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Acute Hepatotoxicity of Ethylene and Halogenated Ethylenes after PCB Pretreatment

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Previous studies from our laboratory have shown that ethylene, vinyl fluoride monomer (VFM), vinyl chloride monomer (VCM), and vinyl bromide monomer (VBM) are all acutely hepatotoxic in rats pretreated with polychlorinated biphenyl (PCB). The time course of hepatic injury development after exposure and several parameters, environmental and chemical, affecting this toxicity were evaluated in the work reported here. Liver injury, as measured by serum alanine-α-ketoglutarate transaminase (SAKT) or sorbitol dehydrogenase (SDH), develops progressively over a 24-hr period following a 4-hr inhalation exposure of PCB-pretreated rats to ethylene or VCM. Environmental temperature during exposure to VCM does not affect hepatotoxicity or mortality below 30.3°C. At 33.8°C, however, mortality and SAKT are dramatically increased. Overnight fasting, which depletes hepatic glutathione (GSH) of PCB-pretreated rats before exposure to ethylene or VCM, significantly increases the hepatotoxicity of these compounds as measured by SDH. The combined effects of fasting and of trichloropropane epoxide (TCPE), an inhibitor of epoxide hydrase (EH), were also examined. TCPE treatment of fasted PCB-pretreated rats immediately before exposure was synergistic in increasing the acute toxicity of ethylene and VCM. TCPE increased mortality in fed or fasted rats exposed to VFM, but there was no effect of fasting alone. Both fasting and TCPE increased the sensitivity of PCB-pretreated rats to VBM, but there was not a clearly synergistic effect of fasting plus TCPE. These data suggest that the acute toxicity of these compounds is mediated through epoxide intermediates.

Introduction

We have reported that ethylene, vinyl fluoride monomer (VFM), vinyl chloride monomer (VCM), and vinyl bromide monomer (VBM) are all acutely hepatotoxic in rats pretreated with polychlorinated biphenyl (PCB) (*1*, *2*). The hepatic lesion which develops after exposure of PCB-pretreated rats to any one of these compounds is similar in histologic appearance. The exposure concentrations sufficient to cause acute hepatic injury are also similar, no matter the compound tested. These compounds are not acutely toxic without PCB pretreatment, which induces the hepatic mixed-function oxidase system (MFOS) (*3*). It is likely, therefore, that the acute toxicity of these compounds is a result of quantitative and/or qualitative changes in their metabolism after PCB pretreatment.

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This report contains the results of further investigations on the acute hepatotoxicity of ethylene, VFM, VCM, and VBM. The time course of hepatic injury development after exposure and the effects of changes in environmental parameters —temperature during exposure and food deprivation—are discussed. Data on the chemical modification of hepatotoxin metabolism are also presented.

Methods

Animals

Male Holtzman rats, 170–250 g, were used. They were housed five or six per cage and supplied with Purina Rat Chow and tap water *ad libitum*. A 12-hr day/night cycle was maintained, 6 AM–6 PM. In studies with fasted rats, food was removed 18 hr before exposure. Fasted animals were allowed water. From the start of exposure until sacrifice 24 hr later, food was removed from all animals but water was allowed.

Treatments

PCB (Aroclor 1254, Monsanto Chemical Company) and trichloropropane epoxide (TCPE, Aldrich Chemical Company) were solubilized in water by 0.5% (v/v) Tween 80 and 0.5% (w/v) methyl cellulose. Between 2 and 5 PM rats were given 300 μmoles PCB/kg by gavage, once daily for 3 days. TCPE, 1 ml/kg of a 10% solution, was given by gavage immediately before exposure to hepatotoxin. Inhalation exposure was on the day following the third PCB pretreatment.

Inhalation Exposure

Inhalation chambers as described by Leach (*4*) were modified to allow regulation of the interior temperature. Polyethylene tubing was wrapped around each chamber and connected to a regulated water supply. This water was heated or cooled as necessary to achieve the desired chamber temperature.

Inhalation exposures used highest purity gases (Matheson Gas Products) and lasted 4 hr, beginning between 9 AM and noon. Chamber concentrations were monitored by using a Hewlett-Packard 5720 A gas chromatograph (GC) with a Porapak Q column and flame ionization detector. The GC signal was quantified by a Hewlett-Packard 3373 B integrator.

Temperature Regulation

An Aminco-Aire 4-5478 D humidity and temperature control apparatus and test chamber were used to maintain specific pre- and post-exposure temperature and relative humidity for some experiments.

Biochemical Assays

Serum alanine-α-ketoglutarate transaminase (SAKT) was measured according to the method of Murphy and Malley (*5*). Sorbitol dehydrogenase (SDH) was determined by the method of Korsrud et al. (*6*). Liver nonprotein sulfhydryl, expressed as glutathione (GSH), was determined by the method of Jaeger et al. (*7*).

Statistics

Data are reported as mean ± standard error of the mean. The independent *t*-test or Mann-Whitney test (*8*) were used to examine differences between means. Fisher's exact probability test (*8*) was used to calculate the probability of increased mortality in some experiments. Analysis of variance was calculated by computer using a DataText program (*9*). A $p < 0.05$ was considered significant.

Results

Time Course of Hepatic Injury Development

VCM. Liver injury as measured by SAKT was not apparent at the end of a 4-hr exposure of PCB-pretreated rats to 30,000 ppm VCM. From 8 through 24 hr from the start of exposure SAKT values increase, and between 24 and 48 hr they decline. These data are presented in Figure 1.

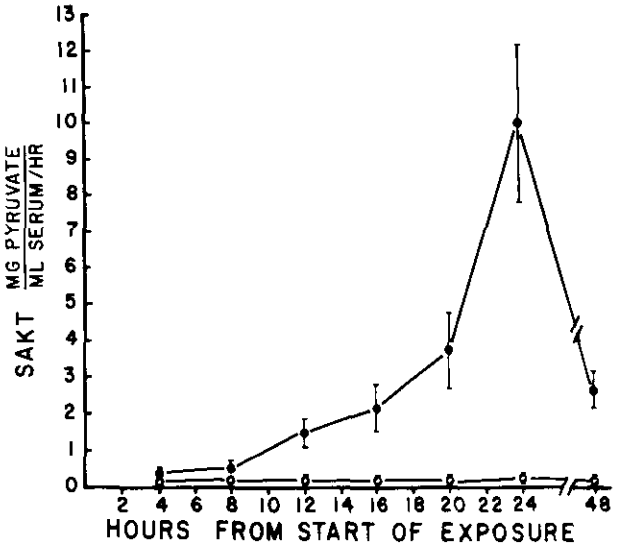

FIGURE 1. Time course of hepatic injury development as measured by SAKT in PCB-pretreated rats exposed to 30,000 ppm VCM for 4 hr: (o) PCB only; (•) PCB + VCM.

Ethylene. Ethylene's acute hepatotoxicity, as measured by SDH, follows a pattern similar to that of VCM for the 24 hr from the start of exposure. There is no measurable SDH elevation during the exposure, but post-exposure SDH values increase up to the 24 hr point. No SDH measurements after 24 hr were made. Figure 2 depicts the ethylene time course data.

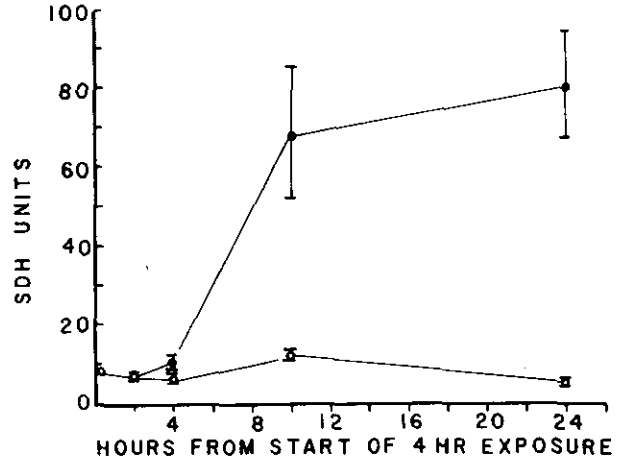

FIGURE 2. Time course of hepatic injury development as measured by SDH in PCB-pretreated rats exposed to 20,000 ppm ethylene for 4 hr: (o) PCB only; (•) PCB + ethylene.

Environmental Factors Affecting Acute Hepatotoxicity

Effect of Temperature. A series of exposures conducted to investigate the effects of differences in environmental temperature on the acute response of PCB-pretreated rats to VCM inhalation. Over a range of exposure temperatures from 12.1 ± 0.1°C to 30.3 ± 0.1°C, response to VCM as measured by SAKT and mortality did not greatly differ. As is shown in Figure 3, however, exposure to VCM at 33.8 ± 0.2°C dramatically increased both mortality and SAKT.

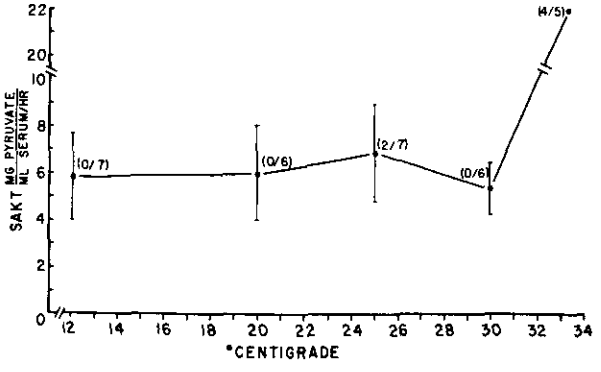

FIGURE 3. Effect of environmental temperature during exposure on SAKT and mortality in PCB-pretreated rats exposed to 25,000 ppm VCM. Mortality is given in parentheses.

Effect of Food Deprivation. Fed and fasted PCB-pretreated rats were exposed to 10,000 ppm VCM. At sacrifice, the mean SDH value for 10 fed rats was 171 ± 54 and for 10 fasted rats was 484 ± 141. The difference between these means is statistically significant. These data are the pooled results of two experiments.

Groups of five or six fed and fasted PCB-pretreated rats were exposed to 10,000, 23,000, or 53,000 ppm ethylene. Analysis of variance after log transformation of the data showed that variation due to the fed–fast difference contributed significantly to the total variation of SDH values in this experiment. These data are given in Figure 4.

Chemical Modification of Hepatotoxin Metabolism

TCPE is an inhibitor of epoxide hydrase (EH) (*10*), an enzyme which may be involved in the metabolism of the hepatotoxins discussed in this report. If epoxide intermediates of these compounds are formed, TCPE pretreatment could increase their acute toxicity through inhibition of epoxide detoxification by EH.

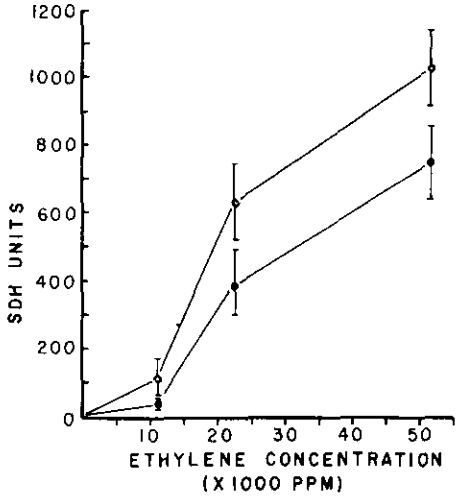

FIGURE 4. Effect of pre-exposure fasting on the acute hepatotoxic response of PCB-pretreated rats to ethylene inhalation: (•) fed + PCB + ethylene; (o) fast + PCB + ethylene. Analysis of variance after log transformation of the SDH values showed that variation due to the fed/fast difference is significant.

Effect of TCPE Pretreatment on Ethylene Hepatotoxicity. Groups of five or six PCB-pretreated rats, with and without TCPE pretreatment, were exposed to 11,000, 22,000, or 51,000 ppm ethylene. At each concentration, hepatotoxicity as measured by SDH did not differ between PCB only and PCB plus TCPE pretreatment groups.

Combined Effects of Fasting and of TCPE on Acute Toxicity. GSH, which is depleted by fasting, may, like EH, be important in the metabolism of these hepatotoxic chemicals. Decreased availability of GSH for GSH-dependent detoxification pathways could increase the acute toxicity of these compounds. Studies were conducted to examine possible additive or synergistic effects of fasting plus TCPE pretreatment in PCB-pretreated rats exposed to hepatotoxin.

As is shown in Table 1, fasting plus TCPE was synergistic in producing acute hepatic injury of PCB-pretreated rats exposed to 10,000 ppm ethylene. In this experiment there was no effect of fasting alone nor of TCPE alone.

Table 1. Effects of fasting and TCPE on the hepatotoxic and lethal responses of PCB-pretreated rats to ethylene inhalation.[a]

	SDH Units	Mortality
Fast + PCB + TCPE (not exposed)	20.0 ± 1.3	0/3
Fed + PCB (exposed)	41 ± 21	0/3
Fast + PCB (exposed)	40 ± 15	0/5
Fed + PCB + TCPE (exposed)	27 ± 3.8	0/5
Fast + PCB + TCPE (exposed)	164 ± 29[b]	0/5

[a]Ethylene concentration 10,000 ppm for 4 hr.
[b]Significantly greater than other groups by the Mann-Whitney test.

No additional effect of fasting was discernible in PCB-pretreated rats exposed to 10,000 ppm vinyl fluoride monomer (VFM). TCPE pretreatment increased mortality in both fed and fasted groups from 0/5 and 0/5 (without TCPE) to 2/6 and 1/6 (with TCPE), respectively. This increase in mortality is not significant. There was no synergistic effect of fasting plus TCPE. These data are given in Table 2.

Table 2. Effects of fasting and TCPE on the hepatotoxic and lethal responses of PCB-pretreated rats to VFM inhalation.[a]

	SDH Units	Mortality
Fast + PCB + TCPE (not exposed)	20.0 ± 1.3	0/3
Fed + PCB (exposed)	1,153 ± 196	0/5
Fast + PCB (exposed)	1,263 ± 109	0/5
Fed + PCB + TCPE (exposed)	993 ± 239	2/6[b]
Fast + PCB + TCPE (exposed)	1,313 ± 171	1/6[b]

[a]VFM concentration 10,000 ppm for 4 hr.
[b]*Total increase in mortality due to TCPE is not significant.*

Table 3 shows that the combination of fasting and TCPE pretreatment had a dramatic effect on the response of PCB-pretreated rats exposed to 10,000 ppm VCM. While no mortality occurred in fed plus PCB, fast plus PCB, nor fed plus PCB plus TCPE groups, the combination of fasting plus TCPE resulted in 4/5 dead.

Table 3. Effects of fasting and TCPE on the hepatotoxic and lethal responses of PCB-pretreated rats to VCM inhalation.[a]

	SDH Units	Mortality
Fast + PCB + TCPE (not exposed)	20.0 ± 1.3	0/3
Fed + PCB (exposed	143 ± 64	0/5
Fast + PCB (exposed)	672 ± 262	0/5
Fed + PCB + TCPE (exposed)	165 ± 26	0/5
Fast + PCB + TCPE (exposed)	466	4/5

[a]VCM concentrations 10,000 ppm for 4 hr.

Exposure of fed and fasted PCB-pretreated rats to 10,000 ppm VBM resulted in significantly higher SDH values for the fasted rats. Though TCPE alone does not appear to elevate SDH levels, this effect may be masked by the mortality (presumably due to massive hepatic injury) which occurred in TCPE pretreated groups (Table 4). The increased mortality due to TCPE was significant. A synergistic effect of fasting plus TCPE is not clear from the data, though mortality is greatest in this group (3/6).

Table 4. Effects of fasting and TCPE on the hepatotoxic and lethal responses of PCB-pretreated rats to VBM inhalation.[a]

	SDH Units	Mortality
Fast + PCB + TCPE (not exposed)	20.0 ± 1.3	0/3
Fed + PCB (exposed)	64 ± 24	0/5
Fast + PCB (exposed)	425 ± 113[b]	0/5
Fed + PCB + TCPE (exposed)	107 ± 26	1/6[c]
Fast + PCB + TCPE (exposed)	430 ± 71	3/6[c]

[a]VBM concentration. 10,000 ppm for 4 hr.
[b]Significantly different from Fed + PCB by the Mann-Whitney test.
[c]Total increase in mortality due to TCPE is significant.

Discussion

Time Course of Hepatic Injury Development

Withey (*11*) has reported on blood levels of VCM during exposure to 7000 ppm. He showed that equilibrium in blood levels is reached within 30 min from the start of exposure and that blood levels fall exponentially on termination of exposure. If acute, VCM-related, hepatic injury is due to production of reactive (and presumably short-lived) metabolites of VCM, injury will occur mainly when VCM is present. If it is assumed that there is a rapid equilibrium between liver and blood levels of VCM, injury caused by VCM metabolites would, therefore, occur during exposure and for a short time thereafter. The rise in SAKT after exposure of PCB-pretreated rats to VCM (Fig. 1) should be thought of as a developing expression of some biochemical lesion which occurred during the exposure period.

It is probable that ethylene, which like VCM has a low aqueous solubility, follows similar uptake and elimination kinetics. The gradual expression of hepatic injury after ethylene exposure (Fig. 2) may therefore, like VCM, reflect production of reactive metabolites during the exposure period.

Environmental Factors Affecting Acute Hepatotoxicity

Effect of Temperature. It is evident that the PCB-pretreated rat in a hot environment is much more susceptible to the acute hepatotoxic effect of VCM (Fig. 3). We assume here that mortality in these studies is an expression of severe hepatic injury, as opposed to depressive effects on the central nervous system. This assumption is supported by the fact that rats exposed to VCM under these hot conditions are normally active at the end of the exposure. The moribund state does not develop until some hours after the end of exposure. It is interesting to speculate on whether or not humans exposed under heat stress would be more susceptible to acute and/or chronic effects of VCM.

Effect of Food Deprivation. Both VCM and ethylene are more acutely toxic in fasted than in fed PCB-pretreated rats. A significant effect of fasting is depletion of hepatic GSH (*7*). These data suggest, therefore, that GSH may be involved in detoxification of toxic chemical species generated through metabolism of VCM and ethylene.

Chemical Modification of Hepatotoxin Metabolism

The importance of TCPE, and hence EH, in

ethylene metabolism in PCB-pretreated rats is suggested in the synergistic effect of combined fasting and TCPE pretreatment on the acute toxic effect of ethylene (Table 1). The fact that fasting alone has a greater effect on acute ethylene toxicity than does TCPE alone suggests that GSH is more important than EH in detoxifying ethylene metabolites. However, EH must be at least a minor pathway for detoxification. This implies in turn that ethylene oxide is an intermediate in the hepatic metabolism of ethylene in PCB-pretreated rats. Acute toxicity of ethylene due to epoxidation of the double bond is a mechanism analogous to that suggested for VCM, the chlorinated analog of ethylene (*12*).

The lack of effect of fasting alone and of a synergistic effect of fasting plus TCPE (Table 2) indicates that GSH may not be important in the detoxification of VFM metabolites in PCB-pretreated rats. The increased mortality in TCPE-pretreated groups suggests that EH may be involved in detoxification of the potential VFM metabolite fluoroethyleneoxide.

The TCPE-fasting data for VCM (Table 3) are analogous to those for ethylene. The effect of fasting alone is greater than that for TCPE alone. The synergistic effect on acute VCM toxicity of fasting plus TCPE strongly suggests that both GSH-dependent pathways and EH are important in the detoxification of VCM metabolites. As seems true for ethylene, GSH-dependent detoxification pathways appear more important than the EH pathway. The relative importance of EH for both ethylene and VCM metabolism must increase as GSH is depleted, however, since fasting alone does not increase acute toxicity nearly as much as does the combination of fasting plus TCPE pretreatment.

The data for VBM (Table 4) suggest that both GSH and EH are involved in the detoxification of VBM metabolites in PCB-pretreated rats. While SDH is significantly higher in fasted groups, mortality is significantly greater in TCPE-pretreatment groups; hence the data on VBM do not offer grounds for meaningful speculation on the relative importance of GSH and EH in VBM metabolism. The data do indicate that, like the other compounds discussed in this report, formation of a reactive epoxide is a significant component of the metabolism which mediates the acute hepatotoxicity of VBM.

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REFERENCES

1. Conolly, R. B., Jaeger, R. J., and Szabo, S. Acute hepatotoxicity of ethylene, vinylfluoride, vinyl chloride, and vinyl bromide after Aroclor 1254 pretreatment. Paper presented at the 16th Annual Meeting, Society of Toxicology, Toronto, Canada, March 27–30, 1977; Toxicol. Appl. Pharmacol. 41: 146 (1977).
2. Conolly, R. B., Jaeger, R. J., and Szabo, S. Acute hepatotoxicity of ethylene, vinylfluoride, vinyl chloride, and vinyl bromide after Aroclor 1254 pretreatment. In: Experimental and Molecular Pathology, in press.
3. Ecobichon, D. J., and Comeau, A. M. Comparative effects of commercial Aroclors on rat liver enzyme activities. Chem. Biol. Interact. 9: 341 (1974).
4. Leach, L. J. A laboratory test chamber for studying airborne materials. AEC Research and Development Report UR-629, University of Rochester 1963, p. 1.
5. Murphy, S. D., and Malley, S. Effect of carbon tetrachloride on induction of liver enzymes by acute stress or corticosterone. Toxicol. Appl. Pharmacol. 15: 117 (1969).
6. Korsrud, G. O., Grice, H. C., and McLaughlin, J. M. Sensitivity of several serum enzymes in detecting carbon tetrachloride-induced liver damage in rats. Toxicol. Appl. Pharmacol. 22: 474 (1972).
7. Jaeger, R. J., Conolly, R. B., and Murphy, S. D. Diurnal variation of hepatic glutathione concentration and its correlation with 1,1-dichloroethylene inhalation toxicity in rats. Res. Commun. Chem. Pathol. Pharmacol. 6: 465 (1973).
8. Armitage, P. Statistical Methods in Research. Blackwell, Oxford and Edinburgh, 1971.
9. Armor, D. J., and Couch, A. Data-Text Primer. The Free Press, New York, 1972.
10. Oesch, F., and Bentley, P. Antibodies against homogeneous epoxide hydratase provides evidence for a single enzyme hydrating styrene oxide and benz(a)pyrene 4,5-oxide. Nature 259: 53 (January 1–8, 1976).
11. Withey, J. R. Pharmacodynamics and uptake of vinyl chloride monomer administered by various routes to rats. J. Toxicol. Environ. Health 1: 381 (1976).
12. Jaeger, R. J., et al. Acute hepatic injury by vinyl chloride in rats pretreated with phenobarbital. Nature 252: 724 (December 20-27, 1974).