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Effects of Chlordane and Heptachlor on the Marine Dinoflagellate, *Exuviella baltica*, Lohmann

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Chlordane and heptachlor are widely used chlorinated cyclodiene insecticides. While the effects of both compounds on certain higher organisms have been studied (EISLER 1970; HARBISON 1973; MEHRLE et al. 1974; MIRANDA and WEBB 1972), little is known of their effects on phytoplankton, the base of marine and freshwater food webs. The present study examined growth, productivity, chlorophyll a content and cell size distribution of a marine dinoflagellate treated with chlordane or heptachlor.

MATERIALS AND METHODS

The marine dinoflagellate Exuviella baltica Lohmann was cultured according to POWERS et al. (1975). To prepare experimental cultures, 150 ml of exponentially growing cells were introduced aseptically to a sterile aspirator bottle containing 2850 ml of medium, yielding a final cell concentration of 6.4 x 10^3 cells/ml. After mixing by magnetic stirrer, cell suspensions were transferred to each of six 500-ml Erlenmeyer flasks fitted with Teflon-lined screw caps (two replicate flasks for each treatment). Cultures were treated with 50 µg/l (ppb) of chlordane or heptachlor dissolved in 50 µl of methanol (POWERS et al. 1975); control cultures received an equal volume of methanol. Experimental cultures were grown as previously described (POWERS et al. 1975).

Technical heptachlor (74% 1,4,5,6,7,8,8a-heptachloro-3a,4,7a-tetrahydro-4,7-methanoindene and 26% related insecticidal compounds) and technical chlordane (60% octachloro-4,7-methanotetrahydroindane and 40% related insecticidal compounds) were provided by Velsicol Chemical Corporation, Chicago, Illinois.

Cultures were sampled shortly after starting the experiment and every 24 to 72 hours thereafter for seven days. Cell numbers were determined optically

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using a Fuchs-Rosenthal counting chamber. Chlorophyll a was determined fluorometrically (LOFTUS and CARPENTER 1971) using a Turner Fluorometer (Turner Designs, Palo Alto, Calif.). For determination of photosynthetic activity, 6 ml of sample were pipetted into each of three vials (two light and one dark) and 0.1 ml of $NaH^{14}CO_3$ (New England Nuclear stock diluted in sterile f/2 medium) was added. Vials were capped and incubated for two hours with gentle shaking every 30 minutes. Photosynthesis was terminated with the addition of 1.0 ml of 0.5 N HCl to each vial. Samples were filtered (0.45-µm Millipore), and filters were dried and placed in scintillation vials containing 5 ml of toluene-based fluor (toluene, 0.3% PPO and 0.01% POPOP). Radioactivity was determined using a Mark II Liquid Scintillation System (Searle Radiographics, Des Plains, Ill.).

Cell size distribution was determined with an ElectroZone/Celloscope® (Particle Data, Inc., Elmhurst, Ill.) particle counting and sizing system equipped with a calibrated 120-µm orifice tube. Five- to 50-ml volumes of E. baltica suspension were diluted with filtered seawater to produce particle concentrations below those at which coincident particle passage through the orifice would occur (SHELDON and PARSONS 1968). This system was adjusted to enumerate particles per ml in 100 channels, ranging in size from 2.4 to 23.4 µm equivalent spherical diameters.

RESULTS AND DISCUSSION

Cell densities in treated cultures were lower than controls throughout the experiment (Fig. 1), resulting in reductions in chlorophyll a concentrations per liter (Table 1). Levels of chlorophyll a per cell were not significantly different, however, in treated and untreated cultures, suggesting that inhibition of ^{14}C uptake per treated cell (Table 1) was due to interference with chlorophyll function rather than its synthesis. Consequently, considerably less carbon was fixed per unit of chlorophyll a in treated cells than in controls. These results generally agree with those of MACFARLANE et al. (1972), who described reduced photosynthesis per cell and per unit of chlorophyll a in DDT-treated cultures of Nitzschia delicatissima. They also observed a reduction in chlorophyll a per cell, however.

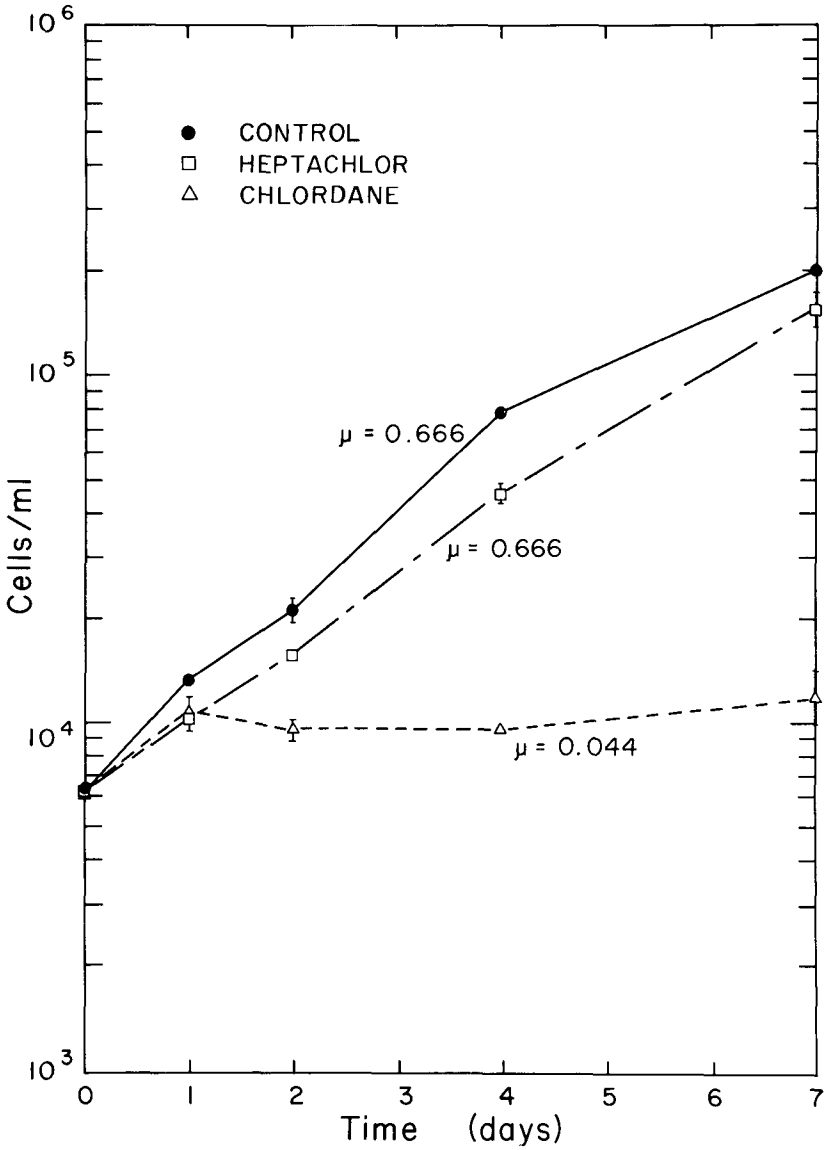

Fig. 1. Cell growth, as determined by optical count, of E. baltica exposed to 50 μg/l of either heptachlor or chlordane. Each point represents the mean of cell counts from two replicate cultures, the actual values indicated by bars above and below the point. Growth rates (μ), expressed as divisions per day between days 1 and 7, were calculated according to the expression $\mu = (35)(\ln n_t - \ln n_{t_0})/(t - t_0)$ where $t - t_0$ represents elapsed time in hours and n_t and n_{t_0} are the population densities at times t and t_0, respectively (EPPLEY and STRICKLAND 1968).

TABLE 1

Biomass and photosynthesis in *E. baltica* exposed to 50 µg/l of heptachlor or chlordane

Samples and Time of sampling		Chlorophyll *a*				Photosynthesis			
		µg/l		µg/cell (x 10^{-6})		Cpm/cell (x 10^{-3})		Cpm/µg chl *a* (x 10^{3})	
	Day	1	2	1	2	1	2	1	2
Control	0	12.89	12.06	2.02	1.89	3.02	3.12	1.50	1.66
	2	41.47	45.24	2.07	1.97	8.83	7.60	4.26	3.86
	4	155.32	177.19	1.97	2.22	11.40	11.30	5.80	5.10
	7	237.51	214.89	1.89	1.07	---	---	--	--
Heptachlor	0	13.50	10.86	2.11	1.70	3.02	1.74	1.43	1.03
	2	29.41	26.92	1.84	1.68	5.73	3.99	3.12	2.45
	4	101.79	79.92	2.12	1.82	9.10	7.08	4.29	3.90
	7	177.19	135.72	1.04	0.97	--	--	--	--
Chlordane	0	12.67	11.99	1.98	1.87	0.67	0.75	0.34	0.40
	2	17.34	16.36	1.87	1.64	1.40	1.46	0.75	0.89
	4	14.33	14.33	1.43	1.52	0.36	1.02	0.25	0.67
	7	19.60	27.14	1.96	1.94	--	--	--	--

Recent experiments utilizing natural phytoplankton isolates exposed to polychlorinated biphenyls (PCB), another group of chlorinated hydrocarbons, revealed a response somewhat different from that reported in this study. Suppressed ^{14}C uptake per treated cell was attributed to less chlorophyll *a* per cell with no loss of carbon-fixing efficiency per unit of existing chlorophyll *a* (POWERS et al. 1978). Inhibition per cell was not detected by FISHER (1975) in three phytoplankton species exposed to DDT or PCB.

No attempt was made to define the mechanism of inhibition of photosynthesis in this study. Interference with electron transport may have been involved, as in algae treated with other chlorinated hydrocarbons (BOWES and GEE 1971; BOWES 1972), since both heptachlor and chlordane inhibited mitochondrial electron transport in mammalian cells (PARDINI et al. 1971).

The effect of chlordane on cell size distribution may be seen in Figs. 2 and 3. Since heptachlor-treated cells differed only slightly from controls, the data are not included. As corroborated by microscope measurements, the peak in particle number (Fig. 2) and particle volume (Fig. 3) between 8 and 16 µm consisted of *E. baltica* cells. Both parameters were suppressed by chlordane in that size range. Concomitantly, both were higher than controls between 4 and 8 µm, particularly on day 4. Microscope examination revealed considerable cellular debris in treated cultures in this size range, suggesting chlordane-induced disruption of cells. A similar effect on this species by dieldrin, another chlorinated cyclodiene, was recently reported (POWERS et al. 1977).

The inhibition of *E. baltica* growth and photosynthesis observed in this study, coupled with the shift in cell size distribution, might have important ecological implications. In nature, such disruptions could influence the availability of food for selectively-feeding herbivores (PARSONS and LeBRASSEUR 1970).

SUMMARY

Chlordane and heptachlor at 50 µg/l reduced cell density, chlorophyll *a* per unit volume of culture, ^{14}C uptake per cell and carbon fixation per unit of chlorophyll *a* in the marine dinoflagellate *Exuviella baltica* Lohmann. The concentration of chlorophyll *a*

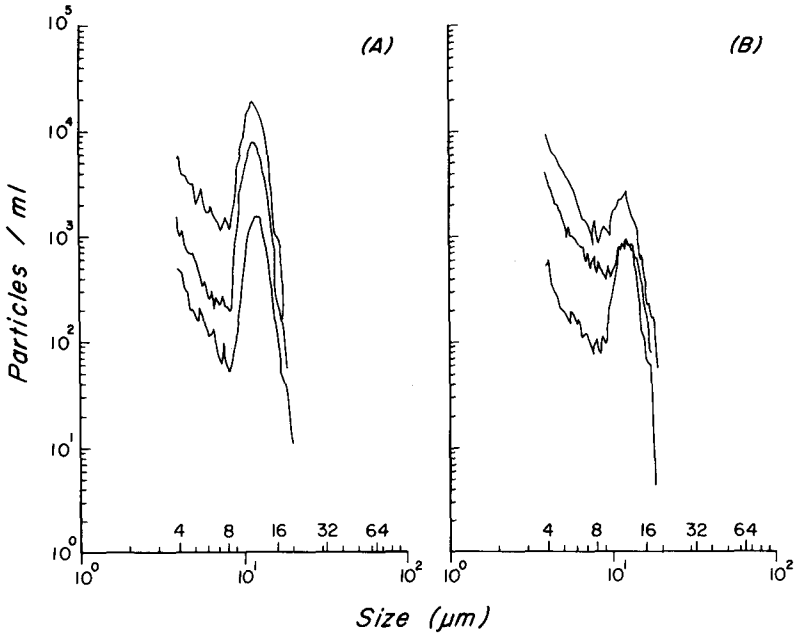

Fig. 2. Particle size distribution, expressed as particles/ml vs. equivalent spherical diameter (in μm), in untreated (A) or 50 μg/l chlordane-treated (B) E. baltica cultures. Lower curve represents day 2; middle curve, day 4; and upper curve, day 7.

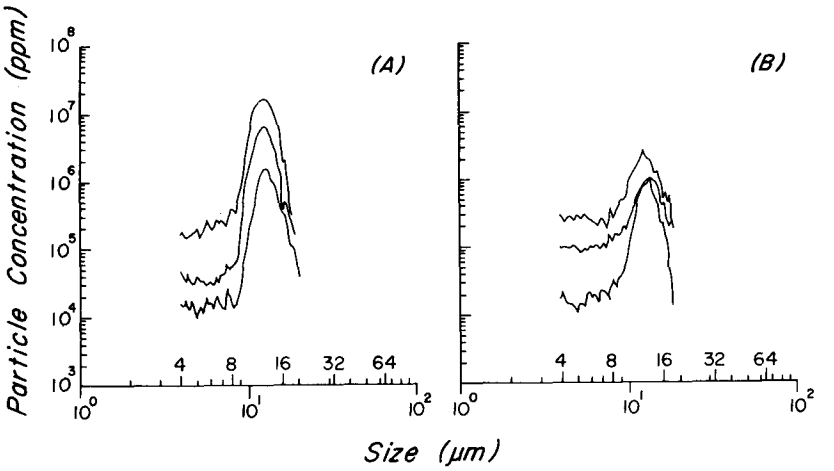

Fig. 3. Particle size distribution, expressed as total cell volume (in ppm or μm^3/ml) vs. equivalent spherical diameter (in μm), in untreated (A) or 50 μg/l chlordane-treated (B) E. baltica cultures. Lower curve represents day 2; middle curve, day 4; and upper curve, day 7.

per cell was not reduced, however, by treatment with either compound. Chlordane was more toxic than heptachlor at this concentration, and caused the disintegration of many cells, thus affecting particle size distribution in the cultures. In nature, such an inhibition and shift in size class distribution could affect the availability of food for particle-feeding herbivores.

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