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Effect of Polychlorinated Biphenyl Formulations on the Growth of Estuarine Bacteria[1]

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Polychlorinated biphenyl formulations inhibited the growth of certain estuarine bacteria. The sensitive strains, although exhibiting some similar physiological characteristics, contained both gram-positive and gram-negative bacteria.

Polychlorinated biphenyl formulations (PBCs) called Aroclors (Monsanto Co., St. Louis, Mo.) have been used commercially as lubricants, plasticizers in paints, plastics, and chlorinated rubbers, as heat-exchange fluids in industrial heating systems, and dielectric compounds in large electrical transformers and capacitors (9). Aroclor formulations are identified by four-digit numbers, the first two indicating the type of molecule and the last two indicating the weight percentage of chlorine in the molecule. PBCs have been reported as environmental pollutants from water, sediment, and biota (2, 3). The ubiquity and significance of various Aroclor formulations have been reviewed (3, 7, 9).

Despite their ubiquity (7) and effects on estuarine and marine organisms (1, 6), only cursory information has been accumulated on the interactions of PCBs and microorganisms. A commercial PCB formulation (Aroclor 1254) has been reported to stimulate in vitro growth of *Escherichia coli*, a bacterium from human intestinal microflora used as an indicator of water quality (4). Inhibition of bacterial growth by PCBs has not been previously reported. This study employs a rapid sensitivity disk assay method to determine the effects of Aroclor 1016 and 1242 on the growth of selected estuarine bacteria.

Eighty-five bacterial isolates from various estuarine environments near Pensacola, Florida, were examined for ability to grow on solid medium in the presence of varying concentrations of Aroclor 1016 and 1242. Each liter of test medium contained 1.0 g of yeast extract (Difco), 5.0 g of beef peptone (Difco) and 20.0 g of agar (BBL), in aged, artificial seawater (Rila Marine Mix, aged 1 month in dark), pH 7.4 and 2.0% salinity. Cells for inocula were grown in this medium without agar for 18 h at 28 C on a rotary shaker. This culture was diluted 1:1 with sterile 2.0% seawater, and 0.1 ml was spread on the agar medium. Absorbant paper disks (Schleicher and Schuell, Inc., no. 740-E, 12-mm diameter) were saturated with 0.1 ml of acetone solutions containing 1.0, 2.5, and 5.0 mg of PCB formulation per ml and air-dried for 24 h at room temperature before use. The PCB-treated paper disks and control disks treated only with acetone were arranged on the agar surface within 2 h of inoculation. Duplicates of each test were prepared. The cultures were incubated for 24 h at 28 C and examined for sensitivity. Sensitivity was defined as a zone of inhibition surrounding the paper disk (Fig. 1).

Of 85 different isolates tested, growth of 26 was inhibited to varying extents by 0.5 mg of either PCB formulation. Zones of inhibition ranged in size from 14 to 20 mm. Cultures sensitive to Aroclor 1242 were also inhibited by Aroclor 1016. Sixty-five percent of the cultures sensitive to 0.5 mg of Aroclor 1242 were still sensitive at 0.1 mg, and 58% of cultures sensitive to Aroclor 1016 at 0.5 mg were sensitive at 0.1 mg.

Identified genera sensitive to PCBs included *Flavobacterium*, *Bacillus*, *Corynebacterium*, *Pseudomonas*, *Achromobacter*, *Micrococcus*, and *Serratia*. Representative cultures of the sensitive bacteria from the disk assay were tested for growth inhibition in liquid culture. Four bacteria—two gram-positive and two gram-negative—were monitored for 30 h in nutrient marine broth and nutrient marine broth plus Aroclor 1241 (10 μg/ml). Growth inhibition of all four test bacteria by Aroclor 1242 is shown in Fig. 2. The nature of the inhibition is unknown; however, the greatly extended lag phase of all test bacteria suggests bacteriostasis.

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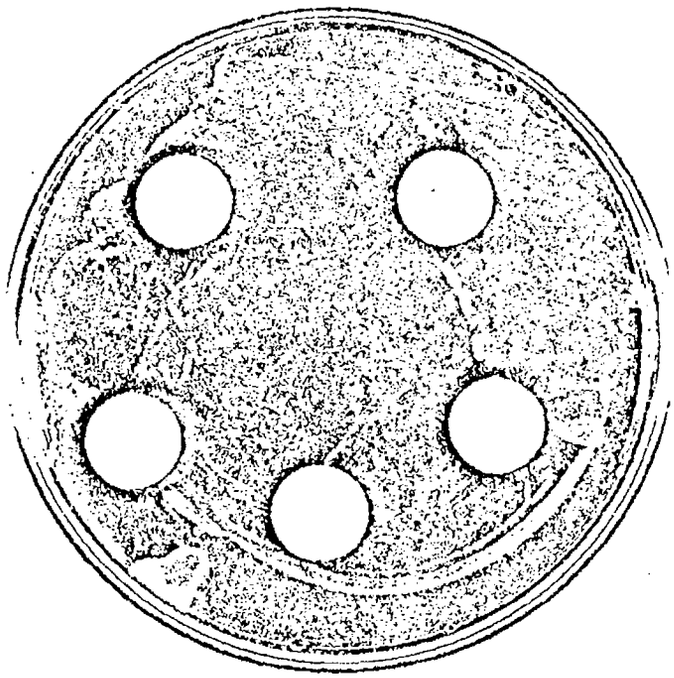


FIG. 1. *Effect of PCB-treated disk on growth of estuarine bacteria. Lower middle disk is acetone control; left to right, 0.5 mg of 1242, 0.5 mg of 1016, 0.5 mg of 1242, 0.5 mg of 1016.*

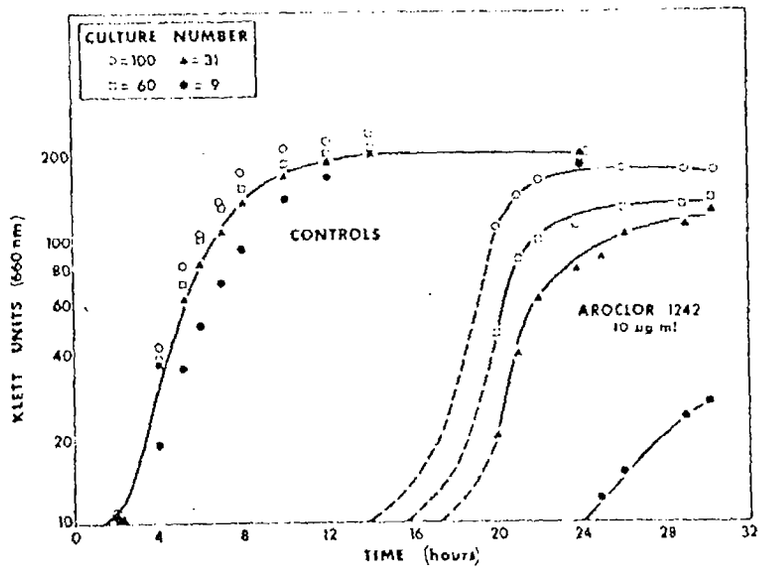


FIG. 2. *Four sensitive bacteria were tested for growth in one-half strength 2216 Marine Broth containing 10 µg of Aroclor 1242 per ml. Symbols: (○) Achromobacter sp., (●) Bacillus sp., (▲) Corynebacterium sp., and (□) Pseudomonas sp. Controls contain one-half strength 2216 Marine Broth only; all salinities were 20‰.*

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TABLE 1. *Comparison of physiological activities of PCB-test bacteria*[a]

Bacteria tested	Production of:					Nitrate reduction	Citrate utilization	Gram reaction
	Urease	Amylase	Lipase	Gelatinase	H_2S			
Sensitive	19	76	33	86	5	29	48	50
Total tested	13	33	23	42	6	37	41	32

[a] Percentage of cultures showing positive reaction.

The PCB-sensitive bacteria included both gram-positive and gram-negative isolates; however, compared to the test population, a slightly greater percentage of the sensitive strains were gram-positive (Table 1). Previous reports indicated that the growth of gram-positive bacteria was inhibited by organochlorine insecticides (technical chlordane), whereas that of gram-negative organisms was unaffected (8, 10). These results suggest that the growth of some gram-negative estuarine bacteria (13 organisms) was sensitive to PCBs, a mixture of organochlorine molecules. When PCB-sensitive and nonsensitive strains were compared (Table 1), the biochemical activities of test bacteria showed two different groups. Amylase was produced by 76% and gelatinase by 86% of the sensitive strains, whereas in the total test population only 33 and 42% showed these activities. Twenty of the 28 amylase producers were also sensitive to PCBs, as were 22 of the 36 gelatinase producers. Further investigation is needed to determine whether the observed prevalence of PCB sensitivity among such amylase and gelatinase producers is significant in relation to total nutrient catabolism.

The sensitivity disk testing procedure commonly used in clinical bacteriology provides a rapid method for detecting estuarine microorganisms that are sensitive to commercial PCB preparations. Although the procedure is qualitative only, it eliminates many hours of tedious experimentation in selecting suitable test organisms for more detailed examinations. These results indicate that a sizeable proportion of estuarine microbes tested were sensitive to commercial PCB formulations. If present in estuaries at the concentrations tested, these PCB formulations could disrupt microbial heterotrophic activity.

PCBs persist in estuarine sediments in relatively high concentrations. Sediments from Escambia Bay, Florida, contained concentrations ranging from 0.6 to 61.0 mg/kg dry weight (2) and sediments at an industrial outfall in the same bay system up to 486 mg/kg dry weight (6). When considering the microenvironment of a sediment core, discrete organic particles, potential microbial substrates, may contain a much greater concentration of PCBs than that reported for the whole core. Such PCB concentrations are similar to, or greater than, concentrations found inhibitory to heterotrophic growth in our study. In nature, PCBs and bacteria are adsorbed on particulate surfaces; consequently, PCBs, by inhibition of specific heterotrophic bacteria attached to organic detritus, could inhibit normal turnover of carbon in estuarine sediments.

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