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Effects of Trichloroethylene, Hexachlorobenzene and Polychlorinated Biphenyls on the Growth and Cell Size of Marine Phytoplankton*

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Although world production of DDT peaked at about 10^5 tons per year in the mid-1960's, annual production of several other organochlorine compounds is currently many times higher (PEARSON and McCONNELL 1975). Some of these chemicals are widespread in the environment, including marine waters and organisms (GOLDBERG 1976), but their biotic effects have not been extensively studied.

Trichloroethylene (TCE) is one of the most heavily produced organochlorine compounds, annual world production being about 10^6 tons (PEARSON and McCONNELL 1975). Hexachlorobenzene (HCB) is a pesticide and a large quantity by-product in the manufacture of many organochlorines (GOLDBERG 1976). Both TCE and HCB are widespread in the marine environment (KOEMAN et al. 1969, PEARSON and McCONNELL 1975, GOLDBERG 1976, U.S. E.P.A. 1976). LU and METCALF (1975) showed that HCB bioaccumulates in aquatic food chains.

We report here the effects of TCE and HCB on marine phytoplankton, using mixed laboratory cultures of an estuarine centric diatom, Thalassiosira pseudonana, and a green alga, Dunaliella tertiolecta, as a test system. The toxicity of these compounds was compared with that of Aroclor 1254, a mixture of polychlorinated biphenyls (PCB) known to inhibit algal growth and photosynthesis (MOSSER et al. 1972a, MOORE and HARRISS 1972, HARDING 1976) and to alter size and species composition of laboratory (MOSSER et al. 1972b, FISHER and WURSTER 1973) and natural (FISHER et al. 1974, O'CONNORS et al., in preparation) phytoplankton assemblages.

MATERIALS AND METHODS

Equal numbers of exponentially growing T. pseudo-

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nana (clone "3H") and D. tertiolecta (clone "Dun"), obtained from R. R. L. Guillard, WHOI, Woods Hole, Mass., and maintained as axenic unialgal cultures, were added aseptically to a sterile aspirator bottle containing 750 ml of medium, yielding a total cell concentration of about 2 x 10^4 cells per ml. After mixing on a magnetic stirrer, 50-ml aliquots of cell suspension were transferred to sterile 200-ml bottles (three replicate bottles for each treatment or control).

In Experiment 1, cultures were treated with 50 or 100 µg/L of TCE or 50 µg/L of Aroclor 1254 dissolved in 10 µl of methanol. Controls received an equal volume of methanol. In Experiment 2, cultures were treated with 50 or 100 µg/L of HCB or 50 µg/L of Aroclor 1254. Since HCB is only slightly soluble in methanol, the HCB and Aroclor were dissolved in 15 µl of acetone; control cultures in Experiment 2 received an equal volume of acetone.

To compare the effects of repeated exposure to these chemicals, 50 or 100 µg/L of TCE or HCB was added daily to other mixed cultures of T. pseudonana and D. tertiolecta. Controls received equal volumes of methanol or acetone daily.

Analytical grade TCE and HCB were obtained from Fisher Scientific Co. and Supelco Scientific Co., respectively. The Aroclor 1254 (Monsanto Co.) came from a laboratory stock provided by the Patuxent Wildlife Research Center, Laurel, Md.

Cultures were grown at 25°C in a temperature-controlled cabinet under constant illumination (2000 lux). They were sampled shortly after starting each experiment and every 24 hr thereafter for three days. Cell numbers were determined using a Levy counting chamber, and species-specific growth rates were calculated as doublings of cell numbers per day, using the method of EPPLEY and STRICKLAND (1968).

Cell size distribution was determined electronically with an Electrozone Celloscope^R (Particle Data, Inc.) particle counting and sizing system. Equipped with a 120-µm orifice, this system enumerated particles per ml in 100 channels, ranging in size from 2.4 to 23.4 µm equivalent spherical diameter (ESD). When growing exponentially in mixed culture, T. pseudonana and D. tertiolecta cells averaged 4.5 and 6.2 µm ESD, respectively, and were resolved as a bimodal distribution (Fig. 1). Data were transformed as square roots of the mean number of particles per channel (mean of three replicates); differences between means were tested for significance using the Pearson X^2 goodness-of-fit test (SOKAL and ROHLF 1969).

RESULTS

In control cultures, T. pseudonana had a higher growth rate than D. tertiolecta (Table 1) and the diatom therefore became dominant, giving the culture a yellow-brown color. Neither TCE nor HCB inhibited algal growth at 50 or 100 µg/L, and these cultures also were dominated by T. pseudonana (Table 1). While cultures were growing exponentially, cell size distributions did not differ significantly from those in the methanol- or acetone-treated controls (Pearson X^2; $p > 0.50$).

Although D. tertiolecta was unaffected, Aroclor 1254 strongly inhibited growth of the diatom T. pseudonana (Table 1 and Fig. 1). By day 2, Aroclor 1254-treated cultures were dominated by D. tertiolecta and were green in color.

Even when TCE or HCB were added daily at concentrations of 50 or 100 µg/L, the cell size distributions did not differ significantly from those in methanol- or acetone-treated control cultures (Pearson X^2; $p > 0.90$). Some T. pseudonana cells aggregated after 24 hr in methanol-treated controls and TCE-treated cultures, however, in response to repeated additions of methanol.

DISCUSSION

Relatively high concentrations (50 or 100 µg/L) of TCE or HCB were employed to determine if these compounds have any effect on phytoplankton. It was assumed that no effects at high concentrations indicate no effects at lower levels.

Neither TCE nor HCB at 50 or 100 µg/L caused any detectable effect on algal growth or size of progeny in a mixed culture containing a sensitive (T. pseudonana) and a resistant (D. tertiolecta) species of marine phytoplankton. This mixed culture system was previously shown to be highly sensitive to PCB, with altered species ratios evident at PCB additions as low as 0.1 µg/L (FISHER et al. 1974). Testing the freshwater alga Chlorella pyrenoidosa, GEIKE and PARASHER (1976) reported that 100 µg/L of HCB slightly reduced dry weight, carbohydrate, chlorophyll a and nitrogen concentrations in exponentially growing cultures.

Such chlorinated hydrocarbons as PCB (MOSSER et al. 1972b, FISHER and WURSTER 1973, O'CONNORS et al., in preparation), DDT (MOSSER et al. 1972b), DDE (POWERS et al. 1975), dieldrin (POWERS et al. 1977) and endrin (MENZEL et al. 1970) are more acutely toxic than TCE or HCB to marine phytoplankton, if results with this mixed culture are representative of other algae. Many cells are killed while growth, photo-

synthesis and cell size among surviving cells are reduced by exposure to less than 50 or 100 µg/L concentrations of these higher molecular weight compounds. If this diatom/green alga system has predictive value, we suggest that a few µg/L of TCE or HCB are unlikely to reduce algal growth or alter species succession within natural phytoplankton assemblages.

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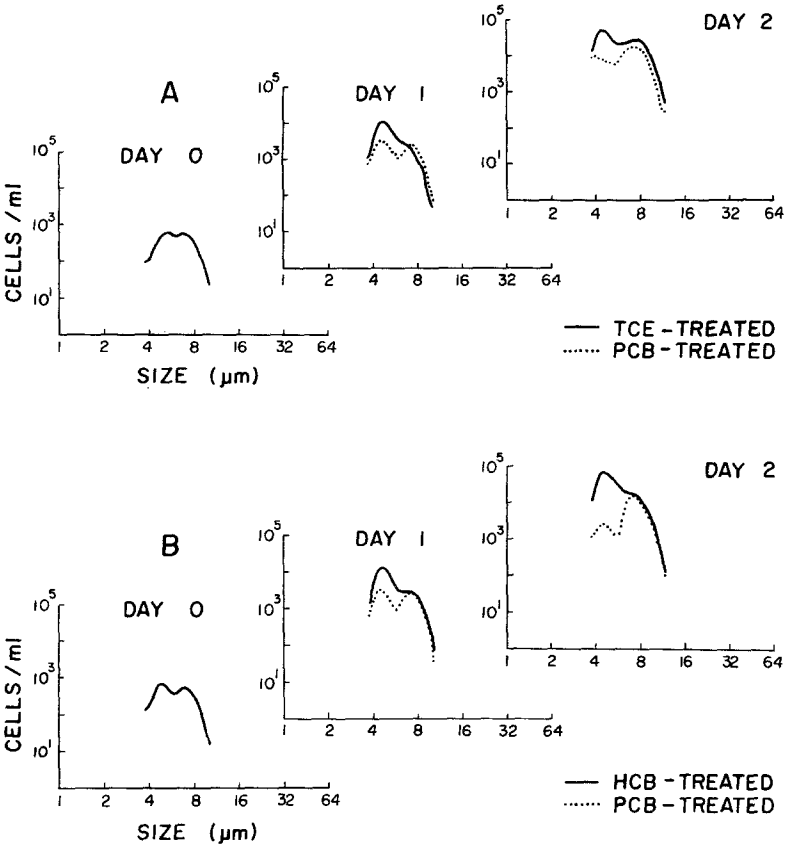

Figure 1. Mean culture density (n = 3) vs. cell size distributions of mixed species algal cultures treated with 50 µg/L of TCE, HCB or Aroclor 1254. A = TCE-treated vs. Aroclor 1254-treated cultures (Experiment 1); B = HCB-treated vs. Aroclor 1254-treated cultures (Experiment 2). Spectra of cultures treated with TCE or HCB did not differ from those of the respective controls.

TABLE 1

Growth rate (doublings of cell numbers per day) ± standard deviation (n = 3) of mixed species algal cultures treated with TCE, HCB or Aroclor 1254. Day 1 = 0 to 24 hours; day 2 = 24 to 48 hours; day 3 = 48 to 72 hours.

		Species-specific Growth Rate				Species-specific Growth Rate	
Day	Treatment	T. pseudonana	D. tertiolecta	Day	Treatment	T. pseudonana	D. tertiolecta
1	methanol	5.0 ± 0.8	2.2 ± 0.7	1	acetone	3.5 ± 0.2	1.3 ± 0.8
	TCE-50[a]	4.6 ± 0.2	1.4 ± 0.2		HCB-50[a]	3.7 ± 0.8	1.5 ± 0.8
	TCE-100[a]	4.7 ± 1.1	1.8 ± 1.0		HCB-100[a]	3.8 ± 0.2	1.0 ± 0.3
	PCB-50[c]	2.7 ± 1.0	2.1 ± 0.4		PCB-50[c]	1.5 ± 0.6	0.1 ± 0.1
2	methanol	3.1 ± 0.2	2.8 ± 0.5	2	acetone	3.1 ± 0.3	1.5 ± 0.3
	TCE-50[b]	3.6 ± 0.2	3.6 ± 0.5		HCB-50[a]	3.2 ± 0.7	2.0 ± 0.2
	TCE-100[b]	3.2 ± 0.5	2.8 ± 0.1		HCB-100[a]	2.9 ± 0.5	2.2 ± 0.5
	PCB-50[c]	0.3 ± 0.1	3.0 ± 0.4		PCB-50[c]	0.2 ± 0.1	3.7 ± 0.5
3	methanol	0.9 ± 0.1	0.3 ± 0.1	3	acetone	0.4	1.5
	TCE-50	0.7 ± 0.2	0.4 ± 0.1		HCB-50	-0.7	-0.1
	TCE-100	1.0 ± 0.3	-0.3 ± 0.1		HCB-100	-0.3	0.1
	PCB-50	-0.2 ± 0.2	1.4 ± 0.3		PCB-50	-	1.2

Footnotes: culture density vs. cell size distribution not significantly different from that of control (Pearson X^2);
a = $p > 0.95$; b = $0.95 > p > 0.5$; c = $p < 0.005$.

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