ISSN 0041-008X

This Number Completes Volume 50

Volume 50, Number 3
September 30, 1979

Toxicology and Applied Pharmacology

Official Journal of the Society of Toxicology

Academic Press
New York and London

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TOXICOLOGY AND APPLIED PHARMACOLOGY 50, 523–531 (1979)

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Acute Hepatoxicity of Vinyl Chloride and Ethylene: Modification by Trichloropropene Oxide, Diethylmaleate, and Cysteine[1]

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Received January 8, 1979; accepted June 16, 1979

Acute Hepatotoxicity of Vinyl Chloride and Ethylene: Modification by Trichloropropene Oxide, Diethylmaleate, and Cysteine. CONOLLY, R. B., AND JAEGER, R. J. (1979). *Toxicol. Appl. Pharmacol.* 50, 523–531. We have reported that vinyl chloride (VC) and ethylene are acutely hepatotoxic in rats pretreated with polychlorinated biphenyl (PCB). The present study used several chemical treatments to investigate the mechanisms of acute VC and ethylene toxicity in PCB-pretreated rats. Trichloropropene oxide (TCPO) depletes hepatic glutathione (GSH) and inhibits hepatic epoxide hydrase (EH). Diethylmaleate (DEM) also depletes hepatic GSH. Cysteine is a rate-limiting precursor in hepatic GSH synthesis. Effects of these various treatments on VC and ethylene toxicity were compared with their influence on hepatic GSH concentrations. Exposures to VC and ethylene were by inhalation at 1000 to 50,000 ppm. TCPO significantly increased VC toxicity in fasted but not in fed rats. Ethylene toxicity was not affected by TCPO. These actions of TCPO were not attributable to changes in hepatic GSH. DEM significantly lowered GSH during exposure, but did not increase the hepatotoxicity of either VC or ethylene. This suggests either that hepatic GSH concentrations are not an important determinant of the acute response to VC and ethylene or that DEM has important effects in addition to hepatic GSH depletion. Cysteine protected significantly, though incompletely, against acute VC hepatotoxicity. There was no effect of cysteine on the toxicity of ethylene. These data indicate that in PCB-treated rats, hepatic GSH and EH influence the acute hepatotoxicity of VC, but not that of ethylene.

Vinyl chloride (VC) and ethylene are acutely hepatotoxic in rats pretreated with polychlorinated biphenyl (PCB) (Conolly *et al.*, 1978). PCB is an inducer of the hepatic mixed-function oxidase system (MFOS) (Alvares and Kappas, 1977). VC and ethylene are not acutely hepatotoxic without such pretreatment. The demonstration of this toxicity, the structural similarity of the two compounds, and their industrial importance (Anonymous, 1978) have lead us to a comparative study of their hepatotoxic mechanisms. Initial results of these studies showed that a number of parameters—environmental (e.g., fed-fast status, exposure temperature) and chemical (e.g., preexposure treatment with trichloropropene oxide)—could affect the hepatotoxicity of VC and ethylene (Conolly and Jaeger, 1977).

[1] Research supported by Grant ES-00002 from the National Institute for Environmental Health Sciences.

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0041-008X/79/120523-09$02.00/0

In the work reported here, we studied the effects of several chemicals—trichloropropene oxide (TCPO), diethylmaleate (DEM), and cysteine—on the acute hepatotoxicity of VC and ethylene in PCB-pretreated rats. TCPO is a potent *in vitro* inhibitor of epoxide hydrase (EH) and an *in vivo* depletor of hepatic GSH (Oesch and Daly, 1972). DEM is an effective depletor of hepatic GSH (Boyland and Chasseaud, 1970). Cysteine is a rate-limiting GSH precursor (Jocelyn, 1972). We investigated the influence of these treatments, TCPO, DEM, and cysteine, on hepatic GSH concentrations and the relationship of changes in these GSH concentrations to the hepatotoxicity of VC and ethylene. Mortality, serum sorbitol dehydrogenase (SDH), and liver weight to body weight ratios were used to measure toxicity.

METHODS

Animals. Male Holtzman rats,[4] 170–250 g, housed five or six per cage, were used. They were supplied with commercial rat chow[5] and tap water *ad libitum*. The animals were maintained on a 12-hr light–dark cycle. In experiments with fasted rats, food was removed 18 hr before VC or ethylene exposure. Fasting animals were allowed water. In all experiments, food and water were not available during inhalation exposure. After exposure the animals were allowed water but not food. Sacrifice was by cervical transection. For GSH determinations, the rats were killed at 2 or 4 hr from the start of a 4-hr inhalation exposure. For determination of SDH activity, sacrifice was 24 hr after the start of exposure (Conolly and Jaeger, 1977).

Treatments. All treatments were by gavage. PCB[6] was solubilized in a vehicle of water containing 0.5% (v/v) Tween 80 and 0.5% (w/v) methyl cellulose. Rats were given 100 mg PCB/kg in a volume of 2.5 ml/kg once daily between 2:00 and 5:00 PM for 3 consecutive days. VC or ethylene exposure was on the day following the third PCB treatment. Cysteine, DEM, and TCPO were given within 0.5 hr before the start of inhalation exposure. Cysteine-HCl[7] was dissolved in water and dosed at 0.5 g cysteine/kg in a volume of 10.0 ml/kg. DEM[8] was dissolved in vehicle and dosed at 0.5 ml DEM/kg in a volume of 2.0 ml/kg. TCPO[9] was dissolved in vehicle and dosed at 0.1 ml TCPO/kg in a volume of 1.0 ml/kg.

Inhalation exposures. All exposures to VC or ethylene were by inhalation. They began between 9:00 and 11:00 AM and lasted 4 hr. The inhalation chambers, gases, and analytical methodology were as previously described (Conolly *et al.*, 1978).

Biochemical assays. Determination of SDH activity used a method modified from that of Gerlach and Hiby (1974). Serum (0.3 ml or an appropriate dilution thereof), triethanolamine buffer (2.3 ml, 0.2 M, pH 7.4), and NADH (0.1 ml, 12 mM in 0.1% $NaHCO_3$) were mixed and incubated at 30°C for 30 min. At the end of this incubation D(−)-fructose (0.3 ml, 4.0 M) was added. The disappearance of NADH absorbance was monitored at 366 nm using a Gilford 240 spectrophotometer equipped with a Gilford digital absorbance meter. Liver nonprotein sulfhydryl, expressed as GSH, was determined by the method of Jaeger *et al.* (1977). Beck *et al.* (1958) have shown about 90% of rat hepatic nonprotein sulfhydryl to be GSH.

Data presentation and analysis. Liver weight data are presented as grams of liver per 100 grams body weight to correct for differences in rat weights. Hepatic GSH concentrations are reported as a percentage of their respective control. The percentages of control values are based on calculation of milligrams of hepatic GSH per 100 g body weight. This transformation corrects for injury-related changes in hepatic weight. For all liver weight and SDH values, statistical evaluation was based on the logarithmic transformation of the raw data to assure similarity of variances. Accordingly, the data are presented as geometric mean and standard error range. The effects of TCPO and cysteine on toxicity were examined at several concentrations of VC and ethylene. These effects were evaluated by two-way analysis of variance (ANOVA) (concentrations of VC or ethylene and treatment vs vehicle) (Armor and Couch, 1972). The F test was used to compare variance due to TCPO or cysteine treatment. The effect on toxicity of changes in VC or ethylene concentration was not tested as this has previously been shown to be dose responsive (Conolly *et al.*, 1978). Data from fed and fasted rats were treated separately in these analyses. Single concentrations of VC and ethylene were used to evaluate the effect of DEM on toxicity. The hypothesis that a difference in toxicity would exist between fed rats given DEM and fasted

[4] Holtzman, Madison, Wis.

[5] Purina Rat Chow, Ralston Purina Co., St. Louis, Mo.

[6] Aroclor 1254, Monsanto Chemical Co., St. Louis, Mo.

[7] Reagent grade, Fisher Scientific Co., Fair Lawn, N.J.

[8] Minimum purity 85%, ICN, K & K Laboratories, Plainview N.J.

[9] TCPO was 99% pure, Aldrich Chemical Co., Milwaukee, Wisconsin.

rats given vehicle was evaluated by independent *t* test (Snedecor and Cochran, 1967). In all experiments mortality figures were tested for significance by both logit analysis (Finney, 1964) and by Fisher's exact test (Armitage, 1971). A p value of less than 0.05 was considered significant.

RESULTS

TCPO—Effects on the Acute Hepatotoxicity of Vinyl Chloride and Ethylene in PCB Pretreated Rats

Table 1 summarizes the data for TCPO effects on rats exposed to VC. ANOVA of the data from fasted rats showed significant increases in liver weight and SDH. No effect of TCPO in fed rats exposed to VC was seen. Mortality was not significantly affected in these experiments. In contrast to its effect on VC toxicity, TCPO did not influence the toxicity of ethylene in either fed or fasted rats (Table 2).

TCPO—Effects on Hepatic Glutathione during Vinyl Chloride or Ethylene Exposure of PCB Pretreated Rats

Exposure of fasted rats to 4500 ppm VC caused minimal injury to the vehicle-treated group, but marked injury to the rats given TCPO (Table 1). Figure 1 shows that this concentration of VC caused significant hepatic GSH depletion in both vehicle and TCPO treated groups, but that there were no differences in GSH concentrations between groups. Figure 2 shows that TCPO treatment of fasted rats lowers hepatic GSH during exposure to 25,000 ppm ethylene. This effect of TCPO, which was significant at 2 hr but not at 4 hr, was not associated with any change in the hepatotoxic response to ethylene (Table 2). Figure 2 also shows that this concentration of ethylene, which caused severe hepatic injury (Table 2), did not itself deplete hepatic GSH.

TABLE 1

EFFECT OF TCPO ON THE ACUTE HEPATOTOXICITY OF VINYL CHLORIDE IN PCB-PRETREATED RATS

Vinyl chloride concentration (ppm)	Fed or fasted	Vehicle or TCPO[a]	Liver weight (g/100g body wt.)[b,c]	Serum SDH activity[c,d]	Mortality
—	Fed	Vehicle	6.29 (5.99–6.60)	22.1 (19.9–24.6)	0/4
—	Fed	TCPO	6.42 (6.31–6.54)	10.1 (8.7–11.7)	0/5
—	Fasted	Vehicle	6.52 (6.32–6.73)	18.7 (17.6–19.9)	0/4
—	Fasted	TCPO	7.23 (6.99–7.48)	15.1 (13.5–17.0)	0/11
1,000	Fasted	Vehicle	6.94 (6.76–7.12)	26.8 (22.9–31.4)	0/10
4,500	Fasted	Vehicle	7.13 (6.93–7.34)	34.2 (29.1–40.3)	0/19
8,000	Fasted	Vehicle	10.09 (9.20–11.07)	487 (283–839)	3/11
1,000	Fasted	TCPO	7.49 (7.34–7.66)	27.2 (20.8–35.5)	0/5
4,500	Fasted	TCPO	7.94 (7.67–8.22)	133 (97.5–182)	1/19
8,000	Fasted	TCPO	11.61 (10.80–12.49)	1,633 (1338–1993)	7/11
10,000	Fed	Vehicle	8.18 (7.70–8.69)	121 (79.6–185)	1/15
25,000	Fed	Vehicle	10.50 (10.12–10.88)	772 (605–986)	3/11
40,000	Fed	Vehicle	10.53 (10.22–10.85)	1,192 (709–2001)	5/12
10,000	Fed	TCPO	8.96 (8.60–9.33)	173 (133–224)	1/15
25,000	Fed	TCPO	10.29 (10.11–10.47)	638 (433–942)	3/11
40,000	Fed	TCPO	10.60 (10.25–10.97)	894 (554–1442)	6/12

[a] Rats were given either vehicle (1.0 ml/kg) or TCPO (0.1 ml/kg) in vehicle (1.0 ml/kg).
[b] Effect of TCPO on liver weight in fasted rats exposed to VC significant ($p = 0.007$ by F test).
[c] Values are expressed as the geometric mean and standard error range.
[d] Effect of TCPO on SDH in fasted rats exposed to VC significant ($p = 0.018$ by F test).

TABLE 2

EFFECT OF TCPO ON THE ACUTE HEPATOTOXICITY OF ETHYLENE IN PCB-PRETREATED RATS

Ethylene concentration (ppm)	Fed or fasted	Vehicle or TCPO[a]	Liver weight (g/100g body wt.)[b]	Serum SDH activity[b]	Mortality
6,000	Fasted	Vehicle	6.38 (6.23–6.52)	10.6 (9.0–12.4)	0/12
12,000	Fasted	Vehicle	7.63 (7.30–7.97)	187 (129–270)	0/12
18,000	Fasted	Vehicle	8.66 (8.44–8.89)	499 (374–667)	0/12
25,000	Fasted	Vehicle	8.95 (8.72–9.20)	833 (712–973)	1/13
6,000	Fasted	TCPO	6.78 (6.62–6.95)	21.0 (17.5–25.1)	0/12
12,000	Fasted	TCPO	7.91 (7.66–8.16)	226 (160–318)	0/12
18,000	Fasted	TCPO	8.17 (7.84–8.51)	399 (305–521)	0/12
25,000	Fasted	TCPO	9.19 (8.96–9.42)	865 (722–1036)	0/14
10,000	Fed	Vehicle	6.73 (6.49–6.98)	52.8 (37.3–75.0)	0/8
25,000	Fed	Vehicle	7.27 (7.09–7.45)	416 (309–559)	0/5
50,000	Fed	Vehicle	8.04 (7.81–8.29)	1,177 (1070–1295)	1/6
10,000	Fed	TCPO	6.64 (6.46–6.83)	42.0 (32.0–55.1)	0/10
25,000	Fed	TCPO	8.28 (7.86–8.73)	599 (409–877)	0/5
50,000	Fed	TCPO	7.44 (7.32–7.56)	1,029 (778–1360)	2/6

[a] Rats were given either vehicle (1.0 ml/kg) or TCPO (0.1 ml/kg) in vehicle (1.0 ml/kg).
[b] Values are expressed as the geometric mean and standard error range.

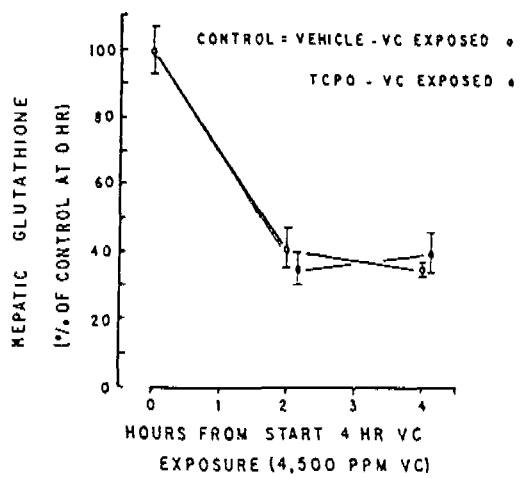

FIG. 1. Effect of trichloropropene oxide (TCPO) on hepatic glutathione during exposure of fasted, PCB-pretreated rats to 4500 ppm vinyl chloride (VC) ($n = 8$). See Table 1 for the corresponding toxicity data.

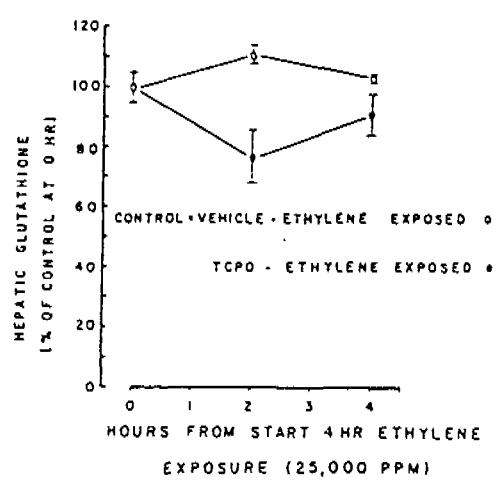

FIG. 2. Effect of trichloropropene oxide (TCPO) on hepatic glutathione during exposure of fasted, PCB-pretreated rats to 25,000 ppm ethylene ($n = 4$). At 2 hr GSH in the TCPO-treated group was significantly lower ($p < 0.02$). See Table 2 for the corresponding toxicity data.

Relationship of Hepatic GSH Depletion by DEM to VC and Ethylene Hepatotoxicity

Immediately before VC or ethylene exposure hepatic GSH concentrations in fed and fasted PCB-pretreated rats were 20.27 ± 0.89 and 15.28 ± 1.70 mg GSH/100 g body wt, respectively. For both of these determinations $n = 3$. Fed rats given DEM and fasted rats given vehicle were sacrificed during exposure to 10,000 ppm VC or 20,000 ppm ethylene and hepatic GSH concentrations determined. Figure 3 shows that 10,000 ppm VC caused significant GSH depletion in both fed-DEM and fast-vehicle groups, and that there were no differences in hepatic GSH concentrations. Table 3 shows that fasting, but not DEM, significantly increased the toxicity of 10,000 ppm VC in PCB-pretreated rats. Furthermore, DEM did not affect the magnitude of the fed-fast difference. The data for ethylene were analogous. Hepatic GSH (Fig. 4) was significantly ($p < 0.01$ at 2 and 4 hr) lower in the fed-DEM group than in the

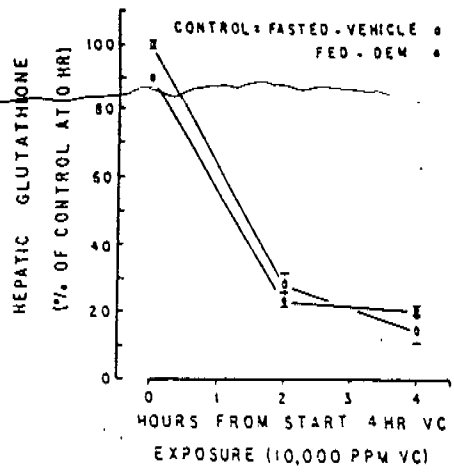

FIG. 3. Effect of diethylmaleate (DEM) on hepatic glutathione during exposure to 10,000 ppm vinyl chloride (VC). Fed, PCB-pretreated rats were given diethylmaleate so that their hepatic GSH during exposure to 10,000 ppm was the same as, or lower than, that of simultaneously exposed, fasted rats. Each datum point has $n = 3$, except for Fed-DEM, 0 hr, which has $n = 2$. See Table 3 for the corresponding toxicity data.

TABLE 3

EFFECT OF DIETHYLMALEATE ON THE ACUTE HEPATOTOXICITY OF VINYL CHLORIDE OR ETHYLENE IN FED AND FASTED PCB-PRETREATED RATS

Inhalation exposure (ppm)	Fed or fasted	Vehicle or DEM[a]	Liver weight (g/100 g body wt.)	Serum SDH activity[b]	Mortality
—	Fed	Vehicle	6.43 (6.28–6.58)	13.1 (12.0–14.3)	0/4
—	Fed	DEM	6.82 (6.62–7.03)	14.7 (11.9–18.1)	0/5
—	Fasted	Vehicle	6.52 (6.32–6.73)	18.7 (17.6–19.9)	0/4
—	Fasted	DEM	6.85 (6.63–7.08)	15.4 (14.5–16.3)	0/5
Vinyl chloride					
10,000	Fed	Vehicle	7.28 (6.92–7.66)	48.8 (34.1–69.8)	0/5
10,000	Fed	DEM	7.63 (7.48–7.79)	41.2 (32.1–52.7)	0/5
10,000	Fasted	Vehicle	10.24 (9.19–11.40)[c,d]	860 (351–2106)[c,d]	0/5
10,000	Fasted	DEM	11.36 (10.48–12.31)[d]	950 (625–1445)[d]	0/5
Ethylene					
20,000	Fed	Vehicle	7.70 (7.43–7.97)	369 (301–453)	0/7
20,000	Fed	DEM	8.30 (8.12–8.48)	205 (183–231)	0/18
20,000	Fasted	Vehicle	8.26 (8.04–8.50)	362 (303–432)	0/18
20,000	Fasted	DEM	9.44 (9.10–9.80)	327 (268–399)	0/13

[a] Rats were given either vehicle (2.0 ml/kg) or DEM (0.5 ml/kg) in vehicle (2.0 ml/kg).
[b] Values are expressed as the geometric mean and standard error range.
[c] Significantly different ($p < 0.05$ by the independent t test) from Fed-Vehicle, VC-exposed.
[d] Significantly different ($p < 0.05$ by the independent t test) from Fed-DEM, VC-exposed.

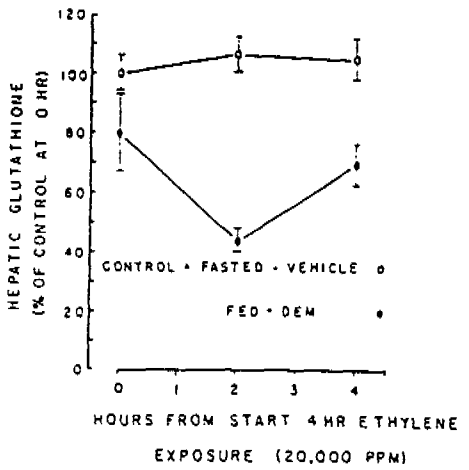

FIG. 4. Effect of diethylmaleate (DEM) on hepatic glutathione during exposure to 20,000 ppm ethylene. Fed, PCB-pretreated rats were given diethylmaleate so that their hepatic GSH during exposure to ethylene was significantly lower than that of simultaneously exposed, fasted rats ($p < 0.01$ at 2 and 4 hr), ($n = 4$). See Table 3 for the corresponding toxicity data.

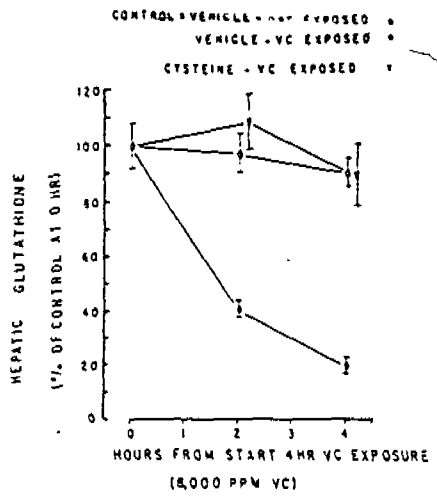

FIG. 5. Effect of cysteine on hepatic glutathione during exposure of fasted, PCB-treated rats to 8000 ppm vinyl chloride (VC). Fasted, PCB-pretreated rats were given cysteine so that their hepatic GSH (total nonprotein sulfhydryl) during exposure to vinyl chloride was the same as or greater than that of nonexposed rats ($n = 6$). See Table 4 for the corresponding toxicity data.

fasted-vehicle group during exposure to 20,000 ppm ethylene. However, in these ethylene-exposed rats, the significantly lower hepatic GSH of the DEM-treated group was not associated with any increase in toxicity (Table 3).

Relationship of Cysteine Treatment to Changes in the Acute Hepatotoxicity of VC and Ethylene

Figure 5 shows that cysteine completely prevented depletion of hepatic GSH during exposure of fasted, PCB-pretreated rats to 8000 ppm VC. ANOVA of the data in Table 4 showed that cysteine protected against the acute hepatotoxic effects of VC in fasted rats. This effect was significant for increases in both liver weight and SDH. It should be noted, however, that this protection was not complete. Significant increases in liver weight and SDH occurred after VC exposure of cysteine-treated rats (Table 4). Mortality was not a significant factor in these experiments.

PCB-pretreated rats given cysteine were exposed to 8,000, 16,000, and 24,000 ppm ethylene. There was no effect of cysteine on the toxicity of ethylene at these concentrations.

DISCUSSION

Trichloropropene Oxide

The increased toxicity of VC in TCPO-treated rats (Table 1) is consistent with literature reports on the biochemical effects of TCPO. It inhibits EH (Oesch and Daly, 1972) and is a substrate for GSH-*S*-alkyl-epoxytransferase (GSH-SAT) (Mukhtar and Bresnick, 1976), while VC metabolism involves epoxide formation (Green and Hathway, 1977) and GSH conjugation (Watanabe *et al.*, 1976b). Figure 1 suggests that differences in hepatic GSH concentrations do not explain this action of TCPO. It is likely therefore, the increased toxicity of VC in TCPO-treated rats is due to inhibition of EH. The action of TCPO in fasted, but not in fed rats might reflect a greater importance of EH

TABLE 4

EFFECT OF CYSTEINE ON THE ACUTE HEPATOTOXICITY OF VINYL CHLORIDE IN PCB-PRETREATED RATS

Vinyl chloride concentration (ppm)	Fed or fasted	Water or cysteine[a]	Liver weight (g/100 g body wt)[b,c]	Serum SDH activity[c,d]	Mortality
—	Fasted	Water	6.94 (6.64–7.26)	18.3 (15.4–21.7)	0/4
—	Fasted	Cysteine	6.74 (6.54–6.93)	22.9 (19.5–27.0)	0/9
5,000	Fasted	Water	8.84 (7.93–9.86)	203 (107–386)	0/6
8,000	Fasted	Water	9.21 (8.90–9.52)	253 (203–315)	1/30
12,000	Fasted	Water	11.69 (11.10–12.32)	634 (573–749)	0/6
17,000	Fasted	Water	11.12 (10.60–11.67)	762 (469–1238)	2/6
5,000	Fasted	Cysteine	6.82 (6.65–7.01)	30.3 (26.6–34.4)	1/6
8,000	Fasted	Cysteine	7.82 (7.52–8.13)	75.9 (57.9–98.1)[e]	2/30
12,000	Fasted	Cysteine	9.49 (8.71–10.34)[e]	423 (226–792)[e]	1/6
17,000	Fasted	Cysteine	9.51 (8.72–10.38)[e]	132 (90.8–194)[e]	0/6

[a] Rats were given either water (10.0 ml/kg) or cysteine (0.5 g/kg) in water (10.0 ml/kg).
[b] Effect of cysteine on liver weight in VC exposed rats significantly different ($p = 0.001$ by F test).
[c] Values are expressed as the geometric mean and standard error range.
[d] Effect of cysteine on SDH in VC exposed rats significantly different ($p = 0.002$ by F test).
[e] Significantly different ($p < 0.02$ by the independent t test) from cysteine treated, not exposed.

when, as in the fasted rat, hepatic GSH is depleted.

TCPO did not affect ethylene toxicity (Table 2), though evidence exists that ethylene is metabolized to ethylene oxide (Ehrenberg *et al.*, 1977; Jerie and Hall, 1978). These data suggest then that EH and GSH-SAT do not act on the oxide. The lack of hepatic GSH depletion by ethylene (Fig. 2) is consistent with a noninvolvement of GSH-SAT in ethylene metabolism. Alternatively, ethylene oxide may not be a toxic metabolite of ethylene.

Diethylmaleate

Jaeger *et al.* (1977) reported that DEM did not increase the hepatotoxicity of VC in phenobarbital-pretreated, normally fed rats. We have shown here the same effect of DEM in both fed and fasted rats pretreated with PCB and exposed to VC or to ethylene (Table 3). In these experiments the hepatic GSH concentrations of DEM-treated fed rats were the same as (VC, Fig. 3), or lower than (ethylene, Fig. 4), the corresponding concentrations in vehicle-treated fasted rats. These results are consistent with our other findings on ethylene but conflict with the data on VC toxicity suggesting involvement of hepatic GSH (Table 4). However, a substantial body of evidence suggests the importance of GSH in detoxification of electrophilic VC metabolites (Green and Hathway, 1975; Watanabe *et al.*, 1976a,b). Hence, we suspect that hepatic GSH concentrations are in fact an important determinant of acute VC toxicity. Some action of DEM in addition to GSH depletion could explain the lack of potentiation of VC toxicity. For example, Chuang *et al.* (1978) have shown that DEM inhibits aryl hydrocarbon hydroxylase (cytochrome *P*-448), an enzyme strongly induced by the PCB mixture Arocior 1254 used in these studies (Alvares and Kappus, 1977).

Cysteine

Leaf and Neuberger (1947) have shown that dietary cysteine, but not glycine or glutamic acid, directly influences hepatic GSH concentrations in rats. Waelsch and Rittenberg

(1942) have estimated the half-life of hepatic GSH to be 2–4 hr. It is likely, therefore, that the lack of "GSH" depletion during VC exposure of treated rats (Fig. 5) in fact represents some combination of increased concentrations of hepatic GSH and of nonprotein sulfhydryl (i.e., cysteine). The combination—GSH and cysteine—may not possess the same potential for detoxification of reactive chemical species as would the same amount of –SH present solely as GSH. This reflects the fact that GSH, but not cysteine, is a substrate for the various GSH transferases. The development of liver injury in the cysteine-treated, VC-exposed group (Table 4) may be due to the fact that much of the –SH was present as cysteine rather than GSH, and hence did not possess the same potential for enzymatically mediated metabolite detoxification.

Cysteine had no effect on the acute hepatotoxicity of ethylene in PCB-treated rats. This is further evidence that GSH does not play an important role in acute ethylene hepatotoxicity.

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