Polychlorinated Biphenyls: Toxicity to Certain Phytoplankters

Abstract. The growth rates of two species of marine diatoms were reduced by polychlorinated biphenyls (PCB's), widespread pollutants of the marine environment, at concentrations as low as 10 to 25 parts per billion. In contrast, a marine green alga and two species of freshwater algae were not inhibited at these or higher concentrations. The sensitivity of these species to PCB's paralleled their sensitivity to DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane].

Polychlorinated biphenyls (PCB's) are industrial chemicals that have become widespread contaminants of the biosphere (*1*, *2*). Their solubility properties, chemical stability, and apparent mobility have resulted in movement through the environment comparable to that of several organochlorine insecticides. Thus PCB's are present in rainwater (*3*), the tissues of marine fish, birds, and mammals (*1*, *4*, *5*), human tissues and milk (*6*), and various other components of the environment.

Knowledge of the biological impact of these widespread pollutants is still limited. Polychlorinated biphenyls are toxic to marine crustaceans, mollusks, and fish at concentrations of a few parts per billion (ppb) (*5*, *7*), and they induce the formation of hepatic hydroxylating enzymes in birds and mammals (*1*, *8*). Unlike several other chlorinated hydrocarbons, however, PCB's do not cause eggshell thinning in birds (*9*). Since certain chlorinated hydrocarbons are toxic to some phytoplankters (*10*), we have investigated the effects of PCB's on the growth of five species of unicellular algae.

Three of the species studied were marine; the two centric diatoms *Thalassiosira pseudonana* and *Skeletonema costatum*, and *Dunaliella tertiolecta*, a green alga, were cultured in half-strength "f-1" medium (*11*). Two freshwater species, *Euglena gracilis* and *Chlamydomonas reinhardtii*, were cultured, respectively, in the media of Edmunds (*12*) and Kates and Jones (*13*). *Skeletonema costatum* was cultured at 17°C and illuminated with 4300 to 5400 lumens per square meter from cool white fluorescent lamps; the other organisms were incubated at 23° to 24°C at an illumination of 7500 to 8600 lumens per square meter.

Methanol solutions of PCB's or DDT (*14*) were diluted 10,000-fold upon addition to cultures containing 10^4 exponentially growing cells per milliliter. Control cultures received equal volumes of methanol. Cells were counted with a Coulter counter after fixation of aliquots with formaldehyde at 4 percent (by volume) (*15*). Culture bottles were tightly sealed between samplings to minimize volatization of the chlorinated hydrocarbons, except for *S. costatum*, which did not grow well under these conditions and was cultured in plastic foam-stoppered flasks. The viability of *T. pseudonana* was determined by spreading a cell suspension on the surface of medium solidified with 1 percent (by weight) Bacto-Difco agar.

Results with *T. pseudonana* and *S. costatum* are shown in Fig. 1. The growth rate of *T. pseudonana* decreased as the concentration of PCB's increased from 25 to 100 ppb, but there was no significant inhibition at 10 ppb. Viability decreased in cultures treated with PCB's at concentrations of 50 and 100 ppb. *Skeletonema costatum* was more sensitive than *T. pseudonana* to PCB's, exhibiting some inhibition of growth at a PCB concentration of 10 ppb. *Skeletonema costatum* may be more sensitive than the data indicate because some of the chlorinated hydrocarbons were probably lost by volatilization. For both species, PCB's were more toxic than the equivalent amount of DDT (Fig. 1).

By contrast, the other three organisms studied were relatively resistant to PCB's and DDT. Concentrations of PCB's or DDT as high as 1000 ppb for

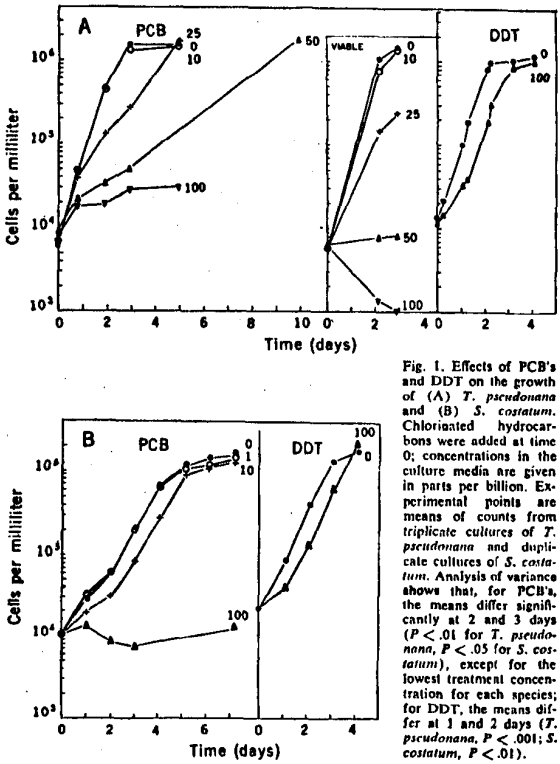

Fig. 1. Effects of PCB's and DDT on the growth of (A) *T. pseudonana* and (B) *S. costatum*. Chlorinated hydrocarbons were added at time 0; concentrations in the culture media are given in parts per billion. Experimental points are means of counts from triplicate cultures of *T. pseudonana* and duplicate cultures of *S. costatum*. Analysis of variance shows that, for PCB's, the means differ significantly at 2 and 3 days ($P < .01$ for *T. pseudonana*, $P < .05$ for *S. costatum*), except for the lowest treatment concentration for each species; for DDT, the means differ at 1 and 2 days (*T. pseudonana*, $P < .001$; *S. costatum*, $P < .01$).

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D. tertiolecta and 100 ppb for *E. gracilis* did not inhibit growth; *C. reinhardtii* was only slightly inhibited at a PCB concentration of 100 ppb.

Data on the amounts of PCB's in natural waters are limited. The low solubility of PCB's in water and their high solubility in lipids indicate that organisms, including algae, will accumulate PCB's from water, and that analysis for PCB's in water is an inadequate criterion for evaluating water quality (*2*). Thus, although PCB's were present in various marine organisms in Scotland, none were detected in the water (*16*). Nevertheless, high concentrations of PCB's in water may occur; PCB's freshly discharged into a Florida river reached concentrations as high as 275 ppb (*5*), well above the concentration that was lethal to the sensitive organisms in our experiments.

Selective inhibition of sensitive phytoplankton species by PCB's, DDT, and other stable pollutants in the environment may alter the species composition of natural algal communities (*10, 17*). Such effects at the base of aquatic or estuarine food webs could profoundly affect higher organisms as well.

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11. *Thalassiosira pseudonana* (strain 3H), formerly *Cyclotella nana* (Q. R. Haxle and B. R. Heimdahl, *Nova Hedwigia* **24**, 359 (1970)], was obtained from C. S. Hegis of the National Marine Water Quality Laboratory, Environmental Protection Agency, West Kingston, R.I.; *S. costatum* (clone "Skel") and *D. tertiolecta* (clone "Dun") were obtained from R. R. L. Guillard, Woods Hole Oceanographic Institution, Woods Hole, Mass.; and *E. gracilis* (strain z) and *C. reinhardtii* (strain 89) were obtained from H. Lyman and D. O'Kane, respectively, of the Division of Biological Sciences of the State University of New York at Stony Brook. For the culture of *T. pseudonana* and *D. tertiolecta*, Instant Ocean (Aquarium Systems, Eastlake, Ohio), adjusted to a salinity of 35 parts per thousand, replaced natural seawater in what was otherwise half-strength f-1 medium of R. R. L. Guillard and J. H. Ryther [*Can. J. Microbiol.* **8**, 229 (1962)]. *Skeletonema costatum* was grown in a medium prepared with water from Long Island Sound. The $NaHCO_3$ concentration was adjusted to 200 mg/liter in both media. Culture media were sterilized by filtration through 0.45- and 0.22-μm membrane filters (Millipore Corporation, Bedford, Mass.). All cultures were grown axenically.
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14. Aroclor 1254 (Monsanto, St. Louis, Mo.), a mixture of PCB's containing 54 percent chlorine (by weight), was kindly provided by the Patuxent Wildlife Research Center, Laurel, Md.; DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane], 99.9 percent pure, was a gift of the Geigy Chemical Corporation, Ardsley, N.Y.
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18. We thank R. Steele, National Marine Water Quality Laboratory, West Kingston, R.I., for advice on seawater culture media. This work was supported in part by NSF grant GB-11902 and by NIH fellowship 1F02ES48112-01 from the National Institute of Environmental Health Sciences.

13 September 1971; revised 13 October 1971

Sulfatide Synthesis: Inhibition by Experimental Allergic Encephalomyelitis Serum

Abstract. *The rate of ^{35}S incorporation into cerebroside sulfate in cultures of embryo mouse spinal cord shows a rapid acceleration at the time of myelin formation. Exposure of cultures to dilute serum from rabbits with experimental allergic encephalomyelitis results in almost complete inhibition of sulfatide synthesis. Within 24 hours after replacement of inhibiting medium with normal medium there is an increase in sulfatide synthesis followed by myelination.*

Incorporation of [^{35}S]sulfate into galactocerebroside to form sulfatide has been used for studying the formation and turnover of myelin and other membranous structures of the nervous system (*1*). Such studies indicate that in the developing rat brain there is a peak incorporation of sulfate to form sulfatide when the rat is about 18 to 20 days of age, the time of maximum myelination. It was further shown by McKhann and Ho (*2*) that the patterns of incorporation of [^{35}S]sulfate into sulfatide both in vivo and by brain homogenate in vitro are similar to the developmental pattern of activity of galactocerebroside sulfotransferase, measured in vitro.

Myelin first appears in cultures of spinal cord tissue of the embryo mouse on about day 8 to day 10 in vitro (*3*). By day 14 or day 15 in vitro the majority of cord cultures have an apparently normal complement of neurons, glia, and myelinated axons. When these cultures are constantly exposed to dilute serum from rabbits with experimental allergic encephalomyelitis (EAE), in the presence of complement, the differentiation of the oligodendroglia and the formation of myelin are totally inhibited. However, differentiation of neuroglia and primary myelination promptly follow the removal of EAE serum (*3*).

We now report that the developmental pattern of sulfatide synthesis in embryonic mouse spinal cord, in relation to myelination, is comparable to that in rat brain. In addition, a marked depression of sulfatide synthesis occurs during EAE serum inhibition with a prompt increase of sulfate incorporation when the nutrient medium containing dilute EAE serum is replaced by normal nutrient medium.

Tissue cultures of 13- to 14-day-old embryo mouse spinal cord were prepared and maintained as described (*3*). All cultures were derived from the same litter (called sister cultures) of CD Swiss mice (Charles River) and each cover slip bore two fragments of spinal cord. At the time of explantation, half of the cultures were fed and subsequently maintained on normal nutrient medium, and half were fed and subsequently maintained on nutri-

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