

Oral Toxicity of 1,1-Dichloroethylene in the Rat: Effects of Sex, Age, and Fasting

by Melvin E. Andersen* and Lawrence J. Jenkins Jr.*

Mortality curves for groups of fasted male rats treated with single, oral doses of 1,1-dichloroethylene (1,1-DCE, vinylidene chloride) were not monotonically increasing sigmoids, but were complex with maxima or extended plateaus in the region of dose between 100 and 700 mg of 1,1-DCE/kg. The exact shape was a function of the size (age) of the rat used. When groups of rats of various sizes were dosed with 50 mg/kg, mortality and hepatotoxicity were greatest for those groups whose average weight was between 100 and 150 g. Smaller and larger male rats were less susceptible to 1,1-DCE intoxication. The toxicity of 1,1-DCE was less severe in female rats and there was no significant effect of rat size on 1,1-DCE toxicity in females. In rats of both sexes the dose dependence of the hepatotoxic response was complex, possessing a threshold level, a region of precipitous increase, and a plateau, where larger doses were ineffective in increasing hepatotoxicity. The threshold in male rats of 100-150 g occurred near 50 mg/kg, and for females it was closer to 100 mg/kg. Considered in their entirety these data suggest that 1,1-DCE is metabolized to a toxic intermediate via some saturable pathway. Based on the effects of pretreatment with microsomal enzyme inhibitors and activators on 1,1-DCE toxicity in rats of various sizes, it appears that there are at least two microsomal reactions involved in 1,1-DCE metabolism.

Introduction

The hepatotoxicity (1, 2) and nephrotoxicity (3) of single, oral doses of 1,1-dichloroethylene (1,1-DCE, vinylidene chloride) in the rat have been previously described. In fasted male rats weighing between 300 and 500 g the acute oral LD₅₀ was 1550 mg of 1,1-DCE/kg (1). However, 1,1-DCE has been found to be much more toxic to immature male rats and dose mortality curves for these smaller animals were complex with extended plateaus or even maxima. A preliminary report of these studies has appeared (4). This present study describes the acute oral toxicity of 1,1-DCE in male and female rats of various sizes and is a part of an extensive reinvestigation of the oral toxicity of this chlorinated ethylene monomer and of those factors which alter its toxicity.

Materials and Methods

Rats

The Holtzman HOT: (SD)BR rats used in these

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experiments were housed singly in wire mesh cages in an air-conditioned room and were provided commercial rat chow and tap water *ad libitum*. Rats were fasted by withdrawing food at 4:00 PM the day previous to treatment, dosed between 8:00 AM and 10:00 AM the next morning and then allowed free access to food 2-4 hr after dosing.

1,1-Dichloroethylene

Gas chromatographic analysis of the stock 1,1-DCE obtained from K & K Laboratories, Plainview, N. Y. showed it to contain 98.5% 1,1-DCE, 0.80% *trans*-1,2-DCE and 0.55% *cis*-1,2-DCE. It was dissolved in corn oil and administered in doses adjusted to a total volume of 2 ml/kg. Control rats received 2 ml of corn oil/kg. In experiments to determine mortality caused by various doses of 1,1-DCE rats were observed for 14 days after dosing, even though over 80% of the mortality was observed in the first 24 hr. Plasma enzyme activities were determined at various times after oral doses of 1,1-DCE. Rats were killed with an intraperitoneal injection of a lethal dose of sodium pentobarbital, the abdomen was rapidly

opened, and the rats were exsanguinated by withdrawing blood from the portal vein. Plasma was prepared from this blood as previously described (5).

Biochemical Determinations

The assay procedures used to determine plasma enzymatic activities of lactic dehydrogenase (LD: L-lactate: NAD oxido-reductase, EC 1.1.1.27), alanine transaminase (ALT; L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2), aspartate transaminase (AST; L-aspartate:2-oxoglutarate aminotransferase, EC 2.6.1.1) and D-sorbitol dehydrogenase (SDH; L-iditol:NAD oxidoreductase, EC 1.1.1.14) have been previously described (3).

Results

Fed-Fasted Differences

Hepatotoxicity, measured as increases in plasma transaminase activities, caused by inhalation of 1,1-DCE, has been shown to be greater in fasted rats than in fed rats (6, 7). When large, mature, male rats were intubated with 400 mg of 1,1-DCE/kg, the hepatotoxic response measured as increased plasma aspartate transaminase (Fig. 1) was 4 to 5 times greater in the fasted rats. Studies on the time

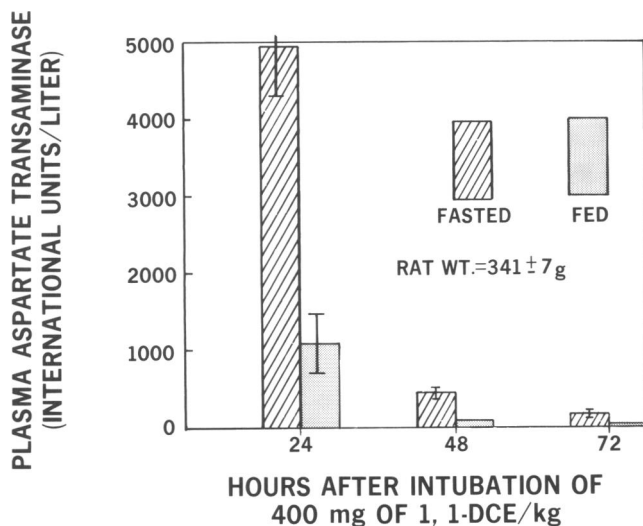


FIGURE 1. Effect of fasting on increases in plasma aspartate transaminase activity following intubation of a single, oral dose of 400 mg of 1,1-dichloroethylene/kg. Plasma aspartate transaminase activity was measured at various times after dosing male rats with 400 mg of 1,1-DCE/kg. The hatched columns are data from rats fasted for 18 hr before dosing; the filled columns, data from rats allowed food *ad libitum* before challenge. Data are means \pm SE for 2-4 rats.

course of the increases in plasma enzyme activities at various times after a single, oral dose of 1,1-DCE in these fasted, large rats showed that the increases at 8 hr or 24 hr were greater than at any other times and were equivalent. As a matter of convenience, plasma enzyme activities in all subsequent studies were measured at 24 hr after 1,1-DCE intubation and all studies were done with fasted rats.

Dose Mortality Curves in Rats of Various Sizes

An attempt was made to determine the oral LD₅₀ of 1,1-DCE in rats of various sizes. The mortality curve for large male rats (395 \pm 11g) increased monotonically from 0 to 100% mortality with doses from 800 to 2000 mg of 1,1-DCE/kg, giving estimates of the acute, oral LD₅₀ which agreed with the earlier report of Jenkins et al. (1). However, even with these larger rats, mortality was consistently observed at doses as low as 50 mg of 1,1-DCE/kg (Fig. 2). With groups of male rats weighing 224 \pm 1 g mortality following doses between 50 and 800 mg of 1,1-DCE/kg varied between 10 and 33%. In weanling rats weighing 73 \pm 1 g, a curve with a maximum of 100% (10/10) mortality at a dose of 300 mg/kg was obtained. Percent mortality then decreased as dose was increased up to 800 mg/kg (Fig. 2). Dose-mortality curves with maxima or extended plateaus between 100 and 700 mg of 1,1-DCE were seen repeatedly in male rats of all sizes. It proved impossible therefore to calculate unambiguously the LD₅₀ of 1,1-DCE in these younger rats.

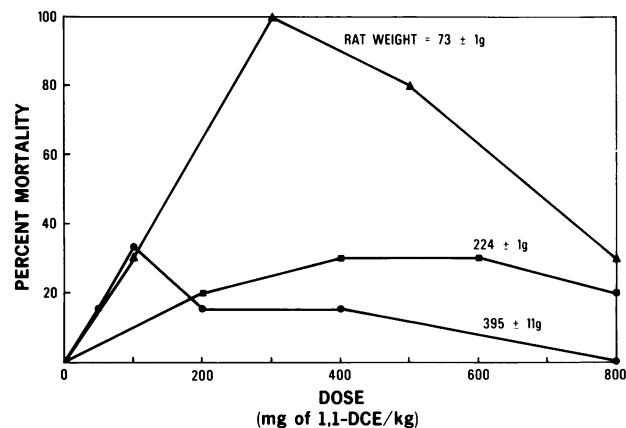


FIGURE 2. Dose-mortality curves for administration of single, oral doses of 1,1-DCE dissolved in corn oil to fasted male rats of various sizes: (●) mortality in groups of six rats [the 30 rats represented by these five points weighed 395 \pm 11 g (mean \pm standard error)]; (▲) mortality in groups of 10 rats for 40 weanling rats weighing 73 \pm 1 g; (■) mortality in groups of 10 rats [the 4D rats weighed 224 \pm 1 g].

1,1-DCE Hepatotoxicity in Male Rats of Various Sizes

Increased mortality in groups of younger rats was accompanied by increased hepatotoxicity of 1,1-DCE in these animals. Thus, when two groups of rats, one whose average weight was approximately 300 g, the second whose average weight was approximately 200 g were challenged with a dose of 25 mg of 1,1-DCE/kg, the smaller rats had increased plasma transaminase levels at 24 hr after dosing while the larger rats were unaffected (Fig. 3). To determine the hepatotoxicity of a dose of 50 mg of 1,1-DCE/kg in male rats of different sizes, 10 groups of rats were used. The rats in each group were the same age and at the time of their delivery from the breeder the individual weights of the rats in any one group were within a 10 g range. Approximately half the rats in each group were dosed with 50 mg of 1,1-DCE/kg and the remainder with corn oil. All rats were sacrificed at 24 hr after dosing. Mortality within the first 24 hr and plasma enzyme activities at 24 hr were greatest in those rats weighing between 130 and 160 g (Table 1). Mortality in the group weighing 138 ± 2 g was 5/6. The LD_{50} of 1,1-DCE in rats of this weight must be less than 50 mg/kg, smaller by a factor of 30 than the oral LD_{50}

in large rats. (A more detailed analysis of a dose mortality curve for rats of this weight might show a mortality maxima somewhere above 50 mg/kg.) The magnitude of the increases in plasma enzymatic activities paralleled mortality in these various groups.

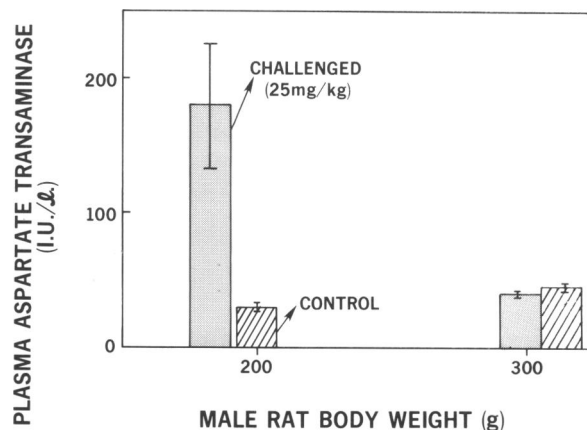


FIGURE 3. Hepatotoxicity of a single, oral dose of 25 mg of 1,1-DCE/kg in male rats of two different sizes. The solid columns represent data from fasted male rats challenged with 25 mg of 1,1-DCE/kg; the hatched columns represent data from control rats given 2 ml of corn oil/kg. Data are mean \pm SE of six rats. Columns are centered on the average weight of six rats in that particular treatment group.

Table 1. Plasma enzyme activities in fasted, male rats of various sizes 24 hr after a single oral dose of 50 mg of 1,1-dichloroethylene/kg dissolved in corn oil.

Weight, g	Treatment	Mortality n^a	AsT, $\mu\text{mole}/\text{min}\cdot\text{l}^b$	AIT, $\mu\text{mole}/\text{min}\cdot\text{l}^b$	SDH, $\mu\text{mole}/\text{min}\cdot\text{l}^b$	LD, $\mu\text{mole}/\text{min}\cdot\text{l}^b$	
83 \pm 6	1,1-DCE	0/6	5	2147 \pm 1593	289 \pm 152	380 \pm 239	746 \pm 544
92 \pm 8	Control	0/5	5	153 \pm 45	64 \pm 34	7 \pm 1.8	194 \pm 75
138 \pm 2	1,1-DCE	5/6	1	8300	680	160	14200
137 \pm 3	Control	0/5	5	141 \pm 62	26 \pm 3	10 \pm 4	194 \pm 75
155 \pm 3	1,1-DCE	3/6	3	3767 \pm 1612	680 \pm 289	900 \pm 372	1122 \pm 731
153 \pm 3	Control	0/5	5	58 \pm 8	29 \pm 5	4.6 \pm 2.0	87 \pm 9.3
180 \pm 2	1,1-DCE	0/6	6	919 \pm 446	287 \pm 145	7.5 \pm 4.8	461 \pm 300
179 \pm 4	Control	0/5	4	57 \pm 9	22 \pm 3	7.75 \pm 3.1	85 \pm 8
197 \pm 6	1,1-DCE	0/6	6	738 \pm 211	521 \pm 127	287 \pm 102	149 \pm 35
231 \pm 10	Control	1/5	4	45 \pm 5	15 \pm 2	3.5 \pm 2	56.5 \pm 7
221 \pm 3	1,1-DCE	0/5	5	1045 \pm 600	255 \pm 114	291 \pm 183	148 \pm 53
217 \pm 10	Control	0/5	5	46 \pm 5	23 \pm 5	9 \pm 2	134 \pm 56
237 \pm 6	1,1-DCE	0/6	6	227 \pm 81	107 \pm 36	66 \pm 25	91 \pm 10
230 \pm 4	Control	0/5	5	44 \pm 3	19 \pm 3	10 \pm 3	97 \pm 22
255 \pm 5	1,1-DCE	1/4	3	47 \pm 17	16 \pm 0	95 \pm 2	117 \pm 49
252 \pm 8	Control	0/4	4	39 \pm 5	24 \pm 3	7 \pm 2	86 \pm 17
275 \pm 8	1,1-DCE	0/6	6	69 \pm 13	63 \pm 31	20 \pm 6	96 \pm 24
271 \pm 10	Control	0/4	4	48 \pm 6	17 \pm 2	6 \pm 1	75 \pm 15
282 \pm 5	1,1-DCE	1/6	5	71 \pm 11	29 \pm 4	20 \pm 6	96 \pm 24
218 \pm 8	Control	0/4	4	49 \pm 4	19 \pm 4	7 \pm 2	93 \pm 11

^a n is the number of rats used in each group in the determination of enzyme activities. (It is in fact the number of survivors at 24 hr.)

^bAsT, aspartate transaminase; AIT, alanine transaminase; SDH, D-sorbitol dehydrogenase; and LD, lactic dehydrogenase. All activities are given as $\mu\text{moles product}/\text{min}/\text{liter}$ of plasma (International Units/l.) at 30°C and data are means \pm SE.

Dose-Effect Relationships in Immature Male Rats

Male rats weighing between 90 and 120 g were dosed in groups of six with various doses of 1,1-DCE between 0 and 200 mg/kg. Survivors were sacrificed at 24 hr after 1,1-DCE administration. Plasma transaminase activities from these rats (Table 2) showed a marked increase as dose was raised from 25 to 50 mg/kg, but further increases in dose had little or no effect on these activities. Plasma lactic dehydrogenase activity, however, doubled between 50 and 125 mg/kg, but was constant thereafter.

Retention of 1,1-DCE Following a Single Oral Dose

Studies were conducted to determine the amount of 1,1-DCE off-gassed by rats after an oral dose of this chemical. Individual rats dosed with various amounts of 1,1-DCE were placed into a 31-liter flow-thru chamber (8), served with compressed air at a constant flow rate. Chamber exhaust was di-

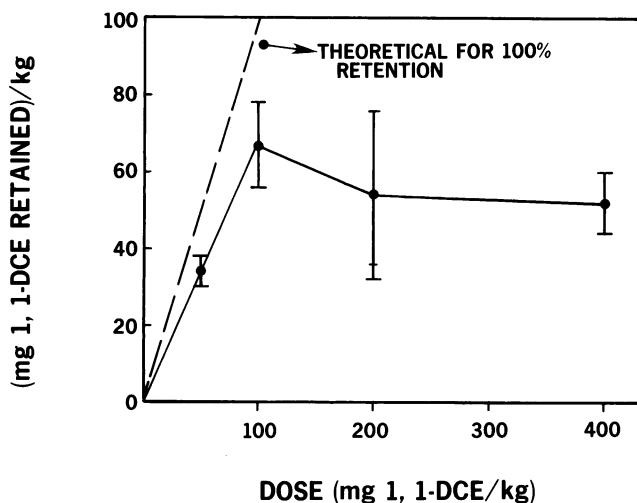


FIGURE 4. Relationship between retention of 1,1-dichloroethylene by the rat and the initial, oral dose. Data are means \pm SE of 3-6 rats. Rats were dosed with 1,1-DCE then housed in flow-thru chambers. The exhaust from the chamber was continuously analyzed for 1,1-DCE by infrared spectrophotometry. The dotted line is that line expected if all the intubated 1,1-DCE were retained. The weights of the rats used for this study varied between 100 and 150 g.

Table 2. Plasma enzyme activities in fasted male rats weighing approximately 100 g 24 hr after intubation of various doses of 1,1-dichloroethylene dissolved in corn oil.

Weight, g	n	Mortality	Dose, mg/kg	AsT, $\mu\text{mole}/\text{min}\cdot\text{l}^{\text{a}}$	AIT, $\mu\text{mole}/\text{min}\cdot\text{l}^{\text{a}}$	SDH, $\mu\text{mole}/\text{min}\cdot\text{l}^{\text{a}}$	LD, $\mu\text{mole}/\text{min}\cdot\text{l}^{\text{a}}$
104 \pm 4	6	0/6	0	59 \pm 6	24 \pm 2	11 \pm 1	110 \pm 25
106 \pm 3	6	0/6	12.5	69 \pm 7	23 \pm 3	9 \pm 4	94 \pm 28
103 \pm 5	6	0/6	25	210 \pm 45	52 \pm 9	65 \pm 21	138 \pm 48
106 \pm 2	6	0/6	50	6929 \pm 746	852 \pm 116	1296 \pm 236	8987 \pm 2291
101 \pm 1	6	4/6	75	4992 \pm 176	1040 \pm 112	1707 \pm 106	9208 \pm 1480
100 \pm 6	6	4/6	100	5848 \pm 3896	936 \pm 648	1135 \pm 308	6528 \pm 4224
101 \pm 4	6	2/6	125	8460 \pm 526	1340 \pm 158	1328 \pm 166	19760 \pm 1918
105 \pm 2	6	4/6	150	6048 \pm 832	976 \pm 592	1311 \pm 431	20720 \pm 6480
108 \pm 5	6	4/6	200	6649 \pm 200	1088 \pm 0	1285 \pm 229	15120 \pm 3440

^aAsT, aspartate transaminase; AIT, alanine transaminase; SDH, D-sorbitol dehydrogenase; and LD, lactic dehydrogenase. All activities are given as $\mu\text{moles product}/\text{min}/\text{liter}$ of plasma (International Units/l) at 30°C and data are means \pm SE.

rected through an infrared spectrophotometer whose monochromator was set at an absorption band of the 1,1-DCE (9.1 μ). Integration of the absorbance output of the infrared spectrophotometer yielded a number proportional to the total amount of 1,1-DCE off-gassed, excreted or passed through the skin. The amount of 1,1-DCE retained was the difference between the total dose and the total amount excreted by these various routes. The amount of 1,1-DCE retained by immature, fasted male rats increased linearly up to a total dose of 100 mg/kg and then levelled off (Fig. 4).

Sex Differences in 1,1-DCE Toxicity

Investigations of the toxicity of single, oral doses of 1,1-DCE were also conducted using female rats. The time course of the hepatotoxic response to 1,1-DCE was essentially the same in the female rat (Fig. 5) as in the male rat. However, females were much less susceptible to 1,1-DCE intoxication. When female rats of various sizes were challenged with 50 mg of 1,1-DCE/kg, no significant differences were observed in plasma transaminase levels between treated and control groups (Fig. 6).

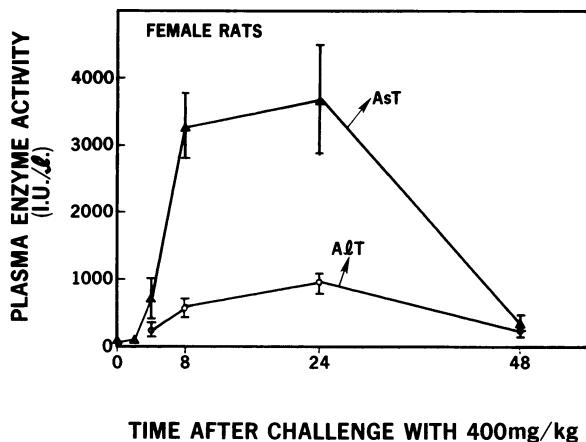


FIGURE 5. Time course of increases in plasma transaminase activities following intubation of female rats with 400 mg of 1,1-DCE/kg. Data are means \pm SE for four rats. AsT is aspartate transaminase; A1T, alanine transaminase.

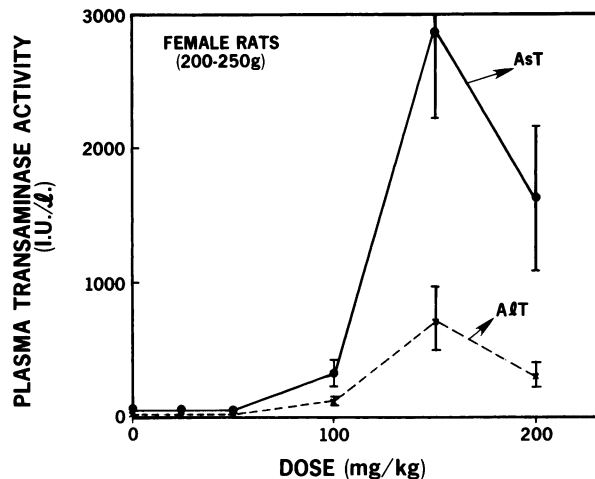


FIGURE 7. Plasma enzyme activities in fasted female rats 24 hr after intubation of various doses of 1,1-dichloroethylene. Data are means \pm SE for four rats. Weight range of the rats used was 200-250 g.

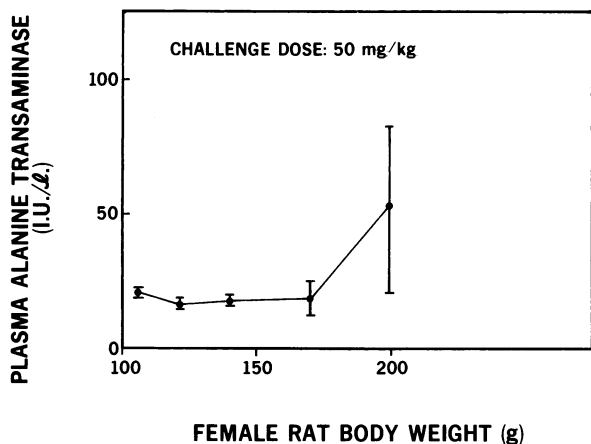


FIGURE 6. Plasma alanine transaminase activities in fasted female rats of various sizes 24 hr after a single, oral dose of 50 mg of 1,1-dichloroethylene/kg. The data points are means \pm SE of five rats. They are located in a position along the ordinate corresponding to the average weight of the rats in the group. In no case was the standard error of the mean of the rat weight of a group greater than 5 g.

Dose Dependence of 1,1-DCE Toxicity in Female Rats

Female rats weighing 200-250 g were dosed in groups of six with various doses of 1,1-DCE between 0 and 200 mg/kg. All of these female rats survived the first 24 hr and were then sacrificed. A large increase in plasma transaminase activities was observed as dose was increased from 100 to 150 mg/kg, while further increases in dose actually produced plasma enzyme activities lower than those observed with a dose of 150 mg/kg (Fig. 7).

Discussion

The dose dependence of the hepatotoxicity of single, oral doses of 1,1-DCE measured as increases in plasma transaminase levels, was complex and appeared to have a threshold. The transition from a dose of 1,1-DCE whose effect was relatively benign to one whose effect was frank, extensive hepatic injury occurred abruptly over a narrow range of dose. In small male rats the threshold occurred near a dose of 25 mg/kg. The hepatotoxicity of bromobenzene also exhibits threshold effects. This compound is metabolized to 3,4-bromobenzene oxide, a reactive intermediate, which can either react preferentially and innocuously with glutathione (GSH) or when cellular GSH is exhausted with vital cellular constituents to produce its toxic effects (9). Threshold effects are manifested at that point where GSH becomes seriously depleted, after which only small increments of ingested bromobenzene produce extensive toxicity (10). The hepatotoxicity of inhaled 1,1-DCE can also be increased by depletion of GSH either by fasting or by pretreating rats with diethylmaleate (6, 7). Similarly, the hepatotoxicity of orally administered 1,1-DCE is also enhanced by pretreating rats with diethylmaleate (11) or by fasting, as shown herein.

The mutagenicity of 1,1-DCE and of vinyl chloride monomer (VCM) were greatly enhanced when the incubation media was supplemented with microsomal protein, O₂ and NADPH regenerating system, indicating that these compounds were microsomally converted to more toxic metabolites (12, 13). Vinyl chloride is metabolized by the rat via

a saturable reaction (14) and simultaneous inhalation of VCM and 1,1-DCE reduced 1,1-DCE hepatotoxicity (15, 16). The dose dependence of the inhibition of 1,1-DCE toxicity by VCM suggested a competitive interaction. Thus VCM and 1,1-DCE appear to be metabolized *in vivo* in similar if not identical reactions. Indeed, the amount of 1,1-DCE retained after a single oral dose becomes constant at challenge doses of greater than 100 mg/kg (Fig. 4), suggesting that the metabolism of 1,1-DCE, just as that of VCM, occurs through a saturable pathway.

In female rats, the threshold dose of 1,1-DCE was approximately 100 mg/kg (Fig. 7) and females were much less susceptible to 1,1-DCE toxicity than were males. Microsomal oxidation of a variety of compounds is greater in male than in female rats (17), and these observed toxicity differences in male and female rats are also consistent with the assumption that 1,1-DCE is metabolized to a more toxic compound. Furthermore, the activity of a number of rat microsomal oxidases reach a maximum in the male rat at 30–40 days (18). Growth curves of the Holtzman rats used in this study showed that rats of this age would weigh 100–150 g, the weight of the rats most susceptible to 1,1-DCE intoxication (Table 1).

The data in this paper describing the oral toxicity of 1,1-DCE are consistent in suggesting that 1,1-DCE must be metabolized *in vivo* by some microsomal enzyme activity to a more toxic intermediate. Based on the dose dependence of the hepatotoxic response in male and female rats, the retention of 1,1-DCE after oral dosing and the unusual shape of the mortality curves, this microsomal reaction converting 1,1-DCE to a more toxic chemical(s) would appear to be saturable and probably inhibitable at higher 1,1-DCE concentrations. While similar effects have not been reported in studies of the inhalation toxicity of 1,1-DCE, they might have been obscured by the custom of conducting exposures for 4 hr. In the investigations of the off-gassing of 1,1-DCE the major portion of the off-gassing occurred between 0.5 and 1.5 hr. Inhalation studies to corroborate these present results would have to focus on 1,1-DCE toxicity after short-term (½ to 2 hr) exposures. Such work is presently under way in our laboratory.

Jenkins et al. (1) and Reynolds et al. (19) have reported that phenobarbital pretreatment, which increased microsomal oxidase activities, protected rats from the hepatotoxic effects of 1,1-DCE. While this at first glance appears to contradict the conclusions of this study, other work on the effect of microsomal inhibitors and activators has clarified this difficulty. Several pretreatment regimens, all of which inhibit Type II microsomal activities, have

been shown to block the hepatotoxicity of 1,1-DCE (4). These are: (1) pyrazole (320 mg/kg); (2) aminotriazole (200 mg/kg); (3) carbon tetrachloride (1500 mg/kg, 1 hr prior to 1,1-DCE or 375 mg/kg at the same time as 1,1-DCE). Phenobarbital pretreatment protects smaller rats but is increasingly less effective as rat weight increases. Conversely, pretreatment with SKF 525-A markedly exacerbates 1,1-DCE toxicity in older, mature rats but does not affect its toxicity in smaller rats (4).

To accommodate these observations of the effects of inducers and inhibitors of microsomal enzyme activities on 1,1-DCE toxicity with a requirement that metabolic activation occur before the toxicity of 1,1-DCE can be expressed at least 2 microsomal reactions must be hypothesized. These two reactions, designated by the numbers 1 and 2, are shown in the metabolic scheme for 1,1-DCE in Figure 8. The first microsomal enzymatic activity (reaction 1) converts 1,1-DCE to a toxic intermediate. It is that activity blocked by pretreatments with CCl₄, pyrazole or aminotriazole. The second microsomal activity (reaction 2), which is absent or present at only low levels in younger rats, is induced by phenobarbital pretreatment in young rats and inhibited by pretreatment with SKF 525-A in older rats. It is responsible for converting the toxic metabolite (or a toxic rearrangement product of a metabolite) to less toxic materials.

PROPOSED SCHEME OF VINYLIDENE CHLORIDE METABOLISM

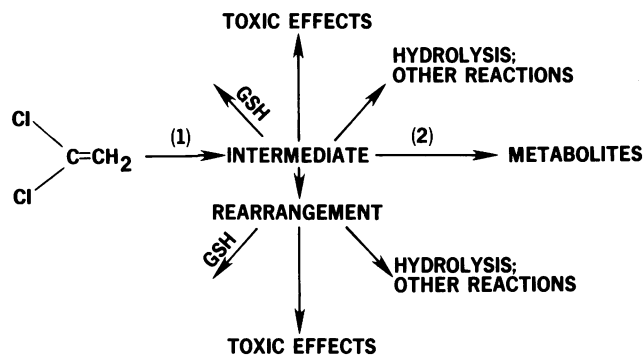


FIGURE 8. Suggested scheme for the metabolism of 1,1-dichloroethylene by the rat.

Based on analogy with the proposed metabolism of other chlorinated ethylenes (20), metabolism of 1,1-DCE has been suggested to produce an epoxide (13, 19). Attempts to synthesize the epoxide of 1,1-DCE have been unsuccessful (13, 21), but from the nature of the reaction products, primarily chloroacetylchloride, the epoxide has been presumed to be a transient, unstable intermediate.

Such an epoxide *in vivo* would be conjugated with GSH by glutathione transferase (22, 23) hydrolyzed by epoxide hydratase (24, 25) or could react with other nucleophiles. If the epoxide rearranged to an acid chloride, it could react with H₂O and any of a variety of nucleophiles including GSH. Glutathione depletion, by pretreatment with diethyl maleate or by fasting then would decrease the capacity of the rat to detoxify the intermediate(s). Careful, detailed *in vitro* studies of the biotransformation of 1,1-DCE are necessary to fully describe the nature of the enzymic activities and the various suspected metabolites of 1,1-DCE.

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