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 volatilization 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

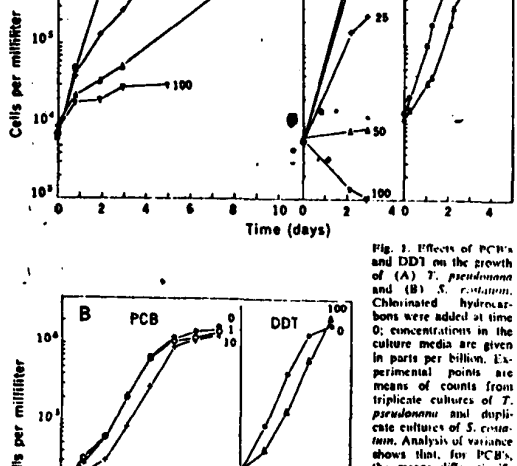

Fig. 1. Effects of PCB's and DDT on the growth of (A) *T. pseudonana* and (B) *S. costatum*. Chlorinated hydrocarbons were added at time 0; concentrations in the culture media are given in parts per billion. Experimental points are means of counts from triplicate cultures of *T. pseudonana* and duplicate 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* [G. R. Hasle and B. R. Heimdahl, *Nova Hedwigia* **31**, 559 (1970)], was obtained from C. S. Hoyt 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 G. R. L. Gaudlad, Woods Hole Oceanographic Institution, Woods Hole, Mass.; and *A. gracilis* (strain 2) and *C. reinhardtii* (strain 89) were obtained from H. Lyman and D. O'Kelley, 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 25 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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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-11982 and by NIH fellowship 1F02ES40112-01 from the National Institute of Environmental Health Sciences.

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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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Chemical Pollution: Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are widely used industrial chemicals that were never intended for release into the environment, but they have nonetheless been found in rainwater, in human tissue, and in many species of birds and fish. How PCBs came to be widely distributed in the environment and what biological effects they have are still largely unknown. But like DDT, PCBs apparently degrade very slowly under natural conditions. They tend to accumulate in the food chain, and once ingested, they are stored in the body's fatty tissues. As is true with many other chlorinated hydrocarbons, even small doses of PCBs can be toxic, although it is not clear that the amounts found in fish or birds have had detrimental effects. The only epidemiological information about PCBs' effects on man comes from an accidental poisoning incident in Japan in which some 1000 persons were affected.

Polychlorinated biphenyls are relatively inert compounds that have low solubilities in water. The PCBs produced commercially are mixtures, incorporating some 50 or more of the 210 different PCB compounds that are theoretically possible from chlorinating biphenyl. The most common industrial mixtures average between 40 and 60 percent chlorine content, but their isomeric composition is only partially known, thus complicating the problems of sampling and analysis. Gas chromatographic techniques are now most commonly used to determine the presence and the amount of PCBs in a sample. The method is not specific for identifying particular PCB compounds because of the lack of samples of the different isomers as reference standards. There appears to be some variability in biological and chemical activities among the compounds; highly chlorinated PCBs seem to persist in the environment the longest and are less toxic than the more rapidly degraded low chlorine forms. There is also evidence that some commercial PCB mixtures, especially those manufactured in Europe or Japan, may contain trace amounts of dibenzofurans or other extremely toxic impurities, which may be the cause of some of the toxic effects ascribed to PCBs themselves.

The major uses of PCBs derive from their nonflammability, high dielectric constant, and plasticizing abilities. These uses include applications as dielectric fluids in capacitors and transformers, as hydraulic fluids, and as heat transfer fluids; also PCBs have been widely used as plasticizers and solvents in adhesives, sealants, paints, and printing inks. Some 95 percent of the large phase correction capacitors used in transmitting electric power, for example, contain PCBs—amounting in 1968 to almost 2.5 million gallons in the United States alone*; replacement of PCBs by other dielectric fluids, according to Martin Broadhurst of the National Bureau of Standards, would require that the capacitors be rebuilt and that they be 50 percent larger. Alternate materials for plasticizer applications of PCBs, on the other hand, are readily available.

Industrial use of PCBs has grown steadily since their introduction in 1930. U.S. sales in 1970 came to about 24,000 tons, with cumulative production over the years amounting to an estimated 4×10^5 tons. (By comparison, U.S. production of DDT in recent years has been about 70,000 tons.) Less, in fact almost nothing, is known about the quantities produced worldwide; in addition to the production in the United States, PCBs are made in Europe, Japan, and the U.S.S.R. The only U.S. producer of PCBs, the Monsanto Chemical Corporation, refused until recently to release production figures, but the company has taken the unusual step of refusing to sell any more PCBs for plasticizer applications and those heat transfer applications involving food preparation. Current annual U.S. sales, as a result, have fallen to about 20,000 tons per year, although exports have continued to increase.

How PCBs are released into the environment is not known, but several scientists believe that sewage outfalls and industrial disposal into waterways are major sources. Rivers and streams may be the primary means of transport for PCBs within the environment, although the atmosphere also appears to be a means of dispersal. Adel Sarofim of the Massachusetts Institute of Technology has estimated that from 1000 to 2000 tons per year escaped into the atmosphere in past years from plasticized materials containing PCBs, and that about 4000 tons per year entered waterways from dumping and leakage of lubricants, hydraulic fluids, and heat transfer fluids. Other sources may include leaching from dumps and landfills, where, Sarofim believes, about half of the PCBs produced eventually end up. Although measurements of PCBs in air and water are scant, the general pattern from these measurements and from more extensive measurements of PCB residues in biotas is consistent, according to Sarofim, with the hypothesis that discharge of PCBs into the environment occurs mainly near industrial areas. A similar pattern was found by Gilman Veith of the University of Wisconsin in his studies of Lake Michigan watersheds; he also detected PCBs in the sewage of all municipalities studied.

Once in the environment, PCBs appear to persist for a long time. Laboratory studies by O. Hutzinger of the National Research Council of Canada in Halifax and others have indicated that PCBs can be dechlorinated by ultraviolet light and lead to the formation of hydroxyl derivatives and other polar compounds that are more readily degraded by microorganisms, but the extent to which this occurs under natural conditions is not known. In many areas, however, PCB residues in fish are equivalent to or higher than DDT and other pesticide residues. David Stalling of the Bureau of Sport Fisheries and Wildlife, Columbia, Missouri, found PCB residues as much as 100 times larger than DDT residues in fish from heavily industrialized areas, with roughly equal residues of both substances in fish from many other regions.

The highest PCB residues in fish seem to occur in highly polluted coastal waters—like Tokyo Bay and Long Island Sound—and in some inland waterways—such as the Great Lakes—where coho salmon have been found with PCB concentrations in excess of 5 parts

* More detailed reference to many of the studies mentioned here can be found in the forthcoming initial issue of *Environmental Health Perspectives*, a journal to be published by the National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

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per million, the limit at which the U.S. Food and Drug Administration attempts to seize contaminated fish and poultry sold in interstate commerce. More typical concentrations, in most species of fish in the North Atlantic, range from 0.01 to 1.0 ppm. Fish taken from undisturbed arctic lakes have been found to contain comparable residues. Both surface-feeding fish and those that dwell at greater depths contain PCBs. Even higher concentrations are found in birds that feed on fish, reaching 300 to 1000 ppm in cormorants and ospreys. According to Robert Risebrough of the University of California's Bodega Marine Laboratory, residues are generally highest in bird specimens from the North Atlantic or from along the California coast, and generally lower in birds from the South Atlantic and Central Pacific Oceans. Residues appear to be lowest in wildlife from the Antarctic region.

Most U.S. inhabitants apparently have some residues, if only traces, of PCBs in their bodies. Preliminary data from a nationwide monitoring program being coordinated by the Environmental Protection Agency show that 33 percent of more than 600 samples of human adipose tissue contained PCB residues of at least 1 ppm. One individual, an old man with no known industrial exposure to PCBs, was found by the Michigan Department of Health to have 100 ppm in his fatty tissues.

The source of PCB residues in human tissue is presumably food. Studies in Sweden have indicated that fish are the primary source of PCBs for most Swedes, with average intake of PCBs from all sources estimated to be about 1 microgram per kilogram of body weight per day. Individuals who ate large amounts of fish consumed about ten times as much PCBs. Babies received the highest doses per kilogram of body weight, largely from milk. Residues of PCBs in food were somewhat smaller than those of DDT and metabolites of chlorinated pesticides.

In the United States, the pattern appears to be the same, with PCBs common in freshwater fish such as coho salmon and lake trout. Small residues in cereals have been traced to the recycled paper used in packaging and thence to the ink in a brand of carbonless carbon paper, among other sources. The ink no longer contains PCBs, and efforts are being made to separate out old stocks of the carbon paper in the manufacture of recycled paper. Several batches of poultry and eggs with high PCB residues have been traced to contamination of the poultry feed by leaking heat exchangers, except in one instance where the source of the PCBs remains undetermined. Residues are present in dairy milk in some localities.

Officials of the Food and Drug Administration characterize PCBs as a "potential but not immediate health hazard," and claim, on the basis of their monitoring programs, that except for his eating of fish the American consumer receives no systematic exposure to PCBs. However, FDA authority extends only to interstate sales of food, and FDA guidelines tend to reflect only the average consumer. It seems likely, because of the localized nature of PCB sources and variations in individual diet, that heavy fish eaters and some other individuals experience more substantial exposures.

The only clinical experience with human illness that is directly traceable to PCB consumption occurred in 1968 in southern Japan. More than 1000 persons who had eaten rice oil contaminated with PCBs that leaked from a heat exchanger during manufacture developed darkened skins, eye discharge, severe acne, and other symptoms of what came to be called Yusho, oil disease. Patients were found to have consumed an average of 2 grams of PCB, with symptoms appearing at a minimum dose of about 0.5 g. The PCBs can readily cross the placental barrier, and several infants were born with Yusho symptoms, some to apparently unaffected mothers. Recovery appears to be difficult, according to Masanori Kuratsune of Kyushu University in Fukuoka, Japan, with symptoms still present in many cases 3 years later; no methods of treatment are known. It is not clear whether any of the subsequent deaths among the patients can be attributed to acute PCB poisoning, and it is still not known whether the commercial PCB mixture involved contained traces of dibenzofurans or other impurities.

Although PCBs have been in use for more than 30 years, detailed studies of their toxic properties have only recently been undertaken. The studies are complicated by variability in the toxicity of different commercial samples of PCBs. Experiments in the Netherlands by J. G. Vos of the University of Utrecht showed liver damage and edema in chickens and skin lesions in rabbits. The worst effects seemed to be caused by an impurity in the European PCB mixture, which Vos believes to be dibenzofuran.

The results of a 2-year study funded by Monsanto showed liver damage and some difference in weight gain in rats and dogs whose feed contained 100 ppm of PCBs, but no teratogenic or mutagenic effects. At a dose rate of 10 ppm, there were apparently no effects of any kind, except for some impairment to the hatching of chicken eggs. Reproduction was affected most by low-chlorine PCB mixtures, but long-term damage to the liver seemed to be due to high chlorine mixtures. By contrast, in one of the field occurrences of PCB contamination, the hatchability of eggs at Holly Farms in North Carolina was noticeably decreased, even though the concentration of PCBs in the feed was less than the no-effect levels reported in the Monsanto study.

Although most birds and mammals appear to be relatively resistant to PCBs, some species, such as mink, seem to be more susceptible. Fish are also more susceptible; Stallings found that bluegill and catfish died when exposed for several weeks to concentrations between 20 and 50 parts per billion, that trout died at about 8 ppb, and that lethal doses for shrimp were as low as 1 ppb.

The biological actions of PCBs inside the body are also relatively unknown. PCBs have been shown to alter liver tissue, inhibit the growth of cultured cells, and affect a variety of enzyme systems; but in most instances the mechanism of action is still uncertain. Paul Lichtenstein of the University of Wisconsin has shown that PCBs interact with DDT and other chlorinated pesticides; nontoxic amounts of PCBs in insects increased the toxicity of DDT and some organo phosphate pesticides by as much as 100 percent.

Much of the research on PCBs has pointed to the similarity of PCBs and DDT in their environmental effects. They are both widely distributed in the environment, they both affect the reproductive system of birds, and they enter the human diet primarily through residues in fish. But PCBs, unlike DDT, were seldom deliberately released into the environment. Their presence and persistence there reemphasize the likelihood that any widely used industrial chemical may become an environmental pollutant, and increase the responsibility for public disclosure of production quantities and use patterns when similar situations occur in the future.

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