Inhibition of Mitochondrial Electron Transport Systems by Phosvel and Some Environmental Conversion Products*

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Previous investigations by our laboratory concerning the chronic toxicology of various pesticides (Pardini and Heidker 1970, Heidker and Pardini 1972) have revealed that the environmental conversion products of certain nonpersistent pesticides show enzyme inhibitory properties that are considerably different than those displayed by the parent compounds.

A study designed to compare the relative effects of DDT and DDE with its photodecomposition products demonstrated that the parent compounds were inhibitory in mitochondrial electron transport systems, whereas the degradation products of these compounds were not (Pardini and Heidker 1970). In the same study, a relatively non-persistent pesticide, Sevin (carbaryl), possessed no inhibitory properties in either mitochondrial succinoxidase or NADH-oxidase system whereas dihydroxynaphthalene, a photodegradative product of Sevin was as inhibitory towards these mitochondrial enzyme systems as the most potent chlorinated pesticide tested (Pardini and Heidker 1970).

Guthion, a relatively persistent organo-phosphate insecticide has been shown to undergo photoinduced decomposition (Heidker and Pardini 1972) and other related phosphonodithioates undergo metabolic desulfuration resulting in their respective oxygen analogues (O'Brien 1967). These oxygen analogues were shown to be more potent inhibitors of acetylcholine esterase than their parent compounds (O'Brien 1967) and the converse was found to be true with respect to mitochondrial enzyme systems in which the phosphonodithioates were more inhibitory than the respective oxygen analogues (Heidker and Pardini 1972).

Recently, the photolytic sequence for Phosvel was shown to include Phosvel-oxon, phenylphosphonic acid, o-methylphenylphosphonothioic acid, and phosvel phenol (Fukuto et al. 1973). It is of interest to note that phosvel phenol, a substituted phenol, resmebles the chlorophenols produced from lindane in rats (Chadwick and Freal 1972) and some of the metabolities proposed for the PCB's (Bache and Lisk 1973). Another substituted phenol, dinitrophenol is a potent uncoupler of oxidative-phosphorylation at low concentrations and a respiratory chain inhibitor at higher concentrations (Hemker 1962). In addition, PCB mixtures (Pardini 1971, toxaphene and other chlorinated chemicals (Pardini et al.

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1971) were reported to be inhibitory towards mitochondrial electron transport systems in vitro. Consequently, we felt that it was important to evaluate the effects of this new chlorinated phosphothioate pesticide and its environmental conversion products on mitochondrial electron transport systems. The findings from these preliminary investigations are herein reported.

Methods and Materials

Heavy Beef HeartMitochondria (HBHM) were obtained as previously described (Pardini et al. 1970). The activities of the HBHM NADH-oxidase and succinoxidase enzyme systems were determined manometrically in the absence and presence of the various test compounds (Pardini et al. 1972). The various compounds were added in ethanol which was maintained at a constant level in each of the reaction flasks (0.1 ml. of ethanol in 3 ml. of reaction mixture). The various test compounds were kindly supplied by Dr. Roy Fukuto, University of California, Riverside, California.

Mitochondrial protein was determined as described (Layne 1953).

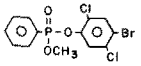
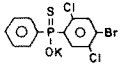
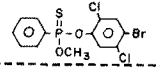
Results and Discussion

In order to classify each compound as an inhibitor or a noninhibitor in the mitochondrial electron transport system, each compound was screened for relative potency at a dose of 1 µmole per flask. The data in Table 1 represent a summary of these experiments for the NADH-oxidase and succinoxidase enzyme systems. It will be noted that three of the environmental conversion products phosvel phenol, phosvel oxon and the potassium salt of phosvel depressed NADH-oxidase activity to 8.9, 9.5 and 12.3% of the uninhibited controls respectively. Phosvel, the parent compound, and the conversion products phenylphosphonic acid and o-methylthiophenylphosphonic acid were relatively uninhibitory as the enzyme activity in their presence was depressed to only 77.5, 92.1 and 81.9% of the uninhibited controls respectively. Test compounds that depressed enzyme activity to 50% of the uninhibited controls at a concentration of 1 µmole/flask were arbitrarily considered inhibitors.

The remaining data presented in Table 1 show that the same three compounds that were inhibitory in the NADH-oxidase system were also inhibitors of the succinoxidase system with phosvel phenol, phosvel oxon and the potassium salt of phosvel depressing activity to 29.2, 10.4 and 19.5% of the uninhibited controls respectively. Phosvel, phenylphosphonic acid and o-methylthiophenylphosphonic acid were noninhibitory with the latter two compounds showing slight stimulatory properties.

In order to compare the relative inhibitory capabilities of the phosvel derivatives, we decided to determine the I_{50} value for each of those chemicals that inhibited electron transport at 1 µmole/flask (Table 1). The I_{50} value will permit a comparison

Table 1. The Effect of Phosvel and its Environmental Conversion Products on Mitochondrial NADH-Oxidase and Succinoxidase Systems.

Compound Added [a]	Structure	NADH-Oxidase		Succinoxidase	
		Specific Activity[b]	Percent Activity[c]	Specific Activity	Percent Activity[c]
Phosvel phenol		.049 ± .005	8.9 ± 0.6	.235 ± .010	29.2 ± 2.1
Phosvel oxon		.055 ± .008	9.5 ± 1.0	.079 ± .008	10.4 ± 1.5
phosvel K Salt		.064 ± .003	12.3 ± 1.0	.159 ± .006	19.5 ± 2.0
O-methyl thiophenyl phosphonic acid		.530 ± .062	81.9 ± 2.4	.869 ± .124	102.8 ± 3.7
Phenyl phosphonic acid		.575 ± .060	92.1 ± 4.0	.858 ± .080	102.8 ± 2.7
Phosvel		.473 ± .065	77.5 ± 3.7	.793 ± .067	94.6 ± 1.6

a. 1 μ mole/flask. Each flask contained .55 - .62 mg mitochondrial protein.

b. microatoms oxygen consumed/min/mg mitochondrial protein.

c. Percent of uninhibited controls.

d. Data presented as mean of 8-12 assays ± standard error of the mean.

of the relative potency of inhibition of each compound towards electron transport in that it represents that dose required to depress enzyme activity to 50% of the uninhibited controls. The titration curves presented in figure 1 demonstrate that the I_{50} values of phosvel phenol, the potassium salt of phosvel and phosvel oxon towards the NADH-oxidase system were 70, 140 and

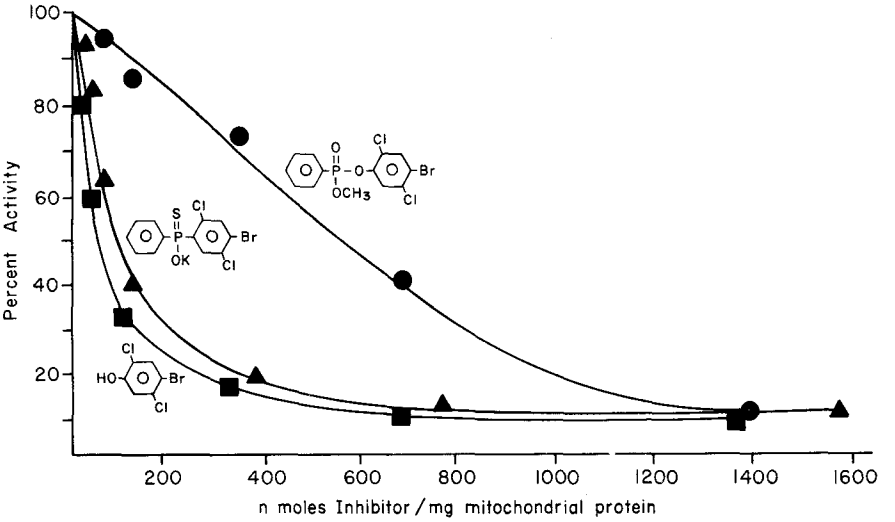


Figure 1. Titration Curves for inhibition of the mitochondrial NADH-oxidase system by environmental conversion products of phosvel.

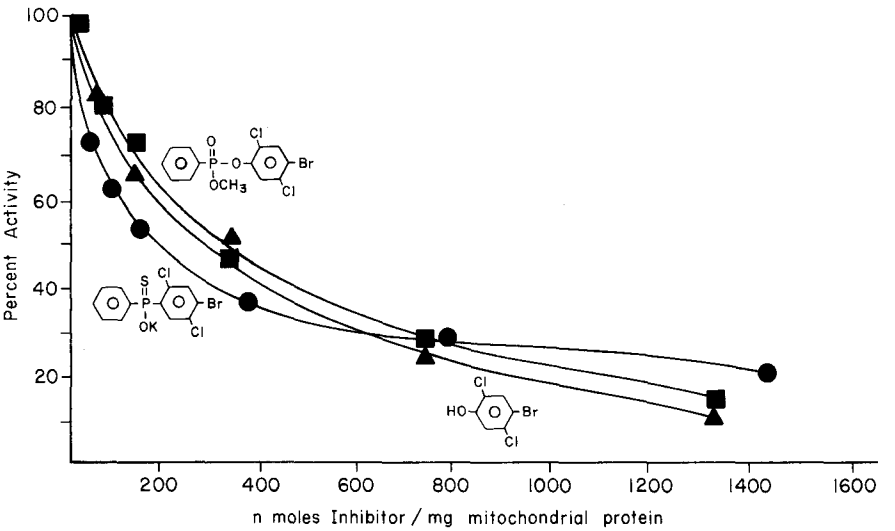


Figure 2. Titration curves for inhibition of the mitochondrial succinoxidase system by environmental conversion products of phosvel.

600 nmoles/ mg. mitochondrial protein respectively. The data in figure 2 indicate that the I_{50} values for phosvel phenol, the potassium salt of phosvel and phosvel oxon towards the succinoxidase system were 310, 210 and 320 nmoles/mg. mitochondrial protein respectively. These data show that phosvel phenol, and the potassium salt of phosvel inhibit preferentially at complex I and secondarily at complex II, since both compounds are more potent inhibitors of the NADH-oxidase than the succinoxidase enzyme system. The lower I_{50} value of phosvel oxon for the succinoxidase enzyme system indicates that this compound is a more potent inhibitor of complex II than complex I. Again this conclusion is justified by comparing the lower I_{50} value towards the succinoxidase (320) when compared to the NADH-oxidase (600) enzyme system. These conclusions are justified if one considers that the succinoxidase and NADH oxidase enzyme systems employ the same electron transfer sequence between cytochromes b and cytochrome oxidase; consequently, this differential toxicity between these two enzyme systems must be related to differential sensitivities of complexes I and II.

The presented data indicate the importance of considering environmental breakdown products as well as the parent pesticide when evaluating the chronic toxicology of an environmental chemical. Our data represent an example of the environmental conversion of a pesticide into a more toxic substance towards two non-target enzyme systems. The data presented herein demonstrate that phosvel is not inhibitory in vitro towards mitochondrial electron transport systems whereas three of its photodegradative products are inhibitory; however, since these investigations were conducted in vitro, the physiological significance of the reported findings remains to be established.

References:

BACHE, C. A. and D. J. LISK: Bull Environ. Contam. Toxicol. 9,315 (1973).
CHADWICK, R. W. and J. J. FREAL: Food Cosmet. Toxicol. 10, 789 (1972).
FUKUTO, R., S. P. SHRIVASTAVA and A. L. BLACK: Arch. Environ. Contam. Toxicol. 1, 133 (1973).
HEIDKER, J. C. and R. S. PARDINI: Bull Environ. Contam. Toxicol. 8, 141 (1972).
HEMKER, H. C.: Biochem, Biophys. Acta. 63, 46 (1962).
LAYNE, E.: Methods in Enzymol. 3, 447 (1953).
O'BRIEN, R. D.: Insecticides, Action and Metabolism pp. 43-46 (1967) Academic Press, N. Y.
PARDINI, R. S. and J. C. HEIDKER: Proc. 25th Annual N. W. Reg. Am. Chem. Soc. pp. 50 (1970).
PARDINI, R. S., J. C. HEIDKER and D. C. FLETCHER: Biochem. Pharmacol. 19, 2695, (1970).
PARDINI, R. S.: Bull. Environ. Contam. Toxicol. 6, 539 (1971).
PARDINI, R. S., J. C. HEIDKER and B. PAYNE: Bull. Environ. Contam. Toxicol. 6, 436, (1971).
PARDINI, R. S., J. C. CATLIN, J. C. HEIDKER and K. FOLKERS: J. Med. Chem. 15, 195 (1972).