Polychlorinated Biphenyls (PCB): Effect on Mitochondrial Enzyme Systems*

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The identification of polychlorinated biphenyls PCBs as contaminants in pesticide residue analysis (1) is responsible for recent concern over environmental pollution by these industrial chemicals (2). Indeed, the PCBs have been detected in the natural environment in the same or greater order of magnitude as the chlorinated pesticides (1,3,4). More recently, an aquatic environment of Florida (5), and eagle eggs (6), and carcasses (7) were found to contain residues of the PCBs. That humans are not exempt from this form of pollution was recently demonstrated by the observation that human adipose tissue also contained residual PCB material (8).

The toxic nature of PCBs is recognized (9-12). Furthermore, reports that dietary PCBs altered serum lipid patterns in mice (13) and may affect steroid hormone metabolism in birds (2) emphasizes our need for further investigations on the toxicology of the PCBs.

Recent reports that DDT (14) and other chlorinated insecticides (15) inhibited mitochondrial electron transport systems provided the incentive for investigating the effect of the PCBs on mitochondrial NADH-oxidase and succinoxidase systems.

METHODS

Heavy beef heart mitochondria (HBHM) were obtained as described (16). Manometric determination of HBHM NADH-oxidase and succinoxidase enzyme systems was conducted in the absence and presence of the various PCB samples (15,17). The PCBs were added to the reaction mixture in ethanol. The same ethanol concentration was maintained in each of the assay flasks (0.1 ml ethanol in 3 ml of reaction buffer). Coenzyme Q was added (100 nmoles per assay flask) to each of

*A contribution of the Nevada Agriculture Experiment Station, Reno, Nevada Journal Series Number 185.

Bulletin of Environmental Contamination & Toxicology,
Vol. 6, No. 6, 1971, published by Springer-Verlag New York Inc.

the reaction flasks used in the NADH-oxidase experiments to insure of maximal enzyme activity.

Mitochondrial protein was determined as described (18).

RESULTS AND DISCUSSION

The effect of the various PCB samples on HBHM, NADH-oxidase and succinoxidase systems was determined and the data presented in Tables I and II respectively. The data presented in Table I demonstrate that all of the PCBs tested are inhibitory to the NADH-oxidase system, since they all depressed enzyme activity to below 20% of the uninhibited controls. Similarly, all of the PCB samples tested depressed succinoxidase activity to below 20% of the uninhibited controls (Table II). These findings suggest that at the concentrations employed, the degree of chlorination of PCB material between the range of 21 - 62% is of minor structural significance in relation to electron transport inhibition. These data however do not permit a strict comparison of potency between the various PCB samples, since potency comparisons would necessitate experiments designed to determine that dose required to elicit a given inhibition response. Titration of enzyme activity with the various PCB mixtures was not conducted during the course of these investigations, but will be conducted in the future employing purified PCB components.

The effect of PCBs on cytochrome oxidase activity was assessed by determining the ability of N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) to bypass the inhibition of NADH-oxidase caused by the PCBs (15,17). That this indirect measurement of cytochrome oxidase inhibition is valid is supported by the finding that the inhibition of NADH-oxidase caused by rotenone, antimycin and 2-N-heptyl-4-hydroxyquinoline-N-oxide, but not cyanide, was bypassed by TMPD (17).

Table 1 - The Effect of Various Polychlorinated Biphenyls on the Mitochondrial NADH-oxidase System

Compound Added[1]	Enzyme Specific Activity (µatoms oxygen consumed/min./mg. protein)						Percent[2]
	I	II	III	IV	V	VI	
0	1.123	1.123	0.902	0.804	0.818	0.749	100
Arochlor 1221	0.069	0.055	0.111	0.125	0.083	0.069	5 - 15
Arochlor 1232	0.055	0.069	0.069	0.125	0.083	0.111	5 - 15
Arochlor 1242	0.069	0.042	0.069	0.014	0.069	0.069	0 - 10
Arochlor 1248	0.055	0.083	0.083	0.083	0.083	0.083	5 - 10
Arochlor 1254	0.055	0.069	0.111	0.139	0.069	0.055	5 - 20
Arochlor 1260	0.111	0.055	0.125	0.097	0.125	0.097	5 - 20
Arochlor 1262	0.055	0.083	0.153	0.097	0.156	0.055	5 - 20
Arochlor 5442	0.139	0.111	0.166	0.111	0.156	0.139	10 - 20

1. All PCB additions were at one µmole per flask. The average molecular weight of the PCB mixtures was calculated based on the percent chlorination designated in the last two digits of the Arochlor number. The mitochondrial protein was at 0.52 mg. per flask. The first two digits of the Arochlor designation represent the following: 12 = biphenyl, 54 = triphenyl.
2. Percent of the uninhibited controls respectively.

Table 11 - The Effect of Various Polychlorinated Biphenyls on the Mitochondrial Succinoxidase System

Compound Added[1]	Enzyme Specific Activity (μatoms oxygen consumed/min./mg. protein)						Percent[2]
	I	II	III	IV	V	VI	
0	0.439	0.735	0.700	0.638	0.735	0.532	100
Arochlor 1221	0.086	0.097	0.097	0.104	0.104	0.043	5 - 20
Arochlor 1232	0.060	0.097	0.104	0.049	0.083	0.077	5 - 15
Arochlor 1242	0.060	0.062	0.097	0.035	0.062	0.069	5 - 15
Arochlor 1248	0.043	0.105	0.097	0.021	0.055	0.120	0 - 20
Arochlor 1254	0.000	0.069	0.111	0.021	0.097	0.052	0 - 15
Arochlor 1260	0.000	0.076	0.118	0.042	0.083	--	0 - 15
Arochlor 1262	0.026	0.083	0.069	0.042	0.049	--	5 - 15
Arochlor 5442	0.089	0.222	0.194	0.118	0.153	--	15 - 30

1. All PCB additions were at one μmole per flask. The average molecular weight of the PCB mixtures was calculated based on the percent chlorination designated in the last two digits of the Arochlor number. The mitochondrial protein was at 0.52 mg. per flask. The first two digits of the Arochlor designation represent the following: 12 = biphenyl, 54 = triphenyl.
2. Percent of the uninhibited controls respectively.

Table III - Effect of N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD) on the Inhibition of Mitochondrial Electron Transport by PCBs

Compound Added[1]	NADH-OXIDASE SPECIFIC ACTIVITY (µatoms oxygen consumed/min./mg. protein)				Percent[2]	
	I		II			
	-TMPD	+TMPD	-TMPD	+TMPD	-TMPD	+TMPD
0	0.610	--	0.593	--	100	--
Arochlor 1221	0.028	0.555	0.097	0.610	5 - 15	90 - 105
Arochlor 1232	0.111	0.596	0.069	0.596	10 - 20	95 - 105
Arochlor 1242	0.125	0.610	0.083	0.610	10 - 20	100 - 105
Arochlor 1248	0.097	0.569	0.115	0.583	15 - 20	90 - 100
Arochlor 1254	0.083	0.596	0.097	0.424	10 - 20	75 - 100
Arochlor 1260	0.139	0.610	0.097	0.525	15 - 25	90 - 100
Arochlor 1262	0.180	0.596	0.111	0.513	15 - 30	85 - 100
Arochlor 5442	0.083	0.624	0.153	0.513	10 - 25	85 - 105

1. All PCB additions were at one µmole per flask. The average molecular weight of the PCB mixtures was calculated based on the percent chlorination designation in the last two digits of the Arochlor number. The mitochondrial protein was at 0.52 mg. per flask. The first two digits of the Arochlor designation represent the following: 12 = biphenyl, 54 = triphenyl.
2. Percent of the uninhibited controls respectively.

The data presented in Table III indicate that inhibition of NADH-oxidase by all of the PCBs tested was bypassed by TMPD; hence, the site of interaction of the PCBs in mitochondrial electron transport systems is on the substrate side of cytochrome c, i.e., NADH-cytochrome c reductase and succinate-cytochrome c reductase.

An earlier report indicated that out of eleven chlorinated insecticides tested, three inhibited both mitochondrial NADH-oxidase and succinoxidase systems (15). In contrast, all of the PCBs tested in the present study inhibited both mitochondrial enzyme systems. Although the physiological significance of these *in vitro* studies remains to be established *in vivo*, these data further demonstrate the toxic nature of the PCBs.

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