Complete Statements of the four EPA Witnesses

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STATEMENT OF THOMAS W. DUKE

My name is Thomas W. Duke. I am the director of the Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory at Gulf Breeze, Florida. I have directed research activities at this laboratory since 1968. The mission of the Gulf Breeze Laboratory is to study the effects of toxic organics on marine organisms.

My educational background is as follows: I received my B.S. degree in Zoology from Texas A & M University in 1953, my M.S. degree in Oceanography from Texas A & M University in 1960 and my Ph.D. in Oceanography from Texas A & M University in 1962. A list of my scientific publications is appended to this testimony.

My testimony will be taken from completed and ongoing research, as well as pertinent results of related studies in the literature. My testimony is concerned with the routes, rates and reservoirs of Aroclor® 1254 in Escambia Bay, Florida and with the effects of various Aroclors on marine organisms as determined in the laboratory.

The following exhibits are included in my testimony:

1. Duke, T. W., J. I. Lowe, and A. J. Wilson, Jr. 1970. A Polychlorinated Biphenyl (Aroclor® 1254) in the Water, Sediment, and Biota of Escambia Bay, Florida. Bulletin of Environmental Contamination and Toxicology, Volume 5, No. 2, pages 171-180.

2. Nimmo, D. R., D. J. Hansen, J. A. Couch, N. R. Cooley, P. R. Parrish, and J. I. Lowe. 1974. Toxicity of Aroclor® 1254 and Its Physiological Activity in Several Estuarine Organisms. Archives Environmental Contamination and Toxicology. (In press)

3. Nimmo, D. R., P. D. Wilson, R. R. Blackman and A. J. Wilson, Jr. 1971. Polychlorinated Biphenyl Absorbed from Sediments by Fiddler Crabs and Pink Shrimp. Nature, Volume 231, No. 5297, pages 50-52.

4. Wilson, A. J., Jr. 1972. Escambia Bay PCB Survey. Unpublished data.

5. Nimmo, D. R., R. R. Blackman, A. J. Wilson, Jr. and J. Forester. 1971. Toxicity and Distribution of Aroclor® 1254 in the Pink Shrimp Penaeus duorarum. Marine Biology, Volume 11, No. 3, pages 191-197.

6. Nimmo, D. R., J. Forester, P. T. Heitmuller and G. H. Cook. 1974. Accumulation of Aroclor® 1254 in Grass Shrimp (Palaemonetes pugio) in Laboratory and Field Exposures. Bulletin of Environmental Contamination and Toxicology. (In press)

7. Staff, Gulf Breeze Environmental Research Laboratory. 1969-1974. Aroclor® 1254 in Escambia Bay System. Unpublished data.

8. Proceedings. 1971. Second Session of Conference in the Matter of Pollution of the Interstate Waters of Escambia River Basin (Alabama-Florida) and the Intrastate Portions of the Escambia Basin and Bay within the State of Florida. Second Session.

9. Proceedings. 1972. Third Session of Conference in the Matter of Pollution of the Interstate Waters of Escambia River Basin and the Intrastate Portions of the Escambia Basin and Bay within the State of Florida. Third Session.

10. Staff, Gulf Breeze Environmental Research Laboratory. 1969-1974. Aroclor® 1254 in Selected Oysters from Escambia Bay, Florida. Unpublished data.

11. Cooley, N. R., J. M. Keltner, Jr. and J. Forester. 1973. The Polychlorinated Biphenyls, Aroclors® 1248 and 1260: Effect on and Accumulation by Tetrahymena pyriformis. Journal of Protozoology, Volume 20, No. 3, pages 443-445.

12. Lowe, Jack I. 1969. Gulf Breeze Environmental Research Laboratory - Bioassay Screening Test on Aroclor® 1248 and 1260. Unpublished data.

13. Cooley, N. R., Keltner, J. M., Jr. and J. Forester. 1972. Mirex and Aroclor® 1254: Effect on and Accumulation by Tetrahymena pyriformis Strain W. Journal of Protozoology, Volume 19, No. 4, pages 636-638.

14. Wildish, D. J. 1972. Polychlorinated Biphenyls (PCB) in Sea Water and Their Effect on Reproduction of Gammarus oceanicus. Bulletin of Environmental Contamination and Toxicology, Volume 7, No. 2/3, pages 182-187.

15. Wildish, D. J. and V. Zitko. 1971. Uptake of Polychlorinated Biphenyls from Sea Water by Gammarus oceanicus. Marine Biology, Volume 9, No. 3, pages 213-218.

16. Parrish, P. R. 1972. Gulf Breeze Environmental Research Laboratory - Bioassay Screening Test on Aroclors® 1242 and 1254. Unpublished data.

17. Bourquin, A. W. 1974. Growth Responses of Estuarine Bacteria to Mirex, Heptachlor and Polychlorinated Biphenyls. Unpublished manuscript.

18. Kinter, W. B., L. S. Merkens, R. H. Janicki and A. M. Guarino. 1972. Studies on the Mechanism of Toxicity of DDT and Polychlorinated Biphenyls (PCB's): Disruption of Osmoregulation in Marine Fish. Environmental Health Perspectives, Volume 1, pages 169-173.

19. Hansen, D. J., P. R. Parrish and J. Forester. 1974. Aroclor®1016: Toxicity to and Uptake by Estuarine Animals. Environmental Research. (In press)

20. Staff, Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory. 1974. Toxicity of Aroclors 1260, 1254, 1248, 1242 and 1016 to Salt Water Organisms in 48- and 96-hour Bioassays. Unpublished summary.

21. Lowe, J. I., P. R. Parrish, J. M. Patrick, Jr. and J. Forester. 1972. Effects of the Polychlorinated Biphenyl Aroclor®1254 on the American Oyster Crassostrea virginica. Marine Biology, Volume 17, No. 3, pages 208-214.

22. Parrish, Patrick R. 1974. Aroclor®1254, DDT and DDD, and Dieldrin: Accumulation and Loss by American Oysters (Crassostrea virginica) Exposed Continuously for 56 Weeks. Proceedings of National Shellfisheries. (In press)

23. Couch, John A. 1974. Free and Occluded Virus, Similar to Baculovirus, in Hepatopancreas of Pink Shrimp. Nature, Volume 147, No. 5438, pages 229-231.

24. Nimmo, D. R. and L. H. Bahner. 1973. Physiological Consequences of Polychlorinated Biphenyl and Salinity-stress in Penaeid Shrimp. Symposium on Pollution and the Physiological Ecology of Estuarine and Coastal Water Organisms, November 14-17, 1973, Hobcaw Barony, South Carolina.

25. Wildish, D. J. 1970. The Toxicity of Polychlorinated Biphenyls (PCB) in Sea Water to Grammarus Oceanicus. Bulletin of Environmental Contamination and Toxicology, Volume 5, No. 3, pages 202-204.

26. Hansen, D. J., P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr. and P. D. Wilson. 1971. Chronic Toxicity, Uptake, and Retention of Aroclor® 1254 in Two Estuarine Fishes. Bulletin of Environmental Contamination and Toxicology, Volume 6, No. 2, pages 113-119.

27. Schimmel, Steven C., David J. Hansen, and Jerrold Forester. 1974. Effects of Aroclor® 1254 on Laboratory-reared Embryos and Fry of Sheepshead Minnows (Cyprinodon variegatus). Transactions of the American Fisheries Society. (In press)

28. Hansen, D. J., S. C. Schimmel and J. Forester. 1974. Aroclor® 1254 in Eggs of Sheepshead Minnows: Effect on Fertilization Success and Survival of Embryos and Fry. Proceedings of the Southeastern Game and Fish Commission. (In press)

29. Johansson, N., S. Jensen and M. Olsson. 1970. PCB - Indications of Effects on Fish. In "PCB Conference, Wenner-Gren Center," September 29, 1970, pages 59-68. National Environmental Protection Board, Stockholm.

30. Hansen, David J. 1974. Aroclor® 1254: Effect on Developing Estuarine Animal Communities in the Laboratory. Contributions to Marine Science. (In press)

31. Hansen, D. J., S. C. Schimmel and J. Forester. 1974. Effects of Aroclor® 1016 on Embryo, Fry, Juvenile and Adult Sheepshead Minnows (Cyprinodon variegatus). Manuscript approved by Gulf Breeze Environmental Research Laboratory.

32. Staff, Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory. 1974. Toxicity of Aroclors 1254 and 1016 to Salt Water Organisms in Flow-through Bioassays Exceeding 96-hour Duration. Unpublished summary.

ROUTES, RATES AND RESERVOIRS OF AROCLOR® 1254 IN ESCAMBIA BAY, FLORIDA

We detected the polychlorinated biphenyl, Aroclor® 1254, in the water, sediment and biota of Escambia Bay in 1969 (Exhibit 1) and continue to follow the movement and accumulation of this chemical within the bay system. Only one source of the chemical, the Monsanto chemical plant on the Escambia River, has been located at this time. The Aroclor reportedly entered the plant's effluent through accidental leakage of a heat exchange fluid. The leakage was stopped after the company was notified of the appearance of PCBs in the bay system. However, a small amount of this chemical continues to enter the system, possibly from leaching from contaminated sediments into the plant's effluent ditch. Several conclusions have been reached from this study:

(1) Much of the chemical entering the water of Escambia River appeared in particulate form in the water samples

(2) Aroclor® 1254 was distributed from the point of entry in the Escambia River to sediment and biota in the Escambia Bay system by physical or biological transport

(3) Some of the organisms containing residues of Aroclor® 1254 are used as seafood by man

(4) Although the quantity of the Aroclor in the Escambia River was reduced drastically, residues continue

to appear in oysters approximately 8 miles downstream.

Exhibit 1 summarizes the history of the discovery of the chemical in the bay. Exhibit 2, Table 1, gives Aroclor® 1254 concentrations in water, sediment and biota from September 1969 to December 1971 after the leakage was stopped. By comparison, samples of sediments had high concentrations ranging from non-detectable to 30.0 mg/kg (dry weight). In the initial survey (1970) of sediments from the river and bay, concentrations were high at the source of the pollutant (Exhibit 3, Fig. 1; see below the industry) and also at the mouth of the river (Exhibit 3, Fig. 1). Other surveys (1970-1972) show that concentrations of Aroclor® 1254 in sediments from three locations in the river and bay appear to decrease with time (Exhibit 2, Fig. 3). Also, the concentrations in sediment strata appear to decrease with depth. While the number of samples was inadequate for absolute comparison, these data indicate a trend. Data from the latest survey (1972) taken in the channel (Exhibit 4, page 4, see Transect A-middle) indicate that in November, 1972, the sediments above the trestle still had considerable amounts of Aroclor® 1254. Concentrations ranged from 0.16 to 9.0 mg/kg (dry.weight).

The sediment reservoir of Aroclor® 1254 is probably a source of this chemical to the biota of the bay because residues continued to appear after the amount in the water was reduced drastically. For example, the initial survey of biota

(Exhibit 1, Table 1) showed biota with levels as high as 12.0 mg/kg. In surveys conducted in 1969-70, shrimp collected far from the original source had whole body concentrations as high as 14 mg/kg wet weight (Exhibit 2, Fig. 4). Concentrations of Aroclor found in species collected in Escambia and East Bays are shown in Exhibit 2, Fig. 5. The biota in East Bay are about 35 kilometers from the original source of the chemical, and yet have detectable levels of the chemical. There is also a higher concentration of Aroclor®1254 in higher trophic levels in both East and Escambia Bays' biota which could implicate a food chain transfer from sediment to large animals.

Three separate studies have been conducted to study the capacity of benthic animals to accumulate Aroclor® 1254 from contaminated sediments. The first of these (Exhibit 3) showed that shrimp placed on various naturally-occurring substrates containing this PCB accumulated the chemical in proportion to the concentration of the chemical in the sediment. The second study showed that penaeid shrimp (Penaeus duorarum), placed on Aroclor®1254-contaminated sediments, in upper Escambia Bay, accumulated the material (Exhibit 5, page 197). Grass shrimp, Palaemonetes pugio, exposed for 3 months to sediments in the bay accumulated Aroclor®1254 to about 0.4 mg/kg whole body (Exhibit 6) in the third study.

Additional data on the levels of Aroclor® 1254 in the water of the Escambia Bay system are shown in Exhibit 7. We analyzed both filtered and unfiltered samples of surface and bottom water and monitored water from the new outfall that the industry constructed in 1971. Duplicate samples were also analyzed periodically, and in general, there was good agreement. The water column was fairly homogeneous judging from the consistency of surface to bottom samples. But in filtered samples of water, little Aroclor® 1254 was recoverable. This observation probably means that most of the chemical was attached to suspended particulates in the river water. Other evidence of this is shown in Exhibit 8, Fig. 1; Exhibit 9, Fig. 1, where waters in Escambia Bay show only trace levels (0.3 to 0.05 µg/l), whereas the levels in the sediments are relatively high (Exhibit 8, Fig. 2; Exhibit 9, Fig. 2).

The Aroclor® 1254 concentrations from a new outfall constructed by the industrial plant in 1971 are listed in Exhibit 7. Levels of this PCB continue to be above 0.03 µg/l but well below those found in the initial surveys (1969). Some Aroclor® 1254 is now found in the river above the industry and we assume this is due to the effects of high tides and winds moving surface waters slightly up the river.

We originally discovered Aroclor® 1254 in the bay in oysters from Devil's Point Light and we have continued to

monitor these mollusks (Exhibit 10). The increases in Aroclor® 1254 levels in May to July each year are probably due to the increase in egg mass which could have a high affinity to these chemicals because of a high lipid content. The levels drop shortly after the oysters spawn. This phenomenon also is observed in the laboratory. If the data from Devil's Point Light are analyzed by linear regression analysis it is projected that these mollusks would reach the <0.03 µg/l level by 1985. This prediction assumes that no more chemical is put into the system and that climate, hydrologic or other factors remain the same.

EFFECTS OF AROCLORS ON MARINE ORGANISMS

At the time Aroclor® 1254 was discovered in Escambia Bay, essentially no data existed on the effects of this chemical or other PCBs on marine organisms. Since it is difficult and sometimes impossible to determine toxic effects of specific chemicals in the natural environment because of the presence of other chemicals and fluctuating environmental conditions, we conducted specific toxicity test with Aroclor® 1254 and other Aroclors under controlled conditions in the laboratory. In order to make our bioassay information as meaningful as possible, we conducted most of our tests with indigenous organisms from Escambia Bay or similar areas, and utilized flowing seawater when possible. The toxicity of the test chemicals was determined for specific marine organisms for a given period of time. Detailed test conditions are given in the exhibits.

Residue analyses were conducted where possible to determine if the animals concentrated the chemical from the water in which they were tested. This is an important aspect of these bioassays because Aroclor® 1254 occurred in animals from Escambia Bay that are used by man for food. Obviously, these animals could serve as a vector for the movement of Aroclor® 1254 from site of application to humans. If residues in marine organisms were to exceed levels deemed harmful for human consumption, the fishery resource could not be harvested and would be adversely affected. Consequently, the well-being of commercial fishermen and those with related occupations would be adversely affected.

The following conclusions were reached from laboratory bioassay experiments:

(1) All of the Aroclors tested are acutely toxic for certain estuarine organisms.

(2) Bioassays lasting longer than 96 hours demonstrate that short-term or acute tests underestimate toxicities of some of the Aroclors.

(3) Bioassays lasting over 96 hours indicate that Aroclor® 1254 is toxic to commercially valuable shrimps at concentrations less than 1 µg/l and its toxicity is greatest to young shrimp and to shrimp that are subjected to stress.

(4) Fishes, particularly sheepshead minnows, are extremely sensitive to Aroclor® 1254. Concentrations

of about 0.1 µg/l in water are lethal to fry and affect reproduction of this fish. About 7 µg/g in eggs of the fish is lethal to fry hatching in water free of this chemical.

(5) The acute or short-term toxicity of Aroclor® 1016 to estuarine organisms is similar to other Aroclors but it appears less toxic to fishes in long exposures than does Aroclor® 1254.

BIOASSAYS OF 96 HOURS OR LESS DURATION

Aroclor 1260: Acute bioassays lasting as long as 96 hours show that Aroclor® 1260 is toxic to a wide variety of animals. Growth of the ciliate, Tetrahymena pyriformis, was reduced after 96 hours in growth media containing 1,000 µg/l of Aroclor® 1260 (Exhibit 11). Pinfish were apparently unaffected and 10% of the pink shrimp died in 100 µg/l of the PCB (Exhibit 12) during a 48-hour test. Oysters, however, were sensitive to Aroclor® 1260 with growth diminished by 44% in 10 µg/l and 52% in 100 µg/l in a 96-hour test.

Aroclor 1254: Acute bioassays indicate that Aroclor® 1254 may be more toxic to some estuarine organisms than Aroclor® 1260. Growth rates of the ciliate protozoan, T. pyriformis, were significantly reduced by 96 hours exposure to 1 µg/l (part per billion) of Aroclor® 1254 in 10 ml of growth medium (Exhibit 13). A concentration of 100 µg/l of Aroclor® 1254 in 48-hour flowing water bioassays appeared to have no effect on

juvenile pinfish, Lagodon rhomboides, but killed 100% of the juvenile pink shrimp, Penaeus duorarum. Shell growth of oysters, Crassostrea virginica, exposed for 96 hours was completely inhibited by 100 µg/l and decreased 41% over that of unexposed controls by 10 µg/l of Aroclor® 1254 (Exhibit 11). All four species accumulated Aroclor® 1254 with quantities in oysters, pinfish, and pink shrimp exposed to 10 µg/l in excess of 1 µg/g (part per million). The Aroclor® 1254 is toxic to the amphipod, Gammarus oceanicus, (Exhibit 14). The amphipod also accumulates Aroclor® 1254 from water (Exhibit 15).

Aroclor 1248: The toxicity of Aroclor® 1248 to T. pyriformis (Exhibit 2) is about 1,000 times less than that of Aroclor® 1254. Its toxicity to shrimp, oysters, and pinfish was similar to that of Aroclor® 1254 (Exhibit 12).

Aroclor 1242: Bioassays lasting 96 hours demonstrate that this PCB is toxic to oysters and shrimps at concentrations of 100 µg/l or less (Exhibit 16). This PCB, like the others tested, had no apparent effect on pinfish. Aroclor® 1242 inhibits the growth of certain strains of estuarine bacteria (Exhibit 17).

Aroclor 1221: In static bioassays lasting 96 hours, 80% of the killifish, Fundulus heteroclitus, exposed to 25,000 µg/l of Aroclor® 1221 died (Exhibit 18). All control and 7,500 µg/l exposed fish survived the 96-hour experiment. Lethal concentrations of Aroclor® 1221 did decrease the ability of this killifish to osmoregulate. The osmolarity and Na concentration of the serum was consistently increased in fish exposed

to lethal levels of Aroclor®1221.

Aroclor 1016: Bioassays lasting 96 hours show that Aroclor®1016, like other PCBs, is acutely toxic to many estuarine organisms at concentrations of 100 µg/l or less (Exhibit 19). About 10 µg/l was estimated to be lethal to 50% of the oysters, brown shrimp (Penaeus aztecus) and grass shrimp (Palaemonetes pugio) in flowing water bioassays. Only 18% of the pinfish exposed to 100 µg/l died in the 96-hour exposure. All four of these species accumulated the PCB with the quantities accumulated being related to concentrations in the test water and apparently not to species of animal. Aroclor®1016 inhibits the growth of certain strains of estuarine bacteria (Exhibit 17).

Data on the acute toxicity PCBs tested is summarized in Exhibit 20.

BIOASSAYS EXCEEDING 96 HOURS DURATION

Aroclor 1254:

Mollusca - Young oysters were continuously exposed to Aroclor®1254 for 24 or 30 weeks in flowing, unfiltered sea water (Exhibit 21). Growth rates (height and in water weights) were significantly reduced in oysters exposed for 24 weeks to 5 µg/l (3.9 µg/l measured), but apparently were not affected by 30 weeks exposure to 1 µg/l (0.64 µg/l measured). Survival of oysters was not affected by these concentrations of the chemical. Oysters accumulated as much as 101,000 X the concentration in the test water. Concentration was <0.3 µg/g in 5 µg/l exposed oysters after 28 weeks of depuration in PCB-free

water and <0.2 µg/g in 1 µg/l exposed oysters after 12 weeks of depuration. Oysters exposed to 5 µg/l for 24 weeks displayed general tissue alterations in the vesicular connective tissue around the digestive diverticula of the hepatopancreas.

In another experiment, oysters were exposed continuously, with no significant mortality, for 56 weeks to 0.01 µg/l of Aroclor® 1254 and showed a concentration factor of 165,000 (Exhibit 22). Maximum concentration of 1.65 µg/g occurred in oysters exposed for eight weeks. Patterns of accumulation and loss were seasonal. Residues decreased 45% to 81% in early July and late October, apparently as a result of spawning, and increased following these periods. This data indicates that life-history of oysters must be known when evaluating residue data from monitoring programs.

Crustacea - blue crabs. Juvenile blue crabs, Callinectes sapidus, exposed for 20 days to 5 µg/l (3.5 to 4.2 µg/l measured) of Aroclor® 1254 were apparently not affected (Exhibit 1). Average concentration in five crabs was 23 µg/g. The Aroclor® 1254 was slow to flush from crabs. After one week in PCB-free water concentrations in a second group of five crabs averaged 22 µg/g and after 4 weeks six crabs contained 11 µg/g.

Fiddler crabs. Fiddler crabs, Uca pugilator, were exposed to Aroclor® 1254 contaminated sediment from Escambia Bay, Florida to determine if the crabs would accumulate the Aroclor®

1254 from the sediment (Exhibit 3). Fiddler crabs accumulated Aroclor in quantities directly related to the amounts in sediments. The chemical was accumulated by ingestion or by its leaching into the water.

Pink shrimp. Pink shrimp were exposed for 20 days to 5µg/l of Aroclor®1254 (3.5 to 4.2µg/l measured) in flowing unfiltered water (Exhibit 1). Seventy-two percent (18 of 25) died during the exposure, no controls died. The first shrimp died on the tenth day of exposure, and a few died each day during the next 10 days. Several died during molting. Symptoms of insecticide poisoning were not exhibited by dying shrimp.

A series of additional bioassays using pink shrimp showed that Aroclor®1254 is differentially toxic to juvenile and adult shrimp (Exhibit 5). In flowing water bioassays, a measured concentration of Aroclor of 0.94µg/l killed 51% of the juvenile shrimp (2.5 to 3.8 cm) within 15 days. Exposure to 3.5µg/l (measured concentration) for 35 days resulted in a mortality of 50% of a group of (9.5 to 12.5 cm) adult shrimp. Dying shrimp exhibited no apparent outward symptoms of poisoning. Mortality was delayed, they died at the rate of one or two per day and seemed most susceptible during molting. Shrimp obtained the PCB from water and food and concentrated it in the greatest amounts in the hepatopancreas. Shrimp placed in PCB-free water lost about 40% of their total body burden of PCB within 5 weeks. If Aroclor®1254 caused shrimp mortality in the environment, it would be unlikely that it could be observed because:

(1) shrimp would die over an extended period of time, (2) dying shrimp would decompose rapidly or be eaten by predators, and (3) dead shrimp would not be visible, because unlike fish, they do not float on the surface of the water.

Pathological examinations of exposed pink shrimp demonstrated that, even if there were no outward symptoms of poisoning, there were alterations at the cellular and sub-cellular level. The hepatopancreas of shrimp exposed to Aroclor® 1254 did exhibit dramatic tissue changes which includes the presence of pyramidal crystalloids in the nuclei of epithelial cells (Exhibit 2). Along with the presence of this crystalloid were rod-shaped, free (non-occluded) and occluded virons which appear similar to the Baculovirus group of invertebrate viruses (Exhibit 17). Aroclor® 1254 may facilitate the transmission or enhance the expression of latent viral infections.

Brown shrimp - adult brown shrimp (Penaeus aztecus) were as sensitive to Aroclor® 1254 as adult pink shrimp (Exhibit 24). Three µg/l was lethal to both shrimps within 30 days. Brown shrimp exposed to a sublethal quantity of the Aroclor® 1254 for seven days died if the salinity of the test water decreased gradually in 8 hours from 30‰ to 10‰ and 7‰. Salinity changes such as this occur during tidal cycles. Osmoregulation seemed impaired because concentrations of most major ions in sera of PCB-exposed shrimp became significantly less as the

ambient salinity decreased. This was reflected as a decrease in the sum of the major ions (e.g. Na, Ca, Mg, K, Cu, and Cl) and particularly in the sodium, chloride, and calcium ions.

Grass shrimp - grass shrimp, Palaemonetes pugio, were exposed to measured concentrations of Aroclor®1254 ranging from 0.17 to 12.5 µg/l for 7 or 16 days (Exhibit 6). A concentration of 4.0 µg/l produced significant mortality but mortality of 1.3 µg/l exposed shrimp was not significantly different from that of control shrimp. Shrimp exposed for seven days to from 0.17 to 9.1 µg/l contained Aroclor in amounts ranging from 3,200 to 11,000 X the measured concentration in the test water. Grass shrimp accumulated the Aroclor® 1254 in ever increasing amounts in exposures lasting as long as 35 days.

Amphipod - the amphipod, Gammarus oceanicus, was exposed to nominal concentrations of Aroclor®1254 of from 1 to 10,000 µg/l for up to 30 days (Exhibit 25). Aroclor®1254 was lethal at concentrations as low as 10 µg/l. Some Gammarus had severely necrosed branchiae. Branchial necrosis was found in some animals exposed to 1 µg/l of the Aroclor®1254.

Fishes - Spot. In three separate flow-through bioassays, 5 µg/l of Aroclor®1254 (measured concentration with ± 20% of nominal) was lethal to juvenile spot (Leiostomus xanthurus) exposed in flowing water for 20, 26 or 45 days (Exhibit 26). Spot exposed to 1 µg/l showed no apparent effects. Affected

spot usually ceased feeding, became emaciated, and developed ragged fins and lesions on the body. In some instances, fish continued to die even though they were place in PCB-free water. Aroclor® 1254 was accumulated by exposed spot with amounts related to duration and extent of exposure and not apparently to mortality. Spot exposed to 1 µg/l of the Aroclor® 1254 for 56 days rapidly stored this chemical, maximum levels were attained in 14 to 28 days. Maximum amount stored in whole fish was 37,000 times that in the test water. After 84 days in PCB-free water, 39% of the Aroclor® 1254 remained in the fish.

Pinfish - in two separate bioassays lasting 14 or 35 days, 5 µg/l of Aroclor® 1254 was lethal to juvenile pinfish (Exhibit 26). Affected fish usually developed fungus-like lesions on the body, especially around the mouth. Concentration of the Aroclor® 1254 in whole pinfish exposed for 35 days to 5 µg/l was 109 µg/g (21,800 X the amount in the test water).

Sheepshead minnows. Sheepshead minnows are the most sensitive estuarine organism to Aroclor® 1254 that we have tested (Exhibit 27). Intermittent flow bioassays lasting three weeks were conducted with embryo, fry, juvenile and adult fish. Newly hatched fry were the most sensitive life-stage tested. Significant fry mortality occurred in 0.3 µg/l (0.16 µg/l measured) and 0.1 µg/l (0.06 µg/l measured) had no apparent effect. Fry did not die immediately upon hatching but were killed over most of the two weeks following hatching. Embryos

and juveniles of this fish were affected by 10 µg/l (3.5 µg/l measured) but this concentration was not lethal to adults. Many fry, juvenile and adult fish developed fin-rot.

Aroclor® 1254 in eggs from adult sheepshead minnows exposed in bioassays to as little as 0.3 µg/l (0.14 µg/l measured) of the Aroclor® 1254 can decrease survival of embryos and fry that are maintained in water free of this chemical (Exhibit 28). Fertilization success was unimpaired by concentrations in eggs as high as 201 µg/g but survival of embryos and fry was significantly reduced. Usually, fry from eggs containing 7.0 µg/g or more began dying 24-48 hours after hatching. Some preliminary research indicates that PCBs in eggs may decrease fertility and survival in early stages of embryonic development in Atlantic salmon, Salmo salar (Exhibit 29).

Communities of Estuarine Organisms:

Aroclor® 1254 affected the composition of communities of estuarine animals that developed from planktonic larvae in salt water that flowed through 10 control aquaria and 10 aquaria contaminated with 0.1, 1, or 10 µg/l of the Aroclor® 1254 (Exhibit 30). Communities that developed in control aquaria and aquaria that received 0.1 µg/l of PCB in water for four months were dominated (>75%) by arthropods, primarily the amphipod Corophium volutator. In aquaria receiving 1 and 10 µg/l, the number of arthropods decreased and the number of chordates, primarily the tunicate Molgula manhattensis, increased; over 75% of the animals in 10 µg/l aquaria were tunicates. Numbers of phyla, species, and individuals (particularly

amphipods, bryozoans, crabs, and mollusks) were decreased, but there was no apparent effect on the abundance of annelids, brachipods, coelenterates, echinoderms or nemerteans. The Shannon-Weaver index of species diversity was not altered by Aroclor®1254.

Aroclor 1016 - Pinfish. The chronic toxicity and uptake of Aroclor®1016 by pinfish was tested in three separate flow-through bioassays lasting 42 days (Exhibit 19). Mortality of pinfish exposed to 32 µg/l (13 µg/l measured in one experiment and 21 µg/l in the second) or 100 µg/l (59 µg/l measured) was significantly greater than mortalities of unexposed pinfish. Mortality of 10 µg/l (about 7 µg/l measured) exposed fish was negligible. Color of most dying fish darkened, they stopped feeding and they swam erratically with their head inclined downward. Difficulty in swimming progressed until the fish swam upside down just prior to death. Pathological examination of 32 µg/l exposed fish revealed several liver and pancreatic alterations that distinguished them from control fish. Concentrations of Aroclor®1016 stored by the pinfish exposed for 42 days ranged from 11,000 to 24,000 times the nominal concentration in the test water. Concentrations in pinfish exposed to 1 µg/l for 56 days increased for three or four weeks and then stablized. When placed in PCB-free water for 56 days, concentrations (µg/g) in these fish decreased by 51%.

Sheepshead minnows: The chronic toxicity of Aroclor® 1016 to fry, juvenile and adult sheepshead minnows was investigated in intermittent flow bioassays lasting 28 days (Exhibit 31). Fry, juvenile, and adult fish were apparently not affected by nominal concentrations of 0.1, 0.32, 1.0, 3.2 or 10 µg/l but died in 32 and 100 µg/l of this chemical. Chemical analyses of the test water showed about 4 µg/l in the 10 µg/l aquaria, 15 µg/l in 32 µg/l aquaria and 42 µg/l in the aquaria contaminated with 100 µg/l of Aroclor® 1016. Responses to poisoning included darkened body coloration, uncoordinated swimming, cessation of feeding, lesions on the body of many juveniles and adults, and death. Sheepshead minnows accumulated the PCB in proportion to its concentration in the test water. Fry contained 2,500 to 8,100 X the nominal concentration in the test water, adults 4,700 to 14,000 X and juveniles 10,000 to 34,000 X. Eggs from exposed adults were fertilized and placed in PCB-free water. As much as 77 µg/g of the PCB in these eggs apparently did not affect survival of embryos and fry for two weeks.

Information on flow-through bioassays lasting longer than 96 hours is summarized in Exhibit 32.

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TOXICITY OF AROCLOR® 1254 AND ITS PHYSIOLOGICAL ACTIVITY IN SEVERAL ESTUARINE ORGANISMS [1]

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®In this paper, Aroclor and PCB are used interchangeably for Aroclor 1254. Aroclor® is a registered trademark of the Monsanto Company, St. Louis, Mo. Reference to commercial products does not constitute endorsement by the Environmental Protection Agency.

[1]Contribution No. 162, Gulf Breeze Environmental Research Laboratory.

Abstract

The occurrence of high concentrations of a PCB (Aroclor 1254) in the Pensacola estuary prompted field and laboratory studies by the Gulf Breeze Environmental Research Laboratory (EPA). Monitoring of the estuary indicates the chemical is present in all components—particularly in sediments and fishes. Residues appear to be diminishing in sediments. Toxicity tests show estuarine species sensitive at parts-per-billion concentrations in water, with a ciliate protozoan Tetrahymena pyriformis W, shrimps Penaeus duorarum, P. aztecus and Palaemonetes pugio, and a fish, Fundulus similis, affected at or near 1.0 ppb. Tissue concentrations of Aroclor 1254 similar to those found in natural populations of shrimps from the contaminated estuary were successfully duplicated in laboratory experiments. Shrimps also concentrated the PCB from very low concentrations (0.04 ppb) in the water. Three estuarine species demonstrated pathologic changes at tissue and cellular level after chronic exposure to the chemical. Oysters, Crassostrea virginica, developed abnormal infiltration of leukocytes in the connective tissue, spot, Leiostomus xanthurus, developed fatty changes in their livers, and shrimp, Penaeus duorarum, developed crystalloides in hepatopancreatic nuclei.

Introduction

Polychlorinated biphenyl (PCB) residues were found in water, sediment and biota of Escambia Bay, Florida, in April 1969 (Duke et al. 1970). Subsequently, investigation into the effects of this chemical on estuarine organisms has been a major research goal of the Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida. In this report, we present data on the chemical in water, sediments and biota of Escambia Bay and adjacent areas, review toxicological data and present some physiological-pathological information. Experimental methods and materials used as well as chemical analyses are given elsewhere (Duke et al. 1970; Cooley et al. 1972; Hansen et al. 1971; Nimmo et al. 1971a; Lowe et al. 1972).

PCB in Escambia Bay

Unlike other studies in which PCBs have been found in the environment, the chemical in Escambia Bay apparently came from a single point-source. Figure 1 is a comparison of chromatograms of (a) an Aroclor 1254 standard, (b) Aroclor 1254 in oysters from a 72-week chronic exposure to the chemical in the laboratory, and (c) PCB isolated from oysters taken from Escambia Bay. PCB concentrations in both oyster samples were similar and both samples and standard were analyzed on the same chromatograph. Earlier, it was established by mass spectroscopy that the PCB in Escambia Bay was Aroclor 1254 (Nimmo et al. 1971a) and in the comparison presented here the similarity is apparent. In laboratory studies with Aroclor 1254, the oysters were continuously exposed to approximately 10 parts per trillion for 72 weeks and the similarity of tissue residues to the PCB found in oysters from Escambia Bay indicates that the chemical

in the Bay has changed little with time.

Monitoring data for the period September 1969 through December 1971 are presented in Table 1. Concentrations of Aroclor in unfiltered water samples from Escambia River downstream averaged 0.6 parts-per-billion. Aroclor was found in 64% of the water samples from the River and in 27% of the water samples from Escambia Bay. Average concentration in sediment samples from the Bay was 2.3 parts-per-million (ppm). PCB in 101 samples of invertebrates, predominately mollusks and crustaceans, averaged 0.8 ppm. Fishes from the Bay had five times as much PCB in their tissues as did invertebrates.

Residues of Aroclor in sediment samples from a survey taken in February 1970 (Nimmo et al. 1971b)are listed in Figure 2. The samples were taken from the upper strata (e. g., upper 10 inches) by corer or by dredge. In this survey, the maximum residue (61 ppm) observed in the River was found at the outfall from the industry; the maximum in Escambia Bay (30 ppm) was found near the mouth of the River.

The amounts of PCB in sediment samples from several locations within the River and Bay have decreased in the ensuing months. The number of samples was inadequate for absolute comparison, but the data indicate a trend. A decrease is especially noticeable in the December 1970 and October 1971 surveys (Fig. 3), in which sediment samples were taken with a corer at three locations in the Bay. Generally, residues in the 1971 survey were about one-tenth the 1970 values, except one sample taken below the trestle in the surface stream. PCB in the lower strata (4-12 inches) in the 1971 survey was non-detectable, except at the outfall of the industry. Later, we examined a deeper core — to 24 inches -- taken

above the trestle but found no residues. Cores taken in a 1972 survey generally indicated less PCB than in 1971.

Whole-body residues of Aroclor 1254 found in shrimps from Escambia Bay and contiguous waters during 1969/1970 are shown in Fig. 4. Each datum represents a composite sample of at least 5 individuals. We show these data to indicate the dispersal of the chemical in an estuarine environment from an apparent point source by either biological or physical transport. Although the material was originally localized in the sediments of upper Escambia Bay, shrimps captured in lower Pensacola Bay also contained significant amounts of the PCB.

The amounts of Aroclor in biota from the estuary remain relatively high and the latest survey showed amounts generally increasing at higher trophic levels (Fig. 5). In the survey of October, 1971, we found no detectable PCB in the sea grasses, Spartina sp. and Zostera marina. The mollusk, Neritina reclivata, contained 0.49 ppm. Of two crustaceans, blue crabs, Callinectes sapidus, had the greater residues (6.9 ppm). Among fishes, one might expect sand seatrout, Cynoscion arenarius, and Atlantic cutlassfish, Trichiurus lepturus, to have the highest residues because these species are predators; instead, the highest residue (10 ppm) was found in silversides, Menidia beryllina, a species whose diet consists mainly of plankton.

In Figure 5, PCB residues are compared in the same species or in species occupying similar trophic levels and captured on the same day in Escambia and East Bays. The collecting site in East Bay is about 35 kilometers from the original source of the chemical. PCB residues in

species from Escambia Bay were 5 to 10 times greater than those found in East Bay but the data clearly show that the chemical was found in species captured distant from the original source of the PCB.

Toxicity of Aroclor 1254 to estuarine organisms

Laboratory research on the toxicity of PCB to estuarine organisms began immediately after the chemical was discovered in the Bay. Animals from several trophic levels have been tested.

Population growth in test-tube cultures of the ciliate protozoan, Tetrahymena pyriformis W, was reduced significantly by exposure to 1 ppb Aroclor 1254 (Table 2). Reduction was measured as effect on population growth rate and on population density at 96 hours. Growth rate was estimated as the quantity b of the least squares estimate of the line $y = a+bx$ for the exponential growth phase of the population growth curve.

These ciliates accumulated the PCB from the test media during the exposures. Cells contained a maximum of 60 ppb (dry-weight basis) of PCB when grown for seven days in medium that contained 1 ppb Aroclor. Because the ciliates can accumulate PCB from a culture medium, they could be a step in the transport of the chemical into aquatic food webs under natural conditions.

Bioassays were conducted in flowing water to determine the toxicity of Aroclor 1254 to a mollusk, three crustaceans and three fishes (Table 3). Growth in oysters, Crassostrea virginica, exposed to 5 ppb for 24 weeks was significantly reduced, but growth in oysters exposed to 1 ppb for 30 weeks was not. Earlier work showed that hydrocarbon compounds inhibited shell deposition significantly at concentrations of 0.1 to 0.5 ppm

in short-term tests (Butler, 1966). In tests lasting about 2 weeks, various shrimps, Penaeus duorarum, Palaemonetes pugio, and Penaeus aztecus, were killed by exposure to 0.9, 1.3, and 1.4 ppb, respectively. In tests lasting 2 weeks or longer this chemical was lethal to longnose killifish, Fundulus similis, at 1 ppb and pinfish, Lagodon rhomboides, and spot, Leiostomus xanthurus, at 5 ppb. Test animals exposed for over one week accumulated the PCB from the water and concentrations ranged from about 10^4 in crustaceans and fishes to 10^5 in oysters over exposure concentrations.

Acute toxicity tests did not show the true sensitivity of marine species to this compound. In comparison to short-term tests lasting 48 hours, Aroclor in chronic bioassays lasting one week or more proved to be 100 times more toxic.

Mortalities were "delayed" as they usually did not begin until after one week of exposure and continued to occur after the animals were removed from the toxicant. This delayed mortality was similar to that observed with the insecticide mirex (Lowe et al. 1971). Gross signs of poisoning varied with species. Fishes typically developed hemorrhagic lesions on the body, ragged fins, and stopped feeding. Shrimps became lethargic and also stopped feeding, thereby mimicking the effects of low concentrations of DDT (1, 1, 1, Trichloro - 2, 2 - bis (p-chlorophenyl) ethane) (Nimmo and Blackman, 1972). Shrimps appear to be most susceptible to the chemical during molting, as previously noted by Duke et al. (1970) and Wildish (1970).

Accumulation of Aroclor 1254 by shrimp

The pathway by which organisms obtain toxicants or the actual effects observed in the laboratory under controlled conditions may or may not approximate those obtained under field conditions. The pathway appears to be an open question in the field of aquatic toxicology and the need for such research was stated by Sodergren et al. (1972) after a study of the accumulation of DDT and PCB by a crustacean.

If we could produce in the laborstory tissue distributions of PCB similar to those found in the field, some insight might be gained into its mode of entry and concentrations present in nature. We began a study by determining PCB residues of shrimp from Escambia and Pensacola Bays (Fig. 6). We administered PCB at three concentrations (0.2, 0.68 and 43 ppm) in food (Fig. 7). PCB was added at 3.0 ppb to seawater filtered through gravel and charcoal and 3.0 ppb was added to unfiltered seawater (Fig. 8). PCB was added to unfiltered seawater at 3.5 and 0.2 ppb (Fig. 9). Analytical methods for PCB were those of Nimmo et al. (1971a). The results of the field surveys and laboratory studies are expressed as the "relative concentration" (Figs. 6, 7, 8, 9):

$$\text{RELATIVE CONCENTRATION} = \frac{\text{ppm in a single tissue or organ}}{\text{ppm in all tissues or organs}} \times 100.$$

The proportion of Aroclor found in tissues of shrimp exposed to 0.2 ppb in water in the laboratory was nearest to that found in shrimp which were exposed naturally in the bays (Fig. 9). We assume that shrimp in the laboratory experiments obtained most of the chemical from the water but

some may have been consumed in detritus that was in the test aquaria. The distribution of PCB in shrimp fed 0.68 ppm in food and distribution in shrimp from the bays were similar (Fig. 7). Even though we varied the time of exposure and conducted the tests at what we considered realistic concentrations, we cannot distinguish the main pathway of PCB into shrimp from these experiments. However, we believe that concentrations of PCB available to shrimp in Escambia and Pensacola Bays were low (e. g., <1.0 ppb in water; <1.0 ppm in food).

In order to determine if there was a concentration below which shrimp could not accumulate the chemical, we tested several hundred grass shrimp, Palaemonetes pugio, at 0.04, 0.09 and 0.62 ppb of Aroclor 1254. The tests were conducted in flowing-water aquaria as before, with one modification. Each tank had a false floor of nylon screen to hold the animals above the detritus. We believe the shrimp obtained more chemical through absorption from the water than in previous experiments.

Within the test concentrations, no threshold level existed below which shrimp did not accumulate the chemical (Fig. 10). Concentrations produced in the shrimp (whole-body) were about 0.2, 1.0 and 10 ppm, respectively; and these were reached between the third and fifth weeks of exposure. Concentrations in the shrimp did not reach equilibrium during the five-week exposure but the rate of accumulation decreased with time. When transferred to PCB-free water, the shrimp lost most of the chemical after four weeks.

Pathology in estuarine organisms

To date, toxicology of PCBs with respect to histopathological effects in estuarine organisms has been little studied. Published sources of information are from studies on mammals and birds (Dalgren et al. 1972; Vos, 1972; Norback and Allen, 1972) and a single study on fishes (Couch, 1972). Structural changes found in tissues of oysters, fish, and shrimp exposed to Aroclor 1254 are characterized below.

Oysters exposed to 5.0 ppb Aroclor 1254 for up to 6 months showed several major tissue changes. Normal structural pattern of oyster vesicular connective tissue (parenchyma) is seen in Fig. 11, and the irregular and broken pattern representative of altered tissue from exposed animals is seen in Fig. 12. In exposed oysters, an abnormal infiltration of leukocytes was found in the vesicular connective tissue (Figs. 13, 14). Sections of digestive glands of normal control oysters (Fig. 15) can be compared to those in exposed oysters (Fig. 16). The epithelia of distal digestive tubules of exposed oysters have undergone atrophy and surround enlarged lumina. Oysters that were removed from Aroclor-contaminated water and allowed to live in natural water for several weeks demonstrated partial or complete tissue recovery.

Spot, an estuarine fish, exposed for two weeks or longer to 5.0 ppb Aroclor, showed fatty changes in their livers. Normal liver tissue has regular distribution of hepatic cells and typical nuclei (Fig. 17). Note the regularity in the orientation of liver cords, cells and uniform scattering of nuclei. In intermediate stages of liver pathogenesis in experimental fish, there are extreme fatty changes characterized by

presence of large vacuoles within hepatocytes and disorientation of liver cord distribution (Fig. 18). Figure 19 shows an advanced stage of pathogenesis in a moribund fish. Note the presence of intracellular PAS-positive bodies (ceroid) and congestion of blood sinuses, and severe vacuolation.

Probably the most dramatic tissue change associated with chronic PCB exposure was observed in shrimp. The normal hepatopancreas, or digestive gland, of shrimp is a tightly packed organ of small elongated tubules (Fig. 23). Figure 24 shows a cross section through one of these tubules. In the hepatopancreas of exposed shrimp, pyramidal crystalloids of various sizes were found as inclusion bodies in the nuclei of epithelial cells (Figs. 24, 25). Free crystalloids are shown in Fig. 26. We know of no other report of the occurrence of precisely shaped crystalloids in hepatopancreatic tissue of crustaceans. We have routinely studied unfixed, fresh exposed shrimp and have found crystalloids in squashes of the tissue (See Fig. 21, 22).

Epithelial cells of the hepatopancreas from shrimp which were exposed to 3 ppb Aroclor for at least 30 days are shown in Figure 20. Exposed shrimp that do not have crystalloids have no conspicuous pathologic tissue signs. In those that have the crystalloids, hypertrophy of the affected nucleus results. Eventually, the growth of the crystalloid inclusion distorts and ruptures the nuclear membrane. Several nuclear membranes and inclosed crystalloids are indicated in Figure 20. The crystalloids are histochemically positive for protein. They occur in widely separated nuclei but may also appear in clusters of adjacent nuclei and are most abundant in epithelial cells of tubules proximal to the main hepatopancreatic ducts.

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These crystalloids were found in individual shrimp before moribundity or death. In certain exposures, crystalloids have been found in up to 80% of the survivors but the incidence is very low over time until about 75% of the test animals have died. Crystalloids were found more often in larger shrimp than in juveniles. At present, we are attempting to establish whether crystalloids occur in individuals from other localities when exposed to PCB, in other species of shrimp, or in shrimp from contaminated areas in nature.

Several possibilities exist as to the origin of the crystalloid inclusions. One suggestion was that they may be the result of sequestering of some normal or abnormal metabolite. Another possibility is that they represent a material produced by a virus* and were produced under PCB stress. In reference to this suggestion, Friend and Trainer (1970) showed that PCB enhanced the pathogenic effects of hepatitis virus in ducks.

*One of us (J. C.) as a result of electron microscope studies has recently found rod-shaped virus-like particles occluded within the crystalloid inclusion bodies. This matter is presently under study.

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Legend for Figures

Figure 1. Chromatograms of an Aroclor 1254 standard, Aroclor 1254 in oysters from a 72-week chronic exposure in the laboratory and PCB in oysters from Escambia Bay.

Table 1. Concentration of Aroclor® 1254 in water, sediment, and biota, September 1969 through December 1971.

Figure 2. Collecting stations in Pensacola Estuary, 24 February 1970. Residues found in sediments at each station are given in parts-per-million (ppm). N. D. = not detected. (After Nimmo et al., 1971b)

Figure 3. Comparison of concentrations of Aroclor 1254 in cores taken 10 months apart in upper Escambia Bay and River. Concentrations given in 1971 above and below trestle are averages of 2 cores each.

Figure 4. Residues of Aroclor 1254 in shrimp (whole body) from Escambia Bay and contiguous waters during 1969/1970. Each datum represents a composite sample of at least 5 individuals.

Figure 5. Comparison of concentrations of Aroclor 1254 found in species collected in Escambia (left side) and East Bays (right side): seagrasses, Spartina sp. and Zostera marina; Rangia clams, Rangia cuneata; olive nerite, Neritina reclivata; brown and white shrimp, Penaeus aztecus and P. setiferus; blue crabs, Callinectes sapidus; bay anchovy, Anchoa mitchilli; sea catfish and gafftopsail catfish, Arius felis and Barge marinus; tidewater silversides, menidia beryllina; silver perch Bairdiella chrysura; sand seatrout, Cynoscion arenarius; spotted seatrout, Cynoscion nebulosus; spot, Leiostomus xanthurus; Atlantic croaker, Micropogon undulatus; hogchoker, Trinectes maculatus;

Figure 15. Normal digestive gland tubules from control oyster. Note the thick epithelia which form normal triradiate lumina. X450. (from Lowe et al., 1972)

Figure 16. Digestive gland tubules of oyster exposed to PCB. Note atrophy (thinning) of tubule epithelium and enlarged, abnormal lumen of tubule. X450.

Figure 17. Liver parenchyma of normal spot (Lagodon rhomboides) from control tank. Note uniform orientation of hepatocytes. X1000.

Figure 18. Liver parenchyma of spot exposed to PCB for several weeks, intermediate pathogenesis. Note large, smooth-edged vacuoles indicative of abnormal fatty-change in hepatocytes. X1000.

Figure 19. Liver parenchyma of spot exposed to PCB until moribund, advanced pathogenesis. Note large vacuoles, amorphous inclusions and sinusoidal congestion. X1000.

Figure 20. Hepatopancreas (digestive gland) tubule from pink shrimp exposed to PCB. Note triangular crystalloids in some hypertrophied nuclei (H); also, normal nuclei (N) with large, prominent endosomes. (Feulgen reaction; DNA appears black in photomicrograph). X1000.

Figure 21. Fresh squash of hepatopancreas from exposed shrimp. Note two crystalloids in center. X1000.

Figure 22. Single, large crystalloid from fresh squash of hepatopancreas of exposed shrimp. X1000.

Figure 23. Longitudinal section of hepatopancreatic duct with branching tubules. In exposed shrimp, the crystalloids appear in the tubule epithelia nearer the main hepatopancreatic ducts. X1000.

Figure 24. Cross-section of hepatopancreas tubule from exposed shrimp. Note crystalloids in several epithelial nuclei; also, normal nuclei with conspicuous endosomes. X450.

Figure 25. Intermediate size crystalloid within hypertrophied epithelial nucleus. X1000.

Figure 26. Pathologic effect of crystalloid in PCB-exposed shrimp. Note rupture of cells and nuclei releasing the crystalloids. X1000.

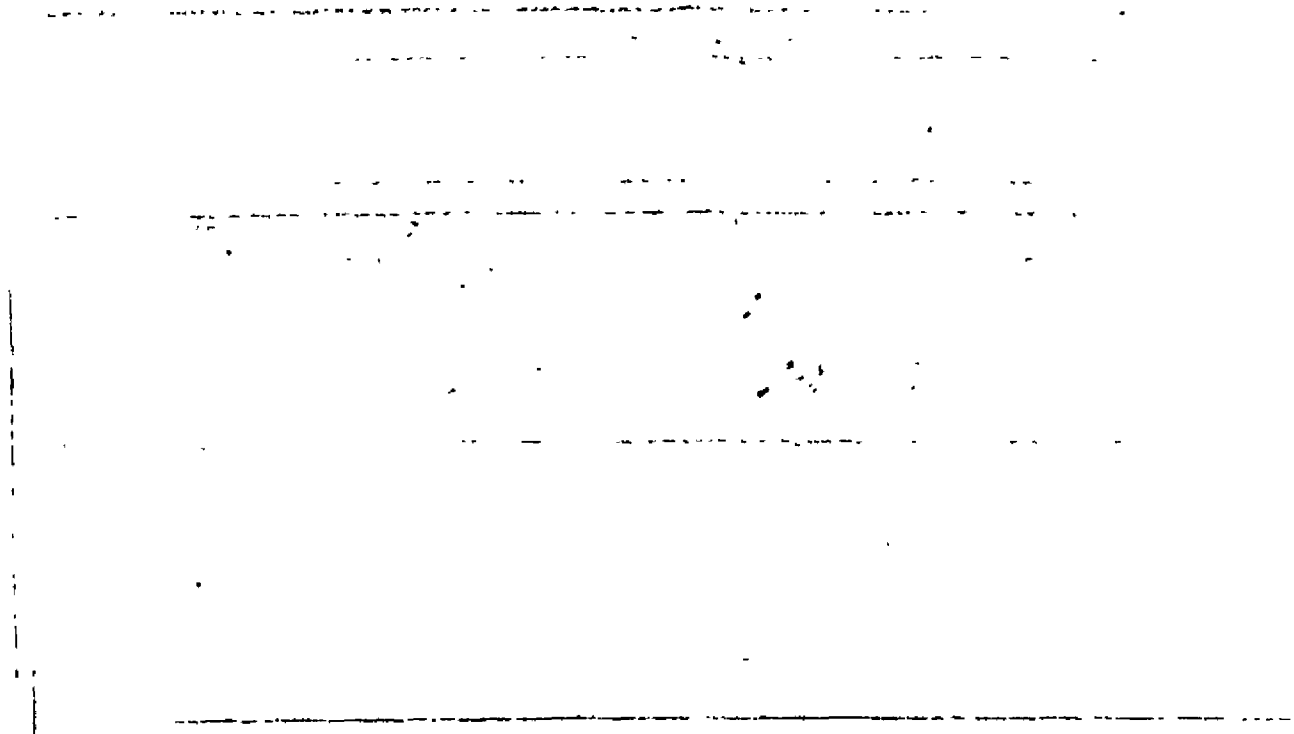
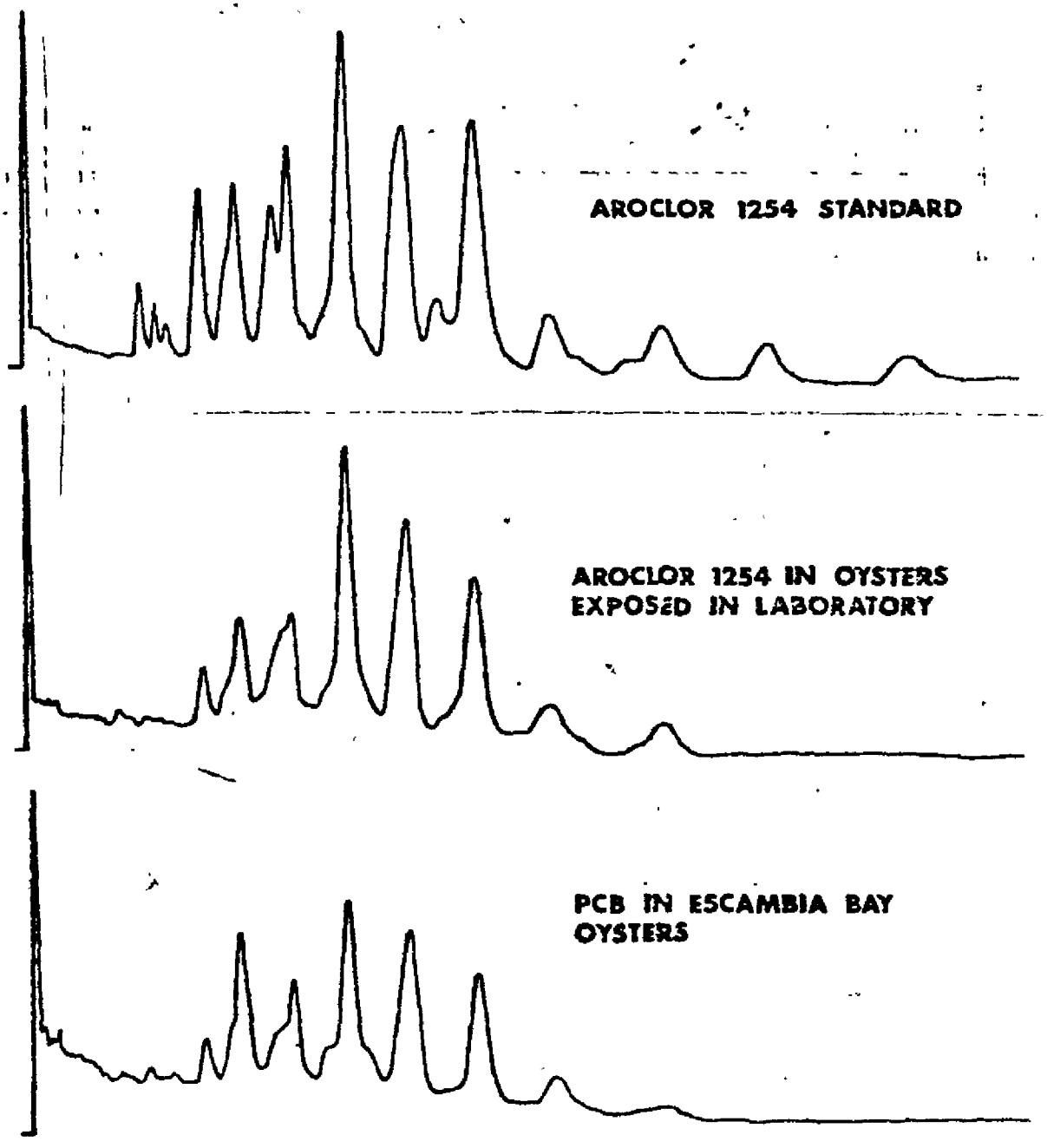


Figure 1. Chromatograms of an Aroclor 1254 standard, Aroclor 1254 in oysters from a 72-week chronic exposure in the laboratory and PCB in oysters from Escambia Bay.


AROCLOR 1254 STANDARD
AROCLOR 1254 IN OYSTERS
EXPOSED IN LABORATORY
PCB IN ESCAMBIA BAY
OYSTERS

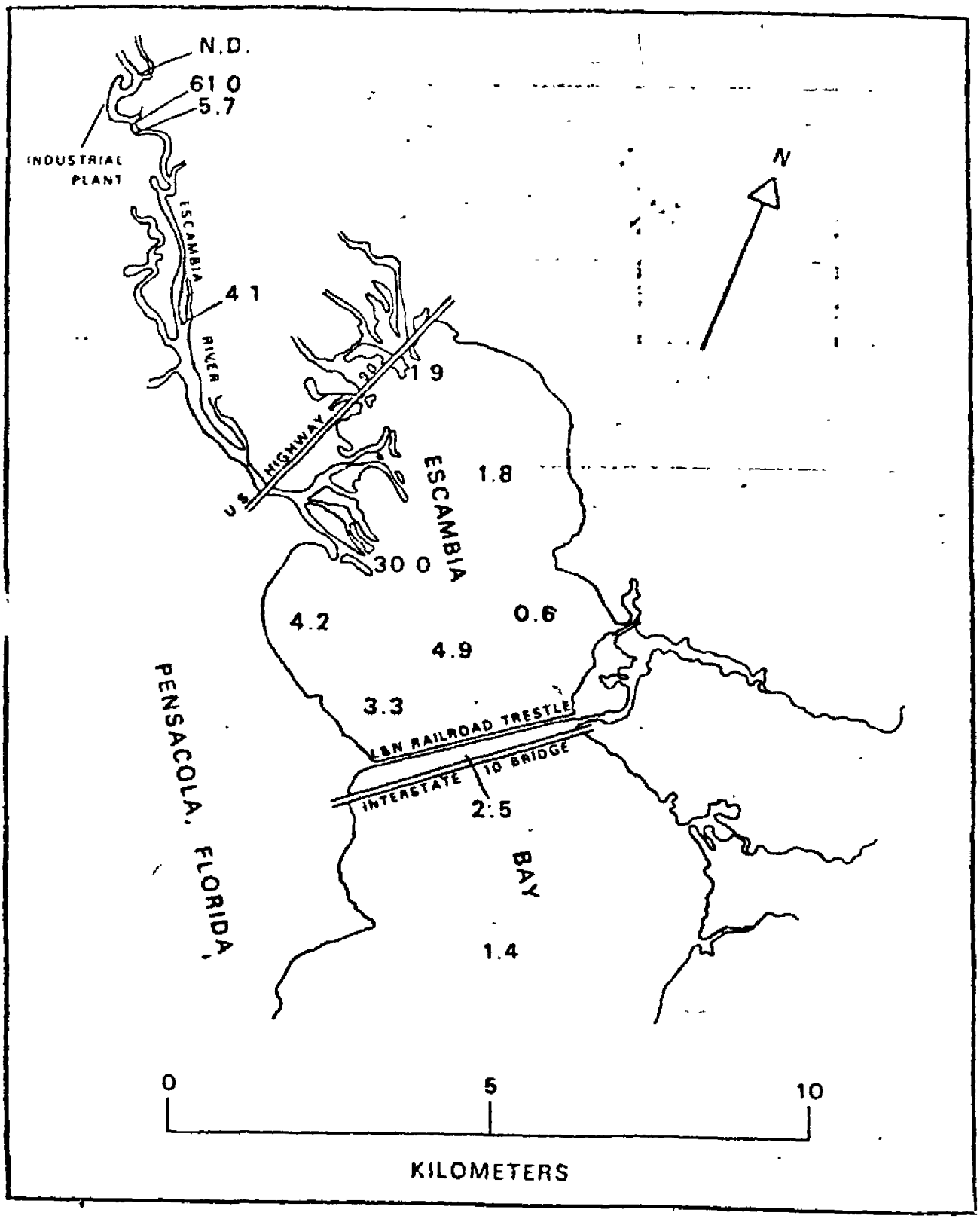
MONS 203453

Table 1. Concentration of Aroclor® 1254 in water, sediment, and biota, September 1969 through December 1971.

Location	Samples			Concentration	
	Type	Total Number	Positive Percent	Average ppm	Range ppm
Escambia River, Fla.	Water	67	64	0.0006	ND- 0.0086
Escambia Bay, Fla.	Water	37	27	ND	ND- 0.00007
Escambia Bay, Fla.	Sediment	56	78	2.33	ND-30.
Escambia Bay, Fla	Invertebrates	101	92	0.81	ND- 6.9
Escambia Bay, Fla.	Fishes	17	100	3.99	0.29-20.

ND = Non-detectable: Water, <0.00003 ppm; Sediment or Biota, <0.01 ppm.

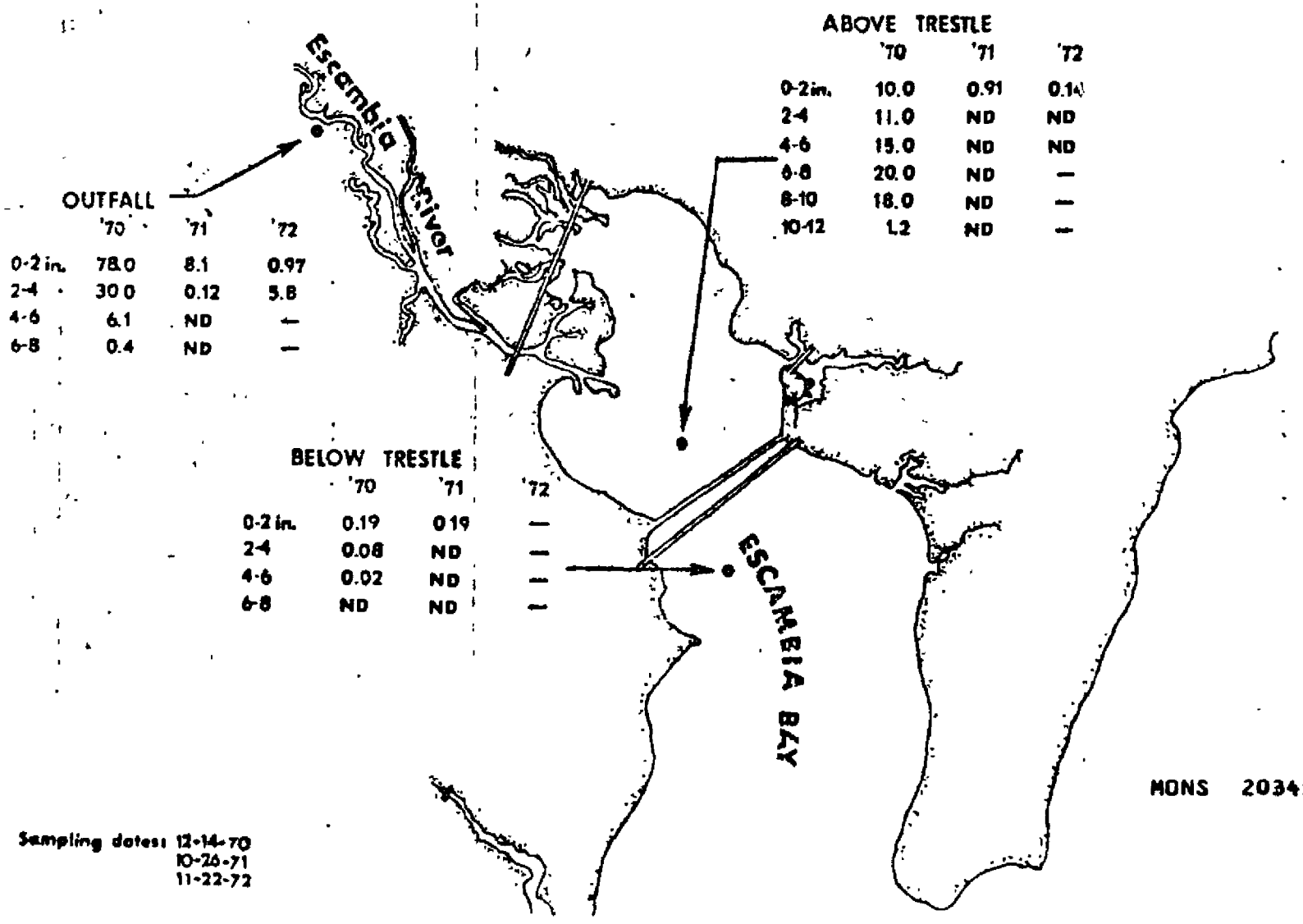
Figure 2. Collecting stations in Pensacola Estuary, 24 February 1970. Residues found in sediments at each station are given in parts per million (ppm). N. D. = not detected. (After Nimmo et al., 6).


N.D.
6.1 0
5.7
INDUSTRIAL PLANT
ESCAMBIA RIVER
4.1
N
1.9
U.S. HIGHWAY 90
ESCAMBIA
1.8
30 0
4.2
0.6
4.9
3.3
L&N RAILROAD TRESTLE
INTERSTATE 10 BRIDGE
2.5
PENSACOLA, FLORIDA
BAY
1.4
0
5
10
KILOMETERS

MONS 203456

Figure 3. Comparison of concentrations of Aroclor 1254 in cores taken 10 months apart in upper Escambia Bay and River. Concentrations given in 1971 above and below trestle are averages of 2 cores each.

SEDIMENT CORE CONCENTRATIONS (ppm) OF AROCLOR



OUTFALL

	'70	'71	'72
0-2 in.	78.0	8.1	0.97
2-4	30 0	0.12	5.8
4-6	6.1	ND	—
6-8	0.4	ND	—

ABOVE TRESTLE

	'70	'71	'72
0-2 in.	10.0	0.91	0.14
2-4	11.0	ND	ND
4-6	15.0	ND	ND
6-8	20.0	ND	—
8-10	18.0	ND	—
10-12	1.2	ND	—

BELOW TRESTLE

	'70	'71	'72
0-2 in.	0.19	0 19	—
2-4	0.08	ND	—
4-6	0.02	ND	—
6-8	ND	ND	—

Sampling dates: 12-14-70
10-26-71
11-22-72

MONS 203458

Figure 4. Residues of Aroclor 1254 in shrimp (whole body) from Escambia Bay and contiguous waters during 1969/1970. Each datum represents a composite sample of at least 5 individuals.

RESIDUES OF AROCLOR 1254 (ppm)

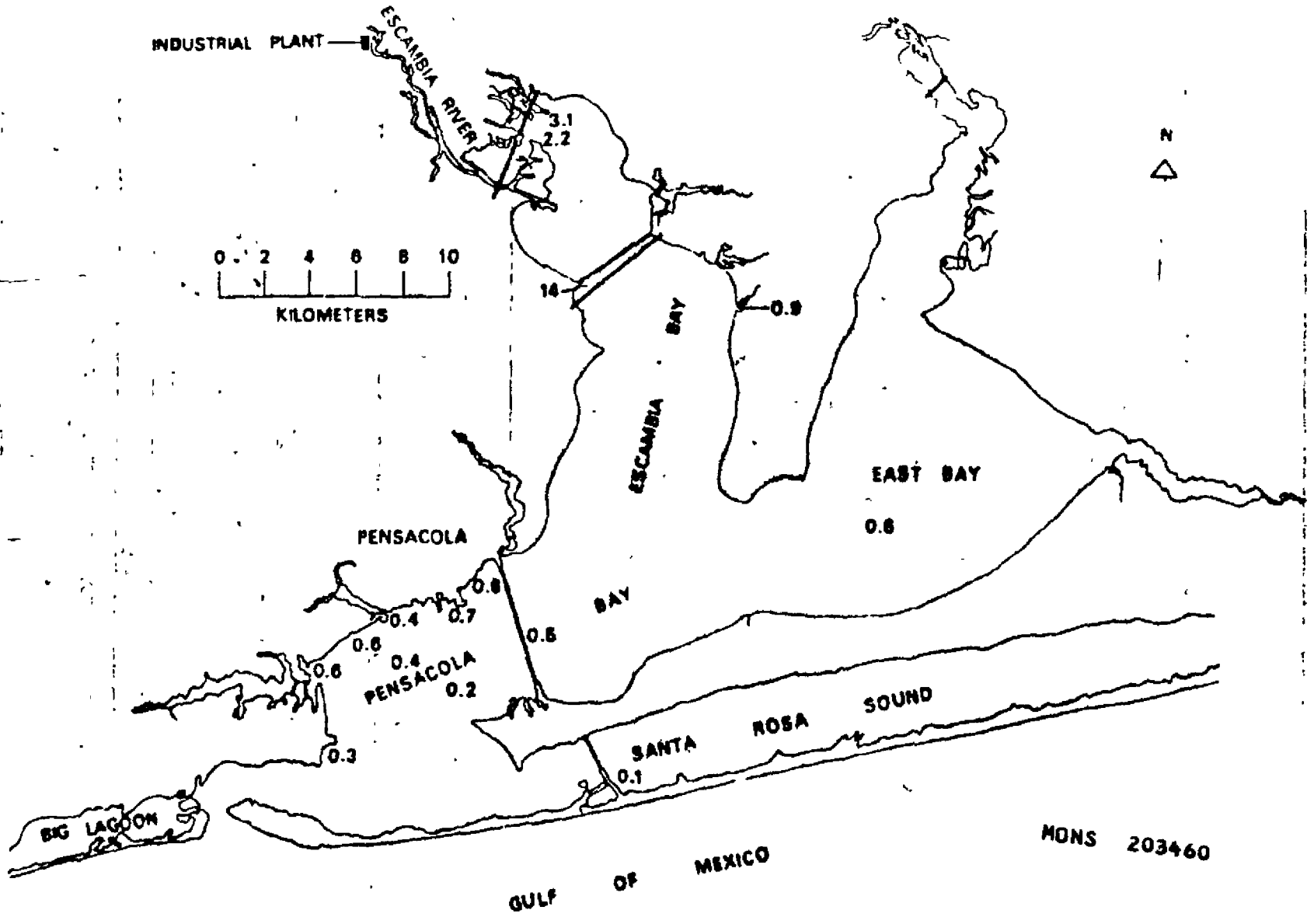


Figure 5. Comparison of concentrations of Arbclor 1254 found in species collected in Escambia (left side) and East Bays (right side): seagrasses, Spartina sp. and Zostera marina; Rangia clams, Rangia cuneata; olive nerite, Neritina reclivata; brown and white shrimp, Penaeus aztecus and P. setiferus; blue crabs, Callinectes sapidus; bay anchovy, Anchoa mitchilli; sea catfish and gafftopsail catfish, Arius felis and Bagre marinus; tidewater silversides, menidia beryllina; silver perch, Bairdiella chrysura; sand seatrout, Cynoscion arenarius; spotted seatrout, Cynoscion nebulosus; spot, Leiostomus xanthurus; Atlantic croaker, Micropogon undulatus; hogchoker, Trinectes maculatus; and Atlantic cutlassfish, Trichiurus lepturus.

AROCLOR® 1254

(ppm)	SPARTINA	ND (ppm)
ND	ZOSTERA	ND
0.49	OLIVE NERITE	ND
ND	RANGIA	ND
0.98	PENAEID SHRIMP	TRACE
6.90	BLUE CRABS	0.46
3.00	BAY ANCHOVY	0.68
3.80	CATFISH	0.58
10.00	TIDEWATER SILVERSIDES	0.95
4.50	SILVER PERCH	0.48
1.50	SAND SEATROUT	---
---	SPOTTED SEATROUT	0.12
1.80	SPOT	TRACE
1.60	ATLANTIC CROAKER	TRACE
1.30	HOGCHOKER	ND
2.90	ATLANTIC CUTLASSFISH	0.72

MONS 203462

Table 2. Effect of Aroclor 1254 on population growth of Tetrahymena pyriformis W.[1]

Toxicant (ug/liter)	Mean Growth Rate* b	Difference %	Mean Population Density (absorbance)	Difference %
0	0.0212		1.044	
0.1	0.0203	-4	0.984	- 6
1.0	0.0195	-8[a]	0.936	-10[b]
10.0	0.0199	-6[a]	0.954	- 9[b]

[1]After Cooley et al.(1972).

[a]$F(3,15) = 6.00$ $(P>0.01)$; [b]$F(3,15) = 23.001$ $(P>0.005)$.

*Means of 6 replicate experiments.

Table 3. Chronic toxicity of Aroclor® 1254 to estuarine animals.*

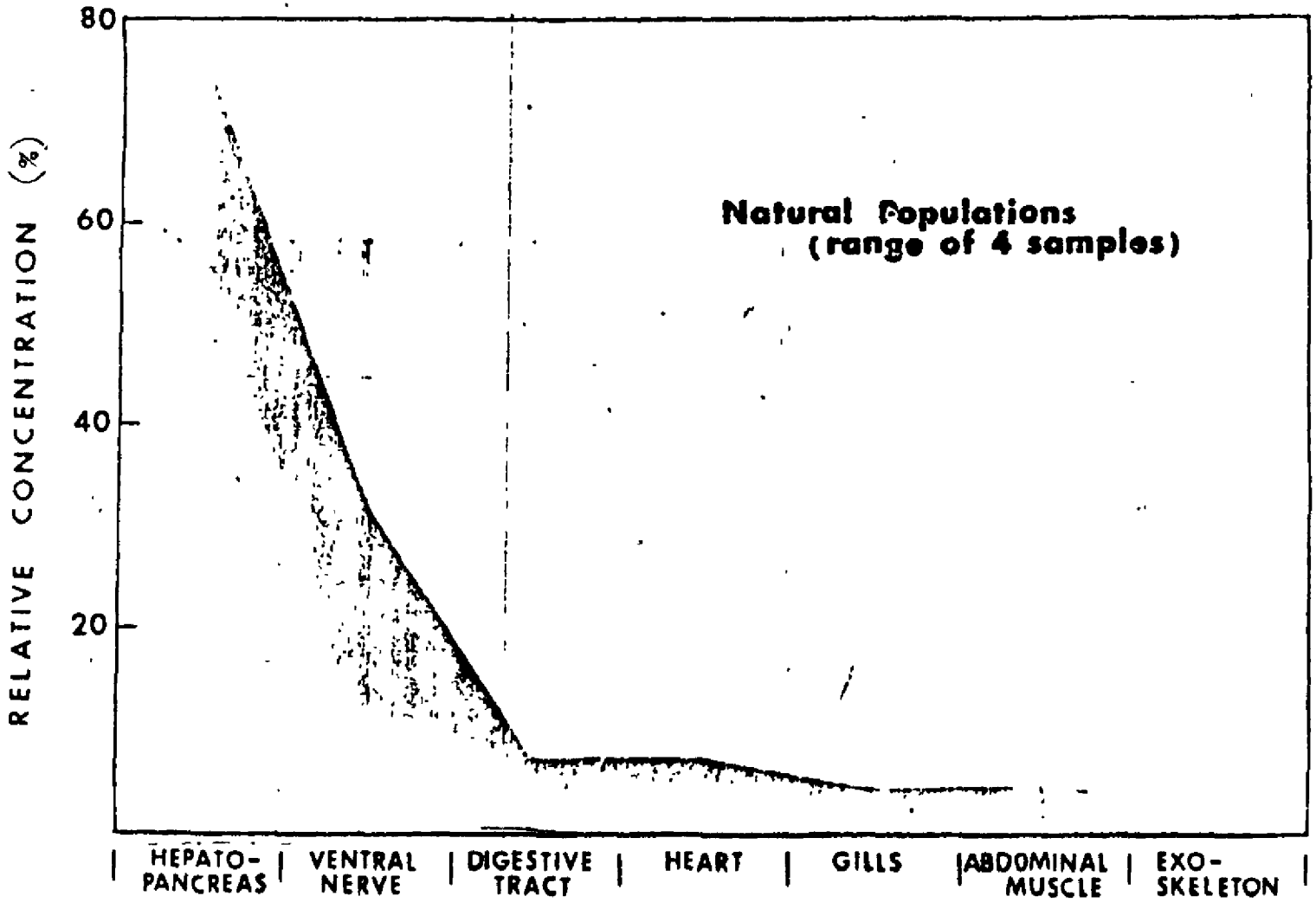
Test Animals	Concentrations (Range)	Minimum Affecting
	ppb (µg/l)	
Pink Shrimp	0.6 - 19.0	0.9
Longnose Killifish	1.0 - 100.0	1.0
Grass Shrimp	0.2 - 12.5	1.3
Brown Shrimp	0.1 - 1.4	1.4
Pinfish	5.0	5.0
Spot	1.0 - 5.0	5.0
Eastern Oyster	1.0 - 5.0	5.0

Controls did not exceed 25% mortality. Toxicity in oysters was measured by reduced shell growth.

*1-30 weeks in flowing water.

Figure 6. Distribution of Aroclor 1254 in tissues of shrimp expressed as relative concentration (%). The grey area represents the range of concentrations in four composite samples of shrimp from different locations in the Pensacola estuary on different dates.

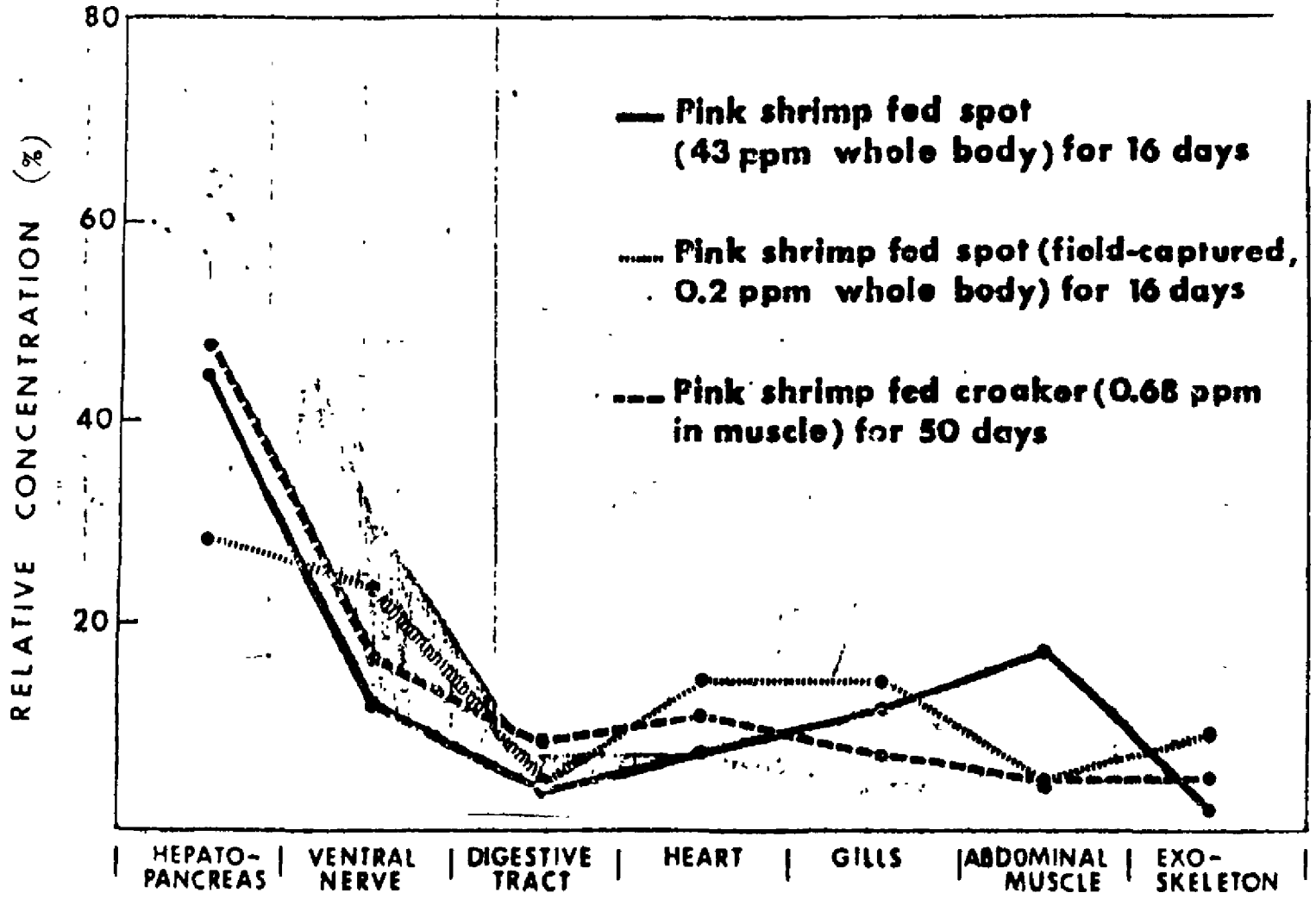
DISTRIBUTION OF AROCLOR 1254 IN TISSUES OF SHRIMP



MONS 203466

Figure 7. Distribution of Aroclor 1254 in tissues of shrimp fed Aroclor 1254-contaminated diets.

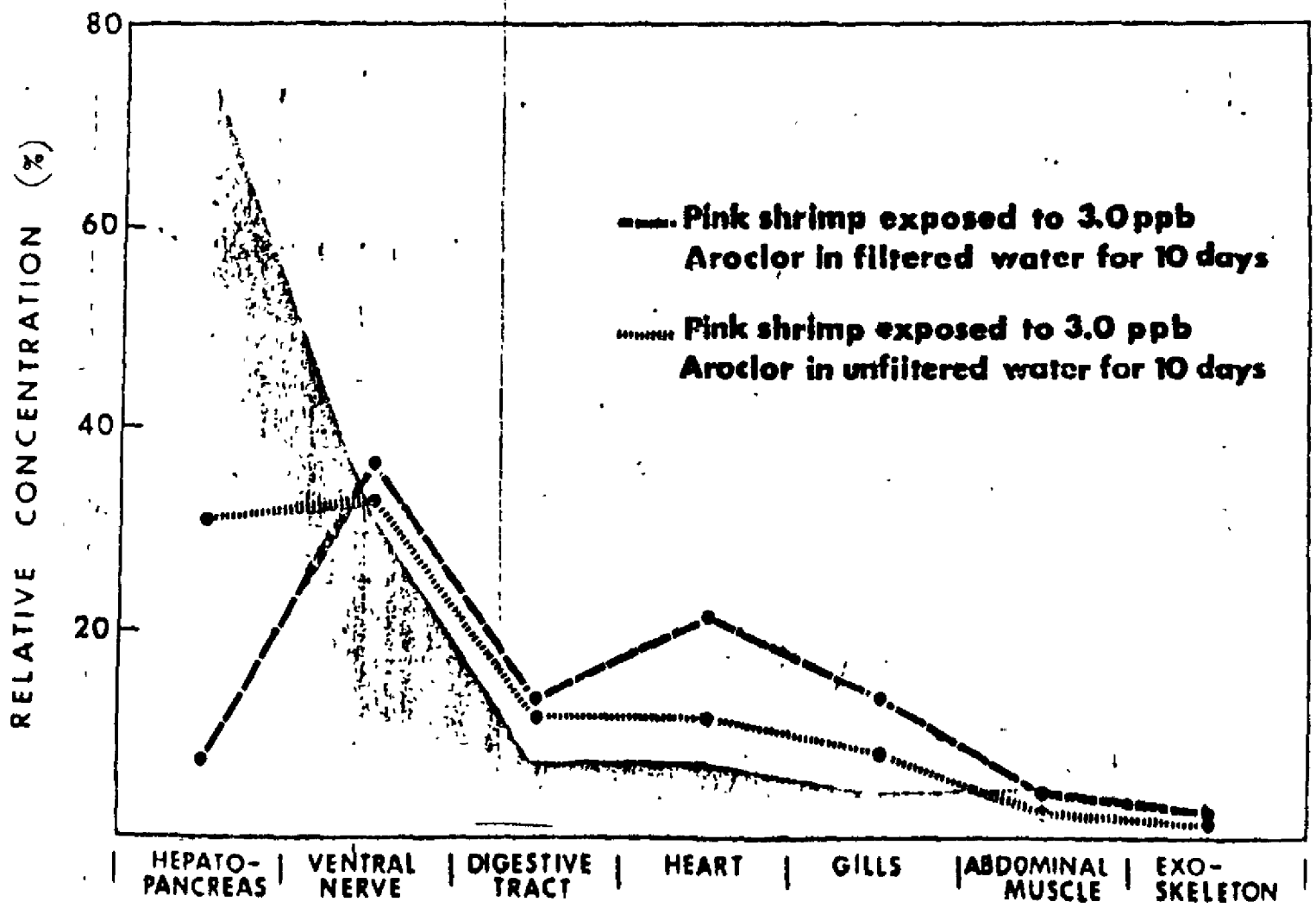
DISTRIBUTION OF AROCLOR 1254 IN TISSUES OF SHRIMP



MONS 203468

Figure 8. Distribution of Aroclor 1254 in tissues of shrimp exposed to Aroclor 1254 in unfiltered and unfiltered seawater.

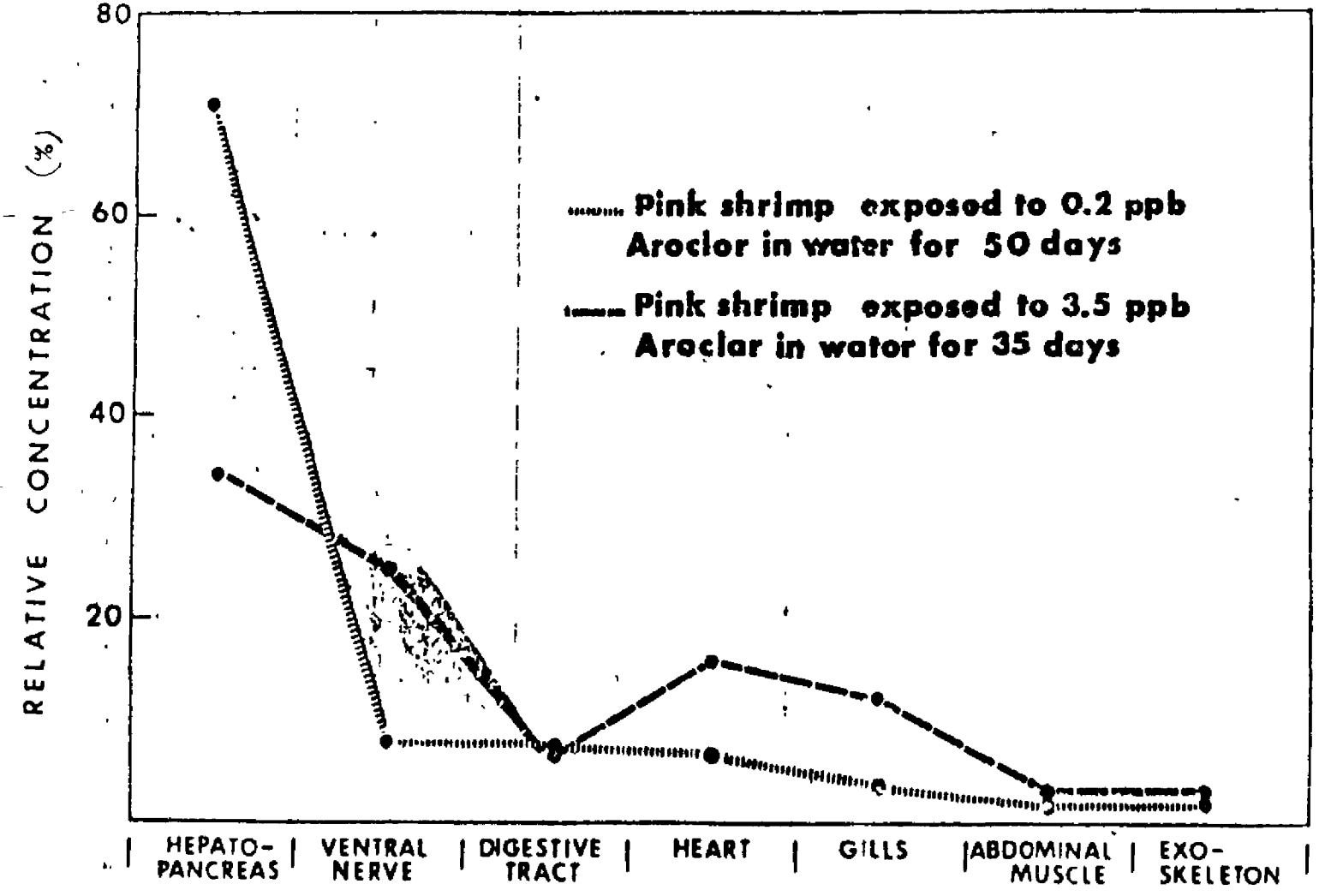
DISTRIBUTION OF AROCLOR 1254 IN TISSUES OF SHRIMP



MONS 203470

Figure 9. Distribution of Aroclor 1254 in tissues of shrimp exposed to the chemical in flowing-water aquaria.

DISTRIBUTION OF AROCLOR 1254 IN TISSUES OF SHRIMP



MONS 203472

Figure 10. Aroclor 1254: Uptake and depuration in grass shrimp exposed to 0.04, 0.09 and 0.62 ppb in water.

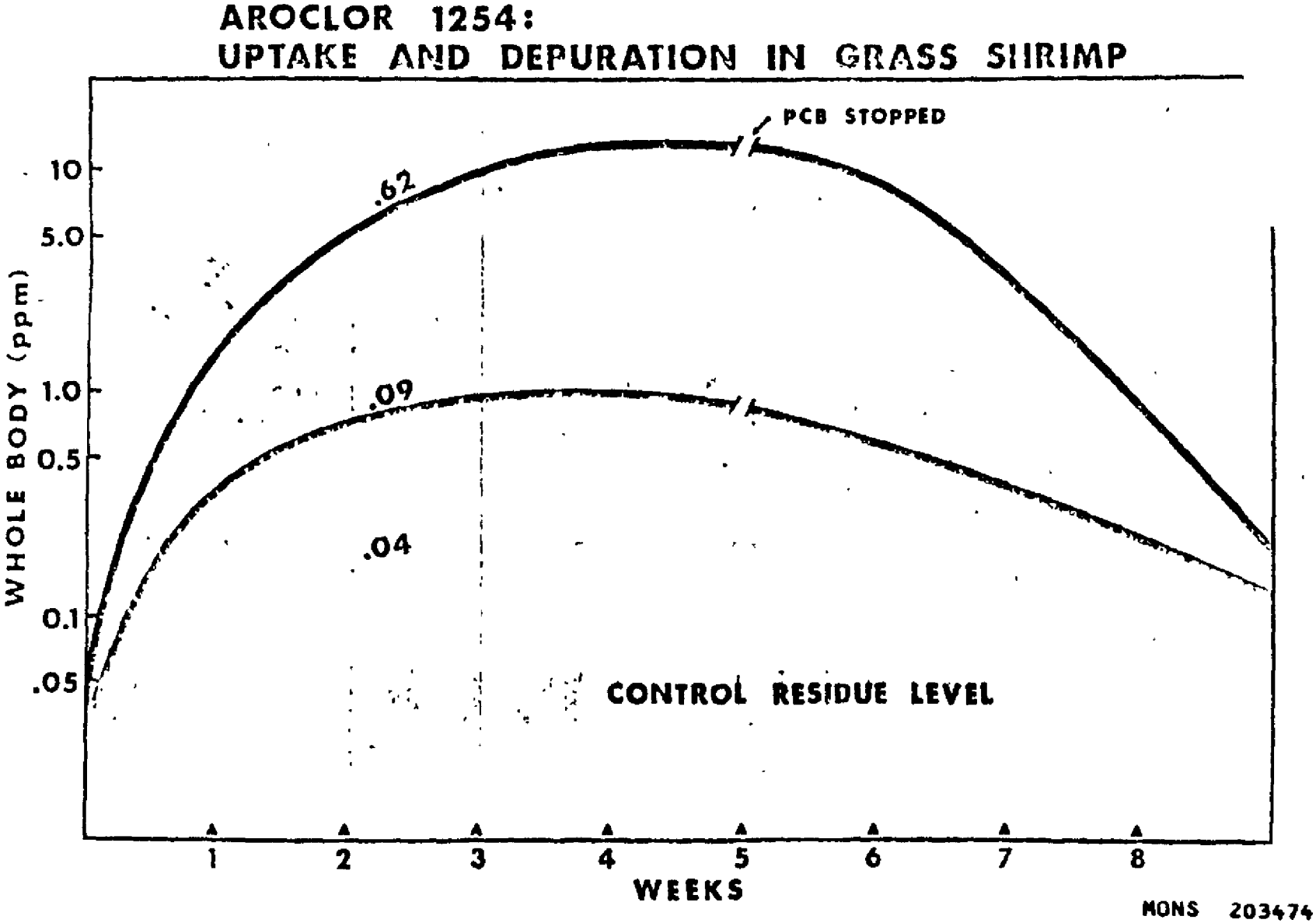

AROCLOR 1254:
UPTAKE AND DEPURATION IN GRASS SHRIMP
PCB STOPPED
.62
.09
.04
CONTROL RESIDUE LEVEL
WHOLE BODY (ppm)
10
5.0
1.0
0.5
0.1
.05
1
2
3
4
5
6
7
8
WEEKS
MONS 203474

Figure 11. Normal vesicular connective tissue (parenchyma) from control oyster. Note uniform cell distribution and paucity of leukocytes. X100.

Figure 12. Vesicular connective tissue from oyster exposed to PCB for six months. Note loss of uniform cell distribution and infiltration by leukocytes. X100.

Figure 13. Normal vesicular connective tissue from control oyster. X450.

Figure 14. Tissue from exposed oyster. Note many leukocytes and degeneration of vesicular connective tissue adjacent to gut epithelium. X450.

Figure 15. Normal digestive gland tubules from control oyster. Note the thick epithelia which form normal triradiate lumina. X450. (from Lowe et al., 5).

Figure 16 Digestive gland tubules of oyster exposed to PCB. Note atrophy of tubule epithelium and enlarged, abnormal lumen of tubule. X450.

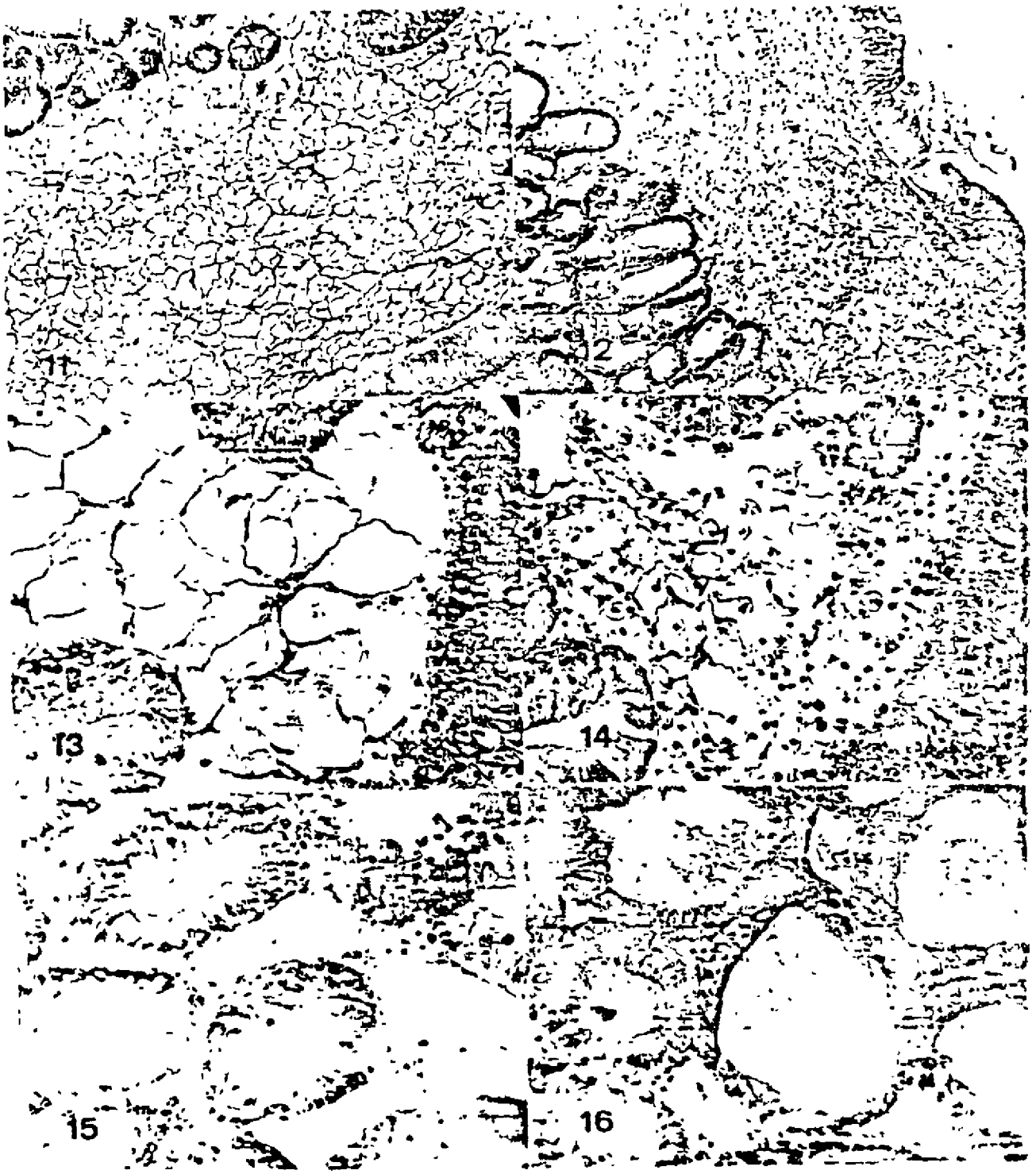


Figure 17. Liver parenchyma of normal spot (Lagodon rhomboides) from control tank. Note uniform orientation of hepatocytes. X1000.

Figure 18. Liver parenchyma of spot exposed to PCB for several weeks, intermediate pathogenesis. Note large, smooth-edged vacuoles indicative of abnormal fatty-change in hepatocytes. X1000.

Figure 19. Liver parenchyma of spot exposed to PCB until moribund, advanced pathogenesis. Note large vacuoles, amorphous inclusions and sinusoidal congestion. X1000.

Figure 20. Hepatopancreas (digestive gland) tubule from pink shrimp exposed to PCB. Note triangular crystalloids in some hypertrophied nuclei (H); also, normal nuclei (N) with large, prominent endosomes. (Feulgen reaction; DNA appears black in photomicrograph). X1000.

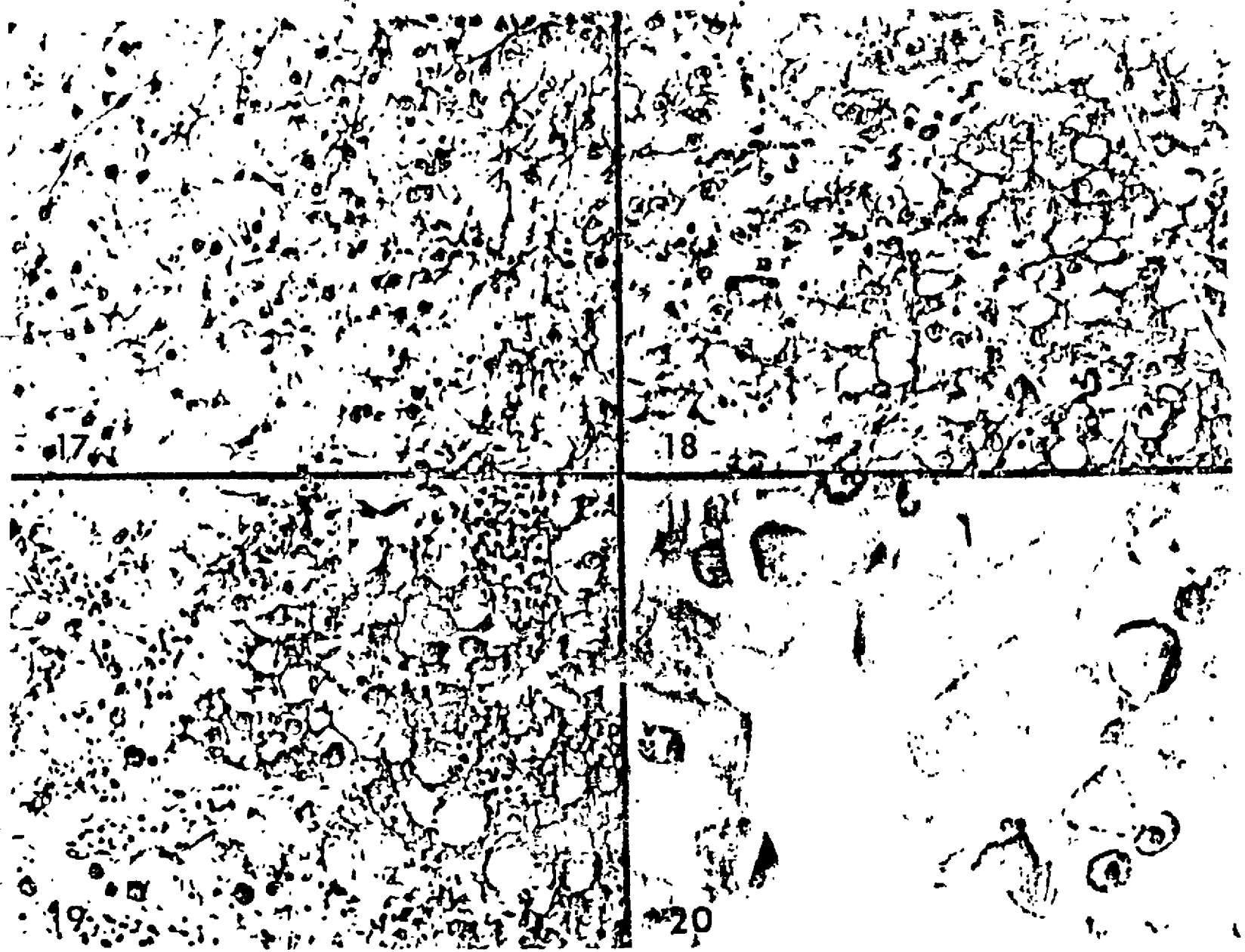

17
18
19
20
MONS 203478

Figure 21. Fresh squash of hepatopancreas from exposed shrimp. Note two crystalloids in center. X1000.

Figure 22. Single, large crystalloid from fresh squash of hepatopancreas of exposed shrimp. X1000.

Figure 23. Longitudinal section of hepatopancreatic duct with branching tubules. In exposed shrimp, the crystalloids appear in the tubule epithelia nearer the main hepatopancreatic ducts. X100.

Figure 24. Cross-section of hepatopancreas tubule from exposed shrimp. Note crystalloids in several epithelial nuclei; also, normal nuclei with large endosomes. X450.

Figure 25. Intermediate size crystalloid within hypertrophied epithelial nucleus. X1000.

Figure 26. Pathologic effect of crystalloid in PCB-exposed shrimp. Note rupture of cells and nuclei releasing the crystalloids. X1000.

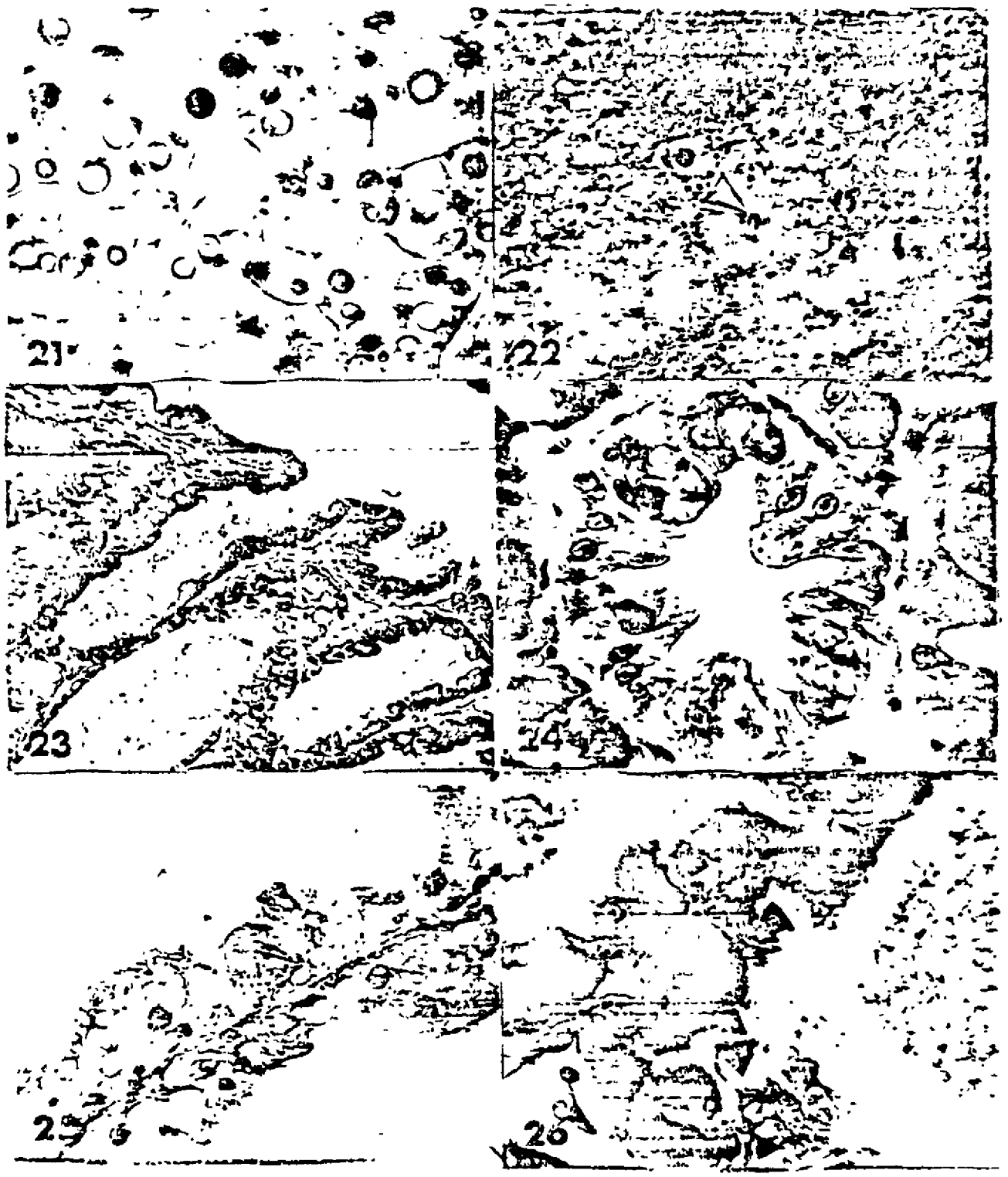


Exhibit 6

ACCUMULATION OF AROCLOR® 1254 IN GRASS SHRIMP (Palaemonetes pugio) IN LABORATORY AND FIELD EXPOSURES

by

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U. S. ENVIRONMENTAL PROTECTION AGENCY

GULF BREEZE ENVIRONMENTAL RESEARCH LABORATORY

SABINE ISLAND, GULF BREEZE, FLORIDA 32561

Results of several experiments indicate that aquatic invertebrates accumulate total body concentrations of polychlorinated biphenyls (PCB) thousands of times greater than that of the surrounding water. For example, Sanders and Chandler (1) showed that fresh water insects and crustaceans rapidly (1 day) accumulated PCB (Aroclor 1254) up to 24,000 times greater than the concentration in the water. Results of similar exposures conducted with estuarine animals showed oysters concentrating 85,000 (2), shrimp 10,000 (3) and fish 30,000 (4) times the amount of PCB in the water.

Although Sanders and Chandler stated that PCBs entering the aquatic environment are below concentrations acutely toxic to invertebrates (1), we have noted that most of the accumulation studies conducted thus far by the investigators cited in the paragraph above have been at concentrations of 1.0 µg/l and above, i.e., concentrations demonstratively toxic to test animals. Little is known about accumulation in marine invertebrates at extremely low concentrations, and with one exception (3), no one to our knowledge has placed PCB-free animals in a natural environment known to have PCBs and followed accumulation with time.

We report here the results of several experiments on chronic toxicity of Aroclor 1254 to Palaemonetes pugio, an estuarine grass shrimp, as well as concentration and loss of the compound from the animals with time. We also exposed grass shrimp for up to 3 months to Aroclor 1254-contaminated sediments in Escambia Bay, near Pensacola, Florida.

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[1] Gulf Breeze Environmental Research Laboratory Contribution No. 170

[2] Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon

...® 1254 IN GRASS SHRIMP (Palaemon...

METHODS AND MATERIALS

With one exception, all laboratory experiments were conducted in 30-ml chambers supplied with flowing water from Santa Rosa Sound. Three sets of 5 chambers each received test concentrations; the fourth set was a control. Each chamber contained 4-10 shrimp, the number depending on the size of the animals. Water flowed continuously through each chamber at a rate of 1.0 l/hr. Aroclor 1254, dissolved in polyethylene glycol (mol. wt. 200), was metered into each mixing tank with a syringe pump before the water entered the test chamber. An equal amount of solvent was added to the water flowing to controls. David J. Hansen of this laboratory, found the sensitivity of a marine fish to Aroclor 1254 [illegible] unchanged when he varied concentrations of polyethylene glycol used to deliver the toxicant (personal communication). The shrimp were fed daily a commercial molly-flake diet (<0.02 mg/kg organochlorine compounds).

The experiment to determine the concentration and [illegible] the tissues of P. pugio was also conducted in a flowing-[illegible] system. We constructed 18-liter aquaria with false [illegible] nylon screen (1/4-inch mesh) to hold shrimp above the [illegible] brought in with the water or produced by the animals. [illegible] fication was intended to prevent the animals from eating [illegible] particles with adsorbed Aroclor 1254. Consequently, we [illegible] that shrimp obtained more of the chemical from the water [illegible] sorption through the gills rather than from ingestion of [illegible] contaminated detritus. The shrimp were not fed during this [illegible] ment.

Concentrations of Aroclor 1254 in tissues by gas [illegible] tography were determined using pooled samples of at least [illegible] shrimp each (5).

P. pugio were exposed to Aroclor 1254-contaminated [illegible] in upper Escambia Bay from November 1971 to February [illegible] shrimp in specially-constructed cages (5) were exposed [illegible] to the sediments. Average concentration of Aroclor 1254 in [illegible] uppermost two inches of sediment in November 1971 was [illegible] (dry weight).

RESULTS OF LABORATORY EXPOSURES

Tests conducted in flowing water showed P. pugio [illegible] were capable to Aroclor 1254 (Table 1). In a 7-day exposure, [illegible] died at 9.1 µg/l, but significant mortality did not occur at 0 and 0.62 µg/l. In the second series of tests lasting [illegible] days, 4.8 and 12.5 µg/l were toxic, but significant mortality did [illegible]

TABLE 1.

MORTALITY AND ACCUMULATION OF AROCLOR 1254 IN *Palaemonetes pugio**

Test Conc. (µg/ℓ)	Days Exposed	Average Mortality (%)**	Body Conc. (mg/kg)	Concentration Factor
CONTROL	7	4(0 - 20)	0.1	———
0.17	7	8(0 - 40)	1.3	7600
0.62	7	4(0 - 20)	5.4	8700
9.2	7	60(20 - 80)***	65.0	7100
CONTROL	16	25(0 - 50)	<0.1	———
1.3	16	40(0 - 100)	18.0	14000
4.0	16	45(25 - 50)***	27.0	6700
12.2	16	55(50 - 75)***	46.0	3700

*All exposures were conducted in flowing seawater: salinity and temperature ranges were 22 to 28‰ and 17 to 28° C.

**5 replicates per concentration: at least 4 shrimp per replication.

***Significant at P >0.05.

TABLE 2.

ACCUMULATION OF AROCLOR 1254 BY
*Palaemonetes pugio**

Test Conc. (µg/ℓ)	Body Conc. (mg/kg)	Concentration Factor
0.17	1.3	7600
0.62	5.4	8700
1.0	3.2	3200
2.3	25.0	11000
2.7	19.0	7000
3.2	15.0	4800
3.2	26.0	8100
5.2	29.0	5600
5.3	16.0	3000
5.3	30.0	5700
9.1	65.0	7100

*7-day exposures conducted in flowing seawater at salinity and temperature ranges of 22 to 28 and 17 to 28° C.

occur in 1.3 µg/ℓ.

At the conclusion of several one-week exposures to a range of concentrations (0.17 to 9.1 µg/ℓ), surviving shrimp from each exposure were analyzed for whole-body residues. Ambient concentration of toxicant in the water and resultant residues in the shrimp were correlated (r=0.91, Table 2). In some cases, duplicate test concentrations produced biological accumulations that differed by a factor of 2. Concentration factors ranged from 3,000 to 11,000. These ranges were similar to those found in tests using penaeid shrimp (3) but were somewhat lower than those found by Sanders and Chandler (1), in tests using several invertebrate species in fresh water.

There appeared to be no threshold below which levels of the chemical added to water failed to produce residues in the tissues (Table 3) in our tests. Whole-body concentrations produced after 5 weeks exposure to 0.04, 0.09 and 0.62 µg/ℓ ranged from 200 to 26,000 times the concentrations in the test water. Concentrations did not reach equilibrium and from 60 to 90 percent of the Aroclor 1254 was lost from the shrimp within 4 weeks after exposure to the chemical was stopped. Test concentrations of the chemical were not significantly toxic to shrimp. Although accumulation increased with increasing concentration of toxicant in this test, this was not observed in earlier studies (see Tables 1 and 2). Implications are to be discussed elsewhere.

RESULTS OF FIELD EXPOSURES

Average whole-body residue of Aroclor 1254 in P. pugio after 1 month was 0.41 mg/kg (0.34 to 0.57); after 3 months, 0.42 mg/kg (0.37 to 0.50). There was no evidence that significant mortality occurred during the exposures of grass shrimp to contaminated sediments.

DISCUSSION

Concentrations of Aroclor 1254 in P. pugio, after exposure to contaminated sediments for 3 months was equivalent to a laboratory-exposure of 0.09 µg/ℓ in water for 2 weeks (Table 3). We expected residues to be higher in caged shrimp since we had found that fiddler crabs exposed in the laboratory accumulated residues equal to or greater than (wet-weight basis) that of the contaminated substratum (dry-weight basis) after 30 days (6). Concentrations of Aroclor 1254 in caged shrimp exposed to contaminated sediments appeared to reach a plateau, but this was not the case in laboratory exposures (Table 3) where an equilibrium

TABLE 3.

ACCUMULATION OF AROCLOR 1254 IN Palaemonetes pugio WITH TIME
AFTER EXPOSURES TO THE CHEMICAL IN WATER AT THREE CONCENTRATIONS (ug/L)
(Each value represents a composite sample of 10 animals)

Length of Exposure (hr/ days)	Control		0.04		0.09		0.62	
	Body Conc. (mg/kg)	Conc. Factor	Body Conc. (mg/kg)	Conc. Factor	Body Conc. (mg/kg)	Conc. Factor	Body Conc. (mg/kg)	Conc. Factor
0	0.1	*	0.1	*	0.1	*	0.1	*
1	---	*	0.1	*	0.1	*	0.1	*
2	---	*	0.1	*	0.1	*	0.1	*
3	---	*	0.1	*	0.1	*	0.1	*
4	---	*	0.1	*	0.1	*	0.1	*
8	---	*	0.1	*	0.1	*	0.12	190
12	---	*	0.1	*	0.1	*	0.14	230
16	---	*	0.1	*	0.1	*	0.26	420
24 / 1	---	*	0.1	*	0.1	*	0.20	320
36 / 1.5	---	*	0.1	*	0.1	*	0.20	470
48 / 2	---	*	0.1	*	0.10	1100	0.37	600
72 / 3	---	*	0.1	*	0.14	1560	0.58	930
96 / 4	---	*	0.1	*	0.15	1670	0.40	650
154 / 6.5	0.1	*	0.1	1590	0.33	3670	1.28	2060
336 / 14	---	*	0.13	3250	0.43	4780	7.40	11930
504 / 21	0.1	*	0.15	3750	0.45	5000	6.67	10900
672 / 28	0.14	*	0.17	4250	1.57	17400	10.82	1745[illegible]
840 / 35	0.10	*	0.21	5250	0.75	8330	16.48	26580
			PCB - STOPPED					

MONS 203487

TABLE 3 (Continued)

1176 / 49	0.1	*	0.1	*	0.12	*	3.24	*
1512 / 63	0.15	*	0.1	*	0.13	*	1.64	*

* Magnification factor not calculated.

was not reached. Therefore, we believe that shrimp exposed to the sediments might have obtained PCB from the water or food singly, but shrimp exposed to Aroclor in the laboratory obtained chemical from two sources, water and food. It might also be more available in the laboratory than the field due to the carrier. In earlier laboratory studies with penaeid shrimp, both water and food appeared to be sources (3).

No significant mortality was observed in caged shrimp and none would be predicted since residues produced in the field were similar to those found in shrimp after laboratory exposures which caused no death.

Penaeid shrimp spend only a fraction of their life cycle in an estuary, moving into oceanic waters after reaching maturity (7), but grass shrimp are endemic in estuaries. Therefore, in relation to time of exposure we would expect grass shrimp to accumulate a pollutant from a contaminated estuary to a greater degree than penaeid shrimps, nevertheless, this is not true. In August 1968, penaeid shrimps (Penaeus duorarum, P. setiferus, and P. aztecus) collected during a survey of Escambia Bay, Florida, had whole-body residues of Aroclor 1254 as high as 14.0 mg/kg (3). In that survey and in subsequent collections, P. pugio had a maximum residue of only 1.4 mg/kg.

Lower residues in P. pugio from Escambia Bay may be due to amounts of PCB in bay sediments and behaviorial patterns of the animals. We noted earlier (3) that residues in species of penaeid shrimp were related to higher concentrations of Aroclor 1254 in sediments that predominate in upper Escambia Bay. We found that penaeid shrimp, as adults, usually are captured in deeper waters and burrow into silty or sandy substrates. In contrast, grass shrimp usually do not burrow, rather are found along shallow sandy beaches and grass beds, where they obtain food that is relatively uncontaminated with PCB.

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Aroclor® 1016: Toxicity to and Uptake by Estuarine Animals[1/]

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U. S. Environmental Protection Agency

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Aroclor 1016: Toxicity to Estuarine Animals

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ABSTRACT

Bioassays were conducted to determine the acute toxicities of the polychlorinated biphenyl (PCB) Aroclor 1016 in flowing sea water to American oysters (Crassostrea virginica), brown shrimp (Penaeus aztecus), grass shrimp (Palaemonetes pugio) and pinfish (Lagodon rhomboides) and to determine its chronic toxicity to and uptake and retention by pinfish. Acute 96-hour EC50/s were: oysters, 10.2 µg/ℓ; brown shrimp, 10.5 µg/ℓ; grass shrimp, 12.5 µg/ℓ. The PCB was not toxic to pinfish at 100 µg/ℓ for 96 hours but significant mortality occurred when pinfish were exposed to 32 µg/ℓ of Aroclor 1016 for 42 days. Pinfish exposed to 1 µg/ℓ for 56 days accumulated the chemical with maximum concentrations attained in whole-fish by 21 to 28 days. Maximum whole-body residue (wet weight) was 17,000 x the nominal concentration in test water. Tissue alterations such as severe vacuolation in the pancreatic exocrine tissue surrounding the portal veins occurred in pinfish exposed to 32 µg/ℓ of Aroclor 1016 for 42 days.

Aroclor 1016: Toxicity to Estuarine Animals

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Polychlorinated biphenyls (PCB's) have been used industrially for over forty years (Broadhurst, 1972), and recently there has been concern about their environmental impact. Because of this concern, manufacture and sale of most PCB's was discontinued and sales restricted for uses that are not likely to produce environmental contamination. A new PCB, Aroclor 1016, is now manufactured in the United States for sale to capacitor manufacturers as a substitute for all other PCB's. This new PCB is similar to Aroclor 1242, except that amounts of isomers containing five or more chlorine atoms per biphenyl group have been considerably reduced. Domestic sales of Aroclor 1016 increased from about 3.3×10^6 pounds in 1971 to 20.9×10^6 pounds in 1972 (W.B. Papageorge, personal communication[2/]).

Our study was conducted to determine the acute toxicity of Aroclor 1016 to the American oyster (Crassostrea virginica), brown shrimp (Penaeus aztecus), grass shrimp (Palaemonetes pugio), and pinfish (Lagodon rhomboides) and to determine its chronic toxicity to and uptake and retention by pinfish.

2/ W. F. Papageorge, Monsanto Industrial Chemicals Company. 800 N. Lindbergh Boulevard, St. Louis, MO 63166, USA.

MATERIALS AND METHODS

Test animals

Test animals were collected near the Gulf Breeze Laboratory and acclimated to laboratory conditions for at least seven days before exposure. If mortality exceeded 1% in the 48 hours immediately preceding the test or if abnormal behavior was observed during acclimation, the animals were not used. Oysters tested were from 35 to 55 mm in height; brown shrimp, 11 to 26 mm rostrum-telson length; grass shrimp, 20 to 32 mm rostrum-telson length; and pinfish 27 to 84 mm standard length. Animals were not fed during acute toxicity tests but they could obtain plankton from the unfiltered sea water. In the chronic exposures and the uptake and retention test, pinfish were fed commercial fish food that contained no detectable PCB (<.01 ug/g).

Acute and chronic tests

Acute toxicity to Aroclor 1016 was determined by exposing ten individual animals to 1, 10 or 100 ug/l for 96 hours in each of two 20l aquaria. Each experiment was conducted twice. The PCB was dissolved in acetone or polyethylene glycol 200 and metered at 30 or 0.1 ml/hr, respectively, into unfiltered sea water that entered each aquarium at 75 l/hr. Two control aquaria received the same quantities of water and solvent. Temperature and salinity of the water flowing into aquaria in replicated tests were similar (± 10%).

Chronic toxicity of Aroclor 1016 to pinfish was determined in three experiments, each lasting 42 days. In each experiment, 50 fish were

placed in each 90ℓ aquarium that received 140 ℓ/hr of sea water. The PCB, dissolved in polyethylene glycol, was metered into the water at 0.083 ml/hr in the first two experiments and 1.04 ml/hr in the third experiment. Control aquaria received the same quantity of water and solvent.

The same exposure techniques that were used in the first two chronic toxicity experiments were used to determine: (1) the rate of uptake and retention of Aroclor 1016 in pinfish exposed to 1 µg/ℓ for 56 days and (2) the rate of depuration of Aroclor 1016 by pinfish in PCB-free water for 56 days.

Effect of Aroclor 1016 was assessed by measuring percentage reduction in shell growth of exposed oysters as compared to control oysters (Butler, 1962), by determining mortality in shrimps and fish, and by pathological examination of chronically exposed fish.

Histopathological examination

Dr. J. A. Couch, pathobiologist at this laboratory, examined viscera from live pinfish from the third 42-day exposure. Viscera were fixed either in 10% neutral buffered formalin or in Davidson's fixative. Those fixed in Davidson's were stored in 70% ethyl alcohol until processed for paraffin sections (7µ) and stained with Harris hematoxylin and eosin (H&E) or Periodic Acid Schiff's (PAS). Viscera fixed in 10% neutral buffered formalin were processed for frozen sections (12µ) and stained with Oil Red O and hematoxylin.

Chemical analyses

Concentrations of Aroclor 1016 in water and animals were determined by electron capture gas chromatography. Unfiltered water samples from each concentration were analyzed once during the 96-hour exposures and weekly during longer exposures. Concentrations in animals that survived the 96-hour exposures were determined as whole-body residues. At the conclusion of each chronic exposure, surviving pinfish were dissected and PCB residues in flesh, flesh and scaleless skin, and remaining tissue determined. Residues in all tissues were summed to compute concentrations of Aroclor 1016 in whole fish. The same procedure was followed in the uptake and retention study, except fish were removed for analysis at selected intervals during exposure and depuration. Also, at the end of the 56-day exposure, brain, gills, heart and liver were removed from exposed fish for residue analysis. All fish samples were composites of 10 individuals.

Tissue samples that weighed more than 5 g were prepared for analysis by mixing them with anhydrous sodium sulfate in a blender. The mixture was extracted for 4 hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated to approximately 10 ml and transferred in 3- to 4-ml portions to a 400 x 20 mm chromatographic column that contained 76 ml of unactiviated Florisil. After each portion settled in the column, vacuum was applied until all solvent was evaporated. This was repeated with three 5 ml

rinses. The residue was eluted from the column with 70 ml of a 9:1 mixture (v/v) of acetonitrile and distilled water. The eluate was evaporated to dryness and the residue transferred to a Florisil column (Mills, et al., 1963) with petroleum ether. Aroclor 1016 was eluted with 6 percent ethyl ether in petroleum ether.

Tissue samples that weighed less than 5 g were analyzed by a modification of the micro method described in the Pesticide Analytical Manual, Volume III (U.S. Food and Drug Administration, 1970). The samples were weighed into a size 23 Duall tissue grinder and extracted three times with 5 ml portions of acetonitrile. The acetonitrile was flooded with 15 ml of 2% (w/v) sodium sulfate in distilled water and extracted with three 5 ml portions of hexane. The hexane was evaporated to approximately one ml and transferred to a 9 x 200 mm Chromaflex column with a 50 ml reservoir that contained 3.3 g of Florisil topped with 3.3 g of anhydrous sodium sulfate. Aroclor 1016 was eluted with 20 ml of 5% ethyl ether in hexane and adjusted to an appropriate volume for analysis.

Water samples were extracted with petroleum ether, dried with anhydrous sodium sulfate and adjusted to an appropriate volume for analysis.

All samples were analyzed by electron capture gas chromatography using a 15 x 3.2 mm glass column packed with 2% OV-101 on 100-120 Gas Chrom Q. Nitrogen flow rate was 25 ml/min, the oven temperature

was 190 C, and the injector and detector temperature was 210 C.

Aroclor 1016 was quantitated by comparing the total height of all peaks in the sample with the total height of all peaks in a standard of known concentration. Recoveries were greater than 80%; data were not adjusted for recovery. All tissue residues were determined on a wet weight basis.

RESULTS AND DISCUSSION

Acute (96-hr) exposure

Aroclor 1016 was acutely toxic to the estuarine organisms tested (Tables 1 and 2). Shell growth in oysters was inhibited greatly by exposure to 100 µg/ℓ for 96 hours. Sensitivities of brown shrimp and grass shrimp were similar and pinfish was the least sensitive species. Acute toxicities of Aroclor 1016 to oysters, brown shrimp and pinfish were similar to that of Aroclor 1242 to these species[3/] and Aroclor 1254 to oysters, pink shrimp (Penaeus duorarum) and pinfish (Duke et al., 1970).

All animals accumulated Aroclor 1016 (Table 1). The quantities accumulated depended on the exposure concentrations and not on species. Whole-body concentrations in live animals ranged from 440 to 4,200 x the nominal concentration in test water and 1,200 to 6,700 x the measured concentration in test water.

3/ P. R. Parrish, unpublished data.

Chronic (42-day) exposure

Toxicity of Aroclor 1016 to juvenile pinfish was greater in tests lasting six weeks than in 96-hour exposures. Pinfish seemed unaffected by 10 µg/ℓ or less, but died in concentrations of 32 and 100 µg/ℓ (Table 3). Mortality began in the second week of exposure. Fifty-percent mortality did not occur in the third exposure, for example, until the 33rd day at 32 µg/ℓ and the 18th day at 100 µg/ℓ. Delayed mortality of pinfish was also observed with Aroclor 1254 (Hansen et al., 1971).

Most of the fish that died in the 42-day exposure exhibited symptoms of poisoning, such as changed appearance and behavior. Initially, their color darkened, they stopped feeding, and they swam erratically with bodies inclined downward. Difficulty in swimming progressed until the fish swam with their tails and dorsal fins breaking the water surface. Finally, the fish lost equilibrium, swam upside down, and died. Affected fish became vulnerable to attack by other pinfish in the tank. In the third experiment, the majority of dying fish exposed to 32 and 100 µg/ℓ lost scales, skin and, finally, flesh in front of the dorsal fin forming lesions sometimes as deep as the neural spine. This condition did not occur in fish exposed to 32 µg/ℓ in the second experiment.

Hepatocytes in liver sections of thirteen control fish showed no unusual characteristics when stained with HHE and PAS or oil Red O. The hepatocytes from six fish demonstrated only a moderate PAS-positive

reaction, indicating moderate-to-light glycogen reserves. In seven other control fish, liver sections stained with oil Red O also showed a broad range of lipid patterns. Structure of livers of control fish was normal, being tubulosinusoidal in nature, with disseminated pancreas prominent along the course of the portal vein (Fig. 1).

Eight fish exposed to 10 µg/ℓ of Aroclor 1016 for 42 days showed no pathologic microscopic visceral characteristics distinguishable from control fish. Half of the fish samples were paraffin-processed; half were prepared for frozen sections.

Pinfish exposed to 32 µg/ℓ of Aroclor 1016 had several liver and pancreatic alterations that distinguished them from control fish and fish exposed to 10 µg/ℓ. Tissues from eight fish were examined; half were paraffin-processed and half were studied as frozen sections. Hepatocytes appeared slightly enlarged and more basophilic than in the control fish (Fig. 2). Normal liver cord orientation was somewhat altered and PAS-positive granules accumulated at the edge of the pancreatic acinar tissue in the liver (Fig. 3). Less lipid material existed in the livers of fish exposed to 32 µg/ℓ than in control fish. This contrasts with the heavy, abnormal accumulation of lipid in the livers of spot exposed to 6 µg/ℓ of Aroclor 1254 for 30 days (Couch, 1973). The most remarkable alteration in the pinfish was the occurrence of severe vacuolation in the pancreatic exocrine tissue surrounding the portal veins (Figs. 2,3). This

vacuolation was distinguishable from normal secretory vacuoles and deposits in pancreatic tissue from control fish because those in exposed fish were small, abundant, and contained no secretory granules (Fig. 1).

After the first exposure, 16 pinfish from each control or Aroclor 1016-contaminated aquarium were held in PCB-free water and the salinity reduced to determine if ability of pinfish to survive osmotic stress had been impaired by exposure to sublethal concentrations of Aroclor 1016. (Aroclor 1254 reduced the ability of pink shrimp, Penaeus duorarum, to withstand stress of decreased salinity—D. R. Nimmo, personal communication.[4/]) Salinity was lowered from an initial salinity of 28 o/oo by 50% on each of four consecutive days (14, 7, 3.5 and 1.8 o/oo). None of the fish died until the salinity fell below 2 o/oo. At that salinity, mortalities of control and PCB-exposed fish were similar.

Pinfish exposed for 42 days to concentrations of Aroclor 1016 between 0.1 and 32 µg/l stored the chemical in proportion to the concentration in test water (Table 3). Concentrations in whole fish renged from 11,000 to 24,000 x the nominal concentration in the test water and 14,000 to 55,000 x the measured concentration in test water, whereas

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[4/] D. R. Nimmo, Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida 32561.

the concentration factor in spot (Leiostomus xanthurus) exposed for 42 days to 1 µg/ℓ of Aroclor 1254 was 30,000 x the nominal concentration (Hansen et al., 1971).

The concentration of Aroclor 1016 in edible tissue was less than that in whole fish. Concentrations in whole fish averaged 2.1 times those in flesh and scaleless skin and 2.8 times those in flesh. Pinfish exposed to 1 µg/ℓ of Aroclor 1016 stored quantities that exceeded the Food and Drug Administration's provisional action-level for all PCB's (5 µg/g) in edible tissues (Table 3).

Chronic (56-day) exposure

Pinfish exposed to 1 µg/ℓ of Aroclor 1016 for 56 days accumulated the chemical, maximum concentrations in whole fish being attained in 21 to 28 days (Table 4). In a similar study with Aroclor 1254, whole-body concentrations of spot stabilize at about the same time, 14 to 28 days (Hansen et al., 1971). Maximum whole-body residue in pinfish was 17,000 x the nominal concentration in test water. Increases in concentrations in edible tissues were also rapid (Table 4), but may not have reached a maximum even after 56 days of exposure.

The quantity of Aroclor 1016 accumulated by pinfish differed in the various tissues and organs. Fish exposed to 1 µg/ℓ for eight weeks accumulated 17 µg/g whole-body residue. Concentrations (µg/g) in other tissues or organs were: gills, 23; skin, 19; liver, 16; brain, 8.7; muscle, 5.9; and remaining tissues, 22.

Aroclor 1016 was lost from the tissues after pinfish were placed

in PCB-free water (Table 4). After 56 days of depuration, concentrations in whole fish decreased by 51%. In an earlier study, spot which had accumulated Aroclor 1254 lost 66% after 56 days in PCB-free water (Hansen et al., 1971).

We examined chromatograms to compare the proportions of nine peaks of Aroclor 1016 in reference standards (Fig. 4) with those peaks from water and pinfish tissue samples (Table 5). Chromatograms of standards, tissue spikes and water samples were similar, while chromatograms of standards and tissue samples were dissimilar. Chromatograms of Aroclor 1016 from different pinfish tissues (flesh, flesh and skin, and rest) were similar. Chromatograms from all tissues had smaller early eluting peaks, numbers 1, 2 and 3. Reduction in early eluting peaks of Aroclor 1254 from shrimp and fish tissue was noted by Nimmo et al. (1971). The relative proportions of the nine peaks found in chromatograms from pinfish tissues early in the 56-day exposure, late in the exposure and throughout the 56-day depuration period were similar. We do not know whether differences between chromatograms from reference standards and chromatograms from tissue samples reflect alterations in Aroclor 1016 molecules, differential solubility, or other factors. It is unlikely, however, that the PCB molecules were altered because no change in relative proportions of peaks was noted throughout the 112-day experiment.

The potential environmental hazard of a chemical is dependent upon its likelihood of entering the environment and its potential

hazard to organisms. Aroclor 1016 is similar to other PCB's in its toxicity to and uptake and retention by estuarine animals. Therefore, its substitution for other PCB's reduces environmental hazard only if the policy of restricting sales for uses not likely to produce environmental contamination is continued.

ACKNOWLEDGEMENTS

We thank Dr. J. A. Couch for histopathological examination of the pinfish, S. S. Foss for preparing illustrations, and the Monsanto Company for providing Aroclor 1016.

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Aroclor 1016: Toxicity to Estuarine Animals

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TABLE 1

Acute toxicity to and uptake of Aroclor 1016 by American oysters (Crassostrea virginica), brown shrimp (Penaeus aztecus), grass shrimp (Palaemonetes pugio), and pinfish (Lagodon rhomboides) in 96-hour exposures. Effect is expressed as percent reduction in shell growth in oysters and death in shrimps and fish. Whole body residues are from animals alive at end of exposure period.

SPECIES	TEST CONCENTRATION (µg/ℓ) Nominal	Measured	EFFECT (%)	WHOLE-BODY RESIDUE (wet weight, µg/g)
C. virginica	Control	ND*	0	ND*
	1	0.6	10	4.0
	10	7.2	38	32.
	100	58.	93	95
P. aztecus	Control	ND	0	ND
	1	0.9	8	3.8
	10	8.9	43	42.
	100	33.	100	-
P. pugio	Control	ND	8	ND
	1	0.4	33	1.1
	10	9.4	38	22.
	100	38.	93	44.
L. rhomboides	Control	ND	2	ND
	1	0.8	5	2.2
	10	6.9	0	21
	100	56.	18	65.

*ND - not detectable; <0.05 µg/ℓ in water; <0.1 µg/g in tissue.

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TABLE 2

Acute toxicity of Aroclor 1016 to American oysters (Crassostrea virginica), brown shrimp (Penaeus aztecus) and grass shrimp (Palaemonetes pugio). Effect is expressed as percent reduction in shell growth in oysters and death in shrimps.

	96-HOUR EC50 (µg/l)	TEMPERATURE (C)		SALINITY °/oo	
	Nominal	Mean	Range	Mean	Range
C. virginica	10.2	30	25-32	29	26-31
P. aztecus	10.5	31	29-32	29	28-30
P. pugio	12.5	30	29-32	29	25-30

TABLE 3

Toxicity and uptake of Aroclor 1016 by pinfish (Lagodon rhomboidas) exposed for 42 days in three separate experiments.

TEST CONCENTRATION (µg/ℓ)		MORTALITY %	CONCENTRATION IN FISH (µg/g, wet weight)		
Nominal	Measured		Flesh	Flesh & skin	Whole fish
Control	ND[1]	36	ND	ND	ND
0.1	0.1	16	0.7	0.8	2.4
1.0	0.8	48	5.1	6.3	11.
10.0	3.0	38	60.	90.	166.
Control	ND	12	0.5	0.6	0.5
1.0	0.9	16	4.0	6.0	17.
3.2	2.5	16	34.	39.	65.
10.0	7.0	28	63.	76.	170.
32.0	13.	44[2]	140.	180.	620.
Control	ND	6	ND	ND	ND
10.0	6.8	6	23.	49.	111.
32.0[3]	21.	50[2]	30.	48.	106.
100.0[3]	59.	50[2]	38.	72.	205.

[1] ND = not detectable: <0.05 µg/ℓ in water; <0.1 µg/g in tissue.

[2] Mortality significantly greater than in control fish, $\alpha = 0.01$.

[3] Exposure terminated and tissues analyzed when 50% of the fish died: 33 days at 32 µg/ℓ and 18 days at 100 µg/ℓ.

TABLE 4

Concentrations of Aroclor 1016 (µg/g wet weight) in pinfish (Lagodon rhomboides) exposed to .1 µg/ℓ of this PCB. Each sample consisted of tissues from ten fish.

DAYS OF EXPOSURE	CONCENTRATION		
	Flesh	Flesh and skin	Whole fish
0	ND[1/]	ND	ND
3	0.8	0.9	1.6
7	1.6	3.4	3.9
14	2.3	3.3	6.5
21	3.2	4.3	9.7
28	3.0	6.8	25.
42	4.0	6.0	17.
56	5.9	8.3	17.
Depuration			
14	4.1	7.8	13.5
28	3.5	6.6	9.3
56	2.2	3.9	6.6

1/ ND - not detectable; <0.1 µg/g.

TABLE 5

Percentages of the nine measured peaks from chromatograms of Aroclor 1016 reference standards and from tissues of pinfish exposed to 1 g/ of Aroclor 1016 for 56 days and then held in PCB-free water for 56 days.

	PERCENTAGE OF PEAK[1/]									CHROMATOGRAMS
	1	2	3	4	5	6	7	8	9	
Reference standard	12.4	11.3	10.0	23.5	13.3	6.4	8.1	8.8	6.2	8
All tissues through 14 days of exposure	2.4	5.3	1.8	39.6	7.7	10.3	12.1	9.4	11.4	7
All tissues, days 21-56 of exposure	2.5	5.0	2.1	33.2	7.8	11.9	11.3	10.3	15.9	12
All tissues during 56-day depuration	1.0	1.7	0.3	38.2	2.8	15.0	12.4	11.9	16.7	7

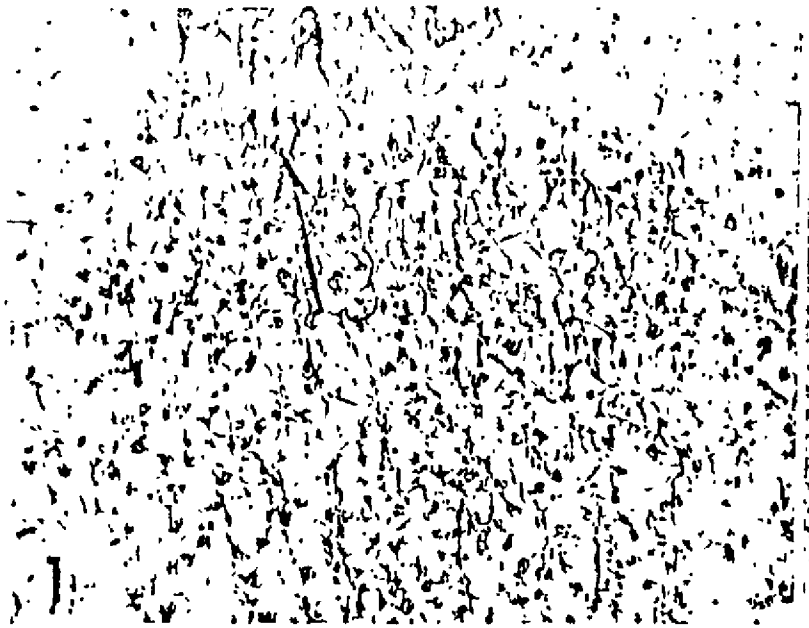
1/ Determined as $\frac{\text{peak height}}{\text{sum of nine peaks}} \times 100$.

LEGEND FOR FIGURES

FIG. 1. Section of normal liver from control pinfish. The vacolues in hepatocytes are results of extraction of lipid and/or glycogen during paraffin processing. Note the normal pancreatic exocrine tissue containing normal secretory deposite (arrows). (X450).

FIG. 2. Section of liver from pinfish exposed to 32 µg/ℓ Aroclor 1016 for 42 days. The hepatocytes appear relatively dense with more prominent nuclei than in Fig. 1. Note the lack of lipid or glycogen vacuoles in hepatocytes. Small, abnormal vacuoles in pancreatic exocrine tissue (arrows) distinguish fish exposed to 32 µg/ℓ from control fish. (X450).

FIG. 3. Pigment deposition (PAS-positive) between pancreatic exocrine tissue and live parenchyma (arrow). Note nature of small vacuoles in pancreatic tissue. This tissue is from fish exposed to 32 µg/ℓ Aroclor 1016. (X1000).



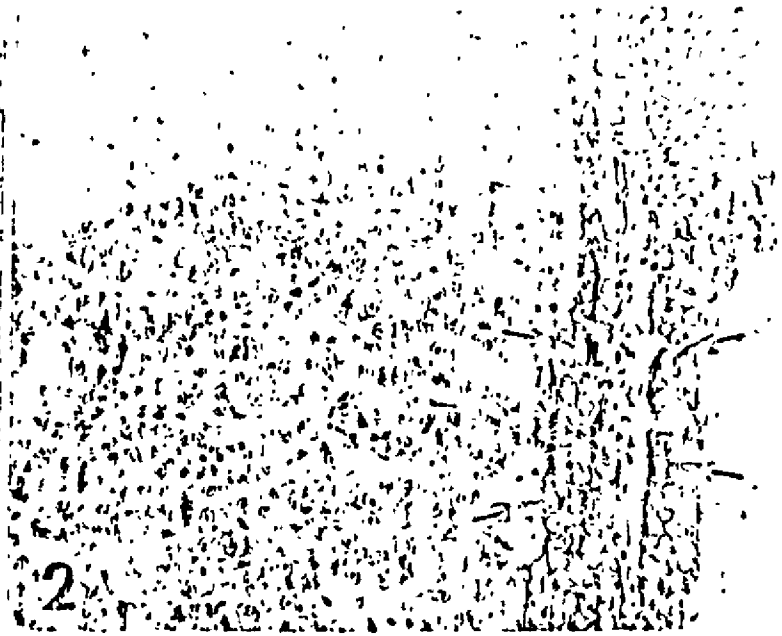


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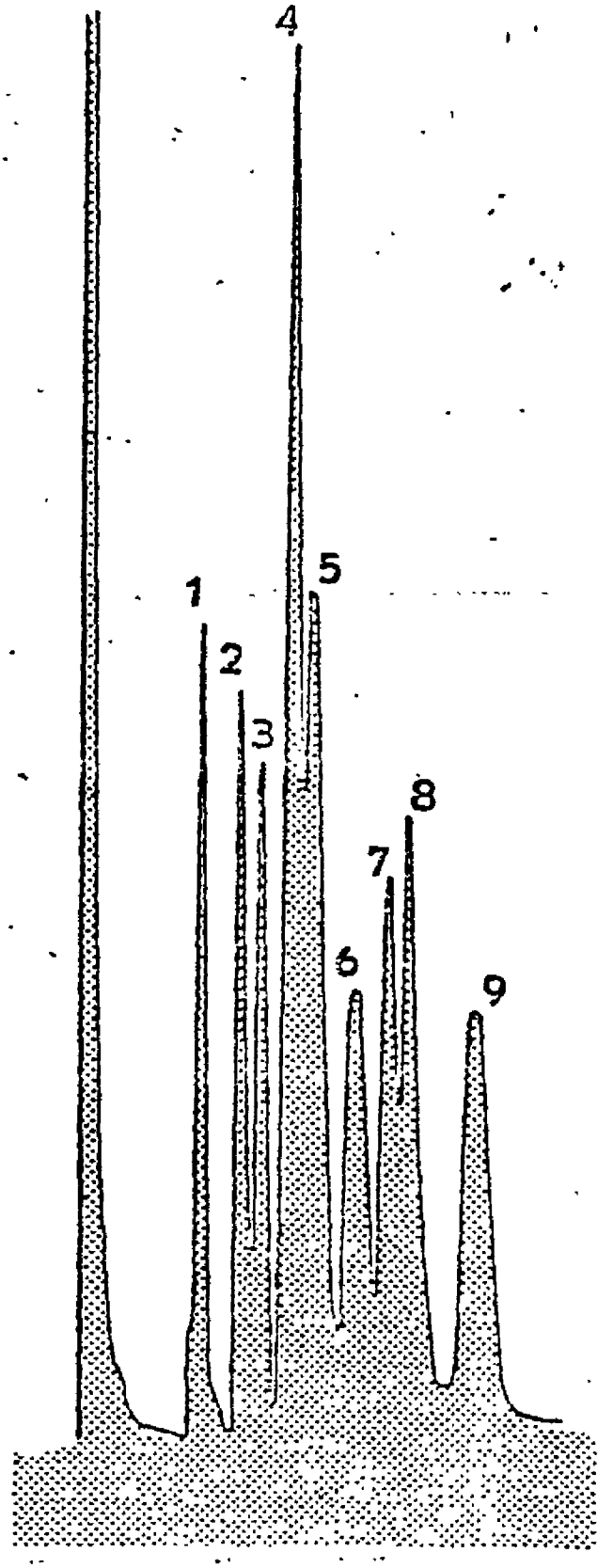
FIGURE 4

Chromatogram of nine peaks of Aroclor 1016 reference standard. Operating conditions: gas flow nitrogen 25 ml/min; injection and detector temperature 210 C; oven temperature 190 C; H^3 electron capture detector; 152.4 x 0.32 cm glass column packed with 2% OV-101 on 100-120 Gas Chrom Q.



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(22)

Aroclor® 1254, DDT and DDD, and Dieldrin: Accumulation and Loss by American Oysters (*Crassostrea virginica*) Exposed Continuously for 56 Weeks[1]

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Separate populations of oysters were exposed continuously for 56 weeks to 0.01 µg/ℓ of Aroclor® 1254, p,p'-DDT and DDD, or dieldrin and sampled at 8-week intervals for residues. Maximum concentrations based on body weight (µg/g) occurred after 8 weeks of exposure, but maximum concentrations based on absolute amount of toxicant accumulated (µg) occurred after 56 weeks of exposure. After 8 weeks, average whole-body residues (wet weight) from five oysters analyzed individually were: Aroclor 1254, 1.65 µg/g, 4.0 µg; DDT (and metabolites DDD and DDE), 0.46 µg/g, 1.0 µg; and dieldrin, 0.08 µg/g, 0.2 µg. After 56

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weeks, residues were: Aroclor 1254, 0.89 µg/g, 25.7 µg; DDT and metabolites, 0.37 µg/g, 7.0 µg; and dieldrin, 0.03 µg/g, 0.6 µg. Seasonal patterns of accumulation and loss of the three toxicants were similar. Residues based on body weight (µg/g) decreased 45% to 81% in early July and late October, apparently as the result of spawning, and increased following these periods. This shows that the life history of oysters must be considered when evaluating residue data from monitoring programs. Growth rate (height and in-water weight) of exposed oysters was not different from that of control oysters (Student's t-test; $\alpha = 0.01$). Mortality was not significant in any group.

PHYSIOLOGICAL CONSEQUENCES OF POLYCHLORINATED BIPHENYL- AND SALINITY-STRESS IN PENAEID SHRIMP

by

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INTRODUCTION

Estuaries are dynamic environments and animals that live in them must be able to cope with change. Some dynamic natural factors are temperature, salinity, currents, hydrostatic pressure, and oxygen or carbon dioxide concentrations. Unfortunately, domestic sewage (nutrients), oils, industrial chemicals, pesticides, metals, or altered temperatures are an influence in estuaries. Unfortunately, the combined effects of the natural and man-introduced factors on estuarine organisms are largely unknown. Exceptions are the estuaries which receive undue amounts of pollutants that for all practical purposes are biologically dead. In contrast, when interactions occur gradually and in combination, the effect could be the death of assemblages of organisms or an entire estuary without our being aware of the trend. Therefore, the problem facing us today is understanding and predicting the interactions of pollutants and natural stresses.

It is common knowledge that the commercial shrimps along the Gulf Coast undertake distinct euryhaline migrations. After adult shrimp spawn in the open Gulf from spring to fall, the post-mysids and juveniles migrate into the fresher waters of bays where they grow rapidly to adulthood before returning to the Gulf. Obviously, these stages of shrimp must be able to adjust to the changing salinities encountered in the estuary and this adjustment requires physiological facilities which must operate at optimum.

One group of chemicals introduced by man that has recently been the concern of many ecologists is the PCBs, or polychlorinated

biphenyls. In 1969, a PCB, identified as Aroclor® 1254 was discovered as a contaminant in water, sediment, and fauna of Escambia Bay, Florida (Duke, Lowe and Wilson, 1970). An early survey indicated that whole body residues of the chemical in feral shrimp were as high as 14 mg/kg whole body (Nimmo et al., 1971a). Subsequent toxicity tests on juvenile pink shrimp (Penaeus duorarum) revealed that about 1.0 µg/ℓ in the water would kill 50% within 15 days (Nimmo et al., 1971b).

While conducting bioassays at our laboratory we noted on several occasions that salinity appeared to affect toxicity. In one instance, we conducted a chronic exposure in which adult pink shrimp, were exposed to a sublethal concentration of the chemical (about 1.0 µg/ℓ). The purpose of the test was to determine if structural damage might occur in gill tissues. On the 27th day of exposure at which time we had recorded no previous deaths from the PCB, the salinity of the incoming water decreased from 20 °/oo to 11 °/oo within 4 hours due to rain, tides and wind. The result was death of ten experimental shrimp before the salinity had returned to 20 °/oo. During the next two days, the salinity was lowered again by aberrant tides and climatic conditions and more experimental, but not control, shrimp died. We, therefore, became interested in the possible interaction of Aroclor® 1254 and environmental stress, particularly the effect of PCB on the ability of shrimp to regulate osmotically and ionically at reduced salinities.

MATERIALS AND METHODS

Adult brown shrimp (Penaeus aztecus), 11.5 - 13.7 cm, rostrum to telson, were captured near Gulf Shores, Alabama and used in our studies. Approximately equal numbers of both sexes were used and the methods of exposure to the Aroclor 1254 were similar to those reported previously (Nimmo et al., 1971a). The modifications were (1) ambient salinity was maintained at 30 ± 1 °/oo and temperature, at 25 ± 2° C, and (2) the exposures to the chemical were "sublethal" and lasted but 7 days. Three µg/ℓ was chosen as the test concentration because previous tests with adult brown shrimp as well as adult pink shrimp (P. duorarum), demonstrated that this concentration would cause 50 °/oo mortality within 30 days.

Following exposure to PCB, equal numbers of PCB-exposed and "control" shrimp were transferred to separate aquaria at ambient temperature and salinity (Fig. 1). While temperature was kept constant, the salinity in each aquarium was gradually lowered during 8 hours to a predetermined level. Since the possibility existed of physiological stress from handling or inherent in the experimental design, both PCB-exposed and control shrimp were analyzed for osmotic and ionic concentrations after going through the procedure without external salinity change (30 °/oo). For the first group, the salinity was maintained at 30 °/oo for 8 hours; the second, salinity was lowered from 30 °/oo to 22 °/oo; the third, from 30 °/oo to 10 °/oo, and the fourth, from 30°/oo to 7 °/oo. Although there was a time differential between groups of shrimp, and therefore, a possible

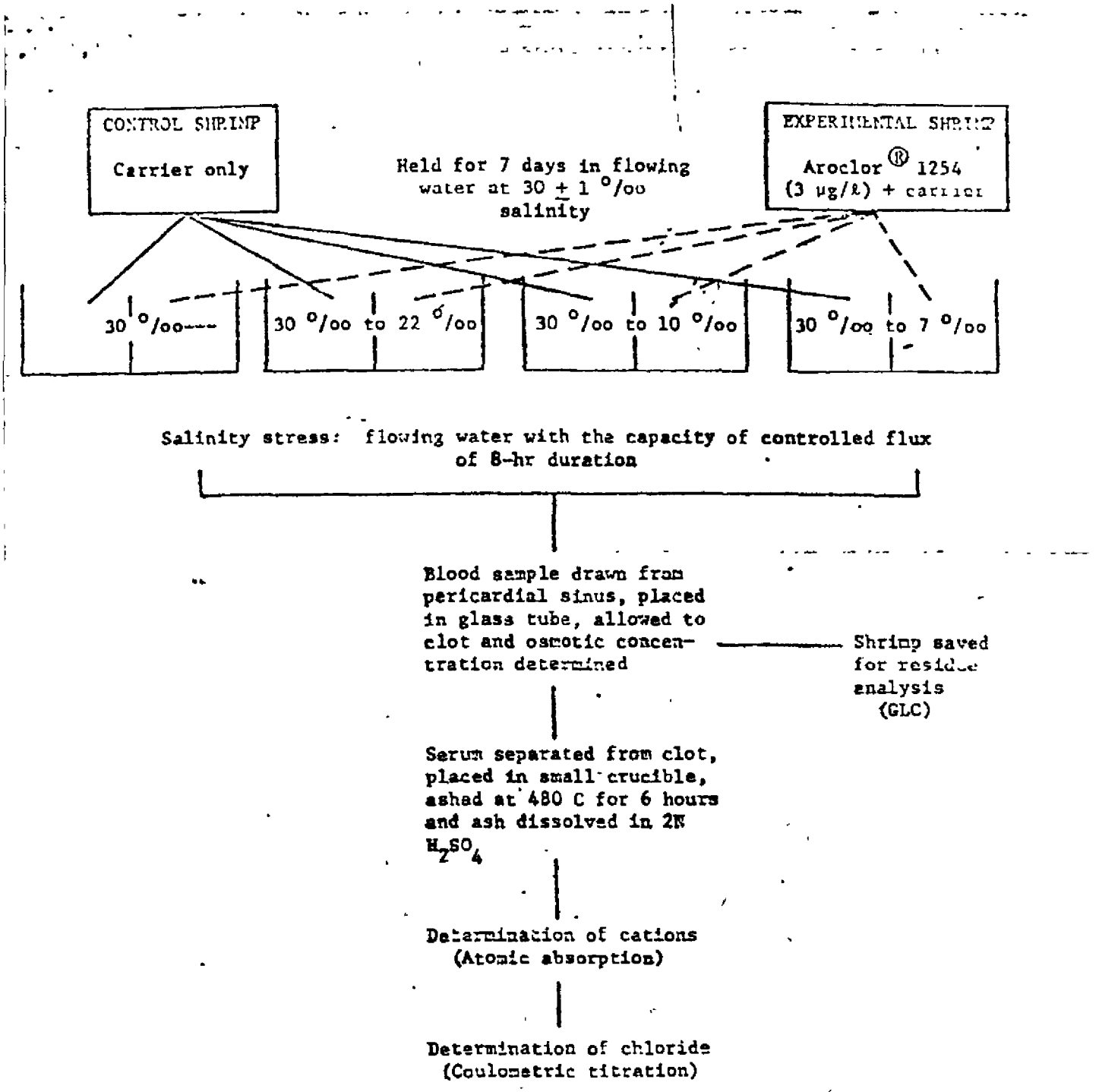


FIGURE 1. FLOW SHEET FOR EXPERIMENTAL PROCEDURE

Table 1. WHOLE BODY CONCENTRATIONS OF AROCLOR® 1254 IN PCB-EXPOSED SHRIMP*

SALINITY °/oo	mg/kg AVERAGE	RANGE
30	9.6	3.0-13.7
22	7.8	1.9-18.8
10	7.1	3.9-14.0
7	8.5	2.8-15.0

*Concentration of Aroclor® 1254 in the test water was 3 ppb; length of exposure, 7 days. Control shrimp had less than 0.1 ppm of Aroclor® 1254.

difference in test animals due to a slight loss of PCB; analyses for the chemical revealed no significant difference in whole body concentrations among groups (Table 1). As in earlier studies, there was a wide range in individual concentrations of PCB (Nimmo et al., 1971b).

Samples of whole blood (hemolymph) for osmotic concentration were removed from each shrimp by pericardial puncture. A ground-glass syringe (1ml), fitted with a #22 gauge stainless-steel needle was inserted into the animal at an oblique angle to obtain at lease 0.3 ml of blood from each animal. The blood was immediately transferred to an osmometer tube and the osmotic concentration determined with a Fisk® Model G-66 osmometer. Since the blood clotted quickly, it was difficult to determine whether we measured osmotic concentration on whole blood or on serum, but on analyzing them separately, we found no significant difference in their osmotic concentrations. Replicate determinations of 10 separate aliquots of pooled sera from several shrimp yielded a standard error of 1.2 (mean concentration = 629 mOs).

Analyses of ions was performed on ashed sera. To prepare the sample, the clot contained in the osmometer tube was squeezed with a small glass rod, the clot was removed and exactly 0.2 ml of the serum was transferred to a small crucible. The crucible was placed in an oven and the contents ashed at 480 C for 6 hr, cooled and the ash was dissolved in 2N H_2SO_4. Analysis of chloride was performed with a Buchler-Cotlove Chloridometer® and cations were determined on a Model 403 Perkin-Elmer® atomic absorption spectrophotometer

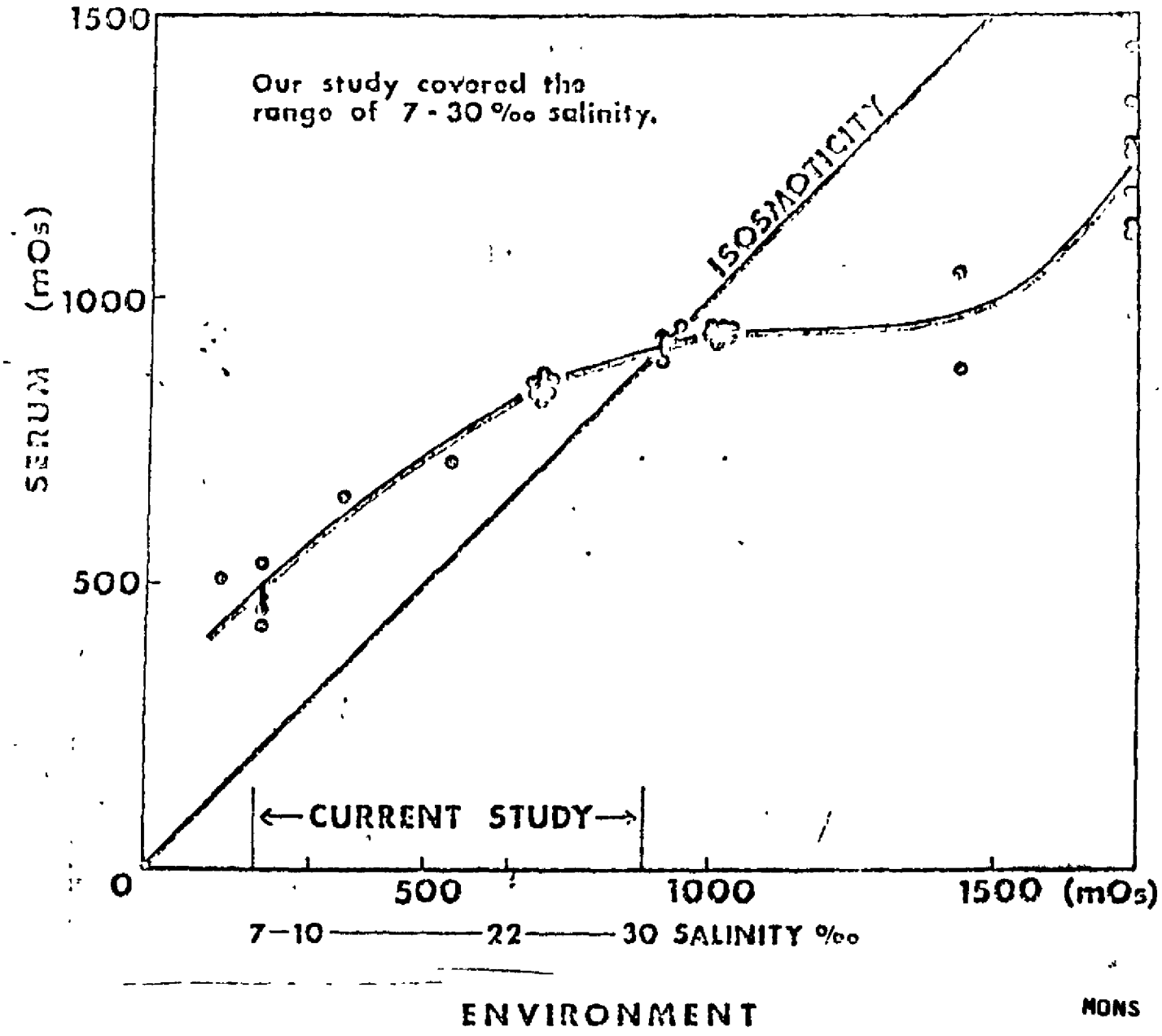
equipped with a deuterium arc-background corrector and an HGA-70 heated graphite atomizer. Cations in standard solutions were in the same proportions as those in the sera. As a check on our methods, an analysis of a single aliquot of serum by emission and atomic absorption yielded identical results for the element; potassium. Five replicates of pooled sera from several shrimp yielded a standard error of 0.24 mEq/l for Cl (mean = 257 mEq/l). Replicate analyses on serum aliquots yielded standard errors in mEq/l of 8.26 for Na (mean = 324.9), of 0.04 for Mg (mean = 16.0), of 0.07 for K (mean = 8.1), of 0.19 for Ca (mean = 4.74), and of 0.10 for Cu (mean = 2.84).

The 95% confidence interval was used to evaluate significance of differences in the data. The 95% intervals are indicated in the graphs by vertical bars on each datum and are listed in each table. It is sometimes difficult to relate "osmolality" or "osmotic concentration" to the environment. For the purpose of our discussion of the marine environment, we expressed the concentration of the environment as salinity. The relationship between mOs and salinity is indicated along the X axis in Figure 2.

Concentrations of Aroclor® 1254 were determined on individual shrimp by gas chromatography, using procedures summarized earlier (Nimmo et al., 1971b).

RESULTS

The most significant result of this study was the discovery that a sublethal concentration of Aroclor 1254 at constant salinity for 7 days became lethal when shrimp were subjected to a


1500
1000
500
0
SERUM (mOs)
Our study covered the
range of 7 - 30 ‰ salinity.
ISOSMOTICITY
←CURRENT STUDY→
500
1000
1500 (mOs)
7—10
22
30 SALINITY ‰
ENVIRONMENT

MONS 203528

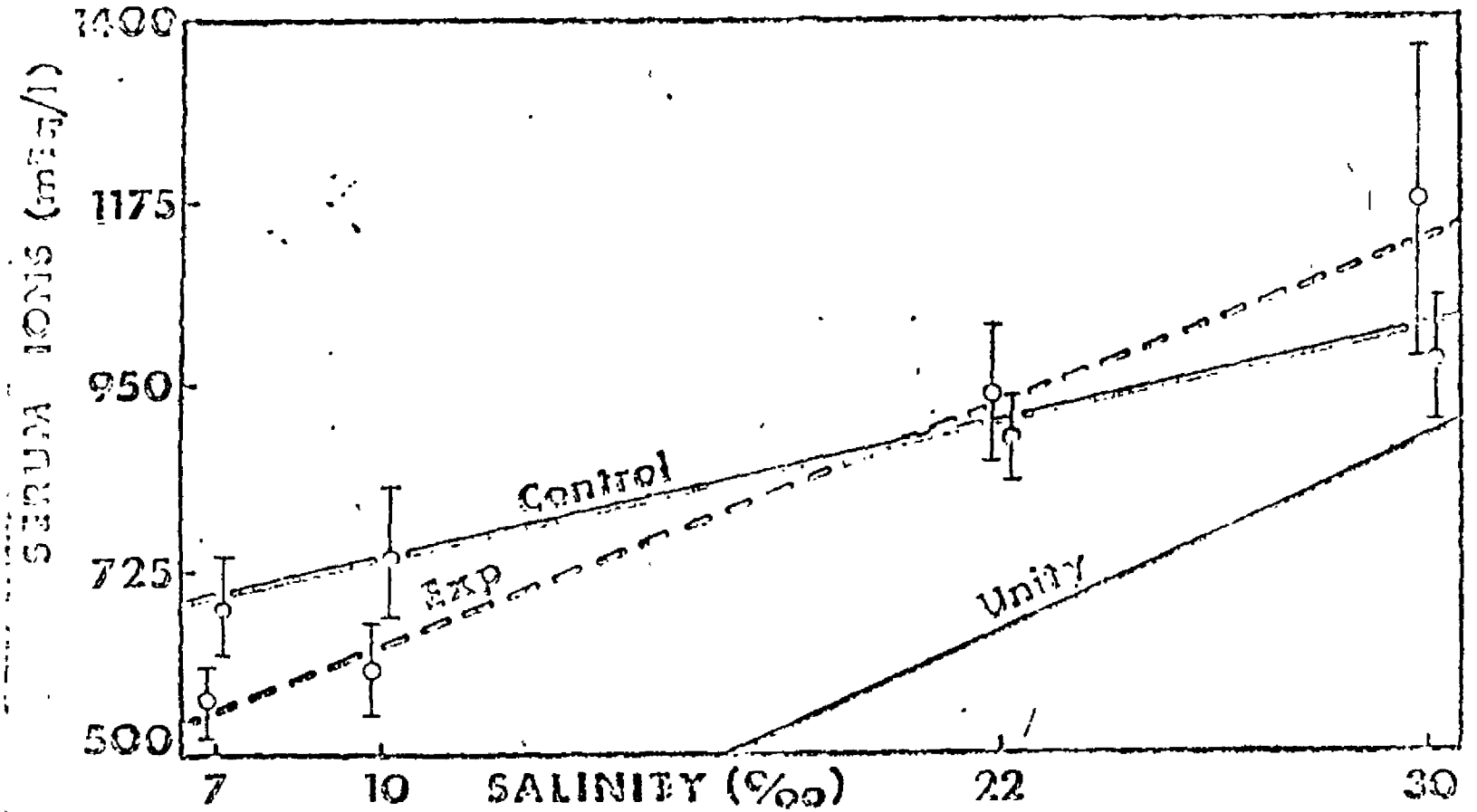
FIGURE 2. OSMOTIC CONCENTRATION: SERUM-ENVIRONMENT IN PENAEUS AZTECUS (after McFarland & Lee, 1963). Our study covered the range of 7 °/oo to 30 °/oo salinity.

gradual decrease in salinity over an 8-hr period. Usually the shrimp exhibited increased swimming activity while the salinity changed from 30 °/oo to 20 °/oo, and this was the only behavioral aberration observed. Deaths in experimental shrimp began at a salinity of about 12-13 °/oo and at 10 °/00 and 7 °/oo, when 8 hours had elapsed, mortality of experimental shrimp was nearly 50%. When 50% of the experimental shrimp had become moribund or had died, living PCB-exposed shrimp were taken for analyses of osmotic concentrations and ion determinations on sera.

The results of these analyses indicated that concentrations of most major ions in the sera of PCB-exposed shrimp became significantly less as the ambient salinity decreased, the sum of major ions (e.g. Na, Ca, Mg, K, Cu, and Cl) being 18% less after ambient salinity reached 10 °/oo or 7 °/oo (Fig. 3). Of this total, sodium was 16% less (Fig. 4), chloride, 19% (Fig. 5) and calcium, 25% (Fig. 6). There was some indication that magnesium decreased, but not significantly (Table 2). No apparent differences in potassium (Table 3), or copper (Table 4) were noted.

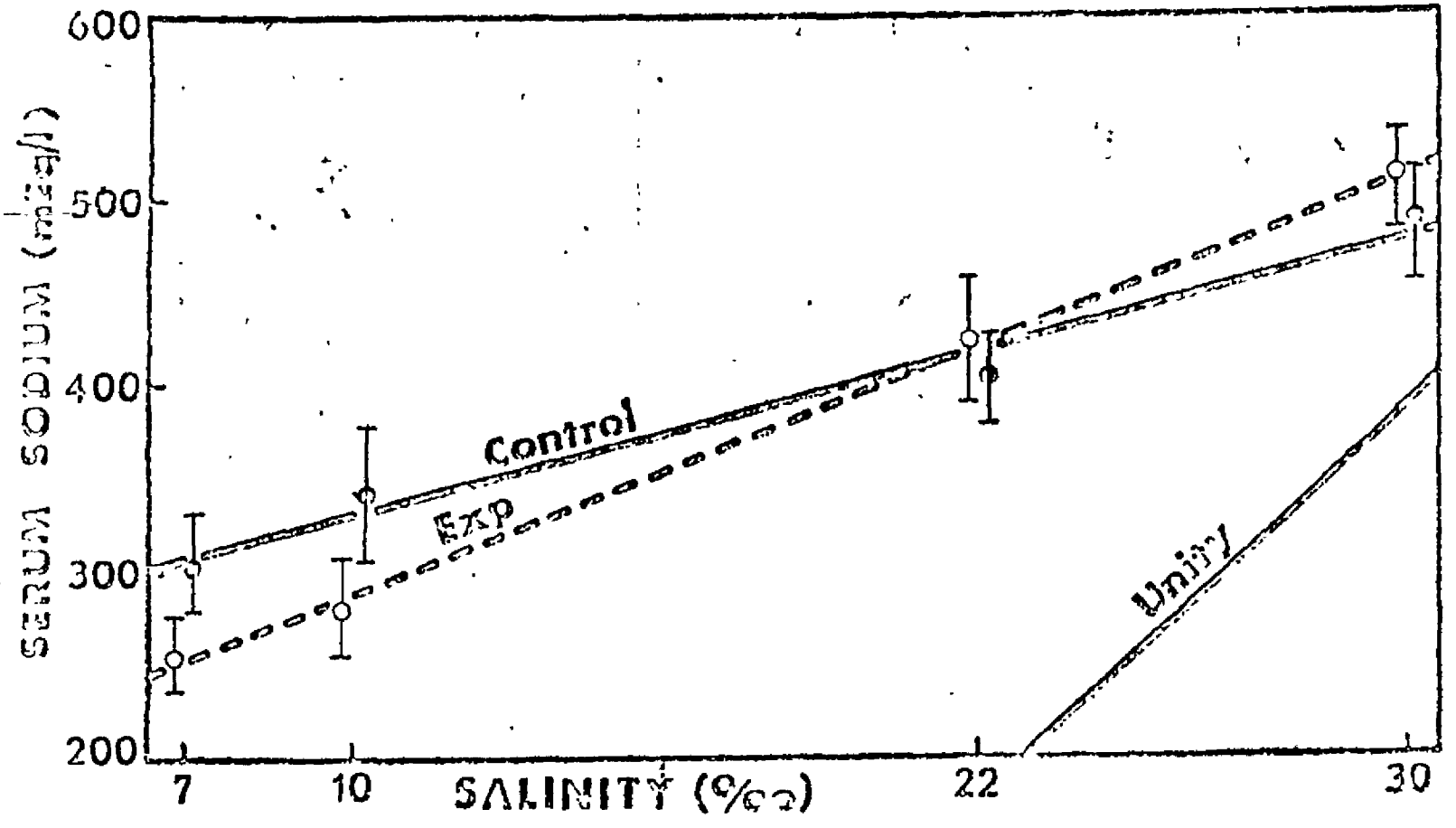
Data for iron (Table 5) are not included in the totals in Fig. 3 because we could not distinguish the divalent from the trivalent form.

Despite significant alterations in the major ion complement or in some major ions, osmotic contentration was not significantly affected by PCB and salinity stress (Table 6). Seemingly, osmotic pressure was less in PCB-exposed shrimp at 10 °/oo or 7 °/oo


1400
1175
950
725
500
SERUM IONS (mEq/l)
Control
Exp
Unity
7
10
SALINITY (‰)
22
30

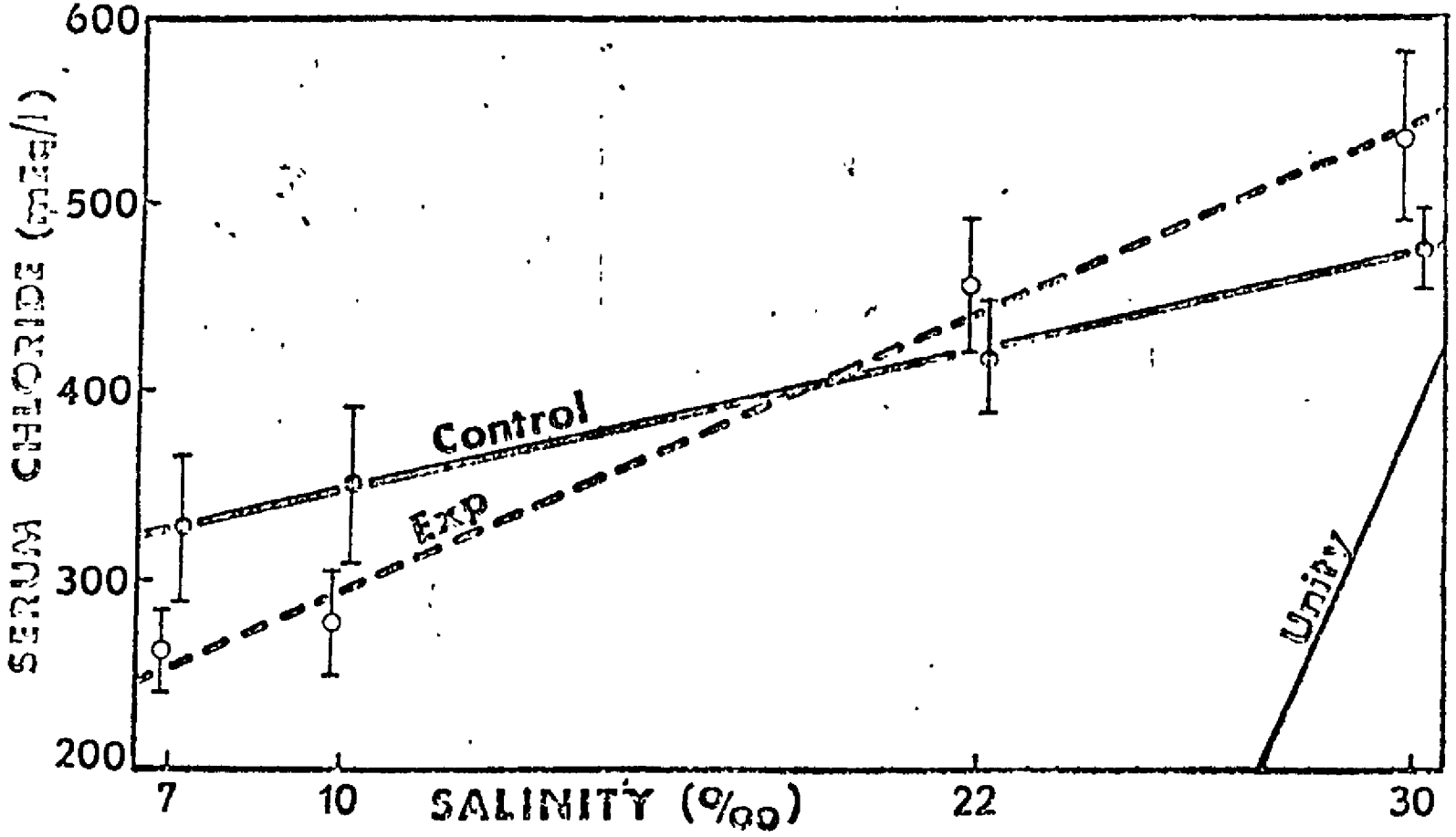
MONS 203531

FIGURE 3. TOTAL IONS IN BROWN SHRIMP SERUM IN RELATION TO SALINITY


600
500
400
300
200
SERUM SODIUM (mEq/l)
Control
Exp
Unity
7
10
22
30
SALINITY (‰)

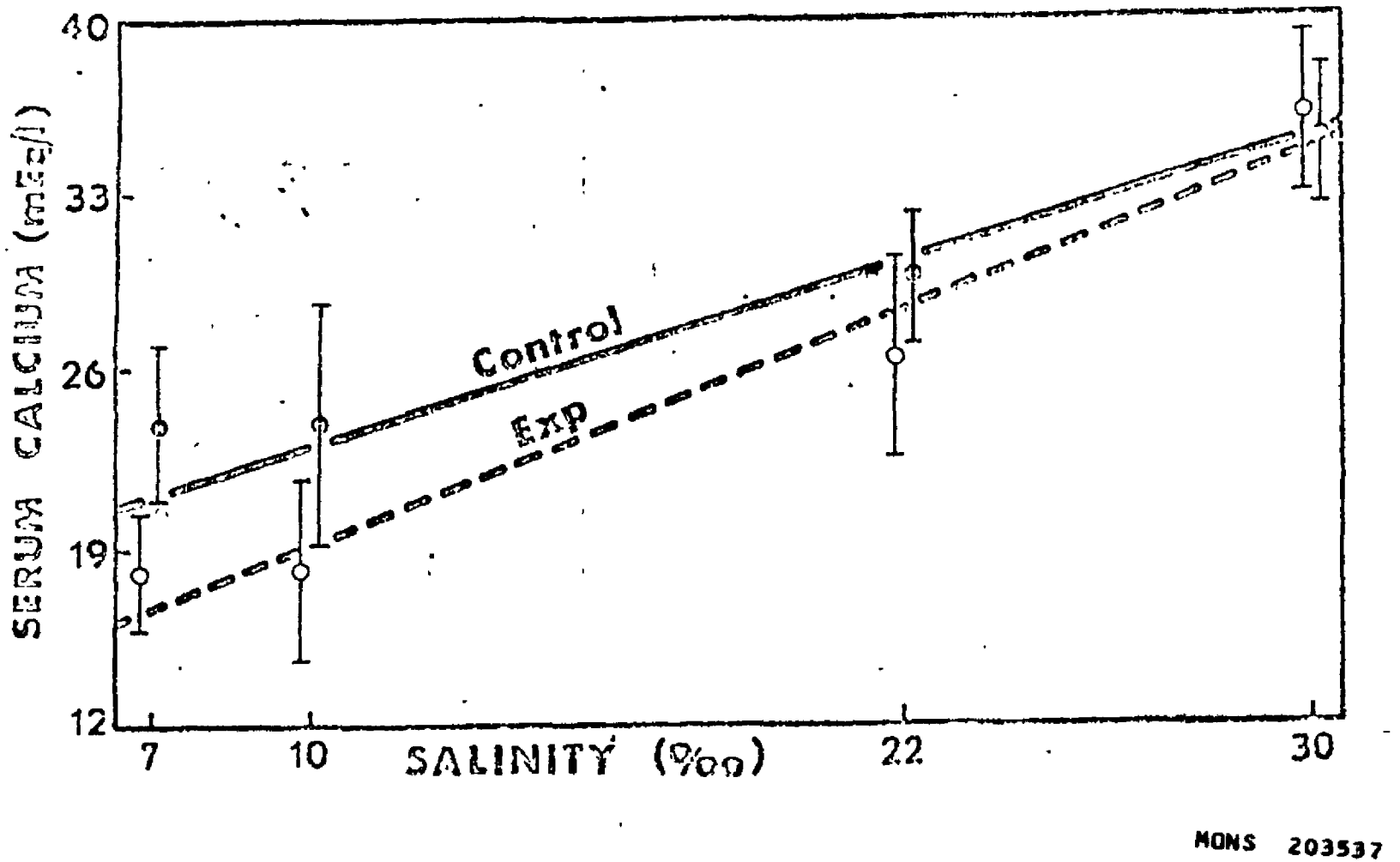
MONS 203533

FIGURE 4. SERUM SODIUM IN BROWN SHRIMP IN RELATION TO SALINITY


600
500
400
300
200
SERUM CHLORIDE (mEq/l)
Control
Exp
Unity
7
10
SALINITY (‰)
22
30

MONS 203535

FIGURE 5. SERUM CHLORIDE IN BROWN SHRIMP IN RELATION TO SALINITY


40
33
26
19
12
SERUM CALCIUM (mEq/l)
Control
Exp
7
10
SALINITY (‰)
22
30

MONS 203537

FIGURE 6. SERUM CALCIUM IN BROWN SHRIMP IN RELATION TO SALINITY

Table 2. AVERAGE SERUM CONCENTRATIONS OF MAGNESIUM IN BROWN SHRIMP IN RELATION TO SALINITY

SALINITY	mEq/l			
°/oo	CONTROL	95% CONFIDENCE INTERVAL	EXPERIMENTAL	95% CONFIDENCE INTERVAL
30	20.1	16.7--23.5	17.3	12.7--21.8
22	16.3	14.2--18.4	14.6	12.3--16.9
10	14.8	12.4--17.2	13.0	11.0--15.0
7	15.0	11.7--18.3	11.5	8.9--14.2

Table 3. AVERAGE SERUM CONCENTRATIONS OF POTASSIUM IN BROWN SHRIMP IN RELATION TO SALINITY

SALINITY °/oo	mEq/l CONTROL	95% CONFIDENCE INTERVAL	EXPERIMENTAL	95% CONFIDENCE INTERVAL
30	14.2	13.5--14.9	14.7	13.6—15.7
22	11.7	11.1--12.3	12.1	11.2--12.9
10	10.4	9.8--11.2	10.1	8.7—11.4
7	9.6	8.4--10.7	10.0	8.4—11.6

Table 4. AVERAGE SERUM CONCENTRATIONS OF COPPER IN BROWN SHRIMP IN RELATION TO SALINITY

SALINITY °/oo	mEq/l CONTROL	95% CONFIDENCE INTERVAL	EXPERIMENTAL	95% CONFIDENCE INTERVAL
30	4.7	3.6—5.9	5.3	4.4--6.3
22	4.0	3.4--4.6	5.9	4.8--7.0
10	5.2	4.0—6.4	4.6	3.8--5.3
7	4.8	3.9—5.7	5.7	5.0--6.4

Table 5. AVERAGE SERUM CONCENTRATIONS OF IRON IN BROWN SHRIMP IN RELATION TO SALINITY

SALINITY	mM/l			
°/oo	CONTROL	95% CONFIDENCE INTERVAL	EXPERIMENTAL	95% CONFIDENCE INTERVAL
30	.23	.17—.29	.31	.15--.48
22	.28	.15--.35	.18	.14--.22
10	.20	.15—.25	.26	.17--.35
7	.24	.14--.33	.33	.24--.41

Table 6. AVERAGE SERUM OSMOTIC CONCENTRATIONS IN BROWN SHRIMP IN RELATION TO SALINITY

SALINITY	MILLIOSMOLES			
°/oo	CONTROL	95% CONFIDENCE INTERVAL	EXPERIMENTAL	95% CONFIDENCE INTERVAL
30	749	728--771	752	726--779
22	687	665--708	688	658--718
10	551	508--593	523	500--546
7	547	521--572	516	495--537

salinity but individual variation was too great to show a significant difference from controls.

DISCUSSION

Knowledge of interactions between toxic compounds and environmental factors is essential for predicting their effects on ecosystems or species. Examples of this need have been demonstrated in both fresh and marine investigations. In fresh water, low-level chronic exposure of the darter (Etheostoma nigrum) to dieldrin greatly affected its ability to survive thermal stress (Silbergeld, 1973). The sublethal effects of mercury on fiddler crabs (Uca pugilator) reduced survival times when crabs were placed under temperature and salinity stress (Vernberg and Vernberg, 1972). Mortality of fiddler crabs previously exposed to cadmium was greatest at high temperatures and low salinites (O'Hara, 1973).

In our studies Aroclor® 1254 possibly interfered with the adenosine triphosphatase (ATPase) activity in gills of shrimp. ATPase activity is associated with active ion transport (Tanaka, Sakamoto and Sakamoto, 1971). Polychlorinated insecticides and the related polychlorinated biphenyls have been shown by several "in vitro" assays to inhibit ATPase in the tissues of fishes (Davis and Hedemeyer, 1971a; 1971b; Davis, Friedhoff and Hedemeyer, 1972; Cutkomp et al., 1972; Yap et al., 1971) and in the nerves of lobsters (Matsumura and Narahashi, 1971). The need for greater efficiency or capacity of ATPase in marine organisms can be inferred from the results of

Pfeiler and Kirschner (1972), who showed that gill ATPase activity of rainbow trout adapted to salt water was greater than in fish adapted to fresh water.

Polychlorinated hydrocarbons have interferred with either osmo- or ionic-regulation in aquatic animals (Eisler and Edmunds, 1966; Grant and Mehrle, 1970; Kinter et al., 1972; Nimmo and Blackman, 1972). The physiological relationship of ionic effects to that of ATPase activity was first reported by Kinter et al. (1972), who postulated that lipophilic agents such as DDT and PCBs, might interact with the phospholipid-activating components of the lipoprotein enzyme. The effect of dieldrin on ion movement in the nervous system of cockroaches showed that dieldrin inhibited binding of calcium to the phospholipid moiety of the enzyme, thus inhibiting the movement of calcium across the nerve membrane (Hayashi and Matsumura, 1967). Calcium salts in fresh water greatly increased the ability of marine and euryhaline animals to survive in that medium (Black, 1957). Toxic symptoms of DDT poisoning in fresh water fish could be alleviated by the addition of calcium salts (Keffler, 1972). In our studies calcium was lowered 25% in the sera of PCB-exposed shrimp at 7 °/oo salinity, and it may be that this reduction was responsible in part for the observed decrease in sodium, chloride, and other ions.

Field observations of juvenile and subadult brown shrimp by several investigators indicate that those shrimp tolerate a wide range of salinities:

Salinity	Location	Observer
0.22 - 0.36 °/oo	St. Lucie estuary, Fla.	Gunter and Hall (1963)
0 - 1.0 °/oo	Mobile Bay, Ala.	Loesch (In Gunter et al. 1964)
0 - 2.0 °/oo	Choctawhatchee Bay, Fla.	Nimmo (unpubl. data)
69 °/oo	Laguna Madre, Texas	(1957)

Gunter, Christmas, and Killebrew (1964), found that in Texas bays, young brown shrimp were most abundant at 10 to 30 °/oo, with greater abundance above 20 °/oo. Zein-Eldin and Aldrich (1965) found that postlarval brown shrimp withstood a wide range of salinity-temperature combinations.

It is evident that adult shrimp are osmoregulaters at all but extremes of salinity (Fig. 2). Williams (1960) gives the isosmotic point of shrimp hemolymph at 26.5 °/oo (788 mOs), as compared to our calculation of 23.4 °/00 (694 mOs) in control shrimp. Nevertheless, there was no significant difference in PCB-exposed and control shrimp (Table 6). Obviously, a slight change in environmental osmotic pressure would not be critical to the osmoregulatory ability of the animals at isosmoticity, but was critical in the dilute environment. Therefore, the salinities where the PCB exerted its

greatest effect in the laboratory (as judged from mortality of shrimp) were well within the range in which brown shrimp occur in nature.

Although no appreciable difference occurred in osmotic concentration, there was significant difference in major ions in the sera of PCB-exposed shrimp (Fig. 3). McFarland and Lee (1963) found that the point of convergence of the total ions in the hemolymph of feral shrimp to that of the environment was 27 °/oo (800 mOs). In our study, by extrapolation, convergence occurred at 34 °/oo (1000 mOs) in controls, whereas in PCB-exposed shrimp showed no convergence and total ions paralleled unity.

In surveys conducted soon after PCB was first discovered in the Pensacola estuary, the distribution of shrimp in relation to salinity seemed to be related the amount of the chemical in the animals (Nimmo et al., 1971b). Of three species captured, brown shrimp had the highest whole-body residues (14 ppm) although most samples were lower. Seemingly, a concentration of 14 ppm PCB in feral shrimp would have been lethal if the animals were subjected to salinity stress such as imposed by our experimental procedure. We have unpublished data that suggest existence of a threshold in average whole-body concentration of PCB (5.6 to 7.8 mg/kg) in pink shrimp (P. duorarum) that would be lethal when superimposed on salinity stress caused by our procedure. However, a recent survey of feral shrimp from the Pensacola estuary showed that young adult shrimp now have only a fraction of PCB concentrations found in 1969/70

periods. For example, a sample taken from Escambia Bay in August, 1973 had a whole-body concentration of only 0.1 mg/kg.

Future studies should include research on interaction of PCB and salinity on juvenile and postlarval shrimp since chronic toxicity tests have shown that these stages were more susceptible to the chemical (Nimmo et al., 1971a). In addition, studies by Dana Beth Tyler-Schroeder, of the Gulf Breeze Laboratory, have shown the susceptibility of larvae of grass shrimp (Palaemonetes pugio) to the PCBs, Aroclors® 1016 and 1242, decreases with age (personal communication). Also, the "in vivo" effect of PCBs on ATPase activity in shrimp should be fully investigated.

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Effects of Aroclor® 1254 on Laboratory-Reared Embryos and Fry of Sheepshead Minnows (*Cyprinodon variegatus*)[1]

(27)

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ABSTRACT

Artificially fertilized sheepshead minnow (Cyprinodon variegatus) eggs were exposed to logarithmic concentrations of Aroclor 1254 (10.0 to 0.1 µg/l) in seawater averaging 30 C and 24 °/oo in an intermittent-flow bioassay. Fertilization was not affected but significantly fewer embryos developed in the 10.0 µg/l and fewer fry survived in concentrations greater than 0.1 µg/l. Fry were more susceptible to Aroclor 1254 than were juveniles or adults.

Sheepshead minnow eggs were artificially fertilized and maintained at temperatures from 15 to 35 C and in salinities from 0 to 35 °/oo to determine efficient culture conditions. Fertilization was not affected by the temperature and salinity ranges chosen, but hatching success was greatest (χ^2; α=0.01) at a temperature range of 24 to 35 C and a salinity range of 15 to 30 o/oo.

INTRODUCTION

Polychlorinated biphenyls (PCB's) occur in estuaries in many states (Butler, 1973), and the occurrence of one, Aroclor 1254, in nearby Escambia Bay and its acute toxicity to estuarine animals has been documented (Duke et al., 1970). Hansen et al. (1971) found 5 µg/l of Aroclor 1254 toxic to the juvenile estuarine fishes, pinfish (Lagodon rhomboides) and spot (Leiostomus xanthurus), in 14 to 45 day bioassays. We are unaware of data on the effects of this PCB on embryonic and larval stages of estuarine fishes.

The sheepshead minnow (Cyprinodon variegatus) is found in brackish waters from Cape Cod, Massachusetts to Brownsville, Texas (Hildebrand, 1917). It is important in estuarine food chains as a voracious omnivore and as food for predators such as croakers (Micropogon undulatus) and spotted seatrout (Cynoscion nebulosus) (Darnell, 1958). The size, hardiness, high fecundity, and generation time of the sheepshead minnow makes it a nearly ideal laboratory test fish.

In this paper, we determined water temperatures and salinities suitable for the culture of embryos and fry of the sheepshead minnow and observed the effect of Aroclor 1254 in water on the various life stages of this fish.

METHODS AND MATERIALS

Temperature and Salinity Studies

Nearly identical methods were used in the temperature and salinity experiments to fertilize eggs. Adult fish were acclimated for one week in salt water averaging 25 C and 20 o/oo. The following week, female fish were given three intraperitoneal injections of 50 I.U. human chorionic gonatrophic hormone to induce egg maturation. Over 70% of the fish produced viable eggs. Eggs were manually stripped and deposited in 40 ml of filtered seawater. Only large, round, clear eggs were selected for fertilization. Testes from 7-10 acclimated males were excised, macerated in 20 ml of filtered seawater and mixed with eggs in a 100 ml beaker, allowing 30 minutes for fertilization. Seawater conditions used for fertilization in the temperature test was 25 C and 20 o/oo salinity while the salinity test conditions were 30 C and the test salinity. Each experiment lasted as long as was required for embryos to hatch plus an additional two weeks to determine survival of fry.

The effect of temperature on hatching was investigated by withdrawing 25 eggs from the 100 ml beaker with a wide-bore pipette and placing them in two, 1" dishes of 25 C, 20 °/oo seawater. Each of the dishes were partially submerged in a bath at 15, 20, 25, 30, or 40 C in one temperature test and 22, 24, 26 or 28 C in another. One hour later, when temperatures in the dishes equalled the bath temperature, fertilization was confirmed microscopically. The criterion for fertilization was cleavage. Dishes were checked daily during the 3-week static tests and dead or non-fertile eggs and larvae were removed. The criteria for embryo mortality was

an opaque, white, fungal growth and for the larvae a white coloration of the trunk musculature. Temperatures were monitored continuously and were within 1 C of the desired temperatures. Salinity alterations due to evaporation in both the salinity and temperature tests were compensated for daily by addition of distilled water.

Fertilization procedures for the salinity study were identical to those of the temperature study except that the number of eggs in each salinity varied from 35-75, depending on egg availability. Filtered seawater from Santa Rosa Sound, Florida was diluted to give salinities of 0, 5, 10, 15, 20, 25, 30 and 35 °/oo. The 30 and 35 °/oo concentrations were attained by adding Rila® salts to the water from the Sound. Salinities, checked daily with a TS® refractometer, were within 2 °/oo of the desired level.

Aroclor 1254 Exposure

An Aroclor 1254, intermittent-flow bioassay was accomplished using suitable culture conditions determined in the temperature and salinity tests. Eggs were fertilized in control water and placed in Petri dishes, four dishes (20 eggs per dish) in each PCB concentration and control. Fertility was confirmed after one hour. Temperatures in both tests averaged 29 C (range 27-31 C) and salinity averaged 24 °/oo (range 16-32 o/oo). The salinity fluctuated with that of Santa Rosa Sound. The toxicant dosing system used in this test was a modification of the apparatus of Brungs and Mount (1970). In our system the toxicant and carrier were injected into the delivery tube leading to each exposure tank (Figure 1). Our apparatus

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allowed us to retain the advantages of the Mount and Brungs (1967) dosing apparatus and to select any concentration of toxicant while maintaining the same concentration of carrier in each toxicant concentration.

Seawater used in this bioassay was pumped from Santa Rosa Round into a constant head box in the laboratory. An oscillating pump (1A), regulated by a rheostat, pumped water from the head box through a 20μ-pore polypropylene filter and into the compartments in the dosing apparatus. After all compartments were filled, the self-starting siphon in the last compartment emptied into a receiving bucket (1D). The weight of the bucket being filled operated two micro-switches. One switch (1a) shut off the oscillating pump and the other (1e) activated a solenoid (1E) that raised the lever of the injection apparatus (1F). This rising lever was connected to gears that forced the barrels of six 50-ml syringes (1G) equal distances. Mixing bottles (1B) received injections from a control syringe containing the carrier (polyethylene glycol 200). Stock solutions of the carrier and Aroclor 1254 were mixed to give concentrations 0.1, 0.32, 1.0, 3.2 and 10.0 μg/l of Aroclor per liter of water. The water then flowed to the distribution boxes (Figure 2) containing one large and four small compartments, each with a standpipe or siphon. Each compartment filled completely during each cycle and the siphon delivered water to the aquarium in which adults and juvenile fish were held. Water remaining in the four small compartments flowed slowly out through submerged holes in the standpipes into each of four containers holding eggs or fry. Each container received 100 ml of water per cycle. The number of cycles per day ranged from 100 to 140 and were sufficient to replace water in each egg container at least three times each day.

toxic effects of polyethylene glycol 200 in this test (7.4 mg/ℓ) were not anticipated because (1) 1% v/v polyethylene glycol was not lethal to juvenile sheepshead minnows in 96 hour tests and (2) in earlier tests at this laboratory, toxicity of 5 µg/ℓ of Aroclor 1254 to pinfish was not changed by an increasing solvent concentration one hundred times (0.1 to 10 mg/ℓ) (Hansen, unpublished data).

Chemical Methods

Aroclor 1254 concentrations in test and control water were determined weekly by gas chromatography. Methods were the same as those of Nimmo et al. (1971), except that an OV-101 column was used and all peak heights were averaged for PCB quantification. Measured PCB concentrations in test water were typically 35% to 60% of nominal concentrations; control water contained no detectable PCB (<.03 µg/ℓ). Recovery efficiency of Aroclor 1254 was greater than 60%. Measured concentrations were not corrected for percentage recovery.

Dissolved oxygen was determined weekly using the modified Winkler method (Strickland and Parsons, 1968). Concentrations seemed adequate and above 50% saturation.

Statistical Methods

The Chi-square (χ^2) test was used in the statistical analysis of the data. In temperature and salinity studies the maximum positive response was compared with all other responses to determine the most efficient culture conditions. Data in the two temperature experiments were analyzed separately.

RESULTS AND DISCUSSION

Temperature Study

Temperature determined the number of days required for hatching (time-to-hatch) and affected the survival of embryos and fry of the sheepshead minnow (Table 1). Sheepshead minnow eggs hatched at water temperatures from 22-40 C but none hatched at lower temperatures. Hatching success was greatest at temperatures ranging from 24-35 C. Fry survival did not differ in the range of 22 to 35 C. The longest time-to-hatch was 15 days at 22 C, and decreased rapidly, leveling off at about 4 days at temperatures ranging from 30 to 40 C.

Salinity Study

Salinity affected survival of embryos and fry but did not affect time-to-hatch (Table 2). Survival to hatching was greater at salinities from 15 to 30 o/oo and fry survival did not differ in that range. Under natural conditions, adult and juvenile sheepshead minnows occur in a wide salinity range (Simpson, 1956). Our data show that embryos and fry also can survive in a wide salinity range.

Aroclor 1254 Study

Aroclor 1254 affected the survival of both embryos and fry of the sheepshead minnow, but had no effect on time-to-hatch. Embryos developed and hatched at all PCB concentrations, but hatching success at 10 µg/ℓ was significantly less than that of control eggs. After the eggs hatched, survival of fry was significantly less than control fry at all PCB concentrations except 0.1 µg/ℓ. Mortality increased with an increasing concentration of PCB. Three-week LC50 in exposed embryos and fry was estimated at 0.93 µg/ℓ (S.E. = 0.17 µg/ℓ). Fry did not die immediately

after hatching but were killed over most of the two-week period of post hatch exposure. Many of the dying fish developed fin rot as previously described in other PCB exposed fishes (Hansen et al., 1971).

Aroclor 1254 was more toxic to fry of sheepshead minnows that it was to juveniles, adults or to the fertilization of eggs (Table 4). In three week exposures to the same concentrations of this PCB, mortality of the juveniles was significantly greater (twenty-four percent) in the 10µg/ℓ concentration. Adult fish were not killed in a three-week exposure but became lethargic and exhibited fin rot. The concentration of PCB in the fry compared to that in the water (concentration factor) was nearly identical with that of adult fish. Concentration factors in fry ranged from 1.6 to 3.2×10^4 and those for adults ranged from 1.1 to 3.2×10^4. A twenty-four hour static test in 10.9µg/ℓ PCB showed no inhibition of fertilization of sheepshead minnow eggs. The greater sensitivity of early life stages of this fish to Aroclor 1254 stresses the need for bioassays designed to assess the effects of such chemical pollutants on these stages.

ACKNOWLEDGEMENTS

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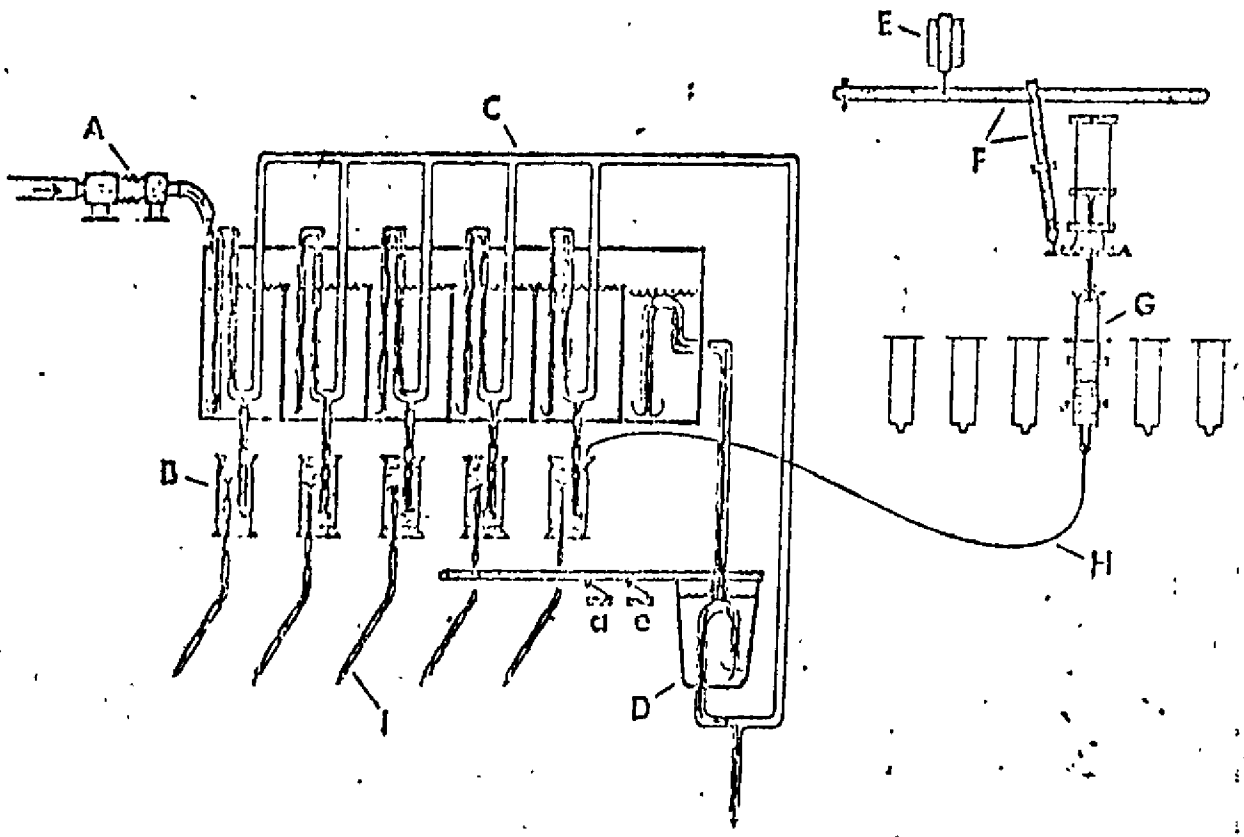

FIGURE 1. Dosing apparatus: A-oscillating pump; a-pump micro-switch; B-mixing bottles; C-vacuum lines; D-receiving bucket; E-solenoid; e-solenoid micro-switch; F-injector lever apparatus; G-50cc. syringe; H-toxicant delivery tubing; I-water delivery tube.

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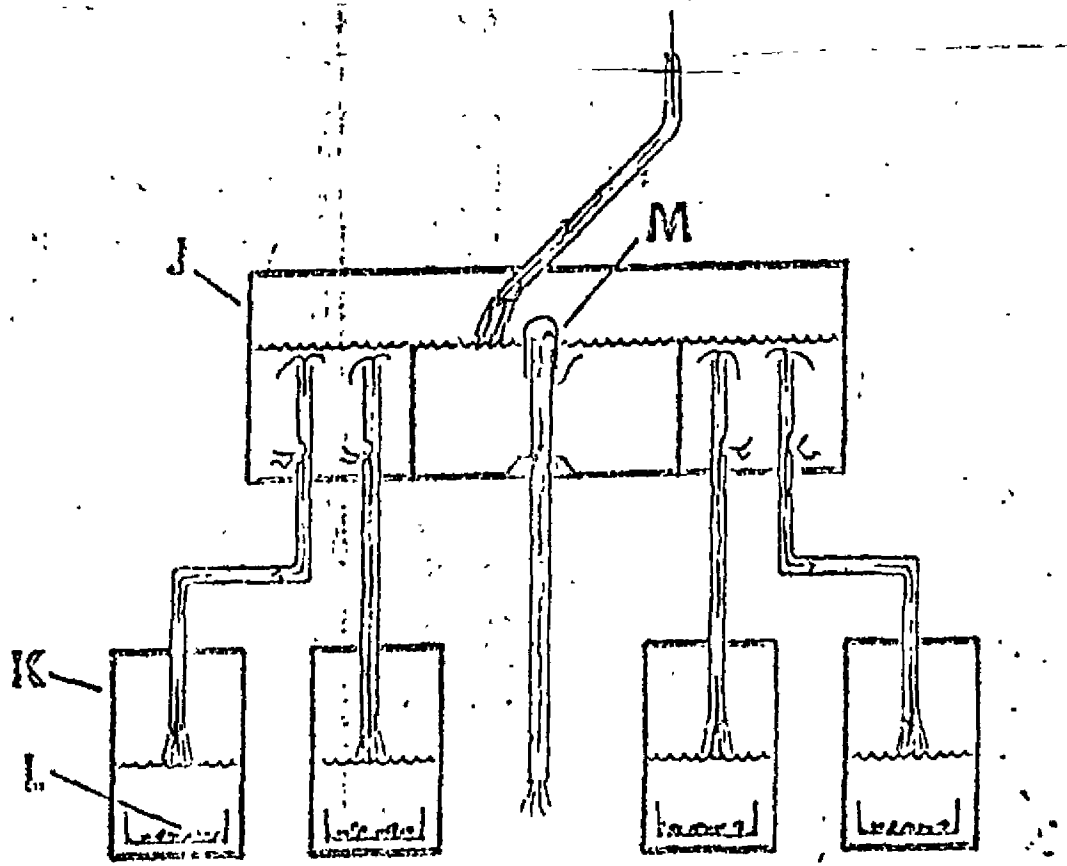

FIGURE 2. Distribution apparatus: J-distribution box; K-egg/larvae trays; L-Petri dishes; M-siphon.

Table 1.—Effect of temperature on [illegible] for two weeks following hatching of sheepshead minnows (Cyprinodon variegatus). Temperatures varied ± 0.5 C; salinity averaged 20 o/oo, ± 2 o/oo.

Temperature (°C)	Eggs		Hatching		Fry
	Number	Fertile %	Days #	Survival %	Survival %
15	50	92	No hatch	0**	0**
20	50	98	No hatch	0**	0**
22	50	100	15.0	28.0**	100
24	50	96	9.0	50.0	100
25	50	98	7.0	83.7	80
26	25*	92	7.0	78.0	100
28	50	90	6.0	55.5	92
30	50	94	4.5	82.9	90
35	50	94	4.0	70.2	100
40	50	94	4.0	8.5**	50

* Loss of 25 eggs due to spillage.

** Significantly less than the greatest fertility or survival (χ^2;α = 0.01).

Table 2.--Effect of salinity on fertility, time-to-hatch, hatch success and survival of fry for two weeks following hatching of sheepshead minnow (Cyprinodon variegatus). Temperature 30 ± 2 C; salinity varied ± 1 o/oo.

Salinity o/oo	Eggs		Hatching		Fry Survival %
	Number	Fertile %	Days #	Survival %	
0	60	35	No hatch	0*	0
5	35	46	7.0	12*	50
10	75	35	7.0	35*	89
15	35	51	5.5	67	92
20	75	44	6.0	67	100
25	35	54	5.5	53	90
30	75	49	5.5	73	93
35	35	37	6.0	30*	50

* Significantly less than the greatest fertility or survival (χ^2; $\alpha = 0.01$).

Table 3.--Effect of Aroclor 1254® on fertility, time-to-hatch, hatch success and survival of fry for two weeks following hatching of sheepshead minnow (Cyprinodon variegatus). Temperature averaged 30 C (range 27-31 C); salinity averaged 24 o/oo (range 16-32 o/oo).

Concentration (µg/ℓ)		Eggs		Hatching		Survival
Nominal	Measured	Number	Fertile %	Days #	Survival %	%
Control	<0.03 µg/ℓ	160	86	7.0	79	89
0.1	0.06	160	86	6.5	69	95
0.32	0.16	160	86	7.0	73	62*
1.0	0.36	160	91	7.0	82	63*
3.2	1.04	160	85	7.0	75	40*
10.0	3.48	160	91	6.5	57*	8*

* Significantly different from control fish ($\chi^2_{;\alpha} = 0.01$).

Table 4.--Relative susceptibility of various life stages of sheepshead minnows (Cyprinodon variegatus) to Aroclor 1254® in a flow-through system. Criteria are infertility of eggs and death of embryos, larvae, juveniles, and adults.

Life Stage	Exposure (days)	Concentration (µg/ℓ)	
		Minimum Affecting	Maximum Not affecting
Egg fertilization*	0.4	----	10.0
Embryos	7	10.0	3.2
Larvae	21	0.32	0.1
Juveniles	21	10.0	3.2
Adults	21	----	10.0

* 24-hour static test.

AROCLOR® 1254 IN EGGS OF SHEEPSHEAD MINNOWS: EFFECT ON FERTILIZATION SUCCESS AND SURVIVAL OF EMBRYOS AND FRY [1]

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ABSTRACT

The effect of the polychlorinated biphenyl (PCB), Aroclor 1254, in eggs of the sheepshead minnow, Cyprinodon variegatus, on fertilization success and survival of embryos and fry was investigated. Adult fish were exposed for four weeks to 0.1, 0.32, 1.0, 3.2 or 10.0 µg/ℓ of PCB, then injected twice with 50 IU of human chorionic gonadotrophin to stimulate egg production. The eggs were fertilized, placed in PCB-free flowing seawater and observed for mortality. Fertilization success was unimpaired by concentrations in eggs as high as 201 µg/g but survival of embryos and fry was reduced. Usually, fry from eggs containing 7.0 µg/g or more began dying 24-48 hours after hatching. If this PCB affects other species similarly, then populations of fish that presently have comparable concentrations in their eggs may be endangered.

INTRODUCTION

Polychlorinated biphenyls (PCB's) have been found frequently in estuarine organisms from many states (Butler, 1973) and in an estuary near the Gulf Breeze Laboratory (Duke et al., 1970). PCB's in seawater are toxic to and accumulated by juvenile shrimp, crabs, oysters and fishes (Nimmo, et al., 1971; Lowe, et al., 1972 and Hansen, et al., 1971). The relationship between the amount accumulated by fish and subsequent effects is poorly understood. However, PCB's in eggs may decrease fertility and survival in early stages of embryonic development in Atlantic salmon, Salmo salar (Johannsson et al., 1970), and PCB's have been implicated in poor reproductive success of striped bass, Morone saxatilis (Anonymous, 1971). Because

reproductive success with both fishes varied, the exact relationship of success to concentration of PCB in eggs remains unclear.

Our study was conducted to determine the effect of one PCB, Aroclor 1254, on fertilization success of eggs of sheepshead minnows, Cyprinodon variegatus, and on survival of embryos and fry. Aroclor 1254 was selected because we found eggs from striped bass that exhibited decreased reproductive success contained a PCB whose chromatograms closely resembled Aroclor 1254. Sheepshead minnows were selected because they can be readily exposed in the laboratory and reproductive success is excellent.

MATERIALS AND METHODS

Test fish

Adult sheepshead minnows were seined from ponds on laboratory grounds and acclimated to laboratory conditions for four days before exposure. During acclimation, mortality was less than 1% and no abnormal behavior was observed. Females averaged 42.5 mm standard length, range 35-52 mm, and males averaged 42.8 mm, range 35-52 mm. During acclimation and exposure, fish were fed commercial fish food that contained no detectable PCB (<0.01 µg/g).

Adult exposure

We exposed 20 female and 10 male fish in aquaria containing none, 0.1, 0.32, 1.0, or 3.2 µg/ℓ of Aroclor 1254 and exposed 25 females and 15 males to 10 µg/ℓ for four weeks in an intermittent-flow bioassay. The apparatus used was a modification of that of Brungs and Mount (1970). In our modification, Aroclor 1254 and carrier, polyethylene glycol 200, were injected into seawater each time the apparatus cycled. Each cycle

siphoned water to six 80ℓ test aquaria. The injection device was operated by a solenoid that raised a lever each cycle turning gears on six injectors and pushing the plungers of six 50cc syringes. Each of the approximately 150 daily cycles delivered 1.5ℓ of filtered 30C seawater, 11 µg of carrier and appropriate amounts of PCB to each aquarium. Water and carrier without PCB were delivered to the control aquarium. Salinity of water averaged 17 o/oo, range 5 to 28 o/oo.

Egg fertilization, embryo and fry survival

The effect of Aroclor 1254 in eggs was determined by enhancing egg production in exposed fish by hormonal injection, fertilizing the eggs artificially and monitoring their development in flowing PCB-free seawater. Female sheepshead minnows were injected intraperitoneally with a 50 I.U. human chorionic gonadotrophic hormone on exposure-days 25 and 27. On day 28, eggs were stripped manually from five females from each aquarium and those from each female placed in individual beakers containing 40 ml of filtered 30C seawater. Ninety-three of 96 females that survived produced eggs. Eggs from a female were fertilized with excised macerated testes from a male from the same aquarium. In addition, eggs from five control fish and two fish surviving exposure to 10 µg/ℓ PCB were fertilized by males exposed to 1.0 µg/ℓ. Twenty-five eggs from each fish were placed in Petri dishes to which a nine cm high collar of 500µ nitex mesh was glued. Dishes were submerged 7 cm in the 80ℓ aquarium which received approximately 225ℓ of filtered PCB-free seawater per day;

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[2]Manufactured by George Frazer, 4528 Pitt Street, Duluth, Minn. 55804

average salinity was 18 o/oo, range 10 - 27 o/oo. Success of fertilization was confirmed by checking microscopically for cleavage 1.5 hours after fertilization. Thereafter, dishes were checked daily to determine survival of embryos and fry. Dishes remained in the aquarium for 34 days. Fry were fed brine shrimp nauplii or dry commercial fish food daily.

Chemical analyses

Concentrations of Aroclor 1254 in water, eggs and fish were determined by electron capture gas-chromatography. Unfiltered water samples from each aquarium were analyzed weekly during the four-week exposures of adults. At the end of the adult exposure, concentrations were determined in the fertilized eggs from each fish and in surviving adult males and females. Also, fry that hatched from these eggs and survived for four weeks in PCB-free water were analyzed for Aroclor 1254 content. Analytical methods for water, eggs and fish were the same as those of Nimmo et al. (1971), except that an OV-101 column was used and all peak heights were summed for PCB quantification. Recovery efficiency of Aroclor 1254 exceeded 80%. Measured concentrations were not corrected for percentage recovery.

Statistical analysis

Probit analysis was used to determine whether increasing concentration of PCB in eggs increased the effect on fertilization success and on survival of embryos and fry. The χ^2 test for independent samples was used to compare data for eggs from individual unexposed and exposed fish. Differences were considered real at $\alpha = 0.05$ for probit analysis and $\alpha = 0.01$ for χ^2 tests.

RESULTS AND DISCUSSION

Aroclor 1254 in water was toxic to and accumulated by adult sheepshead minnows exposed for four weeks (Table 1). Mortality of fish was negligible, except in the aquarium receiving 10 µg/ℓ. Dying fish in this aquarium typically became lethargic, ceased feeding, and some developed fin rot. Fish accumulated the PCB in direct proportion to the concentration in the water and concentrations in fish ranged from 15,000 to 30,000 X the nominal concentration in the water. Concentrations in males and females were similar. Concentrations of the chemical in eggs from exposed adult fish were proportional to the concentrations in the fish and concentrations in female fish were 1.8 to 2.3 times greater than the concentrations in their eggs. The PCB exposure apparently did not alter the percentage of females producing eggs or their fecundity.

Fewer embryos and fry from eggs of exposed fish survived than did embryos and fry from eggs of control fish (Table 2). The percentage of the eggs fertilized was not affected, but survival of embryos to hatching was less in eggs from fish exposed to 10 µg/ℓ. Survival rate of fry in the first week following hatching was less in eggs from fish exposed to 0.32 to 10.0 µg/ℓ than in eggs from unexposed fish. The estimated LC50 was 6.1 µg/g; 95 percent confidence limit equals 3.5 to 11.8 µg/g. Fry typically began to die one or two days after hatching, about the time they started feeding. If fry survived the first week, there seemed to be no additional mortalities

related to PCB during three weeks of additional observation. Concentrations of Aroclor 1254 in surviving fry were similar, 0.26 - 0.56 µg/g, and not proportional to concentrations in eggs.

Embryo survival decreased at the highest concentration of PCB in eggs and fry survival decreased with increasing concentration of PCB in eggs. The amount in eggs was critical because it was the sole source of PCB for the embryos and fry reared in PCB-free water. PCB in milt was probably not critical to fertility and survival because when eggs from control fish were fertilized with milt from either control or 1.0 µg/ℓ exposed males, survival rate of embryos and fry was not altered (Table 2). Survival rate of fry hatched from eggs containing 7.0 µg/g or more of PCB was significantly less than the lowest survival rate of eggs from any of the five control fish (Table 3).

If the effect of PCB in eggs of other fishes is similar to that found with sheepshead minnows — and we have no data to support this view — then variations in published information concerning the chemicals relation to spawning success could be explained. Atlantic salmon eggs containing up to 1.9 µg/g of PCB had decreased fertility and survival of early embryos, but survival of late embryos and sac fry was unimpaired (Johannsson et al., 1970). Chesapeake Bay striped bass eggs containing PCB's had decreased fertility and survival of newly hatched fry (Anonymous, 1971). Our analysis of eggs from eleven striped bass from the Eastern shore of Chesapeake Bay showed that the eggs contained about 2.5 to 8.7 µg/g of a PCB resembling Aroclor 1254. Because concentrations of

PCB in eggs of sheepshead minnows as high as 201 µg/g were not accompanied by decreased fertility and only minimal embryo mortality, it seems unlikely that decreased fertility and embryo survival in Atlantic salmon and striped bass could be related solely to PCB in their eggs. Diminished survival of newly hatched striped bass fry, however, could be PCB-related since concentrations in their eggs were similar to those in sheepshead minnow eggs which produced fry whose survival was poor.

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Table 1. Toxicity and uptake of Aroclor® 1254 by adult sheepshead minnows (Cyprinodon variegatus) exposed for 28 days in an intermittent-flow bioassay. Thirty fish were tested per concentration. Residue analyses are for a minimum of 7 male and 17 female fish and eggs from 5 fish.

TEST CONCENTRATION (µg/l)		MORTALITY	CONCENTRATION IN FISH (µg/g, wet weight)			FEMALES GRAVID	AVERAGE FECUNDITY
Nominal	Measured	%	Males	Females	Eggs	%	No.
Control	ND*	7	0.64	0.47	0.52	100	97
0.1	0.09	13	2.5	1.9	0.88	89	121
0.32	0.14	7	9.7	9.3	5.1	100	110
1.0	0.39	10	—	25.	11.	100	127
3.2	1.1	3	49.	49.	27.	94	152
10.0	5.6	95	—	—	170**	100	138

* ND = not detectable, < 0.03 µg/l.

** Eggs from two fish.

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Table 2. Success of fertilization of eggs from sheepshead minnows exposed to Aroclor® 1254 for four weeks, survival of embryos from fertile eggs until hatching and survival of hatched fry. Eggs are from five fish per concentration (except two fish from 10 µg/ℓ). Percentages are in parentheses.

CONCENTRATION		EGGS			FRY	
Adult Exposure	Eggs Average	Tested	Fertile	Hatched	Survival Week 1	Weeks 2,3,4
(µg/ℓ)	(µg/g)					
Control	0.52	125	125 (100)	116 (93)	111 (95)	106 (96)
0.1	0.88	125	120 (96)	106 (88)	103 (97)	98 (95)
0.32	5.1	126	120 (95)	107 (80)	82 (77)*	76 (93)
1.0	11.	126	121 (96)	118 (98)	31 (26)*	26 (96)
3.2	27.	126	118 (94)	100 (85)	23 (23)*	19 (83)
10.0	170.	50	46 (92)	33 (72)*	0 (0)*	0 —
Control ♀ and 1.0 µg/ℓ ♂	—	128	119 (93)	113 (95)	112 (99)	111 (99)

* Significantly less than control hatching or one week fry survivals (χ^2: $\alpha = 0.01$)

Table 3. Comparison of concentration of Aroclor® 1254 in eggs (wet weight) from sheepshead minnows exposed to the PCB for four weeks and success of fertilization of eggs and survival of embryos and fry.

Concentration	EGGS			FRY		Adults
in Eggs	Tested	Fertile	Hatched	Week 1	Weeks 2,3,4	from Aquaria
(µg/g)						(µg/ℓ)
0.41	25	25	23	23	22	Control
0.44	25	25	24	19	19	"
0.45	25	25	25	25	24	"
0.53	25	25	24	24	23	"
0.57	25	24	19	18	18	0.1
0.76	25	25	20	20	18	Control
0.84	25	23	21	21	19	0.1
0.91	25	25	25	23	23	"
0.98	25	23	17	17	15	"
1.1	25	25	24	24	23	"
3.7	25	23	22	21	20	0.32
4.1	26	26	23	22	21	"
5.4	25	24	19	13	12	"
5.4	25	24	22	21	19	"
7.0	25	23	21	5	4	"
7.1	26	23	22	2	2	1.0
9.5	25	24	23	7	7	"
10.8	25	25	25	8	5	"

Table 3. (Continued)

Concentration in Eggs	EGGS			FRY		Adults from Aquaria
	Tested	Fertile	Hatched	Week 1	Weeks 2,3,4	
(µg/g)						(µg/ℓ)
13.2	25	25	25	9	7	1.0
13.3	25	24	23	5	5	"
23.6	25	23	23	4	4	3.2
25.7	25	24	16	6	6	"
27.9	25	25	23	0	0	"
28.6	25	25	25	5	3	"
28.7	26	21	13	8	6	"
145.	25	23	15	0	0	10.0
201.	25	23	18	0	0	"

AROCLOR® 1254: EFFECT ON COMPOSITION OF DEVELOPING ESTUARINE ANIMAL COMMUNITIES IN THE LABORATORY*

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Running page head: PCB Affects Estuarine Communities

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ABSTRACT

Aroclor® 1254, a polychlorinated biphenyl, affected the composition of communities of estuarine animals that developed from planktonic larvae in salt water that flowed through 10 control aquaria and 10 aquaria contaminated with 0.1, 1 or 10 µg/ℓ of this PCB. Communities that developed in control aquaria and aquaria that received 0.1 µg/ℓ of PCB in water for four months were dominated (>75%) by arthropods, primarily the amphipod Corophium volutator. In aquaria receiving 1 and 10 µg/ℓ, the number of arthropods decreased and the number of chordates, primarily the tunicate Molgula manhattensis, increased; over 75% of the animals in 10 µg/ℓ aquaria were tunicates. Numbers of phyla, species, and individuals (particularly amphipods, bryozoans, crabs, and molluscs) were decreased in this PCB, but there was no apparent effect on the abundance of annelids, brachipods, coelenterates, echinoderms or nemerteans. The Shannon-Weaver index of species diversity was not altered by Aroclor 1254.

INTRODUCTION

Polychlorinated biphenyls (PCBs) have been manufactured for various uses (Broadhurst, 1972) for over 40 years, but their occurrence in aquatic ecosystems was not confirmed until 1966 (Anonymous, 1966). Since then, PCBs have been detected in estuarine organisms from 6 of 15 of the coastal United States (P. A. Butler, 1973). One PCB, Aroclor 1254, was discovered in the water, sediment and biota of Escambia Bay, Florida (Duke et al., 1970).

Chronic and acute toxicity experiments conducted at the Gulf Breeze Laboratory have established that Aroclor 1254 is toxic to some estuarine organisms. A concentration of 100 µg/ℓ of Aroclor was acutely toxic (48 to 96 hours) to pink shrimp, Penaeus duorarum, and oysters, Crassostrea virginica, but not to pinfish, Lagodon rhomboides (Duke et al., 1970). Chronic toxicity was up to 100 times greater than acute toxicity. In exposures lasting more than two weeks, 1 µg/ℓ of Aroclor 1254 killed pink shrimp (Lowe et al., 1971a), whereas 5 µg/ℓ killed pinfish and spot, Leiostomus xanthurus (Hansen et al., 1971) and significantly reduced oyster growth rate (Parrish et al., 1972), but it was not lethal to blue crabs, Callinectes sapidus (Duke et al., 1970). Aroclor 1254 is, therefore, toxic to certain estuarine species exposed separately. However, its effect on communities of estuarine animals is not known. This study reports experiments that determined the effect of this chemical on development of estuarine animal communities in the laboratory.

MATERIALS AND METHODS

I investigated the effect of Aroclor 1254 on development of estuarine communities by comparing the number, species, and diversity of animals that grew from planktonic larvae in apparatuses continuously contaminated with 0.1, 1 or 10 $\mu g/l$ of PCB for four months, 18 May to 25 September 1970, with animals from an identical apparatus that was not contaminated.

The apparatus used in this investigation is illustrated in Fig. 1 (only one of four identical apparatuses is shown). Sea water with its natural component of plankton was pumped from the estuary adjacent to the laboratory into the primary constant head box. Salinity of the water ranged from 10 to 34 °/oo (average, 29.7 °/oo) and temperature ranged from 22 to 33 C (average, 28.5 C). In contaminated apparatuses Aroclor 1254 was added to water after it was siphoned, at the rate of 2,300 ml/min, from the primary into the secondary constant head box; the control apparatus received the same flow of water. Water then flowed from the secondary constant head box to each of 10 adjacent aquaria-10 replicates for each treatment. (Treatment includes control and contaminated apparatuses). Flow rate through each aquarium was maintained at 200 ml/min each by adjusting the height of a 2.7 mm-diameter hole in each of the 10 standpipes in the secondary constant head box. Each aquarium was 44 cm long, 9 cm wide and 14 cm high. Water depth in each aquarium was maintained at 8 cm. PCB-free sand was placed in each aquarium to a depth of 6 cm.

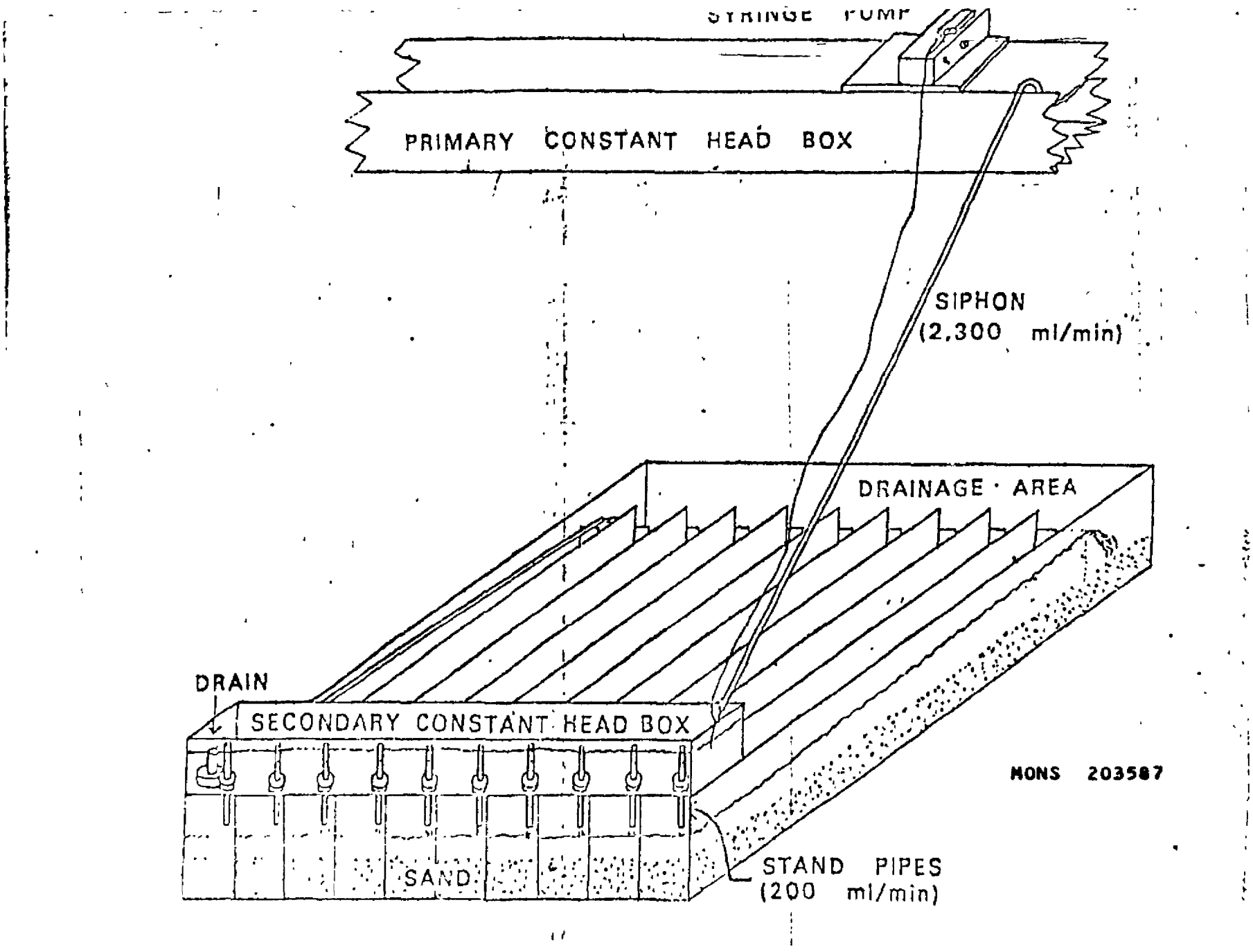
SYRINGE PUMP
PRIMARY CONSTANT HEAD BOX
SIPHON
(2,300 ml/min)
DRAINAGE AREA
DRAIN
SECONDARY CONSTANT HEAD BOX
SAND
STAND PIPES
(200 ml/min)
MONS 203587

Figure 1 -- Apparatus used to test the effect of Aroclor 1254 on composition of estuarine animal communities.

Planktonic larvae colonized this sand and the walls of the aquaria. The siphon and constant head boxes were cleaned weekly so that all aquaria received planktonic larvae from a common source-the incoming water. Water leaving each aquarium flowed through a V-shaped opening and into a common drain for the apparatus.

Aroclor 1254, dissolved in polyethylene glycol 200, was metered by a syringe pump into the water as it entered the secondary constant head box of each experimental apparatus. The same amount of polyethylene glycol (2 ml/day, 0.68 mg/ℓ) was metered into the control apparatus. Solvent-induced effect was not expected because: (1) polyethylene glycol 200 did not affect development of two species of crabs at this concentration (Epifanio, 1971) (2) concentrations up to 1% ($v/_v$) were not lethal to grass shrimp, Palaemonetes pugio, or sheepshead minnows, Cyprinodon variegatus, in 96 hours in static tests (Hansen, unpublished data) and (3) the toxicity of 5 µg/ℓ of Aroclor 1254 to brown shrimp, Penaeus aztecus, and pinfish was not increased by increasing the concentration of solvent up to 100 times (0.1 to 10.0 mg/ℓ) (Hansen, unpublished data).

Concentrations of Aroclor in test water and sediment were determined by gas chromatography. Methods of analysis for water are described by Nimmo et al. (1971a) and for sediment by Nimmo et al. (1971b), except that an OV-101 column was used. Recovery rates were above 70%, but data in this report do not include a correction factor for recovery. Water from the secondary constant head box of each

apparatus was analyzed twice a month (Table 1). Concentrations throughout this box were uniform; water from each standpipe, analyzed once during the 10 µg/ℓ exposure, averaged 7.9 µg/ℓ (range 6.8 – 8.7). Sediment cores from 4 of 10 aquaria from each apparatus were analyzed at the end of the exposure (Table 1).

At the end of the four-month exposure, animals were scraped from the sides of the aquaria and the contents of the aquaria siphoned into a 1 mm mesh sieve. During the last three months of the experiment, animals that left or were lost from the aquaria or were cleaned from the secondary constant head box and the drainage area were also collected in a 1 mm mesh sieve. Animals retained by the sieve were placed in fingerbowls of seawater relaxed with MgCl, preserved in 50% isopropanol and identified.

To determine effect of Aroclor 1254 in each treatment, an index of species diversity as well as the number and percent occurence of various species in each treatment (contaminated and control aquaria) were compared. A species diversity index provides a numerical means of assessing community structure that is independent of sample size, expresses the relative importance of each species and is dimensionless. Modifications of the Shannon-Weaver (1963) formula, $H' = -\sum_{i=1}^{s} p_i \log p_i$, where p_i is the proportion of the i^{th} species in the collection and s = the number of species, have been used in freshwater (Wilhm and Dorris, 1968) and saltwater (Bechtel and Copeland, 1970) to assess effects of pollution on natural communities. In unpolluted

areas, many species of animals are abundant and diversity is high, but pollution can decrease diversity by making a few species very abundant and all others rare. In this study, the Shannon-Weaver formula ($\log_2$) was used to determine the usefulness of the species diversity index in assessing the effect of Aroclor 1254 on community structure in laboratory experiments.

Pooled data from each Aroclor concentration and control were compared statistically using the χ^2 test for independent samples. Data from each of the 10 aquaria receiving one treatment were compared with data from 10 aquaria receiving a different treatment using the Mann-Whitney "U" test (Siegal, 1956). Differences were considered real at $\alpha = 0.01$.

RESULTS

A large number and variety of animals were found in all aquaria (Tables 2 & 3). Of the 67 species from nine phyla, 27 were mollusks (15 pelecypods and 12 gastropods), 23 annelids, 6 arthropods, 3 chordates, 2 coelenterates, 2 echinoderms, 2 nemerteans, 1 brachiopod and 1 bryozoan. Arthropods were most abundant (3,842) of the 5,897 animals followed by chordates (1,302), annelids (448), mollusks (219) and animals from other phyla (86). The two most abundant animals were the amphipod, Corophium volutator (3,770), and the tunicate, Molgula manhattensis, (1,164).

Species composition and abundance of individual species varied

among the 40 aquaria. The number of species in each aquarium ranged from 5 to 23 (average 13). Annelids and mollusks were present in all aquaria. Animals from other phyla were present in from 1 to 36 aquaria (Table 3). Seventeen species were found in all treatments and 26 were in only one.

Aroclor 1254 prevented animals of certain phyla from colonizing (Teble 3). Although nine phyla were found, the number of phyla represented in any aquarium ranged from three to seven. The number of phyla in the control, 0.1 and 1 $\mu g/\ell$ aquaria averaged 5.7, 5.4 and 5.7, respectively. Fewer phyla, average 4.1, were in aquaria contaminated with 10 $\mu g/\ell$ of Aroclor because fewer aquaria contained arthropods, and none contained bryozoans.

The total number of species found in each apparatus ranged from 25 to 52 but the percentage of species in each phylum was similar in all four treatments (Table 4). In each apparatus, more species of mollusks were found than species from any other phylum. The relative numbers of molluscan species were similar for all treatments (40 - 44 percent). The number of annelid species was only slightly less (29 - 35 percent). Although fewer arthropods and chordates were found, the relative numbers of each were similar in all treatments.

The number of species in each aquarium of an apparatus was altered by Aroclor 1254 (Tables 2 & 5). The total number of species and the number of species from each phylum in the ten

control, 0.1 and 1 µg/ℓ contaminated aquaria were similar. However, there were significantly fewer species and the species composition differed in the ten aquaria contaminated by 10 µg/ℓ of the PCB. The greatest shifts in species composition were found in arthropods, bryozoans, and molluska. Although there were significant reductions in the number of molluscan species in the 10 µg/ℓ aquaria, there was no difference in the gastropod - pelecypod ratio.

The total number of animals in each aquarium did not differ significantly among the four treatments; whereas the number and percentage occurrence of species was markedly different (Tables 2 & 6). Arthropods (primarily the tube-dwelling amphipod, Corophium volutator) were the dominant animals in the control (76 percent) and 0.1 µg/ℓ PCB (84 percent) aquaria. In these aquaria, chordates (primarily Molgula manhattensis) were secondarily abundant. Arthropods were also abundant (55 percent) in aquaria that received 1 µg/ℓ but a significant decrease in their abundance and an increase in abundance (31 percent) of chordates occurred. Dominance was different in aquaria contaminated by 10 µg/ℓ; 80 percent of the animals were chordates. This difference from communities dominated by arthropods in control aquaria and in aquaria contaminated by 0.1 µg/ℓ of Aroclor 1254 to communities dominated by chordates in aquaria receiving the highest concentration of this PCB was the most striking PCB-

induced effect in this experiment.

The abundance of animals of other phyla, although less striking, was also altered by PCB. There were more mollusks in the control aquaria than in treated aquaria, but the percentage of their occurrence was not different in the PCB environments. Colonies of the encrusting bryozoan, Membranipora tenuis, were not counted, and therefore their numbers are not adequately represented in Table 6. However, their exclusion from the ten aquaria contaminated with 10 µg/l was significant. Abundance of polychaetes was not altered by any of the three concentrations of PCB.

The Shannon-Weaver (1963) index of species diversity calculated for each aquarium did not differ among the control and three contaminated apparatuses (Table 7). Species diversity is a function of two components, richness (numbers of species (Table 5) and equitability or relative number of each species (Table 7) (Lloyd and Ghelardi, 1964). In my study, species diversity is not correlated ($r = 0.094$) with richness of species, but is correlated ($r = 0.882$) with relative abundance of each species: $J' =$ calculated diversity ÷ maximum diversity (Pielou, 1966). (Maximum diversity is defined as species diversity where all species are equally abundant.) Equitability did not differ between treatments because communities in this study were usually dominated by one species and were not rich in species. Therefore, the effect of Aroclor was not on species diversity but on species composition.

Conclusions based on abundance and diversity of animals collected at the end of the four-month exposure were corroborated by the abundance and diversity of animals that migrated from and were washed from each apparatus during the exposure (Table 8). The total number of animals and species from aquaria with 10 µg/ℓ was markedly lower than those from the other aquaria. Arthropods were abundant in collections from the effluents of control aquaria and aquaria with 0.1 and 1 µg/ℓ but rare from the aquaria with 10 µg/ℓ. The effect of Aroclor on crabs collected from the aquaria could not be assessed at the end of the exposure because only two were found in all aquaria. However, presence of exoskeltons in seven of 10 control, six of 10 0.1 µg/ℓ and eight of 10 1µg/ℓ aquaria and absence of exoskeltons in the 10 µg/ℓ aquaria strongly suggests that crabs were sensitive to highest concentration of Aroclor 1254. This sensitivity was substantiated by collections of crabs from the effluents of the aquaria. Eurypanopeus sp., Neopanope sp., Pinnixa sp., and portunids were abundant in effluents of control, 0.1 and 1 µg/ℓ aquaria but absent from the effluent of the 10 µg/ℓ aquaria. The bryozoan, N. tenuis, was absent in the effluent from the 10 µg/ℓ aquaria, but present in the effluent from the other aquaria. Mollusks were most abundant in effluent from the 10 control aquaria.

DISCUSSION AND CONCLUSIONS

The polychlorinated biphenyl, Aroclor 1254, influenced the composition of animal communities that developed from planktonic larvae in sea water which entered the test aquaria. The primary influence was that the dominant species in the control aquaria was the amphipod, C. volutator, and the dominant species in aquaria receiving 10 µg/ℓ PCB was the tunicate, M. manhattensis. Also, there were fewer species and the number of animals was markedly but not significantly fewer in the 10 µg/ℓ aquaria. The abundance of arthropods, chordates, bryozoans and mollusks differed significantly but abundance of annelids, brachiopods, coelenterates, echinoderms or nemerteans apparently did not differ.

Differences in community structure that were apparent at the lowest concentration (0.1 µg/ℓ) became more pronounced as the concentration of Aroclor 1254 increased to 10 µg/ℓ. Control aquaria were dominated by arthropods (76 percent), with lesser numbers of animals from eight other phyla. Aquaria contaminated by 0.1, 1 or 10 µg/ℓ contained fewer mollusks than did control aquaria; however, the percentage occurrence of mollusks was not altered by the PCB. Aquaria receiving 1 µg/ℓ or 10 µg/ℓ had more tunicates and fewer arthropods than did control aquaria. Aquaria receiving 10 µg/ℓ were dominated by tunicates (80 percent) with lesser numbers of five other phyla; only 1% of the animals were arthropods. Animals most reduced in numbers at the highest PCB concentration included

the amphipod, C. volutator; the xanthid crabs, Eurypanopeus depressus and Neopanope texana; the bryozoan, M. tenuis; the gastropod, Bittium varium; and the pelecypods, Abra aequalis and Tellina alternata.

Few of these changes could have been predicted from information from current literature, because only one species in this study had been challenged previously with PCB and only a few are phylogentically related to previously challenged species. Arthropods, particularly amphipods and crabs, were sensitive to Aroclor 1254 in this experiment and in the experiments by Nimmo et al. (1971a) in which pink shrimp were killed by 1 µg/ℓ. Juvenile blue crabs appeared resistant to 5 µg/ℓ (Duke et al., 1970). In my experiment, the numbers of xanthid crabs was reduced by 10 µg/ℓ indicating that larval stages may be particularly sensitive to PCBs. Sensivity of crab larvae has been shown with the insecticides dieldrin (Epifanio, 1971) and mirex (Boekhout et. al., 1972). Aroclor 1254 was lethal to two estuarine fishes at 5 µg/ℓ (Hansen et. al., 1971) but in this study lower chordates (tunicates) seemed unaffected and were most abundant in the PCB-stressed communities. Lethal effects of PCBs on mollusks are not known. However, growth of oysters was reduced significantly without mortality by 5 µg/ℓ of Aroclor 1254 (Farrish et al., 1972). In my experiment fewer mollusks occurred in all exposure concentrations possibly because the larval stages are sensitive or because

of factors other than the presence of Aroclor. Studies on protozoans (Cooley, Keltner, and Forester, 1972) provided the only other data I am aware of on the sensitivity of estuarine animals of other phyla to PCBs.

The Shannon-Weaver species diversity index has been used as an indicator of the effects of some types of pollution on animal communities in estuaries, but in this experiment, the index was not decreased even though composition of the communities were greatly altered. This species diversity index did not decrease as the concentration of PCB increased because the index was proportional to the relative number of each species present in the aquaria, and communities that developed at each treatment concentration were dominated by one species of animal. If Aroclor 1254 affected the composition of established communities in an estuary as it did the developing communities in this experiment, this species diversity index could not be used to estimate effects of this pollutant in the environment.

One purpose of this experiment was to determine whether the effect of a toxicant on developing estuarine animal communities can be investigated in the laboratory. Preliminary experiments at this laboratory indicated that the structure of communities of organisms setting in aquaria was altered by presence of the insecticide Dursban® (J. I. Lowe, personal communication[2]). My analysis of his data indicated that replicate aquaria were necessary to separate the effects of a toxicant from the effects

of an efficient predator or from the effects of an animal with great reproductive capacity. The use of ten replicate aquaria for each treatment in my experiment readily separated the effect of Aroclor 1254 from other factors that might influence community structure. My experiment also showed that small aquaria can be used, provided that larger animals can emigrate before they drastically affect community structure and thus mask effects of the toxicant. Emigrating animals must be caught and enumerated so the effects on them can be assessed.

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Table 1. Range and average concentration of Aroclor® 1254 in water and sediment from experimental and control apparatuses. Water was analyzed twice monthly and sediment was analyzed at the end of the four month experiment. Limit of quantification was 0.1 µg/ℓ in water and 0.015 mg/kg (dry weight) in sediment. Correction for recovery (>70%) is not included.

Concentration in water, µg/ℓ			Concentration in sediment, µg/g	
Nominal	Measured		Measured	
	Average	Range	Average	Range
Control	None		None	
0.1	<0.1	<0.1 – 0.1	0.1	0.05 – 0.18
1.0	0.6	0.48 – 0.72	0.36	0.25 – 0.42
10.0	6.7	5.2 – 7.8	2.0	1.5 – 2.5

collected from control aquaria and from aquaria contaminated for four months with Aroclor

used for each treatment. Number of animals and number of aquaria from which they were collected are listed.

	Control		Aroclor 1254						Total
			0.1 µg/l		1 µg/l		10 µg/l		
	Animals	Aquaria	Animals	Aquaria	Animals	Aquaria	Animals	Aquaria	Animals
Family									
[illegible] gracilis	8	5	11	4	3	2	0	0	22
Capitella capitata	34	10	30	8	34	9	23	8	121
Cirratulus sp.	1	1	0	0	1	1	0	0	2
[illegible] sp.	0	0	0	0	1	1	0	0	1
Leitoscoloplos acutus	6	5	8	6	11	6	9	6	34
[illegible] p. obtusicola	8	4	13	9	5	4	17	9	43
Heteromastus filiformis	0	0	0	0	2	1	0	0	2
Lumbrineris cruzeri	1	1	0	0	0	0	0	0	1
[illegible] parvapedata	1	1	0	0	0	0	0	0	1
[illegible]	1	1	0	0	0	0	0	0	1
[illegible] californiensis	0	0	0	0	0	0	1	1	1
[illegible] saccata	8	6	11	7	12	6	9	7	40
Notomastus hemipodus	0	0	0	0	1	1	0	0	1
[illegible]	1	1	1	1	0	0	0	0	2
[illegible]	28	10	35	10	51	8	22	4	136
[illegible] sp.	2	2	1	1	1	1	0	0	4
[illegible] sp.	1	1	2	1	0	0	0	0	3
[illegible]	1	1	0	0	0	0	0	0	1
[illegible]	6	4	7	4	3	3	0	0	16
[illegible]	0	0	0	0	0	0	3	1	3
[illegible] sp.	6	1	0	0	0	0	0	0	6
[illegible] sp. #1	2	2	0	0	2	1	1	1	5
[illegible] sp. #2	1	1	0	0	1	1	0	0	2

Table 2. (Continued)

TAXON	Control Animals	Control Aquaria	Aroclor 1254 0.1 µg/l Animals	0.1 µg/l Aquaria	1 µg/l Animals	1 µg/l Aquaria	10 µg/l Animals	10 µg/l Aquaria	Total Animals
Arthropoda									
Balanus sp.	6	4	2	2	9	6	2	1	19
Caprella sp.	0	0	11	1	3	3	0	0	14
Corophium volutator	1338	10	1693	10	736	10	3	3	3770
Neocaprope texana	2	2	0	0	0	0	0	0	2
Lyporeia	0	0	0	0	0	0	1	1	1
Decapod larvae, unident. sp.	0	0	0	0	4	3	0	0	4
Pycnogonidae, unident. sp.	0	0	3	1	28	8	1	1	32
Brachiopoda									
Glottidia pyramidata	1	1	0	0	0	0	0	0	1
Chordata									
Bertrichobranchus bilobatus	100	1	2	2	3	1	29	5	134
Branchiostoma caribaeum	2	1	1	1	0	0	0	0	3
Yoldia sapotteensis	91	7	141	10	434	9	499	10	1164
Coelenterata									
Leptomedusae*, unident. sp.	5	5	4	4	8	8	7	7	24
Zoantharia, unident. sp.	0	0	0	0	3	3	0	0	5

Table 7. (Continued)

TAXON	Control Animal	Control Aquaria	Aroclor 1254 0.1 µg/l Animal	0.1 µg/l Aquaria	1 µg/l Animal	1 µg/l Aquaria	10 µg/l Animal	10 µg/l Aquaria	Total Animals
Echinodermata									
Dendraster excentricus	4	2	17	4	13	6	0	0	1
Amphiodia sp.	1	1	0	0	0	0	0	0	56
Entoprocta									
Membranipora tenuis*	6	6	6	6	4	4	0	0	16
Mollusca									
Acar pectinis	15	8	11	6	5	5	0	0	31
Argopecten papyria	10	6	4	3	0	0	6	6	20
Arcopsis translirata	2	2	0	0	0	0	0	0	2
Astarte transversa	6	4	4	4	3	3	6	5	19
Cerastoderma costata	5	4	1	1	1	1	1	1	8
Crassinella varium	16	7	4	4	7	4	1	1	28
Crenella pulchellum	0	0	2	2	0	0	0	0	2
Crassostrea virginica	4	3	5	4	4	3	4	4	17
Crepidula fornicata	0	0	0	0	0	0	3	3	3
Epitonium humphreysi	0	0	0	0	1	1	0	0	1
Laevicardium mortoni	9	5	3	3	2	2	4	3	16
Lyonsia hyalina	2	1	1	1	1	1	0	0	4
Mysella fragilis	0	0	1	1	1	1	0	0	2
Nuculana lenata	2	2	0	0	1	1	0	0	3
Modiolus americanus	1	1	0	0	1	1	1	1	3
M. demissus	1	1	1	1	2	1	0	0	4

Table 2. (Continued)

TAXON	Control		Aroclor 1254						Total Animals
			0.1 µg/l		1 µg/l		10 µg/l		
	Animal	Aquaria	Animal	Aquaria	Animal	Aquaria	Animal	Aquaria	
Mollusca (Continued)									
Mulinia lateralis	5	3	0	0	1	1	0	0	6
Mya arenaria	7	4	3	2	2	1	3	2	15
Nassarius albus	1	1	0	0	2	2	0	0	3
N. vibex	0	0	0	0	1	1	0	0	1
Ostrea equestris	1	1	0	0	1	1	0	0	2
Retusa canaliculata	1	1	0	0	0	0	0	0	1
Rissoina catesbyana	1	1	0	0	0	0	0	0	1
Tagelus divisus	0	0	1	1	1	1	0	0	2
Tellina alternata	9	7	3	3	7	7	1	1	20
Eulimacea, unident. sp.	1	1	0	0	0	0	0	0	1
Gastropoda, unident. sp.	2	1	0	0	0	0	0	0	2
Nemertinea									
Cerebratulus dorsalis	2	2	0	0	0	0	0	0	2
Unidentified sp.	1	1	0	0	0	0	0	0	1

*Colonies: Counted as one animal.

Table 3. Number of control and experimental aquaria that contained animals, by phylum. Ten aquaria were used for each control and contaminated apparatus. Experimental aquaria were contaminated continuously with Aroclor 1254 for four months.

Phylum	Control Aquaria	Aroclor 1254 Aquaria		
		0.1 ppb	1.0 ppb	10.0 ppb
Annelida	10	10	10	10
Arthropoda	10	10	10	4
Branchiopoda	1	0	0	0
Chordata	7	10	9	10
Coelenterata	5	4	9	7
Echinodermata	3	4	6	0
Ectoprocta	8	6	4	0
Mollusca	10	10	10	10
Nemertea	3	0	0	0
Total phyla in apparatus	9	7	7	6
Average number of phyla per aquarium	5.7	5.4	5.9	4.1

Table 5. Number of species, by phylum, that developed from planktonic larvae in 10 control aquaria and 10 aquaria in each apparatus contaminated continuously for four months with 0.1, 1 or 10 µg/l of Aroclor 1254.

Taxon	Control		Aroclor 1254					
			0.1 µg/l		1.0 µg/l		10.0 µg/l	
	Average number per aquarium	Range	Average number per aquarium	Range	Average number per aquarium	Range	Average number per aquarium	Range
Annelida	5.7	2-10	5.1	3-7	4.5	2-6	3.9	3-5
Arthropoda	1.6	1-2	1.4	1-3	2.8	2-4	0.6	0-3
Chordata	0.9	0-3	1.3	1-2	1.0	0-2	1.3	1-2
Mollusca	6.3	2-10	3.6	1-7	3.9	1-6	2.7	1-5
Other phyla	1.8	0-3	1.4	0-3	2.1	1-4	0.7	0-1
TOTAL	16.3	7-23	12.8	9-18	14.3	7-18	9.4	5-15

Table 4. Number of species, by phylum, that developed from planktonic larvae in control apparatus and in apparatuses contaminated continuously for four months with 0.1, 1 or 10 µg/ℓ of Aroclor 1254. Each apparatus consisted of 10 aquaria.

	Control		Aroclor 1254					
			0.1 µg/ℓ		1 µg/ℓ		10 µg/ℓ	
Taxon	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Annelida	18	34.6	10	29.4	14	32.6	8	32.0
Arthropoda	3	5.8	4	11.8	4	9.3	4	16.0
Chordata	3	5.8	3	8.8	2	4.6	2	8.0
Mollusca	21	40.4	14	41.2	19	44.2	10	40.0
Other phyla	7	13.4	3	8.8	4	9.3	1	4.0
TOTAL	52	100.0	34	100.0	43	100.0	25	100.0

Table 6. Average number per aquarium and average percent frequency per aquarium of animals, by phylum (range in parentheses), that developed from planktonic larvae in 10 control aquaria and 10 aquaria that for four months received 0.1, 1 or 10 µg/l of Aroclor 1254.

Phylum	Control		Aroclor 1254					
			0.1 µg/l		1 µg/l		10 µg/l	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Annelida	11.6 (3-23)	6.5 (1.8-21.4)	11.9 (4-21)	5.8 (1.3-42.8)	12.8 (7-22)	9.0 (3.9-38.5)	8.5 (5-16)	12.9 (5.3-66.7)
Arthropoda	134.6 (6-406)	75.8 (12.7-94.0)	170.9 (14-528)	83.6 (28.6-96.2)	78.0 (8-199)	54.9 (20.5-86.9)	0.7 (0-4)	1.1 (0-2.8)
Chordata	19.3 (0-112)	10.9 (0-64.7)	14.4 (1-52)	7.0 (0.6-19.4)	43.7 (0-130)	30.8 (0-59.1)	52.8 (3-160)	80.4 (25.0-88.0)
Mollusca	10.1 (3-18)	5.7 (0.7-28.6)	4.4 (1-10)	2.2 (0.8-12.2)	4.4 (1-9)	3.1 (1.3-33.3)	3.0 (1-6)	4.6 (1.9-9.0)
Other phyla	2.0 (0-4)	1.1 (0-7.1)	2.7 (0-11)	1.4 (0-6.5)	3.2 (1-7)	2.2 (0.9-7.7)	0.7 (0-1)	1.0 (0-3.6)
TOTAL	177.6 (14-432)	100.0	204.3 (49-589)	100.0	142.1 (24-239)	100.0	65.7 (12-186)	100.0

Table 7. Shannon-Weaver index of species diversity and index of species richness in the ten control aquaria and ten aquaria contaminated with 0.1, 1 or 10 µg/l of Aroclor 1254.

	Control			Aroclor 1254								
				0.1 µg/l			1.0 µg/l			10.0 µg/l		
	Mean	Range	Std. Error	Mean	Range	Std. Error	Mean	Range	Std. Error	Mean	Range	Std. Error
Species diversity	1.80	0.57-2.83	0.24	1.42	0.36-3.17	0.26	2.07	0.94-3.26	0.20	1.62	1.03-2.19	0.13
Equitability (J)	0.47	0.14-0.89	0.07	0.38	0.10-0.81	0.07	0.55	0.25-0.86	0.06	0.53	0.26-0.94	0.06

Table 8. Species and total number of animals collected from the effluents of 10 control aquaria and 10 aquaria contaminated for four months with 0.1, 1 or 10 µg/l Aroclor 1254.

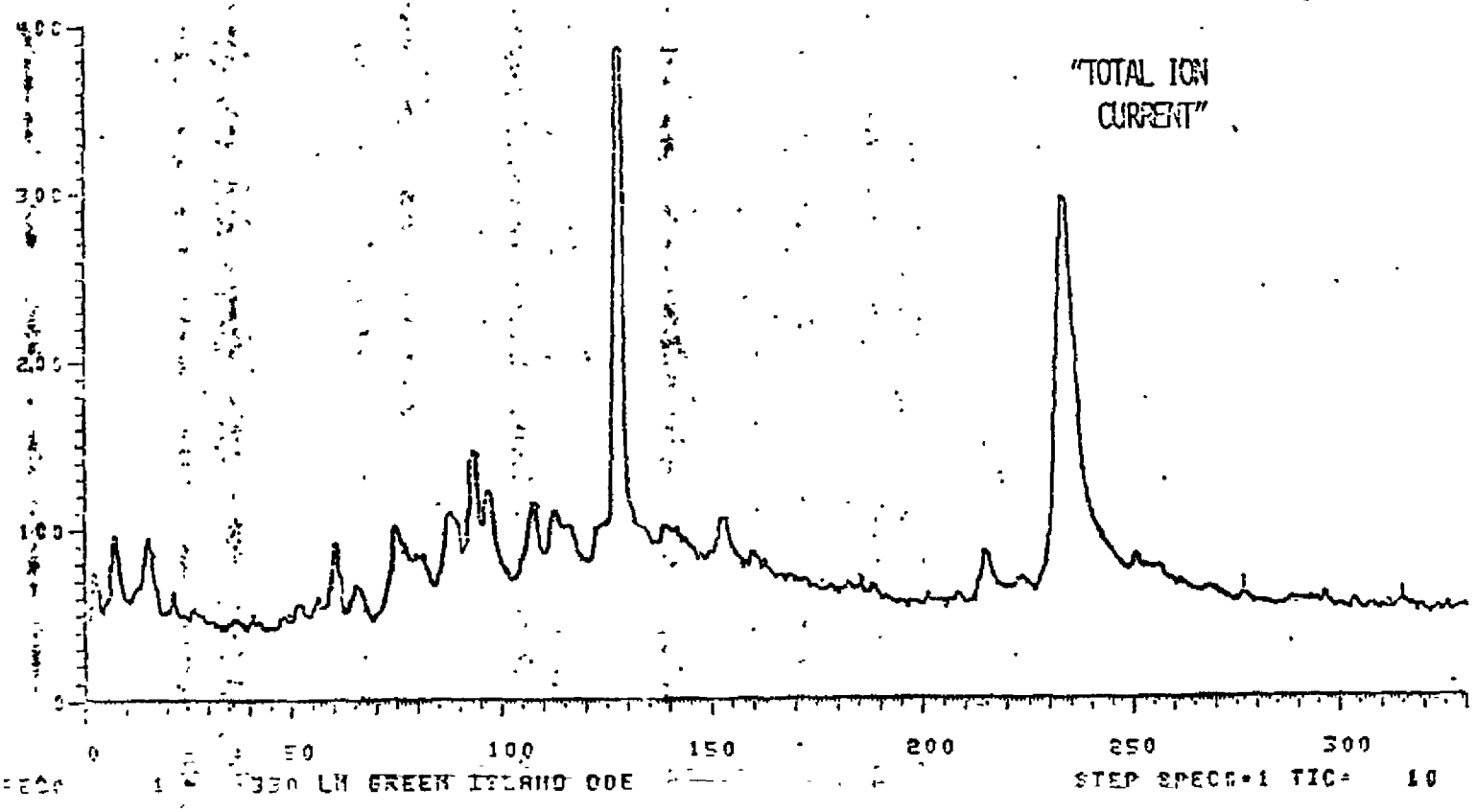
Taxon	Control	0.1 µg/l	1 µg/l	10 µg/l
Annelida				
Eupomatus dianthus	1	1	0	0
E. protulicola	0	1	1	0
Neanthes succinea	1	2	2	0
Total	2	4	3	0
Arthropoda				
Balanus sp.	10	2	2	1
Caprella sp.	0	8	4	0
Elibanarius tricolor	0	1	0	0
Corophium volutator	7	15	32	1
Eurypanopeus depressus	5	2	5	0
Neopanope texana	10	6	3	0
Pagurus longicarpus	1	0	0	0
Pinnixa chaetopterana	1	0	0	0
Upogebia affinis	1	1	0	0
Decapod zoea, unident. sp.	0	0	1	0
Portunidae, unident. sp.	2	1	0	0
Pycnogonidae, unident. sp.	0	0	3	0
Total	37	36	50	2
Chordata				
Boltrichobranchus pilularis	3	0	0	0
Branchiostoma caribaeum	1	0	1	0
Molgula manhattensis	72	103	104	35
Total	76	103	105	35
Coelenterata				
Leptomedusae*, unident. sp.	1	1	1	1
Echinodermata				
Hemipholis elongata	1	0	3	0
Ectoprocta				
*Membranipora tenuis**	1	1	1	0
Mollusca				
Anadara ovalis	1	0	0	0
A. transversa	12	0	4	1
Bittium alternata	0	1	0	0
B. varium	3	0	1	0
Crassostrea virginica	2	0	1	1
Doridella obscura	12	7	2	1
Laevicardium mortoni	1	0	0	0
Mitrella lunata	2	0	2	2

Table 8. (Continued)

Taxon	Control	0.1 µg/ℓ	1 µg/ℓ	10 µg/ℓ
Mollusca (Continued)				
Musculus lateralis	1	0	1	0
Nassarius albus	2	0	0	0
Tagelus divisus	1	2	0	0
Eolidacea, unident. sp.	1	1	1	3
Total	38	5	12	8
TOTALS: Animals	156	150	375	47
Species	27	18	20	10

*Colonies: Counted as one animal.

Figure 16. Total Ion Current Chromatogram of the Polar Gluan. Fraction (Silicic Acid) of a Green Bay Trout Extract



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