g) received, by oral gavage, a single dose or two doses, in the fed state or after an 18-hour fast, of sodium dichloroacetate as an aqueous solution at a dose level of 50 mg/kg body weight. Old animals (aged 16 months, weighing 580 to 690 g) were treated twice in the fed state with a corresponding dose. Groups of 3 or 4 animals were administered 280 to 400 µCi/kg body weight as mixtures of sodium [1-^{14}C]-dichloroacetate or sodium [1,2-^{14}C]-dichloroacetate (respective radiochemical purities > 99 and > 99.5%) and unlabelled sodium dichloroacetate (> 99.8%). The excretion of radioactivity was monitored in the urine, faeces and exhaled air for 24 hours after a single or the last dose and the residual activity subsequently determined in blood, tissues and carcass. One group of fasted young rats was sacrificed early, one hour after administration, in order to determine the distribution of radioactivity in the body. Blood for plasma concentration-time course studies was collected from several animals via an indwelling jugular vein cannula. The reported toxicokinetic parameters are summarised in Table 3 below.

Beginning of Table 3

Table 3. Toxicokinetic parameters for dichloroacetic acid in young and large (old), fed and fasted Sprague-Dawley rats after administration of a single or two oral doses (James et al., 1998)

	Single administration of 50 or 30 mg/kg body weight			Two administrations of 50 or 30 mg/kg body weight		
	Young animals, fasted	Young animals, fasted	Young animals, fed	Young animals, fasted	Young animals, fed	Large (old) animals, fed
Observation period (hours)	1	24	24	24	24	24
Parameters of the plasma concentration curve[1]						
$t_{½}$ abs (hours)	n.a.	0.37 ± 0.03		1.45 ± 0.64		1.30 ± 0.69
V_d (ml/kg)	n.a.	0.68 ± 0.07		0.39 ± 0.14		0.14 ± 0.02
AUC (mg x hour/l)	n.a.	11.72 ± 1.68		240.80 ± 75.4[2]		1509 ± 248[3]
C_{max} (µg/ml)	n.a.	10.19 ± 1.87		27.22 ± 5.54[2]		121.2 ± 20.4[3]
t C_{max} (hours)	n.a.	0.27 ± 0.04		1.13 ± 0.46		2.09 ± 0.96
$t_{½}$ el (hours)	n.a.	0.11 ± 0.02		5.38 ± 0.76[2]		9.72 ± 0.97[3]
$t_{½}$ α el (hours)	n.a.	n.a.		0.16 ± 0.04		0.22 ± 0.05
Excretion (expressed as % of the administered dose)						
In the expired air ($^{14}CO_2$)	6.1 ± 0.5	37.2 ± 1.05[4]	25.5 ± 1.7	43.6 ± 3.0[4]	19.8 ± 2.9	n.a.
In the urine	n.a.	9.8 ± 0.67	12.3 ± 6.5	15.4 ± 3.65	13.4 ± 5.7	28.0 ± 11.41[3]
In the faeces	n.a.	1.39 ± 0.97	n.a.	4.57 ± 0.77	n.a.	5.01 ± 0.74

Table 3. Toxicokinetic parameters for dichloroacetic acid in young and large (old), fed and fasted Sprague-Dawley rats after administration of a single or two oral doses (James et al., 1998)

	Single administration of 50 or 30 mg/kg body weight			Two administrations of 50 or 30 mg/kg body weight		
	Young animals, fasted	Young animals, fasted	Young animals, fed	Young animals, fasted	Young animals, fed	Large (old) animals, fed
Unmetabolised dichloroacetic acid and ^{14}C-containing compounds in urine (expressed as % of the administered dose)						
Dichloroacetic acid	n.a.	0.66 ± 0.51	0.36 ± 0.36	3.21 ± 0.41[2]	0.85 ± 0.25[2]	20.2 ± 12.5[3]
Oxalic acid	n.a.	2.48 ± 0.49	2.57 ± 0.37	4.13 ± 3.04	2.51 ± 0.29	1.97 ± 0.93
Glyoxylic acid and other polar metabolites	n.a.	3.84 ± 0.82	2.61 ± 0.87	3.35 ± 1.91	2.69 ± 0.81	2.12 ± 0.57
Glycine conjugate of not fully elucidated structure	n.a.	0.88 ± 0.73	0.30 ± 0.29[4]	1.69 ± 0.55	0.23 ± 0.18[4]	0.62 ± 0.47
Hippuric acid	n.a.	1.31 ± 0.74	5.66 ± 5.67[4]	0.99 ± 0.62	6.23 ± 5.26[4]	2.36 ± 1.1
Phenylacetylglycine	n.a.	0.45 ± 0.34	0.26 ± 0.29[4]	1.22 ± 0.61	0.06 ± 0.04[4]	0.07 ± 0.1
Residual activity in the organs and tissues (expressed as % of the administered dose)						
Liver	16.3 ± 2.92	8.67 ± 1.12	6.19 ± 0.96	6.16 ± 0.57	7.01 ± 1.21	9.78 ± 4.65
Gastrointestinal tract	9.26 ± 2.13	4.2 ± 1.49	3.74 ± 0.39	2.40 ± 0.89	3.27 ± 0.28	7.97 ± 1.67
Muscle	22.5 ± 4.9	9.36 ± 3.55	11.9 ± 0.74	9.27 ± 2.5	12.8 ± 3.02	11.5 ± 1.92
Fat	4.58 ± 0.78	1.32 ± 0.4	3.87 ± 3.57	1.11 ± 0.29	2.39 ± 1.14	1.18 ± 0.18
Kidney	1.37 ± 0.34	0.65 ± 0.08	0.53 ± 0.05	0.63 ± 0.19	0.55 ± 0.09	0.85 ± 0.32
Other tissues	13.9 ± 1.9	8.38 ± 2.57	9.46 ± 2.37	8.46 ± 2.49	9.33 ± 1.54	7.63 ± 1.97
Total recovery (expressed as % of the administered dose)						
	80.1 ± 6.4	80.9 ± 6.6	73.4 ± 9.3	91.6 ± 10.9	68.6 ± 1.8	71.3 ± 9.24

n.a. not assayed
$t_{½}$ abs absorption half-life
V_d volume of distribution
AUC area under the concentration-time curve
C_{max} maximum plasma concentration
t C_{max} time of peak plasma concentration
$t_{½}$ el half-life of elimination
$t_{½}$ α el α-phase half-life of elimination from plasma
[1] The values showed no differences between fed and fasted animals. The investigators therefore pooled the data for those two groups.
[2] significantly different from singly dosed animals
[3] significantly different from young animals given the same treatment
[4] significantly different from animals given the same treatment without prior fasting for 18 hours

End of Table 3

Table 3 shows that elimination of dichloroacetic acid from the plasma was slower after repeated administration, as found in the studies described above (Barton et al., 1999; Gonzalez-Leon et al., 1997 a, b; Gonzalez-Leon and Bull, 1996; James et al., 1995; Schultz et al., 1999). Moreover, it took markedly longer in older animals than in young animals given the same treatment. Older animals also excreted a markedly higher dose fraction as unchanged compound. The plasma concentration time course was

not affected by the animals' feeding state at the time of dosing. However, the extent to which dichloroacetic acid was metabolised to $^{14}CO_2$ was much greater in animals dosed with the chemical after a prolonged fast than it was in fed animals. The investigators identified compounds that were secondary products of dichloroacetic acid metabolism to glycine – phenylacetylglycine and hippuric acid (benzoylglycine) – which were not detected in the earlier metabolism studies (James et al., 1998).

In order to investigate the binding of dichloroacetic acid to blood proteins, male F344 rat and B6C3F1 mice were given, by gavage, a single oral dose of neutralised [1,2-^{14}C]-dichloroacetic acid at 5 mg/kg body weight (specific activity of the gavage solutions: 11.4 mCi/mg). Analysis of radioactivity compared the amounts of ^{14}C-labelled albumin and haemoglobin with the amounts of the ^{14}C-labelled amino acids serine and glycine. The investigators suggested that these ^{14}C-labelled amino acids are attributable to the formation of the metabolite glyoxylic acid, because during anabolic metabolism, glyoxylic acid undergoes transamination to yield the amino acid glycine, which in turn is converted to the amino acid serine. Expressed as pmol equivalents of dichloroacetic acid/mg protein, respective haemoglobin and albumin values of 7 and 690 were detected in rats 8 hours after administration, with respective fractions of 58 and 67% of activity being accounted for by serine and glycine. The 120-hour value for haemoglobin was 24 (61% glycine and serine) and the 24-hour for albumin was 340 (48% glycine and serine). In the mouse, compared with the rat, the detected pmol equivalents of dichloroacetic acid/mg protein were lower while the percentages of ^{14}C-labelled amino acids were higher, a finding which the investigators attributed to a higher metabolic rate in the mouse (haemoglobin values were 1 and 7 at 8 and 120 hours, with fractions accounted for by glycine and serine being below detection limit and 98%, respectively; albumin values were 490 and 259 at 4 and 24 hours, with fractions accounted for by glycine and serine being 69 and 74%, respectively; Stevens et al., 1992).

Pregnant Long-Evans rats received sodium [1,2-^{14}C]-dichloroacetate at 140 to 3500 mg/kg body weight on gestation day 12 (purity and type of administration unspecified). Peak maternal plasma levels of 1 mg/ml were attained 2 to 4 hours after administration. After that, the plasma concentrations decreased very rapidly. The carbonyl carbon (C1) and the dichloromethyl carbon (C2) of dichloroacetic acid were equally represented in

maternal plasma and liver. Hence, there was no maternal breakdown of the compound. The ^{14}C level in embryonic tissue at 48 hours exceeded maternal plasma levels and remained roughly constant across doses, with C2 fragments dominating (no further details; Roth et al., 1991).

Groups of 6 male New Zealand rabbits were administered sodium dichloroacetate as a bolus intravenous injection of 180 mg/kg body weight (purity unspecified) or by infusion (20 minutes) of 50, 100, 200 or 300 mg/kg body weight. Following intravenous injection, the volume of distribution (V_d) was 0.28 l/kg, the elimination rate constant 1.09/hour, plasma half-life 0.67 hours and clearance 0.31 l/kg/hour. After intravenous infusion over 20 minutes, peak plasma concentrations were reached at the end of the infusion period and exhibited corresponding parallel shifts at dose levels of 50, 100 and 200 mg/kg body weight. The kinetic parameters were practically identical (a volume of distribution of 0.41 to 0.46 l/kg, an elimination rate constant of 0.96 to 0.99/hour, a plasma half-life of 0.70 to 0.72/hour and a clearance of 0.40 to 0.44 l/kg/hour). After administration of 300 mg/kg body weight, the volume of distribution (0.44 l/kg) was unchanged, but there was a significant decrease in elimination rate constant (0.69/hour), a prolongation of half-life (1.01 hours) and a decrease in clearance (0.30 l/kg/hour). This was indicative of nonlinear kinetics at higher doses. According to in-vitro experiments conducted in parallel to investigate serum protein binding, sodium dichloroacetate concentrations of 10 to 300 mg/l serum exhibited 33.1 to 37.2% serum protein binding, whereas a concentration of 600 mg/l showed only 21.4% (Gu et al., 1994).

Further studies are available which were conducted to determine specific toxicokinetic/pharmacokinetic parameters of dichloroacetic acid. These studies were mostly carried out in the context of investigations of sodium dichloroacetate as a therapeutic drug for various metabolic imbalances (see Section 7.11 – Pharmacodynamic effects of dichloroacetic acid) and have been summarised in review articles (Stacpoole et al., 1998 a, b). The determined pharmacokinetic parameters, which were largely in agreement with the toxicokinetic studies discussed in detail above, are listed in Table 4 in the appendix. The elimination of dichloroacetic acid/sodium dichloroacetate depended on the species, age and body weight of the experimental animals and on the duration of administration (Lukas et al., 1980; Yan et al., 1997). It was particularly striking that, as noted in the study by Gonzalez-Leon et al. (1997 a, b) described in detail above, repeated ad-

ministration was associated with a marked increase in plasma half-life when compared with single-dose administration of the same dose, a finding that was also confirmed in clinical studies in humans (see also Section 8). In an early review paper (Stacpoole, 1989), the author suggested that dichloroacetic acid undergoes dechlorination to form glycolic acid and glyoxylic acid, with subsequent metabolism by various microsomal enzyme systems yielding oxalic acid, glycine and carbon dioxide. Cytochrome P450-dependent oxidation, the formation of free radicals via enzymatic oxidation and reduction reactions, conjugation to glutathione and dechlorination via an α-lactone intermediate were discussed as potential catabolic pathways (Stacpoole, 1989; Stacpoole et al., 1998 a, b).

In vitro studies

In-vitro studies with various subcellular fractions obtained from male B6C3F1 mice and male F344 rats showed that dichloroacetic acid was metabolised especially by the liver and lung and to a lesser extent also by the kidney, gastrointestinal tissue and muscle. Lysed whole blood did not measurably degrade dichloroacetic acid. Degradation occurred predominantly in the cytosol (K_M 0.28 mM, V_{max} 11.6 nmol/minute/mg protein) and was dependent on the glutathione level, but involvement of glutathione S-transferase was excluded. Addition of $NADP^+$ and NADPH stimulated the rate of degradation, while NAD^+ and NADH did not. Mitochondria and microsomes were involved only to a minor extent in the metabolisation of dichloroacetic acid, and inhibition of microsomal enzymes (cytochrome P450 by SKF 525-A and carbon monoxide; and alcohol dehydrogenase by isoniazid and pyrazole) did not affect the overall rate of dichloroacetic acid degradation (Lipscomb et al., 1995).

NADPH-dependent oxidation or UDPGA-dependent glucuronidation of dichloroacetic acid failed to be demonstrated in in-vitro studies with phenobarbitone-induced microsomes from male Sprague-Dawley rats. As demonstrated by Lipscomb et al. (1995; see above), metabolism was glutathione and cytosol-dependent (James et al., 1995).

Later in-vitro studies that two research teams conducted with liver cytosol from F344 and Sprague-Dawley rats given single or repeated doses of dichloroacetic acid or sodium dichloroacetate demonstrated dependency of

glycolate, glyoxylate and oxalate on the available amounts of glutathione, and showed, as did the in-vivo studies (Gonzalez-Leon et al., 1997 a, b), that metabolism was slower in cytosol from rats treated with repeated doses of dichloroacetic acid (Cornett et al., 1997; Gonzalez-Leon et al., 1997 b; James et al., 1997).

Dichloroacetic acid inhibited the activities of glutathione S-transferases A, AA, B, C, E and M in rat liver extracts (Dierickx, 1984).

More recent studies have shown that glutathione-dependent oxidation of dichloroacetic acid to glyoxylic acid is catalysed with high substrate specificity by a Zeta-class glutathione transferase. This was demonstrated in in-vitro studies of glutathione transferase Zeta isolated from rat liver cytosol and the recombinant human enzyme. Both purified enzymes had similar activities. When liver cytosol from different species was used, V_{max} values (maximum velocity of the catalysed reaction) and K_m values (substrate concentration, at which the reaction velocity reaches half of its maximum value) for the biotransformation of dichloroacetic acid to glyoxylic acid showed the following order: mouse > rat >> human. Rat liver cytosol converted approx. 20 to 30% of the added [2-^{13}C]-dichloroacetic acid to [2-^{13}C]-glyoxylic acid. [2-^{13}C]-Glyoxylic acid was the only stable metabolite to be formed from [2-^{13}C]-dichloroacetic acid by glutathione transferase Zeta. Conversion required glutathione. The added glutathione, however, was neither consumed nor oxidised to glutathione disulphide, and therefore the investigators suspected that conversion took place via a mechanism that ultimately released glutathione again in a quantitative manner. They suggested that glutathione transferase Zeta presumably catalyses the displacement of one chlorine atom by glutathione to afford S-(α-chlorocarboxylmethyl)glutathione. Subsequent hydrolysis of the intermediate α-chlorosulphide would yield the hemithioacetal S-(α-hydroxycarboxymethyl)glutathione, which may eliminate glutathione to give glyoxylic acid. The activity of glutathione transferase Zeta was inhibited relatively rapidly by dichloroacetic acid but not by glyoxylic acid (Tong et al., 1998 a, b).

In a subsequent experimental study, the team investigated in greater detail the dichloroacetic acid-induced inactivation of glutathione transferase Zeta. The biotransformation of dichloroacetic acid to glyoxylic acid reached a plateau after only 10 minutes in in-vitro studies with rat liver cytosol. The observation that only a second addition of cytosol, but not of dichloroacetic

acid, could restart the reaction led the investigators to conclude that the inactivation of glutathione transferase was irreversible. If the plateau was attained due to equilibrium between substrate and product or due to competitive inhibition of the enzyme, a second addition of dichloroacetic acid should have restarted the reaction. Liver cytosol from male Fischer-344 rats treated with a single intraperitoneal dose of 0.0 (control), 0.15, 0.30 or 0.60 mmol (approx. 18, 39 or 77 mg) dichloroacetic acid/kg body weight exhibited decreases in glutathione transferase Zeta activity (measured as the biotransformation of dichloroacetic acid to glyoxylate upon addition to cytosol) to 79, 55 and 24% of the saline-treated control at 24 hours after administration. Rats treated intraperitoneally at 0.30 mmol/kg body weight showed the highest degree of inactivation of glutathione transferase Zeta and the lowest levels of immunoreactive glutathione transferase Zeta protein at 12 hours after administration. Glutathione transferase Zeta activity and glutathione transferase Zeta protein concentrations did not return to initial values until 10 to 12 days after single-dose treatment. Intraperitoneal administration of 5 daily dichloroacetic acid doses of 0.30 mmol/kg body weight reduced glutathione transferase Zeta activity to only 5% of control values and glutathione transferase Zeta protein to less than 10% of control values. Treatment with dichloroacetic acid decreased both the activity of the enzyme and the amount of the corresponding protein; on the other hand an immunoreactive enzyme with increased molecular weight was detected. These observations led the investigators to suspect the occurrence of an irreversible covalent reaction of dichloroacetic acid with the enzyme. They postulated that the intermediate S-(α-chlorocarboxymethyl)glutathione bound covalently to a nucleophilic portion of the enzyme and the reaction product subsequently underwent degradation by proteolysis (Anderson et al., 1999).

The irreversible covalent binding of an intermediate to glutathione transferase Zeta as postulated by Anderson et al. (1999; see above) was experimentally confirmed by the team in a subsequent in-vitro study with the recombinant human enzyme and ^{14}C-dichloroacetic acid and ^{35}S-glutathione. However, they modified the reaction scheme to the effect that, rather than S-(α-chlorocarboxymethyl)glutathione, it was an instable intermediate (a carbonium-sulphonium compound) formed from that compound by elimination of the chlorine atom that either hydrolysed to glyoxylic acid and glutathione or reacted with a nucleophilic residue of the enzyme, presumably a

sulphydryl group. The inactivated enzyme contained both irreversibly bound dichloroacetic acid-derived ^{14}C and glutathione-derived ^{35}S. The assumption that binding takes place via a nucleophilic amino acid residue on the enzyme was supported by the observation that addition of N-acetyl-L-cysteine to the incubation medium counteracted dichloroacetic acid-induced inactivation of the enzyme. Furthermore, studies in hepatic cytosol from various species showed that the rate constants for dichloroacetic acid-induced inactivation of the enzyme (k_{inact} (minute^{-1}), rat > mouse >> human) and the half-lives ($t_{½}$ (minutes), rat < mouse << human) of the inactivation were species-dependent whereas the half-maximal inhibitory concentrations of dichloroacetic acid (K_{inact} (µM)) were not. The investigators suggested that the differences in the inactivation kinetics of the enzyme in the various species was presumably attributable to their having different polymorphic variants of the enzyme. All investigated polymorphic variants of recombinant human glutathione transferase Zeta were inactivated by dichloroacetic acid in the presence of glutathione, but with different k_{inact} values and half-lives (Tzeng et al., 2000).

Glutathione transferase Zeta is identical to maleylacetoacetate isomerase, a key enzyme of phenylalanine and tyrosine catabolism to acetoacetyl coenzyme A. Maleylacetoacetate isomerase catalyses the isomerisation of maleylacetoacetate to fumarylacetoacetate and the isomerisation of the decarboxylation product of maleylacetoacetate, maleylacetone, to fumarylacetone. Liver cytosol from male Sprague-Dawley rats (3 or 4 rats/group) treated in vivo with dichloroacetic acid as a single dose or 5 daily doses of 4, 12.5, 50, 200 or 1000 mg/kg body weight was assayed for glutathione transferase Zeta activity. A single administration of > 50 mg/kg body weight resulted in significant, concentration-dependent inhibition of the enzyme (measured as conversion to ^{14}C-glyoxylate of sodium ^{14}C-dichloroacetate added to the cytosol) by approx. 50 to 98%. Five treatments were sufficient to cause significant inhibition of the activity of the enzyme even in the lowest dose group, which received 4 mg/kg body weight. Excretion of maleylacetone in the urine was markedly increased in a dose-dependent manner. In vitro, addition of maleylacetone, a physiological substrate of glutathione transferase Zeta, reduced the metabolisation of dichloroacetic acid in human liver cytosol and rat liver cytosol. When hepatic cytosol was preincubated with dichloroacetic acid for 30 minutes and subsequently dialysed overnight to remove unbound dichloroacetic acid, inhibition of glutathione

transferase Zeta by approx. 50% after a second incubation with dichloroacetic acid was noted only in rat liver cytosol. In contrast to the findings of Tzeng et al. (2000; see above), who, however, preincubated the cytosol for up to 55 minutes with dichloroacetic acid, no decrease in glutathione transferase Zeta 1-1 activity was observed here in human liver cytosol after two incubations with dichloroacetic acid. The half-life of inhibition of glutathione transferase determined by Tzeng et al. (2000) for human liver cytosol was 22 minutes, whilst the value for rat liver cytosol was only 5.44 minutes. Moreover, the investigators' suggestion that glutathione transferase Zeta is not inhibited by an intermediate of dichloroacetic acid, but by the alkylating natural substrates maleylacetoacetate and maleylacetone, is not consistent with the findings of Tzeng et al. (2000; see above), who recovered both ^{14}C from ^{14}C-dichloroacetic acid and ^{35}S from ^{35}S-glutathione in the irreversibly inactivated enzyme (Cornett et al., 1999).

The hepatic metabolism and biliary excretion of dichloroacetic acid were investigated in greater detail in a test system of isolated livers from male F344 rats. Livers were perfused for 120 minutes with perfusion medium containing dichloroacetic acid (> 99% pure) at an initial concentration of 25 or 250 µM. More than 50% of the initial amount of dichloroacetic acid was bound to albumin in the perfusion medium. After 5 minutes, the respective amounts of free dichloroacetic acid in the perfusion medium were 43 and 47% of the initial concentration. The concentration of dichloroacetic acid in the recirculating perfusion medium decreased rapidly with respective half-lives of 7 and 32 minutes and respective elimination rate constants (K_{EL} (50)) of 0.062 and 0.022 minute^{-1}. According to the investigators, the markedly lower elimination rate constant observed in the high dose group indicated that metabolism was saturated. By the end of the experiment, the concentration of free dichloroacetic acid in the perfusion medium was down to 7 ± 5 µM in the high dose group. In the low dose group, dichloroacetic acid was completely eliminated from the perfusion medium after 60 minutes. In total, 0.2 and 0.05% of the administered doses of dichloroacetic acid underwent biliary excretion. There was no dichloroacetic acid detectable in livers at the end of the experiment. Liver function (measured as lactate dehydrogenase activity) was not impaired during the experiment (Toxopeus and Frazier, 1998).

Dichloroacetic acid as a metabolite of trichloroethylene, chloral hydrate and trichloroacetic acid

There was no clear answer in the past to the question as to the extent to which dichloroacetic acid is formed as a metabolite of trichloroethylene, tetrachloroethylene, chloral hydrate or trichloroacetic acid and thus contributes to the toxicity, and particularly to the carcinogenicity, of those compounds. Several studies detected dichloroacetic acid as a major metabolite of these compounds whereas other studies found no dichloroacetic acid at all or detected only traces of the compound (see e.g. Bull et al., 1993; Davidson and Beliles, 1991; Dekant et al., 1984, 1986; Larson and Bull, 1992 a, b; Templin et al., 1993; Völkel et al., 1998; Xu et al., 1995). More recent metabolism studies have furnished evidence that dichloroacetic acid is not a major metabolite of these compounds and is formed only in traces, if at all. It was experimentally demonstrated that the large quantities of dichloroacetic acid detected in some studies were artefacts of sample preparation (Barton et al., 1999; Fisher et al., 1998; Greenberg et al., 1999; Ketcha et al., 1996; Merdink et al., 1998, 1999).

7.2 Acute and subacute toxicity

Acute toxicity

The results of the acute toxicity studies of dichloroacetic acid and sodium dichloroacetate are compiled in Table 5 in the appendix.

With LD_{50} values for the rat and mouse of well over 2000 mg/kg body weight, the test chemicals dichloroacetic acid, sodium dichloroacetate and NaOH-neutralised dichloroacetic acid were of very low toxicity following oral and intravenous administration (see Table 5). Even when administered 5 times at intervals of 24 hours, sodium dichloroacetate was of very low toxicity, with LD_{50} values for the male B6C3F1 mouse ranging from 4562 to 6610 mg/kg body weight and those for the female B6C3F1 mouse ranging from 6610 to 7500 mg/kg body weight (Meier et al., 1997). Acute toxicity following inhalation exposure was also low. In the inhalation hazard test in the rat, 8-hour exposure to atmosphere enriched or saturated with dichloroacetic acid at room temperature was not lethal (Smyth et al., 1951). Data that were not consistent with these studies were obtained in the only

dermal study conducted in the rabbit, which showed an LD_{50} of approx. 798 mg (0.51 ml)/kg body weight and hence found dichloroacetic acid to be harmful (Smyth et al., 1951). The only clinical change reported in the context of the acute studies was narcosis (Woodard et al., 1941). The available reports of acute studies provide no necropsy data.

Subacute toxicity

Male and female Sprague-Dawley rats (5 to 7 rats/sex and dose) received dichloroacetic acid at 0 (control), 0.03, 0.125, 0.50 or 1.875 g/l in drinking water for 14 days, corresponding to dose levels of 0 (control), 10, 40, 150 and 600 mg/kg body weight/day according to the investigator. Two additional male rats received 7.5 g/l dichloroacetic acid in drinking water. The only parameters studied were body weight development, food and drinking water consumption, urine volume and osmolality, urinary ammonium and hippurate levels, hepatic and renal enzyme activities and plasma glucose levels. The findings were analysed statistically, but the publication does not always clearly indicate in which sex and from which dose onwards the various findings were significant. The high dose groups showed reduced food and water consumption associated with body weight loss or depressed body weight gain, which, however, were significant only in males. No significant effect was observed on urine volumes, although they were somewhat decreased, consistently with reduced water consumption, in the high dose groups after 7 and less so after 14 days. A trend in both sexes and significant findings in males indicated that urine osmolality and volume were inversely related, which led the investigator to conclude that the kidney's ability to concentrate urine was not impaired by exposure to dichloroacetic acid. Excretion of ammonia in urine was measured on day 14 as an index of renal adaptation to the increased acid load. Increases were only evident in males in all dose groups. In female rats, ammonia excretion decreased at the lower concentrations, in correlation with the activity of phosphate-dependent glutaminase in the kidneys, but increased at higher dose levels of dichloroacetic acid, starting at 0.5 g/l in drinking water. Excretion of hippurate in urine and phosphate-independent glutaminase (glutamyltranspeptidase) activity in the kidneys were not affected by the administration of dichloroacetic acid. Lactate and pyruvate concentrations in the liver and kidneys, measured only for female rats, and plasma glucose levels in

males and females were numerically decreased in the two highest dose groups, but the differences from the controls were not significant. According to the investigator's discussion of the results, they were indicative of renal compensation for the increased acid load caused by the excretion of ammonia and the increased activity of the enzymes involved (Davis, 1986 a, b).

Studies on lipid peroxidation and peroxisome proliferation following acute and subacute administration

A study was conducted to investigate the lipoperoxidative properties of dichloroacetic acid in male B6C3F1 mice and F344 rats (3 to 5 animals per species and dose) treated by oral gavage with a single dose of 100, 300 or 1000 mg/kg body weight, formulated in distilled water and presumably neutralised with NaOH. Rats and mice treated with a single intraperitoneal dose of 1600 mg carbon tetrachloride/kg body weight were included as positive controls. In order to measure lipoperoxidative activity, homogenised livers were assayed for thiobarbituric acid-reactive substances (TBARS, in nmol/g liver) 6 hours after administration, the time point of peak TBARS formation according to preliminary studies. The 300 and 1000 mg/kg dose groups of mice showed significant increases in TBARS levels of up to 129 and 138 nmol/g, respectively, when compared with the water-treated negative control (40 nmol/g). The value for the positive control was 287 nmol/g. In rats, only the 300 mg/kg group showed a significant increase in TBARS (120 nmol/g, negative control 40 nmol/g, positive control 152 nmol/g). The investigators suggested that the lipoperoxidative activity of dichloroacetic acid, which they attributed to the intermediary formation of dechlorinated radicals rather than hydrogen peroxide, could be involved in the formation of lipofuscin and the development of liver nodules in male mice (see Section 7.7 – Long-term studies; Bull et al., 1990, 1993; Washington State University, 1989; Larson and Bull, 1992 a).

A subsequent study was undertaken to investigate whether repeated administration of dichloroacetic acid in drinking water altered the lipoperoxidative activity of acute doses of the compound. To this end, male B6C3F1 mice were given drinking water containing 1 g/l dichloroacetic acid (approx. 99% pure, adjusted to pH 7.0 with NaOH, daily dose approx. 216 mg/kg body weight) for 14 days and then received a single challenge dose of 300 mg/kg body weight (dichloroacetate, again adjusted to pH 7.0 with NaOH)

by oral gavage on day 15. Additional groups received either distilled water (negative control), only 14 days' treatment with drinking water containing the compound or a single dose of 300 mg/kg body weight by gavage without pretreatment. The mouse livers were homogenised and assayed for thiobarbituric acid reactive substances (TBARS, indicators of lipid peroxidation), palmitoyl-coenzyme A oxidase and catalase activities (markers of peroxisome proliferation), laurylic acid hydroxylase activity (a marker of cytochrome P450 4A activity), hydroxylation of p-nitrophenol (a marker of cytochrome P450 2E1 activity) and total cytochrome P450 and individual cytochrome P450 isoenzyme levels. Fourteen-day administration of drinking water containing dichloroacetic acid at 1 g /l had no effect on body weight gain, food and water consumption or relative liver weights in mice. The acute challenge dose, in contrast to an earlier study by the same research group (see above; Larson and Bull, 1992 a), only slightly increased TBARS levels in non-pretreated animals (approx. 70 nmol/g wet weight, negative control approx. 50 nmol/g wet weight, difference statistically non-significant). In pretreated animals, an additional dose of 300 mg/kg body weight administered by gavage caused a significant increase in TBARS levels to approx. 130 nmol/g wet weight as compared with the negative control and the control that was only pretreated (pretreated control approx. 55 nmol/g wet weight). Repeated administration resulted in no increase in palmitoyl-coenzyme A oxidase activity and a significant 1.3-fold increase in catalase activity relative to the control. Lauric acid hydroxylase activity and p-nitrophenol hydroxylation did not differ from the control levels. Total cytochrome P450 levels and the levels of cytochrome P450 isoenzymes 4A, 4A1, 4A2, 4A3, 2E1, 1A1/2, 2B1/2 and 3A1 were also unchanged. Pretreatment with dichloroacetic acid, when compared with the single challenge dose, thus resulted in a significant increase in lipoperoxidative activity which was not associated with an increase in cytochrome P450 2E1, as the investigators had originally expected, or an increase in any cytochrome P450 isoenzyme studied (Austin et al., 1995).

Another study in male and female Sprague-Dawley rats found that the amount and activity of cytochrome P450 isoenzyme 2E1 was increased 3 hours after the last administration of dichloroacetic acid (2.45 mmol/kg body weight (approx. 3.16 g/kg), 3 administrations by gavage within 24 hours). Females additionally showed increases in the total content of cytochrome P450 and the activity of NADPH-dependent cytochrome P450 re-

ductase. The amount and activity of cytochrome P450 2B were unaffected in both sexes and the activity of ethoxyresorufin dealkylase was reduced in males (Yang et al., 1996).

The extent to which the administration of dichloroacetic acid results in oxidative damage to DNA in addition to lipid peroxidation was investigated by the same research group in another study in male B6C3F1 mice treated by gavage with a single oral dose of dichloroacetic acid (adjusted to pH 7.0 with NaOH) at 300 mg/kg body weight. Oxidative damage to the DNA was assessed by measuring the levels of 8-hydroxydeoxyguanosine (8-OHdG) in the liver. 8-OHdG levels were significantly higher than control at 4 and 6 hours after administration (with peak values of approx. 150% of the normal value) but returned to the normal range by 8 hours after administration. In addition, but without giving further details, the investigators reported that repeated administration of dichloroacetic acid did not result in any increase in 8-OHdG levels in livers from B6C3F1 mice. The investigators suggested that oxidative damage to DNA may play a role in the carcinogenic activity of dichloroacetic acid because of the capability of 8-OHdG to induce guanine $\rightarrow$ thymine and adenine $\rightarrow$ cytosine transversions (Austin et al., 1996).

The peroxisome proliferative effect was investigated in male Sprague-Dawley rats and B6C3F1 mice given dichloroacetic acid (≥ 99% pure, adjusted to pH 6.8 to 7.2 with NaOH) at concentration levels of 8, 16 or 39 mM (1, 3 or 5 g/l) in their drinking water for 14 days. According to the investigators, this corresponded to daily dichloroacetic acid doses of 166, 294 and 666 mg/kg body weight for rats and 90, 166 and 346 mg/kg body weight for mice. Control animals received drinking water containing 34 mM sodium chloride. Body weight gain was dose-dependently depressed in rats, whereas it remained unaffected by treatment with dichloroacetic acid in mice. Relative liver weights were dose-dependently increased in mice but not in rats. Both species showed dose-related increases in the activity of hepatic carnitine acetyl-coenzyme A transferase, a marker enzyme of peroxisomal proliferation. In the high dose group, the respective activity levels in rats and mice were 1033% and 450% of the controls. The activity of another marker enzyme of peroxisomal proliferation, cyanide-insensitive palmitoyl-coenzyme A oxidase, was enhanced to 430% of the controls in the intermediate and high dose groups of mice. In rats, a significant increase in the activity of this enzyme to 243% of the control occurred only in the high dose group. A further indication of peroxisome proliferation was

provided by the presence of a PP-A protein, a peroxisome proliferation-associated protein with a molecular weight of 80000 kDa, at the high dose level in both species. Quantitative morphometry by electron microscopy revealed increased numbers of peroxisomes/100 µm³ cytoplasm at the high dose level in both species (mouse 30.77, control 6.89; rat 16.75, control 6.60). According to the investigators' discussion of their results, the mouse proved the clearly more sensitive species with respect to the peroxisome proliferative effect of dichloroacetic acid (DeAngelo et al., 1986, 1989).

In the absence of further details, it was reported that the administration of dichloroacetic acid was demonstrated by electron microscopy to induce hepatic peroxisome proliferation in mice (Tamirisa, 1997).

7.3 Skin and mucous membrane effects

A primary skin irritation index reported for dichloroacetic acid without further details was given as grade 7 on a scale from 1 to 10 (Smyth et al., 1951). According to an earlier publication by the same investigators on the evaluation of skin irritation tests, an irritation index of 7 indicates that the application of undiluted compound causes necrosis of the rabbit skin and the application of a 1-percent solution in acetone gives no reaction more severe than oedema (Smyth et al., 1949). Dichloroacetic acid can therefore be assumed to be corrosive to the skin of the rabbit.

In vitro, the corrosivity of dichloroacetic acid was tested in a three-dimensional human skin model (Skin^2 ZK 1350^{TM}) obtained by growing neonatal human skin cells on an inert nylon mesh and consisting of dermal, epidermal and stratum corneum-like layers. In this model, test substances are applied to the epidermal side of the skin cultures for 10 seconds and the percent cell viability is determined after 24 hours as the criterion of corrosivity. Dichloroacetic acid at a concentration of 36.1% resulted in a cell viability of 21.8 ± 14.3% and was evaluated as corrosive, based on the classification that viability rates below 80% were taken to represent a corrosive effect (Liebsch et al., 1995).

Another in-vitro test system known as the Corrositex test, which is used for classification of chemicals as stipulated in the packaging guidelines, also classified dichloroacetic acid as a corrosive (Packaging Group II). The test system measures the time a chemical takes to destroy a protein-coated

cellulose membrane, referred to by the investigators as a biobarrier, and therefore appears in the adjacent medium, causing a colour change of the pH indicator contained in the medium (Gordon et al., 1998).

The eye irritancy of dichloroacetic acid was assigned a value of 10 on a 10-point scale, the report giving no further details (Smyth et al., 1951). According to an earlier publication by the investigators on the conduct and evaluation of the test, this corresponds to corneal opacity affecting more than 50% of the area (88 to 100% after fluorescein staining) and marked damage to the conjunctivae and iris 24 hours after application of a 1% solution in propylene glycol (Carpenter and Smyth, 1946). Dichloroacetic acid must therefore be assumed to have a severely corrosive effect on the eye.

An alternative eye irritation test (HET-CAM Test) was employed to test a number of organochlorine compounds, including dichloroacetic acid. The lowest irritant concentration of dichloroacetic acid in water was 100 mg/l (no irritancy data given for higher concentrations). The test was performed on the chorioallantoic membrane of 9-day incubated hens eggs, a tissue well supplied with blood vessels and similar in texture to a mucous membrane. The test substances were applied to the membrane for 60 minutes. The signs of irritation observed included hyperaemia (vascular dilation), lysis (vessels no longer macroscopically detectable), haemorrhage (leakage of blood from the vessels) or hyperaemic reaction of the vessels (capillary injection and/or a more prominent pattern of the capillaries) and coagulation of proteins and blood (Erdinger et al., 1997/98).

7.4 Sensitisation

No information available.

7.5 Subchronic and chronic toxicity

Groups of 10 male and 10 female Sprague-Dawley rats were given sodium dichloroacetate (99.5 to 100.7% pure) daily by oral gavage at dose levels of 0 (controls), 125, 500 or 2000 mg/kg body weight for 13 weeks. An additional 5 animals/sex were included in the control group and the high dose group for observation during a 4-week recovery period. The scope of the study and data analysis procedures largely met the requirements of OECD

guideline No. 408. Two males and 2 females treated at 2000 mg/kg body weight died. All dose groups showed dose-dependent depression of body weight gain in conjunction with decreased food consumption. Observed clinical signs of toxicity included hindlimb paralysis and frequent urination in the high dose group. Dose-dependent findings in all dose groups included slight decreases in red blood cell counts and/or haemoglobin and haematocrit values and marked decreases in blood glucose and lactate levels. Furthermore, the blood levels of creatinine were increased in females from 500 mg/kg body weight onwards; in males, iron levels were reduced at all dose levels, bilirubin levels were increased and triglycerides decreased from 500 mg/kg body weight onwards and the levels of creatinine, sodium and potassium were elevated and total protein and calcium reduced at 2000 mg/kg body weight. There were dose-dependent increases in relative liver (both sexes) and kidney (females) weights in all dose groups and increases in adrenal weights in male rats from 500 mg/kg body weight and in female rats in the high dose group relative to controls. Histopathologically, the brain and testes were the principal target organs. Brain lesions occurred at all dose levels tested, affected all animals from the intermediate dose level onwards and were characterised by vacuolation of the myelinated tracts of the cerebrum and, to a lesser extent, the cerebellum. The sciatic nerve and the optical nerve were normal. Degenerative changes in the testicular germinal epithelium were observed in 40% of males in the intermediate dose group. In the top dose group, all males had small testes at gross pathology and degenerative changes with syncytial giant cell formation in the germinal epithelium at histopathology. The testes and epididymides were both devoid of spermatogonia and spermatozoa, respectively. During the recovery period, the clinical signs of intoxication, depressions in body weight gain and changes in haematology and clinical chemistry parameters were reversible, whereas the histopathological findings were irreversible or reversible only to a minor extent (Katz et al., 1981). Even at the lowest dose level of sodium dichloroacetate tested, 125 mg/kg body weight/day, there were substance-related findings including histopathological lesions in the CNS, and therefore a *no observed effect level* (NOEL) can not be established on the basis of this study.

Groups of 10 male Sprague-Dawley rats received 90-day treatment with drinking water containing dichloroacetic acid (99% pure) at concentration levels of 0 (controls), 50, 500 or 5000 ppm, adjusted to pH 7.0 to 7.5 with

NaOH. Based on the water consumption data, the animals' daily intake levels of dichloroacetic acid were 0 (controls), 3.9, 35.5 or 345 mg/kg body weight. Drinking water intake and body weight gains were depressed in the two highest dose groups. Relative liver and kidney weights in the two highest dose groups and relative spleen weights in the top dose group were increased. In serum, total protein levels were dose-dependently depressed in all dose groups and alkaline phosphatase activity was elevated in the two highest dose groups while alanine aminotransferase activity was elevated in the highest dose group. In the 5000 ppm group, hepatic peroxisomal β-oxidation (measured as ^{14}C-palmitoyl-coenzyme A oxidation) was increased whereas the amount and activity of hepatic cytochrome P450 was unchanged. There were no indications of effects on immunological parameters (antibody production, delayed hypersensitivity, cytotoxicity of natural killer cells, production of prostaglandin PGE_2 and interleukin IL_2). No haematology examinations were carried out. Enlargement of the livers of rats treated at 5000 ppm was grossly evident, and histopathology revealed focal areas of enlarged cells containing significant amounts of glycogen. In addition, the kidneys of animals in the top dose group exhibited a diffuse degeneration of the tubular epithelium and cells of the glomeruli. No further pathological changes were observed in the comprehensive histological studies. Furthermore, the investigators also pointed out that, similarly to the studies described above (Katz et al., 1981) and below (Yount et al., 1982), another, at the time unpublished 6-month drinking water study they conducted with dose levels of 500 or 5000 ppm also found rear limb weakness and testicular atrophy (Mather et al., 1990). At the lowest dose level tested, 50 ppm (equivalent to 3.9 mg/kg body weight/day) in drinking water, depressed total serum protein levels were the only finding (low observed effect level). Hence, a *no observed effect level* was not found in this study.

A group of 6 male Wistar rats received sodium dichloroacetate in their diet at a level of 0.04 mol/kg over a period of 12 weeks. Based on the animals' food consumption, intake varied from 4 mmol/kg body weight/day at the beginning of the study to 2.5 mmol/kg body weight/day at the end of the study, equivalent to approx. 604 and 377 mg/kg body weight/day, respectively. A control group also consisting of 6 animals received normal diet. Rats showed depressed body weight gain in conjunction with significantly decreased food consumption. One animal died. At 2 to 4 weeks into the

study, hindlimb weakness and abnormal gait developed. This was associated with decreased nerve conduction velocity and tibial nerve diameter. Lipid and protein levels in the sciatic nerve did not differ from those seen in the controls. At the end of the study, the relative organ weights of the adrenal glands, brain, kidneys and liver were significantly increased, as were plasma ketone body levels. Macroscopic enlargement of the liver was noted. Histopathology revealed inhibition of spermiogenesis and degeneration of germ cells in one rat, increased amount of haemosiderin in the spleen in another rat and atrophic muscle fibres in a third rat (Yount et al., 1982).

A group of 5 male Sprague-Dawley rats received 90-day treatment with drinking water containing 80.5 mM (10.38 g/l) dichloroacetic acid (> 99% pure), adjusted to pH 7.2 to 7.4 with NaOH. Based on the animals' consumption of drinking water, this corresponded to a daily dose of approx. 1100 mg/kg body weight, or about ¼ of the acute LD_{50}. The treatment caused severely decreased body weight gain from the beginning of the study. By the end of the study, body weights were down to 66% of the controls treated with distilled water. Absolute and relative liver weights were increased whereas absolute and relative testes weights were decreased. Histopathological examination revealed changes in the lungs, liver, testes and brain. Liver changes were located primarily in the portal triads and were characterised by bile duct proliferation, enlarged portal veins, collagen deposition, oedema and scattered foci of inflammation. The lungs had foci of perivascular inflammation. The testes showed moderately to severely atrophic seminiferous tubules, which contained enlarged Sertoli cells, multinucleated giant cells, very few spermatocytes and no mature spermatozoa, and hyperplasia of the interstitial cells. Focal vacuolation and gliosis were seen in large areas of the brain, particularly in the forebrain, brain stem, cervical spinal tracts and basal ganglia (Bhat et al., 1991).

For special neurotoxicology studies in Fischer-344 and Long-Evans rats given dichloroacetic acid by gavage or in drinking water in numerous acute, subchronic and chronic substudies and observed to develop neurotoxicity even in the lowest dose range of approx. 16 mg/kg body weight/day, see Moser et al. (1999) in Section 7.10.

Groups of 3 male and 3 female beagle dogs received 0 (controls), 50, 75 or 100 mg sodium dichloroacetate (99.5 to 100.7% pure) in gelatine capsules

for 13 weeks. One extra animal of each sex was included in the control group and the high dose group for observation during a 5-week recovery period. The scope of the study and data analysis procedures largely met the requirements of OECD guideline No. 409. One male in the 100 mg/kg dose group and one female in the 75 mg/kg dose group died. There were dose-dependent body weight losses along with depressed feed consumption at all dose levels in both sexes. Clinical signs of toxicity included emesis from 75 mg/kg body weight onwards and bloody stools and hindlimb paralysis in the high dose group. Ophthalmological examination revealed bilateral lenticular opacities, conjunctival hyperaemia and superficial corneal vascularisation and signs of keratoconjunctivitis sicca at all dose levels in both sexes. Haematology results showed depressed erythrocyte counts, haematocrits and haemoglobin levels at all dose levels in both sexes. Furthermore, blood levels of glucose, lactate and pyruvate were substantially reduced in all treated animals while calcium and potassium levels were slightly decreased. Urine analyses were unremarkable. Gross pathology revealed lung consolidation in all animals treated with sodium dichloroacetate. Dose-dependent histopathological findings in all dose groups included vacuolation of the myelinated tracts of the cerebrum and, to a lesser extent, the cerebellum (the optic and sciatic nerves were unremarkable), prostate glandular atrophy and degenerative changes in the germinal epithelium of the testes with giant cell formation and vacuolation of the Leydig cells, cystic mucosal hyperplasia in the gall bladder and increased incidence rates of haemosiderin-laden Kupffer cells in the liver. Several animals had pulmonary nematode infections and concomitant inflammatory changes. The histopathological and ophthalmological lesions were not, or only to a small extent, reversible during the recovery period (Katz et al., 1981). Even at the lowest dose level of sodium dichloroacetate tested, 50 mg/kg body weight/day, there were substance-related changes including histopathological lesions in the CNS, prostate, testes, gall bladder and liver, and therefore a *no observed effect level* (NOEL) can not be established on the basis of this study. The investigators also made reference to a study they had previously conducted in beagle dogs treated intravenously with sodium dichloroacetate at 10, 40 or 100 mg/kg body weight/day for 1 month, which gave comparable results for haematology parameters and gall bladder findings, but not for ocular lesions or histopathological brain, prostate and testicular findings (no further details; Katz et al., 1977).

In a further study, groups of 5 male and 5 female beagle dogs (4 months old, initially weighing 6.1 to 13.6 kg one month before the beginning of dosing) received daily doses of dichloroacetic acid ("reagent grade", adjusted to pH 7.4 with NaOH) of 0 (controls), 12.5, 39.5 or 72 mg/kg body weight in gelatine capsules for 90 days. Animals in the top and intermediate dose groups exhibited clinical signs including diarrhoea, dyspnoea and conjunctivitis with clear to purulent ocular discharge. Hindlimb partial paralysis occurred in several dogs in the top dose group beginning at day 50 of the study. Two high-dose male dogs died at days 51 and 74 and one female dog of the top dose group died at day 50. Necropsy identified pneumonia and severe dehydration as the causes of death. Associated with large individual variation, food and water consumption were reduced in all dose groups. Males and females of the intermediate and top dose groups exhibited losses in body weight in the range from 9 to 16% in the course of the study. Haematology tests, which were repeated at 15-day intervals, showed reduced erythrocyte counts and haemoglobin levels for animals in the top dose group from study day 30 and for animals in the intermediate dose group from study day 45. Serum lactate dehydrogenase activity was increased in males at the end of the study and in females at the day 30 and 45 assessments. Serum alanine and aspartate aminotransferase activities in the top dose group were increased only at the day 60 assessment and only in males. Increased relative liver weights were observed in all dose groups, increased kidney weights in the intermediate and high dose groups and increased brain and lung weights in the high dose group in both sexes. Gross pathology revealed changes in the lungs, trachea, kidneys and liver of animals treated at 72 mg/kg body weight per day. As observed in the earlier study by Katz et al. (1977; see above), there were severe histopathological lesions in the CNS and the testes. There was vacuolisation of the myelinated tracts of cerebrum, cerebellum and/or spinal cord particularly in male dogs of the top dose group, findings which were also noted in dogs from the intermediate and low dose groups. Only the low-dose females had no histopathological changes in the CNS. Male dogs of all dose groups showed dose-dependent degeneration of the testicular germinal epithelium with syncytial giant cell formation and, in the intermediate and high dose groups, prostatic glandular atrophy. High-dose males additionally exhibited thymic atrophy, characterised by depletion of lymphoid tissue. Hepatic vacuolisation was observed in all groups, including the control. Mucosal epithelial vacuolisation and hyperplasia of the gall

bladder were prominent in all dichloroacetic acid-treated groups, as were signs of chronic inflammation of the liver and haemosiderosis in the two highest dose groups. Pneumonia and pancreatitis were observed in many high-dose and several intermediate-dose animals. A *no observed effect level* (NOAEL) was not determined in this study. The investigators considered the pathological changes in the various organ systems to be the consequence of the activation of pyruvate dehydrogenase by dichloroacetic acid (see also Section 7.11 – Pharmacodynamic effects of dichloroacetic acid) and the associated effects on lipid and amino acid metabolism, the induction of thiamine deficiency apparently being of particular importance (Cicmanec et al., 1991).

Further subchronic studies in the mouse are currently being conducted in the context of the NTP in the United States of America (NTP, 2003).

7.6 Genotoxicity

7.6.1 In vitro

The numerous studies on the in-vitro genotoxicity of dichloroacetic acid and sodium dichloroacetate are summarised in Tables 6 and 7.

Beginning of Table 6

Table 6. In-vitro genotoxicity tests with dichloroacetic acid or sodium dichloroacetate in bacteria

Test system	Concentration range tested (µg/plate)	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
1 Gene mutation					
1.1 Salmonella/microsome assay systems					
1.1.1 Salmonella/microsome assay as a standard plate incorporation test					
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537; assay according to a standardised and validated protocol with a minimum of 3 plates/concentration	333 to 5000[1], not toxic to bacteria up to the highest concentration	S9 mix from rat liver (presumably Aroclor 1254-induced)	negative	negative	Fox et al., 1996 a
[1] The sodium dichloroacetate tested was ≥ 99.5% pure (the only impurity being ≤ 0.5% trichloroacetate).					
Salmonella typhimurium TA 102, 3 plates scored per concentration	1–1000[1], no bacteriotoxicity data	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Meier et al., 1997
[1] Tests were carried out with > 99% pure dichloroacetic acid which was adjusted to pH 7.0 with NaOH.					

Table 6. In-vitro genotoxicity tests with dichloroacetic acid or sodium dichloroacetate in bacteria					
Test system	Concentration range tested (µg/plate)	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
Salmonella typhimurium TA 102, TA 2638; assay with independent repeat and reproduction of results by another laboratory	313–5000[1], bacteriotoxicity tested	S9 mix from phenobarbitone- and 5,6-benzoflavone-induced rat liver	negative	not tested	Watanabe et al., 1996
[1] No details as to the purity of the dichloroacetic acid used.					
Salmonella typhimurium TA 100	no information	S-9 mix (no further details)	negative	negative	Matsuda et al., 1991
Salmonella typhimurium (5 strains)	no information[1]	no information	positive (TA 1538; no indication whether performed with and/or without metabolic activation)		Herbert et al., 1978
[1] Tests were carried with dichloroacetate (presumably dichloroacetic acid neutralised with NaOH), purity unspecified. The revertant count for strain TA 1538 was reported to be 10 times higher than control, thus indicating a clear mutagenic effect (no further details). The finding was not confirmed by a later study that the investigators conducted (see below; Herbert et al., 1979 a, b, c, 1980).					
Salmonella typhimurium TA 98[1]	0, 1, 5 and 10[1], no bacteriotoxicity data	S9 mix from Aroclor 1254-induced rat liver	weakly positive[2]	weakly positive[2]	Herbert et al., 1979 a, b, c, 1980
[1] A preliminary test was carried out with dichloroacetic acid (purity unspecified), adjusted to pH 3, 7 or 9 with NaOH, in strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538. The preliminary studies showed increased revertant counts only in strains TA 98 and TA 1538, but the increases were concentration-dependent only in strain TA 98 and only in the tests using the pH 7-adjusted, i.e. neutralised, compound (no further details). The main study was only conducted in strain TA 98 with compound that was adjusted to pH 7. [2] There was no minimum two-fold increase in revertant count either with or without metabolic activation. The evaluation as weakly positive by the investigators is based on a slight concentration-dependent increase in revertant counts by factors of 1.08, 1.26 and 1.36 (without metabolic activation) and 1.37, 1.47 and 1.69 (with metabolic activation), which were calculated from 3 or 4 independently conducted test.					
Salmonella typhimurium, TA 98, TA 100, TA 1535, according to Herbert et al. (1980)	tested up to the bacteriotoxic concentration[1]	S9 mix from Aroclor 1254- and phenobarbitone-induced rat liver	weakly positive or negative[2]	not tested	Waskell, 1978
[1] Purity unspecified. According to Herbert et al. (1980), practical-grade, distilled, ethanol-recrystallised dichloroacetic acid and the potassium salt prepared from it were used for testing. [2] The investigator reported that the practical-grade and distilled samples were weakly mutagenic, whereas the recrystallised sample did not produce genotoxicity (no further details). According to Herbert et al. (1980), the practical-grade dichloroacetic acid tested by Waskell (1978) was weakly positive in strains TA 100 and TA 1535 but negative in strain TA 90 whereas the ethanol-recrystallised sample and the potassium salt prepared from it were no longer mutagenic (Herbert et al., 1980).					
1.1.2 Salmonella/microsome assay as a preincubation test					
Salmonella typhimurium TA 98, TA 100	up to 4000[1], tested up to the bacteriotoxic concentration	S9 mix[1]	negative	positive (TA 100)	Kato et al., 1999; Saito et al., 1995
[1] Tests were carried out with dichloroacetic acid ≥ 97% pure. Apart from the positive result obtained with strain TA 100 at a concentration of 2560 µg/plate without metabolic activation, the publications, written mostly in Japanese, provide no further details.					

Table 6. In-vitro genotoxicity tests with dichloroacetic acid or sodium dichloroacetate in bacteria

Test system	Concentration range tested (µg/plate)	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
1.1.3 Salmonella/microsome assay in a closed test system for testing volatile substances					
Salmonella typhimurium TA 100; assay with at least one independent repeat	100–600 ppm (ca. 526–3156 mg/m^3)[1], tested up to the bacteriotoxic concentration	S9 mix from Aroclor 1254-induced rat liver	positive[2, 3]	positive[2, 3]	DeMarini et al., 1994
[1] The dichloroacetic acid tested was 99% pure.					
[2] Fuscoe et al. (1996) reported that, according to a personal communication from DeMarini, dichloroacetic acid produced a negative result when tested on strain TA 100 in the standard plate incorporation test.					
[3] Sequence analysis of revertant DNA amplified by polymerase chain reaction showed that the dichloroacetic acid-induced base substitution at the hisG46 allele, in deviation from the background mutation spectrum, primarily consisted in CCC → CTC transitions and to a lesser extent in CCC → CAC transversions. The investigators proposed, as a likely mechanism, the formation of an etheno adduct on cytosine, causing the DNA polymerase to misincorporate primarily adenine and, to a lesser extent, thymine instead of guanine opposite the adducted cytosine. Upon replication of the strand containing adenine or thymine, the G : C → A : T transitions and G : C → T : A transversions become manifest.					
1.1.4 Salmonella/microsome assay as a fluctuation test					
Salmonella typhimurium TA 100; assay with at least one independent repeat	30–3000 µg/ml (– S9 mix) and 300–1000 µg/ml (+ S9 mix)[1], toxic to bacteria from 2000 µg/ml (– S9 mix) and 10000 µg/ml (+ S9 mix)	S9 mix from Aroclor 1254-induced rat liver	positive	positive	Giller et al., 1997
[1] The dichloroacetic acid tested was > 97% pure.					
1.1.5 Salmonella/microsome assay as a microsuspension assay					
Salmonella typhimurium TA 98, TA 100	no data	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Kohan and Huggins-Clark, 1998
1.2 Further gene mutation tests in bacteria					
Escherichia coli WP2 uvrA; tryptophan reversion test, assay according to a standardised and validated protocol with a minimum of 3 plates/concentration	333–5000[1], not toxic to bacteria up to the highest concentration	S9 mix from rat liver (presumably Aroclor 1254-induced)	negative	negative	Fox et al., 1996 a
[1] The sodium dichloroacetate tested was ≥ 99.5% pure (the only impurity being ≤ 0.5% trichloroacetate).					
Escherichia coli WP2/pKM101 and WP2 uvrA/pKM 101; assay with independent repeat and reproduction of results by another laboratory	313–5000[1], bacteriotoxicity tested	S9 mix from phenobarbitone- and 5,6-benzoflavone-induced rat liver	negative	not tested	Watanabe et al., 1996
[1] No details as to the purity of the dichloroacetic acid used.					

Table 6. In-vitro genotoxicity tests with dichloroacetic acid or sodium dichloroacetate in bacteria

Test system	Concentration range tested (µg/plate)	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
2 DNA damage					
2.1 DNA repair test systems					
Salmonella typhimurium hisGr (repair proficient) and TS 24 recA, TA 2322 polA and TA 1950 uvrB (repair deficient); DNA repair test as a diffusion test using filter paper discs	31 mg/filter paper disc (6 mm in diameter)[1]	not tested	not tested	negative	Waskell, 1978
[1] The dichloroacetic acid tested was presumably of practical grade.					
2.2 Tests to study induction of an SOS response					
Salmonella typhimurium TA 1535/pSK 1002; umu test with measurement of β-galactosidase activity	58, 5 µg/ml[1]	S9 mix from phenobarbitone- and 5,6-benzoflavone-induced rat liver	positive[2]	negative	Ono et al., 1991
[1] No details as to the purity of the dichloroacetic acid used. [2] There was a 1.5-fold increase in enzyme activity relative to the initial value. Positive and/or negative controls were not included.					
Escherichia coli PQ37, SOS chromotest; assay with at least one independent repeat	10–10000 µg/ml[1], toxic to bacteria from 750 µg/ml (– S9 mix) and 3000 µg/ml (+ S9 mix)	S9 mix from Aroclor 1254-induced rat liver	negative	weakly positive[2]	Giller et al., 1997
[1] The dichloroacetic acid tested was > 97% pure. [2] Maximum induction factor 1.56 (at 500 µg/ml); the investigators scored a result as positive when the induction factor was 1.5 or greater.					
Escherichia coli WP2s lambda (lon11, sulA1, trpE65, uvrA155, lamB$^+$), prophage lambda induction test as a Microscreen assay with a minimum of one independent repeat	up to 5000 µg/ml (– S9 mix) or 10000 µg/ml[1] (+ S9 mix), tested up to the bacteriotoxic concentration	S9 mix from rat liver (no details of the chemical used for induction)	weakly positive[2]	negative	DeMarini et al., 1994
[1] The dichloroacetic acid tested was 99% pure. [2] 3.7- to 4.3-fold increases in plaque-forming units (PFU) relative to background values. The investigators' criterion for a positive effect was a minimum 3-fold increase relative to the background value and they classed dichloroacetic acid as having a weak effect in the prophage lambda induction assay, compared with more than 100 positive substances.					
3 Other tests					
Klebsiella pneumoniae, induction of uracil and/or proline independence	0.01% (v/v)[1]	not tested	not tested	negative	Voogd et al., 1972
[1] No details as to the purity of the dichloroacetic acid used.					

End of Table 6

Beginning of Table 7

Table 7. In-vitro genotoxicity tests with dichloroacetic acid or sodium dichloroacetate in mammalian cells

Test system	Concentration range tested (µg/ml)	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
1 Gene mutation					
V79/HPRT test, 6-thioguanine resistance, Chinese hamster lung fibroblasts (V79); test according to OECD guideline No. 476 with an independent repeat	30–1000 (– S9 mix) and 30–600 (+ S9 mix)[1], top concentrations cytotoxic	S9 mix from Aroclor 1254-induced rat liver	negative	negative	RBM, 1994 a
[1] The dichloroacetic acid tested was 99.5% pure and not neutralised. In the preliminary test for cytotoxicity, pH values of < 3.6 were measured at dichloroacetic acid concentrations of 2000 to 5000 µg/ml in the incubation medium after an incubation period of 2 hours.					
L5178Y/TK assay, trifluorothymidine resistance assay, mouse lymphoma cells (L5178Y/TK$^{+/-}$-3.7.2C); assay according to a standardised and validated protocol with a minimum of 3 plates/concentration	125–5000[1], not cytotoxic up to the highest concentration	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Fox et al., 1996 a
[1] The sodium dichloroacetate tested was ≥ 99.5% pure (the only impurity being ≤ 0.5% trichloroacetate).					
L5178Y/TK assay, trifluorothymidine resistance assay, mouse lymphoma cells (L5178Y/TK$^{+/-}$-3.7.2C), clone -3.7.2C; assay with 1 plate/concentration and an independent repeat	100–1000 in the first test and 200–900[1] in the repeat, concentration-dependent cell survival rates of 100–2% and 97–7%	not tested	not tested	weakly positive[2]	Harrington-Brock et al., 1998
[1] No details as to the purity of the dichloroacetic acid used, which was not neutralised. The pH of the incubation medium ranged between 7.2 and 6.1, depending on the concentration. The investigators therefore did not exclude a pH-related positive effect. [2] In an earlier publication, the investigators also reported that they had obtained a positive result in this test system at a dichloroacetic acid concentration of 1200 µg/ml (tested concentration range: 100 to 1200 µg/ml) and a cell survival rate as low as 10% (cell survival rates over the entire concentration range 100 to 10%; no further details; Harrington-Brock et al., 1992).					
2 Chromosome damage					
Chromosome aberration test, Chinese hamster lung fibroblasts (V79); test according to OECD guideline No. 473 with an independent repeat, 200 metaphases examined per concentration	150–1500 (– S9 mix) and 150–3000 (+ S9 mix)[1], cytotoxic at the highest concentration in both cases	S9 mix from Aroclor 1254-induced rat liver	negative	negative	RBM, 1994 b
[1] The dichloroacetic acid tested was 99.5% pure and not neutralised. In the preliminary cytotoxicity test, a marked decrease in pH was measured in the incubation medium at a dichloroacetic acid concentration of 5000 µg/ml.					

Table 7. In-vitro genotoxicity tests with dichloroacetic acid or sodium dichloroacetate in mammalian cells

Test system	Concentration range tested (µg/ml)	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
Chromosome aberration test, Chinese hamster ovary (CHO WBL) cells; test according to a standardised and validated protocol, 100 metaphases per concentration examined at two sampling times	500–5000[1], cytotoxicity at the highest concentration	S9 mix from Aroclor 1254-induced rat liver	negative	negative	Fox et al., 1996 a
[1] The sodium dichloroacetate tested was ≥ 99.5% pure (the only impurity being ≤ 0.5% trichloroacetate).					
Chromosome aberration test, Chinese hamster lung fibroblasts (CHL); test according to a standardised and validated protocol, 100 metaphases per concentration examined at two sampling times	250–2000[1], cytotoxicity tested	not tested	not tested	positive[2]	JETOC, 1996
[1] No details as to the purity of the dichloroacetic acid used, which was not neutralised. [2] Positive from 1500 µg/ml (preparation after 24 hours) and at 2000 µg/ml (preparation after 48 hours), analysis including and excluding gaps.					
Combined chromosome aberration and micronucleus test, mouse lymphoma cells (L5178Y/TK$^{+/-}$-3.7.2C), analysis of 1000 cells/concentration in the micronucleus test and 100 metaphases/concentration in the chromosome aberration test; cultures were parallel cultures from the above-described L5178Y/TK test by the investigators	600 and 800[1], cell survival rates of 42% and 28%	not tested	not tested	positive[2]	Harrington-Brock et al., 1998
[1] No details as to the purity of the dichloroacetic acid used, which was not neutralised. [2] The number of aberrations (excluding gaps) increased from 8 in the control to 22 and 28 at the two concentrations tested. Analysis for significant induction of micronuclei gave a negative result. Furthermore, the investigators pointed out that aneuploidy was also not induced. No information given as to whether the findings were confirmed in an independent repeat experiment.					
3 DNA damage					
SCE test, Chinese hamster ovary (CHO-K1) cells, at least 50 metaphases examined per concentration	500–10000[1], cytotoxic from 2000 (– S9 mix), and not cytotoxic up to and including 10000 (+ S9 mix)	S9 mix from Aroclor 1254-induced rat liver	negative	weakly positive[2]	Meier et al., 1997
[1] Tests were carried out with > 99% pure dichloroacetic acid which was adjusted to pH 7.0 with NaOH. [2] The result was evaluated as weak by the investigators because a slight but concentration-dependent significant increase in the number of SCEs/cell (14.58 as opposed to 10.02 in the solvent control and 25.48 in the positive control) was observed only at the very high concentration of 2000 µg/ml.					

Table 7. In-vitro genotoxicity tests with dichloroacetic acid or sodium dichloroacetate in mammalian cells					
Test system	Concentration range tested (µg/ml)	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
DNA single-strand breaks, alkaline elution ("alkaline unwinding assay"), rat hepatocytes (F344)	ca. 129–1289 (1–10 mM)[1], no cytotoxicity (measured as lactate dehydrogenase activity)	primary hepatocytes	negative	–[2]	Chang et al., 1992
[1] No details as to the purity of dichloroacetic acid, which was adjusted to pH 7.0 with NaOH. [2] Not applicable since primary hepatocytes per se are metabolically competent.					
DNA single-strand breaks, alkaline elution ("alkaline unwinding assay"), mouse hepatocytes (B6C3F1)	ca. 12.9–1289 (0.1–10 mM)[1], no cytotoxicity (measured as lactate dehydrogenase activity)	primary hepatocytes	negative	–[2]	Chang et al., 1992
[1] No details as to the purity of dichloroacetic acid, which was adjusted to pH 7.0 with NaOH. [2] Not applicable since primary hepatocytes per se are metabolically competent.					
DNA single-strand breaks, alkaline elution ("alkaline unwinding assay"), human lymphoblasts, CCRF-CEM cells	ca. 129–1289 (1–10 mM)[1], no cytotoxicity (measured as lactate dehydrogenase activity)	not tested	not tested	negative	Chang et al., 1989, 1992
[1] No details as to the purity of dichloroacetic acid, which was adjusted to pH 7.0 with NaOH.					

End of Table 7

Tests for mutagenic activity

The mutagenic potential of dichloroacetic acid and sodium dichloroacetate in procaryotes was tested in Salmonella/microsome assays and in *Escherichia coli*. Tests carried out as standard plate incorporation tests in *Salmonella typhimurium* strains TA 98, TA 100, TA 102, TA 1535, TA 1537, TA 1538 and TA 2638 and in *Escherichia coli* WP2 strains gave negative results both in the absence and presence of metabolic activation (S-9 mix from rat liver induced with Aroclor or phenobarbitone and benzoflavone; see Table 6). A test carried out as a microsuspension test in strains TA 98 and TA 100 was negative both with and without metabolic activation (Kohan and Huggins-Clark, 1998).

The positive results of several older standard plate incorporation tests were based on only a slight, but less than twofold increase in revertant counts, they were not confirmed in later studies by the same research group or they were obtained with only technical-grade dichloroacetic acid and no longer observed upon purification of the sample by recrystallisation (Herbert et al., 1978, 1979 a, b, c, 1980; Waskell, 1978). In the preincubation test in strain TA 100, the result was negative in the presence and positive in the absence of metabolic activation (Kato et al., 1999; Saito et al., 1995). The results for the strain were also positive in the fluctuation test and the test modified for volatile substances with and without metabolic activation (S-9 mix from Aroclor 1254-induced rat liver; DeMarini et al., 1994; Giller et al., 1997). Another finding in support of a positive effect of dichloroacetic acid in strain TA 100 was that a sequence analysis of revertant DNA amplified by polymerase chain reaction produced a base substitution at the hisG46 allele which deviated from the background mutation spectrum. The investigators suggested that primarily CCC → CTC transition and to a lesser extent CCC → CAC transversion were induced by the formation of an etheno adduct on cytosine (see also Table 6; DeMarini et al., 1994). The HPRT test of non-neutralised dichloroacetic acid (99.5% pure) performed on Chinese hamster lung fibroblasts (V79 cells) in accordance with OECD guideline No. 476, and the L5178Y/TK test of sodium dichloroacetate (≥ 99.5%) on mouse lymphoma cells (L5178Y/TK$^{+/-}$-3.7.2C), also performed according to a standardised and validated protocol, gave no indication of mutagenic potential for the compounds (RBM, 1994 a; Fox et al., 1996 a). In contradiction with these findings, a positive result was obtained with non-neutralised dichloroacetic acid of unknown purity in the L5178Y/TK test on mouse lymphoma cells (L5178Y/TK$^{+/-}$-3.7.2C) without metabolic activation. Only one plate was scored per concentration, but the result was confirmed in an independent repeat of the experiment. It is unlikely that the positive result was induced by shifts in the pH of the medium. The pH of the incubation medium ranged between 7.2 and 6.3, depending on the concentration (Harrington-Brock et al., 1998).

Tests for chromosome-damaging activity

Chromosome aberration studies of sodium dichloroacetate (≥ 99.5% pure) on Chinese hamster CHO cells and of non-neutralised dichloroacetic acid (99.5% pure) on Chinese hamster V79 cells which were conducted in com-

pliance with current standards gave no indication of any chromosome-damaging potential of the compounds either in the absence or in the presence of metabolic activation (S-9 mix from Aroclor 1254-induced rat liver; Fox et al., 1996 a; RBM, 1994 b). In contrast, chromosome aberration tests performed on Chinese hamster CHL cells in accordance with the Japanese guidelines for testing and on mouse lymphoma cells (L5178Y/TK$^{+/-}$-3.7.2C) were positive in the absence of metabolic activation. Both tests were only performed with non-neutralised dichloroacetic acid (purity unspecified) in the absence of metabolic activation (JETOC, 1996; Harrington-Brock et al., 1998). An in-vitro study with mouse lymphoma cells (L5178Y/TK$^{+/-}$-3.7.2C), performed as a micronucleus test, and only without metabolic activation, produced a negative result (Harrington-Brock et al., 1998).

Tests for DNA-damaging activity

A DNA repair test performed only in the absence of metabolic activation on the DNA repair-proficient *Salmonella typhimurium* strain hisGr and the repair-deficient strains TS24recA, TA 2322 polA and TA 1950 uvrB yielded no indication of any DNA-damaging potential of dichloroacetic acid (Waskell, 1978). Test systems utilising the induction of an SOS response as a parameter for a DNA-damaging effect (the umu test on *Salmonella typhimurium* TA 1535/pSK 1002, the SOS chromotest on *Escherichia coli* PQ37 and the prophage lambda induction test on *Escherichia coli* WP2 lambda) yielded a weakly positive result with or without metabolic activation (Ono et al., 1991; Giller et al., 1997; DeMarini et al., 1994). Dichloroacetic acid was also evaluated by the investigators as weakly positive in an SCE test on Chinese hamster ovary (CHO) cells in which there was a slight but concentration-dependent, significant increase in the number of SCEs/cell at the very high concentration of 2000 µg/ml of NaOH-neutralised dichloroacetic acid in the absence of metabolic activation (Meier et al., 1997). Neutralised dichloroacetic acid induced no DNA single-strand breaks in primary rat or mouse hepatocytes or in human lymphoblasts (CCRF-CEM cells) in the alkaline elution test (“alkaline unwinding assay”; Chang et al., 1989, 1992).

7.6.2 In vivo

The data on the genotoxicity of dichloroacetic acid and sodium dichloroacetate in in-vivo test systems are summarised in Table 8.

Beginning of Table 8

Table 8. In-vivo genotoxicity tests with dichloroacetic acid or sodium dichloroacetate

Test system	Dose, regimen	Toxicity	Result	Reference
1 Chromosome damage				
Micronucleus test, rat (CR:CD (SD) BR), 5 rats/sex and dose; 1000 polychromatic erythrocytes/rat (bone marrow) were examined; test according to a standardised and validated protocol	275, 550 or 1100 mg/kg body weight[1], intravenous, 3 doses at intervals of 24 hours (sampling time points not specified)	ataxia and prostration after every administration at the top concentration; dose levels > 1100 mg/kg body weight were lethal in a preliminary study	negative	Fox et al., 1996 a
[1] The sodium dichloroacetate tested was ≥ 99.5% pure (the only impurity being ≤ 0.5% trichloroacetate).				
Micronucleus test, mouse (Swiss-Webster), 6 mice/sex, dose and sampling time point; a minimum of 500 polychromatic erythrocytes/mouse (bone marrow) were examined	1125, 2250 or 4500 mg/kg body weight[1], by oral gavage, 2 doses at an interval of 24 hours, assessment at 48 or 72 hours after the first administration	the LD_{50} in the preliminary study in B6C3F1 mice was 5697 to 7500 mg/kg body weight; 2 females in the top dose group died and the percentage of polychromatic erythrocytes out of the total number of erythrocytes was decreased in the males and females from the top dose group	negative	Meier et al., 1997
[1] Tests were carried out with > 99% pure dichloroacetic acid which was adjusted to pH 7.0 with NaOH.				
Micronucleus test, mouse (B6C3F1), 10 males/group; 1000 polychromatic erythrocytes from the peripheral blood were examined per mouse	0.5, 1, 2 or 3.5 g/l in drinking water[1], administered orally for 9 days; another group treated at 3.5 g/l additionally received vitamin E (100 mg/kg body weight/week, intraperitoneally)	no dose-dependent significant change in the number of polychromatic erythrocytes/1000 erythrocytes	weakly positive in the highest dose group[2]	Fuscoe et al., 1996; Salman et al., 1996
[1] The dichloroacetic acid tested was ≥ 99% pure. The drinking water was adjusted to pH 6.8 to 7.4 with NaOH. [2] Slight, but statistically significant increase in micronucleated polychromatic erythrocytes to 180% of the untreated control. Additional administration of vitamin E in the 3.5 g/l group had no effect on the number of micronucleated polychromatic erythrocytes.				
Micronucleus test, mouse (B6C3F1), 10 males/group; 1000 to 2000 polychromatic and 1000 to 2000 normochromatic erythrocytes from the peripheral blood were examined per mouse	0.5, 1, 2 or 3.5 g/l in drinking water[1], administered orally for 28 days; another group treated at 3.5 g/l additionally received vitamin E (100 mg/kg body weight/week)	no significant change in the number of polychromatic erythrocytes/1000 erythrocytes	negative[2]	Fuscoe et al., 1996; Salman et al., 1996
[1] The dichloroacetic acid tested was ≥ 99% pure. The drinking water was adjusted to pH 6.8 to 7.4 with NaOH. [2] Additional administration of vitamin E had no effect on the number of micronucleated polychromatic erythrocytes.				

Table 8. In-vivo genotoxicity tests with dichloroacetic acid or sodium dichloroacetate

Test system	Dose, regimen	Toxicity	Result	Reference
Micronucleus test, mouse (B6C3F1), 10 males/group; 1000 to 2000 polychromatic and 1000 to 2000 normochromatic erythrocytes from the peripheral blood were examined per mouse	3.5 g/l in drinking water[1], administered orally for 10, 26 or 31 weeks; all groups were examined after a total study period of 31 weeks	no significant change in the number of polychromatic erythrocytes/1000 erythrocytes	weakly positive[2]	Fuscoe et al., 1996; Salman et al., 1996

[1] The dichloroacetic acid tested was ≥ 99% pure. The drinking water was adjusted to pH 6.8 to 7.4 with NaOH.

[2] For all treatment periods, there were slight, but statistically significant increases in micronucleated normochromatic erythrocytes to 170, 180 and 200% of the control. The percentage of micronucleated polychromatic erythrocytes did not differ significantly from the control. The investigators suggested that possibly the slight increase in micronucleated normochromatic erythrocytes was not a consequence of direct genotoxicity of the compound but rather may also have been a secondary consequence of the systemic toxicity of the administered dose and that dichloroacetic acid only had a very weak genotoxic effect, if any.

2 DNA damage				
SCE test, mouse	no data	no data	negative	Meier, year not given
DNA single-strand breaks, alkaline elution ("alkaline unwinding assay"), liver DNA, rat (Sprague-Dawley), 5 males/group	ca. 0.1– ≥ ca. 3.9 mmol (12.9–503 mg)/kg body weight[1], single dose by oral gavage, sacrifice 4 hours after administration	no hepatotoxicity (activity levels of serum aspartate and alanine aminotransferase not increased in a parallel group treated with 3.8 mmol/kg body weight 24 hours after administration)	positive (from 0.23 mmol (ca. 30 mg)/kg body weight)	Nelson and Bull, 1988; Washington State University, 1989

[1] The dichloroacetic acid tested was > 99% pure. No information whether the Tween 80-formulated compound was neutralised prior to administration. It remains unclear what the highest administered dose was, which can only be read from the graphical representation of the data. Though identical in shape, the curves in the two publications have different abscissas, indicating maximum administered doses of approx. 3.9 (Washington State University, 1989) and approx. 15 mmol/kg body weight (Nelson and Bull, 1988).

DNA single-strand breaks, alkaline elution ("alkaline unwinding assay"), liver DNA, rat (Sprague-Dawley), 4 males/group	ca. 3.9 mmol (503 mg)/kg body weight[1], single dose by oral gavage, sacrifice 0.5, 1 or 2 hours after administration	no information	positive (at all sacrifice times)	Nelson and Bull, 1988; Washington State University, 1989

[1] The dichloroacetic acid tested was > 99% pure. No information whether the Tween 80-formulated compound was neutralised prior to administration.

DNA single-strand breaks, alkaline elution ("alkaline unwinding assay"), liver DNA, mouse (B6C3F1), 5 males/group	ca. 0.006– ≥ ca. 3.9 mmol (12.9–503 mg)/kg body weight[1], single dose by oral gavage, sacrifice 4 hours after administration	no hepatotoxicity (activity levels of serum aspartate and alanine aminotransferase not increased in a parallel group treated with 3.9 mmol/kg body weight 24 hours after administration)	positive	Nelson and Bull, 1988; Washington State University, 1989

[1] The dichloroacetic acid tested was > 99% pure. No information whether the Tween 80-formulated compound was neutralised prior to administration. It remains unclear what the highest administered dose was, which can only be read from the graphical representation of the data. Though identical in shape, the curves in the two publications have different abscissas, indicating maximum administered doses of approx. 3.9 (Washington State University, 1989) and approx. 15 mmol/kg body weight (Nelson and Bull, 1988).

Table 8. In-vivo genotoxicity tests with dichloroacetic acid or sodium dichloroacetate				
Test system	Dose, regimen	Toxicity	Result	Reference
DNA single-strand breaks, alkaline elution ("alkaline unwinding assay"), liver DNA, mouse (B6C3F1), 6 males/group	0.08 or 3.9 mmol (ca. 10 or 503 mg)/kg body weight[1], single dose by oral gavage, sacrifice 1, 2, 4, 8 or 24 hours after administration	no peroxisome proliferation (no increase in ^{14}C-palmitoyl-coenzyme A oxidation as a measure of increased β-oxidation rate after single-dose administration; after repeated doses of 500 mg/kg body weight over 10 days: increased liver weights, enlarged hepatocytes with glycogen deposition, and higher numbers of peroxisomes and increased oxidation of palmitoyl-coenzyme A (approx. 160% of control))	positive (0.08 and 3.9 mmol/kg body weight at sacrifice times 1 (maximum), 2 and 4 hours)	Nelson and Bull, 1988; Nelson et al., 1988, 1989; Washington State University, 1989
[1] The dichloroacetic acid tested was > 99% pure. No information whether the Tween 80-formulated compound was neutralised prior to administration.				
DNA single-strand breaks, alkaline elution ("alkaline unwinding assay"), liver DNA, rat (F344), 4 males/group, DNA measured in 3 samples/rat	1 or 5 mmol (ca. 129 or 645 mg)/kg body weight[1], single dose by oral gavage, sacrifice 4 hours after administration	administration of ¼ to ⅓ of the LD_{50}	negative	Chang et al., 1989, 1992
[1] No details as to the purity of dichloroacetic acid, which was adjusted to pH 7.0 with NaOH.				
DNA single-strand breaks, alkaline elution ("alkaline unwinding assay"), liver DNA, rat (F344), 5 males/group, DNA measured in 3 samples/rat	0.05, 0.5 or 2 g/l in drinking water[1] administered orally for 30 weeks	Peroxisome proliferation (the activity of cyanide-insensitive palmitoyl-coenzyme A oxidase, a marker enzyme, was increased in the high dose group to 364% of the control value in liver)	negative	Chang et al., 1992
[1] No details as to the purity of dichloroacetic acid, which was adjusted to pH 7.0 with NaOH.				
DNA single-strand breaks, alkaline elution ("alkaline unwinding assay"), DNA from liver, spleen, stomach and intestines, mouse (B6C3F1), 4 males/group, DNA measured in 3 samples/mouse and tissue	1, 5 or 10 mmol (ca. 129, 645 or 1289 mg)/kg body weight[1], single dose by oral gavage, sacrifice 1 or 4 hours after administration	administration of ¼ to ⅓ of the LD_{50}	negative	Chang et al., 1989, 1992
[1] No details as to the purity of dichloroacetic acid, which was adjusted to pH 7.0 with NaOH.				

Table 8. In-vivo genotoxicity tests with dichloroacetic acid or sodium dichloroacetate

Test system	Dose, regimen	Toxicity	Result	Reference
DNA single-strand breaks, alkaline elution ("alkaline unwinding assay"), liver DNA, mouse (B6C3F1), 3 males/group, DNA measured in 3 samples/mouse	0.5 or 5 g/l in drinking water[1] administered orally for 7 weeks and 14 days, respectively	Peroxisome proliferation (the activity of cyanide-insensitive palmitoyl-coenzyme A oxidase, a marker enzyme, was increased in the high dose group to 652% (7 days) and 90% (14 days) of the control value in liver)	negative	Chang et al., 1992
[1] No details as to the purity of dichloroacetic acid, which was adjusted to pH 7.0 with NaOH.				
DNA single-strand breaks, alkali-labile DNA lesions, incomplete repair, cross-links, modified alkaline elution ("alkaline single cell gel electrophoresis assay"), leucocyte DNA, mouse (B6C3F1), 9 or 10 males/group, 25 cells/mouse examined	0.5, 1, 2 or 3.5 g/l in drinking water[1], administered orally for 28 days; another group treated at 3.5 g/l additionally received vitamin E (100 mg/kg body weight/week, intraperitoneally)	no information	negative for induction of DNA breaks, alkali-labile lesions and incomplete repair, weakly positive for cross-links in the high dose group[2]	Fuscoe et al., 1996; Salman et al., 1996
[1] The dichloroacetic acid tested was ≥ 99% pure. The drinking water was adjusted to pH 6.8 to 7.4 with NaOH. [2] No significant differences were noted between the group additionally treated with vitamin E and the untreated control.				
3 Other tests				
Abnormal spermhead morphology, mouse (B6C3F1), 12 mice/dose and sampling time point; 500 sperm cells/mouse were examined	0, 1125, 2250 or 4500 mg/kg body weight[1], by oral gavage, daily for 5 days, assessment at 21 or 35 days after the first administration	The LD_{50} value for 5 doses was 4562 to 6610 mg/kg body weight	positive[2]	Meier et al., 1997
[1] Tests were carried out with > 99% pure dichloroacetic acid which was adjusted to pH 7.0 with NaOH. [2] The percentage of sperm cells with abnormal heads was significantly increased in all dose groups at the day 21 examination and in the top dose group at the day 35 examination. Furthermore, the numbers of sperm cells in the epididymides were significantly decreased in all dose groups after 35 days. Testicular weights in all dose groups and at all sampling times were similar to those of the controls. The investigators suggested that, taking into account the negative result of their micronucleus test (see above) and the testicular findings noted after repeated administration of the compound (Katz et al., 1981; see Section 7.5), the observed sperm-head abnormalities and the decreases in epididymal sperm counts were more likely to be attributable to the compound having a nonspecific toxic effect on the testes rather than a genotoxic effect.				

End of Table 8

Tests for chromosome-damaging activity

Micronucleus tests in CR:CD rats given three intravenous doses of up to 1100 mg/kg body weight and in Swiss-Webster mice given two oral administrations of up to 4500 mg/kg body weight both gave no indication of clas-

togenic activity of sodium dichloroacetate or NaOH-neutralised dichloroacetic acid (≥ 99% pure) when erythrocytes from the bone marrow were examined. Inconstant findings were obtained in the male B6C3F1 mouse in a series of micronucleus tests with examination of erythrocytes from the peripheral blood. Animals received drinking water (adjusted to pH 6.8 to 7.4 with NaOH) containing 0.5, 1, 2 or 3.5 g/l dichloroacetic acid (≥ 99% pure) over a period of 9 or 28 days in one substudy and drinking water with 3.5 g/l dichloroacetic acid for 10, 26 or 31 weeks in another substudy. In the second substudy, all animals were examined after 31 weeks, i.e. some of the animals were observed for 21 weeks or 5 weeks. Examination after 28 days' administration yielded no increase in the number of micronucleated polychromatic or normochromatic erythrocytes in any dose group. The investigators evaluated as weakly positive the findings in the group treated with 3.5 g/l for 9 days (increase in micronucleated polychromatic erythrocytes to 180% of the untreated control) and in the group treated with 3.5 g/l for 10, 26 or 31 weeks (respective increases in micronucleated normochromatic erythrocytes to 170, 180 and 200% of control, no increase in micronucleated polychromatic erythrocytes). The investigators suggested that possibly the slight increase in micronucleated normochromatic erythrocytes was not a consequence of direct genotoxicity of the compound but rather may also have been a secondary consequence of the systemic toxicity of the administered dose and that dichloroacetic acid only had a very weak genotoxic effect, if any (Fuscoe et al., 1996; Salman et al., 1996).

Tests for DNA-damaging activity

Whereas a weakly positive result was obtained with dichloroacetic acid in vitro in the SCE test on Chinese hamster CHO cells at high concentrations without metabolic activation (see above; Meier et al., 1997), an in-vivo SCE test which the investigators performed in the mouse reportedly was negative (no further details; Meier, not dated).

Diametrically opposite results were obtained by two research groups in series of tests for induction of DNA single-strand breaks in the alkaline elution test ("alkaline unwinding assay"). The first research team administered dichloroacetic acid (> 99% pure) by gavage in single oral doses of up to 3.9 mmol (approx. 500 mg)/kg body weight to male Sprague-Dawley rats and male B6C3F1 mice. They observed significant increases in DNA single-

strand breaks in liver DNA from both species at sampling times up to and including 2 hours (rat) and 4 hours (mouse) after administration. Findings at 8 and 24 hours after administration were negative in the mouse (Nelson and Bull, 1988; Nelson et al., 1988, 1989; Washington State University, 1989). The second research team administered dichloroacetic acid (purity unspecified), adjusted to pH 7.0 to 7.4 with NaOH, to male F344 rats and male B6C3F1 mice either by oral gavage in single doses of up to 645 (rat) and up to 1289 mg/kg body weight (mouse) or orally in drinking water at dose levels of up to 2 g/l for 30 weeks (rat) or 0.5 or 5 g/l for 7 or 14 days (mouse). None of the substudies showed any increase in DNA single-strand breaks in liver DNA, and the acute substudy in the mouse also showed no single-strand breaks in DNA from the spleen, stomach or intestines (Chang et al., 1989, 1992). In the modified alkaline elution test ("alkaline single cell gel electrophoresis assay"), analysis of leucocyte DNA from male B6C3F1 mice after 28-day administration of drinking water containing dichloroacetic acid (≥ 99% pure) at 0.5, 1, 2 or 3.5 g/l (adjusted to pH 6.8 to 7.4 with NaOH) yielded no indication of DNA strand break induction or alkali-labile lesions or of incomplete DNA repair. As DNA migration in the electric field was decreased, the investigators suspected the formation of cross-links (Fuscoe et al., 1996; Salman et al., 1996).

Other tests

Sperm-head abnormalities and epididymal aspermia were observed in the B6C3F1 mouse after 5 oral administrations of dichloroacetic acid (> 99%, adjusted to pH 7.0 with NaOH) by gavage at dose levels of 1125 to 4500 mg/kg body weight (examinations at 21 or 35 days after the first administration). The investigators suggested that, taking into account the negative result of their micronucleus test (see above) and the testicular findings noted after repeated administration of the compound (see Section 7.5), the observed sperm-head abnormalities and the decreases in epididymal sperm counts were more likely to be attributable to the compound having a nonspecific toxic effect on the testes rather than a genotoxic effect (Meier et al., 1997).

A study in transgenic male Big Blue B6C3F1 mice harbouring the *Escherichia coli* lacI gene showed slight increases in mutation frequency. In order to determine potential mutations, the liver DNA was isolated from animals treated with dichloroacetic acid at 0 (control), 1 or 3.5 g/l in drinking water

for 4, 10 or 60 weeks and the lacI gene transferred to lambda phages. *Escherichia coli* bacteria were then infected with the phages and their β-galactosidase activity was determined. There was no increase in β-galactosidase activity after 4 or 10 weeks of treatment. After a treatment period of 60 weeks, increases by 30% and 133% over control were noted in the 1 g/l and 3.5 g/l dose groups, respectively. Sequence analysis revealed an increase in mutations at the A:T base pair as compared with the control (Leavitt and Ross, 1997; Leavitt et al., 1997).

7.7 Carcinogenicity

Long-term studies

The carcinogenicity of dichloroacetic acid > 99% pure) in rats was investigated in a study which was conducted as two substudies in male rats of the F344 strain. Animals in the first substudy (50 to 60 per group, only indirectly deducible from the publications) received dichloroacetic acid at 0.05, 0.5 or 5.0 g/l in their drinking water, adjusted to pH 6.9 to 7.1 with NaOH, for up to 100 weeks. The high 5.0 g/l concentration was reduced to 2.5 g/l after 9 weeks, to 2.0 g/l after 23 weeks and to 1.0 g/l after 52 weeks because the rats developed peripheral hind leg neuropathy. These animals were sacrificed at 60 weeks due to irreversibility of the neuropathy even after dose reduction and were not included in the analysis of the tumour data. In the second substudy, an initial 78 animals were administered dichloroacetic acid in their drinking water at 2.5 g/l over a total treatment period of 103 weeks. The concentration was lowered to 1.5 and 1.0 g/l at 8 and 26 weeks, respectively, because rats showed mild signs of neurotoxicity, which were largely reversible upon reduction of the concentration (the mean concentration over the entire study period was 1.6 g/l). The controls in the first substudy received drinking water containing 2 g NaCl/l, which was approximately isomolar (32 mM) to the drinking water given to the high dose group (39 mM), while the controls in the second substudy were offered deionised water. Based on the water consumption data and the analytically determined concentration levels in the drinking water, the animals' daily intake levels of dichloroacetic acid were 0 (controls), 3.6, 40.2 or 139 mg/kg body weight. Interim necropsies of 5 to 7 animals/group (only indirectly deducible from the publications) included determinations of body, liver, kidney, testes and spleen weights and examination for gross lesions and were car-

ried out at 15, 30, 45 and 60 weeks (first substudy) or at 14, 26, 52 and 78 weeks (second substudy). Animals examined at the end of the study underwent complete necropsy including fixation of organs and tissues generally examined in carcinogenicity studies. Liver, kidneys, spleen and testes and any gross lesions were histopathologically examined. Additionally, for each sampling time point, liver homogenate was assayed for activity of cyanide-insensitive palmitoyl-coenzyme A oxidase as a measure of peroxisome proliferation, and hepatocyte proliferation was measured after prior administration of [^{3}H]-thymidine (first substudy) or bromodeoxyuridine (second substudy). Furthermore, for all groups and scheduled necropsies in the first substudy, the livers were immunohistochemically analysed for glutathione S-transferase P-positive foci (GST-P-positive foci) and for tumour markers (oncoproteins p21 ras, p39 c-jun and p55 c-fos, tumour-associated aldehyde dehydrogenase, glutathione S-transferase P and α-foetoprotein) in small foci, hyperplastic nodules, adenomas and carcinomas. Survival in the treatment groups did not differ from that in the controls. Animals in the high dose group exhibited depressed body weight gain, increased relative liver and kidney weights and decreased absolute testes weight (as determined at study week 78). Animals in the intermediate dose group had increased absolute and relative testes weights. Compound-related neoplastic changes were observed exclusively in the liver (see Table 9).

Table 9. Hepatocarcinogenicity of dichloroacetic acid in male F344 rats after oral administration in drinking water – numbers of animals[1]/group with hepatocellular lesions (% of animals examined; DeAngelo et al., 1996)

Treatment group	Substudy 1			Substudy 2	
	2 g/l NaCl (control)	0.05 g/l DCA	0.5 g/l DCA	Water (control)	1.6 g/l DCA
Number of animals examined	23	26	29	33	28
Hyperplastic nodule	1 (4.4)	0	3 (10.3)	1 (3.0)	1 (3.6)
Adenoma	1 (4.4)	0	5 (17.2)	0	3 (10.7)
Carcinoma	0	0	3 (10.3)	1 (3.0)	6 (21.4)[2]
Combined adenoma and carcinoma	1 (4.4)	0	7 (24.1)[2]	1 (3.0)	8 (28.6)[3]
Combined hyperplastic nodules, adenoma and carcinoma	2 (8.7)	0	10 (34.9)[2]	2 (6.1)	9 (32.1)[3]

DCA dichloroacetic acid
[1] Animals were only examined if they survived for more than 78 weeks.
[2] $p \leq 0.05$
[3] $p \leq 0.01$

As shown in Table 9, there were significant dose-dependent increases in the prevalence of combined neoplastic hepatocellular lesions (combined adenoma and carcinoma) and combined proliferative hepatocellular lesions (combined hyperplastic nodules, adenoma and carcinoma) in the intermediate and high dose groups and in the prevalence of carcinoma in the high dose group. Apart from numbers of animals with the above-mentioned lesions, the numbers of lesions/animal were also increased in the intermediate and high dose groups. The low and intermediate dose groups exhibited neither peroxisome proliferation nor hepatocyte proliferation. In the top dose group, the activity of cyanide-insensitive palmitoyl-coenzyme A oxidase was increased and the percentage of bromodeoxyuridine-labelled hepatocytes was significantly depressed, or showed a trend towards depression, from study week 14 onwards. The immunohistochemical measurements performed in the first substudy yielded an increased number of GST-P-positive foci/cm² in all dose groups at study week 45 and dose-independent increases in the area percent of GST-P-positive altered foci at study week 60 and at the end of the study. No GST-P-positive foci tested positive with respect to any other tumour marker. Of the 27 top-dose animals sacrificed at study week 60 that were not included in the tumour analysis in the first substudy, 19 had hyperplastic nodules, 7 had hepatic adenomas and one had hepatocarcinomas. All these lesions were GST-P-negative, whereas they were positive for the other 5 tumour markers, with percentages ranging from 37 to 89% (hyperplastic nodules) and 43 to 86% (adenoma and carcinoma), depending on marker. All other hepatocellular lesions (cytoplasmic vacuolation, increased glycogen deposition, sinusoidal dilation and cystic degeneration (no further details)) were not considered compound-related by the investigators. The investigators did not report any data on non-neoplastic changes in other organs, except for an indication that alterations were observed in the myocardium (no further details). The *no observed effect level* (NOEL) was determined as 0.05 g/l (equivalent to 3.6 mg/kg body weight/day). The investigators suggested that GST-P-positive foci were not preneoplastic alterations in dichloroacetic acid-induced hepatocarcinogenesis and that peroxisome proliferation also did not play a direct role in hepatocarcinogenesis. Rather, they suspected that dichloroacetic acid depressed natural protective mechanisms (e.g. apoptosis) leading to the destruction of initiated cells, thereby enhancing spontaneous carcinogenic events (DeAngelo et al., 1996; DeAngelo and Daniel, 1992; Richmond et al., 1995).

When treated for 60 weeks with 2.5 g/l dichloroacetic acid, a dose known to be carcinogenic, approx. 70% of male F344 rats (controls 8%) developed hepatic tumours, the majority being carcinomas. A marked inhibition of mitochondrial mRNA transcription of the NADH dehydrogenase subunit 4 (ND4) gene was measured in the tumours. Animals without hepatic tumours had a ND4 transcript level comparable to that in the controls. The transcription of subunits ND4L and ND5, which are located on the same RNA strand, was increased, whereas the transcription of subunit ND6, which is located on a different RNA strand, was not increased. Following treatment for 26 or 52 weeks (interim sacrifices), when tumours were not yet observed, ND4 transcription was greatly increased. The investigators interpreted the change in the expression of these genes as a consequence of the activation of the pyruvate dehydrogenase complex (see Section 7.11 – Pharmacodynamic effects of dichloroacetic acid). It remains unclear, according to the investigators, how this change contributes to the hepatocarcinogenicity of dichloroacetic acid (Kandala et al., 1997).

The hepatocarcinogenicity of dichloroacetic acid in the B6C3F1 mouse has been investigated in numerous individual studies conducted by various research groups.

The hepatocarcinogenicity of dichloroacetic acid in the male B6C3F1 mouse was first demonstrated by a research group from the US Environmental Protection Agency. Designed as an initiation-promotion assay, the study was conducted in 16 to 32 male B6C3F1 mice/group dosed intraperitoneally with ethylnitrosourea at 2.5 µg/g body weight at age 15 days and given drinking water containing dichloroacetic acid (≥ 99% pure) at a concentration level of 2 or 5 g/l for 61 weeks, starting at age 4 weeks. The drinking water was adjusted to pH 6.5 to 7.5 with NaOH. In deviation from the initial assumption that administration of 2 or 5 g/l dichloroacetic acid in drinking water corresponded to respective ingested doses of 400 and 1000 mg/kg body weight/day, a later study by the investigators reported that 2 and 5 g/l corresponded to approx. 230 and 486 mg/kg body weight/day (see DeAngelo et al., 1991; below). Following prior or no pretreatment with ethylnitrosourea, controls received drinking water containing NaCl at 2 g/l or only dichloroacetic acid at 5 g/l. At 16 weeks, an interim necropsy was performed on 5 to 9 animals/group. The scope of the examination was limited to the determination of body, liver and kidney weights, liver lesions, the activity of hepatic cyanide-insensitive palmitoyl-coenzyme A oxidase as a

marker enzyme of peroxisome proliferation, the number of peroxisomes per unit of area of cytoplasm and the percentage of cytoplasm occupied by peroxisomes. Only the groups treated with the 5 g/l concentration of dichloroacetic acid exhibited depressed body weight gains and decreased absolute kidney weights. All dichloroacetic acid-treated groups had markedly increased absolute and relative liver weights. Of the animals in the group given 5 g/l dichloroacetic acid without prior ethylnitrosourea treatment, 96% (25/26) had liver adenomas (NaCl control 9% (2/22), ethylnitrosourea control 5% (1/22)) and 81% (21/26) had liver carcinomas (NaCl control 0%, ethylnitrosourea control 5% (1/22)). Among animals treated with ethylnitrosourea plus 2 or 5 g/l dichloroacetic acid, respective incidences were 76% (22/29) and 97% (31/32) for adenomas and 66% (19/29) and 72% (25/32) for carcinomas. The number of adenomas/animal and the number of carcinomas/animal were also increased relative to both the NaCl control and the ethylnitrosourea control. In the 2 g/l dichloroacetic acid-treated group, the activity of hepatic cyanide-insensitive palmitoyl-coenzyme A oxidase was not increased at study week 12 whereas it was 79% higher than the NaCl control at the end of the study. In the group treated with dichloroacetic acid at 5 g/l, the activity levels of hepatic cyanide-insensitive palmitoyl-coenzyme A oxidase determined at 12 and 61 weeks exceeded the control value by 122% and 216%, respectively (no indication as to whether or not prior treatment with ethylnitrosourea was administered). The number of peroxisomes and percentage of cytoplasm occupied by peroxisomes were unchanged compared with the control. In view of the marked increases in tumour incidences observed both with and without prior administration of ethylnitrosourea, the investigators discussed the possibility that dichloroacetic acid functioned as a complete carcinogen, but also pointed out that the strain of mouse used in the study (B6C3F1) had a high incidence of spontaneous tumours and that dichloroacetic acid possibly also acted as a promoter for spontaneously initiated liver cells. Furthermore, they discussed the finding that regression analysis of palmitoyl-coenzyme A oxidase activity versus tumour burden (liver tumours/mouse) failed to indicate a clear relationship between the two parameters and concluded from this that peroxisome proliferation might not be the mechanism of tumorigenesis (Herren-Freund et al., 1987; DeAngelo and McMillan, 1990).

In a subsequent study, groups of 50 male B6C3F1 mice (initial age 28 days) received drinking water containing dichloroacetic acid (≥ 99% pure)

at concentrations of 0.05, 0.5, 3.5 or 5 g/l over a period of 60 to 75 weeks. The drinking water supplied to these animals was also adjusted to pH 6.8 to 7.2 with NaOH. The controls were treated with drinking water containing 2 g/l NaCl or 1.5 g/l acetic acid (refers only to the control for the 3.5 g/l dose group). Based on drinking water consumption and body weight data, the animals had a daily intake of 7.6, 77, 410 or 486 mg/kg body weight (DeAngelo and Daniel (1990) give the respective intakes for the 0.05, 0.5 and 5 g/l groups as 7, 74 and 465 mg/kg body weight). Interim necropsies were performed on 5 animals/group at 4, 15, 30 and 45 weeks. The two higher dose groups in the study were terminated after 60 weeks and the two lower dose groups were terminated partly after 60 weeks (9 animals/group) and partly after 75 weeks. The liver, kidneys, testes and spleen were weighed and examined for gross lesions and, where necessary, by histopathology. Treatment had no effect on mortality. Drinking water intake and body weight gains were depressed in the two highest dose groups by up to 40% (water consumption) and by 17% and 13% (respective body weight losses). Increases by 18, 130 and 251% in relative liver weights were observed in the three highest dose groups relative to the controls. A slight increase in relative kidney weight was noted only in the 3.5 g/l dose group. The incidences of neoplastic lesions were 80% (adenoma) and 83% (carcinoma) in the 5 g/l group and 100% (adenoma) and 67% (carcinoma) in the 3.5 g/l group. In addition, 58% of animals treated at 3.5 g/l and 83% of those treated at 5 g/l exhibited hyperplastic nodules. Tumour incidences in the groups receiving dichloroacetic acid at 3.5 or 5 g/l differed significantly from those in the control groups (adenoma + carcinoma, NaCl 7.1%, acetic acid 0%). Tumour incidences in the 0.05 and 0.5 g/l dose groups (combined adenoma and carcinoma 24.1 and 11.1%, respectively) did not differ significantly from the tumour incidence among the controls. Non-neoplastic liver changes reported for the 5 g/l group included transient peroxisome proliferation, which was no longer observed at study week 60, and chronic inflammation with severe liver necrosis. Hepatic inflammation and necrosis were less severe in the 0.5 g/l group. Data on non-neoplastic liver changes in the other dose groups were not provided. Furthermore, in the group given 5 g/l, tumour markers (oncoproteins p21 ras and p39 c-jun, phosphotyrosine, tumour-associated aldehyde dehydrogenase and α-foetoprotein) were immunohistochemically determined in normal liver tissue, small foci, hyperplastic nodules, adenomas and carcinomas at all scheduled sacrifices. Normal liver was negative for tumour

markers. Only very few small foci were detected by means of markers and histochemical staining methods and, moreover, were observed only up to study week 30. The majority of hepatic adenomas and carcinomas were positive for the markers, whilst the percentage of marker-positive hyperplastic nodules was considerably smaller. As a rule, all cells in the adenomas and carcinomas showed a marker-positive reaction. Hyperplastic nodules that gave a positive reaction contained small nests of positive cells to which the reaction was limited. This observation led the investigators to conclude that the nests of positive cells contained in some hyperplastic nodules represented neoplastic changes which would subsequently develop into adenomas and carcinomas. Hence, dichloroacetic acid also proved carcinogenic in this study in male mice, the *no observed effect level* (NOEL) for 75-week administration in drinking water being 0.5 g/l (77 mg/kg body weight/day; DeAngelo and Daniel, 1990; DeAngelo et al., 1991; Richmond et al., 1991).

The same research team exposed 33 male B6C3F1 mice (initial age 28 days, treated as two groups of 23 and 10 mice) to drinking water containing 0.5 g/l dichloroacetic acid (≥ 95%; neutralised to pH 6.8 to 7.2 with NaOH) over a period of 104 weeks. Based on drinking water consumption and body weight data, this corresponded to a dose level of 88 mg/kg body weight/day. An interim necropsy of 5 animals was carried out at 30 weeks. The 33 control animals (also divided into two groups of 23 and 10 animals) received distilled water. In contrast to most other carcinogenicity studies of dichloroacetic acid, in which only the liver was comprehensively examined, this study included a comprehensive terminal examination with a full necropsy, fixation of 40 organs and tissues and histopathological assessment of the liver, kidneys, testes, spleen and any gross organ lesions. The animals showed no clinical signs of toxicity. Mortality, water consumption and the development of body weight and organ weights, with the exception of liver weight, did not differ from the respective data for the controls. The absolute and relative liver weights were significantly increased even at the interim necropsy. At the end of the study, the (absolute and relative) liver weights were approx. 50% higher than in the controls. In the 24 animals examined, gross and histopathological changes were found exclusively in the liver. Non-neoplastic changes included hepatocellular necrosis (8/24, control 1/20), hepatocellular hyperplasia (8/24, control 0/20), cytoplasmic vacuolisation (24/24, control 19/20), cytomegaly (22/24, control 1/20,

probably referring to very severe hypertrophy with severe glycogen deposition; cf. Bull et al., 1990, 1993; Washington State University, 1989; below) and chronic active inflammation (11/24, control 7/20). All of these non-neoplastic changes were considerably more severe in dichloroacetic acid-treated animals than they were in the controls. Out of the 24 dichloroacetic acid-treated animals that were examined, 15 had hepatocellular carcinomas, 10 had hepatocellular adenomas and 2 had hyperplastic nodules in the liver, whereas in the control group only 3/20 animals had proliferative liver changes. No animal examined at the interim necropsy had any proliferative changes in the liver. In addition, there is a published abstract (Snyder et al., 1995 b) according to which liver tissue, hyperplastic nodules, hepatocellular adenomas and hepatocellular tumours obtained from male B6C3F1 mice treated with dichloroacetic acid for 100 weeks exhibited a significant, dose-related decrease in apoptotic activity. The investigators suggested that the carcinogenic activity of dichloroacetic acid was likely attributable to this inhibition of apoptosis (programmed cell death; cell death which, in contrast to necrosis, is regulated by the cell's own genetic information and results in degradation) and the ensuing inability to clear spontaneously initiated cells (Daniel et al., 1992; Snyder et al., 1995 c).

Lastly, the same research group also conducted another carcinogenicity study in male B6C3F1 mice, which underwent lifetime exposure to dichloroacetic acid (> 99% pure) levels of 0.05, 0.5, 1, 2, or 3.5 g/l in their drinking water and were comprehensively examined. From the age of 28 to 30 days, 35 to 71 animals/group were treated for 90 to 100 weeks with drinking water solutions, adjusted to pH 6.9 to 7.1 with NaOH. The study first started with the ≥ 0.5 g/l dose groups, the 0.05 g/l dose group and a second control group being added one month later. A total of 88 controls were treated with normal drinking water. Based on the water consumption data and the analytically determined concentration levels in the drinking water, the animals' daily intake levels of dichloroacetic acid were 0 (controls), 8, 84, 168, 315 or 429 mg/kg body weight. Interim necropsies at 26, 52 and 78 weeks were performed on 10 animals/group, except in the low dose group. The animals were examined for gross lesions and their whole body, liver, kidney, testes and spleen weights determined. After 100 weeks all remaining animals in the study underwent complete necropsy including fixation of organs and tissues generally examined in carcinogenicity studies. Liver, kidneys, spleen and testes and any gross lesions from all dose

groups were histopathologically examined, as were all fixed tissues obtained from 5 top-dose animals. Liver changes were analysed with respect to the prevalence and multiplicity of neoplastic changes and the extent of foci of necrotic hepatocytes. At all scheduled examinations, liver homogenate was analysed in addition to determine the activity of cyanide-insensitive palmitoyl-coenzyme A oxidase as a measure of peroxisome proliferation. The animals for the interim necropsies at study weeks 26 and 52 received prior treatment with [^{3}H]-thymidine, and the labelling index was then determined as a parameter of hepatocyte proliferation. Serum enzyme activities were measured at study day 30 for alanine aminotransferase and at study weeks 26 and 52 for lactate dehydrogenase. The treatment groups receiving 2 or 3.5 g/l initially showed a decrease in water consumption and a trend towards increased early mortality, and body weights were 18% lower than controls at the end of the study. All dose groups showed dose-related increases in relative and absolute liver weights at 26 and 52 weeks. At the end of the study, increases in absolute and relative liver weights were noted only in the 2 and 3.5 g/l groups. Kidney, spleen and testes weights were not affected by treatment with dichloroacetic acid. Compound-related neoplastic changes were observed exclusively in the liver (see Table 10).

Beginning of Table 10

Table 10. Hepatocarcinogenicity of dichloroacetic acid in the male B6C3F1 mouse after oral administration in drinking water for up to 100 weeks (DeAngelo et al., 1999)

Treatment group	Controls	0.05 g/l	0.5 g/l	1 g/l	2 g/l	3.5 g/l
Number of animals used	88	35	55	71	55	46
Number of animals at interim sacrifices	35	0	30	30	30	30
Number of animals that died	3	2	1	9	11	8
Number of animals at terminal necropsy	50	33	24	32	14	8
Hepatocellular carcinomas (prevalence (% of animals examined) and multiplicity (number of carcinomas/animal))						
After 26 weeks	0%	-	0%	0%	0%	0%
After 52 weeks	0%	-	0%	0%	20% 0.20±0.13	50% 0.70±0.25
After 78 weeks	10% 0.10±0.10	-	0%	20% 0.20±0.13	50% 1.0±0.47	70[1]% 1.20±0.37[1]
After 100 weeks	26% 0.28±0.07	33% 0.58[1]	48% 0.69±0.17[1]	71[1]% 1.29±0.17[1]	95[1]% 2.47±0.29[1]	100[1]% 2.90±0.40[1]

Table 10. Hepatocarcinogenicity of dichloroacetic acid in the male B6C3F1 mouse after oral administration in drinking water for up to 100 weeks (DeAngelo et al., 1999)

Hepatocellular adenomas (prevalence (% of animals examined) and multiplicity (number of adenomas/animal))						
After 26 weeks	0%	-	0%	0%	0%	10% 0.10±0.09
After 52 weeks	0%	-	10% 0.10±0.09	10% 0.10±0.09	0%	50% 0.80±0.31[1]
After 78 weeks	10% 0.10±0.09	-	10% 0.10±0.09	20% 0.20±0.13	50% 1.00±0.42	50% 1.00±0.42
After 100 weeks	10% 0.12±0.05	n.d.	20% 0.32±0.14	51.4[1]% 0.80±0.17[1]	42.9[1]% 0.57±0.16[1]	45[1]% 0.64±0.23
Large preneoplastic altered foci of cells[2] (multiplicity (foci/animal))						
After 52 weeks	n.d.	n.d.	0.1	0.1	0.2	0.16
After 100 weeks	n.d.	0.03[1]	n.d.	0.06[1]	0.14[1]	0.27[1]
Hepatic necrosis severity score[3]						
After 26 weeks	0.10±0.10	n.d.	0.20±0.13	1.20±0.38[1]	1.20±0.39[1]	1.10±0.28[1]
After 52 weeks	0	n.d.	0	0.20±0.13	0.40±0.22	1.10±0.43[1]
After 78 weeks	0	n.d.	0	0	0.30±0.21	0.20±0.13
After 100 weeks	0.20±0.16	n.d.	0.20±0.08	0.42±0.15	0.38±0.20	1.38±0.42[1]
Estimate of effective dose in liver, AUCL[4] (mg x hour/l)						
100 weeks	0	0.041	0.72	15.8	417	1064

n. d. no data
[1] $p \leq 0.05$
[2] corresponds to the changes referred to as hyperplastic nodules in other studies by the same research group (Daniel et al., 1992; DeAngelo et al., 1991, 1996; DeAngelo and Daniel, 1990, 1992; Richmond et al., 1991, 1995; Snyder et al., 1995 c)
[3] on a scale from 0 to 4 (0 = no changes, 1 = ≤ 25%, 2 = 25 to 50%, 3 = 50 to 75%, 4 = 100% of liver sections showed foci of necrotic hepatocytes containing inflammatory cell infiltrates)
[4] area under the concentration-time curve in the liver according to Barton et al. (1999; see Section 7.1)

End of Table 10

As shown in Table 10, there was a trend at the end of the study towards an increase in the prevalence of animals with hepatocarcinoma from the lowest test dose of 0.05 g/l (8 mg/kg body weight/day) onwards and a statistically significant increase starting at the 1 g/l dose (168 mg/kg body weight/day). The carcinoma multiplicities observed in all dose groups were dose-dependently and statistically significantly higher than the control value. The activity of cyanide-insensitive palmitoyl-coenzyme A oxidase, a marker of peroxisome proliferation, did not correlate with tumour prevalence. Increased activity was seen only in the 3.5 g/l-treated group, attaining statistical significance only in animals examined at 26 weeks. In dichloroacetic acid-treated animals the labelling indexes for liver tissue outside of proliferative lesions were in the range of the control values. From 1 g/l onwards, there were marked necrotic lesions, which, however, were not strictly time- or dose-dependent. In the dose range below 1 g/l, hepatocel-

lular necrosis was observed to be mild and, moreover, not present at all sampling times. Lactate dehydrogenase activity was increased in a dose-related manner in all dose groups at the week 26 examination, but the increase attained statistical significance only in the top dose group and returned to control levels by 52 weeks. There was an increase in alanine aminotransferase activity in the dose range from 0.5 to 2 g/l at study day 30. Hepatocellular hypertrophy (which the investigators referred to as cytomegaly) and cytoplasmic vacuolisation with massive glycogen deposition in the cells were dose-related and significant in all dose groups. Without giving further details, Carter et al. (1998) reported in an abstract that they had performed immunohistochemical determinations of proliferative and apoptotic cells in premalignant hepatic lesions in comparison with adjacent normal liver from the animals in this study (DeAngelo et al., 1999). The percentages of proliferative cells in altered foci, hyperplastic nodules and adenomas were negatively correlated with dose. At study week 100, no dose-dependent suppression of cell proliferation was detected in normal liver or altered foci. Apoptotic cells were increased in adenomas in the controls but not in adenomas in dichloroacetic acid-treated animals (see also Section 7.7 – Studies investigating dichloroacetic acid-induced biochemical and morphometric changes with respect to the extent of their relevance to carcinogenicity). Using a toxicokinetic model, Barton et al. (1999; see Section 7.1) estimated that the animals had AUCL (area under the concentration-time curve in liver) values of 0 (control), 0.041, 0.72, 15.8, 417 or 1064 mg x hour/l during the treatment phase. The investigators concluded that a *no observed effect level* (NOEL) was not attained in the study because carcinoma prevalences were dose-dependent, showing a trend towards an increase in all dose groups and significant increases from 1 g/l onwards, and carcinoma multiplicities were dose-dependently and significantly higher than the control values from the lowest dose tested (Carter et al., 1998; Barton et al., 1999; DeAngelo et al., 1999).

From 7 to 8 weeks of age, female B6C3F1 mice were treated with dichloroacetic acid at 2, 6.67 or 20 mmol/l (approx. 0.26, 0.86 or 2.6 g/l) in drinking water (adjusted to pH 6.5 to 7.5 with NaOH) for 217, 360 or 576 days. Additional animals were intermittently treated at 20 mmol/l for 360 or 576 days (24 days of exposure to dichloroacetic acid, 48 days without exposure to the chemical) such that the total dose of chemical they received corresponded to the continuous administration of 6.67 mmol/l. In an asso-

ciated initiation-promotion study mice were initiated on day 15 of age with a single intraperitoneal dose of 25 mg/kg body weight of N-methyl-N-nitrosourea and then administered the above-mentioned doses of dichloroacetic acid in drinking water for 31 or 52 weeks (217 or 360 days). A number of N-methyl-N-nitrosourea-initiated animals treated at 20 mmol/l were removed from dichloroacetic acid exposure after 31 weeks and held for an additional recovery period of 21 weeks. Control groups were treated with N-methyl-N-nitrosourea and/or 20 mmol NaCl/l in drinking water. Assessments included only drinking water and food consumption, body weights, gross and histopathological examination of hepatic lesions and evaluation for immunohistochemical liver parameters. Only the dose groups treated at 20 mmol/l showed initial decreases in water consumption and reduced body weights from study weeks 27 and 35. All dichloroacetic acid-treated animals exhibited a linearly dose-dependent increase in relative liver weights and hepatocellular vacuolisation. In the absence of N-methyl-N-nitrosourea initiation, animals treated with dichloroacetic acid for 31 weeks had neither foci of altered hepatocytes nor hepatic tumours. The incidences of proliferative lesions observed following an exposure period of 360 or 576 days are presented in the following Table 11.

Beginning of Table 11

Table 11. Hepatocarcinogenicity of dichloroacetic acid in female B6C3F1 mice after oral administration in drinking water for 360 or 576 days (Pereira, 1996)

Treatment groups (mmol/l)	Number of animals examined (N)	Foci of altered cells[1]	Adenomas	Carcinomas
	(% and number of animals with lesions/number of animals examined)			
Treatment administered for 360 days				
20.0	20	40 (8/20)[2]	35 (7/20)[2]	5 (1/20)
20.0, intermittent	15	0	0	0
6.67	20	5 (1/20)	15 (3/20)	0
2.0	40	0	0	0
NaCl control	40	0	2.5 (1/40)	0

Table 11. Hepatocarcinogenicity of dichloroacetic acid in female B6C3F1 mice after oral administration in drinking water for 360 or 576 days (Pereira, 1996)				
Treatment groups (mmol/l)	Number of animals examined (N)	Foci of altered cells[1]	Adenomas	Carcinomas
	(% and number of animals with lesions/number of animals examined)			
Treatment administered for 576 days				
20.0	19	89.5 (17/19)[2]	84.2 (16/19)[2]	26.3 (5/19)[2]
20.0, intermittent	34	41.2 (14/34)[2]	8.8 (3/34)	2.9 (1/34)
6.67	28	39.3 (11/28)[2]	25.0 (7/28)[2]	3.6 (1/28)
2.0	50	14.0 (7/50)	6.0 (3/50)	0
NaCl control	90	11.1 (10/90)	2.2 (2/90)	2.2 (2/90)

[1] Foci of altered cells which the investigators evaluated as proliferative lesions contained 6 or more cells, had an increased mitotic index and slightly compressed the surrounding tissue. The distinction from adenomas was based upon size (diameter < 1 mm) and the extent of tissue compression. Most likely the lesions referred to here as foci of altered cells are identical to the changes referred to as hyperplastic nodules by the investigators of the rat and mouse studies described above (see above; Daniel et al., 1992; DeAngelo et al., 1991, 1996, 1999; DeAngelo and Daniel, 1990, 1992; Richmond et al., 1991, 1995; Snyder et al., 1995 c).

[2] $p \leq 0.05$

End of Table 11

As is evident from Table 11, only those animals that were continuously treated at 20 mmol/l exhibited a significant increase in foci of altered cells and adenomas after 360 days. After a treatment period of 576 days, the groups that were continuously or intermittently treated at 20 mmol/l and those exposed to 6.67 mmol/l exhibited significantly increased incidences of foci of altered cells, whereas a significant increase in carcinomas was observed only in the group that was continuously treated at 20 mmol/l. The adenoma incidences were significantly increased only in the groups that continuously received 20 or 6.67 mmol/l. As regards the low carcinoma incidences, there was no significant difference between the treatment group that continuously received 6.67 mmol/l and the group that was intermittently exposed to 20 mmol/l (both groups received the same total dose). Exposure to the tumour initiator N-methyl-N-nitrosourea caused the administration of 20 mmol/l dichloroacetic acid to induce foci of altered cells and adenomas in 80% and 50% of animals, respectively, after only 31 weeks and in 50% and 73.1% of animals, respectively, after 52 weeks. The overall incidence of lesions (foci of altered hepatocytes, adenomas and carcinomas) and the number of lesions/animals found after 52 weeks did

not significantly differ from the data for 31 weeks. However, both the incidence and the number of altered foci/mouse decreased with the duration of administration while the corresponding values for adenomas increased. This finding led the investigators to suggest that the foci of altered cells possibly progressed to adenomas. Strikingly, the group that was initiated with N-methyl-N-nitrosourea, treated at 20 mmol/l and placed under observation for 21 weeks showed greatly decreased incidences and multiplicities of lesions that were not significantly increased as compared with the values obtained after 31 weeks without a recovery period. Using high-resolution magnetic resonance imaging in studies conducted in the male B6C3F1 mouse, another research group confirmed the ability of dichloroacetic acid-induced tumours to regress (see below; Miller et al., 2000). The investigators also considered it possible that the regression of foci of altered cells and tumours upon cessation of exposure to dichloroacetic acid also resulted from activation of apoptosis, which is inhibited by dichloroacetic acid (see also Section 7.7 – Studies investigating dichloroacetic acid-induced biochemical and morphometric changes with respect to the extent of their relevance to carcinogenicity; Snyder et al., 1995 a, b, c). The groups with significant increases in the incidences of foci and/or tumours also had increased numbers of these lesions/animal as compared with the controls. The concentration-response relationship for total lesions (foci of altered cells plus tumours) was represented by a second-order function in the presence and absence of N-methyl-N-nitrosourea pretreatment. Almost all foci of altered cells and tumours observed in the N-methyl-N-nitrosourea-initiated groups and 80% and 90% of these lesions observed in the two highest continuously treated dose groups were eosinophilic, whilst in the intermittent treatment group 43% of lesions were eosinophilic and 56% were basophilic. The eosinophilic lesions were almost all immunohistochemically positive for glutathione S-transferase-π (GST-π). The lesions from N-Methyl-N-nitrosourea-initiated animals were predominantly basophilic and GST-π-negative. Immunohistochemical analysis of foci of altered cells and tumours from animals randomly selected from the N-methyl-N-nitrosourea-initiated group exposed to 20 mmol/l dichloroacetic acid showed that these lesions stained positive not only for GST-π but also for TGF-α, c-jun, c-myc, CYP 2E1 and CYP 4A1 and were negative for c-fos and TGF-β. Loss of heterozygosity on chromosome 6 (polymorphic loci D6mit1, D6mit9, D6mit204 and D6mit323) and, hence, loss of tumour suppression genes was not detected when polymerase chain reaction-amplified

tumour DNA was sequenced (Tao et al. 1996). Following prior administration of bromodeoxyuridine, hepatocyte proliferation was determined in 10 animals from each of the continuously treated dose groups, with 2000 hepatocytes/animal being scored at treatment days 5, 12 and 33. After an initial significant dose-related increase, proliferation no longer differed from the control at 12 and 33 days. The investigators concluded from their findings that dichloroacetic acid was to be considered a tumour promoter, but that tumorigenesis was unlikely to be related to hepatocellular vacuolisation (different dose-response curves) or peroxisome proliferation (peroxisome proliferator-induced tumours are basophilic; Pereira, 1994, 1996; Pereira and Phelps, 1996; Tao et al. 1996; Latendresse and Pereira, 1997).

The findings of the initiation-promotion study described above were again confirmed when the investigators conducted a subsequent study in which the main focus was on investigating the synergistic effects of dichloroacetic and trichloroacetic acid. This study in female B6C3F1 mice also began with their initiation on day 15 of age with 25 mg/kg body weight N-methyl-N-nitrosourea, followed by exposure for 44 weeks from 6 weeks of age to drinking water containing dichloroacetic acid at 7.8, 15.6 or 25.0 mmol/l (approx. 1.0, 2.0 or 3.2 g/l). Approximately 30 weeks upon commencement of dichloroacetic acid administration, the two higher dose groups showed decreases in body weight. Relative liver weight was increased in all dose groups in a linear dose-related manner. Animals exposed to 25 mmol/l exhibited significantly increased incidences and multiplicities of foci of altered liver cells and hepatic adenomas. As in the previous study, these proliferative changes were almost exclusively eosinophilic and GST-π-positive. In the groups treated with 15.6 or 7.8 mmol/l dichloroacetic acid, the incidences of foci of altered hepatocytes and hepatic tumours were not significantly different from the corresponding control values (Kramer et al., 1996; Pereira et al., 1997).

Without prior administration of N-methyl-N-nitrosourea, 25 female B6C3F1 mice/group were treated with 0.5 or 3.5 g/l dichloroacetic acid in drinking water (neutralised with NaOH to pH 6.9 to 7.1) for 104 weeks beginning at 28 days of age. The 39 control animals were treated with 1.5% neutralised acetic acid. On average, the animals ingested doses of 0 (controls), 94 or 438 mg/kg body weight/day. In the top dose group, body weight was decreased, relative liver weight was elevated by more than 250% and the incidence of hepatic carcinomas was increased from 2.6% (0.05 tumours/ani-

mal) in the control group to 92% (2.96 tumours/animal). The low dose group treated with 0.5 g/l exhibited no significant differences from the control group with regard to body weight gain, relative liver weight and incidence of hepatic carcinomas (4%, 0.04 tumours/animal). Other assessments were not performed, or not reported, with the exception of the characterisation of tumours with respect to H-ras proto-oncogenes (Schroeder et al., 1997).

Another study with the primary objective to investigate liver tumours with respect to the activation of the H-ras proto-oncogene (see also Section 7.7 – Studies on the activation of proto-oncogenes and the loss of tumour suppressor genes) was also carried out in male B6C3F1 mice, which were treated with dichloroacetic acid (1 and 3 g/l, neutralised with NaOH to pH 6.8 to 7.2, the control group receiving neutralised isomolar acetic acid) for 104 weeks starting on day 28 of age. The incidences of liver carcinoma of 100% (5.06 tumours/animal) and 70.6% (1.29 tumours/animal) were significantly increased in both dose groups relative to the control (19% (1.0 tumours/animal); no further details; Ferreira-Gonzalez et al., 1995).

K-ras and H-ras proto-oncogenes (see Section 7.7 – Studies on the activation of proto-oncogenes and the loss of tumour suppressor genes) were also studied in tumours from male B6C3F1 mice which were treated with 5 g/l dichloroacetic acid in their drinking water (adjusted to pH 6.5 to 7.5 with NaOH) for 76 weeks and of which 93% (4.98/animal) had hepatic adenomas and 74% (1.73/animal) had carcinomas. The controls, which were supplied with normal drinking water, were examined after 134 weeks and found to have 8% adenomas and 8% carcinomas (no further details; Anna et al., 1994).

The hepatocarcinogenicity of dichloroacetic acid in the B6C3F1 mouse was also confirmed by another research group who administered dichloroacetic acid (analytical grade) to male and female B6C3F1 mice at concentrations of 1 or 2 g/l in drinking water for up to 52 weeks. Drinking water containing 1 g/l was supplied to 11 males for 52 weeks. The 2 g/l treatment was administered to a total of 50 males and 10 females. Groups of 5 males were examined at 5, 24 and 37 weeks, 11 males received dichloroacetic acid for 37 weeks and were subsequently placed under observation for 15 weeks (total study duration: 52 weeks) and the remaining 24 males and the 10 females received 2 g/l dichloroacetic acid for 52 weeks. The drinking water

was adjusted to pH 6.8 to 7.2 with NaOH. Appropriate controls were given ordinary tap water. Treatment had no effect on mortality, body weight, food or water consumption or kidney weight. Hepatic lesions were dose-dependent. Non-neoplastic liver changes noted in males treated at 1 g/l for 52 weeks included increased relative and absolute liver weights, multifocal necrosis with frequent lymphocyte infiltration particularly on the surface of the liver, as well as very marked hepatocellular hypertrophy, evenly distributed throughout the liver, with massive hepatocellular glycogen accumulation (referred to by the investigators as cytomegaly). In a later study published only as an abstract, the investigators attributed the increased accumulation of glycogen to an impairment in glycogen degradation rather than to an increase in glycogen synthesis (Kato and Bull, 1995). The dose groups given 2 g/l showed qualitatively identical changes and, in addition, had centrilobular basophilic foci of glycogen-poor cells from week 24 of treatment onwards and slight lipofuscin accumulations after the total treatment duration of 52 weeks. Increased liver weight and hepatocellular hypertrophy were clearly reversible in the recovery group that was observed for 15 weeks. Hepatoproliferative lesions (hyperplastic nodules, adenomas and carcinomas) were observed in 2/11 males of the 1 g/l dose group (hyperplastic nodules), in 7/11 males of the 2 g/l group treated for 37 weeks and then kept for observation for 15 weeks (predominantly hyperplastic nodules (no further details)) and in 23/24 males (predominantly hyperplastic nodules and carcinomas (no further details)) and 3/10 females (hyperplastic nodules) from the 2 g/l group treated for 52 weeks. One male out of a total of 35 male and 10 female control animals bore hyperplastic nodules. The incidence data for the various proliferative lesions are inaccurate due to the fact that out of a total of 120 individual hepatoproliferative lesions only 45 randomly selected lesions, obtained from a total of 20 mice, were histopathologically examined. Strikingly, males exposed to 2 g/l dichloroacetic acid for only 37 weeks and then observed during a 15-week recovery period had predominantly hyperplastic nodules, few adenomas and no carcinomas, whereas 5/10 males exposed to 2 g/l dichloroacetic acid had carcinomas upon examination. A later study by the investigators (see below; Miller et al., 2000) confirmed by high-resolution magnetic resonance imaging that dichloroacetic acid-induced tumours of the male B6C3F1 mouse can regress. In a parallel exploratory study conducted in the Sprague-Dawley rat, treatment of 3 males and 2 females with 5 g/l dichloroacetic acid (analytical grade) in drinking water (neutralised to pH 6.8 to 7.2 with

NaOH) for 12 months also resulted in hepatocellular hypertrophy with glycogen accumulation, albeit of much lesser severity than in the B6C3F1 mouse (Bull et al., 1990, 1993; Washington State University, 1989).

As in the earlier studies reported by Pereira and Phelps (1996), Bull et al. (1990, 1993) and Washington State University (1989; see above), it was found, or indications were obtained, that dichloroacetic acid-induced hyperplastic nodules and adenomas could regress when further exposure to the chemical was suspended. This was confirmed by means of an in-situ examination method in a study in male B6C3F1 mice. Out of 30 mice which were 6 weeks old at treatment initiation and received 2.0 g/l dichloroacetic acid in drinking water (neutralised with NaOH to pH 6.8 to 7.2) for 48 weeks, 10 developed a total of 13 hepatic tumours. The tumours were examined in situ in the living animals under anaesthesia with ketamine hydrochloride (120 mg/kg body weight, administered intraperitoneally) and xylazine (7.2 mg/kg body weight, administered intraperitoneally) by means of high-resolution magnetic resonance imaging. Five of the 10 animals whose exposure to dichloroacetic acid was suspended for a recovery period of 2 to 3 weeks showed a decrease in individual tumour size, whilst the remaining 5 animals, which continued exposure to the chemical, showed an increase in size. The presence of tumours was confirmed by subsequent histopathological examination (Miller et al., 2000).

Studies investigating the mechanism of carcinogenicity

- **Peroxisome proliferation studies**

Dichloroacetic acid induces lipid peroxidation and peroxisome proliferation following acute and subacute administration (see Section 7.2). According to one in-vitro study, dichloroacetic acid binds to a ligand-activated transcription factor, “peroxisome proliferator-activated receptor α”, in the liver, thus inducing peroxisome proliferation (Zhou and Waxman, 1998). Initial assumptions that the formation of neoplastic lesions was the consequence of chronic tissue damage due to the release of reactive oxygen molecules as a result of peroxisome proliferation were not confirmed. In long-term studies (see Section 7.7 – Long-term studies), the induction of peroxisome proliferation in the male F344 rat and the male B6C3F1 mouse required higher dose levels than did the induction of neoplastic lesions (Herren-Freund et

al., 1987; DeAngelo et al., 1996; DeAngelo and Daniel, 1992; DeAngelo and McMillan, 1990; Richmond et al., 1995). Moreover, peroxisome proliferation was no longer detectable in the male B6C3F1 mouse after a prolonged exposure period (DeAngelo and Daniel, 1990; DeAngelo et al., 1991; Richmond et al., 1991) and statistical analysis failed to reveal an unequivocal correlation between peroxisome proliferation and the neoplastic effect (Herren-Freund et al., 1987; DeAngelo and McMillan, 1990; DeAngelo et al., 1999).

As described above, numerous in-vivo studies have demonstrated dichloroacetic acid to have a peroxisome proliferative effect, which did not, however, correlate with the chemical's tumorigenic effect. Peroxisome proliferation was also detected in vitro in primary hepatocytes from male Long-Evans rats and male B6C3F1 mice. The cells were incubated for 72 hours in medium containing dichloroacetic acid levels of 0.1 to 2.0 or 4.0 mmol/l. Subsequently, the activity of a marker enzyme of peroxisome proliferation, palmitoyl-coenzyme A oxidase, was determined and the amount of "peroxisome proliferator-activated receptor α"-induced peroxisomal bifunctional enzyme measured using monoclonal antibodies. Furthermore, hepatocytes were incubated at 2.0 mmol/l and examined by light microscopy for peroxisomes. Hepatocytes from both species exhibited similar concentration-dependent increases in palmitoyl-coenzyme A oxidation and peroxisomal bifunctional enzyme protein levels. Hepatocytes incubated with 2.0 mmol/l dichloroacetic acid showed marked peroxisome accumulation (Everhart et al., 1998; Walgren et al., 2000).

Whilst the investigators succeeded in demonstrating that dichloroacetic acid induced peroxisome proliferation in vitro in rat and mouse hepatocytes (Everhart et al., 1998; Walgren et al., 2000; see above), they failed to do so in a subsequent study in human hepatocytes. Both primary and long-term cultures of hepatocytes were used, which were obtained from 3 male and 3 female donors (aged 56 to 67 years and 32 to 55 years, respectively). The palmitoyl-coenzyme A oxidation rate was below the limit of detection in hepatocytes incubated with dichloroacetic acid at 4 mmol/l for 72 hours. Measured by monoclonal antibodies, the levels of CYP4A11, the gene assumed by the investigators to activate the "peroxisome proliferator-activated receptor α", were slightly, but not statistically significantly increased to 1.3 to 1.4 times the control value. Following incubation with 1 mmol/l dichloroacetic acid, DNA synthesis, as determined by the incorpo-

ration of [^{3}H]-thymidine, was significantly decreased in primary human hepatocytes, whilst it was not inhibited in hepatocytes cultured for several cell cycles (Walgren et al., 2000).

- **Genotoxicity studies**

Many studies addressing the question as to whether the carcinogenic effect of dichloroacetic acid could result from mutagenic changes in the genetic information did not yield unequivocal results. The majority of genotoxicity tests, comprising numerous test systems, largely produced negative results (see Section 7.6 and Tables 6, 7 and 8). The majority of investigators, and research groups, who obtained positive genotoxicity results suggested that the genotoxic effect of dichloroacetic acid, if it existed at all, was low and unlikely to be a prerequisite for tumorigenic lesions caused by the chemical (see e.g. Carter et al., 1995; DeAngelo et al., 1991, 1999; ILSI, 1997; Latendresse and Pereira, 1997; Pereira, 1996; Pereira and Phelps, 1996; Snyder et al., 1995 a, b, c; Stauber and Bull, 1997; Stauber et al., 1998; Tao et al., 1996, 1998, 2000). Studies on the activation of proto-oncogenes (see Section 7.7 – Studies on the activation of proto-oncogenes and the loss of tumour suppressor genes) also suggest a non-genotoxic mechanism of carcinogenicity.

- **Studies on the activation of proto-oncogenes and the loss of tumour suppressor genes**

Liver obtained from the male B6C3F1 mice in the 37-week and 52-week studies described above (cf. Bull et al., 1990; Washington State University, 1989) was studied for the expression of the c-myc-ras and c-H-ras proto-oncogenes in the mRNA using an in-situ hybridisation technique. At 52 weeks it was found that there was a significant, approximately 3-fold increase in c-myc expression in the carcinomas and preneoplastic nodules as compared with normal liver tissue. Significant expression of c-H-ras, about 4 times higher than in normal liver, was observed only in carcinoma tissue, not in the preneoplastic nodules (Nelson et al., 1990).

A more detailed study was carried out to investigate whether the hepatocarcinogenic effect of dichloroacetic acid results from the activation of ras proto-oncogenes by chemical-induced mutations. For this purpose, DNA

was isolated from the tumours obtained in the study by Anna et al. (1994; see above) and analysed by molecular genetic techniques (sequencing analysis (Southern blot) of the polymerase chain reaction-amplified DNA) for H-ras, K-ras, raf and c-met proto-oncogenes. The tumours (adenomas, carcinomas, combined adenomas and carcinomas) from the dichloroacetic acid-treated animals almost exclusively showed mutations at codon 61 of the H-ras proto-oncogene. The overall mutation rate of this proto-oncogene that also occurs in spontaneous tumours of the male B6C3F1 mouse (cf. Maronpot et al., 1987, 1995) did not differ from the values obtained for tumours from the concurrent study controls and historical controls (dichloroacetic acid group 62%, study control 69%, historical controls 70%). Analysis of the individual mutations revealed a shift in the mutational spectrum. Compared with the controls, which predominantly exhibited CAA $\rightarrow$ AAA mutations (58%) and fewer CAA $\rightarrow$ CGA (27%) and CAA $\rightarrow$ CTA mutations (14%), CAA $\rightarrow$ AAA mutations in tumours from dichloroacetic acid-treated animals were decreased (28%) whilst CAA $\rightarrow$ CTA (38%) and CAA $\rightarrow$ CGA mutations (35%) were increased. The investigators overall evaluation was that mutations at the ras proto-oncogenes was not essential to the hepatocarcinogenic effect of dichloroacetic acid. Rather, they suggested that dichloroacetic acid provides a selective growth advantage for cells with spontaneous mutations at codon 61 of the H-ras proto-oncogene (Anna et al., 1994).

The findings by Anna et al. (1994) were largely confirmed in an independent study, in which hepatic tumours obtained from male B6C3F1 mice treated with 1.0 or 3.5 g/l dichloroacetic acid for 104 weeks were examined with respect to activation of ras proto-oncogenes (see Section 7.7 – Long-term studies; Ferreira-Gonzalez et al., 1995). In total, 89 hepatocellular carcinomas were examined for K-ras and H-ras mutations (32 spontaneous tumours in control animals, 33 tumours in the 3.5 g/l group and 13 tumours in the 1.0 g/l group). The incidences of base mutations on the K-ras and H-ras proto-oncogenes were similar in all groups and were found to be 58% in the spontaneous tumours, 46% in the 1 g/l group and 50% in the 3.5 g/l group (sequence analysis of the polymerase chain reaction-amplified DNA). The investigators explicated that genotoxic hepatocarcinogens were found, as a rule, to increase the frequency of mutations in the ras proto-oncogenes whereas they remained unchanged, or decreased, relative to the controls in the case of nongenotoxic hepatocarcinogens. In addition to

determining the total number of mutations in the K-ras and H-ras proto-oncogenes, their mutational spectrum was also analysed. Again, mutations were observed almost exclusively at H-ras codon 61, with the respective frequencies in 3.5 g/l or 1.0 g/l dichloroacetic acid-treated mice being 21 and 16% for CAA → AAA mutations, 50 and 50% for CAA → CGA mutations and 29 and 34% for CAA → CTA mutations. In the spontaneous tumours of the controls, CAA → AAA and CAA → CGA mutations accounted for 80% and 20% of mutations, respectively, whilst CAA → CTA mutations were not detected among the controls. Due to the shift in the mutational spectrum of H-ras codon 61, the investigators suspected that the activation of ras-proto-oncogenes could nonetheless be important in dichloroacetic acid-induced carcinogenicity (Ferreira-Gonzalez et al., 1995). However, the relatively high level of CAA → CGA mutations is not in support of a mutagenic effect on the part of dichloroacetic acid, because it is precisely that mutation which occurs spontaneously in the livers of young male B6C3F1 mice and is also detected even in young animals of the parent strains of B6C3F1 mice (C3H/He and C57BL/6). Rather, it has been suggested that the carcinogenicity of chemicals which induce tumours with a relatively high level of this mutation occurs via a nongenotoxic mechanism, by facilitating a clonal expansion of cells bearing the spontaneous mutation (cf. Moulds and Goodman, 1994). As observed in various studies, dichloroacetic acid inhibits apoptosis (see Carter et al., 1995; Snyder et al., 1995 c), thereby possibly enhancing a clonal expansion of spontaneously initiated cells.

Male B6C3F1 mice were treated with 2 g/l dichloroacetic acid in drinking water for up to 50 weeks and then administered water containing 0 (controls), 0.02, 0.5, 1 or 2 g/l dichloroacetic acid (> 99% pure, NaOH-neutralised to pH 6.8 to 7.2) for another 2 weeks. They were then examined for cell proliferation rate, immunohistochemical presence of the oncoproteins c-jun and c-fos and the staining behaviour of the proliferative lesions. Hepatocyte proliferation rate was increased relative to the control 2 weeks after the beginning of administration, but markedly inhibited at 4 and 40 weeks. It was found in animals treated at different concentration levels during the last 2 weeks of the study that proliferation rates were dose-dependently increased in gross, c-jun-positive hepatic lesions. Gross tissue lesions predominantly stained immunohistochemically positive for the c-jun and c-fos oncoproteins (found mostly in the cytoplasm and cell nucleus, respectively), and they were predominantly basophilic and contained very little

glycogen relative to unchanged tissue. The investigators suggested that c-jun and c-fos, the expression of which is also closely linked to the expression of H-ras in spontaneous tumours of the male B6C3F1 mouse, belong to a family of transcription factors that are involved in the control of cell division. The activities of these factors are regulated via different phosphorylation reactions. They suspected that dichloroacetic acid, which is known to inhibit pyruvate dehydrogenase kinase activity by inhibiting phosphorylation, thereby activating the pyruvate dehydrogenase complex (see Section 7.11 – Pharmacodynamic effects of dichloroacetic acid), also inhibits the kinases involved in the regulation of cell growth (Stauber and Bull, 1997).

In a subsequent study, the investigators demonstrated in vitro that dichloroacetic acid produced a concentration-dependent positive effect in a modified cell transformation assay system (expansion of anchorage-independent hepatocytes in soft agar; hepatocytes from male B6C3F1 mice were incubated with 0 to 2.0 mM dichloroacetic acid for 10 days) and that the transformed hepatocytes were c-jun-positive. Concentration dependence was expressed not in terms of the number of transformed colonies, but solely as the decrease in the latency of colony formation. Non-transformed hepatocytes showed no expression of c-jun. When hepatocytes were used which were obtained from male B6C3F1 mice after pretreatment with 0.5 g/l dichloroacetic acid for 2 weeks, marked acceleration of noncontrolled cell growth was observed. This finding led the investigators to conclude that dichloroacetic acid promoted the growth and survival of initiated cells. The investigators suspected that during the early increase in cell proliferation that follows the administration of dichloroacetic acid, initiated cells expand clonally and escape the mitoinhibitory effects observed as exposure progresses (Stauber et al., 1998).

In contrast to B6C3F1 mice, the females of this strain have only a very low spontaneous incidence of liver tumours. However, their tumours exhibit H-ras codon 61 mutation rates (42 to 67%) and mutation spectra (consisting mostly of CAA $\rightarrow$ AAA transversions, fewer CAA $\rightarrow$ CGA transitions, followed by CAA $\rightarrow$ CTA transversions) similar to those found in males. Out of a total of 22 tumours isolated from female B6C3F1 mice treated with 3.5 g/l dichloroacetic acid in drinking water for 104 weeks, only one tumour carried an H-ras codon 61 mutation, which was a CAA $\rightarrow$ CTA transversion (as identified by sequence analysis of polymerase chain reaction-amplified tumour DNA). According to the investigators' discussion, this finding also

argues against the carcinogenic activity of dichloroacetic acid being associated with mutational activation of the H-ras codon 61 proto-oncogene (Schroeder et al., 1997).

One hour after intraperitoneal administration of NaOH-neutralised dichloroacetic acid at 1000 mg/kg body weight, male Sprague-Dawley rats were detected (by differential analysis after amplification by polymerase chain reaction) to express a gene referred to as IC1 in RNA isolated from their liver. The gene revealed 50% homology with a mouse fibroblast growth factor mRNA. The investigators suspected that the growth factor stimulated DNA synthesis in rat hepatocytes and that the induction of IC1 by dichloroacetic acid was involved in the nongenotoxic processes through increasing hepatocellular proliferation (Choi and Park, 1996).

By means of a subtraction hybridisation method, it was found that liver from male B6C3F1 mice administered dichloroacetic acid exhibited increased expression of cDNA (cdca2m), which showed 98.7% homology with rat mRNA for S-adenosylmethionine synthetase and 82.4% homology with human mRNA for S-adenosylmethionine synthetase. cDNA (cdca2m) expression was markedly suppressed in hepatocellular carcinoma tissue (no further details given in the English abstract of the publication written mostly in Korean; Shin and Guntaka, 1995).

A genotoxic mechanism is also argued against by the findings of a study that measured the level of the methylated base 5-methylcytosine (5MeC). According to the investigators, 5MeC plays a role in regulation of gene expression, hypomethylation of DNA via decrease of 5MeC has been hypothesized to be an epigentic, nongenotoxic mechanism involved in tumour promotion by facilitating aberrant gene expression. Female B6C3F1 mice were treated on day 15 of age with a single intraperitoneal dose of N-methyl-N-nitrosourea at 25 mg/kg body weight and from 6 weeks of age onwards received drinking water containing 25 mmol/l dichloroacetic acid (neutralised with NaOH, pH 6.5 to 7.5) for 43 or 44 weeks. Some animals received the dose of dichloroacetic acid over periods of 11 days or 44 weeks without pretreatment with N-methyl-N-nitrosourea. After 11 days, the levels of 5MeC in hepatic DNA were significantly decreased in the groups that were not pretreated with N-methyl-N-nitrosourea, and after 44 weeks they were comparable with the control values. In N-methyl-N-nitrosourea-treated animals, the level of 5MeC in unchanged liver tissue did not differ

significantly from the controls but was decreased in the adenomas from those animals. In adenomas from animals pretreated with N-methyl-N-nitrosourea and then treated with dichloroacetic acid for 43 weeks, the 5MeC level was no longer decreased at 44 weeks, when they were examined. The research group (Pereira and Phelps, 1996; see Section 7.7 – Long-term studies) had previously shown that dichloroacetic acid-induced adenomas and hyperplastic nodules regressed upon cessation of exposure to the chemical (Ge et al., 1998; Tao et al., 1998).

In a subsequent study, the same research group demonstrated in female B6C3F1 mice treated with dichloroacetic acid (neutralised to pH 6.5 to 7.5 with NaOH) at 500 mg/kg body weight/day by oral gavage for 5 days that the promoter regions of the c-jun and c-myc proto-oncogenes were hypomethylated and expression of their mRNA and levels of c-jun and c-myc proteins were increased. Methionine dose-dependently prevented hypomethylation and subsequent expression of the corresponding mRNA and proteins when given intraperitoneally at doses of 100 mg/kg body weight or higher 30 minutes after each administration of dichloroacetic acid. The investigators suggested that dichloroacetic acid possibly induces hypomethylation of the c-jun and c-myc proto-oncogenes by depleting S-adenosylmethionine, thus altering the regulation of cell proliferation and apoptosis and ultimately acting as an epigenetic carcinogenic (Tao et al., 2000).

- **Studies investigating dichloroacetic acid-induced biochemical and morphometric changes with respect to the extent of their relevance to carcinogenicity**

When administered in carcinogenic doses, dichloroacetic acid led to a severe accumulation of glycogen in the liver, whilst the initiated cells and tumour cells themselves were low in glycogen. Furthermore, when male B6C3F1 mice were treated with carcinogenic dose levels (0.2 to 2.0 g/l in drinking water) for 2 weeks and tumours from long-term studies (DeAngelo et al., 1999; see Section 7.7 – Long-term studies) were examined, it was found that both initiated cells and tumour cells had increased levels of insulin receptor protein whereas the corresponding levels in noninitiated liver and serum decreased severely. The investigators suspected that these differences contributed to the carcinogenic activity of dichloroacetic acid (Kato-Weinstein et al., 1998; Lingohr et al., 2001).

The investigators noted in subsequent in-vitro studies conducted in B6C3F1 mouse hepatocytes that dichloroacetic acid-induced glycogen accumulation did not result from a decrease in glycogen degradation but rather that it was independent of the available amount of insulin and proceeded via a phosphatidylinositol-3' kinase-dependent mechanism (Lingohr et al., 2002).

A more detailed study of hepatocellular lesions induced by dichloroacetic acid (> 99% pure) was conducted in male B6C3F1 mice exposed to concentrations of 0 (controls), 0.3, 1.0 or 2.0 g/l in their drinking water (adjusted to pH 6.8 to 7.2 with NaOH) for 2, 5 or 14 days. Body and kidney weights were unchanged by treatment. There was a dose-related increase in relative liver weight in the intermediate dose group after 14 days and in the top dose group after 5 days and 14 days of treatment. After 14 days, hepatocytes were enlarged and exhibited severe glycogen accumulation in the two higher dose groups. In addition, these dose groups showed localised areas of coagulative necrosis with acute inflammation and multiple mitotic figures. The increases in hepatic weight were generally accompanied by decreases in the concentrations of DNA. The percentage of [^{3}H]-thymidine-labelled cells ([^{3}H]-thymidine administered 2 hours prior to sacrifice) in the top dose group was markedly increased after 14 days, particularly around the necrotic areas. Measurements of [^{3}H]-thymidine incorporation into whole liver DNA revealed a significantly increased value in the top dose group at 5 days and a significantly decreased value in the low dose group at 14 days of treatment. As far as they were examined, male and female Swiss-Webster mice treated at 1 or 2 g/l in an exploratory study exhibited similar changes. Based on their findings, the investigators concluded that reparative hyperplasia resulting from tissue necrosis contributed significantly to the hepatocarcinogenicity of dichloroacetic acid (Sanchez and Bull, 1989, 1990).

The latter conclusion, however, is not consistent with the findings of a later study by other investigators. Groups of 28-day-old male B6C3F1 mice received up to 30 days' treatment with dichloroacetic acid at 0.5 or 5.0 g/l in drinking water (neutralised with NaOH to pH 6.8 to 7.2; controls were supplied distilled water). From day 5 onwards, the animals were administered a total of 200 µCi [^{3}H]-thymidine in vivo via implanted minipumps prior to each sacrifice. In the top dose group, body weight development was decreased and water consumption markedly reduced. Based on water consumption and body weights and depending on the duration of exposure,

mean daily doses in the 0.5 g/l group ranged between 109 (after 5 days) and 87 mg/kg body weight (after 30 days) and in the 5.0 g/l group between 420 (after 5 days) and 490 mg/kg body weight (after 30 days). Every 5 days, 5 animals from each dose group were sacrificed and their absolute and relative liver weights determined. They were further examined histopathologically for lesions in liver sections, histomorphometrically for hepatocyte density (cellularity), nuclear size, numbers of mononuclear and multinucleated cells and apoptosis (programmed cell death; cell death which, in contrast to necrosis, is regulated by the cell's own genetic information, resulting in degradation), autoradiographcially for cell proliferation (determination of labelling index, the percentage of cells with [^{3}H]-thymidine-labelled nuclei) and for DNA density and specific [^{3}H]-thymidine activity (dpm/µg DNA) in liver homogenate. Treatment with dichloroacetic acid resulted in a significant dose- and time-dependent increase in absolute and relative liver weights. The low dose group exhibited increased relative liver weight from 10 days of treatment onward without exceeding 116% of the control at any time of examination. In the top dose group, relative liver weight was 16% higher than the control on day 5 of treatment, increasing to twice the control from day 20 of treatment onwards. The specific activity of [^{3}H]-thymidine in the DNA from the 5 g/l group initially showed a marked decrease after 5 days of treatment (42.8% of the control) then increased markedly after 15 and 20 days of treatment (265 and 245% of the controls, respectively), returning to control levels by treatment days 25 and 30. The investigators suggested that an increase in specific [^{3}H]-thymidine activity in liver homogenate can not be conclusively interpreted as indicative of an increased hepatocellular proliferation rate, because Kupffer cells, for instance, which are very highly labelled with [^{3}H]-thymidine, are also measured, resulting in a distortion of the result. A parameter that is more suitable for the assessment of hepatocellular proliferation is the labelling index. This index was markedly decreased at days 5, 10, 20 and 25 of treatment (down to 8.5% of the control value at 5 days). At day 30, the decrease was no longer significantly different from the control. Moreover, liver cellularity and DNA concentration in the liver were reduced, large numbers of mononucleated cells were present whose nuclei were enlarged and, according to the investigators, probably tetraploid, hepatocytes exhibited marked glycogen accumulation and apoptosis was greatly reduced. In the 0.5 g/l group, the same trends were discernible in the various parameters, but the changes were not always significant. These findings led the investigators to

conclude that hepatomegaly was attributable to hypertrophy rather than hyperplasia, that the mitotic activity of hepatocytes was greatly reduced by dichloroacetic acid rather than their proliferation rate being increased, as suspected by other investigators (Bull et al., 1990; Sanchez and Bull, 1989, 1990), and that protective mechanisms for the elimination of initiated cells (apoptosis) were suppressed – changes which according to the investigators were frequently observed with nongenotoxic tumour promoters (Carter et al., 1991, 1995; DeAngelo and Chavis, 1991; Snyder et al., 1995 a, b, c; see also Section 7.7 – Long-term studies, Carter et al., 1998; DeAngelo et al., 1999).

The mitoinhibitory effect was confirmed in an independent experiment the research group conducted in male B6C3F1 mice and F344 rats. Mice received dichloroacetic acid in their drinking water at 0 (controls), 0.5, 1, 2 or 3.5 g/l for 5, 15 or 30 days and rats were exposed to 0 (controls), 0.05, 0.5 or 1.5 g/l for 5, 10, 15 or 28 days. The labelling index values measured after pretreatment with the appropriate label were found to be significantly decreased in mice from the 2 and 3.5 g/l groups at 5 and 15 days and in mice from the 0.5 and 1 g/l groups at 15 days. By 30 days, the mice no longer exhibited changes in labelling index. In rats, the labelling index was significantly depressed in the top dose group at 5 and 10 days and in the intermediate dose group at 10 days. The labelling index was not increased at any time point. Levels of hepatic glucocorticoid receptor activity in male B6C3F1 mice were also measured in the context of this trial and found to be decreased in the cytosolic fraction and increased in the nuclear fraction after administration of doses > 0.5 g/l (no further details; DeAngelo and Eldrich, 1996; DeAngelo and McFadden, 1995).

Cholesterol synthesis (incorporation of [2-^{14}C]-acetate into hepatocyte cholesterol) and DNA synthesis (incorporation of [^{3}H]-thymidine into hepatocyte DNA) were studied as markers of cell proliferation. Primary cultures of hepatocytes isolated from male F344 rats were incubated with 1 mM dichloroacetic acid for 48, 60 or 72 hours. At 48 and 72 hours, cholesterol synthesis in the cultures was significantly increased. DNA synthesis was in the range of the control (DMSO) at 48 hours but significantly elevated at 60 and 72 hours (Reddy et al., 1992).

The incorporation of [^{3}H]-thymidine into liver DNA was measured in male B6C3F1 mice offered drinking water containing 3.5 g/l dichloroacetic acid

for up to 90 days. Additionally, primary hepatocyte cultures were prepared and studied in vitro for the effects of epidermal growth factor, hepatocyte growth factor acidic fibroblast growth factor and mitoinhibitory transforming growth factor β. Up to 20 days after administration, there were no differences in [^{3}H]-thymidine incorporation between dichloroacetic acid-treated animals and the controls, after which time incorporation was significantly decreased. With regard to the growth factors, there were no differences between livers from animals treated with dichloroacetic acid and control livers. The data suggested that the growth factors are not involved in dichloroacetic acid-induced carcinogenesis (Tsai and DeAngelo, 1993, 1996).

Dichloroacetic acid was investigated in vitro with regard to its effect on intercellular communication (gap junction intercellular communication) in Clone 9 rat hepatocytes. According to the investigators, the disruption of intercellular communication between initiated cells and surrounding normal cells can cause initiated cells to be released from the growth control constraint exerted by neighbouring cells, thereby facilitating clonal expansion of initiated cells and, ultimately, tumour formation – a mechanism thought to be relevant to nongenotoxic carcinogens. The concentrations employed were 0 (controls), 5, 10 and 50 mM (approx. 645, 1289 and 6447 µg/ml) and the exposure periods were 1, 4, 6, 24, 48 and 168 hours. The 5 mM concentration showed no effect. Significant levels of inhibition of intercellular communication relative to the control were observed for an incubation period of 6 hours at 10 mM and for all durations of exposure at 50 mM. A 50% decrease in intercellular communication lasting 24 hours required a concentration of 41 mM (5285 µg/ml; Benane et al., 1994, 1996).

Further studies are planned in the context of the US National Toxicology Program in order to investigate the biochemical, cellular and tissue effects of dichloroacetic acid (NTP, 2003).

7.8 Reproductive toxicity

In an embryotoxicity/teratogenicity study, Long-Evans rats received dichloroacetic acid (> 99% pure) by oral gavage at 0, (control), 14, 140, 400, 900, 1400, 1900 or 2400 mg/kg body weight as aqueous solutions, adjusted to pH 7 with NaOH, on days 6 to 15 of gestation. The dams were killed and examined on day 20 of gestation. Mortality was increased in the

3 highest dose groups, in which 1 out of 19, 2 out of 19 and 5 (one accidental death) out of 21 pregnant dams died. Compared with the water-treated control group, maternal body weight gain was dose-dependently reduced in all dose groups except the lowest one. Administration of dichloroacetic acid resulted in an increase in relative liver weight in all dose groups, and the dose groups treated at ≥ 400 mg/kg body weight also had increased relative kidney and spleen weights. The percentage of postimplantation loss was higher in the dose groups given ≥ 900 mg/kg body weight, and the number of live foetuses/litter was reduced in the highest dose group. Foetal body weight and length were reduced at and above a dose of 400 mg/kg. The ratio of male to female foetuses was significantly increased in the top dose group. Dichloroacetic acid dose-dependently induced visceral malformations, with 2.6 to 73% of foetuses in the 140 to 2400 mg/kg dose groups being affected in a dose-related manner. The by far most frequent abnormality was seen in the cardiovascular system, primarily a defect between the right ventricle and the ascending aorta. Other dose-related alterations affected the urogenital system (hydronephrosis) and occurred at dose levels ≥ 1400 mg/kg body weight. The incidences of skeletal malformations were not increased. Malformations occurred only at maternally toxic dose levels (maternal *no observed adverse effect level* (NOAEL) < 14 mg/kg body weight (lowest dose tested)). The NOAEL for reproductive toxicity was 14 mg/kg body weight (Randall et al., 1991; Roth et al., 1991; Smith et al., 1992).

A subsequent study was conducted in groups of 7 to 10 Long-Evans rats. They were treated with dichloroacetic acid at 1900 mg/kg body weight on gestation days 6 to 8, 9 to 11 or 12 to 15, at 2400 mg/kg body weight on gestation days 10, 11, 12 or 13 or at 3500 mg/kg body weight on gestation days 9, 10, 11, 12 or 13. Six rats were treated at 1900 mg/kg body weight on gestation days 6 to 15. As in the previous study, dichloroacetic acid (≥ 99% pure) was administered by oral gavage as an aqueous solution adjusted to pH 7 with NaOH. There were no changes in maternal body or organ weights in any of the dose groups. The foetuses were examined only with respect to malformations of the cardiovascular system. No malformations occurred following administration of 1900 mg/kg body weight on days 6 to 8. When dams were treated with that dose on gestation days 9 to 11, 7.2% of foetuses had malformations, and after dosing on gestation days 12 to 15, 15.1% of foetuses had malformations. Treatment at 1900 mg/kg

body weight on gestation days 6 to 15 resulted in malformations in 45% of foetuses. Following administration of 2400 mg/kg body weight on gestation days 10 or 12 or of 3500 mg/kg on gestation days 9, 10 or 12, the percentage of foetuses with malformations was observed to be as low as 2.5 to 3.6%, whilst the foetuses from dams given a single dose on gestation day 11 or 13 showed no pathological changes in the cardiovascular system. Malformations occurred mainly as intraventricular septum defects at the level of the semilunar valves (Epstein et al., 1992; Roth et al., 1991).

Published only as an abstract, there are data showing that oral administration by gavage of dichloroacetic acid at 0 (controls), 0.23, 0.47, 0.93, 1.9, 3.7, 5.6 or 7.4 mmol/kg (approx. 30 to 954 mg/kg) body weight/day on gestation days 6 to 15 failed to induce significant reproductive toxicity in the CD-1 mouse. According to the investigators, there were suggestive trends of delayed parturition and increased perinatal loss in the two highest dose groups (no further details). Pups were observed until postnatal day 6. Spina bifida aperta was noted in one pup each in the 1.9 and 7.4 mmol/kg dose groups, a finding which the investigators suggested may have been substance related. The only signs of maternal toxicity noted were observations of piloerection (no further details; Narotsky et al., 1996).

CD-1 mouse embryos explanted on gestation day 9 were incubated in heat-inactivated rat serum with dichloroacetic acid at concentrations ranging from 734 to 14680 µM (approx. 95 to 1893 mg/l) for 24 to 26 hours. Concentration levels ≥ 5871 µM (approx. 757 mg/l) induced neural tube defects, and higher concentrations also caused defects in the heart, eye and pharyngeal arch. The defects were not due to generation of free radicals (Hunter and Rogers, 1995; Hunter et al., 1996).

A subsequent study in 3 to 6 somite staged explanted CD-1 mouse embryos conducted by the same research team investigated the effects of dichloroacetic acid on cell cycle and apoptosis. Test concentrations were selected to induce 100 percent embryotoxicity with no consequent embryolethality (no further details). The cell cycle was not perturbed by treatment with dichloroacetic acid. However, there were indications of cell death, particularly in the region of the head. Apoptosis was increased in the prosencephalon. No apoptosis was observed in the region of the heart. The investigators suspected altered apoptosis to be relevant to dichloroacetic acid-induced embryotoxicity (Ward et al., 1998).

A 48-hour incubation of Sprague-Dawley rat embryos explanted on gestation day 10 with 1 to 10 mM dichloroacetic acid (99% pure, 128.95 to 1289.5 mg/l) resulted in concentration-dependent delay in embryonic development and, starting at 2.5 mM (approx. 322 mg/l), malformations of the heart and cranofacial region (Saillenfait et al., 1995).

In an in-vitro fertility study in oocytes and sperm from B6D2F1 mice (C57BL/6 x DBA/2), incubation with 100 or 1000 ppm dichloroacetic acid reduced fertilisation rates in a concentration-independent manner (control 87%, 100 ppm dichloroacetic acid 67.3%, 1000 ppm dichloroacetic acid 71.8%; Cosby and Dukelow, 1992).

As reported in Section 7.5, subchronic oral administration of dichloroacetic acid to dogs and rats caused dose-related degenerative changes in the testes, as characterised by small testes, degeneration of the germinal epithelium and syncytial giant cell formation, enlarged Sertoli cells, atrophy of the seminiferous tubules and absence of spermatogonia and spermatozoa in the testes and epididymides. Histopathologically, testicular lesions and prostate glandular atrophy were observed even at the lowest test dose of 12.5 mg/kg body weight/day (administered in gelatine capsules) in dogs. In the rat, testicular lesions occurred only after administration (by gavage) of 500 mg/kg body weight (see Section 7.5; Cicmanec et al., 1991; Bhat et al., 1991; Katz et al., 1981).

A more detailed study of the gonadal damage induced by dichloroacetic acid was conducted in groups of 18 or 19 male Long-Evans rats (initial age 100 days) given sodium dichloroacetate at daily dose levels of 0 (controls), 31.25, 62.5 or 125 mg/kg body weight by oral gavage for 10 weeks. After the last treatment, the males were each allowed to mate with one untreated female and then terminated after 5 days. The females were sacrificed on day 14 of gestation and their uteri examined. Assessments included determinations of body weight gain, organ weights of the liver, kidneys, spleen, testes, accessory sex organs (prostate and seminal vesicles), preputial glands and epididymides, sperm counts and sperm motility assessments and histopathological examination of the testes and epididymides. Dose-related effects noted even at the lowest dose level tested, 31.25 mg/kg body weight, included slowed body weight gain, increased relative liver weight and decreased absolute preputial gland and epididymis weights. From 62.5 mg/kg body weight onwards, there were reductions in the rela-

tive weights of the epididymis and preputial glands, increases in relative kidney and spleen weights and absolute liver weight, and from 125 mg/kg body weight onwards, reductions in the absolute weights of the accessory sex organs and increases in relative testis weights. Total sperm count, sperm motility and the percentage of normal intact sperm were reduced in the two highest dose groups. Histopathological examination revealed that spermiogenesis was inhibited in the two highest dose groups. However, atrophic seminiferous tubules, Leydig cell hyperplasia or hypoplasia and epididymal changes were not observed. Fertility was impaired in the highest dose group, in which the numbers of pregnant females and implants/dam were reduced (Toth et al., 1992).

A further study to investigate the testicular toxicity of dichloroacetic acid was conducted in groups of 6 to 8 male Sprague-Dawley rats/dose and time point of analysis. The rats were treated with a single dose of 1500 or 3000 mg/kg body weight, 1440 mg/kg/day on two days, 480 or 1440 on 5 days, 160, 480 or 1440 mg/kg/day on 9 days or 18, 54, 160, 480 or 1440 mg/kg body weight/day for 14 days. The compound, > 99% pure, was adjusted to pH 6.5 with NaOH and administered by oral gavage. Animals treated with a single dose were sacrificed 2, 14 or 28 days after administration and those given multiple doses were sacrificed 24 hours after the last administration. Assessments encompassed body weights, testicular and epididymal weights, sperm counts and sperm motilities, spermatogenesis staging by histopathological examination after special preparation of the testes and epididymides, and determination of serum testosterone levels. Body weight gain was dose-dependently decreased, except after administration of 18 or 54 mg/kg body weight. No treatment-related adverse changes in testicular weights were noted. Epididymal weights were significantly decreased only in animals treated at 480 or 1440 mg/kg body weight/day for 14 days. The spermiograms showed dose-related and duration of exposure-related reductions in sperm count, sperm motility and/or sperm maturation in all groups treated with dichloroacetic acid. Serum testosterone levels were unchanged in all dose groups. Sperm head abnormalities occurred after 14-day administration of 480 or 1440 mg/kg body weight and fused sperm were observed in the epididymides after 5-day treatment at dose levels ≥ 160 mg/kg body weight. Sperm maturation was still delayed 28 days after a single dose of 1500 mg/kg body weight (Linder et al., 1997).

7.9 Effects on the immune system

Immunological parameters (antibody production, delayed hypersensitivity, cytotoxicity of natural killer cells, production of prostaglandin PGE_2 and interleukin IL_2) investigated in the context of a subchronic study in male Sprague-Dawley rats administered up to 5000 ppm dichloroacetic acid in their drinking water (approx. 345 mg/kg body weight/day) gave no indication of a harmful effect of dichloroacetic acid on the immune system (see Section 7.5; Mather et al., 1990).

Mouse lymphocytes isolated from the spleen were incubated in vitro with dichloroacetic acid at concentrations pf 10^{-10} to 10^{-4} mol/l for 8 hours. The T-cell mitogen concanavalin A (2 µg/ml) or the B-cell mitogen lipopolysaccharide (100 µg/ml) was also added to the incubation media. The inhibition of T cell proliferation (B cells remained unaffected) led the investigators to conclude that dichloroacetic acid possibly impairs the cellular immune response (no further details; Ueno et al., 1999).

7.10 Neurotoxicity

Dichloroacetic acid induced degenerative CNS changes in subchronic studies in the rat and dog. Even the lowest dose levels tested, 12.5 mg/kg body weight/day in the dog (administered in gelatine capsules) and approx. 16 mg/kg body weight/day in the rat (administered in drinking water in a specific 12-week neurotoxicity study), caused nonreversible degenerative CNS lesions. The dose-related lesions in the CNS manifested clinically as hindlimb paralysis and histopathologically as vacuolisation of the white myelinated tracts of the cerebrum, cerebellum and/or spinal cord. The peripheral nervous system was free of histopathological changes, as was the optical nerve (see e.g. Bhat et al., 1991, and Katz et al., 1981, in Section 7.5).

The United States Environmental Protection Agency (EPA) conducted numerous studies to investigate the neurotoxicity of dichloroacetic acid (≥ 99% pure, adjusted to pH 7 using NaOH) after subchronic studies, particularly in the dog and rat, demonstrated degenerative changes in the central nervous system, with some studies also showing hindlimb paralysis, and clinical trials of dichloroacetic acid as a drug for the treatment of metabolic disorders detected peripheral neuropathy in study subjects (see Stacpoole et al., 1979, Kurlemann et al., 1995, and Moore et al., 1979, in Sec-

tion 8). Young and old rats of the Fischer-344 and Long-Evans strains received acute, subchronic or chronic treatment with the compound by oral gavage or in their drinking water and underwent comprehensive functional observational battery testing and motor activity assessments. Clinical signs, body temperature and body weight data were recorded in addition. The study designs and detailed findings from the individual studies are summarised in Table 12 in the appendix. Even after acute administration by oral gavage, doses from 300 mg/kg body weight resulted in short-term decrease in hindlimb grip strength, a characteristic sign of dichloroacetic acid neurotoxicity. In all studies, subchronic and chronic administration of dichloroacetic acid in the drinking water resulted in neurological changes which manifested, in particular, as gait impairment and weakness of the hindlimbs down to the lowest dose range tested (approx. 16 mg/kg body weight/day over a period of 12 weeks and 137 mg/kg body weight/day over a period of 2 years). Further findings included decreased fore- and hindlimb grip strength, abnormal body position, increased landing foot splay when dropped from a height of 30 cm, impairment or loss of righting reflex and pupil response and mild tremor. The higher dose groups usually displayed retardation of body weight gain. Following ingestion of comparable doses, administration in drinking water resulted in more intense changes than administration by oral gavage, changes observed in young animals (28 to 30 days old at the beginning of the study) were more marked than in older animals (68 to 80 days old at the beginning of the study) and F344 rats reacted more sensitively than did Long-Evans rats. The incidence and intensity of the individual changes correlated with dose level and duration of treatment. Neurohistopathological examinations, which were performed only in a small number of animals in a chronic study, revealed pathological changes in a very limited area of the spinal cord. The fasciculus gracilis of the posterior column in the grey spinal cord exhibited marked gliosis with loss of myelinated axons and large granular structures. The investigators tentatively identified the lesions as dystrophic axons. The peripheral nervous system showed no pathological alterations and also there was no vacuolation in the brain. The neurological changes occurred slowly and were not completely reversible, as seen in animals observed for 14 weeks after administration of 172 mg/kg body weight/day for 3 months and animals observed for 18 months after administration of 235 mg/kg body weight/day for 6 months. A *no observed effect level* (NOEL) for neurological changes in rats can not be established on the basis of these

studies, because even the lowest test dose of 16 mg/kg body weight/day, which was administered in drinking water for 12 weeks, produced pathological changes (Moser et al., 1999).

Published only as an abstract, one study reported that the administration of sodium dichloroacetate in drinking water at a level of 2 g/l for periods of up to 20 weeks caused widespread vacuolation of myelin and dystrophic and degenerative changes in scattered axons in the brain and spinal cord of male CD-CRL:COBS CD (SD)BR rats. Damage in the peripheral nervous system was minimal. The investigators also pointed out that these lesions may have been caused by an energy deficit in the nerves (no further details; Spencer et al., 1981)

A study was conducted to investigate whether these lesions were caused by dichloroacetic acid-induced thiamine deficiency. To this end, groups of 15 male Sprague-Dawley rats were administered sodium dichloroacetate (> 99% pure) in drinking water at 0 (controls), 50, 1000 or 1100 mg/kg body weight/day over a period of 7 weeks. Additional groups received the same doses plus 0.6 mg thiamine (vitamin B1) intraperitoneally three times weekly or the same dose of thiamine as daily oral administrations. Thiamine status was monitored by measurement of transketolase activity. The 50 mg/kg dose caused no signs of neurotoxicity (hindlimb weakness) and no changes in transketolase activity. In contrast, 1100 mg/kg body weight resulted in severe clinical signs and lowered enzyme activity by 25%. Co-administration of thiamine was associated with markedly reduced neurological signs and normal transketolase activity. The investigators considered that their findings confirmed their opinion that the neurotoxic effects caused by sodium dichloroacetate were, at least partially, the consequence of thiamine deficiency, which in turn was the consequence of dichloroacetic acid-induced activation of the pyruvate-dehydrogenase complex (Stacpoole et al., 1984, 1990).

7.11 Other effects

Pharmacodynamic effects of dichloroacetic acid

Dichloroacetic acid inhibits pyruvate dehydrogenase kinase, thus interfering with a central mechanism of metabolism, the oxidative decarboxylation

of pyruvate to acetyl coenzyme A. The irreversible decarboxylation of pyruvate to acetyl coenzyme A, in the process of which nicotinamide adenine dinucleotide (NADH) is formed, takes place via the pyruvate dehydrogenase system, a mitochondrial multi-enzyme complex with thiamine diphosphate as coenzyme. The activity of the enzyme complex is regulated by a reversible phosphorylation reaction, which is catalysed by a phosphatase and a kinase, which in turn are normally regulated in their activity by the NADH/NAD^+ and acetyl coenzyme A/coenzyme A ratios and both belong to the complex. High levels of NADH or acetyl coenzyme A activate the kinase, resulting in phosphorylation and, consequently, inactivation of the multi-enzyme complex (see textbooks of biochemistry). Dichloroacetic acid inhibits the kinase, thus locking the enzyme complex in its unphosphorylated, catalytically active form. This increase in pyruvate dehydrogenase activity caused by the inhibition of pyruvate dehydrogenase kinase has been demonstrated in numerous in-vitro and in-vivo studies (see the review article by Crabb et al., 1981; Peer and Graf, 1990; Stacpoole, 1989; Stacpoole et al., 1998 a, b). Both pyruvate and acetyl coenzyme A are central metabolic products and substrates of carbohydrate, lipid and protein metabolism. Therefore, by inhibiting pyruvate decarboxylase kinase, dichloroacetic acid affects numerous intermediary metabolic processes, something that likely explains the toxicodynamic but also the pharmacodynamic effects of the compound (cf. Crabb et al., 1981; Stacpoole, 1989; Stacpoole et al., 1998 a, b).

Based on this effect of dichloroacetic acid, potential therapeutic uses in pathological metabolic alterations have for many years been closely investigated in in-vitro, animal and human studies, particularly by US research teams at the University of Florida in Gainesville and at Indiana University in Indianapolis. The very numerous studies and publications on the pharmacodynamic properties of dichloroacetic acid have been comprehensively reviewed in the above-mentioned review articles (see Crabb et al., 1981; Stacpoole, 1989; Stacpoole et al., 1998 a, b). Therefore the following will provide only a brief summary of the findings.

Effects of dichloroacetic acid on hyperglycaemia

Several studies in the 1960s and early 1970s demonstrated the blood glucose-lowering effect of diisopropylammonium dichloroacetate. Subsequent

studies showed that the effect was attributable to dichloroacetic acid itself and was due to the inhibition of pyruvate dehydrogenase kinase in the pyruvate dehydrogenase complex (Stacpoole, 1969, 1989). Dichloroacetic acid and sodium dichloroacetate lower the blood glucose levels in laboratory animals with induced diabetes and ketonuria and increase the survival rate of animals with ketoacidotic coma. The compound has little or no effect on blood glucose levels in animals not rendered diabetic. It has been shown that dichloroacetic acid counteracts hyperglycaemia, on the one hand by accelerating glycolysis and on the other by inhibiting gluconeogenesis. The permanent activation of the pyruvate dehydrogenase complex in the absence of regulation by pyruvate dehydrogenase kinase leads to an increase in the demand for pyruvate, which is met by increasing the conversion of the pyruvate substrates glucose, alanine and lactate. This explains the marked blood glucose-lowering effect of dichloroacetic acid, as on the one hand, degradation of glucose to pyruvate is augmented by increased glycolysis, while on the other hand gluconeogenesis, the essential substrates of which are lactate and alanine, is suppressed. It is further assumed that other gluconeogenic enzymes are altered in their activity due to altered levels of NAD^+/NADH (glyceraldehyde-3-phosphate dehydrogenase) and accumulation of the dichloroacetic acid metabolite oxaloacetate (pyruvate carboxylase). In clinical studies, dichloroacetic acid significantly reduced hyperglycaemia as well as blood lactate and alanine levels in patients with type II diabetes mellitus. In healthy subjects, short-term administration of dichloroacetic acid had no glucose-lowering effect but caused a strong decrease in blood lactate levels (Brown and Gore, 1996; Diamond et al., 1980; Enser and Whittington, 1983; Park et al., 1983; Pegorier et al., 1978; Stacpoole, 1989, 1997; Stacpoole and Greene, 1992; Stacpoole et al., 1978, 1998 a, b).

Effects of dichloroacetic acid on lactic acidosis

Dichloroacetic acid leads to a severe decrease in the amounts of lactate present in tissues and circulating in the blood. The increased degradation of pyruvate by the pyruvate-dehydrogenase complex also accelerates the catabolism of lactate, the precursor of pyruvate.

The beneficial effect in congenital or acquired general or local metabolic acidosis of treatment with dichloroacetic acid in addition to, or in replace-

ment of, sodium hydrogencarbonate therapy has been demonstrated in numerous studies in both experimental animals and human subjects. The administration of dichloroacetic acid markedly improved the prognosis particularly of patients in whom treatment with sodium bicarbonate was not or not sufficiently effective (Aynsley-Green et al., 1984; Blackshear et al., 1982; Burlina et al., 1993; Coude et al., 1978; Crabb et al., 1981; Fox, 1996; Fox et al., 1996 b; Henderson et al., 1997 b; Irsigler et al., 1979; Jahoor et al., 1994; Kodama et al., 1986; Krishna et al., 1994, 1995, 1996; Kuroda et al., 1986; Ludvik et al., 1991; McCormick et al., 1985; Mc Khann et al., 1980; Naito et al., 1989; Okabe et al., 1986; Saijo et al., 1991; Scheid et al., 1985; Shangraw et al., 1992; Stacpoole, 1989, 1993, 1997; Stacpoole et al., 1983, 1988, 1992, 1997, 1998 a, b; Wargovich et al., 1988). Further clinical studies are currently underway, including studies in neonates and children with congenital lactic acidosis due to various enzyme defects, patients undergoing liver transplantation and patients with liver cirrhosis, stroke, heart attack or *Plasmodium falciparum* infections (malaria), who frequently develop severe lactic acidosis (Bersin et al., 1994; Fox et al., 1996 b; Marangos et al., 1999; Saitoh et al., 1998; Shangraw et al., 1994 a, b; Shangraw and Fischer, 1996, 1999; Stacpoole et al., 1997; Takanashi et al., 1997; Tóth et al., 1993; Zeman et al., 1998).

Other pharmacodynamic of dichloroacetic acid, e.g. in ischaemia or altered lipid or lipoprotein metabolism, will not be further discussed here. These actions have also been investigated by Stacpoole and associates and comprehensively discussed in the above-mentioned review articles (see Stacpoole, 1989, 1997; Stacpoole et al., 1998 a, b).

Cytotoxicity of dichloroacetic acid

The cytotoxicity of dichloroacetic acid was investigated in vitro in HEP G2 cells (a cell line derived from human hepatoma cells) by measuring cellular protein content. Dichloroacetic acid was tested together with 113 other substances of diverse chemical structure. The parameter measured was the PI_{50}, i.e. the molar concentration which reduces protein content by 50% after a 24-hour incubation period. It was 24 mM for dichloroacetic acid as compared with 57 mM and 118 mM for acetic acid and sodium acetate, respectively (Dierickx, 1989).

The cytotoxicity of dichloroacetic acid was also investigated in liver slices from male B6C3F1 mice. Potassium content and enzyme leakage of serum lactate dehydrogenase, alanine aminotransferase and aspartate aminotransferase served as the study parameters. In addition, the metabolic competence of the liver slices was studied by analysing 7-ethoxycoumarine metabolism. The EC_{50} values determined for potassium content were 9360 µg/ml and 64 mM and those for serum lactate dehydrogenase leakage were 8890 µg/ml and 69 mM (a minor difference in the data reported in the two available publications based on the study). Alanine aminotransferase and aspartate aminotransferase leakage and metabolic competence of liver slices were not altered by dichloroacetic acid (Pravecek et al., 1994, 1996).

Sprague-Dawley rats and B6C3F1 mice were pretreated in vivo with clofibric acid (a peroxisome proliferator), or remained untreated. The cytotoxicity of dichloroacetic acid (> 99%) was then tested in vitro in hepatocyte suspensions, with lactic acid release and/or trypan blue exclusion serving as the criteria for cytotoxicity. Dichloroacetic acid had no cytotoxic properties up to a concentration of 5.0 mM. The investigators concluded from the studies that the necrotic areas observed following high doses of dichloroacetic acid in vivo were not due to cytotoxicity (Bruschi and Bull, 1993).

Impact on the intestinal flora

Administration of dichloroacetic acid in drinking water (1 g/l) to male Fischer-344 rats over a period of 5 weeks resulted in changes in the intestinal flora, which, according to the investigators, could influence bioactivation of promutagens and procarcinogens. Changes in the intestinal flora were measured by determining the activities of various enzymes (beta-glucuronidase, beta-galactisodase, beta-glucosidase, nitroreductase, azoreductase and dechlorinase) in the caecum and the small and large intestines (George et al., 2000).

8 Experience in humans

The data on the human toxicokinetics/pharmacokinetics, metabolism and toxicodynamics/pharmacodynamics of dichloroacetic acid that were exclusively gained from research on the use of the compound as a therapeutic drug for various metabolic diseases and disorders are discussed in com-

prehensive recent review articles (cf. Henderson et al., 1997 a; Peer and Graf, 1990; Stacpoole, 1989; Stacpoole et al., 1998 a, b). Beyond that, the following will address only a few points that are of particular toxicological relevance.

Toxicokinetics and metabolism

Selected studies in which human pharmacokinetic parameters of dichloroacetic acid were determined are summarised as examples in Table 13 in the appendix. The following discusses in detail a study that observed marked changes, as seen in animal studies, in the metabolism and excretion of dichloroacetic acid following repeated administration in comparison with single-dose administration.

Male and female volunteers (aged 18 to 45 years) were given either a single dose or multiple doses of sodium dichloroacetate at 50 mg/kg body weight intravenously or orally in gelatine capsules. Intravenous doses were administered over 30 minutes using an infusion pump. Single oral administration of 50 mg/kg body weight produced, e.g., a maximum plasma concentration of about 90 µg/ml at approx. one hour, the concentration dropping to near zero at approx. 12 hours. The maximum plasma concentration observed after single intravenous administration of 50 mg/kg body weight over 30 minutes, for instance, was 170 µg/ml, the concentration dropping to near zero at approx. 9 hours. Calculations using all the data for oral administration yielded a maximum concentration of 111.7 µg/ml at 1.24 hours, a volume of distribution of 19.9 l, a plasma clearance value of 102.13 ml/minute and renal clearance values of 42.87 and 53.00 ml/hour (obtained by two different methods). Bioavailability and excretion (only oxalate was determined in urine) exhibited no significant difference between the oral and intravenous routes. Further experimental subjects were administered up to four doses of sodium dichloroacetate at 50 mg/kg body weight, the intervals between administrations ranging between 2 and 8 weeks. Subjects given a total of two treatments at an interval of 2 weeks exhibited prolongation of plasma half-life from 1.75 hours after the first administration to 2.65 hours after the second administration. Subjects with a 4-week interval between the two administrations showed a prolongation of plasma half-life from 2.28 to 3.9 hours. There were great individual differences. Two subjects who were treated twice at an interval of 8 weeks did not exhibit

any prolongation of plasma half-life. When the same subjects received additional treatment after 6 weeks, or 6 and 2 weeks, they also showed prolongation of plasma half-life. The investigators had already found in an earlier study that repeated administration was associated with a marked increase in half-life. Subjects given infusions of sodium dichloroacetate at 50 mg/kg body weight at 2-hour intervals showed increases in half-life from 63.3 minutes (range: 15.0 to 112.2 minutes) after the first administration to 374.0 minutes (range: 37.8 to 1386.0 minutes) after the fifth administration (Curry et al., 1985, 1991).

This prolongation of plasma half-life following repeated administration was also observed in early clinical trials of the compound for treatment of lactic acidosis (Stacpoole et al., 1983).

Dermal absorption of dichloroacetic acid appears to be possible in humans. Subjects exposed for 30 minutes to chlorinated swimming pool water containing a mean dichloroacetic acid concentration of 484 µg/l (range: 52 to 647 µg/l) excreted, within 3 hours, quantities of dichloroacetic acid in their urine which could not be explained by any pool water they may have swallowed while they were in the pool. The investigators estimated that approx. 6 µg dichloroacetic acid had been absorbed via the skin (Kim and Weisel, 1998).

As shown in in-vitro studies in rat liver cytosol (see Section 7.1; Cornett et al., 1997; Gonzalez-Leon et al., 1997 b; James et al., 1997), glutathione-dependent metabolism to glyoxylate and oxalate was also demonstrated in vitro in human hepatic cytosol (James et al., 1997).

Toxicodynamic effects

Similarly, the experience regarding the effects of dichloroacetic acid in humans is limited to the findings in clinical study subjects participating in the clinical investigation of the compound.

Short-term administrations of dichloroacetic acid in studies conducted in healthy subjects have not, so far, been observed to produce relevant adverse effects (for details, see the review article by Stacpoole et al., 1998 a, b and Peer and Graf, 1990). However, in chronic diseases, long-term therapy with dichloroacetic acid is contra-indicated due to severe adverse effects

(see below; Stacpoole et al., 1979; Moore et al., 1979; Kurlemann et al., 1995). Dichloroacetic acid has been patented for use as an acute therapeutic agent to compensate short-term acid-base imbalances due to acute local underoxygenation and is in the continued process of clinical investigation (see Section 7.11 – Effects of dichloroacetic acid on lactic acidosis).

An investigation of the sodium salt of dichloroacetic acid for long-term therapy of hypercholesterolaemia, in which a 21-year-old patient with severe familial hypercholesterolaemia received a 50 mg/kg daily oral dose of sodium dichloroacetate, had to be discontinued after 16 weeks due to the emergence of severe adverse neurological effects. Under sodium dichloroacetate treatment, the plasma lipoprotein concentrations fell markedly, but a polyneuropathy developed in parallel, characterised by weakness of the facial, finger and lower-extremity muscles. In addition, the deep tendon reflexes were diminished and the nerve conduction velocity was slowed. The neurological findings are in agreement with the adverse changes observed in animal studies. The treatment was discontinued and dichloroacetic acid evaluated as unsuitable for long-term therapy. After withdrawal of sodium dichloroacetate, the patient's neurological condition gradually improved over a period of 6 months (Stacpoole et al., 1979; Moore et al., 1979).

Long-term treatment with dichloroacetic acid of a 13-year-old female patient, who had developed severe lactic acidosis with ensuing metabolic encephalopathy due to mitochondrial complex I deficiency, had to be discontinued owing to the emergence of adverse neurological effects. Initially, the patient was treated three times daily with 80 mg/kg dichloroacetic acid. This resulted in normal lactate levels, which remained in the normal range when the dose was reduced to 60 mg/kg body weight after 8 weeks. Lactate levels rose again markedly after a further dose reduction to 25 mg/kg body weight and they did not return to normal again even when the dose was once more increased to 50 mg/kg body weight. Except for a clear improvement in her ability to concentrate, the patient, co-medicated with 100 mg thiamine/day, showed no change in her clinical condition. At week 20 of therapy, adverse effects of the treatment emerged; the patient exhibited a shuffling gait and her deep tendon reflexes were diminished. At week 24 of therapy, ankle and knee-jerk reflexes were reduced, the motor nerve conduction velocity of the tibial nerve was reduced and that of the peroneal nerve was not detectable; evoked potentials and sensory nerve conduction velocity of the sural nerve were in the normal range. The treatment was

discontinued. The overall average dose of dichloroacetic acid administered during the 24-week treatment period was 55.8 mg/kg body weight three times daily. Medication with thiamine was continued, and the patient's gait and her nerve conduction velocity returned to normal again within 6 months (Kurlemann et al., 1995).

9 Classifications and threshold limit values

The United States Environmental Protection Agency (EPA) has classified dichloroacetic acid as a "probable human carcinogen" (Group B2; EPA, 2001; ILSI, 1997).

In the Federal Republic of Germany, the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") of the Deutsche Forschungsgemeinschaft has listed dichloroacetic acid and its sodium salt in the "Yellow Pages" ("Substances being Examined for the Establishment of MAK Values and BAT Values") of the 2005 List of MAK and BAT Values on the suggestion of BG Chemie in order that the carcinogenic potential be examined (DFG, 2005).

Table 4. Pharmacokinetic parameters of sodium dichloroacetate in experimental animals (Based on Stacpoole et al., 1998 a)

Species, strain, sex[1]	Dose (mg/kg body weight), route of administration	C_{max} (µg/ml)	$t_{½}$ (hours)	V_d (ml/kg)	CL (ml/kg/minute)	Reference
Rat, Sprague-Dawley, male (230 g)	50, single or repeated doses, oral	–	0.6 and 3.0, respectively	–	–	Yan et al., 1997
Rat, Sprague-Dawley, male (632 g)	50, repeated oral doses	–	10.1	–	–	Yan et al., 1997
Rat, Sprague-Dawley, 3 males	100 (^{14}C-NaDCA), single intravenous dose	120–164	2.1–4.4	701–1080	1.84–5.94 (plasma)	Lukas et al., 1980
Dog, beagle, 2 males	100 (^{14}C-NaDCA), single intravenous dose	447 and 508	17.1 and 24.6	249 and 262	0.123 and 0.168 mg (plasma)	Lukas et al., 1980

[1] where specified
– no data
NaDCA sodium dichloroacetate
C_{max} maximum concentration in plasma/blood
$t_{½}$ half-life in plasma/blood
V_d volume of distribution
CL clearance

Table 5. Acute toxicity studies of dichloroacetic acid and sodium dichloroacetate

Species, strain, sex[1]	Route	Dose (mg/kg body weight)	Compound, purity[1]	Effect	Observation period	Reference
Rat	oral	> 5000	NaDCA	LD_{50}	n.d.	Traina et al., 1977
Rat	oral	4480	NaDCA	LD_{50}, narcosis	6 days	Woodard et al., 1941
Tests were carried out with dichloroacetic acid which was adjusted to pH 6 to 7 with NaOH.						
Rat	oral	2820	DCA	approximate LD_{50}	14 days	Smyth et al., 1951
Mouse, B6C3F1, male, female	oral	5697–7500	NaDCA, > 99%	LD_{50}	14 days	Meier et al., 1997
Tests were carried out with dichloroacetic acid which was adjusted to pH 7 with NaOH.						
Mouse	oral	5520	n.d.	LD_{50}, narcosis	6 days	Woodard et al., 1941
Tests were carried out with dichloroacetic acid which was adjusted to pH 6 to 7 with NaOH.						
Mouse	oral	> 5000	NaDCA	LD_{50}	n.d.	Traina et al., 1977
Mouse, ICR, male, female	oral	ca. 4844 (32.1 mmol)	NaDCA	LD_{50}	7 days	Yount et al., 1982
Mouse, B6C3F1, male	oral	4562–6610, 5 doses at intervals of 24 hours	NaDCA, > 99%	LD_{50}	14 days	Meier et al., 1997
Tests were carried out with dichloroacetic acid which was adjusted to pH 7 with NaOH.						
Mouse, B6C3F1, female	oral	6610–7500, 5 doses at intervals of 24 hours	NaDCA, > 99%	LD_{50}	14 days	Meier et al., 1997
Tests were carried out with dichloroacetic acid which was adjusted to pH 7 with NaOH.						
Dog	oral	1000	NaDCA	not lethal	n.d.	Traina et al., 1977
Rabbit	dermal	ca. 798 (0.51 ml)	DCA	LD_{50}	14 days	Smyth et al., 1951
Rat	inhalation (hazard test)	8-hour exposure to atmosphere enriched or saturated at room temperature	DCA	mortality not increased	n.d.	Smyth et al., 1951
Rat	intravenous	> 2200	NaDCA	LD_{50}	n.d.	Traina et al., 1977
Mouse	intravenous	2852	NaDCA	LD_{50}	n.d.	Traina et al., 1977
Dog	intravenous	> 1000	NaDCA	LD_{50}	n.d.	Traina et al., 1977

[1] where specified
NaDCA sodium dichloroacetate
DCA dichloroacetic acid
n.d. no data

Beginning of Table 12

Table 12. Rat neurotoxicity of dichloroacetic acid after acute, subchronic or chronic oral administration by gavage or in drinking water (Moser et al., 1999)

Strain, sex, age at study initiation and number of animals	Dose[1] (mg/kg body weight/day)	Route of administration, duration of treatment	Scheduled assessment times after the beginning of administration	Findings for FOB (Functional Observational Battery) and motor activity assessments	Other findings
Study 1, Long-Evans, male, female, 76 to 89 days, 9 or 10 rats/group	100, 300, 1000, 2000	gavage, single dose	4, 24, 168, 336 hours	100 mg/kg body weight: no findings; ≥ 300 mg/kg body weight: hindlimb grip strength in males decreased by 20 to 24% at 4 hours; ≥ 1000 mg/kg body weight: lowered motor activity; all findings reversible after 168 hours	body weight decreases in all dose groups after 24 hours
Study 2, Long-Evans, male, 80 days, 9 or 10 rats/group	30, 100, 300, 1000	gavage, 10 weeks, treatment 5 days a week	2, 4, 7, 11 weeks	≤ 100 mg/kg body weight: no findings; ≥ 300 mg/kg body weight: abnormal gait (with a score of 2 on a 4-point scale) from week 7; 1000 mg/kg body weight: tremor in 30 to 50% of animals from weeks 2 to 7, hypotonia in 40% of animals at week 7, progressive decrease in forelimb grip strength (maximum decrease of 30% at week 11); gait abnormalities seen in the 300 mg/kg group at week 11 (one week after the end of administration) were readily reversible	≥ 100 mg/kg body weight: retarded body weight gain; week-11 body weights were 88, 79 and 78% of controls; one top-dose animal died after 4 weeks

Table 12. Rat neurotoxicity of dichloroacetic acid after acute, subchronic or chronic oral administration by gavage or in drinking water (Moser et al., 1999)

Strain, sex, age at study initiation and number of animals	Dose[1] (mg/kg body weight/day)	Route of administration, duration of treatment	Scheduled assessment times after the beginning of administration	Findings for FOB (Functional Observational Battery) and motor activity assessments	Other findings
Study 3, F344, male, 28 days, 18 rats/group (24 controls)	137, 235 (2.5 → 2 → 1.5 g/l and 3.5 → 2.5 → 0)*	drinking water, 104 or 27 weeks*	1, 2, 3, 3.5, 4.5, 5.5, 7, 8, 9, 10, 11, 12, 14, 16, 18, 20, 22, 24 months	≥ 137 mg/kg body weight: progressive gait abnormalities (up to a score of 4 on a 4-point scale) noted from the first assessment onwards; decreased grip strength in the forelimbs and hindlimbs (to 45 and 23% of control, respectively); reduced righting reflex, tremor, inhibition of pupil response to light, abnormal chest clasping response in 23/24 animals from the high and 8/24 animals from the low dose group when suspended by the tail; increase in landing foot splay when dropped from a height of 30 cm; findings in the high dose group were not, or only partially, reversible during the 18-month treatment-free period; neurohistological examination of 3 animals/group revealed marked gliosis with loss of myelinated axons and large granular structures in the fasciculus gracilis in the posterior columns of the grey spinal cord, a finding which the investigators tentatively interpreted as dystrophic axons; the peripheral nervous system showed no pathological changes and no vacuolation was detected in the brain	no increase in mortality, retarded body weight gain (reversible in the high dose group only after 18 months after the end of administration; retarded body weight gain in the low dose group during the first year, then decrease in body weight to 72% of the control by the end of the study, ocular opacity (90 to 100% in the treated groups, 46% in the control group)

* Due to toxicity, the high test concentration was lowered at 6 week from 3.5 g/l to 2.5 g/l, and treatment was discontinued completely after another 21 weeks. The low concentration, initially 2.5 g/l was lowered to 2 g/l at week 6 and then to 1.5 g/l after another 4 weeks, the latter concentration being maintained until the end of the study after a total of 104 weeks. The animals represented satellite groups for the carcinogenicity study by DeAngelo et al., 1996, which is described in Section 7.7.

Table 12. Rat neurotoxicity of dichloroacetic acid after acute, subchronic or chronic oral administration by gavage or in drinking water (Moser et al., 1999)

Strain, sex, age at study initiation and number of animals	Dose[1] (mg/kg body weight/day)	Route of administration, duration of treatment	Scheduled assessment times after the beginning of administration	Findings for FOB (Functional Observational Battery) and motor activity assessments	Other findings
Study 4, F344, male, 30 days, 9 or 10 rats/group	162, 308 (2 and 4 g/l)*	drinking water, 12 or 3 weeks*	3, 6, 9, 12, 17 weeks	162 mg/kg body weight: progressive gait abnormalities (up to a score of 3 on a 4-point scale), reduced righting reflex, tremor, decreased fore- and hindlimb grip strength (to 35 and 78% of the control), lowered motor activity (to 46% of the control), abnormal chest clasping response in 78% of animals when suspended by the tail, findings not reversible 5 weeks after the end of dosing; 308 mg/kg body weight: findings at 3 weeks (at the end of treatment) were largely identical to those seen in the low dose group at 12 weeks; findings were not reversible (gait abnormalities) or only very slowly reversible (abnormal chest clasping response when suspended by the tail) during the 14-week observation period	retarded body weight gain (maximum values in the low and high dose groups were 84 and 74%, respectively, of body weight gain seen in the control)
* Due to toxicity, the high dose group was treated only for 3 weeks.					
Study 5a, F344, male, 28 to 29 days, 12 rats/group	16, 66, 172 (0.2, 0.75 and 2 g/l)	drinking water, 12 weeks	3, 6, 9, 13, 16, 19, 23, 27 weeks	≥ 16 mg/kg body weight: progressive gait abnormalities (dose-dependent up to scores of approx. 1.7, approx. 2.1 and approx. 2.7 on a 4-point scale), dose-dependently impaired righting reflex; ≥ 66 mg/kg body weight: decreased motor activity (to 69 and 52% of the control), increase in landing foot splay when dropped from a height of 30 cm; 172 mg/kg body weight: tremor, decreased grip strength in the forelimbs and hindlimbs (by 21 and 28% relative to control), abnormal chest clasping response in 42% of animals when suspended by the tail, absence of pupil response in 42% of animals; findings were not or only slowly reversible during the 14-week observation period, gait abnormalities in the low dose group were more slowly reversible than in the intermediate dose group	retarded body weight gain in the top dose group (body weight was 83% of the control at the end of dosing), not reversible within 14 weeks after the end of dosing

Table 12. Rat neurotoxicity of dichloroacetic acid after acute, subchronic or chronic oral administration by gavage or in drinking water (Moser et al., 1999)

Strain, sex, age at study initiation and number of animals	Dose[1] (mg/kg body weight/day)	Route of administration, duration of treatment	Scheduled assessment times after the beginning of administration	Findings for FOB (Functional Observational Battery) and motor activity assessments	Other findings
Study 5b, F344, male, 28 to 29 days, 9 rats/group	246 at the beginning, 176 later on*	gavage, 12 weeks, treatment 5 days a week	3, 6, 9, 13, 16, 19, 23, 27 weeks	progressive gait abnormalities (maximum score of approx. 2.5 on a 4-point scale), tremor only in 2/9 animals, no changes in grip strength, slightly decreased motor activity; all findings reversible (e.g. gait abnormalities by week 19)*	retarded body weight gain reversible after 10 weeks (body weight at the end of the study was 86% of the control)
* Mean dose in mg/kg body weight/day, based on the 5 administrations/week regimen. The dose was adjusted at 2-week intervals, based on the intake in drinking water observed for the high dose group in study 5a. Overall, findings were significantly less pronounced and more readily reversible than in the animals in study 5a that were treated with a corresponding dose in their drinking water.					
Study 6a, Long-Evans, male, 28 to 29 days, 9 or 10 rats/group	17, 88, 192 (0.2, 1 and 2 g/l)	drinking water, 13 weeks	3, 6, 9, 13 weeks	≥ 17 mg/kg body weight: dose-dependent, progressive gait abnormalities (maximum scores of approx. 1.8, approx. 2.2 and approx. 2.5 on a 4-point scale); ≥ 88 mg/kg body weight: dose-independent reduction in hindlimb grip strength to approx. 80 and approx. 81% of the control; 192 mg/kg body weight: tremor (10 to 20% of animals), hypotonia (10 to 20% of animals), absence of pupil response in 20% of animals; no impairment of motor activity, righting reflex or forelimb grip strength; no change in landing foot splay when dropped from a height of 30 cm, no abnormal chest clasping response when suspended by the tail	≥ 88 mg/kg body weight: retarded body weight gain (body weight at the end of the study was 84 to 73% of the control)
Study 6b, F344, male, 28 to 29 days, 9 or 10 rats/group	16, 89, 173 (0.2, 1 and 2 g/l)	drinking water, 13 weeks	3, 6, 9, 13 weeks	≥ 16 mg/kg body weight: dose-dependent, progressive gait abnormalities (maximum scores of approx. 1.8, approx. 2.1 and approx. 3.3 on a 4-point scale), 173 mg/kg body weight: decrease in hindlimb grip strength to 60% of the control, tremor (20 to 50% of animals), hypotonia (20 to 70% of animals), absence of pupil response in 70% of animals; impaired motor activity (in 57% of the control), impaired righting reflex, decreased forelimb grip strength (approx. 80% of the control); 26% increase in landing foot splay when dropped from a height of 30 cm, abnormal chest clasping response in 50% of animals when suspended by the tail*	173 mg/kg body weight: retarded body weight gain (body weight at the end of the study was 81% of the control)
* Compared with the identically treated Long-Evans rats in study 6a, the F344 rats' reaction was significantly more sensitive.					

Table 12. Rat neurotoxicity of dichloroacetic acid after acute, subchronic or chronic oral administration by gavage or in drinking water (Moser et al., 1999)

Strain, sex, age at study initiation and number of animals	Dose[1] (mg/kg body weight/day)	Route of administration, duration of treatment	Scheduled assessment times after the beginning of administration	Findings for FOB (Functional Observational Battery) and motor activity assessments	Other findings
Study 7a, Long-Evans, male, 68 to 69 days, 9 or 10 rats/group	23, 122, 220 (0.25, 1.25 and 2.5 g/l)	drinking water, 8 weeks	2, 5, 8, 10 weeks	23 mg/kg body weight: no findings; ≥ 122 mg/kg body weight: progressive gait abnormalities, decreased hindlimb grip strength (85 and 65% of the control); 220 mg/kg body weight: decreased forelimb grip strength (80% of the control) and lowered motor activity (65% of the control) only at the end of the study; no increase in landing foot splay when dropped from a height of 30 cm, impaired righting reflex in 33% of animals, no abnormal body position when suspended by the tail and no impairment of the pupil response; impairment of gait and grip strength not reversible 2 weeks after the end of treatment	≥ 122 mg/kg body weight: retarded body weight gain (maximum decrease in body weight was to 81 to 84 or 71% of the control)
Study 7b, F344, male, 28 to 29 days, 9 or 10 rats/group	18, 91, 167 (0.25, 1.25 and 2.5 g/l)	drinking water, 13 weeks	2, 5, 8, 10 weeks	18 mg/kg body weight: some gait abnormalities; ≥ 91 mg/kg body weight: progressive gait abnormalities (more pronounced than in the Long-Evans rats investigated in the parallel study 7a), decreased hindlimb grip strength (85 and 65% of the control); 167 mg/kg body weight: all assessments yielded decreased forelimb grip strength (85% of the control), lowered motor activity (60% of the control); a 40% increase in landing foot splay when dropped from a height of 30 cm, impaired righting reflex in 40% of animals, abnormal chest clasping response in 50% of animals when suspended by the tail, absence of pupil response; impairment of gait and grip strength not reversible 2 weeks after the end of treatment*	167 mg/kg body weight: retarded body weight gain (maximum decrease in body weight was to 81 to 84% of the control)

* Compared with the identically treated Long-Evans rats in study 7a, the F344 rats exhibited a significantly greater reduction in grip strength.

[1] Dose levels given in mg/kg body weight/day for administration in drinking water, with body weight and liquid consumption taken into account.

End of Table 12

Table 13. Pharmacokinetic parameters of sodium dichloroacetate in humans

Study subjects	Dose (mg/kg body weight), route/mode of administration	C_{max} (µg/ml)	AUC (µg/ml/hour)	$t_{½}$ (hours)	V_d (ml/kg)	CL (ml/kg/minute)	Reference
Two healthy male subjects (42 and 38 years, approx. 70 kg)	10 (^{14}C-NaDCA), single infusion over 20 minutes	19.9 and 24.7	15.46 and 14.35	0.33 and 0.36	308 and 366	10.86 and 11.76 (plasma)	Lukas et al., 1980
Two healthy male subjects (52 and 26 years, 80 and 83 kg)	20 (^{14}C-NaDCA), single infusion over 20 minutes	57.3 and 74.9	59.85 and 93.61	0.41 and 0.61	186 and 195	3.53 and 5.58 (plasma)	Lukas et al., 1980
Sixteen healthy subjects (15 males and 1 female, 25 to 45 years)	1 to 50 NaDCA, single infusion over 30 minutes	dose-dependently linear from ca. 5 to ca. 100 for doses up to 30 mg/kg body weight; disproportionate increase to ca. 300 at doses from 35 mg/kg body weight	no information	0.27 to 0.84 (mean value ca. 0.5), no dose-related trend	no information	0.57 to 0.08 l/kg/hour with a trend towards weight loss at dose levels > 20 mg/kg body weight	Wells et al., 1980
Thirteen patients with severe malaria (27 ± 8 years, 54 ± 5.8 kg)	46 NaDCA, single infusion over 30 minutes	78 ± 23	261 ± 155	2.3 ± 1.8	750 ± 350	320 ± 160	Krishna et al., 1994
Eleven patients with severe malaria (32 ± 10 years, 57 ± 10 kg)	46 NaDCA, two 30-minute infusions at an interval of 12 hours	103 ± 26 (106 ± 28 after the first dose)	no information	4.4 ± 2 (3.4 ± 2.2 after the first dose)	440 ± 200	130 ± 27	Krishna et al., 1996
Nine children with severe malaria (39 ± 10 months, 11.7 ± 3.4 kg)	50 NaDCA, single infusion over 10 minutes	170 ± 52	378 ± 65	1.8 ± 0.4	323 ± 97	161 ± 50	Krishna et al., 1995

NaDCA sodium dichloroacetate
C_{max} maximum concentration in plasma/blood
AUC area under the plasma concentration-time curve
$t_{½}$ half-life in plasma/blood
V_d volume of distribution
CL clearance

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