Inhibition of ATPases Activity in Channel Catfish Brain by Kepone® and Its Reduction Product[1]

by D. DESAIAH and R. B. KOCH
Department of Biochemistry
Mississippi State University
Mississippi State, Miss. 39762

Modern pest control methods by chemicals cause contamination of world waterways and thus endanger the life of aquatic fauna, including fish. Although voluminous information is available on the residual toxicity and other environmental factors of the so-called hard pesticides, there is little information available on the mechanism of toxic action of these organochlorine pesticides. In recent years, the ATPase enzyme system(s) has been proposed by us (KOCH 1969b, KOCH 1969/70, CUTKOMP et al. 1971a) and others (MATSUMURA et al. 1969, AKERA et al. 1971, DAVIS et al. 1972) as a possible target site for these organochlorine compounds. Kepone[R], an organochlorine insecticide has been widely used for controlling leaf-eating insects. However, the mode of action of this compound, like other chlorinated hydrocarbons, is not clearly understood to date.

In the present work, we have examined the effects of Kepone[R] and its reduction product[2] on channel catfish brain ATPases in vitro.

Materials and Methods

The enzyme source was brain tissue from pond-cultured channel catfish, Ictalurus punctatus. The preparation of enzyme samples and enzyme assays were done as reported earlier (KOCH 1969a, DESAIAH and KOCH in this issue). Protein concentration was measured by the method of LOWRY et al. (1951). Stock solution of Kepone[R] (60 mM) and DCPD (7.5 mM) were prepared using a 50:50 mixture of acetone:ethanol. Further dilutions were made only by using ethanol. One to four microliters of the above solutions were added to the reaction mixture using a Hamilton microsyringe. The solvent at the doses used in this study had no detectable effect on ATPase activity.

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[2]decachloropentacyclo-decan-5-ol; referred to hereafter as DCPD.

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The reduced product of Kepone^{R} was obtained by the reduction of Kepone^{R} with lithium aluminum hydride (Fig. 1). It was a chromatographically-pure sample obtained from Dr. B. R. Layton, Mississippi State Chemical Laboratory, Mississippi State University.

KEPONE^{R} KEPONE^{R} REDUCTION PRODUCT

Fig. 1. Structural formulas of Kepone^{R} [1,2,3,5,6,7,8,9,10,10-decachloro-pentacyclo (5.2.1.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$)-decane-4-one] and its reduction product [1,2,3,4,6,7,8,9,10,10-decachloro-pentacyclo (5.3.0.$0^{2,6}$.$0^{3,9}$.$0^{5,8}$)-decan-5-ol].

Results and Discussion

The sensitivity of ATPases from channel catfish brain to Kepone^{R} and DCPD were determined in vitro, and the results are tabulated in Tables I, II, and III. The inhibition of Na^{+}-K^{+} ATPase by Kepone^{R} and DCPD was quite similar and there was increased inhibition with increased concentration of the inhibitors (Table I). We observed a similar response with dicofol (Kelthane^{R}) on bluegill brain Na^{+}-K^{+} ATPase (KOCH 1969/70, CUTKOMP et al. 1971a). However, a number of other organochlorines which inhibited Na^{+}-K^{+} ATPase did not show such a progressive response. Instead, a maximum inhibition of 30-50 per cent was observed (KOCH 1969/70, CUTKOMP et al. 1971a, CHU and CUTKOMP 1971, DESAIAH et al. In Press 1974).

Oligomycin-sensitive (mitochondrial) Mg^{2+} ATPase from catfish brain showed the greatest sensitivity to Kepone^{R} and DCPD (Table II). The inhibition by both compounds was very similar. The 50 per cent inhibition levels with the two compounds (Table II) were close to that of DDT in bluegill fish brain (CUTKOMP et al. 1971b).

TABLE I

Sensitivity of channel catfish brain Na^+-K^+ ATPase to Kepone^R and decachloropentacyclo-decanol (DCPD). Standard errors were calculated based on the mean of three separate enzyme determinations. Specific Activity = μmoles Pi mg^{-1} Protein hr^{-1}.

Concentration (μM)	Kepone^R		DCPD	
	Specific Activity ± S.E.	% Inh.	Specific Activity S.E.	% Inh.
none	18.51 ± 0.56		18.75 ± 0.64	
0.62	16.66 ± 0.86	9.9	15.93 ± 0.93	15.0
1.25	15.41 ± 0.60	16.7	15.13 ± 0.53	19.3*
2.5	13.50 ± 0.53	27.0**	14.29 ± 0.45	23.7**
5.0	10.51 ± 0.71	41.6***	10.78 ± 1.05	42.5**
10.0	8.05 ± 0.12	56.5***	6.39 ± 1.19	65.9***
20.0	4.93 ± 1.28	73.4***	---	---

A 3-ml reaction mixture for the continuous method contained 4.3 mM ATP, 135 mM imidazole buffer (pH 7.3), 0.2 mM NADH, 0.5 mM phosphoenolpyruvate (PEP), 0.02 per cent bovine serum albumin, 5 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+ (all three as chlorides), approximately 9 units of pyruvate kinase, 12 units of lactic dehydrogenase, and 100 μl of the B fraction. Absorbance changes were measured at 340 nm using a Gilford 2400 automatic recorder spectrophotometer with temperature controlled at 37°C. Mean values of absorbance change per minute were used for calculating specific activity.

Mg^{2+} ATPase activity was measured when 1 mM ouabain was present in the reaction mixture containing Na^+, K^+, and Mg^{2+}. Na^+-K^+ ATPase is the total ATPase activity minus Mg^{2+} ATPase activity. Mg^{2+} ATPase was further delineated into oligomycin-sensitive (mitochondrial) and oligomycin-insensitive portions by adding 0.15 μg oligomycin per ml reaction mixture. The oligomycin contained 15% of oligomycin A and 85% of oligomycin B.

Statistically significant when determined by Student's t test: *P<.05; **P<.01; ***P<.001.

TABLE II

Sensitivity of channel catfish brain oligomycin-sensitive (mitochondrial) Mg^{2+} ATPase to Kepone^R and decachloropentabyclo-decanol (DCPD). Standard errors were calculated based on the mean of three separate enzyme determinations. Specific Activity = μmoles Pi mg^{-1} Protein hr^{-1}.

Concentration (μM)	Kepone^R		DCPD	
	Specific Activity ± S.E.	% Inh.	Specific Activity ± S.E.	% Inh.
none	4.96 ± 0.59		4.58 ± 0.44	
0.62	3.56 ± 0.34	28.2	3.38 ± 0.62	26.2
1.25	2.73 ± 0.46	44.9*	2.76 ± 0.54	39.7
2.5	2.07 ± 0.42	58.2*	2.20 ± 0.47	51.9*
5.0	1.38 ± 0.36	72.1**	1.20 ± 0.35	73.8**
10.0	0.91 ± 0.22	81.6**	0.58 ± 0.07	87.3***
20.0	0.45 ± 0.05	90.9**	---	---

See the footnote of Table I for reaction conditions.

Statistically significant when determined by Student's t test: *P<.05; **P<.01; ***P<.001.

Oligomycin-insensitive Mg^{2+} ATPase activity from catfish brain also showed similar sensitivity to Kepone^R and DCPD (Table III) and the inhibition was less than that for mitochondrial Mg^{2+} ATPase. The responses of oligomycin-insensitive Mg^{2+} ATPase have been shown to vary with different types of organochlorine pesticides; for example, DDT (CUTKOMP et al. 1971b) and some acaricides (DESAIAH et al. 1972b, CUTKOMP et al. 1972) have a low inhibitory effect on this enzyme activity. Polychlorinated biphenyls, on the other hand, had a high inhibitory effect on the oligomycin-insensitive Mg^{2+} ATPase activity (DESAIAH et al. 1972a). However, Kepone^R and DCPD were intermediate in their action on this enzyme activity. The latter two compounds, like chlordane (unpublished data), were effective (greater than 50%) inhibitors of the three types of ATPase activities. The inhibition in all cases (see Tables I, II, and III) were highly significant.

TABLE III

Sensitivity of channel catfish brain oligomycin-insensitive Mg^{2+} ATPase to Kepone^{R} and decachloropentacyclo-decanol (DCPD). Standard errors were calculated based on the mean of three separate enzyme determinations. Specific Activity = μmoles Pi mg^{-1} Protein hr^{-1}.

Concentration (μM)	Kepone^{R}		DCPD	
	Specific Activity ± S.E.	% Inh.	Specific Activity ± S.E.	% Inh.
none	11.13 ± 0.47		11.16 ± 0.33	
0.62	9.03 ± 0.49	18.8	8.90 ± 0.45	20.2*
1.25	8.26 ± 0.51	25.7*	8.21 ± 0.30	26.4**
2.5	7.04 ± 0.64	36.7**	7.34 ± 0.41	34.2**
5.0	6.36 ± 0.60	42.8**	6.16 ± 0.50	44.8***
10.0	5.65 ± 0.50	49.2**	5.13 ± 0.84	54.0
20.0	3.84 ± 0.07	65.5***	---	---

See the footnote of Table I for reaction conditions.

Statistically significant when determined by Student's t test: *P<.05; **P<.01; ***P<.001.

Oligomycin-sensitive (mitochondrial) Mg^{2+} ATPase activity was more sensitive to Kepone^{R} and DCPD as compared to the other two ATPase activities tested on catfish brain homogenate. The inhibition of the mitochondrial Mg^{2+} ATPase by these compounds could possibly cause physiological responses of the types described below due to a reduction in energy (ATP) supply. ELA et al. (1970) observed a stimulation in the conversion of glucose-1-^{14}C and glucose-6-^{14}C to $^{14}CO_2$ in DDT-poisoned roaches. Non-toxic analogues of DDT, such as DDE, had no effect on these pathways. On the basis of the results obtained, ELA et al. (1970) suggested that DDT could act in vivo as an inhibitor of oxidative phosphorylation. KACEW and SINGHAL (1973a, 1973b) found a significant increase in blood glucose in rats treated with DDT and chlordane. Their results showed that the insecticide-induced stimulation of hepatic and renal gluconeogenic enzymes, as well as an increase in blood urea. These responses according to the authors, were a physiological reaction of the animal to meet the demand for energy (KACEW and SINGHAL 1973a, 1973b). Our recent findings (DESAIAH et al. In Press 1974, DESAIAH et al. 1974) showed a greater inhibition of mitochondrial Mg^{2+} ATPase of fish brain both *in vitro* and *in vivo*

by DDT. The present results indicate a similar response of mitochondrial Mg^{2+} ATPase in catfish brain by Kepone^{R} and DCPD, the greater sensitivity (I_{50}<2.5 μM) of mitochondrial Mg^{2+} ATPase indicates that the initial toxic action may be a reduction of ATP synthesis by inhibition of oxidative phosphorylation in the mitochondria.

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