Metabolism of Diazinon by Fish Liver Microsomes[1]

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Although the metabolism of certain organophosphate insecticides by fish liver preparations has been reported (1,2,3,4), diazinon apparently has not been studied. The metabolic fate of diazinon and diazoxon in vitro was recently investigated in rat liver (5,6) and in house flies, Musca domestica (7). Accordingly, we examined the metabolism of diazinon-^{14}C in vitro by hepatic subcellular preparations from channel catfish, Ictalurus punctatus.

MATERIALS AND METHODS

Ethoxy-^{14}C-diazinon (specific activity 4.3 uCi/mg) and nonradioactive samples of diazinon and diazoxon were supplied by Geigy Chemical Corporation. Diethyl phosphorothioic acid was provided by Dr. W. C. Dauterman, North Carolina State University, and diethyl phosphoric acid was obtained from Eastman Organic Chemicals, Rochester, N. Y.

Male channel catfish (200 to 300 g) were obtained from the Fish Farming Experimental Station, Bureau of Sport Fisheries and Wildlife, Stuttgart, Arkansas. The spinal cord of the fish was severed, and the liver was excised. Hepatic subcellular fractions were prepared by differential centrifugation as previously described (5).

Unless otherwise stated the incubation system for in vitro diazinon metabolism was as follows: 3 umoles of NADPH, 0.03 umoles of ethoxy-^{14}C-diazinon, and enzyme preparation equivalent to 200 mg of liver. The volume of the incubation system was adjusted to 2.0 ml with 0.05 M Tris-HCl buffer (pH 7.4). Incubation was usually for 2 hr with shaking in a water bath at 25° C. Following incubation, diazinon and its metabolites were partitioned between water and ethyl acetate.

Diazinon and certain metabolites were tentatively identified by cochromatography with authentic standards. Diazinon and diazoxon in the ethyl acetate fraction were separated by TLC on a 0.25-mm silica gel plate containing a fluorescent indicator; the chromatograms were developed with ethyl acetate:n-hexane (2:1). Water-soluble metabolites were resolved using a modification (6) of the ion exchange chromatographic method of Plapp and Casida (8). Standards were detected thru the use of the phosphorus detection method of Allen (9).

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Liquid scintillation spectrometry was used to quantify the radiocarbon-containing compounds.

RESULTS AND DISCUSSION

Metabolism of diazinon increased throughout the 2-hr incubation period; however, the rate of metabolism was most rapid during the initial 30 min. Table 1 shows that all subcellular fractions possessed some capability for diazinon metabolism when exogenous NADPH was present. However, maximum activity was evident with the microsomes; 1.6 nmoles of diazoxon and 3.8 nmoles of water-soluble metabolites were formed when 30 nmoles of diazinon were incubated with the microsomal preparation from channel catfish liver (Table 1). Thus, the total diazinon metabolism by the microsomes was about 15%; however, total metabolism was found to vary considerably with liver microsomes prepared from different channel catfish.

TABLE 1

Metabolism of diazinon-^{14}C by various channel catfish liver subcellular fractions in the presence of NADPH

	nMoles formed/2 hr/200 mg of liver		
Fraction	Diazoxon	Water-soluble	Total
Nuclei	1.1	2.2	3.3
Mitochondria	1.5	1.3	2.8
Microsomes	1.6	3.8	5.4
Microsomes (-NADPH)	0.1	0.1	0.1
Soluble	0.1	0.5	0.5
Control (no enzyme)	0.1	0.1	0.1

Table 2 shows the effect of reduced glutathione (GSH, 5 umoles) on diazinon metabolism by channel catfish liver microsomal and soluble enzymes. There was only a slight change in diazinon metabolism by both microsomal and soluble fractions in the presence of GSH. This was evidenced by an increase in water-soluble diazinon metabolites with this cofactor (Table 2).

TABLE 2

Effect of reduced glutathione on the metabolism of diazinon-^{14}C by channel catfish liver enzymes

Enzyme source	Cofactors	nMoles formed/2 hr/200 mg of liver		
		Diazoxon	Water-soluble	Total
Microsomes	None	0.2	0.1	0.3
	GSH	0.2	0.2	0.4
	NADPH	2.2	6.6	8.8
	NADPH+GSH	2.0	7.6	9.6
Soluble fraction	None	0.2	1.7	1.9
	GSH	0.2	2.4	2.6
	NADPH	0.2	1.4	1.6
	NADPH+GSH	0.1	2.2	2.3

The effect of air, carbon monoxide, and nitrogen on the metabolism of diazinon by channel catfish liver microsomal fraction is given in Table 3.

TABLE 3

Effect of air, carbon monoxide, and nitrogen on the metabolism of diazinon-^{14}C by channel catfish liver microsomal fraction

Gas	nMoles formed/2 hr/200 mg of liver		
	Diazoxon	Water-soluble	Total
None	3.7	9.5	13.2
Air	3.9	9.8	13.7
Carbon monoxide	0.5	2.2	2.7
Nitrogen	2.8	6.7	9.5

These results show that carbon monoxide is a potent inhibitor of diazinon metabolism and that oxygen is required.

In addition to the formation of diazoxon, the oxygen analog of diazinon, by channel catfish liver enzymes additional polar metabolites were also present. Anion exchange chromatography of the radioactive components in the aqueous fraction revealed at least two major compounds; one cochromatographed with diethyl phosphorothioic acid and the other with diethyl phosphoric acid. Moreover, the ratio of diethyl phosphorothioic acid to diethyl phosphoric acid was approximately 3 to 1.

A similar but abbreviated series of experiments were conducted in which microsomes isolated from bluegill, Lepomis

macrochirus, liver were incubated with ethoxy-^{14}C-diazinon. The results of these experiments indicated that diazinon was metabolized by the bluegill liver microsomal enzyme system in the presence of NADPH and oxygen and that diazoxon and water-soluble metabolites were formed.

CONCLUSIONS

The results of these experiments indicate that the in vitro metabolism of diazinon is accomplished by enzyme preparations from fish liver homogenates, with the microsomal fraction being most active. The enzyme system responsible for diazinon metabolism in channel catfish liver microsomes required NADPH and oxygen for the oxidative desulfuration of diazinon to diazoxon, and probably for cleavage of diazinon and diazoxon to diethyl phosphorothioic acid and diethyl phosphoric acid, respectively. Thus, these reactions are presumed to be catalyzed by the NADPH-cytochrome P_{450} mixed function oxidase system. The only slight increase in diazinon metabolism by channel catfish hepatic microsomal or soluble enzymes with exogenous GSH suggests that GSH-dependent enzymes were not very active.

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