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Biochemical Toxicology of Unsaturated Halogenated Monomers

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Previous inhalation toxicity studies from our laboratory have shown that 1,1-dichloroethylene (1,1-DCE), 1,1-dibromoethylene (1,1-DBE), and 2-chloro-1,3-butadiene (2-CBD) are more toxic to fasted rats than to fed rats. Vinyl chloride monomer (VCM) and 1,1-difluoroethylene (1,1-DFE) were not acutely hepatotoxic at 46,500 and 82,000 ppm, respectively, in normal male rats, whether fed or fasted. On a molar basis, 1,1-DBE and 1,1-DCE have similar toxicities while 2-CBD is less toxic. All three compounds produce similar elevation of serum transaminase and bloody ascites, although at differing times following differing exposure concentrations.

1,1-DCE produces massive midzonal hepatic necrosis with hepatic thrombosis and chromatolysis within 2 hr after a 4 hr exposure of fasted rats to 200 ppm. Subsequent to formation of this midzonal lesion, the central portion of the lobule collapses, accompanied by congestion, ascites, and an increased hematocrit in the rat. Serum transaminase and sorbital dehydrogenase are greatly elevated at 6 hr. This effect in fasted rats is associated with glutathione (GSH) depletion. Diethyl maleate (DEM) which depletes GSH in fed rats potentiates the injury associated with 1,1-DCE exposure as well as that produced by 2-CBD. Rats fed *ad libitum* and exposed to 1,1-DCE or 2-CBD at night, a time of low hepatic GSH concentration, exhibit enhancement of hepatotoxic response when compared to animals exposed during the day when GSH is high.

Introduction

This communication presents results of our studies on the hepatotoxic effects in male rats following inhalation exposure to 1,1-dichloroethylene (1,1-DCE), 2-chlorobutadiene (2-CBD), or vinyl chloride monomer (VCM). Additionally, toxicological interactions between vinyl chloride monomer and 1,1-dichloroethylene will be reported

In our earlier studies we found that inhalation exposure to 1,1-DCE was uniformly hepatotoxic and fatal to fasted rats at concentrations which were not fatal or hepatotoxic to fed rats (*1*). In an attempt to elucidate the reasons for this enhanced response in fasted animals, biochemical comparisons were made between the two nutritional states (i.e., fed vs. fasted), and it was observed that glutathione (GSH) content was diminished in the livers from fasted rats. Other investigators (*2*) had reported a similar observation previously. Previous reports (*3–5*) had shown that glutathione was an important site of detoxification following exposure of rats to bromobenzene. From this, an initial hypothesis was developed which postulated glutathione as a site of detoxification of 1,1-DCE. When this hypothesis was tested, both *in vivo* and in the isolated perfused rat liver, it was found that those animals with a diminished hepatic glutathione concentration were significantly more susceptible to 1,1-DCE exposure (*1*).

In the present report we will detail our ob-

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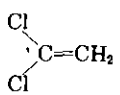
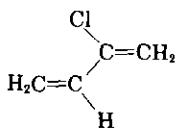
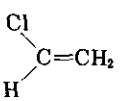
servations on the biochemical toxicology of 1,1-DCE, 2-CBD, and VCM. While the results of these biochemical observations in rats may not be directly relatable to the consequences of human occupational exposure, it is hoped that they will provide some indication for direction for future epidemiologic and industrial hygiene investigations.



1,1-Dichloroethylene (1,1-DCE)



2-Chlorobutadiene (2-CBD)



Vinyl Chloride Monomer (VCM)

Materials and Methods

Adult male Holtzman rats weighing 250–350 g were used. They were housed in an air conditioned room having a daily light-dark cycle (12 hr, 6 AM to 6 PM Eastern Daylight Saving Time). They were supplied with Purina laboratory chow and water *ad libitum*. In the case of fasted rats, food was removed from the animals at 4 PM on the day prior to exposure. The animals were allowed free access to water. During exposure, no food or water was available. Following exposure, fasted animals were deprived of food while fed animals were allowed access to food. The animals were sacrificed by cervical transsection using sharp scissors, and blood was collected from the cut vessels.

Inhalation exposures were conducted as previously described (*1,6*) by using the dynamic inhalation chamber described by Leach (*7*). Determinations of chamber concentrations were made gas chromatographically using a Varian-1700 (*1*). This method was also used for determination of chlorobutadiene and vinyl chloride monomer concentrations. In these cases, conditions were optimized for either of the monomers being tested. It was found that 1,1-DCE and VCM could be separated readily by standard techniques. However, acetone interfered with the determination of 1,1-DCE and in the experiment where acetone was administered with or prior to 1,1-DCE, acetone was vaporized in an air stream of known 1,1-DCE content and concentrations calculated on the basis of the metered volumes of acetone in atmosphere of known 1,1-DCE content.

For morphologic studies, fasted or fed rats, 3 per group, and exposed to 1,1-DCE (200 ppm) for the times specified, were anesthetized with sodium pentobarbital (50 mg/kg). Isoprel (0.2 mg/kg) was given IP, the animal opened with a midline incision, and 3 ml of blood was drawn from the abdominal aorta. An additional 1 mg of Isoprel was given IV immediately thereafter. The portal vein was cannulated and the liver rinsed by perfusion with warm Ringers–lactate solution and immediately perfusion fixed with buffered 1% glutaraldahyde. Livers were removed, sliced, and selected blocks post-fixed in osmium tetroxide and uranyl acetate. The slices were embedded in Epon, sectioned, appropriately stained, and examined by electron microscopy. Comparable liver slices, embedded in paraffin, were examined by conventional histologic techniques.

Glutathione concentrations and serum enzyme activities were determined (*1,6*). Tests of significance were done either parametrically when the F ratio indicated that this was permissable or nonparametrically. The parametric test used was Student's t-test, while the nonparametric test was the Mann-Whitney U test. In all cases, a p value less than or equal to 0.05 was considered significant.

Results

Figure 1 shows the variation of hepatic glutathione (GSH) concentration in rat liver as a function of the time of day. Food was withdrawn from animals at 4 PM, and groups of rats were sacrificed at 3 hr intervals. In a second experiment, a group of rats was fasted from 4 PM on day 1 and these animals were sacrificed beginning at 4 PM on day 2. The data show that GSH concentration, expressed as milligrams per 100 g of body weight, decreases precipitously after 4 PM in fed rats and reaches a minimum between 7 and 10 PM.

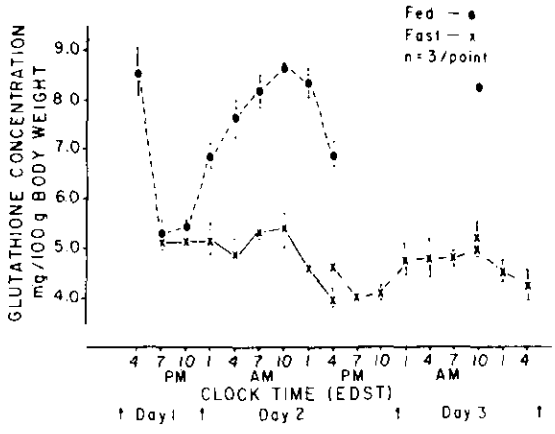


FIGURE 1. Circadian rhythm of hepatic glutathione concentration in fed or fasted rats. See text for details.

The maximum is achieved between 10 AM and 4 PM of the next day. Fasted rats sustained a diminished hepatic GSH concentration which did not show an increase; rather, the level remained low and fell again in the interval of 1 PM to 4 PM on day 2. The data for the second group of animals fasted for 24 hr suggests an endogenous rhythm independent of the night-time ingestion of food.

When rats were exposed to 1,1-DCE during time of high or low GSH concentration (AM versus PM exposure) as shown in Table 1, it became clear that the serum alanine α-ketoglutarate transaminase (AKT) activity as well as lethality were dependent on the time of exposure. Daytime (AM–PM) exposure resulted in a slight but significant elevation of serum AKT with no deaths whereas night-time (PM–AM) exposure resulted in a much greater (approximately 60–80 fold) elevation of AKT and two deaths of the five exposed. Similar results were also obtained with 2-CBD (data not shown). Depletion of liver glutathione levels in fed rats following diethyl maleate treatment potentiated both 1,1-DCE and 2-CBD toxicity.

Because fasting is also associated with ketosis and since acetone, a ketone, is a commonly encountered industrial solvent, we questioned whether acetone might enhance the hepatotoxic response associated with 1,1-DCE exposure. Table 2 shows that acetone (10,000 ppm), either for 2 hr prior to 1,1-DCE exposure or during exposure resulted in a significant

Table 1. Effect of time of exposure on hepatotoxic and lethal response to 1,1-DCE exposure.[a]

Time	Serum AKT, mg pyruvate/ml-hr[b]	Deaths
10 AM–2 PM	0.80±0.25	0/5
10 PM–2 AM	26.09±8.30[c]	2/5

[a] Blood was taken at sacrifice (23 hr) or when the rat was in extremis. Exposure was 2000 ppm of 1,1-DCE for 4 hr.
[b] Control range: 0.20–0.40 mg/ml-hr.
[c] $p < 0.05$ when compared nonparametrically with daytime exposed controls.

Table 2. Effect of acetone exposure on the serum AKT response to inhaled 1,1-DCE in fed rats.[a]

	N	Serum AKT, mg pyruvate/ml-hr[b]	
		6 hr	24 hr
Air+1,1-DCE	10	0.37±0.05	0.43±0.03
Acetone before 1,1-DCE	5	1.10±0.20[c]	1.39±0.34[c]
Acetone with 1,1-DCE	5	1.02±0.41[c]	0.54±0.15
Fasted rats	3	13.45±1.92[d]	—

[a] Acetone was ca. 10,000 ppm for 2 hr before or simultaneous with 1,1-DCE; 1,1-DCE concentration was 2000 ppm for 4 hr.
[b] Control AKT: 0.20–0.40 mg pyruvate/ml-hr.
[c] $p < 0.05$ when compared to air+1,1-DCE.
[d] $p < 0.01$ when compared to air+1,1-DCE.

increase in serum AKT activity at 6 hr in fed rats. In the animals exposed to acetone during 1,1-DCE exposure, the 6-hr enhancement of serum AKT activity returned to normal value by 24 hr. For comparison data from fasted animals is also shown. These data demonstrate clearly at these concentrations, previous or concomitant exposure to acetone is not as potent a modifier of the hepatotixic response as fasting alone.

Other factors which might affect the hepatotoxic response of rats following 1,1-DCE exposure could be related to the activity of the drug metabolizing enzymes. Because of the GSH dependence, administration of sulfur-containing amino acids might modify the toxicity of 1,1-DCE. Therefore, two experiments were conducted. In the first, rats were pretreated with SKF-525A. In the second experiment, fed or fasted rats were pretreated with cysteine, a sulfur containing amino acid found in GSH. The data shown in Table 3 suggests that SKF-525A did not affect the response of fed animals to exposure to 1,1-DCE nor were fasted animals

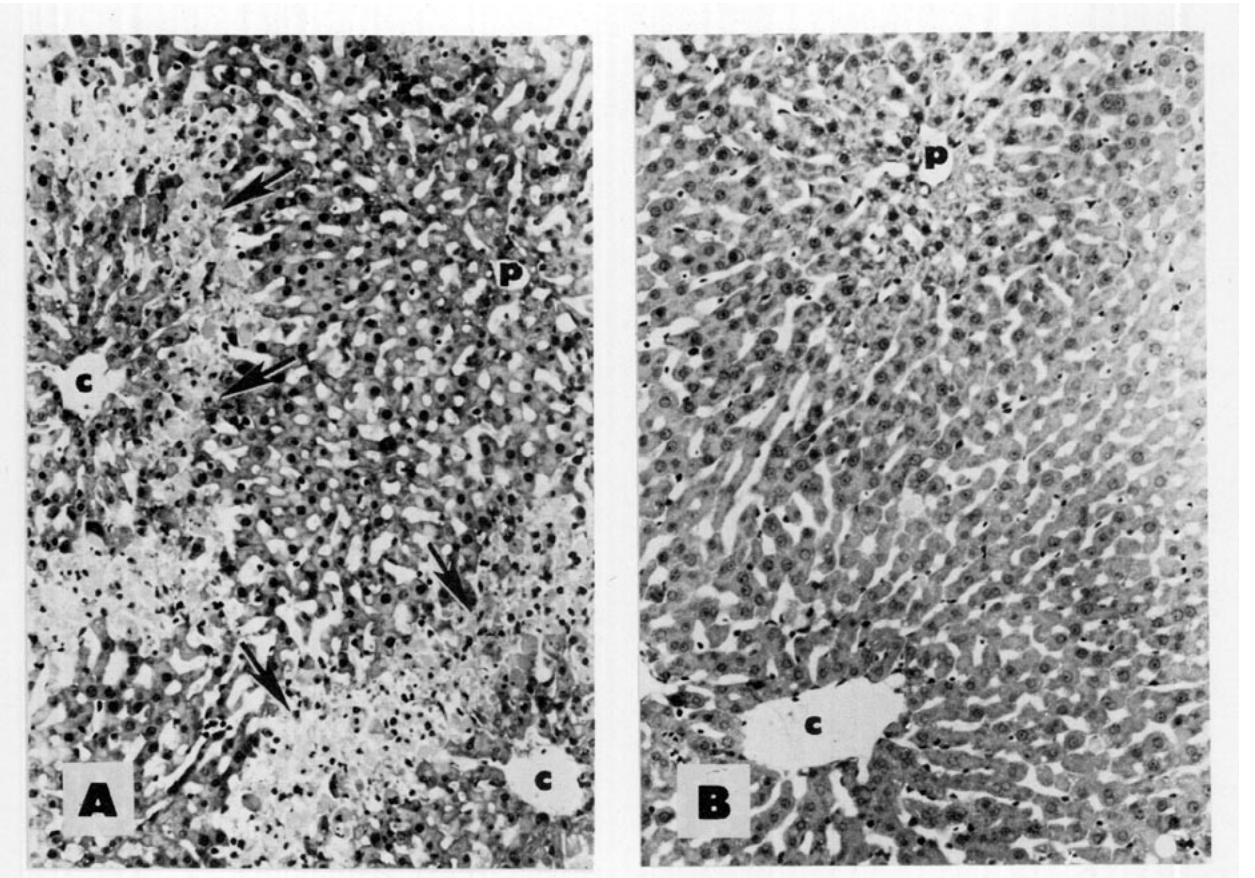


FIGURE 2. Liver, 6 hr after the onset of a 4-hr exposure to 200 ppm of 1,1-DCE: (*A*) fasted rat has prominent "stripes" of mid-zonal necrosis at 6 hr (arrow); (*B*) fed animal reveals no necrosis. C = central vein; P = portal vein; magnification 192×.

protected from the hepatotoxic effects of 1,1-DCE. Cysteine increased slightly the toxic effect of 1-1-DCE in fed rats. This slight elevation while statistically significant was not meaningful. Fasted animals were protected from the hepatotoxic and lethal effects of 1,1-DCE exposure by pretreatment with cysteine.

Prominent "stripes" of midzonal necrosis were consistently present in livers of fasted animals within 2 hr following the end of a 4-hr exposure to 200 ppm 1,1-DCE (Fig. 2*A*). These stripes, consisting of fragments of necrotic parenchyma, thrombus, and a few inflammatory cells, were not present in livers of fed animals similarly exposed to 1,1-DCE (Fig. 2B). Isolated clusters of necrotic cells were occasionally seen in the mid zone at this time. Fatty infiltration does not occur as a consequence of poisoning with 1,1-DCE.

Single altered parenchymal cells may be seen in the midzone of liver lobules as early as 2 hr following the onset of 1,1-DCE exposure in fasted animals. These cells (see insert, Fig. 3) show sharp retraction of cell borders with the formation of a pericellular lacunae which may contain cytoplasmic protrusions, red blood cells, and clotted fibrin. Nuclear changes in such cells are conspicuous, with loss of perinucleolar chromatin, clumping, and coalescense of perinucleolar chromatin into crescentric deposits of electron opaque material against the nuclear membrane. With time these deposits disappear, and involved nuclei appear empty.

Cytoplasmic borders of retracted parenchymal cells become smooth; they lose their microvilli, and fibrin is deposited on the outer aspect of the plasma membranes (Fig. 3). Golgi cysternae delaminate and, vacuolate, and mitochrondria show variable increases in electron lucency of their matrix. In contrast the

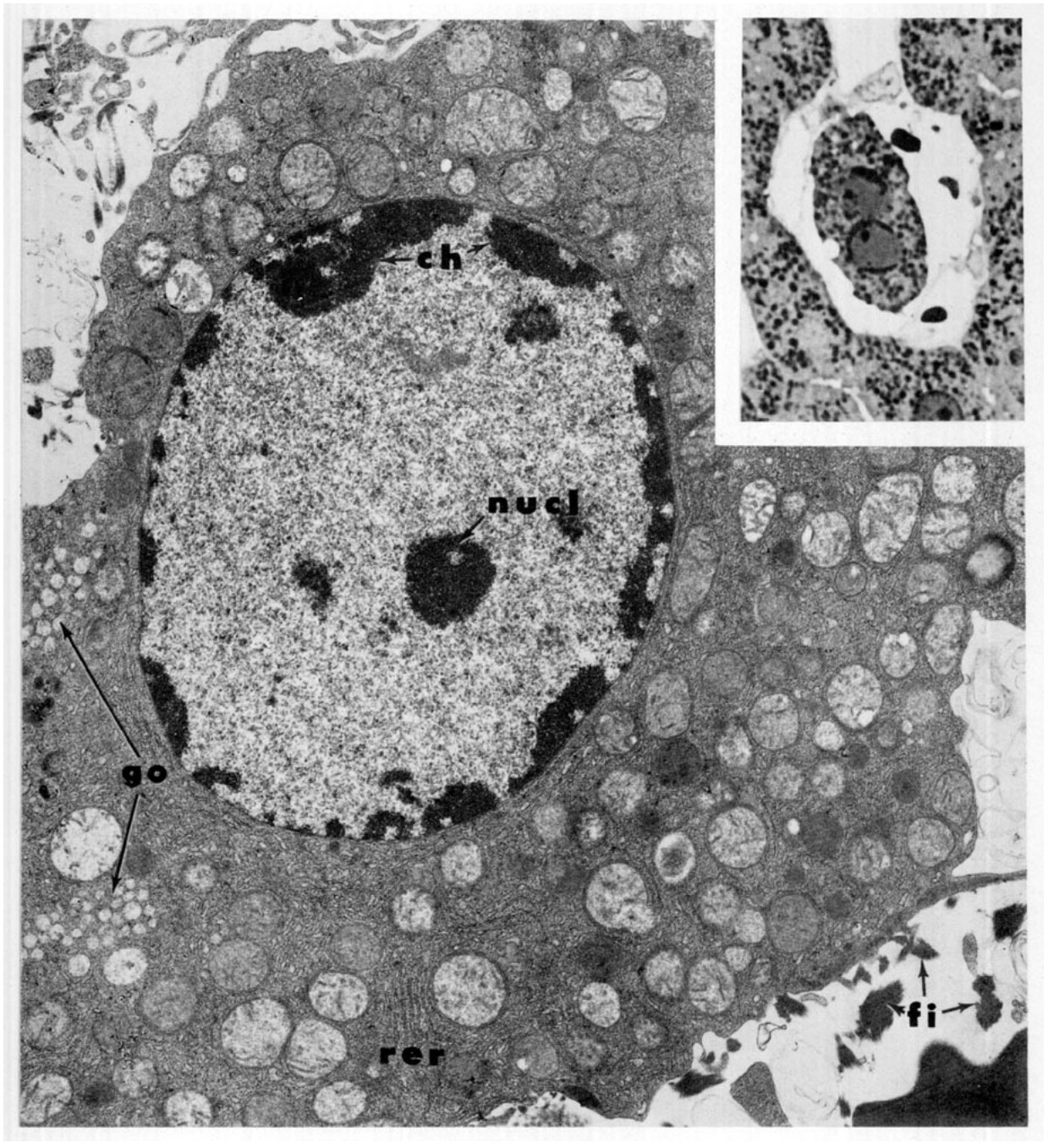


FIGURE 3. Midzonal parenchymal liver cell 2 hr (inset) and 4 hr following onset of exposure to 200 ppm 1,1-DCE. Electron micrograph of the cell shows retracted cell borders. Fibrin (fi) can be seen adjacent to the cell borders at the lower right. Nucleolus (nucl) shows loss of perinucleolar chromatin. Chromatin (ch) is marginated against the nuclear envelope and is partially clumped. Golgi (go) is diffusely vacuolated. Mitochondria show variable increases in electron lucency of matrix material. Rough endoplasmic reticulum (rer) appears unaffected. 1200×. *Inset.* Light micrograph of a contracted binucleated liver parenchymal cell in the lacunae of a hepatic cord. Nuclear chromatin shows early crescent formation. 1200×.

ergastoplasm does not readily disperse early in the course of cellular injury following 1,1-DCE, nor do its component cysternae of rough endoplasmic reticulum degranulate.

The dose–response relationship for serum AKT and death following inhalation exposure to 2-chlorobutadiene (2-CBD) is shown in Figure 4. It can be seen that exposure to 500, 1000, or 2000 ppm of 2-CBD was without effect in fed rats. These concentrations produced increases in serum AKT activity and caused death in each group of fasted rats. At 10,000 ppm, the fed–fasted difference following 2-CBD exposure disappeared. In Figure 5, the time–response relationship of serum AKT following inhalation exposure to 2-CBD (4600 ppm) is shown. It can be seen from this data that 12 hr was sufficient time for development of the difference in hepatotoxic response in fed or fasted rats to be observed. At 18 and 24 hr, there was a significantly greater enhancement of hepatotoxicity following 2-CBD exposure in fasted rats. These data are consistent with 2-CBD being a slower, less potent hepatotoxin than is 1,1-DCE.

Vinyl chloride (VCM) is not known to produce an immediate hepatotoxic response. This observation is confirmed by the results in Table 4. Fed or fasted animals exposed to VCM at approximately 10,500 and 46,500 ppm did not have elevated serum AKT activity when sacrificed. It should be noted that 46,500 ppm of

Table 3. Effect of SKF-525A or cysteine on serum AKT in rats exposed to 1,1-DCE (1000 ppm, 4 hr).

Expt.		Serum AKT, mg pyruvate/ml-hr	
		Fed	Fasted
I	Saline	0.54±0.07 (5)[a]	9.88± 4.96[b] (4)[a] (1.22–23.58)[c]
	SKF-525A[d]	0.60±0.08 (5)[a]	18.34 (2)[a] (9.10+27.58)[c]
II	Saline	0.32±0.04 (5)	17.72±1.47[b] (3)[a] (2 died)
	Cysteine[e]	0.75±0.09: (5)[a]	2.43±0.75[d, f, g] (5)[a]

[a] Numbers in parentheses indicate number of animals in groups *N*.
[b] $p < 0.05$ when compared to fed rats.
[c] Values in parentheses are ranges.
[d] 50 mg/kg, IP.
[e] 500 mg/kg, IP.
[f] $p < 0.05$ when compared to saline control rats.
[g] $p < 0.05$ when compared to fed saline pretreated rats.

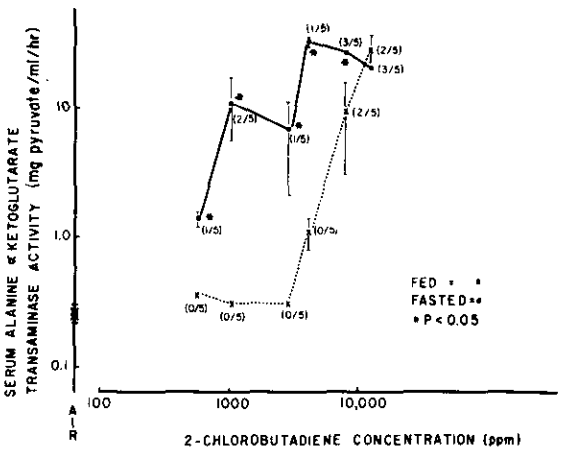


FIGURE 4. Dose responses relationship for 2-chlorobutadiene exposure and its effect on serum AKT activity in fed or fasted rats sacrificed at 24 hr. See text for details.

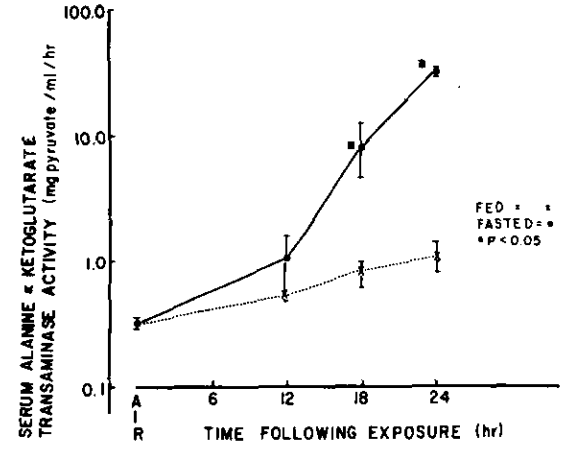


FIGURE 5. Time response relationship for 2-chlorobutadiene exposure in fed or fasted rats and its effects on serum AKT activity. See text.

VCM was within the lower explosion limit for this compound (*8*).

Because 1,1-DCE and VCM are chemically similar and are used simultaneously in the manufacture of certain copolymers, the question of the possible toxic interaction between these compounds was investigated. In Table 5, it can be seen that 1,122 ppm of VCM was without hepatotoxic effect in fasted rats at 6 hr. 1,1-DCE (205 ppm) caused a slight but not

Table 4. Acute inhalation toxicity of vinyl chloride monomer.[a]

VCM concentration, ppm	Serum AKT activity, mg pyruvate/ml-hr	
	Fasted rats	Fed rats
0 (air)	0.20–0.40[b]	
10,522± 204	0.29±0.02	0.36±0.02
46,554±1158	0.31±0.02	0.35±0.02

[a] Rats were sacrificed at 24 hr.
[b] Control range; $N = 5$ per group.

Table 5. Interactions between 1,1-DCE and VCM and their effect on serum AKT in fed or fasted rats.

Atmospheric concentration, ppm		Serum AKT activity, mg pyruvate/ml-hr	
VCM	1,1-DCE	Fed rats	Fasted rats
0	0	0.20–0.40[a]	
1122± 46	—	—	0.24±0.01
0	205± 7	0.80±0.35	16.00±8.95[b]
1056± 68	195±15	0.21±0.03	0.16±0.02
671±116	210± 9	0.21±0.01	0.21±0.02
201± 12	190± 7	0.24±0.05	2.00±1.46
0	1980±76	0.22±0.01	9.73±2.45[b]
12,093±929	1971±50	0.21±0.01	0.18±0.03

[a] Control range.
[b] $p < 0.05$ compared to controls; $N = 5$ per group.

significant elevation in serum AKT in fed rats. Fasted rats, however, had 30–60 fold elevation of serum AKT. The simultaneous exposure of fasted male rats to approximately 1000 ppm of VCM with a hepatotoxic concentration if 1,1-DCE (200 ppm) completely prevented the anticipated hepatotoxic response.

Vinyl chloride monomer at 671 ppm also prevented the increase in serum AKT expected in fasted 1,1-DCE-exposed rats. Only when VCM was reduced to 201 ppm was the hepatotoxic response to 1,1-DCE again demonstrated. When 1,1-DCE concentrations were increased 10-fold (approximately 2000 ppm), fasted animals were sacrificed *in extremis* before 6 hr, and they had large elevations of serum AKT activity. Vinyl chloride monomer at a concentration of 12,000 ppm completely prevented this increase of serum AKT activity. These results suggest that the protection resulted from a competitive interaction between the compounds.

Previous experiments showed that 1,1-DCE exposure caused liver levels of GSH to be reduced. When rats were exposed to VCM,

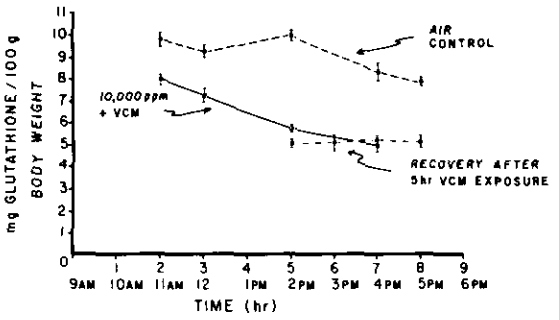


FIGURE 6. Effect of VCM exposure on hepatic glutathione concentration in fed rats. See text for details.

it was observed that liver glutathione became depleted as a result of the exposure. These data are shown in Figure 6. VCM caused a steady reduction in liver GSH concentration during an 8-hr exposure interval. Rats removed from the chamber at 5 hr did not demonstrate a recovery of liver GSH concentration to the level of nonexposed controls. Because of our previous observations which showed that diminished GSH concentrations resulted in an enhanced hepatotoxic response after exposure to 1,1-DCE, we hypothesized that VCM depletion of liver glutathione should exert a similar effect even though simultaneous VCM exposure prevented the hepatotoxic response associated with exposure to 1,1-DCE. When this hypothesis was tested, the results confirmed that diminished glutathione concentration was associated with an increase in hepatotoxic response. Results of this experiment, shown in Table 6, demonstrate the influence of a prior 5 hr VCM exposure (10,600 ppm) on the effect of a 4 hr 1,1-DCE exposure (2000 ppm) on serum AKT and SDH activity. In normal rats, 1,1-DCE did

Table 6. Effect of prior VCM exposure on serum AKT+SDH after 1,1-DCE exposure in fed rats.

Exposure conditions	N	Serum AKT, mg pyruvate/ml-hr[a]	Serum SDH, units/ml serum-min[a]
Unexposed controls	50	0.20–0.40	5–10
Air+1,1-DCE	5	0.38±0.01 (0.35–0.43)	6.2±1.0 (4.4–10.0)
VCM (5 hr)+1,1-CDE	5	3.77±1.39[b] (0.94–8.22)	548±190[b] (42–1178)

[a] Values in parentheses are ranges.
[b] $p < 0.05$ compared to animals also given 1,1-DCE but previously exposed to air. See text for details.

not cause any change in serum AKT or SDH activity. However, prior exposure to VCM for 5 hr did result in a significant enhancement of the serum AKT and SDH response. The data thus suggest that vinyl chloride monomer may protect against 1,1-DCE hepatotoxicity when given simultaneously with 1,1-DCE, but prior administration of VCM resulted in a diminished glutathione concentration and an enhanced degree of liver injury.

Conclusion

We have shown that fasted, male rats were markedly more sensitive to the hepatotoxic effects of 1,1-DCE and 2-CBD. At times of diminished GSH content, e.g. during the night, the animals were more susceptible to 1,1-DCE and 2-CBD injury. The morphologic changes observed in fasted rats were distinctive and did not resemble those which occur following CCl_4, $CHCl_3$, CHI_3, halothane, or vinyl chloride. Early changes do not involve the endoplasmic reticulum but the striking changes in the nucleus, mitochondria, and/or plasma membrane suggest that one or all three of these organelles are primarily affected by 1,1-DCE or its toxic metabolites. Acetone, a ketone, caused enhanced hepatotoxic response while cysteine, a sulfur containing amino acid found in glutathione, afforded protection against both lethality and serum AKT elevation. Data obtained in limited experiments suggested that SKF-252A was without effect at the doses employed.

Rats exposed to 2-CBD, like 1,1-DCE exposure, had a fed–fast difference associated with exposure. However, both the time course and the dose response data suggested 2-CBD to be a less potent and a less rapid-acting hepatotoxin than 1,1-DCE.

Vinyl chloride monomer was not acutely hepatotoxic in normal rats. Modest doses of VCM given simultaneously with 1,1-DCE afforded significant protection against 1,1-DCE induced hepatic injury. However, preexposure to concentrations of VCM which depleted hepatic glutathione concentration significantly enhanced the early acute hepatotoxic response to 1,1-DCE in fed rats. This synergism is consistent with a hypothesis of a common pathway which involves glutathione for both 1,1-DCE and VCM toxicity or metabolism.

Three suggestions for future investigation may be taken from this research. First, if man, like the rat, has a circadian period of hepatic glutathione concentration, night shift exposure to hepatotoxic chemicals like 1,1-DCE or 2-CBD may present a greater than normal risk to workers. Unusual patterns of food intake may add to this risk. Second, the simultaneous exposure of workers to other environmental chemicals may alter the toxic response to a hepatotoxin like 1,1-DCE. Third, alcoholism and diabetes are disease states in man that have a degree of ketosis associated with them. Alloxan diabetes in rats has been shown to enhance liver injury (*9*). If fasting or acetone exposure results in an elevated ketone level which is *per se* responsible for the enhanced response following exposure to hepatotoxins, then exposed workers with either of these disease states may have an increased risk of injury.

Acknowledgement

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