The Effect of DDT and PCB on Lipid Metabolism in *E. coli* and *B. fragilis*

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DDT and PCB, chlorinated hydrocarbons similar in structure and physiological effects (LICHTENSTEIN, et al, 1969), are of environmental concern because of their ubiquity, magnification and effects in natural food chains (COX, 1970; MACEK and KORN, 1970; GOULD, 1972; McLANE and HALL, 1972). Recent work has also suggested that DDT could be unrecognized source of PCB (MAUGH, 1973). These chemicals have been of concern in human health because of their accumulation in the fatty tissues of man (DEICHMAN, RADOMSKI, 1968). A number of workers have shown a statistical association between DDT and its metabolites to risk factors of heart disease (ROAN, MORGAN, 1973; SANDIFER, KEIL, 1972) and to other pathological states (HEINRICHS, et al, 1971; O'LEARY, et al, 1970a; O'LEARY, et al, 1970b). Conversely, DDT and its metabolites have been screened as chemotherapeutic agents for neoplastic diseases (WALKER, et al, 1970; TEMPLE, et al, 1969).

In this experiment Escherichia coli and Bacteroides fragilis were selected as models to observe the effects of DDT and PCB on lipid metabolism in bacteria. Since these species comprise about 90% of the intestinal bacteria in areas where lipid absorption occurs (DAVIES, et al, 1970), it was also of interest to study the metabolism of DDT and PCB in these organisms.

Methods

E. coli was cultured in one liter Erlenmeyer flasks containing 500 ml Trypticase Soy Broth. B. fragilis was grown in 60 ml Scott Anaerobic Broth bottles. Toxicant treatment solutions were prepared with Geigy 99.9% pp'DDT and PCB (Aroclor 1242 ®) dissolved in acetone, (Nanograde, Mallinckrodt Chemical Works).

Each species of bacteria was treated with .01 ppm DDT, .1 ppm DDT, .01 ppm PCB, .1 ppm PCB and acetone (solvent control). Each treatment consisted of four replicates. E. coli and B. fragilis cultures were incubated at 35C for 24 and 49 hours, respectively. Culture flasks were agitated every four hours during the incubation period. Upon completion of incubation, each

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replicate culture was centrifuged at 2000 xg and the pellet was lyophilized and weighed. Total lipids were extracted from the lyophilized samples (VORBECK, MARINETTI, 1965) and the solvent was evaporated under a stream of nitrogen in tared vials. The residue was weighed, brought up to a volume of 5 ml in chloroform-methanol (2:1) and stored at 4C in Teflon-lined screw-cap vials. Total lipids were determined from aliquots containing approximately 100 ug of total lipid by chromic acid oxidation (PANDE, et al, 1963) using a standard consisting of a known concentration of total lipids extracted from a large culture of E. coli. All solvents were distilled.

DDT and its metabolites were quantitated by gas-liquid chromatography on two different columns. Aliquots of the samples in chloroform-methanol were evaporated to dryness under a stream of nitrogen and were made up to volume in hexane (Matheson, Coleman and Bell, nanograde). Determinations were obtained on a Tracor 220 gas chromatograph fitted with two 6 ft x 2 mm ID glass columns and tritium-foil electron capture detectors. Primary determinations were obtained with a column packed with a mixed stationary phase consisting of 1.5% OV-17 and 1.95% QF-1 on 100/120 Chromasorb WHP. Determinations were confirmed on another column packed with a mixed stationary phase consisting of 4% SE-30 and 6% QF-1 on 80/100 Chromosorb WHP. Operating parameters were: inlet 235C, column oven 200C, and detector 205C. The flow-rate of the nitrogen carrier gas was 70 cm^3/min for the OV-17, QF-1 column and 80 cm^2/min for the SE-30, QF-1 column.

Results

One way analysis of variance allowed calculation of least significant difference at the 95% probability level, for different numbers of replicate comparisons.

As shown in Table 1, only the .01 ppm DDT treatment group showed a significant increase in dry weight in the E. coli series. This parallels the findings of Keil, et al (KEIL, et al, 1972). There was no significant difference in total lipid yields of any treatment group as compared to the acetone control. PCB had no detectable effect on E. coli growth or lipid yield.

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TABLE 1

Harvest weights, toxicant residues, and lipid yields of *Escherichia coli* cultures treated with DDT and PCB

Treatment	No. Replicates	Mean Harvest Weight (mg)	Mean Toxicant Residues (ug)	Mean Lipid Yields (ug/mg dry wt)
.01 ppm DDT	4	311.3	2.05**	38.9
.1 ppm DDT	4	219.5	14.97**	36.6
.01 ppm PCB	4	257.3	N.D.	36.8
.1 ppm PCB	3	288.9	N.D.	37.7
Control	3	235.9	0	31.9
Acetone Control	4	220.1	0	35.3
LSD_{05}*	4/4	51.6		4.8
	3/4	31.8		9.5

*LSD = Least significant difference at 95% probability level calculated from one way variance analysis.
**Total DDT = DDT + 1.114 (DDE + DDD)
N.D. = Not Determined

As shown in Table 2, .01 ppm DDT significantly depressed the growth of *B. fragilis* and .1 ppm PCB significantly stimulated growth. Neither dosage of DDT had any effect on *B. fragilis* lipid synthesis, but PCB caused a significant and apparently dose-related depression of lipid synthesis.

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TABLE 2

Harvest weights, toxicant residue, and lipid yields of Bacteriods fragilis cultures treated with DDT and PCB

Treatment	No. Replicates	Mean Harvest Weight (mg)	Mean Toxicant Residues (ug)	Mean Lipid Yields (ug/mg dry wt)
.01 ppm DDT	4	93.0	4.2**	36.9
.1 ppm DDT	4	99.0	68.38**	36.7
.01 ppm PCB	4	137.4	N.D.	23.2
.1 ppm PCB	2	210.4	N.D.	16.2
Control	4	119.7	0	34.9
Acetone Control	4	109.9	0	35.7
LSD_{05}*	4/4	26.0		7.8
	2/4	31.8		9.5

*LSD = Least Significant Difference at 95% probability Level Calculated from one way analysis of variance
**Total DDT = DDT + 1.114 (DDE + DDD)
N.D. = Not Determined

TABLE 3

Comparison of DDT metabolites in E. coli and B. fragilis

DDT Media Concentration	Escherichia coli		Bacteroides fragilis	
	DDD	DDE	DDD	DDE
.01 ppm DDT	95%	5%	100%	0%
.1 ppm DDT	98%	2%	93%	7%

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In Table 3 it is shown that of the DDT metabolites, DDD was the principal metabolite in both species, although DDE was present in small to trace quantities.

Discussion

An important finding was that conversion of DDT by E. coli and B. fragilis was primarily to DDD, a metabolite easily hydroxylated and excreted in urine, thus demonstrating that these key aerobic and anaerobic human bacteria are capable of participating in the degradation of this insecticide.

Although neither DDT nor PCB significantly altered total lipid levels in these organisms, there remains the possibility of changes in ratios of lipid classes. There appears to be no relationship between DDT residues and lipid levels although both PCB treatments significantly depressed lipid production by B. fragilis. While these findings are not conclusive, it does suggest that if there is a relationship between DDT exposure and blood pressure and cholesterol, it may be via a mechanism other than stimulation of lipid synthesis.

Values approaching the extreme limits of $LSD_{.05}$ were discounted because of the inaccuracies arising from the small number of treatments.

Summary

Escherichia coli and Bacteroides fragilis, principal human enteric aerobic and anaerobic bacteria, were cultured with suitable controls in the presence of .01 and .1 ppm of DDT and PCB. The bacteria were assayed for dry weight and total lipids. DDT increased the growth of E. coli at the .01 ppm level but had no detectable effect at .1 ppm. Total lipid content was not significantly altered by either DDT or PCB.

DDT at .01 ppm depressed B. fragilis growth, but had no significant effect on lipid levels. PCB increased the growth of B. fragilis significantly at the .1 ppm level, but significantly depressed lipid synthesis at both concentrations. The effect was apparently dose dependent. All cultures metabolized DDT to DDD. Trace amounts of DDE were also found in several replicates.

DDT/PCB are statistically associated with cardiovascular disease risk factors, but the mechanism does not appear to be that of increased lipid anabolism by enteric bacteria.

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