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Mirex and Aroclor® 1254: Effect on and Accumulation by *Tetrahymena pyriformis* Strain W*

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SYNOPSIS. Effects of 2 toxicants, Mirex and Aroclor 1254, on *Tetrahymena pyriformis* strain W in axenic cultures were investigated. Mirex is a chlorinated hydrocarbon effective against the fire ant, and Aroclor 1254 is a compound structurally related to DDT and used extensively in various industrial processes. Both toxicants reduced growth rates and population densities of *T. pyriformis* grown at 26 C generally in proportion to concentrations of the chemicals, their effects becoming statistically significant ($P < 0.05$) at 0.9 μg/liter for Mirex and 1.0 and 10.0 μg/liter for Aroclor 1254. Ciliates exposed to the toxicants for 7 days concentrated Mirex 193 X and Aroclor 60 X as compared to the initial concentrations of these compounds. It is suggested that the chief effect of the 2 toxicants on populations of *T. pyriformis* and of similarly responding ciliates in nature would be to reduce the availability of these protozoa as food organisms and nutrient regenerators. The ability of the ciliates to concentrate the tested compounds would permit the toxicants to enter into and to be translocated through aquatic food chains. In this manner the compounds could exert toxic effects at higher trophic levels.

Index Key Words: *Tetrahymena pyriformis* strain W; axenic cultivation; toxicants; Mirex; Aroclor 1254; bioaccumulation by ciliates.

PROTOZOA, algae, and bacteria form the broad base of aquatic food chains. Ciliated protozoa are the most numerous organisms of the estuarine benthos (1, 6) and may be more important as nutrient regenerators, particularly of nitrogen and phosphorus, than are bacteria (9, 10). Further, some ciliates are able to concentrate certain persistent pesticides (7) and thereby aid in translocating them. Thus, it is possible that effects of such toxicants could be exerted at higher trophic levels either through disruption of nutrient cycles or through translocation and biological concentration of the toxicants in the food chain (2, 3).

The dearth of published information concerning the effect of pesticides and related chemicals on ciliates led us to investigate the effects of Mirex[1] and Aroclor 1254 on populations of *Tetrahymena pyriformis* strain W. This species was chosen because it occurs in freshwater and in salt marshes, has world-wide distribution, is readily grown in axenic culture, and its physiology has been studied extensively (see references in 4).

Mirex (dodecachlorooctohydro-1, 3, 4-metheno-2H-cyclobuta [cd] pentalene), a chlorinated hydrocarbon insecticide used to control infestations of the imported fire ant (*Solenopsis saevis-*

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[1] Trade name, Allied Chemical Corp., Agricultural Div., Jackson, Miss. 39213. Mention of commercial products does not constitute endorsement by the Environmental Protection Agency.

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sima richteri) in the United States, has been considered relatively harmless for animals other than fire ants and a few closely related species. According to recent laboratory experiments, however, Mirex has a delayed toxicity for juvenile blue crabs (*Callinectes sapidus*) and juvenile pink shrimp (*Penaeus duorarum*) (11). These studies indicated also that pinfish (*Lagodon rhomboides*), although apparently not affected by the toxicant, concentrated large amounts of the chemical (30-40 μg/kg) in their tissues. Eight weeks after exposure, approximately half of the amount taken up was retained, indicating that Mirex was not easily metabolized or eliminated from the body.

Aroclor 1254, a polychlorinated biphenyl (PCB) compound, has been widely used as a dielectric and industrial heat-exchange fluid, as a plasticizer in paints and plastics and in many other applications. Structurally related to DDT, it was found recently in water, sediments, and biota of Escambia Bay, Florida, near our laboratory (5). It is toxic to and accumulated by shrimp, oysters and fishes (5, 8, 12).

MATERIALS AND METHODS

Tetrahymena pyriformis strain W was used in all experiments. The ciliate was grown axenically in proteose peptone [2% (w/v)] medium, supplemented with 0.1% (w/v) dehydrated yeast extract and 0.5% (w/v) glucose, at 26 C. Optically matched 18 × 150 mm glass culture tubes were employed. The tubes, each containing 10 ml of the medium, were slanted at 60° during incubation to enhance aeration of the ciliate cultures.

Stock solutions of Mirex and of Aroclor 1254 (henceforth referred to as Aroclor) were prepared in acetone and polyethylene glycol 200, respectively. The stock solutions were used to obtain the desired final concentrations of Mirex and 0.1% (v/v) acetone or of Aroclor and 0.1% (v/v) polyethylene glycol in the proteose peptone medium. Growth of *T. pyriformis* in the presence of either solvent at 0.1% concentration was not significantly different from that of this ciliate in the solvent-free control cultures. Mirex was used at concentrations of 0.009, 0.09, and 0.9 μg/liter, and Aroclor at 0.1, 1.0, and 10.0 μg/liter. Chemical analyses indicate that concentrations of Mirex measured in the test media were within 20% of those desired. Concentrations of Aroclor were more variable, deviating from those desired by from 15% at 1,000 μg/liter to 33% at 1 μg/liter. Part of this error may be caused by losses during extraction of the Aroclor residues since the mixture of proteose peptone medium, polyethylene glycol and Aroclor forms a heavy emulsion with the extraction solvent. Ten culture tubes were prepared for each concentration tested.

The inoculum for each tube was 1 ml of a 65-hr culture diluted 3:10 with sterile medium, the final volume in each tube being 10 ml. Population density was measured in a spectrophotometer as absorbance at 540 nm. The blanks consisted of sterile proteose peptone medium with the appropriate concentrations of the toxicant and carrier. Because conversion of absorbance values to absolute numbers of ciliates is precluded by variation in volume of individuals with the growth period (13), mean absorbance was used to estimate differences in population densities of control and experimental cultures.

The effect of Mirex or Aroclor on growth rate and population density was estimated from absorbance measurements of control and experimental cultures at 0, 4, 8, 16, 24, 36, 48, 60, 72, 84, and 96 hr. These data were analyzed to determine the period of exponential growth for each population. Since growth in this period is logarithmic, growth rate was estimated by the quantity b of the least squares estimate by the line $y = a + bx$. The calculated line gave a close fit to the experimental data (for Mirex, $r \geq 0.76$ for 1 experiment and ≥ 0.95 for 4 others; for Aroclor, $r \geq 0.94$). Differences in maximum densities of populations in control and experimental cultures were determined at 96 hr. Five replicate experiments were performed with Mirex, and 6 with Aroclor 1254. In view of a large variation among replicates, the effect of Mirex or Aroclor on growth rate and population density of *T. pyriformis* was analyzed statistically by the analysis of variance with two-way classification ($\alpha \leq 0.05$). Subsequently, individual comparisons among means were made by Scheffé's test (14). Variation among replicates was significant ($P < 0.05$).

To determine whether *T. pyriformis* could accumulate Mirex or Aroclor 1254, the ciliates were grown for 7 days in a toxicant-containing medium, using 10 cultures per concentration tested. The cultures were then concentrated by centrifugation at 1,000 *g* and washed twice with toxicant-free medium. The wet packed cells were stored in sealed tubes at −20 C or lower until toxicant residues could be analyzed by a modification of the Enos micromethod [R. F. Enos (Environmental Protection Agency, Perrine Primate Laboratory, P. O. Box 490, Perrine, Fla. 33157), 1967. Personal communication to A. J. Wilson, Jr., Environmental Protection Agency Laboratory, Gulf Breeze, Fla. 32561]. Each sample was weighed into a Duall® tissue grinder (Kontes Glass Co., Vineland, N. J.) and extracted with three 2.0-ml portions of acetonitrile. The combined acetonitrile extract was diluted with 6 ml of 2% (w/v) Na_2SO_4 in distilled water, shaken, and extracted with three 2-ml portions of hexane. The combined hexane extract, concentrated to ~0.5 ml by evaporation with a Snyder column, was transferred to a size "B" Chromaflex® column (Kontes) containing 1.5 g of Florisil and 1.5 g of anhydrous Na_2SO_4. The extract was eluted from the column with 20 ml of 1% (v/v) ethyl ether in hexane. The eluate was adjusted to an appropriate volume for analysis by gas chromatography, using a Varian Aerograph model 1400 gas chromatograph equipped with an electron capture detector and a 6-foot, ⅛-inch glass column packed with 2% OV-101 on Gas Chrom Q. Operating parameters were: injector temperature, 210 C; column temperature, 192 C; detector temperature, 210 C; and gas flow, 25 ml/min. Mirex was quantitated by comparison with the peak height of a known concentration standard. Aroclor was quantitated by averaging the 5 major peaks and comparing them with the same peaks of a known concentration standard. Recovery rates for both toxicants were >90%. All residues were calculated on a wet-weight basis, but were not corrected for recovery. Duplicate cultures, dried in an oven at 60 C for approximately 65 hr and then in a desiccator to constant weight, were used to determine percentage of solids to convert wet-weight values to dry-weight basis.

RESULTS

Mirex

Effect on Population Growth.—Mirex reduced growth rates of *T. pyriformis* populations during exponential growth in proportion to concentration of the chemical (Table 1). The mean growth rate of populations exposed to 0.9 μg/liter was significantly less than that of controls ($P < 0.05$), but growth rates of populations exposed to lower concentrations were not ($P > 0.05$). The F value for treatment was highly significant ($P < 0.005$).

In cultures exposed to Mirex for 96 hr, population density decreased as concentration of the toxicant increased. Mirex reduced significantly population density at 0.09 and 0.9 μg/liter

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TABLE 1. *Comparisons of the effect of Mirex and Aroclor 1254 on population growth of* T. pyriformis *W at 26 C.*

Toxicant (μg/liter)	Mean growth rate* (*b*)	Difference (%)	Mean population density* (absorbance)	Difference (%)
Mirex				
0	0.0150		0.861	
0.009	0.0139	−7	0.825	−4
0.09	0.0135	−10	0.809	−6‡
0.9	0.0100	−33†	0.721	−12‡
Aroclor 1254				
0	0.0212		1.044	
0.1	0.0203	−4	0.984	−6
1.0	0.0195	−8§	0.954	−9§
10.0	0.0199	−6§	0.936	−10§

* Means of 5 replicate experiments with Mirex, and of 6 replicate experiments with Aroclor 1254.
† $F_{(3, 11)} = 26.666$ ($P < 0.005$); ‡ $F_{(3, 11)} = 23.001$ ($P < 0.005$); § $F_{(3, 11)} = 6.000$ ($P < 0.01$); ‖ $F_{(3, 11)} = 23.001$ ($P < 0.005$).

($P < 0.05$), but not at 0.009 μg/liter, when compared with controls ($P > 0.05$). The density of populations exposed to 0.9 μg/liter was significantly less than those exposed to all other concentrations. The *F* value for treatment was significant at the 0.005% level.

Accumulation and Biological Concentration.—*T. pyriformis* accumulated Mirex from medium that contained 0.9 μg/liter during the 7-day exposure. In 2 experiments, we recovered 115.4 ng/g and 173.7 ng/g of cells (dry-weight basis, uncorrected for recovery). Thus, the concentration factor ranged from 128 to 193. Over 82% of the available toxicant was taken up by the ciliate.

Aroclor 1254

Effect on Population Growth.—Mean growth rates of *T. pyriformis* during exponential growth declined as concentration of Aroclor increased (Table 1). Exposure to 1.0 and 10.0 μg/liter reduced significantly the growth rate compared to that of control populations ($P < 0.05$), but growth rates of populations exposed to 0.1 μg/liter were not significantly affected ($P > 0.05$). Growth rates of populations exposed to 1.0 and 10.0 μg/liter were not significantly different from each other. The *F* value for treatment was significant ($P < 0.01$).

After 96 hr exposure to Aroclor, mean population densities of the ciliate decreased in an inverse proportion to the toxicant concentration, populations exposed to 1.0 or 10.0 μg/liter of the PCB compound being significantly less dense than those in control cultures ($P < 0.05$). Concentrations of 0.1 μg/liter had no significant effect upon population density. The *F* value for treatment was highly significant ($P < 0.005$).

Accumulation and Biological Concentration.—*T. pyriformis* accumulated Aroclor from media that contained 10, 100, and 1,000 μg/liter during the 7-day exposure. Depending on the original concentration in the medium, cells contained as much as 59.8 μg/gram (dry-weight basis, uncorrected for recovery). The concentration factor, therefore, ranged to approximately 60. Ciliates exposed to 10 μg/liter absorbed 9.5% of the available toxicant; those exposed to 1,000 μg/liter absorbed 51% of the available toxicant. The reason for this difference in absorption is not clear. It may be due in part to sorption of the PCB compound to the wall of the culture tube and to binding of the toxicant to proteinaceous materials in cells and medium.

DISCUSSION

Both Mirex and Aroclor 1254 reduced significantly growth rate and population density of *T. pyriformis* W. The ciliate absorbed the toxicants from the medium, concentrating Mirex up to 193 times and Aroclor up to ~60 times. At the concentrations tested, the chief effect of these compounds on natural populations of *T. pyriformis* and ciliates that respond similarly appears to be reduction in numbers. This would reduce their availability as food organisms and as nutrient regenerators. The survivors' ability to concentrate Mirex and Aroclor could provide yet another pathway for their entry into aquatic food chains, thereby permitting the toxicants to pass to, and possibly exert their effect at, higher trophic levels.

REFERENCES

1. Borror, A. C. 1963. Morphology and ecology of the benthic ciliated protozoa of Alligator Harbor, Florida. *Arch. Protistenk.* 106, 465-534.
2. Burdick, G. E., Harris, E. J., Dean, H. J., Walker, T. M., Skea, J. & Colby, D. 1964. The accumulation of DDT in lake trout and the effect on reproduction. *Trans. Am. Fish. Soc.* 93, 127-36.
3. Butler, P. A. 1969. Monitoring pesticide pollution. *BioScience* 19, 889-91.
4. Corliss, J. O. 1970. The comparative systematics of species comprising the hymenostome ciliate genus *Tetrahymena*. *J. Protozool.* 17, 198-209.
5. Duke, T. W., Lowe, J. I. & Wilson, A. J., Jr. 1970. A polychlorinated biphenyl (Aroclor 1254®) in the water, sediment, and biota of Escambia Bay, Florida. *Bull. Environ. Contam. Toxicol.* 5, 171-80.
6. Fenchel, T. 1967. The ecology of marine microbenthos. I. The quantative importance of ciliates as compared with metazoans in various types of sediments. *Ophelia* 4, 121-37.
7. Gregory, W. W., Jr., Reed, J. K. & Priester, L. E., Jr. 1969. Accumulation of parathion and DDT by some algae and protozoa. *J. Protozool.* 16, 69-71.
8. Hansen, D. J., Parrish, P. R., Lowe, J. I., Wilson, A. J., Jr. & Wilson, P. D. 1971. Chronic toxicity, uptake, and retention of Aroclor® 1254 in two estuarine fishes. *Bull. Environ. Contam. Toxicol.* 6, 113-19.
9. Johannes, R. E. 1965. Influence of marine protozoa on nutrient regeneration. *Limnol. Oceanogr.* 10, 434-42.
10. ———. 1968. Nutrient regeneration in lakes and oceans, *in* Droop, M. R. & Ferguson Wood, E. J., eds., *Advances in Microbiology of the Sea*, Academic Press, New York, 1, 203-13.
11. Lowe, J. I., Wilson, P. D., & Davison, R. B. 1970. Effects of mirex on crabs, shrimp, and fish. *Prog. Rep. Bur. Comm. Fish. Center Estuarine and Menhaden Res., Pesticide Field Sta., Gulf Breeze, Fla. 32561, F. Y. 1969, U. S. Dept. of Interior Circ.* 335, 22-3.
12. Nimmo, D. R., Wilson, P. D., Blackman, R. R. & Wilson, A. J., Jr. 1971. Polychlorinated biphenyl absorbed from sediments by fiddler crabs and pink shrimp. *Nature* 231, 50-2.
13. Slater, J. V., & Elliott, A. M. 1951. Volume change in *Tetrahymena* in relation to age of the culture. *Proc. Am. Soc. Protozool.* 2, 20.
14. Snedecor, G. W. & Cochran, W. G. 1971. *Statistical methods.* 6th ed. Iowa State University Press, Ames.

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