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Induction of Lipid Peroxidation by Hexachlorocyclohexane, Dieldrin, TCDD, Carbon Tetrachloride, and Hexachlorobenzene in Rats

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Hexachlorobenzene (HCB), 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), hexachlorocyclohexane (HCCH) and dieldrin are all halogenated lipophilic environmental contaminants (Murphy and Henry, 1985; Chadwick et al., 1979). A common biologic property of these compounds is their ability to induce hepatic microsomal drug metabolizing enzymes (Campbell et al., 1983, Viviani et al., 1978). Furthermore, exposure of laboratory animals to these xenobiotics elicits a number of similar effects including porphyria, hypothyroidism, a wasting syndrome and lethality (Rozman et al., 1986; Poland and Knutson, 1982; Taira et al., 1980). The causal relationships between the above responses and the ultimate causes of death is unknown (Rozman et al., 1986; Poland and Knutson, 1982; Chadwick et al., 1983). Perturbation of membrane lipids (Bach and Sela, 1984) and lipid peroxidation (Junqueira et al., 1986) may be responsible for at least part of the toxic effects of HCCH. TCDD has been shown to induce lipid peroxidation in hepatic and extrahepatic tissues (Al-Bayati et al., 1987). Recent studies have also shown that hepatic lipid peroxidation is induced in rats 60 days after low dose administration of HCB (Alleman et al., 1985). Shorter periods of time and high doses were not examined.

Based on the similar toxic manifestations of HCB, HCCH, TCDD and dieldrin, we have examined the effects of these xenobiotics on hepatic lipid peroxidation following an acutely toxic dose. Lipid peroxidation was assessed by determining the content of thiobarbituric acid reactive substances (TBARS) in the liver, employing malondialdehyde as the standard. Animals were also treated with carbon tetrachloride, a well known inducer of lipid peroxidation (Mico and Pohl, 1983), as a positive control. Furthermore, the ability of these xenobiotics to inhibit selenium dependent glutathione peroxidase (GSHPX) activity was determined.

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MATERIALS AND METHODS

Female Sprague-Dawley rats, weighing 140-160 gm, were obtained from Sasco Co. Inc., Omaha, NE. The animals were maintained on standard laboratory chow and water ad libitum, with a 12 hr lightand dark cycle at 21° C. The animals were acclimated to the environment for 3-5 days prior to experimental use. All animals were killed by decapitation between 7:00-8:00 a.m. to eliminate possible effects due to diurnal variation.

With the exception of HCB, all xenobiotics were dissolved in corn oil and given P.O. HCB was administered as a fine, sonicated suspension in corn oil. Control animals received the vehicle. The doses used for each compound are given in Table 1, and with the exception of hexafluorobenzene constitute approximately 56% of the reported LD_{50} values.

Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) in liver homogenates according to the method of Uchiyama and Mihara (1978). Malondialdehyde (MDA) was used as the standard. The homogenates were prepared in cold 1.15% KCl using a Potter-Elvehjem homogenizer. The extinction coefficient for MDA was 1.56×10^5 $M^{-}CM^{-1}$. Selenium dependent glutathione peroxidase activity (GSHPX) was determined by the coupled assay procedure of Paglia and Valentine (1976) as modified by Lee et al. (1981) on the cytosol fractions of liver. Hydrogen peroxide (0.25 mM) was employed as the substrate. The change in absorbance at 340 nM was measured. Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as the standard. Significant differences ($P<0.05$) between mean values were analyzed by Student's t-test.

RESULTS AND DISCUSSION

Various halogenated xenobiotics were administered to rats orally in corn oil, and the effects on hepatic lipid peroxidation and selenium dependent glutathione peroxidase (GSHPX) activity were determined 24 hours after administration (Table 1). Carbon tetrachloride is a well known hepatotoxic compound which as been shown to be a potent inducer of hepatic lipid peroxidation through the formation of trichloromethyl peroxyl radical via metabolic activation (Mico and Pohl, 1983). Lipid peroxidation (TBARS content) is expressed both as nmoles/mg protein and nmoles/g wet weight. The administration of 0.40 ml carbon tetrachloride/100 g (2740 mg/kg) resulted in greater than a 4.5-fold increase in lipid peroxidation. Treatment of rats with 50 mg/kg hexachlorocyclohexane (lindane, HCCH) produced approximately a 3.8-fold increase in TBARS content. However, massive doses of hexachlorobenzene (HCB), and hexafluorobenzene had no effect on hepatic lipid peroxidation 24 hrs after administration. Treatment of rats with a single dose of 26 mg dieldrin/kg resulted in approximately a 3-fold increase in lipid peroxidation. Twenty-four hours after the administration of 50 μg TCDD/kg a 25%

Table 1. Induction of Hepatic Lipid Peroxidation by Halogenated Xenobiotics in Female Rats

Compound	Dose (mg/kg)	Lipid Peroxidation (Malondialdehyde Content) nmoles/mg protein	Lipid Peroxidation (Malondialdehyde Content) nmoles/g wet weight	Glutathione Peroxidase Activity (nmoles/min/mg protein)
Control	-	0.36 ± 0.05	80.2 ± 13.5	0.31 ± 0.03
Carbon Tetrachloride	2740	1.64 ± 0.15*	393.4 ± 39.1*	0.21 ± 0.04*
TCDD	0.05	0.48 ± 0.04*	104.1 ± 10.3*	0.24 ± 0.02*
Hexachlorocyclohexane	50	1.32 ± 0.17*	307.8 ± 27.5*	0.30 ± 0.03
Hexachlorobenzene	5000	0.37 ± 0.09	79.1 ± 22.4	0.30 ± 0.05
Hexafluorobenzene	8000	0.39 ± 0.08	98.5 ± 17.6	0.29 ± 0.04
Dieldrin	26	0.94 ± 0.07*	274.5 ± 13.7*	0.32 ± 0.03

Rats were given the indicated compound P.O. in corn oil. Control animals received the vehicle. All animals were killed 24 hrs after treatment. Each value is the mean of 4-8 animals.
*$P<0.05$ with respect to the control group.

increase in hepatic lipid peroxidation was observed.

Previous studies have shown that the administration of polychlorinated biphenyls (Immani et al., 1979) and TCDD (Stohs et al., 1986) induce hepatic lipid peroxidation and inhibit selenium-dependent GSHPX activity. Therefore, the effects of the various xenobiotics on GSHPX activity were determined (Table 1). Only carbon tetrachloride and TCDD administration resulted in a decrease in the activity of this enzyme. HCCH, HCB, hexafluorobenzene and dieldrin had no effect on GSHPX activity.

The time courses of effect of carbon tetrachloride, TCDD, HCCH, HCB and dieldrin administration on hepatic lipid peroxidation are presented in Figure 1. Carbon tetrachloride, dieldrin and HCCH resulted in significant increases in TBARS content 12 hours after oral administration. For all three xenobiotics maximum lipid peroxidation was produced 24 hours after administration and decreased thereafter. Following a single dose of TCDD, maximum increase in hepatic lipid peroxidation was not observed until 6 days post-treatment and decreased thereafter. For HCB, a 40% increase in hepatic TBARS content was observed 9 days after treatment, the only time point at which a significant increase in lipid peroxidation was observed. Later time points were not examined.

The effects of various concentrations of HCCH 24 hrs post-treatment on lipid peroxidation are presented in Table 2. A dose of 25 mg/kg HCCH produced a 2.7-fold increase in TBARS content while doses of 50 and 100 mg/kg resulting in 3.9-fold increases in lipid peroxidation. None of the doses of HCCH altered GSHPX activity. All doses of HCCH decreased body weight, with similar results being produced by doses of 50 and 100 mg/kg.

The results demonstrate that carbon tetrachloride, HCCH, HCB, TCDD, and dieldrin induce lipid peroxidation as determined by an increase in TBARS, using MDA as the standard. With the exception of hexafluorobenzene, the dose of each of the xenobiotics which was administered was approximately 56% of the LD_{50} (Table 1 and Figure 1). Due to the low toxicity of hexafluorobenzene, no LD_{50} has been reported.

Although HCCH, carbon tetrachloride, dieldrin, TCDD and HCB all induce lipid peroxidation, the time courses of induction are quite different (Figure 1). HCCH, carbon tetrachloride and dieldrin produce maximum lipid peroxidation at approximately 24 hours post-treatment, while TCDD induced maximum lipid peroxidation at 6 days post-treatment. HCB produced a small but significant increase in hepatic lipid peroxidation 9 days after treatment. Differences in the mechanisms of initiation of lipid peroxidation may exist between these compounds. HCCH, carbon tetrachloride and dieldrin may undergo rapid metabolic activation giving rise to reactive or free radical intermediates which initiate lipid peroxidation (Junqueira et al., 1986; Haake et al., 1987). HCB is a very

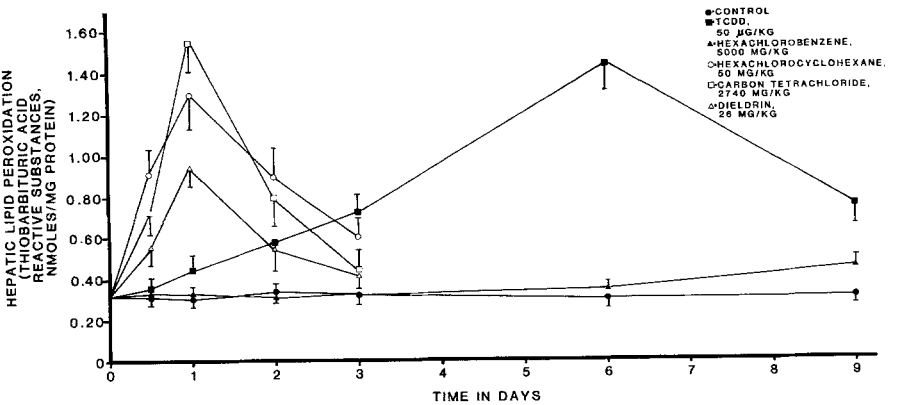

Figure 1. Hepatic lipid peroxidation (TBARS content) following the administration of various halogenated xenobiotics. Each chemical was given as a single dose in corn oil. Values are the mean ± S.D. of 4-6 animals.

stable compound which is slowly metabolized. One of the metabolites of HCB is HCCH (Debets et al., 1980), which may be further metabolized to a reactive intermediate. Due to the poor rate of metabolism of TCDD, it does not appear likely that direct metabolic activation of this xenobiotic is involved in its induction of lipid peroxidation. However, reactive oxygen species (Stohs et al., 1986) and iron (Al-Bayati and Stohs, 1987) are involved in TCDD-induced lipid peroxidation.

The results suggest that a correlation may exist between the ability to induce lipid peroxidation and other toxic manifestations as porphyria, wasting syndrome and lethality. However, it is not clear whether lipid peroxidation is a primary or a secondary effect associated with the toxicity of these compounds.

Previous studies in our laboratories (Stohs et al., 1986) have shown that TCDD inhibits the selenium dependent enzyme GSHPX. This enzyme plays an important role in the removal of hydrogen peroxide and lipid hydroperoxides from tissues. The lipid peroxidation induced by TCDD may be due in part to the inhibition of this enzyme, resulting in the accumulation of hydrogen peroxide. In this study, TCDD produced a 22% inhibition of this enzyme. Carbon tetrachloride also partially inhibited the activity of this enzyme. The other xenobiotics which were examined had no effect of the activity of GSHPX. Thus, hepatic lipid peroxidation induced by HCCH and dieldrin were not due to or associated with the inhibition of this enzyme.

Table 2. Effect of Hexachlorocyclohexane on various parameters in female rats

Dose (mg/kg)	Lipid Peroxidation (nmoles/mg protein)	Glutathione Peroxidase Activity (nmoles/min/mg protein)	Body Weight
0	0.35 ± 0.04	0.32 ± 0.03	176.8 ± 8.5
25	0.96 ± 0.13*	0.34 ± 0.03	161.5 ± 7.7*
50	1.38 ± 0.21*	0.34 ± 0.04	150.8 ± 5.9*
100	1.37 ± 0.28*	0.35 ± 0.06	149.3 ± 7.1*

Rats were treated with hexachlorocyclohexane in corn oil P.O. Control animals received the vehicle. All animals were killed 24 hrs post-treatment. Each value is the mean ± S.D. of 4-6 animals. *$P<0.05$ with respect to the control group.

The role of iron in the initiation of lipid peroxidation is well documented. Depletion of body iron stores is effective therapy for porphyria, and iron deficiency protects mice against porphyria caused by TCDD (Rowley and Sweeney, 1984). The administration of an iron deficient diet results in a marked decrease in TCDD-induced hepatic lipid peroxidation (Shara et al., 1987). Iron overload and iron depletion, respectively, potentiate and diminish the porphyrinogenic effect of HCB in rats (Blekkenhorst et al., 1980). Alleman et al., (1985) have shown that the induction of porphyria by HCB is dependent on the presence of iron, and lipid peroxidation is increased in rat liver tissue of porphyric rats. In addition, inhibition of hepatic drug metabolism or addition of compounds which are known to trap electrophiles or radicals protect against the prophyrinogenic action of HCB as well as polybrominated biphenyls (Debets et al., 1980). These investigators proposed that a reactive intermediate formed by the metabolism of the polyhalogenated hydrocarbon may react with the catalytic sulfhydryl-containing part of the enzyme uroporphyrinogen decarboxylase, inhibiting the enzyme and resulting in the formation of porphyria.

The above results suggest that a relationship may exist between lipid peroxidation, iron, porphyria and the ultimate toxicity of polyhalogenated xenobiotics.

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