EPA-600/3-77-070
June 1977

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EFFECTS AND INTERACTIONS OF POLYCHLORINATED BIPHENYL (PCB) WITH ESTUARINE MICROORGANISMS AND SHELLFISH

Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Gulf Breeze, Florida 32561

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EFFECTS AND INTERACTIONS OF POLYCHLORINATED BIPHENYL (PCB) WITH ESTUARINE MICROORGANISMS AND SHELLFISH

by

Rita R. Colwell and Gary S. Sayler
University of Maryland
College Park, Maryland 20742

Grant No. R-803300-01-0

Project Officer

A. W. Bourquin
Environmental Research Laboratory
Gulf Breeze, Florida 32561

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FOREWARD

The protection of our estuarine and coastal areas from damage caused by toxic organic pollutants requires that regulations restricting the introduction of these compounds into the environment be formulated on a sound scientific basis. Accurate information describing dose-response relationships for organisms and ecosystems under varying conditions is required. The Environmental Research Laboratory, Gulf Breeze, contributes to this information through research programs aimed at determining:

- the effects of toxic organic pollutants on individual species and communities of organisms;
- the effects of toxic organics on ecosystem processes and components;
- the significance of chemical carcinogens in the estuarine and marine environments.

The role of microorganisms in the mobilization, transport, and possible removal of organic pollutants is an important aspect for consideration in the proper regulation of these compounds in the ecosystem. Additionally, microorganisms from sewage outfalls, etc., can and do act as pollutants to shellfish in the estuarine environment. The secondary effects of toxic organic pollution on the accumulation and depuration of enteric bacteria by shellfish is an important area of research given little attention. This report contributes to our knowledge on the interactions of biotic and abiotic pollutants.

Al W. Bourquin, Ph.D.
Research Microbiologist

ABSTRACT

The role of estuarine bacteria in the mobilization, transport, and removal of polychlorinated biphenyls (PCB) was investigated in estuarine environments. A main objective of this investigation was to determine a secondary impact of PCB contamination of estuarine systems. The specific secondary effect was the PCB-stress-induced accumulation and depuration of enteric bacteria by shellfish, i.e., the Chesapeake Bay oyster, Crassostrea virginica.

For this report, bacteria uninhibited by PCB, but capable of growth in the presence of PCB, are defined as PCB-resistant. In this regard, PCB-resistant bacteria were found to be distributed ubiquitously throughout estuarine and marine environments sampled in this study. The residence time of PCB in estuarine and marine environments is concluded to be sufficiently long to induce stress upon estuarine animals.

This study was completed October 31, 1975. The project was supported by EPA Grant R-803300-01-0, Maryland Department of Natural Resources, Westinghouse Agency Contract No. 34-A-03427, and National Oceanographic Atmospheric Administration Sea Grant No. 04-5-15811.

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ACKNOWLEDGMENTS

The technical assistance and contributions of the following individuals and organizations are gratefully acknowledged: Ms. Andi Hirsh and Mr. Mark Shon, Department of Microbiology, University of Maryland; Dr. R. Thomas, Mr. B. Olive and Mr. R. L. Marshall, U.S. Environmental Protection Agency, Chemical and Biological Investigations Branch, Beltsville, Maryland; and the crews of the R/V RIDGELY WARFIELD, Chesapeake Bay Institute, Johns Hopkins University, and R/V EASTWARD, Duke University Marine Laboratory. Conjoint support for this study was made available through Maryland Department of Natural Resources Grant, Westinghouse Agency, No. 34-A-03427 and National Oceanographic Atmospheric Administration Sea Grant No. 04-5-15811. Shiptime was made available through National Science Foundation Grant No. GD-31707. Computer time and facilities were made available through the University of Maryland Computation Center, Grant No. 204104.

SECTION I

INTRODUCTION

The occurrence of polychlorinated biphenyls (PCB) in freshwater, estuarine, and marine environments has been documented (5, 7, 13, 15, 34). These industrial compounds are recognized as persistent pollutants of global importance (24, 25). They have been shown to be toxic to aquatic invertebrates and vertebrates (7, 11) and can be transferred and accumulated in food webs which may include man's (26). At the microbial level, PCB's have been reported to inhibit growth of phytoplankton populations (8, 22) and to interfere with protozoan chemotaxis (39). They also can stimulate or inhibit bacterial growth (4, 18).

Recent evidence has indicated a reduction in the concentration of PCB's in some marine environments (13). It was concluded that the decline in PCB levels was due primarily to reduction in the use of PCB's, mandated by the federal government. Significant biodegradation was assumed to be nonexistent. However, stimulatory and inhibitory effects of PCB formulations on bacterial growth and activity have been reported (4, 18), as has microbial degradation of PCB's (1, 17, 40).

Hypothetically, microbial degradation of chlorinated biphenyls is a potential mechanism for their removal from the aquatic environment. Therefore, a primary objective of this investigation was to assess the potential for degradation of PCB by estuarine bacteria of Chesapeake Bay. In addition, the metabolic fate of PCN was determined in order to assess ecological effects of PCB contamination on heterotrophic bacterial populations in the estuarine environment.

Investigations in our laboratory have focussed on the biodegradation of three ubiquitous pollutants: mercury compounds, petroleum hydrocarbons, and polychlorinated biphenyls (PCB) (23, 29, 36). These pollutants commonly are localized in the sediments of the aquatic environment. Resultant concentrations and interactions among these components are relatively unknown. Available evidence indicates a propensity of petroleum hydrocarbons to concentrate chlorinated hydrocarbon pesticides (12, 30) and mercury compounds (37), but the sequestering of both of these pollutants by petroleum in a fresh or marine system has yet to be shown.

Little information is available concerning secondary levels of impact of PCB contamination on estuarine and marine animals. A secondary level of impact includes PCB-induced stress, altering the normal physiology of the animal, and rendering it vulnerable to invading parasites or pathogens.

An objective of this investigation was to determine whether PCB-induced stress on the oyster, Crassostrea virginica, caused it to accumulate enteric bacteria. A study was undertaken to test the hypothesis that PCB concentrations commonly encountered by estuarine invertebrates may result in reduced bacteriological quality of a commercially important shellfish. Other investigators have shown that the oyster can effectively filter pathogenic bacteria and viruses from overlying waters and accumulate significant quantities of these microorganisms in tissue and on gill surfaces (9, 14). Retention of enteric or pathogenic bacteria in stressed oysters could lead to serious economic, as well as public health situations, if commercial oyster beds are closed as a result of high coliform counts from PCB or other stress, excluding sewage contamination.

SECTION II

CONCLUSIONS

At low concentrations (<100 μg l^{-1}), the effect of PCB may be stimulatory to heterotrophic bacterial growth. PCB stress on estuarine invertebrates is such that an improved bacteriological quality of commercially important shellfish may be deceptive since, in fact, it may be the result of preferential effect of PCB on enteric bacteria. Additional study should be undertaken to evaluate the full impact of PCB contamination on the ecology of aquatic microorganisms.

SECTION III

RECOMMENDATIONS

Results of this study, namely that PCB contamination has a detectable impact on the microbial activity of selected estuarine and marine bacteria, suggest that environmental discharges of PCB, including incineration and other forms of release, should be restricted and be subject to critical monitoring. The impact of PCB on autochthonous, heterotrophic microorganisms should also be monitored, both under *in situ* and laboratory conditions to describe accurately total impact of PCB. Effects of PCB in microbial ecology should receive greater attention. Predation of higher trophic levels on bacteria, nutrient cycling by bacteria, and changes in species diversity of microorganisms can provide indices of environmental quality. These indices should be further investigated so their potential can be developed. Secondary stress on higher organisms as a consequence of PCB contamination, such as bacterial invasion and pathogenesis, also should be investigated. Synergistic effects on heterotrophic processes, as co-contamination of PCB and heavy metals, appear significant and should be investigated further.

SECTION IV

MATERIALS AND METHODS

PCB EFFECT UPON ACCUMULATION OF ENTERIC BACTERIA BY SHELLFISH

Culture Conditions

Laboratory investigations were conducted by using three bacterial strains; one an indicator of contamination by domestic sewage and the other two, known pathogens: Escherichia coli type I and Salmonella enteritidis, isolated from Upper Chesapeake Bay and a laboratory stock culture of Salmonella typhimurium.

Bacterial cultures were harvested by centrifugation at 16,300 x g after growth for 48 hr in nutrient broth. Pelleted cells were resuspended in sterile salts broth. The resuspended cells were divided into equal portions and used for inoculation of aquarium water in tanks containing oysters.

Analysis of shellfish tissue, following dosing with the bacteria, was according to American Public Health Association (APHA) procedures (3). Oyster shell surfaces were disinfected with 2.5% hypochlorite in an ice bath. Oysters and clams were shucked, and their tissues were excised, rinsed with phosphated buffered saline, weighed, and homogenized with 100 ml 0.5% peptone.

Total viable bacterial counts (TVC) of both the oyster homogenate and the aquarium water were performed by using UBYE agar and appropriate dilutions of the samples. Quantitative E. coli determinations were made by employing MacConkey agar. Since there was an absence of lactose-fermenting organisms prior to E. coli dosing, all lactose-positive cultures growing on MacConkey agar were recorded as E. coli. At high concentrations of E. coli, water or tissue dilutions were plated directly on MacConkey agar. As the number of E. coli dropped, membrane filters (Millipore Corp., New Bedford, Mass.) were used to concentrate the bacteria. The filters were placed on the surface of MacConkey agar plates and incubated at 37°C for 24 to 48 hr.

A similar procedure was used for estimation of Salmonella typhimurium, except that Bismuth Sulfate Agar (BSA) was used for enumeration. Green colonies on BSA, after 24 hr incubation at 41°C, were recorded as S. typhimurium. Additional confirmatory tests were made on Kliegler iron agar, as warranted, to determine if biochemical alteration of the Salmonella resulted from exposure to PCB. Salmonella enteritidis was enumerated with similar methods and by using Brilliant Green Agar (Difco Laboratories, Detroit, Mich.) as the selective differential plating medium.

PCB stress was simulated by using Aroclor 1254[R], coated on diatomaceous earth (Celite) (J. T. Baker Chemical Co., Phillipsburg, N.J.). PCB dosing was maintained at 10 mg per liter (100 mg per liter Celite) for all experimental work.

Oyster Maintenance

Oysters (Crassostrea virginica) used in this study were dredged from Tolly Bar in the lower part of Upper Chesapeake Bay, near Annapolis, Maryland. This area of Chesapeake Bay, including water, sediment, and oysters harvested in the area, has been found free of enteric pathogens and is judged fit for shellfish harvesting (28). Each animal collected received a preliminary cleaning aboard ship to remove mussels and associated animals from the shell. All oysters were transported to the laboratory and stored at 6°C within 6 hr of collection. Experimental work was initiated within 72 hr of collection. Experiments using the soft shell clam, Mya arenaria, were similar to those with oysters.

Oysters were maintained in 60 gallon, custom-designed, recirculating refrigerated aquaria (Sea Lake Systems, Inc., Euclid, Ohio). Operating temperature was maintained at 15°C. Each aquarium was sterilized by autoclaving in an AMSCO steam autoclave (American Sterilizer Corp., Erie, Pa.). Two hundred liters of steam-distilled water were filtered through 0.45 µm, 90 mm Millipore membrane filters and added aseptically by gravity flow to each aquarium. Artificial sea salt (Sea Lake Systems) was autoclaved in the dry state and was added to each aquarium, to a final salinity of 12 °/oo, equal approximately to the in situ salinity at Tolly Bar. Each aquarium was fitted with glass covers to reduce, or eliminate, potential contamination. Refrigerant coils and air lines were disinfected with 2.5% hypochlorite prior to each experiment.

One hundred randomly sized oysters were selected from the total set of oysters collected. Shell surfaces were thoroughly cleaned with a wire brush and each animal was surface-disinfected in an ice bath, followed by an iced 2.5% hypochlorite bath for 3 to 5 min. Icing insured that each animal remained tightly closed, hence preventing the disinfectant from reaching the tissue of the animal. In each two aquaria were placed 50 cleaned and disinfected oysters. The oysters were retained in the aquaria for 48 hr so they would become equilibrated to the system.

Following the equilibration period, one group of oysters received a dose of 10 mg per liter Aroclor 1254 coated on 100 mg per liter Celite. The duplicate aquarium received a placebo of 100 mg per liter Celite and, therefore, served as the control for the experiment. Both sets of oysters were held under identical conditions except that stress was induced in one aquarium by addition of PCB. Ninety-six hours after PCB dosing, five oysters were aseptically removed from each tank, disinfected, and assayed for bacterial quality, according to APHA procedures (3). After removal of the five control

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Aroclor 1254[R], Registered Tradename, Monsanto Industrial Chemicals, St. Louis, Mo.

oysters, both tanks received a dose of a washed bacterial suspension, after which five oysters were removed from each tank, disinfected, and assayed for accumulated bacteria. Sampling of oysters and water from both aquaria proceeded at established time intervals for 12 days, after which the remaining oysters from both tanks were removed, surface-disinfected, and placed in separate sterile aquaria. Elimination of the accumulated bacteria was monitored in water of the aquaria to which the oysters had been transferred; purging of bacteria from the animals was determined by periodic sampling of the oysters.

PARTITIONING OF PCB AND HG

Chemicals

Isotopically labeled $^{203}HgCl_2$ (Amersham Searle Corp., Arlington Heights, Ill.) and {U-^{14}C} 2, 4, 5, 2', 4', 5' hexachlorobiphenyl (HCB) (New England Nuclear Corp., Boston, Mass.), 98% purity as determined by thin layer chromatography, were employed in all partitioning studies. Artificial seawater was prepared with Tri Sea Salts (Sea Lake Systems, Inc.). Sediment was simulated with the diatomaceous earth, Celite (J. T. Baker Chemical Co.).

Experimental Design

An experimental outline describing the partitioning of Hg and HCB between three phases of an oil, water and sediment system is given in Table 1. In order to assess the various partitioning of each phase in the presence of $HgCl_2$ and HCB, separately or in combination, experimental test systems were established which included water, water and oil, water and sediment, and water, oil and sediment for both freshwater and seawater.

TABLE 1. OUTLINE OF THE EXPERIMENTAL DESIGN FOR ASSESSMENT OF THE PARTITIONING OF MERCURY AND HCB IN WATER, OIL AND SEDIMENT

I. Water types
   a. Fresh
   b. Marine

II. Isotopic Assessment
   a. $HgCl_2$
   b. $HgCl_2$ in the presence of HCB
   c. HCB
   d. HCB in the presence of $HgCl_2$

III. Phase
   a. Water
   b. Water and oil
   c. Water and sediment
   d. Oil and water
   e. Oil, water and sediment
   f. Sediment and water
   g. Sediment, water and oil

The experimental test systems in the laboratory consisted of 130 mm x 15 mm sterile screw-capped test tubes containing 10 ml of fresh (tap) water or artificial seawater. To appropriate tubes were added 40.0 µg (3.2 µCi) $^{203}HgCl_2$, or 10.0 µg (0.14 µCi) {U-^{14}C} HCB, or both. Control tubes received no amendments. Additional test environments were established by adding 100 mg Celite, or 10% (v/v) Kuwait crude oil, or a combination of both to the initial freshwater and seawater systems. The test tubes containing the components were tightly capped and mixed for 5 sec in a vortex mixer. Following mixing, the test tubes were placed in a 15°C chamber and gently shaken at 100 rpm for 24 hr.

After incubation, the water, sediment, and oil phases were separated, collected, and assayed for radioactivity. Crude oil layers were separated and collected by pipette, followed by centrifugation (2100 x g) of the remaining water and sediment, to pellet any suspended sediments. The aqueous phase was removed by pipetting and the remaining sediment was washed, centrifuged, resuspended, and harvested.

One ml of the aqueous phase or sediment resuspended in water was placed in 10 ml dioxane-based Omnifluor (New England Nuclear Corp.) cocktails; ^{14}C radioactivity was measured with an Intertechnique liquid scintillation counter Model SL-40 (Teledyne Corp., Westwood, N.J.), employing a standard ^{14}C window setting. Counting efficiency was 94%. Beta emission from ^{203}Hg was also measured, with a standard ^{14}C window as a reference for total radioactivity measured in the double label, ^{203}Hg + ^{14}C-HCB, test systems. One ml of the 1/100 dilutions of oil was placed in toluene-based Omnifluor (NEN) cocktails and counted in the same manner as the water samples. There was no significant quenching effect observed in any of the liquid scintillation counting systems.

Gamma emission from the decay of ^{203}Hg (279.2 KeV) was measured in a Packard Tri-Carb scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.), equipped with an auto-gamma spectrometer. Harvested samples of sediment, oil, and water were placed directly into gamma tubes following appropriate dilution. Quantitative ^{203}Hg determinations were thus based on gamma emission rather than beta emission. By comparing the ratio ^{203}Hg β : γ to ^{14}C β emission in the double label experiments, it was possible to segregate Hg and HCB partitioning in the various phases.

Sampling

Estuarine samples for enumeration of PCB-resistant bacteria were collected over a 9-month sampling period, October 1974 to June 1975, aboard the R/V RIDGELY WARFIELD. Estuarine samples analyzed for PCB content were collected in June 1975. Marine samples for enumeration of PCB-resistant bacteria and analysis for PCB were collected in November 1974 during R/V EASTWARD Cruise E-16B-74. The ocean sampling stations were located in the southeast Atlantic outer continental shelf area, extending from Miami, Florida, to Cape Hatteras, North Carolina (Fig. 2). Estuarine samples were collected at stations located along the entire length of Chesapeake Bay, from the Susquehanna River to the Atlantic Ocean (Fig. 1).

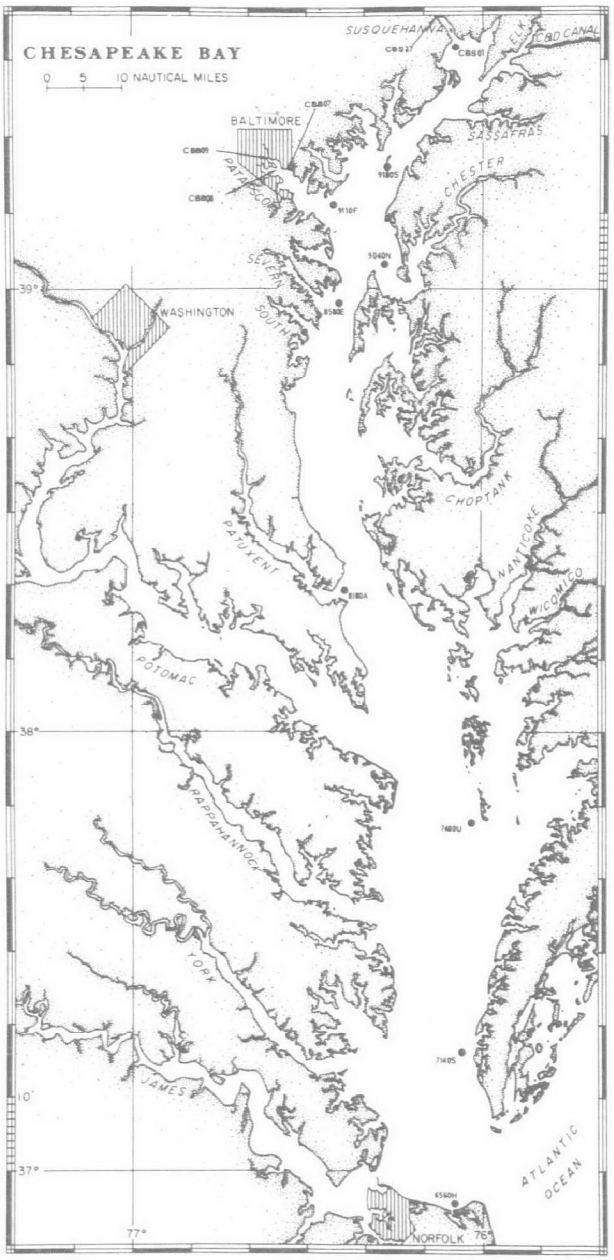

Figure 1. Chesapeake Bay sampling stations.

Water samples for microbiological analysis were collected by using a Niskin sterile bag sampler (General Oceanics Inc., Miami, Fla.). Estuarine and marine sediment samples were collected by means of non-aseptic Ponar and Shipeck grabs, respectively. Sediment samples for bacteriological analysis were taken aseptically from the subsurface of the grab sample. Surface water samples for PCB analysis were collected by using shipboard submersible pumps. Methods for the determination of physical and chemical parameters at the time of sample collection are reported in detail elsewhere (27). Phosphate and nitrate measurements of estuarine samples were measured according to the methods of Strickland and Parsons (32).

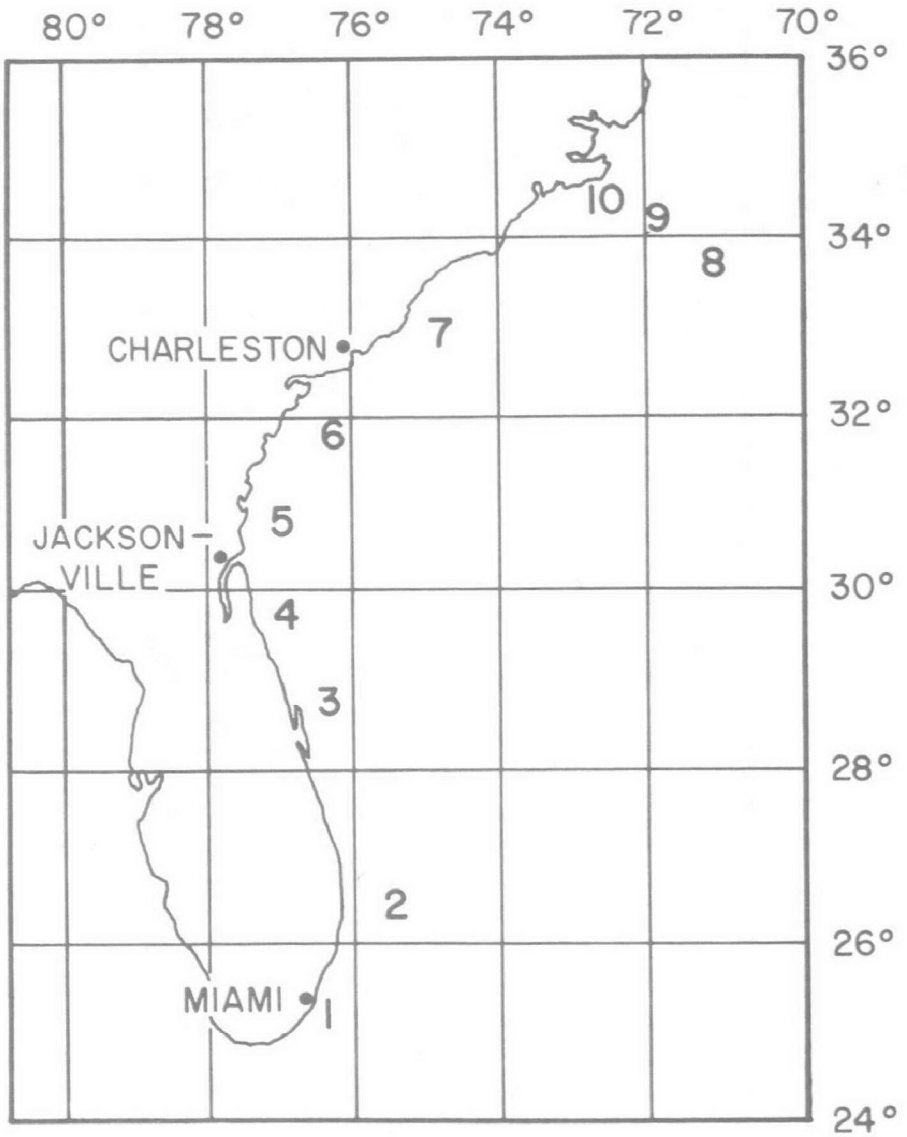

Figure 2. Southwest Atlantic stations sampled during R/V EASTWARD Cruise, E-16B-74, November 1974.

Methods for determining salinity, phosphate, and productivity for marine samples were according to Strickland and Parsons (32). Ammonia nitrogen content of the marine samples was determined using the method of Koroleff (20). Transparency was measured by using a Secchi disc. Dissolved oxygen concentration was determined by titration, employing the Alsterberg modification of the Winkler method (32).

Bacterial Enumeration

Total viable bacterial counts of samples containing less than 20 °/oo salinity were obtained by using Upper Bay yeast extract agar (UBYE) (15). Total viable counts of samples of salinities greater than 20 °/oo were obtained by using marine agar 2216 (Difco). Fungi and yeasts were enumerated for

selected samples by plating on Sabouraud dextrose agar (Difco) and Littman Oxgall agar (Difco). Presumptive counts of fungi and yeasts were obtained by examination of colonial morphology and microscopic observation. Indication of PCB degradation, with the PCB present as a primary carbon source, was obtained by plating on 1254 agar (29), formulated as follows: NaCl, 23.4 g l^{-1}; $MgSO_4 \cdot 7H_2O$, 6.9 g l^{-1}, and KCl, 0.9 g l^{-1}. The latter was used for marine samples.

Samples were enumerated by spread plate count following appropriate dilution, and inoculated media were incubated at 25°C. Counts were made at 2 and 4 weeks. An enrichment broth containing marine salts (vide supra) or estuarine strength salts (15) was supplemented with NH_4NO_3, 0.2 g l^{-1} and Aroclor 1254, 1.0 g l^{-1} coated on Celite, 1.0 g l^{-1} (J. T. Baker Chemical Co.) or 3 mm glass beads, 10.0 g l^{-1}. Each flask containing 100 ml enrichment broth was inoculated with 1.0 ml of a 1/10 dilution of bottom sediment or 1.0 ml of surface or bottom water and the inoculated flasks were incubated at 15°C for 4 weeks. Isolated colonies picked from count plates and streak plates prepared from the enrichment broths were purified on 1254 agar or UBYE agar. The pure cultures were presumptively identified to genus following the scheme of Johnson and Colwell (16).

Extracts of Polychlorinated Biphenyls

Polychlorinated biphenyls were extracted with hexane (Burdick and Jackson Laboratories Inc., Muskegon, Mich.) from 10-liter water samples, following the method of Vieth and Lee (35). Hexane extracts were concentrated on board ship by using a gentle stream of warm air. Extracts concentrated to 10 ml were returned to the laboratory in acetone-washed, screw-capped tubes for liquid column chromatographic clean-up prior to further chemical analysis.

Marine sediment samples were placed in acetone-washed jars and were frozen on board ship. Later, the samples were thawed in the laboratory and 100 g subsamples were dried at 100°C for 12 hr to provide dry weight data. Each marine sediment sample was extracted for 12 hr with 200 ml of a (1:1) hexane and acetone (Burdick and Jackson) mixture. Sediment extracts were evaporated to dryness at 60°C in a rotary evaporator and were reconstituted in 30 ml of hexane.

Estuarine sediment samples were batch-extracted with 200 ml of hexane and acetone mixture by shaking for 12 hr on an orbital shaker. These extracts were handled like the marine sediment extracts.

Column Chromatography of Hexane Extracts

Each extract was mixed with 10 g anhydrous Na_2SO_4 (Fischer Scientific Co., Fair Lawn, N.J.) and filtered through glass fiber filters. The extracts were evaporated to dryness, resuspended in 10 ml of hexane, and applied to the top of a 10 cm x 25 mm (19 g) activated fluorsil (J. T. Baker Chemical Co.) column topped with 10 g (2.5 cm) of Na_2SO_4. Samples were eluted with 200 ml hexane, followed by elution with 200 ml 20% ethyl ether (Fischer) in hexane. Each fraction was concentrated to 10 ml in a rotary evaporator and stored in the dark in acetone washed screw-capped tubes.

Analytical Analysis

Concentrated sample extracts were analyzed for total PCB's by gas liquid chromatography (GLC). GLC analysis was performed on a Shimadzu CG-4BM PF gas chromatograph (American Instrument Co., Silver Spring, Md.), equipped with a 3% OV-1, 80-100 mesh Shimalite W column (1500 mm x 3 mm) and a ^{63}Ni electron capture detector. Operating conditions were maintained as follows: injection and detector temperature, 285°C; column temperature, 240°C; and nitrogen carrier gas flow rate, 50 ml per min. Relative peak height, area, and retention times were measured with a Shimadzu R-201 recorder and a Hewlett Packard digital integrator, Model 3373B (Hewlett Packard Analytical Instruments, Avondale, Pa.).

Computerized gas chromatography-mass spectrometry (GC/MS) analysis of extracted samples was performed by using a Hewlett Packard Model 5930A GC/MS data system. Initial separation of PCB components was obtained with a 5% OV-17 60-80 mesh AW chromosorb W, 4 ft x 1/8 in pyrex column.

Individual PCB components were also identified with thin layer chromatography (TLC), following the method described by the Environmental Protection Agency (33) and GLC, employing a Perkin-Elmer Model 3920 gas chromatograph (Perkin-Elmer, Norwalk, Conn.). The instrument was equipped with dual ^{63}Ni (EC) electron capture detectors and 5% OV-17 and 20% SE30, 60-80 mesh, AW chromosorb W, 5 ft x 1/4 in pyrex columns.

All glassware used in the procedures, viz., extraction of PCB from the samples, extract concentration and clean-up, and analytical analysis, was boiled in detergent, distilled water rinsed, and hexane and acetone washed to eliminate PCB contamination from outside sources. All reagents were of spectrograde suitable for pesticide analysis. Samples were extracted in diffuse light or in the dark; extracts were stored in the dark to eliminate potential photo-decomposition (6).

SECTION V

RESULTS

EFFECT OF PCB ON ENTERIC ACCUMULATION IN THE OYSTER AND THE SOFT SHELL CLAM

Three groups of experiments investigated accumulation, retention, and survival of enteric bacteria. The first set examined the accumulation and elimination of E. coli in the oyster, Crassostrea virginica, under PCB stress and non-stress conditions. Additionally, the removal of E. coli from aquaria water and survival of E. coli in aquaria water, were investigated. An outline of the experimental procedure is given in Table 2.

TABLE 2. EXPERIMENTAL OUTLINE FOR ASSAY OF ENTERIC BACTERIA ACCUMULATED BY THE OYSTER, CRASSOSTREA VIRGINICA, FOLLOWING ACUTE PCB STRESS

	Aquaria	
Days	Stress	No stress
-2	50 random oysters 48-hr equilibration	50 random oysters 48-hr equilibration
0	PCB stress	No stress
4	Bacterial dose--under stress	Bacterial dose
4-11	Survival and accumulation --under stress	Survival and accumulation
12	Transfer to fresh aquarium --post-stress	Transfer to fresh aquarium
12-19	Survival and elimination --post-stress	Survival and elimination

Due to a faulty aquarium, the elimination of Salmonella typhimurium by Crassostrea virginica could not be assessed in a group of experiments similar to the E. coli accumulation experiments. However, the experimental

procedure allowed for the study of accumulation and retention of Salmonella and a partial study of the elimination of Salmonella by Crassostrea virginica.

In the third group of experiments, the accumulation, retention, survival, and elimination of Salmonella enteritidis by the soft shell clam, Mya arenaria, were investigated. The experimental procedures were identical to those used in the oyster studies, with minor modification in the initial cleaning of the clams' exterior surfaces.

PCB Stress and the Accumulation of E. coli

The effect of Aroclor 1254 on the survival of E. coli in aquarium water is demonstrated in Fig. 3. After E. coli addition (four days post PCB dosing), an E. coli concentration of 10^6 cells per liter was reached in both the PCB-dosing aquarium and control aquarium. This concentration was maintained for 24 hr in the control tank; however, there was ca. 99% reduction in E. coli concentration in the PCB-dosed aquarium water. Within 48 hr, 90% of the E. coli added to the control aquarium were no longer detectable. The bacteria rapidly declined in the water column in both aquaria thereafter, although the decline was slightly less pronounced in the control aquarium. Six days following addition of E. coli to the PCB-stressed oyster aquarium, E. coli concentrations dropped to undetectable levels (less than 1 per ml). E. coli were detectable in the control aquarium for an additional four days, indicating a slightly longer survival in the non-PCB-stressed environment. Comparison of the survival of E. coli with fluctuations in total viable counts (TVC), as shown in Fig. 3, indicated trends similar to that demonstrated by E. coli, except that there was no immediate marked loss of TVC from the water column. Twelve days after dosing, oysters were removed from the aquaria (Fig. 3). Absence of the oysters apparently had only a negligible effect on E. coli. However, the TVC increased after the oysters were removed from the aquaria, suggesting growth of heterotrophic bacteria introduced into the aquaria with the oysters. It is impossible to eliminate all bacteria from the oysters without killing the animals. Therefore, a background TVC, as indicated in counts at the outset, must be established for experimental work of this kind.

Oyster tissues assayed for E. coli when oysters were removed to fresh aquaria (day 12) were found to have accumulated large numbers of E. coli (Fig. 4). There was no significant difference between accumulation of E. coli, after exposure for 12 days, by the stressed and non-stressed oysters. Both groups of oysters accumulated ca. 10 times more E. coli than the number of E. coli added to the aquarium water (see Figs. 3 and 4). There was significantly greater survival of E. coli in the non-stressed oysters, from day 12 through day 19, compared with stressed oysters, which eliminated all accumulated E. coli by day 19. The peak in E. coli accumulation, at day 14 in the non-stressed oysters, was most likely due to experimental error; growth of E. coli in the oyster tissue, is doubtful, but not an impossible situation.

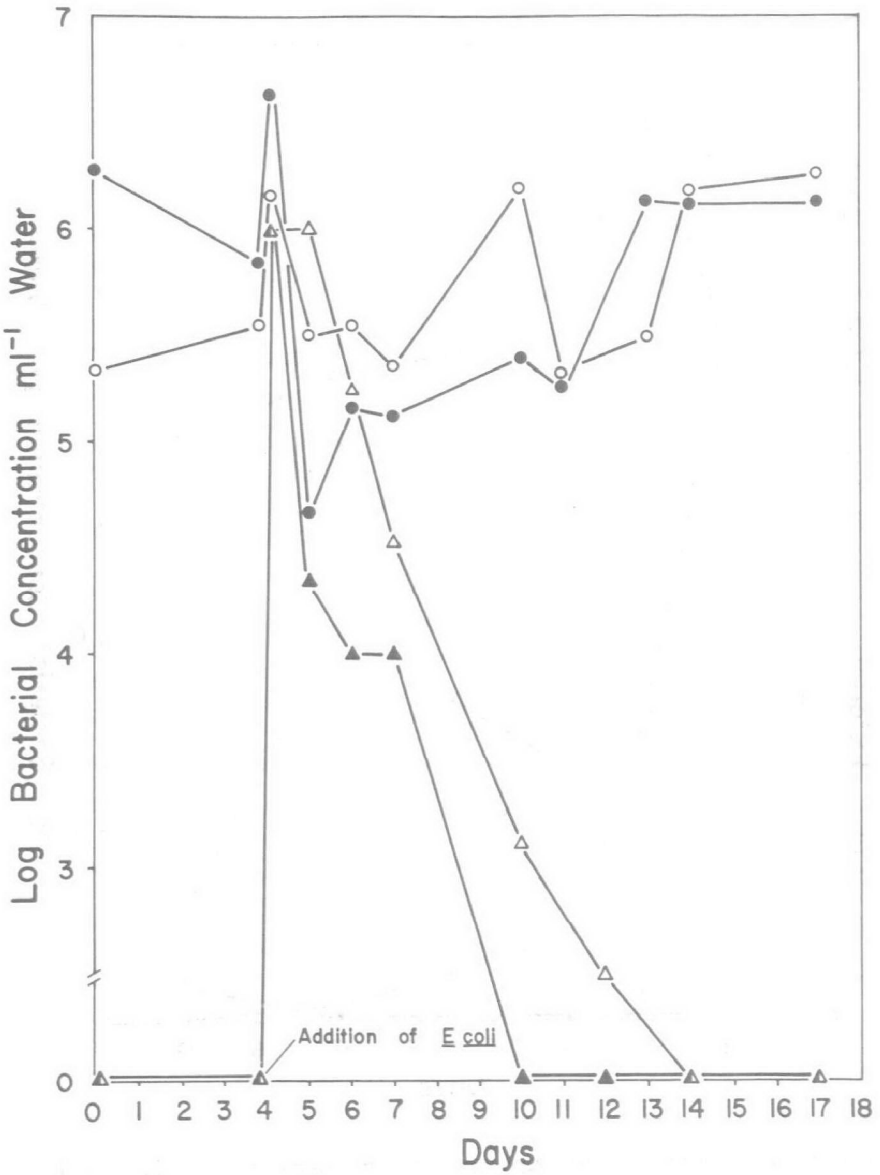

Figure 3. Survival of total viable bacteria (TVC) and E. coli in aquaria water under PCB stress and non-stress conditions. (▲ E. coli - PCB stress, Δ E. coli - no stress, ● TVC - PCB stress, o TVC - no stress; oysters were removed from the aquaria at day 12).

Elimination of E. coli, as seen in Fig. 4, was interesting, in that E. coli lost from stressed oysters were not recovered in the aquarium water. The resulting conclusion is that these cells were no longer viable. However, in non-stressed oysters, E. coli was recovered in the water at day 14, corresponding to the marked loss of E. coli from non-stressed oyster tissue. Although E. coli recovered from aquarium water were insignificant in the total accumulation of E. coli by the oysters (<1.0%), it was significantly more that that recovered from oysters dosed with Aroclor 1254.

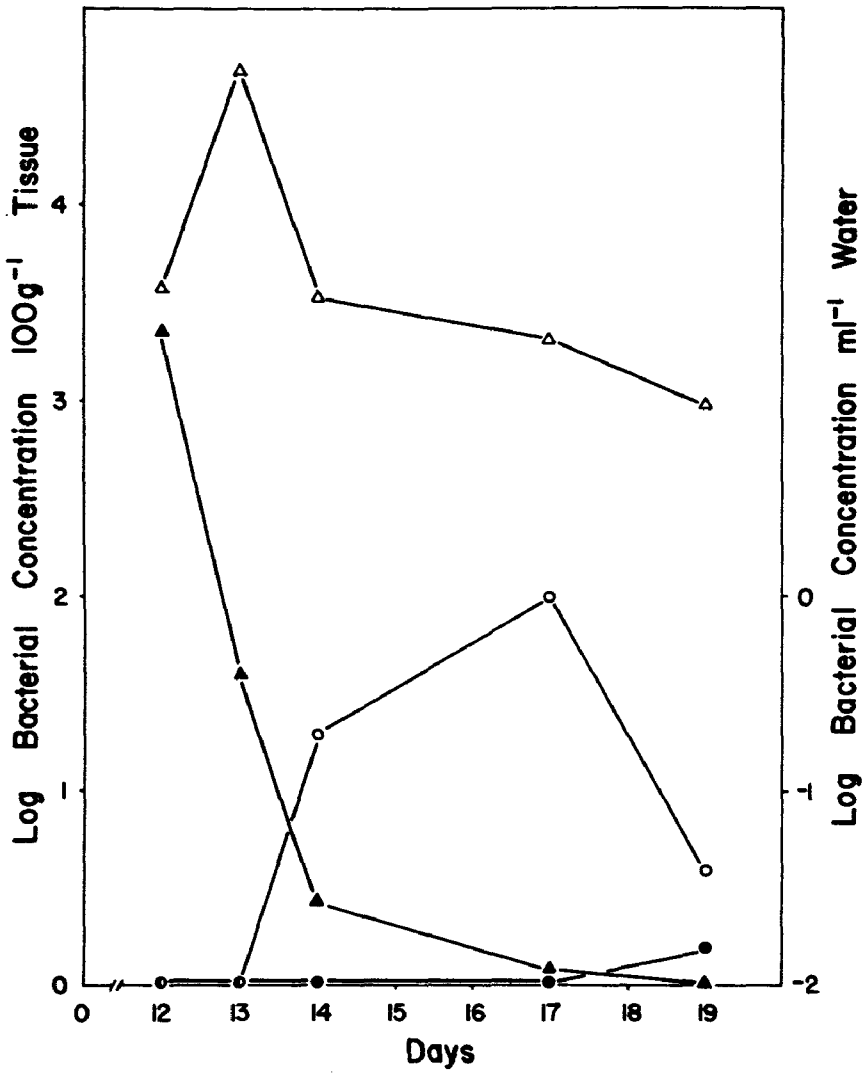

Figure 4. Accumulation and elimination of E. coli by oysters following PCB stress and non-stress conditions. (▲ E. coli tissue accumulation--PCB stress, Δ E. coli tissue accumulation--no stress, ● E. coli elimination--PCB stress, o E. coli elimination--no stress).

The depressive effect of Aroclor 1254 on the elimination, or depuration, by the oyster of total viable bacteria was clearly evident (Table 3). Elimination of the viable heterotrophic bacteria by PCB-stressed oysters amounted to a maximum of 3.8% of the total bacteria accumulated, compared with 407% for the control oysters, even though initial accumulation of TVC was approximately the same.

TABLE 3. ELIMINATION[a] OF TOTAL VIABLE BACTERIA (TVC) FROM OYSTERS FOLLOWING PCB STRESS AND *E. COLI* DOSING

	Stressed			Non-stressed		
Day	Water[b]	Tissue[c]	Percent Eliminated[d]	Water[b]	Tissue[c]	Percent Eliminated[d]
12	2.5×10^2	2.8×10^5	.007	3.5×10^2	2.6×10^5	.01
13	5.3×10^3	1.2×10^4	1.9	5.6×10^3	3.6×10^5	1.5
14	4.8×10^5	2.2×10^5	2.0	2.3×10^5	1.2×10^5	190.0
17	1.5×10^6	3.4×10^6	3.8	2.2×10^6	5.0×10^4	407.0

[a]Elimination from oysters to water, assuming no growth in water.

[b]TVC per ml.

[c]TVC per 100 g tissue.

[d]Cumulative percent eliminated, Σ water/Σ tissue x 100.

These data support two theories: 1) E. coli is sensitive to Aroclor 1254 and 2) the ability of the oyster to accumulate bacteria is not inhibited by PCB but depuration is diminished.

PCB Stress and the Accumulation of Salmonella typhimurium

Accumulation of S. typhimurium by stressed and non-stressed oysters revealed patterns similar to those of E. coli, with some exceptions. It was immediately obvious that the quantitative rate of recovery of Salmonella by using Bismuth Sulfite agar was much less than that of E. coli. This was evident from the discrepancy observed between TVC and numbers of recovered Salmonella following addition of $>10^6$ cells per ml to the water of each aquarium (Fig. 5). However, results for groups of oysters receiving the same treatment would not be affected by the problem of quantitation.

As noted for E. coli (Fig. 3), the number of Salmonella in the aquarium water decreased rapidly, starting with the addition of the bacteria four days after PCB dosing (Fig. 5). There was a slight difference between decline in Salmonella levels between 8 and 12 days, as the aquarium water without PCB showed what could be interpreted as growth of the Salmonella, paralleled with a rise in total viable bacteria. Both increases ceased at day 13, with a precipitous drop in the number of S. typhimurium in the control aquaria. In general, the decline in the number of Salmonella was much less gradual than that noted for E. coli although the length of time during which a detectable number of viable cells could be recovered was approximately the same, i.e., 10 days. The total viable counts followed closely the trends observed for Salmonella, with higher TVC concentrations detected in the non-stressed environment.

It was not possible to follow depuration of S. typhimurium because of a defect that in one aquarium prohibited removal of the oysters to a fresh, sterile environment for purging experiments. It was possible, however, to assay accumulation of S. typhimurium in the presence of low levels of residual Salmonella in the initial dosing tanks.

At day 6, oysters in both environments accumulated approximately 1/10th the concentration of cells as was present in the surrounding water (Figs. 5 and 6). Minor loss of Salmonella from control oysters, between days 6 and 12, may have been responsible for the observed increase in concentration of Salmonella in the water, as shown in Fig. 5. As the concentration of Salmonella in the water declined, following day 12 (Fig. 5), a dramatic reduction occurred in the concentration of Salmonella in the tissues (Fig. 6). The results indicated depuration of Salmonella by the oyster.

Comparisons between accumulation of Salmonella by stressed and non-stressed oysters are presented in Table 4. There was little difference noted, both in absolute accumulation of Salmonella, or in relative percent accumulation of Salmonella, between the groups. One difference, however, was the high initial rate of accumulation of bacteria by non-stressed oysters at day 6. Interestingly, only deaths of the oysters occurring throughout all the experiments were between days 6 and 14 for the control oysters dosed with

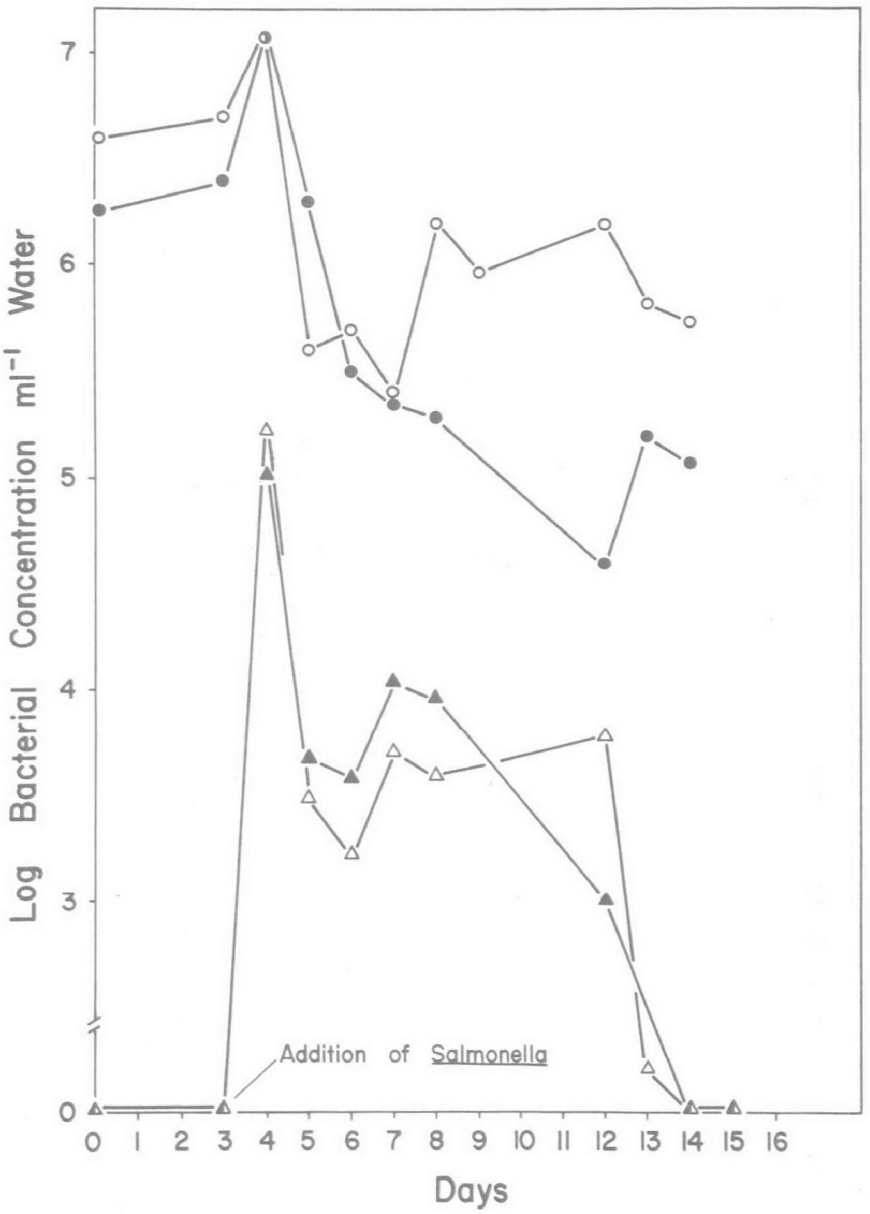

Figure 5. Survival of total viable bacteria (TVC) and Salmonella typhimurium in aquarium water under stress and non-stress conditions. (▲ Salmonella - PCB stress, Δ Salmonella - no stress, ● TVC - PCB stress, o TVC - no stress; oysters removed at day 12).

Salmonella. Salmonella typhimurium was recovered from the gut of one of the dead animals. The evidence is suggestive, but not conclusive, of Salmonella-induced death.

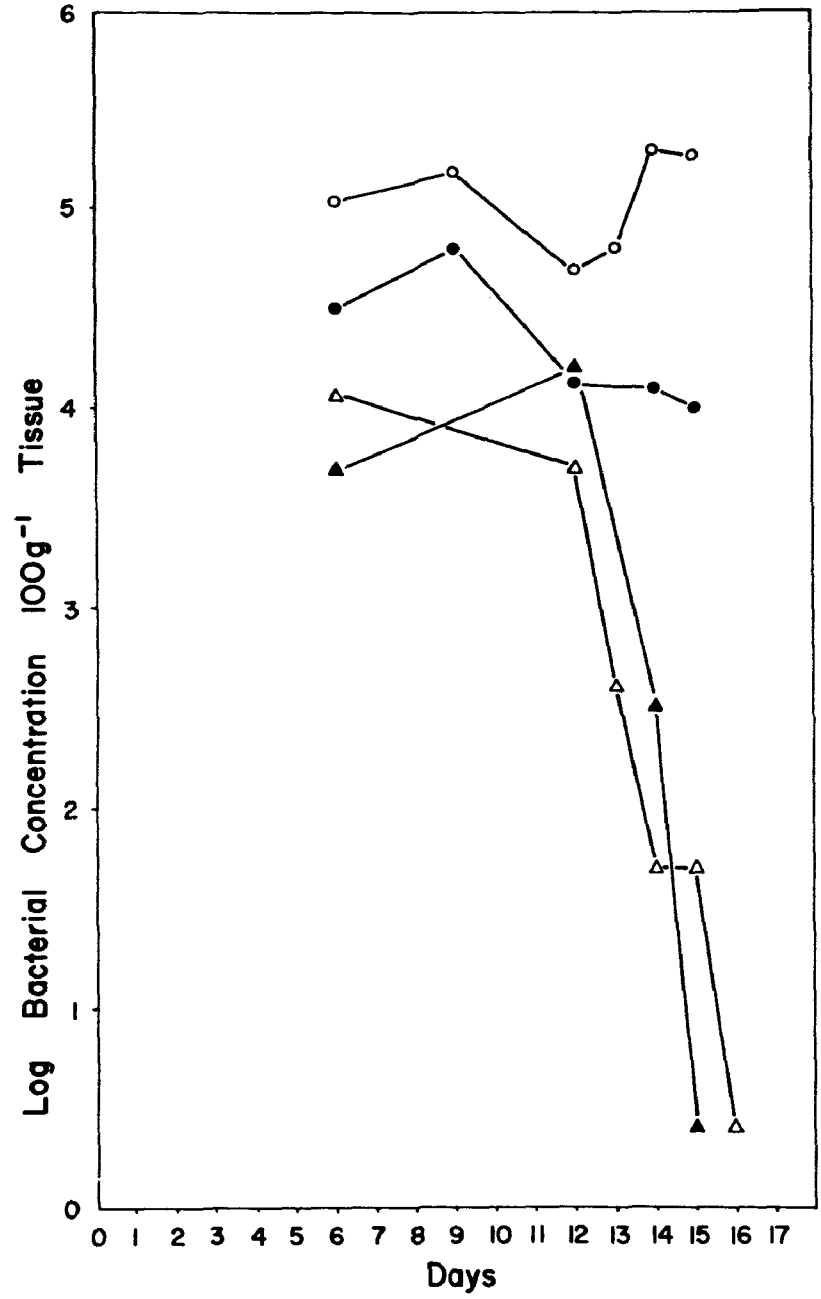

Figure 6. Accumulation of total viable bacteria (TVC) and Salmonella typhimurium by the oyster, Crassostrea virginica, following PCB stress. (● TVC - PCB stress, o TVC - no stress, ▲ Salmonella - PCB stress, Δ Salmonella - no stress).

TABLE 4. ACCUMULATION[a] OF *SALMONELLA TYPHIMURIUM* IN OYSTER TISSUE FOLLOWING PCB STRESS

	Stressed			Non-stressed		
Day	Water[b]	Tissue[c]	Accumulated[d]	Water[b]	Tissue[c]	Accumulated[d]
6	4.4×10^3	4.8×10^3	109.0	1.7×10^3	1.3×10^4	764.7
12	4.7×10^3	1.9×10^4	261.5	7.5×10^3	5.5×10^3	201.1
14	1.0×10^1	3.0×10^2	264.5	2.0×10^2	4.0×10^2	201.1
15	1.0×10^0	2.8×10^1	265.0	3.0×10^1	5.0×10^1	201.0

[a]Accumulation, assuming no growth of *Salmonella* in tissue.

[b]*Salmonella* per ml aquarium water.

[c]*Salmonella* per 100 g oyster tissue.

[d]Cumulative percent accumulated, Σ tissue/Σ water x 100.

Accumulation of total heterotrophic bacteria in oyster tissue (Fig. 6) was greater in non-stressed oysters. This observation was made for TVC in the E. coli accumulation experiments. In terms of relative percent accumulation of TVC, accumulation by the control oysters ranged from 26% to 11.4% of the total number of Salmonella, compared with 10.0% to 8.5% for PCB-stressed oysters (Table 5).

Janssen (14) reported oyster retention of 2.8×10^4 S. typhimurium per oyster from water containing 2×10^5 cells per ml after 48 hr exposure. Although it is difficult to compare the results reported by Janssen, on the basis of per oyster accumulation, it does appear that the results of this investigation are comparable in non-stressed oysters.

Several preliminary conclusions can be drawn from the results of these investigations to date. As expected, the oyster, Crassostrea virginica, demonstrated an ability to concentrate enteric bacteria. The effect of PCB stress on oysters apparently is a more complex process than considered initially, in terms of bacterial accumulation and depuration. A stress appears to be imposed on the bacterial population, as well as on the oyster, the end result of which is that PCB stress artificially produced what superficially could be considered, from bacteriological criteria, to be oysters of higher bacteriological quality than was, in fact, the case. This observation was totally unexpected, as can be judged from the above hypothesis that assumed PCB stress would result in poorer bacteriological quality. On the other hand, indications are that PCB stress may result in a lessening of the ability of the animal to purge itself of bacteria. These observations require further study before they can be accepted as fact. In the interim, experimental work was done with another estuarine invertebrate, the soft shell clam, to determine whether the effects observed for the oyster are specific or are applicable to other estuarine animals.

PCB STRESS AND EFFECT ON SALMONELLA ENTERITIDIS ACCUMULATION IN THE CLAM

Total viable aerobic heterotrophic bacterial counts made of the control and PCB-stressed clams showed a marked drop after dosing with Salmonella at day 2. The drop in the count was a sharp deflection in the TVC survival curve, shown in Fig. 7. The number of total viable aerobic heterotrophic bacteria stabilized at day 6 and 7, respectively, in the PCB-stressed and control aquaria waters. However, the TVC associated with PCB-stressed clams were found to be 10 times higher than those in the control clams (Fig. 7). This observation held true at day 12, two days after removal of the clams from the dosing tanks to the purging tanks. Furthermore, release of TVC into the aquarium water where the clams had been placed for purging was 10 to 100 times greater in the control clams, compared with PCB-stressed clams.

Survival of Salmonella enteritidis in the dosing water was similar under both control and PCB stress conditions (Figs. 8 and 9). However, PCB-stressed clams initially accumulated greater than 10 times as many Salmonella per 100 g of tissue than control clams. (See Figs. 8 and 9.) The accumulation of Salmonella by stressed and control clams, relative to the concentration of Salmonella in the aquaria water, was 20 to 50 times greater in the stressed oysters during the following 2 through 6 days of Salmonella

TABLE 5. ACCUMULATION OF TOTAL VIABLE BACTERIA (TVC) IN OYSTER TISSUE FOLLOWING PCB STRESS AND SALMONELLA DOSING

	Stressed			Non-stressed		
Day	Water[a]	Tissue[b]	Accumulated[c]	Water[a]	Tissue[b]	Accumulated[c]
6	3.1×10^5	3.1×10^4	10.0	4.6×10^5	1.2×10^5	26.0
9	8.0×10^5	6.3×10^4	8.5	8.0×10^5	1.6×10^5	22.2
12	4.8×10^4	1.4×10^4	9.3	1.6×10^6	5.6×10^4	11.7
13	1.6×10^5	1.6×10^4	9.4	6.8×10^5	6.7×10^4	11.4
14	1.2×10^5	1.3×10^4	9.3	5.6×10^5	2.0×10^5	14.7

[a] TVC per ml.

[b] TVC per 100 g tissue.

[c] Cumulative percent accumulated, Σ tissue/Σ water x 100.

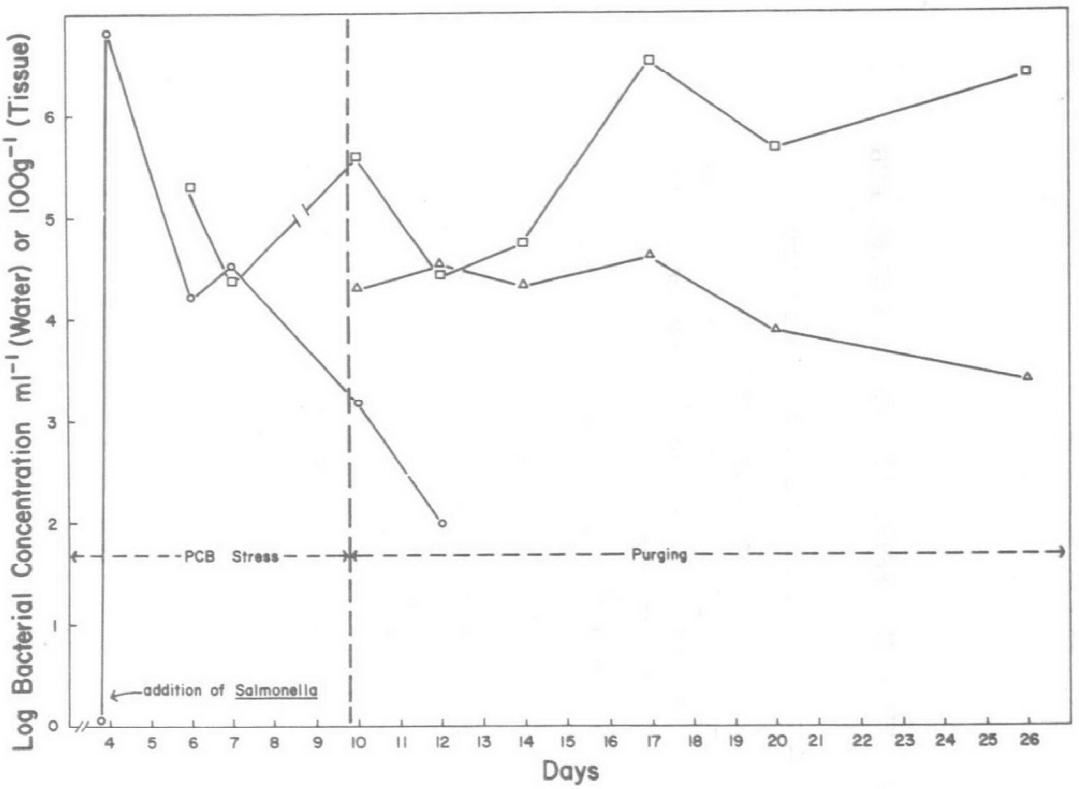

Figure 7. Comparative survival and release of bacteria, measured as total viable bacterial counts (TVC), under control and PCB-stressed conditions. (Survival of the total aerobic heterotrophic bacteria in the dosing tanks: ● = PCB stress, o = control, release of TVC: ▲ = PCB stress, Δ = control).

dosing (Table 6). At day 10, at which time the clams were placed in the purging aquaria, there was no significant difference in tissue accumulation of Salmonella in PCB-stressed or control clams. Retention of accumulated Salmonella by both stressed and control clams remained at high levels, i.e., 10^5 to 10^6 Salmonella per 100 g throughout the period of purging. In comparison, the level of release of Salmonella, which was 10^4 organism per ml, was high in both experimental groups (Figs. 8 and 9). These results suggest growth may occur in the clam tissue, as well as prolonged survival of Salmonella under purging conditions. There was, however, no significant difference in the relative elimination of Salmonella from clam tissue during purging of both control or PCB-stressed oysters (Table 7).

As shown in Fig. 10, survival of pure cultures of Salmonella enteritidis placed in flask cultures containing artificial seawater of various salinities extended beyond the period of dosing and purging employed in these studies. These data show little or no effect of PCB on the survival of S. enteritidis at salinities of 10 °/oo or greater. Furthermore, the data suggest that, in fact, there may be a potentially beneficial effect of PCB on S. enteritidis survival at salinities of <10 °/oo.

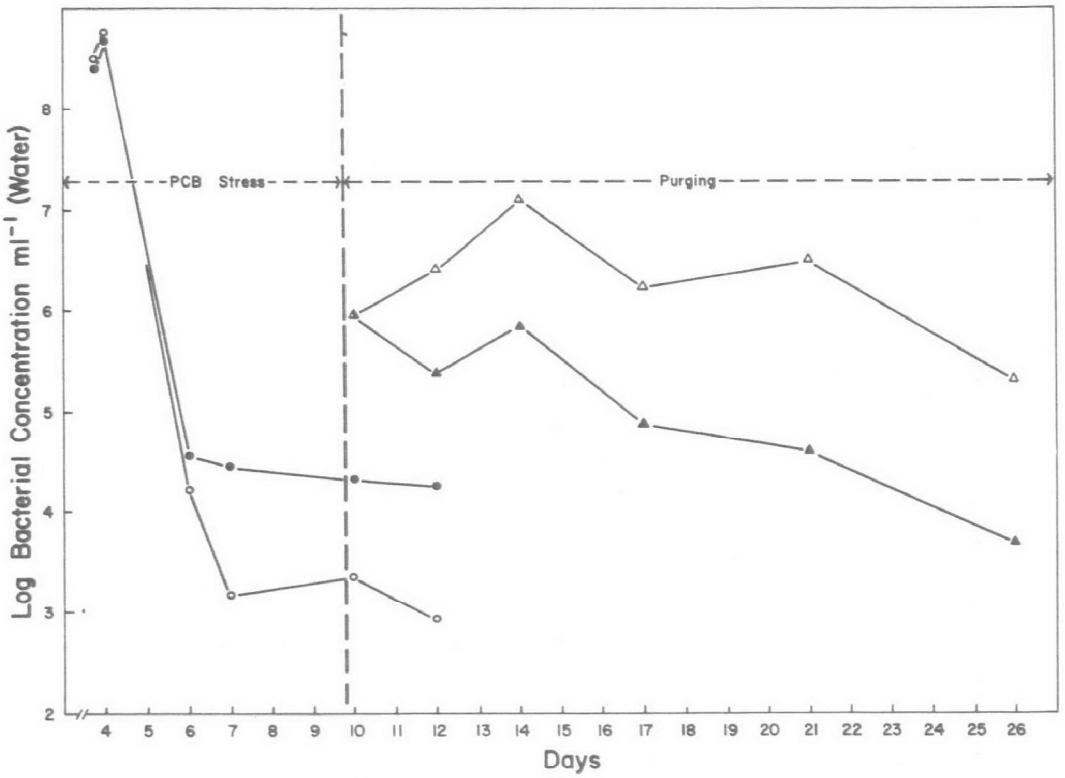

Figure 8. Accumulation and retention by the clam, and survival of Salmonella enteritidis under control conditions. (o = survival in water of the dosing tank, □ = accumulation and retention in oyster tissue, Δ = release of Salmonella into the purging aquarium water) (Celite placebo added in place of PCB).

Some general observations can be made concerning results of studies on the accumulation of enteric bacteria in oysters and clams. Accumulation and retention of bacteria, measured by TVC counts, and of Salmonella in the clam are significantly greater than in the oyster under both PCB stress and control conditions. However, the release of bacteria accumulated by the clam was remarkably low. Physiological differences between clams and oysters are sufficiently great that the effects of PCB stress on these animals should be expected to be different.

The patterns observed for Salmonella enteritidis accumulation and release by soft shell clams and accumulation and release of E. coli by oysters were very similar (Figs. 5, 8 and 9). In both cases, the total bacterial accumulation was approximately the same for each group of animals under both PCB stress and control conditions. However, survival of E. coli and Salmonella enteritidis released from the animals during depuration appeared to be reduced in the presence of PCB.

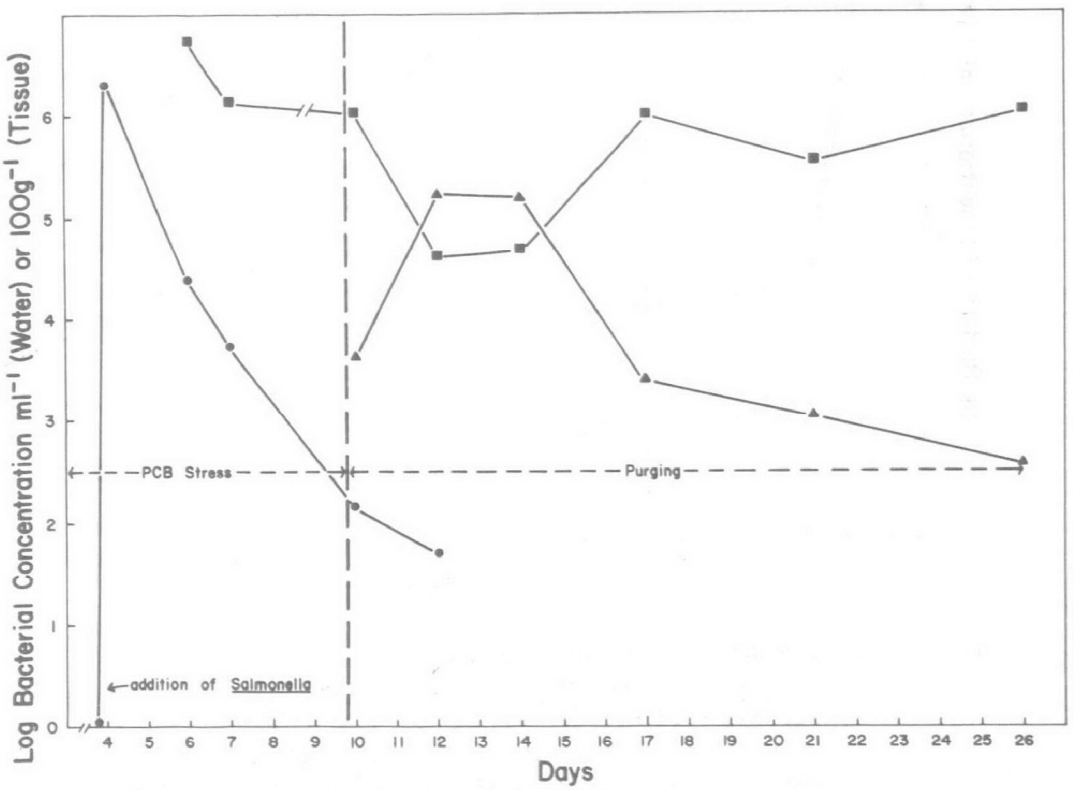

Figure 9. Accumulation and retention by the clam, and survival of Salmonella enteritidis under PCB-stressed conditions. (● = survival in dosing aquarium water, ■ = accumulation and retention in clam tissue, ▲ = release of Salmonella enteritidis into purging aquarium water.)

TABLE 6. ACCUMULATION[a] OF *SALMONELLA ENTERITIDIS* IN CLAM TISSUE FOLLOWING PCB STRESS

	Stressed			Non-stressed		
Day	Water[b]	Tissue[c]	Accumulated[d]	Water[b]	Tissue[c]	Accumulated[d]
2	2.5×10^4	6.0×10^6	24,000	1.5×10^4	2.0×10^5	1,333
3	5.6×10^3	1.2×10^6	23,500	3.5×10^4	2.6×10^4	452
6	1.5×10^3	1.1×10^6	25,000	1.4×10^3	4.0×10^5	1,217

[a] Accumulation, assuming no growth of *Salmonella* in tissue.

[b] *Salmonella* per ml aquarium water.

[c] *Salmonella* per 100 g oyster tissue.

[d] Cumulative percent accumulated, Σ tissue/Σ water x 100.

TABLE 7. ELIMINATION[a] OF *SALMONELLA ENTERITIDIS* FROM CLAM TISSUE FOLLOWING PCB STRESS AND DEPURATION

	Stressed			Non-stressed		
Day	Water[b]	Tissue[c]	Percent Eliminated[d]	Water[b]	Tissue[c]	Percent Eliminated[d]
6	5.7×10^3	1.1×10^6	0.5	2.0×10^4	4.0×10^5	5.0
8	1.9×10^5	4.3×10^4	17.1	3.5×10^4	2.9×10^4	12.8
10	1.4×10^5	5.2×10^4	27.8	2.1×10^4	5.6×10^4	15.7
13	2.6×10^3	1.2×10^6	28.7	4.1×10^7	2.7×10^6	2.5
17	1.2×10^3	3.6×10^5	12.0	7.8×10^3	4.9×10^5	2.4
22	4.2×10^2	1.2×10^6	8.5	2.5×10^3	2.3×10^6	1.5

[a] Elimination from clam to water.

[b] *Salmonella* per ml aquarium water.

[c] *Salmonella* per 100 g clam tissue.

[d] Cumulative percent excreted, Σ water/Σ tissue x 100.

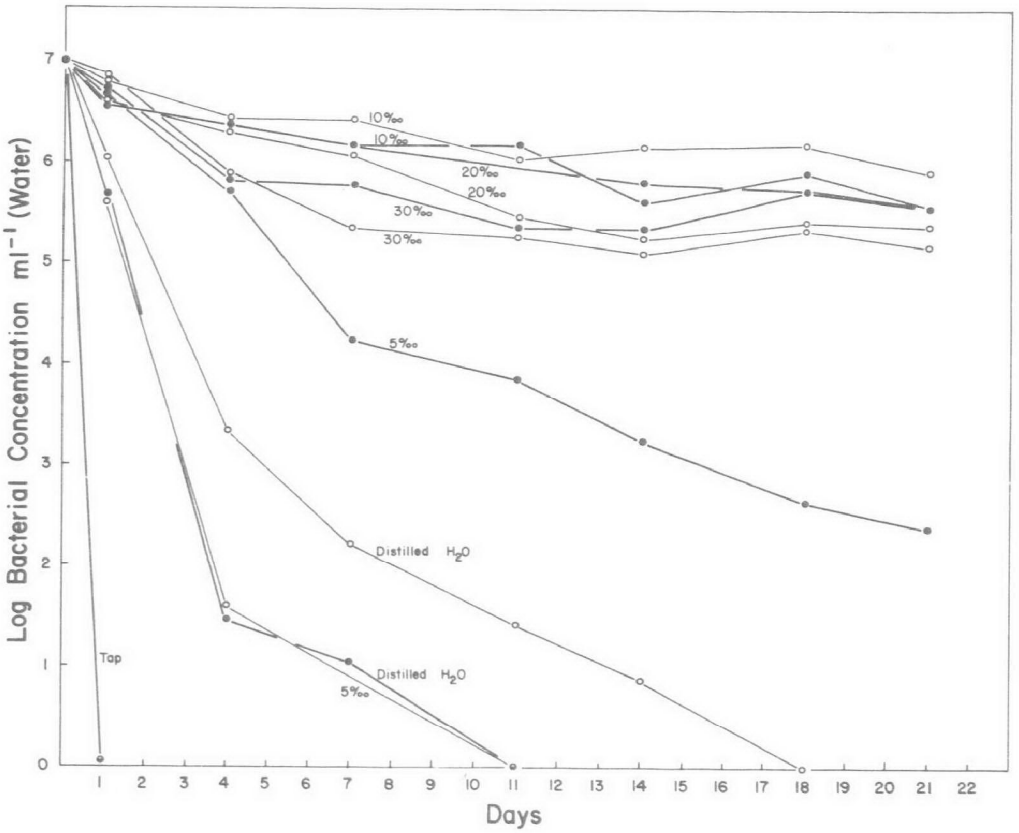

Figure 10. Comparative survival of Salmonella enteritidis at various salinities during control and PCB stress conditions. (o = PCB stress, o = control).

PARTITIONING OF HG AND PCB BETWEEN OIL, WATER AND SUSPENDED SEDIMENT

Results of a factorial analysis of variance, assuming all major sources of variation to be fixed treatment effects, showed significant variation between freshwater and seawater and between the various oil, water, and sediment phases (Table 8). No significant variation was attributable to the individually labeled compounds being partitioned. Variation attributed to all treatment effect interactions, except that between water and labeled compounds, was found to be significant. These results refute the hypothesis that there is no significant change in HCB or Hg concentration in the water column. Furthermore, salinity and partitioning of the various phases were found to be significant.

A comparison of the mean percent partitioning of ^{203}Hg and ^{14}C-HCB by the various phases in the freshwater and seawater systems was revealing (Tables 9 and 10). From the data given in Tables 9 and 10, it is evident that oil is an extremely effective partitioning agent for both HCB and $HgCl_2$ even in the presence of sediment. There appears to be a significantly greater percent partitioning of HCB by crude oil, compared with $HgCl_2$, in both freshwater and seawater. This effect was also observed in the significant variation found for the Phase X Compound interaction (Table 8). In all cases, sediment was

TABLE 8. FACTORIAL ANALYSIS OF VARIANCE[a] (2x8x4) OF THE PARTITIONING OF $^{203}HgCl_2$ AND {U-^{14}C} HEXACHLOROBIPHENYL (HCB), BETWEEN WATER, OIL AND SEDIMENT

Source of variation	d.f.	SS	MS	Fs
Water (W)	1	879	879	19.8*[b]
Phase (P)	7	151769	21681	488.0*
Compound (C)	3	21	7	0.2
WXP	7	1025	146	3.3*
WXC	3	1	0.6	0.0
PXC	21	16765	798	18.0*
WXPXC	21	4740	226	5.8*
Within subgroups	64	2839	44	
Total	127	178040		

[a] ANOVA, Model 1 with replication (α = .05).

[b] * indicates variability significantly greater than within subgroup variation.

much less effective in partitioning mercury and HCB from the water column, compared with the crude oil. The partitioning of HCB and mercury by sediment, in the presence of oil, was from the oil rather than the water, which was surprising, considering the distance between the sediment pellet and the floating oil layer (approx. 7 cm).

The difference between the freshwater and seawater was less apparent than that observed for the various phases. Thus, the ability of seawater to retain both HCB and mercury is greater than freshwater. This was not an unexpected observation, in view of the ionic nature of $HgCl_2$ and the number of chlorine residues of the hexachlorobiphenyl.

Data on mean percent loss of radioactive label for both freshwater and seawater were pooled to determine potential loss of both HCB and mercury from the water column to the oil and sediment phases (Table 11). Eighty-five percent of the HCB and mercury was removed from the water column by both the oil phase, alone, or the oil in conjunction with sediment. However, sediment removed only 28.4% of the HCB and mercury. The remainder was found in the water. Greater selective partitioning of HCB by crude oil, compared with

TABLE 9. RELATIVE PERCENT[a] PARTITIONING OF ^{203}Hg AND ^{14}C-HCB RADIOACTIVITY IN A THREE-PHASE WATER SYSTEM

	Percent radioactivity partitioned		
	Water	Oil	Sediment
$^{203}HgCl_2$	19.0	72.8	8.2
	23.5	85.5	--
	75.7	--	24.4
	100.0	--	--
$^{203}HgCl_2$ (HCB)	28.4	63.1	8.5
	32.9	67.1	--
	85.7	--	14.3
	100.0	--	--
^{14}C-HCB	0.9	92.4	6.8
	0.4	99.5	--
	74.8	--	25.2
	100.0	--	--
^{14}C-HCB ($HgCl_2$)	2.7	93.7	2.6
	2.5	97.5	--
	71.2	--	28.8
	100.0	--	--

[a]Mean of duplicate observations relative to the total radioactivity in the water column.

mercury, (ca. 97% removal vs. 74%) was an expected result, considering the affinity of HCB for non-polar solvents.

The relative percent partitioning, or removal, of mercury and HCB by sediment was less than for crude oil (Tables 9, 10, and 11). However, when the data are expressed in terms of net concentration of mercury or HCB in the oil layer, compared with the sediment, sediment was highly efficient in concentrating both mercury and HCB (Table 12). Efficiency of sediment partitioning was slightly reduced in the presence of oil. Concentrations of HCB in sediment increased 13.6 fold and 28.1 fold, respectively, for sediment in the presence of oil and sediment alone. About a ninefold increase was noted for the oil.

Less dramatic results were obtained for concentration of mercury in oil or sediment. Separately, oil and sediment were found to be nearly equal in ability to accumulate mercury. However, in a three-phase system, oil was a better competitor in the partitioning of mercury, with a sixfold increase, compared with a twofold increase in mercury in suspended sediment.

TABLE 10. RELATIVE PERCENT[a] PARTITIONING OF $^{203}HgCl_2$ AND ^{14}C-HCB RADIOACTIVITY IN A THREE-PHASE SEAWATER SYSTEM

	Percent radioactivity partitioned		
	Water	Oil	Sediment
$^{203}HgCl_2$	28.3	54.9	16.8
	17.2	82.4	--
	61.5	--	38.5
	100.0	--	--
$^{203}HgCl_2$ (HCB)	30.0	61.9	8.1
	30.9	69.1	--
	76.4	--	23.6
	100.0	--	--
^{14}C-HCB	10.7	81.1	8.3
	0.9	99.1	--
	69.3	--	30.7
	100.0	--	--
^{14}C-HCB ($HgCl_2$)	4.1	85.4	10.5
	1.2	98.8	--
	95.9	--	4.1
	100.0	--	--

[a]Mean of duplicate observations relative to the total radioactivity in the water column.

TABLE 11. MEAN PERCENT LOSS OF $^{203}HgCl_2$ AND ^{14}C-HCB RADIOACTIVITY FROM THE WATER COLUMN TO THE OIL AND SUSPENDED SEDIMENT PHASES

	Radioactive label			
	$^{203}HgCl_2$	^{14}C-HCB	Composite	
Phases			$\bar{Y}$	s
Oil + sediment	73.6	95.4	84.5	12.4
Oil	73.9	98.7	85.4	12.9
Sediment	23.2	22.2	28.4	18.8
Water	0.0	0.0	0.0	

[a]Relative to the total radioactivity in the water for both the freshwater and seawater systems.

TABLE 12. CONCENTRATION OF $HgCl_2$ AND HCB IN OIL AND SUSPENDED SEDIMENT FOLLOWING PARTITIONING FROM THE WATER PHASE[a]

	$HgCl_2$			HCB		
Phase	mg l^{-1}	s	Percent[b] increase	mg l^{-1}	s	Percent[b] increase
Oil	30.4	0.4	2640	9.5	0.001	857
Oil (sediment)[c]	25.3	0.4	2030	8.8	0.07	782
Sediment	24.8	3.9	2080	28.1	1.2	2710
Sediment (oil)[d]	10.4	1.8	640	13.6	0.3	1260

[a]Mean of eight observations. s, standard deviation.

[b]Percent increase relative to the initial concentration of $HgCl_2$ (4 mg l^{-1}) or hexachlorobiphenyl (1 mg l^{-1}) in the water column.

[c]Oil partitioning in the presence of sediment.

[d]Sediment partitioning in the presence of oil.

Differences in partitioning efficiency, between sediment and oil, arise from the highly non-polar nature of crude oil and the resulting partitioning on the basis of surface adsorption occurring on the relatively large surface area of the Celite.

Kenega (19) reported that adsorption of chlorinated pesticides in some environmental systems is 50% complete within a few hours. Thus, the findings of the short-term (24-hr) laboratory experiments in this study can be extrapolated to the natural environment. Maximum partitioning of DDT (structurally similar to HCB) has been reported to occur at ca. 10 days (12). Greater partitioning of PCB and mercury would be expected to occur as the time of incubation increased. Hartung (12) reported steady-state oil partition coefficients for DDT that exceed 10^6, indicating an even greater potential for chlorinated residues, such as PCB, to accumulate in areas continually receiving oil and PCB.

Since no significant difference was noted between mercury and PCB partitioning, it is doubtful that organic forms of mercury would be accumulated to any less extent than the $HgCl_2$ and HCB employed in this study. However, Hg^o volatilization is a microbial metabolic pathway shown to occur in the aquatic environment and is a mechanism of escape of mercury from a combined Hg-oil environment (21). Also, water-extractable material in oil (31) can be a potential mercury volatilizing mechanism (2).

It is evident from the data presented here that concentration of heavy metals and chlorinated hydrocarbons in sediment and/or oil can result in a highly toxic environment, i.e., inhibitory to microorganisms capable of degrading each of the components separately but inhibited by the high concentrations in combination. The relatively exotic substrates thereby occurring in sediment must eventually be mineralized by microorganisms. Thus, spilled, dumped, or seeping oil reaching marine or estuarine sediments may be rendered impervious to microbiological attack (38). Furthermore, application of degradation kinetics, established for any one of the individual pollutants discussed here, to a multiple-contaminated environment will not be valid. The consequences of impeded or inhibited microbial degradation of the components of a mixed-pollutant system are serious and should be investigated.

DISTRIBUTION OF PCB-RESISTANT BACTERIA AND PCB IN ESTUARINE AND MARINE ENVIRONMENTS

Physical and chemical parameters measured at the time of collection of the samples from Chesapeake Bay and the Southeast Atlantic Coast are given in Tables 13 and 14. The marine surface water samples collected in Miami Beach Harbor and in the shallow water of Cape Hatteras, North Carolina, revealed the highest concentrations of NH_4-N and PO_4-P (Table 13). Coincident with the values for these nutrients was the relatively high level of heterotrophic activity (^{14}C uptake) and chlorophyl-a content observed at Miami Beach and, to a lesser extent, off Cape Hatteras. In general, nutrient concentrations and net activity of the surface waters was lower off the continental shelf extending to the deep station, #8, farthest from shore, i.e., ca. 200 mi off Cape Hatteras.

Fewer nutrient data were available for samples collected in Chesapeake Bay, but the data obtained indicated a high level of nutrient input in the Upper Bay, from Annapolis, Maryland to the Susquehanna River. The nutrient concentrations observed were most likely due to the influence of the Baltimore Harbor area and the Susquehanna River (Table 14). Previously reported data indicated domestic sewage point source contamination at Chesapeake Bay stations CBS01 and CBB09 (15, 16). In general, stations located in the open bay yield lower levels of contamination relative to both Baltimore Harbor and the Susquehanna River areas. Salinities measured at the stations in Chesapeake Bay included in this sutdy ranged from essentially freshwater to ca. 70% seawater.

Recovery of PCB and PCB-Resistant Bacteria

Polychlorinated biphenyls were recovered from all of the marine water and the sediment samples collected during the study. However, in 9 of the 14 samples analyzed, the concentrations of PCB detected in the samples were below the sensitivity limits of the methods employed, i.e., ca. 10 µg/kg sediment and 0.1 µg/1000 ml H_2O. Samples collected in Miami Beach Harbor, at stations 5 and 7, and off Cape Hatteras were all found to contain significant levels of PCB. Qualitatively, the presence of Aroclor 1254 was detected at station 10 off Cape Hatteras, North Carolina. However, sediment samples collected in Miami Beach Harbor contained significant levels of polychlorinated biphenyl.

TABLE 13. PHYSICAL AND CHEMICAL PARAMETERS FOR ALL STATIONS SAMPLED ON R/V EASTWARD CRUISE E16B-74, NOVEMBER 16-21, 1974

Station	Location	Depth (m)	Salinity (°/oo)	Temp. (C)	DO (mg/l)	PO_4-P (ugat/l)	NH_4-N (ugat/l)	^{14}C (mg/m^3/day)	Chl-a (mg/m)
1	Miami Beach Harbor	0	34.1	23.4	3.0	0.02	2.10	32.0	25.6
		11	34.2	--	3.1	0.06	2.25	92.8	30.8
2	26° 30.0'N, 79° 20.0'W	0	36.1	26.7	3.0	0.0	0.01	0.09	0.18
		460	35.7	13.3	2.5	0.24	0.03	--	--
3	29° 05.6'N, 80° 05.0'W	0	36.1	25.5	2.9	0.03	0.49	0.12	0.17
		528	36.4	19.5	2.1	0.13	0.15	--	--
4	30° 05.6'N, 80° 15.0'W	0	36.2	25.8	5.5	0.0	0.35	4.50	0.09
		160	36.0	14.4	2.0	0.22	0.06	--	--
5	31° 00.0'N, 80° 00.0'W	0	36.1	24.2	3.5	0.0	0.68	5.4	0.13
		160	35.7	15.8	2.0	0.26	0.37	--	--
6	32° 05.0'N, 79° 05.4'W	0	36.2	24.6	2.9	0.0	0.27	0.2	0.27
		200	35.0	8.3	1.9	0.44	0.17	--	--
7	33° 00.4'N, 77° 40.0'W	0	36.2	25.7	2.9	0.0	0.17	0.4	0.25
		152	36.1	13.3	2.1	0.17	0.12	--	--
8	33° 53.5'N, 74° 55.0'W	0	36.3	24.0	2.9	0.01	0.17	1.5	0.18
		3199	35.0	2.5	3.7	0.01	0.24	--	--
9	34° 19.8'N, 76° 04.7'W	0	36.3	22.4	3.0	0.0	0.05	0.03	0.34
		50	36.2	20.1	3.1	0.3	0.001	--	--
10	34° 37.3'N, 76° 33.1'W	0	35.5	15.6	3.6	0.04	0.57	26.3	0.19

TABLE 14. PHYSICAL AND CHEMICAL PARAMETERS MEASURED AT THE CHESAPEAKE BAY STATIONS INCLUDED IN THIS STUDY

Station[a]	Location	Depth (m)	Salinity (°/oo)	DO (mg/l)	Transparency (m)	Temp. (C)	PO_4-P (mg/l)	NO_3-N (mg/l)
CBS01	Havre de Grace	12.0	0.0	8.8	1.5	22.8	.028	3.10
CBS27	Conowingo Dam	2.0	0.0	--	0.3	--	.066	2.02
CBB07	Colgate Creek[b]	9.1	3.9	8.5	0.8	22.5	.048	1.71
CBB08	Fort McHenry[b]	6.5	3.9	10.3	0.7	21.1	.076	3.72
CBB09	Jones Falls[b]	2.6	1.4	6.9	0.6	22.6	--	--
9180S	Pooles Island	6.7	4.3	10.7	2.0	19.5	.048	2.20
9110F	Baltimore Harbor	4.5	3.2	6.8	2.0	18.6	.048	2.50
9040N	Chester River	10.0	8.6	9.5	2.0	14.5	--	--
8580E	Tolly Bar	6.5	7.0	6.2	1.8	15.1	.038	1.17
818A	Solomons	9.7	10.9	8.5	1.7	23.0	--	--
7480U	Tangier Island	6.1	14.0	3.6	2.3	23.0	.19	--
7140S	Cape Charles	28.0	25.8	7.4	3.3	--	--	--
6560H	Little Creek[c]	8.2	20.3	5.8	1.7	25.4	--	--

[a]Dates of sample collection: September and October 1974; June 18 and 19, 1975.

[b]Baltimore Harbor.

[c]Norfolk, Virginia.

Bacteria capable of growth on media containing Aroclor 1254 as the primary carbon source were recovered from water and sediment samples collected at all stations sampled (Table 15). In general, the number of PCB-degrading/resistant bacteria was higher in the sediment than in the water column. However, the relative proportions of PCB degraders making up the TVC were higher in the water than in the sediment. Total numbers of PCB-resistant bacteria were highest at shallow stations and appeared to decrease with increasing depth and distance from shore.

TABLE 15. ENUMERATION OF PCB-RESISTANT BACTERIA IN ATLANTIC OCEAN SURFACE WATER AND SEDIMENT SAMPLES

Station	Sample type[a]	TVC	PCB resistant	Percent composition	Total PCB g l^{-1} (kg^{-1})
1	W	$5.7 \times 10^{2}_{5}$	$1.3 \times 10^{2}_{3}$	22.8	0.3
	S	2.1×10^{5}	9.5×10^{3}	4.5	12.0
3	W	$2.0 \times 10^{3}_{4}$	$1.0 \times 10^{1}_{2}$	0.5	<0.1
	S	1.2×10^{4}	3.5×10^{2}	2.9	<10.0
5	W	$1.6 \times 10^{3}_{4}$	$1.0 \times 10^{2}_{3}$	6.2	0.5
	S	2.6×10^{4}	4.5×10^{3}	17.3	<10.0
7	W	$3.8 \times 10^{3}_{4}$	$1.0 \times 10^{2}_{3}$	2.7	0.5
	S	2.6×10^{4}	1.2×10^{3}	4.6	<10.0
8	W	$7.2 \times 10^{0}_{2}$	$7.2 \times 10^{0}_{2}$	100.0	<0.1
	S	3.6×10^{2}	1.2×10^{2}	33.3	<10.0
9	W	$3.0 \times 10^{1}_{3}$	$3.0 \times 10^{1}_{4}$	100.0	<0.1
	S	4.0×10^{3}	5.4×10^{4}	13.5	<10.0
10	W	$7.7 \times 10^{2}_{5}$	$7.0 \times 10^{1}_{3}$	9.0	0.7
	S	1.2×10^{5}	3.9×10^{3}	3.2	<10.0

[a] W = water; S = sediment.

An apparent inconsistency in the data was observed concerning the levels of PCB-resistant bacteria at station 8 and station 9. The recovered PCB-degrading bacteria accounted for 100% of the total viable heterotrophic bacterial population. This result could be interpreted as indicating a higher level of PCB contamination, thereby inducing larger populations of bacteria to metabolize PCB. However, the concentration of PCB at these stations did not support such a hypothesis (Table 15). It is more likely that the microorganisms recovered on the low nutrient-containing PCB medium were adapted to the low nutrient concentration prevalent in their environment and, consequently, gave a better growth response on the 1254 medium than on the richer 2216 marine agar used to enumerate the TVC.

In Chesapeake Bay, the PCB-resistant bacteria and TVC were observed to be present at exponentially higher levels than in the marine bacterial

populations (Table 16). As in the case of the seawater and deep ocean sediment bacterial populations, there was a marked variability in the numbers of PCB-resistant bacteria and in the proportion of the TVC observed for the Chesapeake Bay samples. Nine samples were assayed for PCB in June 1975. These samples were found to contain low concentrations of PCB in the water column, ranging from 0.01 to 0.14 µg per liter. However, the PCB concentrations in the sediment ranged from 4.0 to 400 µg per kg. Sediment samples collected in Baltimore Harbor, station CBB09, and near Norfolk, Virginia, station 6560H, both industrialized sites, contained 400 and 125 µg per kg sediment, respectively, i.e., up to 100-fold greater concentrations than those observed for samples collected at stations in the open bay. Suspended sediment samples collected in the Upper Chesapeake Bay have been reported to contain PCB in concentrations as high as 2 mg per kg (personal communication, T. O. Munson, Westinghouse Ocean Research Laboratory, Annapolis, Md.). Such data indicate that large influxes of polychlorinated biphenyls into Chesapeake Bay may occur sporadically, with subsequent contamination of the Atlantic Ocean via tidal outflow.

Levels of polychlorinated biphenyl contamination of the aquatic environment can be traced directly to domestic and industrial pollution. Duke et al. (7) reported PCB concentrations in water and sediment reaching 275 µg/l and 486 µg/kg, respectively, in areas directly polluted by PCB leaks from industrial heat exchangers into waters of Escambia Bay, Florida. Elimination of the source of pollutant resulted in an immediate decline in PCB concentrations in the water column and sediment. It can be concluded that significant concentrations of the contaminant were subsequently carried to the ocean. Halcrow et al. (10) reported that PCB content of marine sediment samples collected off the coast of Scotland ranged from 26 µg/kg to 1000 µg/kg, mainly from sewage and garbage dumping. Recently data were published by Harvey et al. (13), indicating lower PCB concentrations in the North Atlantic, with surface water PCB concentrations showing highest levels, i.e., 4.3 µg/l, and decreasing concentrations with depth.

Contamination of the freshwater environment is no less severe that that of the marine and estuarine environment. Vieth and Lee (35) reported PCB concentrations in Milwaukee River water in the range, .05 µg/l to .1 µg/l. Waste effluents of the same region carried PCB concentrations from .04 µg/l to 2.5 µg/l. Crump-Wiesner et al. (5) published results of surveys of PCB contamination undertaken throughout the United States. Freshwater samples were found to contain PCB at concentrations of 0.3 µg/l to 4.0 µg/l, whereas sediment samples were found to contain PCB at concentrations from non-detectable levels to 2400 µg/kg.

The published data on PCB occurrence and concentration indicate the ubiquitous nature of PCB contamination in the aquatic environment. Presumably much of this material is deposited in the sediments, is lost to the atmosphere through co-distillation at the air-water interface, or accumulates in plant and/or animal life. Recent evidence suggests that bacterial decomposition and natural weathering can be a significant factor in the elimination of PCB from the environment (17, 29). Wong and Kaiser (40) found significant bacterial populations resistant to chlorinated biphenyls. Ahmed and Focht (1)

TABLE 16. ENUMERATION OF PCB-RESISTANT BACTERIA FROM CHESAPEAKE BAY BOTTOM WATER AND SEDIMENT SAMPLES

Station	Sample type[a]	TVC	PCB metabolism	Percent composition	Total PCB $\mu g\ l^{-1}$
CBS01	W	--	--	--	--
	S	1.0×10^6	10^2	.01	--
CBS27	W	1.0×10^4	10^2	10.0	--
	S	--	--	--	--
CBB07	W	1.4×10^6_7	1.7×10^3_6	0.2	--
	S	1.7×10^7	1.2×10^6	7.1	--
CBB08	W	1.0×10^7_7	2.2×10^4_6	0.2	--
	S	1.1×10^7	1.7×10^6	16.3	--
CBB09	W	4.0×10^6_7	8.0×10^4_5	2.0	0.14
	S	3.3×10^7	1.0×10^5	0.3	400.0
9180S	W	5.5×10^2_4	3.5×10^1_2	6.3	--
	S	1.2×10^4	4.2×10^2	3.6	--
9110F	W	1.6×10^5_6	1.1×10^4_5	7.2	--
	S	2.2×10^6	4.5×10^5	20.2	--
9040N	W	6.0×10^4_6	4.8×10^3_5	7.8	--
	S	3.1×10^6	6.0×10^5	19.0	--
8580E	W	2.3×10^5_6	1.6×10^3_6	0.8	--
	S	6.2×10^6	1.2×10^6	28.0	--
8180E	W	1.8×10^4_6	7.3×10^3_4	38.8	0.01
	S	4.1×10^6	8.0×10^4	1.9	--
7480U	W	5.0×10^4_6	0.0	0.0	0.01
	S	2.9×10^6	0.0	0.0	4.0
7140S	W	2.2×10^5_6	5.5×10^3_4	2.5	0.06
	S	2.3×10^6	6.5×10^4	2.8	6.0
6560H	W	3.3×10^4_7	1.2×10^3_5	3.6	0.01
	S	1.5×10^7	1.2×10^5	0.7	125.0

[a] W = water; S = sediment.

reported the isolation of an Achromobacter sp. from sewage that was capable of degrading PCB.

In the study reported here, significant numbers of PCB-metabolizing bacteria were recovered from all of the Chesapeake Bay stations sampled. All of the samples collected at the southeastern coastal Atlantic Ocean stations also contained PCB-metabolizing bacteria, although in lower numbers than in the Bay (Table 17). In the marine environment, PCB resistant bacterial populations were found to be greater than both the fungal and yeast populations of Atlantic Ocean surface waters and sediment.

TABLE 17. CORRELATION OF MICROBIAL POPULATIONS WITH PCB CONCENTRATIONS IN ESTUARINE AND MARINE ENVIRONMENTS SAMPLED[a]

	Correlation coefficient (r)		
Environment sampled	TVC	PCB degraders	PCB degraders/10^6 TVC
Marine	.54[b]	-.24	-.38
Estuarine	.98[c]	.62	-.20

[a]Sediment, surface and/or bottom water.

[b]Significant correlation, critical r = .53; α = .05.

[c]Significant correlation, critical r = .67; α = .05.

To determine whether the PCB-resistant bacteria were representative of only one or a few microbial groups, predominantly allochthonous organisms capable of PCB degradation, or were typical of the larger microbial populations in the estuary and ocean, pure cultures were identified to genus. A significant difference was noted in the composition of the marine and estuarine bacterial flora capable of degrading PCB. Pure cultures of PCB-resistant bacteria isolated from seawater and ocean sediment were predominantly Pseudomonas and Vibrio spp. Of 44 pure cultures presumptively identified to genus, approximately 50% were Pseudomonas spp. and the remaining were Vibrio spp. There was no immediate discernible difference, based on the limited number of isolates examined, in the distribution of these two genera from station to station. However, Pseudomonas spp. representing Groups 1 and 2 were isolated less frequently in samples collected northward along the outer continental shelf.

A greater diversity of bacterial genera was observed for the strains isolated from Chesapeake Bay samples. Seven bacterial genera were represented among the 25 PCB-resistant bacterial strains examined. The small sample size, multiple sample types and enrichment methods, and the limited geographical areas examined prevented further extrapolations concerning generic distribution. However, it should be noted that Gram positive bacteria were isolated

from samples collected at the lower end of the Upper Chesapeake Bay. The occurrence of the Gram-positive PCB-resistant bacteria may be related to salinity or nutrient and pollutant influx. However, further study will be required to resolve this point.

It is apparent from the data that resistance to PCB is not restricted to strains of bacteria representing a single genus. In addition, all of the samples collected in this study were found to contain microorganisms capable of growth on PCB medium. Thus, the bacterial populations of waters may contain a small, but persistent fraction of bacteria capable of PCB degradation. Even more important, the possibility may exist that a low level of PCB contamination in the environment results in a corresponding level of induced PCB-resistant bacteria.

In conclusion, PCB contamination was found to be higher in areas of urbanization and industrialization. Such contamination was found to decrease with distance from probable sources of contamination. Although not statistically significant because of small sample sizes, correlations of PCB levels and PCB-degrading bacteria suggest that the latter occur ubiquitously, along with PCB residues. More extensive sampling of areas receiving high levels of PCB contamination should be undertaken, if the hypothesis that increased numbers of PCB-degrading bacteria occur as a direct response to increased PCB contamination is to be established. If such a hypothesis is proven, it will then be possible to develop a PCB-degrading bacterial index of PCB contamination in the estuary and ocean. The concentrations of PCB observed for samples examined in the study reported here were relatively low. However, certain of the sediment samples did contain PCB levels reported by other workers to be toxic to selected estuarine invertebrates (7). It should be emphasized that very low concentrations of PCB in the aquatic environment can provide a reservoir for bioaccumulation via bacteria, zooplankton, and higher life forms. Experiments in progress in our laboratory suggest that, indeed, such bioaccumulation can occur.

SECTION VI

SUMMARY

Polychlorinated biphenyls (PCB) were found to be present in samples of Chesapeake Bay and southeastern Atlantic surface water and sediment. PCB concentrations ranged from 0.01 μg l^{-1} to 0.3 μg l^{-1}, for surface water, and 4.0 μg g^{-1} to 400 μg g^{-1} for sediments. Although detectable in all samples analyzed in this study, PCB was observed to be correlated with urbanized areas.

Partitioning of PCB residues in suspended sediments, oil-contaminated sediments, or surface films may result in elevated PCB levels at some localities. Under laboratory conditions, both PCB residues and mercury compounds were effectively partitioned and concentrated by suspended sediments and by petroleum hydrocarbons. Under the experimental conditions employed in this study, up to 99.5% of the PCB in the water column was partitioned into an oil phase during 24 hr incubation.

Bacteria capable of growth on, or in the presence of, high (500 ppm) concentrations of Aroclor 1254 were recovered from all but one sampling area examined, the latter a station in Chesapeake Bay. The total viable bacterial population capable of growth on PCB ranged from <.1% to 100%. The variability observed in this study was attributed to localized environmental and nutrient conditions. In Chesapeake Bay, the number of bacteria grown in the presence of PCB was found to be positively correlated with presence of PCB in the water or sediment.

Acute PCB stress was reflected in the bacteriological quality of the oyster, Crassostrea virginica. A decrease in depuration of fecal coliforms (E. coli) and the human enteric bacterial pathogen (Salmonella typhimurium) was observed. However, net accumulation of these organisms was not affected by PCB stress. PCB stress did effect a reduced long-term viability of enteric bacteria accumulated by the oyster.

PCB-stressed soft shell clams, Mya arenaria, accumulated >10-fold more Salmonella enteritidis, relative to the water column concentration, than did control clams. Depuration rates, however, for both stressed and control animals remained approximately the same. As in the case of the oyster, long-term in vivo survival of Salmonella was reduced in stressed clams, resulting in a superficially improved bacteriological quality of the shellfish.

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EPA-600/3-77-070		

4. TITLE AND SUBTITLE	5. REPORT DATE
Effects and Interactions of Polychlorinated Biphenyls (PCB) with Estuarine Microorganisms and Shellfish	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Rita R. Colwell and Gary S. Sayler	8. PERFORMING ORGANIZATION REPORT NO. ERL/GB 324
9. PERFORMING ORGANIZATION NAME AND ADDRESS University of Maryland Department of Biology College Park, Maryland 20742	10. PROGRAM ELEMENT NO. 1EA615 11. CONTRACT/GRANT NO. R-803300-01-0
12. SPONSORING AGENCY NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory Gulf Breeze, Florida 32561	13. TYPE OF REPORT AND PERIOD COVERED final 14. SPONSORING AGENCY CODE EPA/ORD
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

The role of estuarine bacteria in the mobilization, transport, and removal of polychlorinated biphenyls (PCB) was investigated in estuarine environments. A main objective of this investigation was to determine a secondary impact of PCB contamination of estuarine systems. The specific secondary effect was the PCB-stress-induced accumulation and depuration of enteric bacteria by shellfish, i.e., the Chesapeake Bay oyster, Crassostrea virginica.

For this report, bacteria uninhibited by PCB, but capable of growth in the presence of PCB, are defined as PCB-resistant. In this regard, PCB-resistant bacteria were found to be distributed ubiquitously throughout estuarine and marine environments sampled in this study. The residence time of PCB in estuarine and marine environments is concluded to be sufficiently long to induce stress upon estuarine animals.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
PCB contamination Effects of Bacteria Marine Bacteria-PCB Interactions Shellfish-Microorganism-PCB Interactions	PCB-resistant Bacteria Estuarine Bacteria Shellfish Depuration and Accumulation	

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