The Effect of Some Cyclodiene Pesticides, Benzenehexachloride and Toxaphene on Mitochondrial Electron Transport*

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Recent concern over the widespread use of persistent pesticides (1,2) prompted our investigation on the effect of various cyclodiene insecticides, benzenehexachloride and toxaphene on mitochondrial electron transport systems. Although acute toxicity data for laboratory animals are available for all of the chemicals currently in use in agriculture (3), our understanding of the long term chronic effects of some of the persistent chemicals is not complete. In fact the mechanism of action for some of the chlorinated pesticides remains to be elucidated.

Since the cellular role of the mitochondria includes such important functions as supplying energy for other cellular processes (4), maintaining appropriate oxidation-reduction state of cellular pyridine nucleotides (5), control of cellular metabolic processes (6), and active ion translocation (7), any alteration of normal mitochondrial function on an acute or chronic basis could have profound cellular implications.

Furthermore, since mitochondrial membranes are rich in phospholipids, it seems possible that those pesticides that accumulate in body fat (8) may also be found in the mitochondrial membrane and consequently alter mitochondrial function. A classic example of a pesticide that alters mitochondrial function is Rotenone (9, 10).

These preliminary investigations were designed to evaluate the effect of various chlorinated insecticides on beef heart mitochondrial electron transport.

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Methods and Materials

Heavy beef heart mitochondria (HBHM) were obtained as previously described (11). The activity of the reduced nicotine adenine dinucleotide-oxidase (NADH oxidase) and succinoxidase systems were determined manometrically or polarographically in the absence and presence of the various pesticides (12, 13). The various pesticides and electron transport carriers were added in ethanol or water depending on their solubility. The same ethanol concentration was maintained in each of the reaction flasks (0.1 ml of ethanol in 3 ml of reaction mixture).

Mitochondrial protein was determined as described (14).

Photoaldrin and Photodieldrin were kindly supplied by J. Rosen, Department of Agricultural Chemistry, Rutgers State University, New Brunswick, New Jersey 08903.

Results and Discussion

The data presented in Table 1 indicate that heptachlor, chlordane and toxaphene depressed the mitochondrial succinoxidase system to 5.8, 21.2 and 24.1% of the uninhibited controls respectively whereas lindane, photoaldrin, dieldrin and heptachlor epoxide did not depress enzyme activity. Benzenehexachloride, aldrin, photodieldrin and endrin were considered as marginal inhibitors as their presence only depressed succinoxidase activity to 70.4, 83.1, 85.7 and 80.6% of the uninhibited controls respectively. Any pesticide that depressed enzyme activity to 25% or less of the uninhibited controls at the concentration employed (1 µmole/0.58 mg. protein or 1.7 µmoles/mg. protein) was arbitrarily considered to be an inhibitor. Any compound that depressed enzyme activity from 25-85% of the uninhibited controls was arbitrarily considered a marginal inhibitor. The remainder were considered to be non-inhibitory.

The data presented in Table II demonstrate that the addition of benzenehexachloride, aldrin, heptachlor, chlordane, and toxaphene depressed mitochondrial NADH-oxidase activity to 5.8, 10.3, 8.6, 10.1 and 3.9% of the uninhibited controls respectively whereas dieldrin and heptachlor epoxide did not depress enzyme activity. Lindane, photoaldrin, photodieldrin and endrin were considered as marginal inhibitors since their presence only depressed NADH-oxidase activity to 45.2, 46.1, 46.2 and 80.9 percent of the uninhibited controls respectively.

The effect of the NADH-oxidase inhibitors on cytochrome oxidase activity was determined by employing N,N,N^1,N^1-tetramethyl-p-phenylenediamine (TMPD) as previously described (15). The principle of employing TMPD stems from the observations that NADH non-enzymatically reduces TMPD which in turn shunts its electrons back into the electron transport chain after the cytochrome b step (16), permitting cytochrome c to participate in terminal electron transport. It then follows that inhibition of electron transport at a site on the substrate side of cytochrome c will be bypassed by the addition of TMPD whereas inhibition on the oxygen side of cytochrome c will not. The recent report (15) that demonstrates that the inhibition of mitochondrial electron transport by antimycin, rotenone, and 2-N-heptyl-4-hydroxy quinoline-N-oxide was bypassed by TMPD whereas the inhibition by cyanide was not, verifies the validity of this line of reasoning.

The data presented in Table III demonstrate that the inhibition of NADH-oxidase by lindane, heptachlor, aldrin, chlordane and toxaphene was bypassed by TMPD whereas the inhibition by benzenehexachloride was not. These data indicate that benzenehexachloride interacts at the cytochrome oxidase step whereas lindane, heptachlor, aldrin, chlordane and toxaphene inhibit electron transport on the substrate side of cytochrome c.

TABLE I

The Effect of Various Chlorinated Pesticides on the Beef Heart Mitochondrial Succinoxidase System

Compound added (1μmole/flask)	Enzyme Specific Activity (μatoms oxygen consumed/min/mg. protein		Percent
	Mitochondrial Batch[1] I	Mitochondrial Batch[1] II	
0	0.760 ± 0.026[2]	0.847 ± 0.047[2]	100[3]
Benzenehexachloride	0.535 ± 0.100	------	70.4 ± 13.2
Lindane	0.863 ± 0.036	------	113.6 ± 4.7
Aldrin	0.631 ± 0.037	------	83.1 ± 4.9
Photoaldrin[4]	------	0.860 ± 0.118	101.5 ± 13.9
Dieldrin	0.861 ± 0.070	------	113.3 ± 9.3
Photodieldrin	------	0.726 ± 0.089	85.7 ± 10.4
Endrin	0.612 ± 0.031	------	80.6 ± 4.1
Heptachlor	0.044 ± 0.010	------	5.8 ± 1.3
Heptachlor-epoxide	1.00 ± 0.049	------	131.7 ± 6.5
Chlordane	0.161 ± 0.009	------	21.2 ± 1.2
Toxaphene	0.183 ± 0.012	------	24.1 ± 1.6

1. Protein from mitochondria batches I and II were adjusted to 0.58 mg./flask.
2. Average from 6 to 15 determinations ± standard error of the mean.
3. Percent of uninhibited controls ± standard error of the mean.
4. Because of solubility difficulties, only 0.5 μmoles of photoaldrin were added in each assay flask.

TABLE II

The Effect of Various Chlorinated Pesticides on the Beef Heart Mitochondrial NADH-Oxidase System

Compound added (1µmole/flask)	Enzyme Specific Activity (µatoms oxygen consumed/min/mg. protein)		Percent
	Mitochondial Batch[1] I	Mitochondrial Batch[1] II	
0	1.317[2] ± 0.211	1.012[2] ± 0.081	100[3]
Benzenehexachloride	0.077 ± 0.009	-----	5.8 ± 0.7
Lindane	0.595 ± 0.036	-----	45.2 ± 2.7
Aldrin	0.136 ± 0.018	-----	10.3 ± 1.4
Photoaldrin[4]	------	0.466 ± 0.100	46.1 ± 9.9
Dieldrin	1.672 ± 0.362	-----	126.9 ± 27.5
Photodieldrin	------	0.468 ± 0.168	46.2 ± 16.6
Endrin	1.065 ± 0.032	-----	80.9 ± 2.4
Heptachlor	0.114 ± 0.018	-----	8.6 ± 1.4
Heptachlor epoxide	1.552 ± 0.271	-----	117.8 ±20.6
Chlordane	0.133 ± 0.027	-----	10.1 ± 2.1
Toxaphene	0.051 ± 0.014	-----	3.9 ± 1.0

1. Protein from mitochondria batches I and II were adjusted to 0.58 mg./flask.

2. Average of from 3 to 5 determinations ± standard error of the mean.

3. Percent of uninhibited controls ± standard error of the mean.

4. Because of solubility difficulties only 0.5 µmoles of photoaldrin were added in each assay flask.

TABLE III

Effect of N,N,N^{1},N^{1}-tetramethyl-p-phenylenediamine (TMPD) on The Inhibition of Mitochondrial Electron Transport by Various Chlorinated Pesticides

Compound added (2.5 μmoles/flask)	Percent Enzyme Activity[1]	
	0	+ TMPD
0	100[2]	
Benzenehexachloride	5.5	36.4
Lindane	6.7	147.1
Heptachlor	5.9	88.2
Aldrin	6.7	83.3
Chlordane	14.6	111.1
Toxaphene	7.3	115.0

1. Percent of uninhibited controls.

2. These values represent single determinations determined polarographically; hence, the values for uninhibited, inhibited and inhibited + TMPD were obtained consecutively in the same flask for each of the pesticides tested. The uninhibited value was set at 100%.

An important comparison may be made between benzenehexachloride (a mixture of isomers) and lindane (benzenehexachloride γ-isomer). Benzenehexachloride inhibits both the NADH-oxidase and succinoxidase systems to a greater extent than does lindane at the concentration employed. In addition, when the insecticide concentrations are increased lindane does not appear to affect cytochrome oxidase activity whereas benzenehexachloride is inhibitory to this terminal electron transport system. These preliminary findings suggest that isomers of benzenehexachloride may differ in their toxicity towards mitochondrial electron transport and may interact at different loci in the electron transport sequence.

Photodieldrin, photoaldrin and aldrin depressed NADH-oxidase activity to below 50% of the uninhibited controls but did not inhibit succinoxidase activity at the concentration employed. This observation suggests that the site of interaction of these compounds is at complex I (NADH-Coenzyme Q Reductase) since complex I is unique to the NADH-oxidase system.

Heptachlor, chlordane and toxaphene were the only pesticides tested that inhibited both NADH-oxidase and succinoxidase activity. Since these insecticides did not interact after cytochrome c (Table III), the site of interaction may be either at complex III (Coenzyme Q - Cytochrome c Reductase) or at complexes I and II (NADH-Coenzyme Q Reductase and Succinate-Coenzyme Q Reductase respectively). The presented data do not differentiate inhibition at complex III from inhibition at complexes I and II.

Although these preliminary studies suggest that some of the chlorinated insecticides alter mitochondrial electron transport in vitro, further investigations must be conducted to determine if this demonstrated enzyme inhibition is in fact a significant factor in the toxicology of these insecticides in the intact organism.

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