The Effects of Pesticides, Polychlorinated Biphenyls and Metals on the Growth and Reproduction of *Acanthamoeba castellanii*

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Despite the large number of investigations on the effects of environmental toxicants on organisms, relatively little effort has been devoted to studies of protozoan responses to potentially toxic pollutants. We have selected the free-living amoeba, Acanthamoeba castellanii for a study of pollutant effects on protozoa. Acanthamoeba is widespread in soil and fresh water, it can be axenically cultured in semidefined or defined media, and enough is known of its metabolism to provide a basis for the future study of the mechanism of any toxic effects which might be observed. The effects of a few pesticides on the growth of Acanthamoeba cultures have been described in a prior publication (PRESCOTT and OLSON, 1972). This work was undertaken in order to expand our knowledge of the sensitivity of this protozoan to potentially toxic materials which are commonly found in the environment.

METHODS

The effects of eight pesticides from several different chemical classes have been studied--the insecticides dieldrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-exo-1,4-endo-5,8-dimethanonaphthalene), aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-exo-1,4-endo-5,8-dimethanonaphthalene), and sevin or carbaryl (1-naphthyl N-methylcarbamate); and the herbicides linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea), stam F-34 or propanil (3',4'-dichloropropionanilide), IPC (O-isopropyl N-phenyl carbamate), atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), and simazine (2-chloro-4,6-bis (ethylamino)-1,3,5-triazine). Samples of pure pesticides were kindly provided by the following companies: E. I. Dupont (linuron), Rohm and Haas (stam F-34), Union Carbide (sevin or carbaryl), and the Shell Chemical Company (dieldrin and aldrin). Atrazine, simazine, and IPC were purchased from the K & K laboratories. Atrazine and simazine were further purified before use by treatment with activated charcoal and recrystallization from ethanol. Aroclor 1254, a polychlorinated biphenyl (PCB) preparation, was contributed by the Monsanto company. Reagent grade metal salts were obtained commercially. The I-12 strain of Acanthamoeba castellanii used in these studies was provided by Dr. Robert J. Neff.

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Bulletin of Environmental Contamination & Toxicology, Vol. 18, No. 1 © 1977 by Springer-Verlag New York Inc.

The pesticides and Aroclor 1254 were dissolved in reagent grade acetone. Experimental Acanthamoeba cultures were incubated at 30°C in 125 ml Erlenmeyer flasks, each containing 20 ml of Neff's optimal growth media (PRESCOTT, 1964) prepared by the method of BYERS, et al. (1969). Prior to inoculation, each flask received 10 µl of acetone solution containing enough of the proper pesticide to give the proper final concentration. Control flasks received 10 µl of acetone alone. The effects of metals were determined by dissolving each in distilled water, combining 10 µl of the aqueous metal solution with 2.0 ml of glucose stock solution, and autoclaving this glucose-metal combination. The sterile glucose-metal mixture was then aseptically added to the remainder of the media components which had been separately autoclaved.

At zero time, each flask was inoculated with a 1 ml. aliquot of a six day old culture to give an initial concentration of approximately 0.5-1.0 X 10^4 amoebae/ml. Duplicate counts were made of each culture flask at zero time and after 6 days incubation using a 0.2 mm deep Fuchs-Rosenthal hemocytometer. Prior experiments have established that culture growth has reached a plateau after 144 hours under these conditions. Each experiment was normally carried out with three to five control flasks and three experimental flasks for each toxicant. Counts from these flasks were averaged, and the average cell counts were used to calculate the degree of growth inhibition for each toxicant in terms of percent of control values. The significance of the data was determined by the use of both the Student's t-test and the sign test.

RESULTS AND DISCUSSION

The sensitivity of A. castellanii to pesticides varies a good deal. As seen in Table 1, the population growth of this amoeba is inhibited by linuron, stam F-34 IPC, sevin and atrazine at a level of 10 mg/l. The polychlorinated biphenyl Aroclor 1254 has no significant effect at a concentration of 0.01 mg/l (10 ppb).

The atrazine results are quite different from those published previously (PRESCOTT and OLSON, 1972). In the earlier studies we found that 0.1 ppm reduced growth and reproduction by 62%, while 1 ppm atrazine reduced growth by 96%. As can be seen from the atrazine data published here, we have not been able to reconfirm this result. Purified atrazine does inhibit, but much less effectively than our earlier data indicated. Its toxicity is similar to that of simazine, another triazine herbicide which also interferes with plant photosynthesis (CORBETT, 1974). We have obtained the same results with unpurified commercial atrazine as well. The effects of 0.1 and 1 mg/l concentrations of atrazine have also been redetermined and fit with the data presented in Table 1. In two experiments we found that growth averaged 95% of control levels in the presence of 0.1 ppm atrazine and 86% of control levels in the presence of 1 ppm atrazine. We

have no plausible explanation for this discrepancy other than the presence of an unknown toxic contaminant in the original experimental flasks.

TABLE 1

Effects of Pesticides and PCBs on the Growth and Reproduction of Acanthamoeba castellanii

Toxicant	Concentration (mg/l)	Number of Experiments	Percent of Control*
Linuron	10	5	62 ± 17†
Stam F-34	10	4	40 ± 9†
IPC	10	3	66 ± 2†
Sevin	10	5	59 ± 8†
Dieldrin	10	3	88 ± 9
Aldrin	10	4	89 ± 18
Atrazine	10	3	60 ± 8†
	4	3	85 ± 15
Simazine	4	3	86 ± 11
Aroclor 1254	0.01	2	97 ± 2

*The average number of amoebae per ml. in experimental flasks after incubation divided by the cell count in 6 day control flasks times 100 is the percent of control. Standard deviations for these average values are also included.

†Experimental data significantly different from controls at P = 0.05 by Student's t-test.

It is difficult to compare the pesticide sensitivity of A. castellanii with that of other protozoa since so little data is available (MITCHELL, 1972). It appears to be no more sensitive than those protozoa and algae which have been studied to any extent. There is somewhat more basis for comparison in the instance of sensitivity to the presence of PCBs. Tetrahymena pyriformis is significantly inhibited by 10 µg/l Aroclor 1254, but not by Aroclor 1248 or 1260 at the same concentration (COOLEY, et al., 1972; COOLEY, et al., 1973). Some marine and estuarine

TABLE 2

Effects of Metals on the Growth and Reproduction of Acanthamoeba castellanii

Metal	Concentration (mg/l)	Number of Experiments	Percent of Control*
$CuSO_4 \cdot 5H_2O$	1	3	101 ± 7
	10	2	96 ± 2
$ZnSO_4 \cdot 7H_2O$	1	2	97 ± 2
	50	2	112 ± 10
$Pb(NO_3)_2$	1	3	99 ± 10
	10	3	58 ± 8†
	20	2	26 ± 9†
$HgCl_2$	0.05	2	100 ± 2
	0.2	2	73 ± 11
	0.4	2	34 ± 2†
	1	2	1.2 ± 0.4†

*The average number of amoebae per ml. in experimental flasks after 6 days incubation divided by the cell count in 6 day control flasks times 100 is the percent of control. Standard deviations are also included.

†Experimental data significantly different from controls at P = 0.05 by Student's t-test.

diatoms are also adversely affected by polychlorinated biphenyls (MOSSER, et al., 1972; FISHER, et al., 1974) whereas Chlorella pyrenoidosa, a fresh water alga, is relatively resistant to a variety of Aroclors at levels of 100 ppb of less (HAWES, et al., 1976). We tested the toxicity of Aroclor 1254 only at a concentration of 10 µg/l. Although experiments were not carried out at higher PCB concentrations, our results do indicate that A. castellanii is less sensitive to this PCB fraction than is the ciliate T. pyriformis and several species of diatoms. Such variations in sensitivity may result in the alteration of the composition of a microbial population in the presence of toxicants such as polychlorinated biphenyls.

The results of experiments on the response of Acanthamoeba castellanii to the presence of several common metal ions are presented in Table 2. This amoeba is unaffected by moderately high levels of copper and zinc, but is--not surprisingly--sensitive to the presence of lead and mercuric ions. It is difficult to interpret these results with complete confidence because of the large

amount of metal ions which are bound by media components (RAMAMOORTHY and KUSHNER, 1975). Effects may be attributed to very small concentrations of free metal ions or to the uptake of metal ion-organic molecule complexes. The total concentrations of metals used in these experiments are above those which one might expect to normally observe in natural waters (LELAND, et al., 1975).

Acanthamoeba seems to be more resistant to copper and zinc than most protozoan species which have been tested (RUTHVEN and CAIRNS, 1973). It is not possible to directly compare lead toxicity due to the fact that Cairn's experimental exposure times were of shorter duration. A. castellanii is more sensitive to mercuric chloride than the ciliate Tetrahymena pyriformis (TINGLE, et al., 1973).

ACKNOWLEDGEMENT

This research has been partially funded by the Brown-Hazen Fund of the Research Corporation. It was also supported by National Science Foundation Undergraduate Research Participation Grants.

LITERATURE CITED

BYERS, T. J., V. L. RUDICK and M. J. RUDICK: J. Protozool. 16, 693 (1969).

COOLEY, N. R., J. M. KELTNER and J. FORESTER: J. Protozool. 19, 636 (1972).

COOLEY, N. R., J. M. KELTNER and J. FORESTER: J. Protozool. 20, 443 (1973).

CORBETT, J. R.: The Biochemical Mode of Action of Pesticides. 1 ed. New York: Academic Press 1974.

FISHER, N. S., E. J. CARPENTER, C. C. REMSEN and C. F. WURSTER: Microbial Ecol. 1, 39 (1974).

HAWES, M. L., J. C. KRICHER and J. C. UREY: Bull. Envir. Contam. Toxicol. 15, 14 (1976).

LELAND, H. V., E. D. COPENHAVER and D. J. WILKES: Jour. Water Poll. Control Fed. 47, 1635 (1975).

MITCHELL, R. (ED.): Water Pollution Microbiology. 1 ed. New York: John Wiley & Sons 1972.

MOSSER, J. L., N. S. FISHER, T.-C. TENG, C. F. WURSTER: Science 175, 191 (1972).

PRESCOTT, D. M. (ED.) Methods in Cell Physiology. Vol. 1. 1 ed. New York: Academic Press 1964.

PRESCOTT, L. M. and D. L. OLSON: Proc. S. Dakota Acad. Sci. 51, 136 (1972).

RAMAMOORTHY, S. and D. J. KUSHNER: Microbial Ecol. 2, 162 (1975).

RUTHVEN, J. A. and J. CAIRNS, JR.: J. Protozool. 20, 127 (1973).

TINGLE, L. E., W. A. PAVLAT and I. L. CAMERON: J. Protozool. 20, 301 (1973).