Water Research Pergamon Press 1972. Vol. 6, pp. 837–841. Printed in Great Britain.

DDT AND POLYCHLORINATED BIPHENYL (AROCLOR 1242®) EFFECTS OF UPTAKE ON *E. COLI* GROWTH

J. E. KEIL* and S. H. SANDIFER

Section of Preventive Medicine, Medical University of South Carolina

C. D. GRABER

Microbiology Department, Medical University of South Carolina

and

LAMAR E. PRIESTER

Environmental Health Laboratories, State Board of Health, Columbia, South Carolina

(*Received* 1 *February* 1972)

Abstract—DDT at 0.01 and PCB at 0.01 and 0.1 ppm consistently stimulated *E. coli* growth *in vitro*. Differences between treatments and controls were statistically significant at the 95 per cent confidence level.

E. coli uniformly converted DDT to DDD (75 per cent) and DDE (25 per cent).

There was no difference in nucleic acids content between treatments and controls at the conclusion of 24 hours incubation although increased uridine uptake was noted in all DDT and PCB cultures after 5 h of incubation.

INTRODUCTION

DDT AND polychlorinated biphenyls (PCBs), similar to each other in structure and physiological effects (LICHTENSTEIN *et al.*, 1969), are of concern to the scientific community because of their ubiquity (RISEBOROUGH *et al.*, 1968) and persistence (WURSTER and WINGATE, 1968). The alleged lack of biodegradability of these environmental pollutants has caused alarm and bans of these useful chemicals. Work as early as 1963 by COPE and SANDERS indicated that DDT levels could be significantly lowered by several species of bacteria. Subsequently, other workers (CHACKO, LOCKWOOD and ZABIK, 1966; STENEVSEN, 1965; WEDEMEYER, 1966) reported degradation of DDT by a variety of bacteria and fungi. KEIL's work (1969) also pointed to the metabolism of this insecticide by marine diatoms. With the thesis established that DDT was indeed biodegradable, the authors postulated that human flora may play an important part in the metabolism of DDT, PCB and other ecological contaminants.

Research was performed to study the effects of these chemicals *in vitro* on a facultative organism, common to human intestinal flora, that is the prime indicator of fecal contamination.

MATERIALS AND METHODS

The study organism, *Escherichia coli*, was selected because it is a common autochthonous, human intestinal aerobe, easily cultured, and is an accepted standard indicator of fecal contamination.

Initial work screened a wide range (0–1000 ppm) of DDT and PCB concentration, against *E. coli* by use of impregnated paper filter discs similar to those used to test antibiotic sensitivity. Impregnated discs with DDT and PCB concentrations of 0,

® Registered Trade Mark, Monsanto Chemical Co., St. Louis, Mo.

* JULIAN E. KEIL, M.S., Associate in Preventive Medicine, Medical University of South Carolina, 80 Barre Street, Charleston, South Carolina, 29401, U.S.A.

DSW 031132

STLCOPCB4015094

0.001, 0.01, 0.1, 10, 100, and 1000 ppm were placed on trypticase soy broth (TSB) agar plates which had been inoculated with *E. coli*. Experimentation indicated that 6 mm diameter discs cut from No. 2 Wattman filter paper would absorb 0.02 ml of the acetone solvent. The discs were dried prior to use.

For the first experimental series (I) twenty-eight 1-l. Erlenmeyer flasks, each with 500 ml TSB, were inoculated with *E. coli*. Four flasks served as controls, four for each of two levels of DDT and PCB treatments, and four as acetone controls since acetone was the suspending vehicle for the toxicants. Additionally, four flasks were used as uridine controls. One flask, containing only media, was used as a growth check. Each flask was vigorously agitated every 2 h during the first and last 8 h of culture time.

At the conclusion of a 24-h incubation period, flask contents were centrifuged, the resulting pellet lyophilized, weighed and assayed for DDT, PCB, nucleic acids and tritiated uridine (^3H_U) uptake.

A second experiment (Series II) identical in treatments except for uridine, was conducted to confirm stimulation patterns observed in Series I. Series II measured lyophilized harvest weights only.

A third trial (Series III) identical in design to Series I, assayed tritiated uridine uptake after five hours and eight hours incubation time to determine if assimilation rates were affected by DDT or PCB during the logrithmic growth phase. Analytical grade p,p DDT (99.9 per cent) and Aroclor 1242® (PCB—42 per cent chlorine) were used as sources of active ingredients in these experiments.

Bacteria samples were extracted with nanograde hexane and a 5 μl aliquot was injected into the gas chromatograph for analysis.

Two column systems were used: a 6 ft $\times$ $\frac{1}{4}$ in. o.d. glass column packed with 3 per cent SE-30 on 60–80 ABS chromosorb W and 2 per cent QF-1 on 60–80 ABS chromosorb W. The operating parameters were: (1) inlet temperature 235°C; (2) column temperature 200°C isothermal; (3) detector temperature 240°C; (4) flow-rate 70 cm^3 min^{-1} N_2.

Qualification was accomplished by measurement of the absolute and relative retention times of five major peaks of the PCB. Quantitation was accomplished by determination of the area under the curve of the five major peaks. Utilizing this technique, recoveries of 92.4 per cent of the PCB were experienced.

DDT and analogues were determined by direct hexane extraction of the lyophilized bacteria by gas chromotography, utilizing the identical system as described for the PCB.

RNA and DNA were measured using a modification of Agranoff's procedure (AGRANOFF and SANTEN, 1963). Radio assays utilized the Nuclear-Chicago Mark I Liquid Scintillation Spectrometer.

RESULTS AND DISCUSSION

As may be seen from FIG. 1 concentrations up to 1000 ppm of DDT or PCB impregnated into filter paper discs failed to inhibit *E. coli* growth on TSB agar.

As shown in TABLE 1 (Series I) the addition of 0.01 and 0.1 μg ml^{-1} DDT and PCB to TSB markedly stimulated *E. coli* growth above control levels. This is consistent with other microorganism work (KEIL and PRIESTER, 1969; KEIL, PRIESTER and

DSW 031133

STLCOPCB4015095

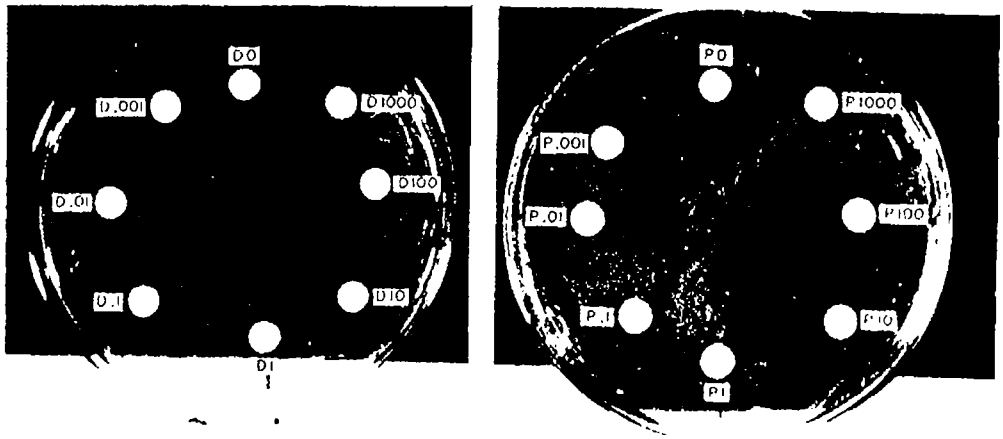

FIG. 1. Sensitivity screening of *E. coli* on TSB to 0, 0.001, 0.01, 0.1, 1, 10, 100 and 1000 ppm of A, DDT; B, PCB.

(*Facing p.* 838)

DSW 031134

STLCOPCB4015096

TABLE 1. LYOPHILIZED WEIGHTS AND TOXICANT UPTAKE BY *Escherichia coli* CULTURES EXPOSED TO DDT AND PCB (BACTERIAL SERIES I)

Treatment	Mean* harvest weights (mg)	Mean residue ($\mu g\ g^{-1}$)
DDT 0.1 $\mu g\ ml^{-1}$	51.1	11.2‡
DDT 0.01 $\mu g\ ml^{-1}$	115.9	2.2‡
PCB 0.1 $\mu g/ml^{-1}$	86.0	13.6
PCB 0.01 $\mu g\ ml^{-1}$	90.4	4.4
Control	44.5	0
Acetone control	27.2	1.2
Uridine control	41.6	0
LSD_{05}†	13.6	10.8

* Mean of four replicates.
† LSD_{05} = Least significant at 95 per cent probability level calculated from one way analysis of variance.
‡ Total DDT = DDT + 1.114 (DDE + DDD).

SANDIFER, 1971) showing that DDT and PCB are concentrated up to 1000 times by marine diatoms. The data also suggest that the concentration factor may be inversely related to dosage.

TABLE 2. LYOPHILIZED WEIGHTS OF *Escherichia coli* CULTURES EXPOSED TO DDT AND PCB (BACTERIAL SERIES II)

Treatment	Mean* harvest weights (mg)
DDT 0.01 $\mu g\ ml^{-1}$	126.7
DDT 0.01 $\mu g\ ml^{-1}$	176.0
PCB 0.1 $\mu g\ ml^{-1}$	186.4
PCB 0.01 $\mu g\ ml^{-1}$	153.2
Control	160.8
Acetone control	133.6
LSD_{05}†	23.6

* Mean of four replicates.
† LSD_{05} = Least significant difference at 95 per cent probability level calculated from one way analysis of variance.

TABLE 2 presents data from a duplicate experiment (Series II) but where only harvest weights were studied. This second series confirmed apparent stimulatory effects of all levels of PCB and the lowest dosage (0.01 $\mu g\ ml^{-1}$) of DDT. The highest DDT dosage did not stimulate *E. coli* growth as significantly as in experimental Series I. FIGURE 2 presents growth levels of *E. coli* in both experimental series I and II and, although culture yields differed markedly, the DDT effects parallel each other.

The results of *E. coli* residue analyses from Series I are presented in TABLE 3 and

DSW 031135

STLCOPCB4015097

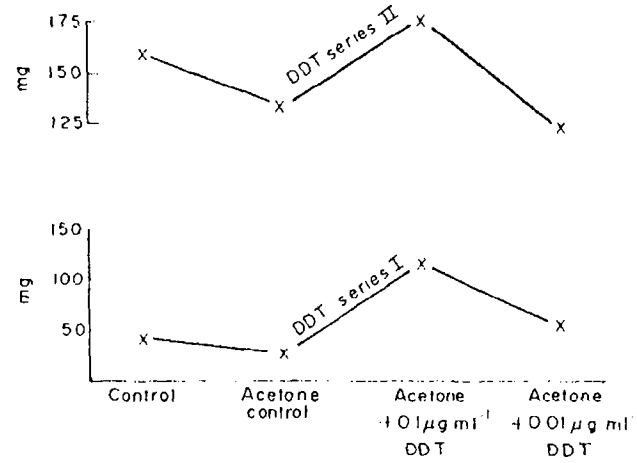

FIG. 2. *E. coli* culture yields in Experimental Series I and II showing harvest weight obtained from each treatment.

show that the organism uniformly converted DDT to DDD (75 per cent) and DDE (25 per cent). Metabolism to DDD is particularly interesting inasmuch as this step is considered to be reductive dechlorination. Aerobic and anaerobic conversion has been previously reported (MALONE, 1970; PARR, WILLIS and SMITH, 1970; WEDEMEYER, 1966), but to the authors' knowledge, this is the first indication of aerobic degradation to DDD by *E. coli*.

TABLE 3. p,p' DDT UPTAKE AND METABOLISM BY *Escherichia coli* (BACTERIAL SERIES I)

DDT in media	Residues			
	DDT	DDD	DDE	Total DDT*
	($\mu g\ g^{-1}$—mean of four replicates)			
0.1 $\mu g\ ml^{-1}$	2.1	6.0	2.3	11.2
0.01 $\mu g\ ml^{-1}$	0	1.5	0.5	2.2

* Total DDT = DDT + 1.114 (DDD + DDE).

There were no differences between treatments in DNA and RNA levels after 24 h incubation. The significance of this is not clear, since it had been anticipated that increased RNA levels would accompany growth increases. However, it is thought that differences may have occurred earlier than the 24 h time.

Data showing uridine uptake by DDT and PCB treated *E. coli* at several phases of growth are presented in TABLE 4. No significant differences in uptake between treatments and controls were evident except at 5 h incubation time, when there was a diminished ^3H_U uptake in all experimental treatments. Statistically significant diminution occurred only in PCB dosed bacteria. While not consistent with increased culture yields, the lack of change in uptake between chemically treated *E. coli* and controls does coincide with chemical assays indicating no increased levels of nucleic acids.

DSW 031136

STLCOPCB4015098

TABLE 4. TRITIATED URIDINE UPTAKE BY DDT AND PCB TREATED *Escherichia coli* CULTURES DURING EXPONENTIAL AND DECLINE GROWTH PHASES

Treatment	Counts min^{-1}* 5 h	8 h	24 h
DDT 0.1 μg ml^{-1}	5789	3284	2075
DDT 0.01 μg ml^{-1}	6165	2845	2391
PCB 0.1 μg ml^{-1}	4342	2250	2192
PCB 0.01 μg ml^{-1}	4756	2367	2462
Uridine control	8020	2675	2389
LSD_{05}†	2800	979	859

* Mean of four replicates.
† Least significant difference at 95 per cent probability level calculated from one way analysis of variance.

Results from these experiments indicate that DDT and PCB stimulate *in vitro* growth of *E. coli*. If this phenomena occurs *in vivo* further concern is justified since any environmental contaminant which affects indicator organisms may result in a distorted portrayal of an actual situation.

Acknowledgements—This research was supported by the Community Pesticide Study of the Environmental Protection Agency—Contract No. PH21-2017. Appreciation is also expressed for technical facilities afforded by Training Grant No. AMO5078-NIAMD and NIH General Research Support Grant 5420. Monsanto Chemical Company kindly supplied the PCB and Geigy Chemical Company the DDT used in this work.

REFERENCES

AGRANOFF B. W. and SANTEN R. J. (1963) Studies on the estimation of deoxyribonucleic acid and ribonucleic acid in rat brain. *Biochem. Biophys.* **72**, 251.
CHACKO C. I., LOCKWOOD J. L. and ZABIK M. (1966) Chlorinated hydrocarbon pesticides: degradation by microbes. *Science* **154**, 893–895.
COPE O. B. and SANDERS H. O. (1963) Microorganism and hydrocarbons. U.S.D.I. Fish and Wildlife Circ. *Pesticides—Wildlife Studies* **167**, 27.
KEIL J. E. and PRIESTER L. E. (1969) DDT uptake and metabolism by a marine diatom. *Bull. Environ. Contam. Tox.* **3**, 169–171.
KEIL J. E., PRIESTER L. E. and SANDIFER S. H. (1971) Polychlorinated biphenyl (Aroclor 1242®) effects of uptake on growth, nucleic acids, and chlorophyll of a marine diatom. *Bull. Environ. Contam. Tox.* **6**, 156–159.
LICHTENSTEIN E. P., SCHULZ K. R., FOHREMANN T. W. and LIANG T. T. (1969) Biological interaction between plasticizers and insecticides. *J. econ. Ent.* **62**, 761–765.
MALONE THOMAS C. (1970) *In vitro* conversion of DDT to DDD by the intestinal microflora of the northern anchovy, *Engraulis mordax*. *Nature, Lond.* **227**, 848–849.
PARR J. F., WILLIS G. H. and SMITH S. (1970) Soil anaerobiosis: II. Effect of selected environments and energy sources on the degradation of DDT. *Soil Sci.* **110**, 306–312.
RISEBROUGH P. W., RIECHE P., HERMAN S. G., PEAKALL D. B. and KIRVEN O. N. (1968). Polychlorinated biphenyls in the global ecosystem. *Nature, Lond.* **220**, 1098–1102.
STENEVSEN J. H. V. (1965). DDT metabolism in resistent and susceptible stable-flies and in bacteria. *Nature, Lond.* **207**, 660–661.
WEDEMEYER G. (1966) Dechlorination of DDT by aerobacter aerogenes. *Science* **152**, 647.
WURSTER C. F. JR. and WINGATE D. B. (1968) DDT residues and declining reproduction in the Bermuda petrel. *Science* **159**, 979–981.

DSW 031137

STLCOPCB4015099