Copyright 1973 by the Society of Protozoologists
Reprinted from THE JOURNAL OF PROTOZOOLOGY.
J. PROTOZOOL., 20(3), 443–445 (1973).
Made in United States of America

The Polychlorinated Biphenyls, Aroclors® 1248 and 1260: Effect on and Accumulation by *Tetrahymena pyriformis**

NELSON R. COOLEY, JAMES M. KELTNER, JR. and JERROLD FORESTER†

Environmental Protection Agency, Gulf Breeze Environmental Research Laboratory, Sabine Island, Gulf Breeze, Florida 32561; Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon

SYNOPSIS. Effects of 2 polychlorinated biphenyls, Aroclor 1248 and 1260, on axenic *Tetrahymena pyriformis* strain W were investigated and compared with published data on Aroclor 1254. Aroclors 1248 and 1260 at 1 mg/liter in the presence of 0.1% (v/v) polyethylene glycol 200 reduced significantly ($P < 0.005$) growth rates and 96-hr populations of *T. pyriformis* grown at 26 C. Both toxicants were ~ 0.001 as toxic as Aroclor 1254. Ciliates were exposed for 7 days to concentrated Aroclors 1248 40×, 1254 60×, and 1260 79× over initial concentrations in the media. Accumulation of Aroclors increased with increased chlorination. It is suggested that if levels in the environment reached those used in these studies, the chief ecologic effect of Aroclor 1254 would be reduction of availability of the ciliates as food and as nutrient regenerators, but with Aroclors 1248 and 1260, this effect would be secondary to accumulation of the toxicants by the ciliates. Accumulation of polychlorinated biphenyls by ciliates would permit the toxicants to enter aquatic food chains. Thus the compounds could exert toxic effects at higher trophic levels.

Index Key Words: *Tetrahymena pyriformis* strain W; axenic cultivation; Aroclor 1248; Aroclor 1254; Aroclor 1260; toxic effect on, and accumulation by ciliates.

THE Aroclors are mixtures of polychlorinated biphenyls (PCBs) or terphenyls. The PCBs are related structurally to DDT and some are toxic to and accumulated by animals and man (17). Aroclor 1254, reported from water, sediment, and biota of Escambia Bay, Florida (5), in laboratory experiments is toxic to and accumulated by the ciliate, *Tetrahymena pyriformis*, and by shrimp, fiddler crabs, oysters, and fishes (4, 5, 9, 10, 13, 14). The ubiquity and significance of these and related PCBs in the environment have been reviewed (6, 5, 17).

Individual Aroclors are identified by 4-digit numbers, the first 2 indicating the type of molecule and the last 2, the weight percentage of chlorine in the molecule. For example, Aroclor 1248 is a polychlorinated biphenyl that contains 48% chlorine. The Aroclors have been used in lubricants; as plasticizers in paints, plastics, and chlorinated rubbers; as heat-exchange fluids in industrial heating systems; and as dielectric compounds in large electrical transformers and capacitors (17). Recently, use of these compounds has been restricted to their dielectric application (17).

We report here the effects of Aroclors 1248 and 1260 on growth of populations of *T. pyriformis* and the accumulation of these compounds by this ciliate. The data are compared with our previous observations on response of *T. pyriformis* to Aroclor 1254 (4).

MATERIALS AND METHODS

Tetrahymena pyriformis strain W was grown in optically matched culture tubes containing 10 ml of proteose peptone [2% (w/v)] medium, supplemented with 0.1% (w/v) dehydrated yeast extract and 0.5% (w/v) glucose, at 26 C. Culture tubes were slanted at 60° to enhance aeration.

Stock solutions of toxicants in polyethylene glycol 200 were prepared, and chemical residue analyses in media and cells were performed by methods already described (4), with one exception. Previously, petroleum ether was used in extraction before gas-chromatographic analysis. In this study, a mixture of equal parts of ethyl and petroleum ethers was used.

Aroclors were tested in the presence of 0.1% (v/v) polyethylene glycol 200 for effect on *Tetrahymena* growth and for accumulation by the ciliates. This concentration of polyethylene glycol had no toxic effect on the ciliates (4) and we had no reason to suspect that effects of polyethylene glycol and Aroclors were additive. Aroclor 1248 was tested at 0.01, 0.1, and 1 mg/liter. Aroclor 1260 was tested for effect on population growth at 0.001, 0.01, 0.1, 1, and 10 mg/liter and for accumulation at 0.001, 0.01, 0.1, and 1 mg/liter. Each concentration was tested for effect on population growth in 6 replicate 96-hr experiments and for accumulation in 3 replicate 7-day experiments. Initial concentrations of each Aroclor in the media were confirmed by electron-capture gas chromatography, and were within 17% of the desired concentrations.

Growth was measured as absorbance at 540 nm. Observations were made at 0, 4, 8, 16, 24, 36, 48, 60, 72, 84, and 96 hr and the data graphed. Exponential growth rate of each population was estimated as the quantity b of the least squares estimate of the line $y = a + bx$ of the exponential part of the growth curve. The calculated regression lines for this segment of the growth curves closely fitted the experimental data—for Aroclor 1248, $r \geq 0.89$; for Aroclor 1260, $r \geq 0.87$. In addition, populations were compared at 96 hr, when densities of control populations were maximal. Values for all treatments in 6 replicates were subjected to analysis of variance with 2-way classification and individual treatments were compared by Scheffé's test (16). Differences were considered significant at $P < 0.05$.

RESULTS

Aroclor 1248

Effect on population growth.—Exponential growth rate of the ciliate (Table 1) was reduced significantly by Aroclor 1248 [variance ratio, $F_{6,30} = 6.77$; $P < 0.005$], growth rates of populations exposed to 1 mg/liter of the compound being 18.9%

® Registered Trademark, Monsanto Co., 800 N. Lindbergh Blvd., St. Louis, Mo. Mention of commercial products does not constitute endorsement by the Environmental Protection Agency.

* Contribution No. 158, Gulf Breeze Environmental Research Laboratory.

† We thank Dr. John O. Corliss for the cultures of *T. pyriformis* strain W from which our stock was derived, the Monsanto Company for the samples of Aroclors 1248 and 1260 used in this investigation, and David J. Hansen and Dr. Alvin L. Jensen for valuable advice on the statistical analysis of our data.

DSW 027636

STLCOPCB4011598

TABLE 1. *Comparison of the effect of Aroclors 1248 and 1260 on growth of* T. pyriformis *strain W at 26 C.*

Toxicant	Concentration (mg/liter)	Mean growth rate* (k)	Difference (%)	Mean population density at 96 hr* (absorbance)	Difference (%)
AROCLOR 1248	0	0.0164		0.7732	
	0.01	0.0168	+ 2.2	0.7883	+ 1.9
	0.1	0.0161	− 1.8	0.7915	+ 2.4
	1.0	0.0133	−18.9†	0.6992	− 9.6‡
AROCLOR 1260	*Series 1*				
	0	0.0168		0.8472	
	0.001	0.0176	+ 4.8	0.8487	+ 1.8
	0.01	0.0179	+ 6.5	0.8533	+ 7.2
	0.1	0.0171	+ 1.8	0.8317	−18.3
	1.0	0.0126	−25.0§	0.6577	−22.4‖
	Series 2				
	0	0.0194		1.0175	
	1.0	0.0157	−19.1**	0.8788	−13.6††
	10.0	0.0113	−41.1**	0.7075	−30.5††

* Means of 6 replicate experiments.
† $F_{(a,\infty)} = 6.77$, $P < 0.005$; ‡ $F_{(a,\infty)} = 9.35$, $P < 0.005$; § $F_{(a,\infty)} = 5.85$, $P < 0.005$; ‖ $F_{(a,\infty)} = 6.06$, $P < 0.005$; ** $F_{(a,\infty)} = 485.00$, $P < 0.005$; †† $F_{(a,\infty)} = 217.90$, $P < 0.005$.

less than those of control populations. There was no statistically significant difference between growth rates of control and experimental populations exposed to lower concentrations or among growth rates of experimental populations exposed to lower concentrations.

Population density at 96 hr (Table 1) was reduced (9.6%) significantly by 1 mg/liter [$F_{(a,\infty)} = 9.35$; $P < 0.005$], but not by lower concentrations of the toxicant.

Accumulation.—Tetrahymena pyriformis accumulated Aroclor 1248 from media containing 0.01, 0.1, or 1 mg/liter during 7-days exposure. Uptake of the toxicant from the medium was linear with increasing concentration. When initial concentration in the medium was plotted against mean concentration in the cells, the data agreed closely ($r = 0.998$) with the calculated regression line $\bar{Y}_g = 0.9322X - 1.4746$. Concentration factors ranged from 14.8 to 40.6. Ciliates exposed to 0.01 mg/liter absorbed 15.1-25.5% ($\bar{x} = 21.6\%$) of the toxicant in the medium, those exposed to 0.1 mg/liter absorbed 13.4-19.2% ($\bar{x} = 15.7\%$), and those exposed to 1 mg/liter absorbed 14.9-19.2% ($\bar{x} = 16.8\%$).

We observed no indication of change in isomeric composition of Aroclor 1248 as was reported for Aroclor 1254 in tissues of fish (9) and shrimp (14).

Aroclor 1260

Effect on population growth.—Exponential growth rate of the ciliate (Table 1) was reduced significantly by Aroclor 1260 [$F_{(a,\infty)} = 5.85$, $P < 0.005$, in the 1st series; $F_{(a,\infty)} = 485.00$, $P < 0.005$, in a 2nd series], growth rates of organisms exposed to the highest concentrations being 19.1-25.0% less at 1 mg/liter and 41.1% less at 10 mg/liter than those of control populations. There was no statistically significant difference between growth rates of control populations and populations exposed to lower concentrations of the toxicant.

~~Mean population density was reduced significantly~~ [$F_{(a,\infty)} = 6.06$, $P < 0.005$, in the 1st; and $F_{(a,\infty)} = 217.9$, $P < 0.005$ in the 2nd experimental series]. ~~The reduction ranged 13.6-22.4% in ciliate populations exposed to 1 mg/liter and 30.9%~~ in those exposed to 10 mg/liter. No significant reductions in population density were observed in the presence of a lower concentration of the toxicant.

Accumulation.—Tetrahymena pyriformis accumulated Aroclor 1260 from medium that contained 0.001, 0.01, 0.1, or 1 mg/liter during 7-days exposure. Uptake of the toxicant from the medium was linear with increasing concentration. When initial concentration in the medium was plotted against mean concentration in the cells, the data agreed closely ($r = 0.917$) with the calculated regression line $\bar{Y}_g = 0.06967X - 0.2191$. Concentration factors ranged 21-79. Ciliates exposed to 0.001 mg/liter absorbed amounts that ranged from detected-but-unquantifiable to 61.7% ($\bar{x} = 38.2\%$) of the toxicant in the medium; those exposed to 0.01 mg/liter, 16.4-49.3% ($\bar{x} = 36.8\%$), those exposed to 0.1 mg/liter, 15.1-68.1% ($\bar{x} = 41.7\%$), and those exposed to 1 mg/liter, 26.4-81.0% ($\bar{x} = 53.3\%$).

We observed no indication of change in isomeric composition of Aroclor 1260 as was reported for Aroclor 1254 in tissues of fish (9) and shrimp (14).

Comparison of accumulation of Aroclor 1248 and Aroclor 1260 by *T. pyriformis* by the *t*-test (differences considered significant at $P \leq 0.05$) (16) revealed that (a) when concentration of each compound in the medium increased, concentrations of Aroclor 1248 in the cells increased at the same rate as did Aroclor 1260 ($P > 0.05$) and (b) the mean amounts of Aroclor 1248 and Aroclor 1260 accumulated were not significantly different ($P > 0.05$).

DISCUSSION

Protozoa, algae, and bacteria form the broad base of aquatic food chains. Ciliates are among the most numerous organisms of the estuarine benthos (1, 7) and may be more important as nutrient regenerators, particularly of nitrogen and phosphorus, than are bacteria (11, 12). Also, some ciliates, including *T. pyriformis*, can concentrate certain persistent pesticides and PCBs (4, 8), and thus help translocate them up the trophic pyramid. It has been shown that PCBs build up in food chains (6). It is possible, therefore, that PCBs acting on or through these ciliates could be toxic at higher trophic levels either

1000 ppb ug/l

DSW 027637

STLCOPCB4011599

through disruption of nutrient cycles or through translocation and bioaccumulation in the food chain (2, 3, 17).

Tests of Aroclor 1254 included concentrations found in natural waters and sediments (5). Testing similar concentrations of Aroclor 1248 and 1260 permitted comparison of effects and accumulation of the 3 compounds. All were toxic to and accumulated by *T. pyriformis*, although in different degree. Aroclor 1254 was the most toxic, as judged by significant reduction of both growth rate and 96-hr population density at 0.001 mg/liter concentration (4), whereas Aroclors 1248 and 1254 did not reduce significantly either population growth or 96-hr population density at concentrations below 1 mg/liter. Concentration of these PCBs by *T. pyriformis* increased with increasing chlorination of the toxicants: Aroclor 1248, 40 ×; Aroclor 1254, 60 ×; and Aroclor 1260, 79 ×. This trend appears to be in agreement with the suggestion that PCBs with fewer chlorines tend to be metabolized or excreted faster than those more chlorinated, so that the latter compounds tend to increase in food chains (10, 17).

At the concentrations tested, the chief effect of Aroclor 1254 on natural populations of *T. pyriformis* and ciliates that respond similarly might be reduction of population density. This would reduce their availability as food organisms and nutrient regenerators. Conversely, with Aroclors 1248 and 1260, reduction of population density is secondary in importance to accumulation of the toxicants by the ciliates. The ability of these ciliates to concentrate them would enable these PCBs to enter 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. Cooley, N. R., Keltner, J. M., Jr. & Forester, J. 1972. Mirex and Aroclor® 1254: Effect on and accumulation by *Tetrahymena pyriformis* strain W. *J. Protozool.* **19**, 636-8.

5. Duke, T. W., Lowe, J. I. & Wilson, A. J., Jr. 1970. A polychlorinated biphenyl (Aroclor® 1254), in water, sediment, and biota of Escambia Bay, Florida. *Bull. Environ. Contam. Toxicol.* **5**, 171-80.

6. Dustman, E. H., Stickel, L. F., Blus, L. J., Reichel, W. L. & Wiemeyer, S. N. 1971. The occurrence and significance of polychlorinated biphenyls in the environment. *Trans. 36th N. Am. Wildl. Nat. Resour. Conf.* 118-33.

7. Fenchel, T. 1967. The ecology of marine microbenthos. I. The quantitative importance of ciliates as compared with metazoans in various types of sediments. *Ophelia* **4**, 121-37.

8. 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.

9. 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**, 113-9.

10. Jensen, S., Johnels, A. G., Olsson, M. & Otterlind, G. 1969. DDT and PCB in marine animals from Swedish waters. *Nature* (London) **224**, 247-50.

11. Johannes, R. E. 1965. Influence of marine protozoa on nutrient regeneration. *Limnol. Oceanogr.* **10**, 434-42.

12. ——— 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.

13. 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.

14. ———, Blackman, R. R., Wilson, A. J., Jr. & Forester, J. 1971. Toxicity and distribution of Aroclor® 1254 in pink shrimp *Penaeus duorarum*. *Mar. Biol.* (Berlin) **11**, 191-7.

15. Peakall, D. B. & Lincer, J. L. 1970. Polychlorinated biphenyls: another long-life widespread chemical in the environment. *BioScience* **20**, 958-64.

16. Snedecor, G. W. & Cochran, W. G. 1971. *Statistical Methods*. 6th ed. Iowa State Univ. Press, Ames.

17. U. S. Interdepartmental Task Force on PCBs. 1972. *Polychlorinated Biphenyls and the Environment*. Final Report. COM-72-10419. 192 pp. (National Technical Information Service, U. S. Dept. of Commerce, Springfield, Va. 22151.)

DSW 027638

STLCOPCB4011600