Arch Toxicol (1989) 63: 445–449

Archives of
Toxicology

Liver toxicity due to 1,2-dichloropropane in the rat*

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Abstract. The effect of 1,2-dichloropropane on rat liver was studied after short (5 days) and long term (4 weeks) i.p. administration. Animals were injected daily with 10–500 mg/kg body wt 1,2-dichloropropane and biochemical and histological changes of liver were investigated. Treatment was monitored by measuring urinary mercapturic acid excretion. A significant increase of mercapturate excretion was observed at all dose levels, with no further increase during the treatment; at lower doses a return to baseline values occurred within 48 h after the end of treatment. Mercapturate excretion at the end of weeks 2, 3 and 4 of treatment was significantly lower than that observed at the end of week 1. The liver reduced glutathione content was different after single or repeated injections. A dose-dependent decrease of liver reduced glutathione was observed after a single injection and a dose-dependent increase after 4 weeks. The liver biochemical pattern after 4 weeks of treatment (characterized by a decrease of cytochrome P-450 and by an increase of reduced glutathione and glutathione S-transferase activity) suggests a hyperplastic evolution of the liver cells, probably a repair mechanism induced by early depletion of reduced glutathione. Light microscopy confimrs that the prevalent alterations are regenerative in type (atypical mithosis and hyperplastic nodules). Areas of focal necrosis are isolated, and trend to disappear after long term treatment.

Key words: 1,2-Dichloropropane – Liver metabolism – liver toxicity

Introduction

1,2-Dichloropropane (1,2 D) is an aliphatic halogenated saturated hydrocarbon largely used in industry as solvent and degreaser. It is known for the acute toxicity occurring in accidental or voluntary ingestion and/or inhalation.

Several years ago the toxicity of 1,2 D was recognized in different experimental animals (Heppel et al. 1946 a, b; Highman and Heppel 1946; Heppel et al. 1948). Acute toxicity in man was also reported (Chiappino and Secchi 1968), liver (Chiappino and Secchi 1968; Secchi et al. 1968) and kidney (Locatelli and Pozzi 1983) being the target organs. Recently a haemolitic syndrome (Locatelli and Pozzi 1983; Pozzi et al. 1985) and a disseminated intravascular coagulation syndrome were also reported (Perbellini et al. 1985; Pozzi et al. 1985).

Oral and dermal LD^{50} in rats and rabbits are approximately 2.3 and 10.4 g/kg body wt, respectively, (Smyth et al. 1969). The inhalation LC_{50} in mice is 720 ppm for 10 h or 1500 ppm for 4 h (Carpenter et al. 1949; Torkelson and Rowe 1981).

Subchronic inhalation studies with low doses (50 ppm) of a mixture of 1,2 D and *cis* and *trans* isomers of the 1,3-dichloropropene (Parker et al. 1982) did not show signs of toxicity in rats and mice. Recently, slight hepatic changes were observed in rats after subchronic oral treatment with different doses (100–1000 mg/kg) of 1,2 D (Ramanathan et al. 1986).

1,2 D is excreted in rat urine as two mercapturic acids (Jones and Gibson 1980), the peak of excretion occurring 9 h after i.p. administration (Trevisan et al. 1988).

The aim of this work is to investigate the effects of 1,2 D on rat liver after 5 days and 4 weeks of i.p. treatment with doses between 1/100 and 1/2 LD_{50}.

Methods

Groups of five Albino, male, Wistar rats (Morini, S. Polo d' Enza, Italy), starting weight 200 ± 10 g, were treated i.p. (0.5 ml) with different doses (10, 25, 50, 100, 250, and 500 mg/kg body wt) of 1,2 D (Merck, Darmstadt, FRG, purity 97%) dissolved in corn oil for 5 days (once a day) or for 4 weeks (once a day, 5 days/week). The two control groups were treated for the same time with corn oil (0.5 ml) only. The rats were located in metabolic cages, allowed to eat and drink ad lib. The urine was collected over a period of 24 h, daily during the first 5 days, and then twice a week (at the end of the 5-day treatment and 48 h later). The urine collectors were plunged in an ice bath and a small amount of L-ascorbic acid was added to the urine. The concentration of urinary mercapturic acids was measured according to Vainio et al. (1978), slighty modified (Rizzi et al. 1987). The values are expressed as mmole-SH groups for mmole creatinine (determined with a commercial kit by Boehringer, Mannheim, FRG).

* Some results were presented at the 1st Italian Symposium on: Mechanism of activation and toxicity of xenobiotics, Rome, 26–27 March 1987, and at the 50th National Congress of the Italian Society of Occupational Health and Industrial Hygiene, Rome, 21–24 October 1987

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Twenty-four hours after the last treatment, the rats were fasted overnight, and killed by decapitation; liver was then removed and perfused with cold saline.

Reduced glutathione content (GSH) was measured as non-protein sulfhydryl groups, according to Sedlak and Lindsay (1968). Glutathione S-transferase (GST) activity was measured according to Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene as substrate. Cytochrome P-450 (Cyt P-450) activity was measured according to Omura and Sato (1964). Protein concentration of liver homogenates was determined according to Miller (1959).

In a separate experiment, GSH content was determined after single i.p. injections of 1,2 D dissolved in corn oil (0, 50, 100, and 250 mg/kg body wt). Four rats of each treated group and of a control group were sacrificed at different times from 8 a.m. to 8 p.m.

For light microscopy, liver was fixed in buffered 10% formaldehyde. The fomaldehyde-fixed fragment was embedded in paraffin, and sections were stained with hematoxylin and eosin, Van Gieson stain for the collagen fibers, Gomori silver impregnation for the reticular fibers, PAS reaction and Perls method for iron.

Variance analysis was used for statistical evaluation.

Results

General findings

One rat died during treatment after the 15th injection of the 500 mg/kg dose. According to previous determinations, i.p. LD_{50} in the rat for 1,2 D is 1100 mg/kg body wt.

A dose-dependent decrease of body weight gain was observed only after 4 weeks' treatment.

Mercapturic acids excretion

Figure 1 shows the time course of excretion of mercapturic acids after 1,2 D. The daily excretion of mercapturic acids is dose-dependent and shows a statistically significant increase, as compared to the control, at any dose level. Daily treatment with 1,2 D does not further increase the excretion of mercapturic acids, which returns to baseline values within 48 h after the end of low dose treatments. The return to baseline values of mercapturic acids excretion is not complete after 48 h at the highest doses (250 and 500 mg/kg). Furthermore, the excretion at the end of weeks 2, 3 and 4 of treatment is significantly lower than that observed at the end of week 1 ($p < 0.02$).

Biochemical findings

GSH liver content decreases after a single injection (Fig. 2) in a dose-dependent manner. The peak of depletion is delayed 2, 4, and 6 h; the higher the dose the more delayed the peak. The values return to baseline about 12 h after the injection.

After 5 days of treatment, GSH content and Cyt P-450 activity do not show significant changes, and GST activity increases significantly only after the 500 mg/kg dose ($p < 0.025$). After 4 weeks of treatment (Table 1) a significant dose-dependent increase of the GSH content and GST activity was observed from the 50 mg/kg dose up; only the lowest dose (10 mg/kg body wt) caused a significant, unexplained, decrease of GSH content and GST activity. Cyt P-450 activity shows a significant decrease after the 250 and 500 mg/kg doses.

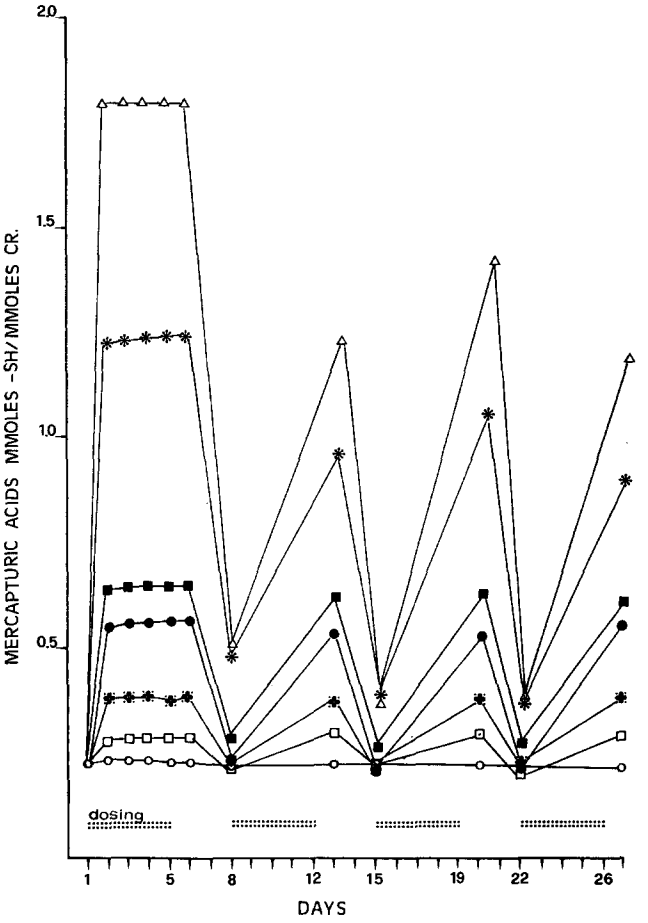

Fig. 1. Time course of the urinary mercapturic acids excretion after dosing with 1,2 D. Control: ○; 10 mg/kg: □; 25 mg/kg: ■; 50 mg/kg: ●; 100 mg/kg: ■; 250 mg/kg: ★; 500 mg/kg: △

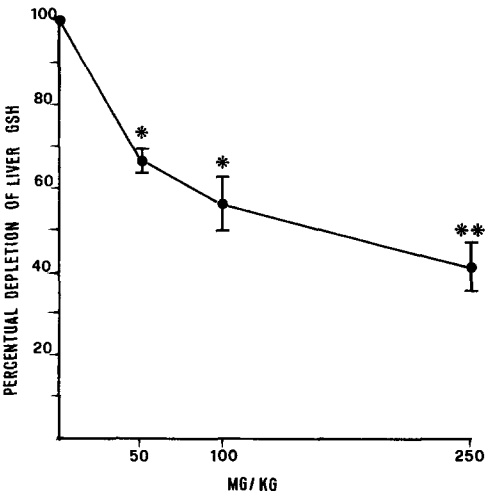

Fig. 2. Depletion of the liver GSH content after dosing with single doses of 1,2 D. $*p < 0.005$, $**p < 0.001$ compared to controls at the same time. The GSH depletion is 42, 57, and 67% of the control, respectively

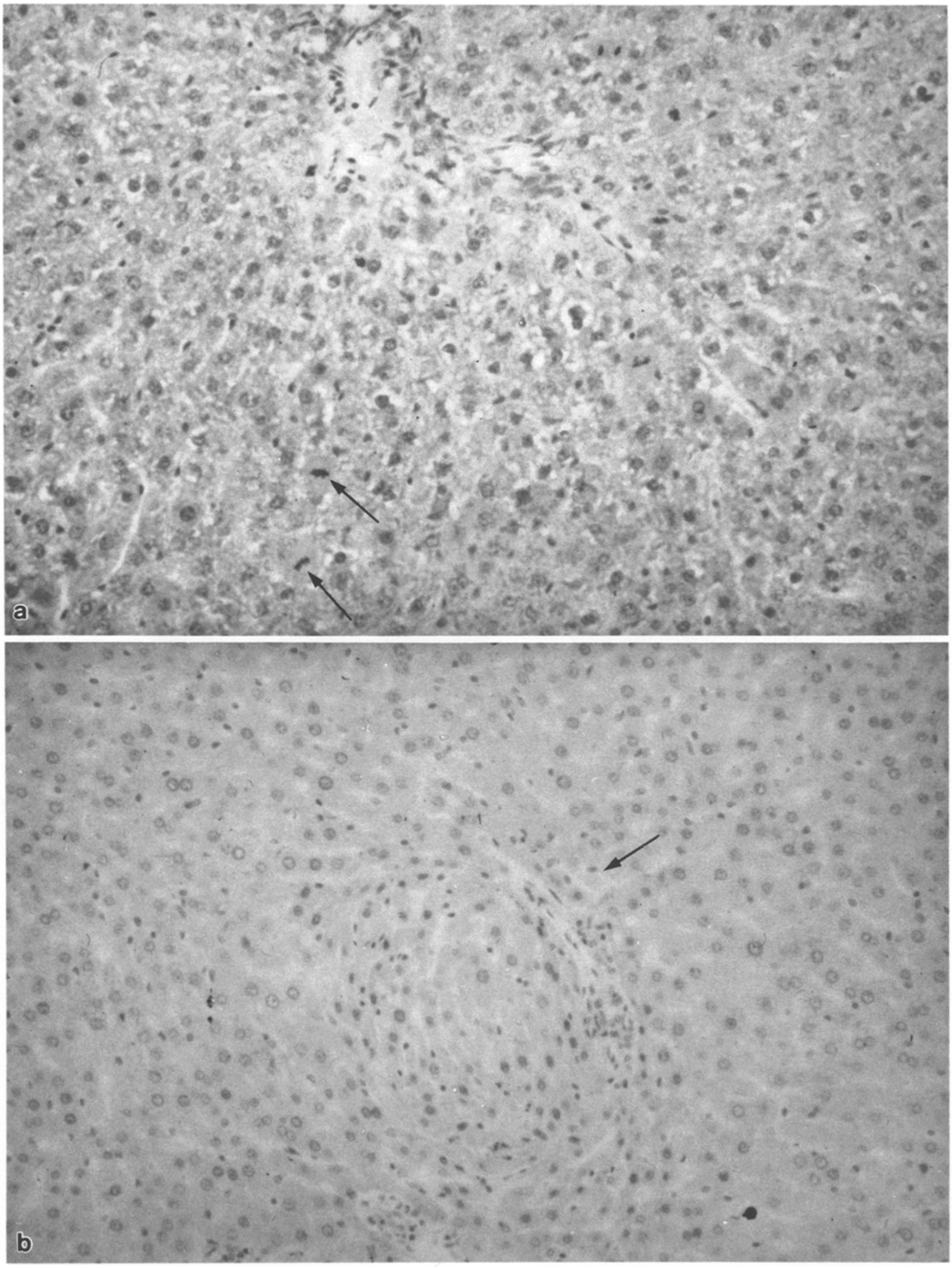

Fig. 3.a Atypical mithosis after 50 mg/kg (hematoxylin-eosin, 25×); **b** Hyperplastic nodule after 100 mg/kg (hematoxylin-eosin, 25×)

Table 1. Liver GSH content, GST and Cyt P-450 activities after 4 weeks of treatment (mean ± standard deviation)

Dose (mg/kg)	GSH nmol/mg tissue	GST mcmol/mg prot.	Cyt P-450 nmol/mg prot.
0	5.30 ± 0.46	243.3 ± 11.2	0.53 ± 0.05
10	4.31 ± 0.45*	199.0 ± 25.7*	0.54 ± 0.02
25	5.49 ± 0.38	220.7 ± 19.8	0.54 ± 0.01
50	6.68 ± 0.34*	348.7 ± 17.9*	0.55 ± 0.10
100	6.65 ± 0.53*	357.9 ± 35.6*	0.48 ± 0.13
250	7.66 ± 0.77*	378.3 ± 60.2*	0.39 ± 0.03*
500	8.86 ± 0.39*	428.5 ± 19.7*	0.34 ± 0.04*

* $p < 0.025$ or more as compared with control

Histological findings

The histological alterations are mainly hyperplastic; the severity of hyperplasia increases with the dose and length of treatment. Atypical mithosis (Fig. 3 *a*) and hyperplastic nodules (Fig. 3 *b*) are evident in some cases. Focal necrosis is isolated, and decreases with the duration of treatment; slight perivenular steatosis is also evident. No fibrosis and iron accumulation were found. Table 2 summarizes the histological findings.

Discussion

The return of mercapturic acid excretion to baseline values after the end of the treatment and the absence of a progressive increase with daily dosing suggest that 1,2 D does not accumulate and that its metabolism is fast.

However, the treatment induces biochemical and histological changes in rat liver.

Changes of liver GSH content differ according to the treatment. The depletion of GSH content after a single injection of 1,2 D is dose related and indicates a possible role of GSH in the acute toxicity of the liver as has been reported for other chemicals (Chasseaud 1973; Gillette 1977). At these doses, the GSH content is quickly restored, and no further decrease is observed after 5 days of administration. On the contrary, after 4 weeks GSH content increases with the dose (from 50 mg/kg) and parallels the increase of GST activity. This treatment also causes a significant decrease of Cyt P-450 activity after 250 and 500 mg/kg doses. The decrease of phase I components (Cyt P-450) and the increase of phase II components (GSH content and GST activity) is consistent with liver hyperplasia (Eriksson et al. 1983; Columbano et al. 1987).

Light microscopy shows the hyperplastic evolution of liver cell damage and supports biochemical data. The mild focal necrosis observed after 5 days is self-limiting 4 weeks thereafter. These observations agree with previous results (Highman and Heppel 1946), suggesting a possible increased resistance to 1,2 D during daily treatments.

The biochemical and the histological picture agree with the hyperplastic changes induced by repeated doses of the solvent. 1,2-Dichlorethane, another halogenated saturated compound, causes similar biochemical effects. After subchronic (30 days) inhalation (up to 450 ppm), it produces in fact a dose-dependent increase of liver GSH content and a dose-independent decrease of liver Cyt P-450 activity (Igwe et al. 1986b). The histological findings were midzonal necrosis, swelling of the cytoplasm, and proliferation of bile ducts (Igwe et al. 1986a). After a single oral dose (4 mmole/kg body wt) 1,2-dichloroethane induces a decrease (about 50% of the control) of the GSH content (Johnson 1965). In conclusion, the liver biochemical effects induced by 1,2-dichloroethane are similar to these of 1,2 D but not the histological findings.

The long term effects of 1,2 D are also different from the centrilobular hepatic necrosis caused by acute poisoning as reported in experimental (Heppel et al. 1948) and clinical studies (Secchi et al. 1968; Baerg and Kimberg 1970). The necrosis might be due to the depletion of GSH content or to active metabolites which overwhelm the GSH-dependent detoxification system. Repair mechanism might explain the hyperplastic alterations associated with biochemical signs of cellular hyperactivity, and with self-limitation of necrosis. In contrast, with previous reports (Heppel et al. 1946a; Parker et al. 1982), biochemical and histological alterations are also produced at low doses (50 mg/kg, less than 1/20 of the LD_{50} i.p.).

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Table 2. Histological findings after treatment with 1,2 D. Necrosis was always a slight focal lesion; steatosis had a perivenular localization; hyperplasia is represented by regenerative alterations as atypical mithosis and hyperplastic nodules

Dose (mg/kg)	Necrosis		Steatosis		Hyperplasia	
	5 days	4 weeks	5 days	4 weeks	5 days	4 weeks
0	0/5	0/5	0/5	0/5	0/5	0/5
10	1/5	1/5	0/5	0/5	5/5	5/5
25	1/5	1/5	0/5	0/5	5/5	5/5
50	2/5	1/5	0/5	1/5	5/5	5/5
100	2/5	0/5	3/5	2/5	5/5	5/5
250	3/5	2/5	5/5	5/5	5/5	5/5
500	1/5	2/4	5/5	3/4	5/5	4/4

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Received March 9, 1989/Received after revision June 11, 1989/Accepted June 12, 1989