figures 6-7, 8, 9
residues from environ - laboratory

2

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.

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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.

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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

DSW 027592

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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

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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

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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

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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).

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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 laboratory 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

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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.

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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

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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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References

Butler, P. A.: Pesticides in the marine environment. J. Appl. Ecol. 3 (suppl.), 253 (1966).

Cooley, N. R., J. M. Keltner, Jr., and J. Forester: Mirex and Aroclor® 1254: Effect on and accumulation by Tetrahymena pyriformis W. J. Protozool. 19(4), 636 (1972).

Couch, J. A.: Histopathologic effects of pesticides and related chemicals on the livers of fishes. Proc. Fish Disease Symposium. Armed Forces Inst. Path., Univ. of Wisconsin Press (In press) (1972).

Dahlgren, R. B., R. L. Linden, and C. W. Carlson: Polychlorinated biphenyls: their effects on penned pheasants. Environ. Health Perspect. 1, 89 (1972).

Duke, T. W., J. I. Lowe, and A. J. Wilson, Jr.: A polychlorinated biphenyl (Aroclor® 1254) in the water, sediment, and biota of Escambia Bay, Florida. Bull. Environ. Contam. Toxicol. 5, 171 (1970).

Friend, M., and D. O. Trainer: Polychlorinated biphenyl: interaction with duck hepatitis virus. Science 17, 1314 (1970).

Hansen, D. J., P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr., and P. D. Wilson: Chronic toxicity, uptake, and retention of Aroclor® 1254 in two estuarine fishes. Bull. Environ. Contam. Toxicol. 6, 113 (1971).

Lowe, J. I., P. R. Parrish, J. M. Patrick, Jr., and J. Forester: Effects of the polychlorinated biphenyl Aroclor 1254 on the oyster Crassostrea virginica. Mar. Biol. 11(3), 209 (1972).

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Lowe, J. I., P. R. Parrish, A. J. Wilson, Jr., P. D. Wilson and T. W. Duke: Effects of mirex on selected estuarine organisms. Trans. 36th N. Am. Wildl. Nat. Resour. Conf., p. 171 (1971).

Nimmo, D. R. and R. R. Blackman: Effects of DDT on cations in the hepatopancreas of penaeid shrimp. Trans. Am. Fish. Soc. 101(3), 547 (1972).

___, R. R. Blackman, A. J. Wilson, Jr., and J. Forester: Toxicity and distribution of Aroclor® 1254 in the pink shrimp Penaeus duorarum. Mar. Biol. 11(3), 191 (1971a).

___, P. D. Wilson, R. R. Blackman and A. J. Wilson, Jr.: Polychlorinated biphenyl absorbed from sediments by fiddler crabs and pink shrimp. Nature 231 (5297), 50 (1971b).

Norback, D. H., and J. R. Allen: Chlorinated aromatic hydrocarbon induced modifications of the hepatic endoplasmic reticulum: concentric membrane arrays. Environ. Health Perspect. 1, 137 (1972).

Sodergren, A., Bj. Svensson, and S. Ulfstrand: DDT and PCB in South Swedish streams. Environ. Pollut. 3, 25 (1972).

Vos, J. G.: Toxicology of PCBs for mammals and for birds. Environ. Health Perspect. 1, 105 (1972).

Wildish, D. J.: The toxicity of polychlorinated biphenyls (PCB) in sea water to Gammarus oceanicus. Bull. Environ. Contam. Toxicol. 5, 202 (1970).

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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): sea-grasses, 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 sea-trout, Cynoscion nebulosus; spot, Leiostomus xanthurus; Atlantic croaker, Micropogon undulatus; hogchoker, Trinectes maculatus;

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and Atlantic cutlassfish, Trichiurus lepturus.

Table 2. Effect of Aroclor 1254 on population growth of Tetrahymena pyriformis W. (After Cooley et al., 1972)

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

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.

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

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

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

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

Figure 11. Normal vesicular connective tissue (parenchyma) from control oyster. Note uniform cell patterns and distribution 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 many 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.

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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.

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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.

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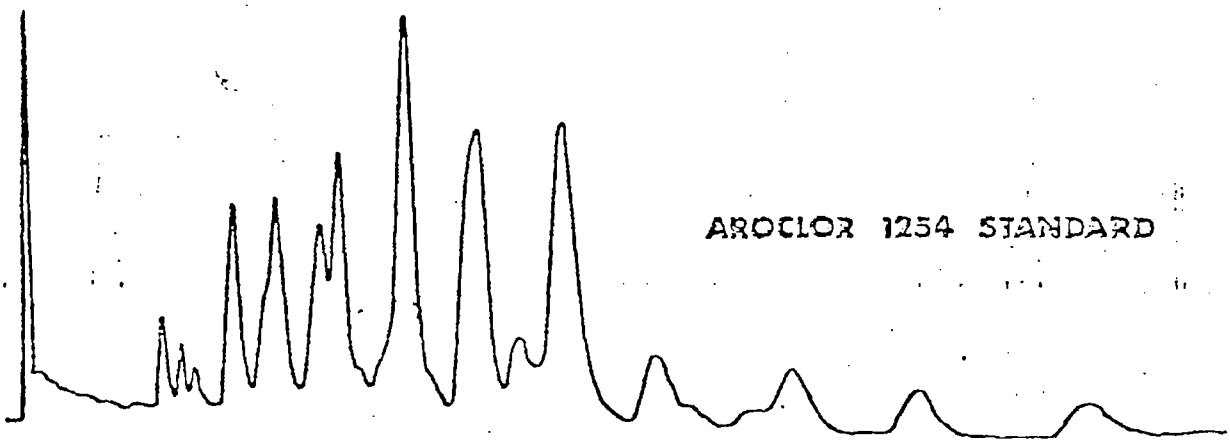
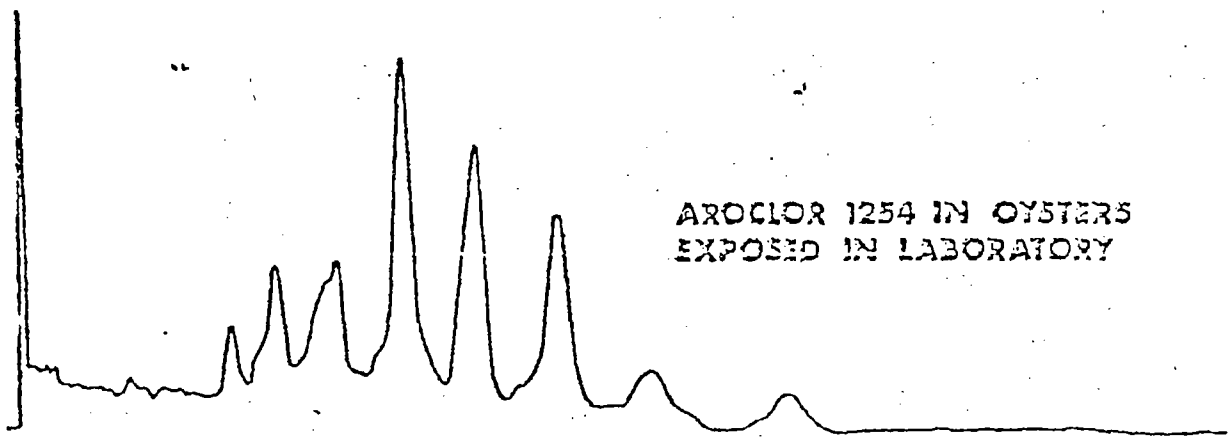
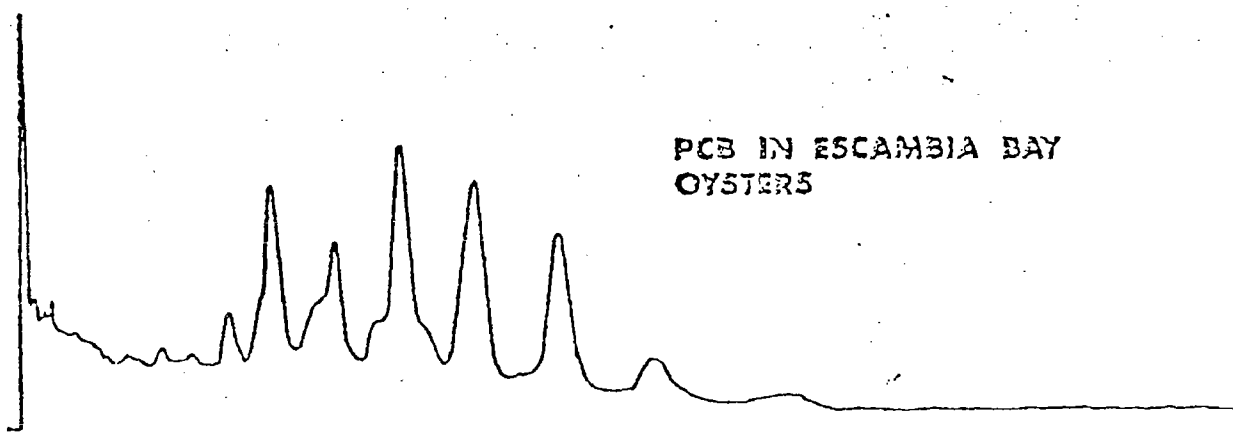


AROCLOR 1254 STANDARD



AROCLOR 1254 IN OYSTERS
EXPOSED IN LABORATORY



PCB IN ESCAMBIA BAY
OYSTERS

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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 2.3×10^{1}
Escambia Bay, Fla.	Fishes	17	100	3.99	0.29-20. 6.7×10^{1}

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

$\frac{.81 \text{ ppm}}{.03}$ $\frac{8.1 \times 10^{-1}}{3 \times 10^{-5}}$ 2.7×10^{4} 27,000

2.3

2.3×10^{4}

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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 Mimmo et al., 6).

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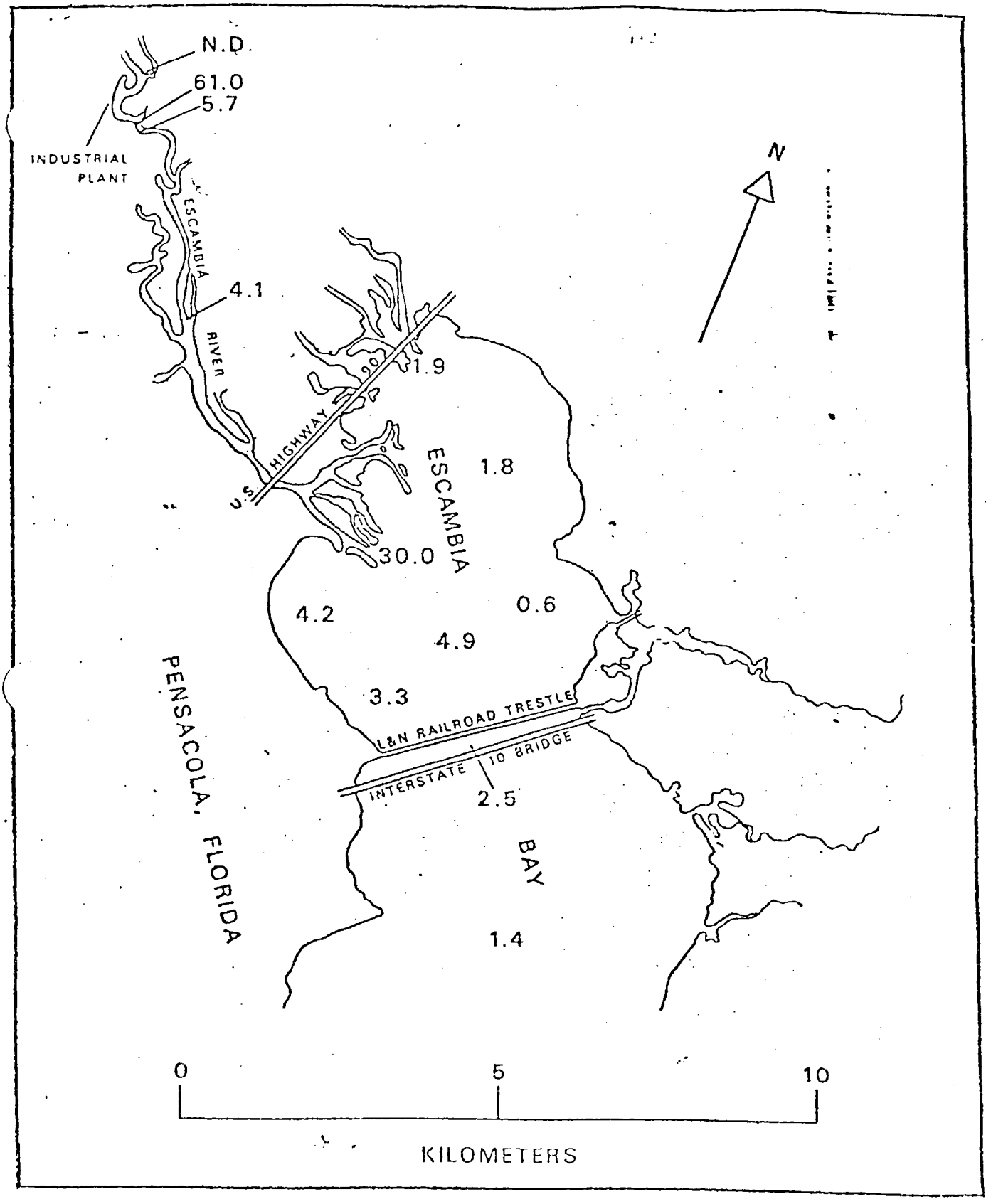

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Figure 3. Comparison of concentrations of Aroclor 1254 in cores taken 10 months apart in upper Escambia Bay and River. Concentrations given in 1971 above and below trestle are averages of 2 cores each.

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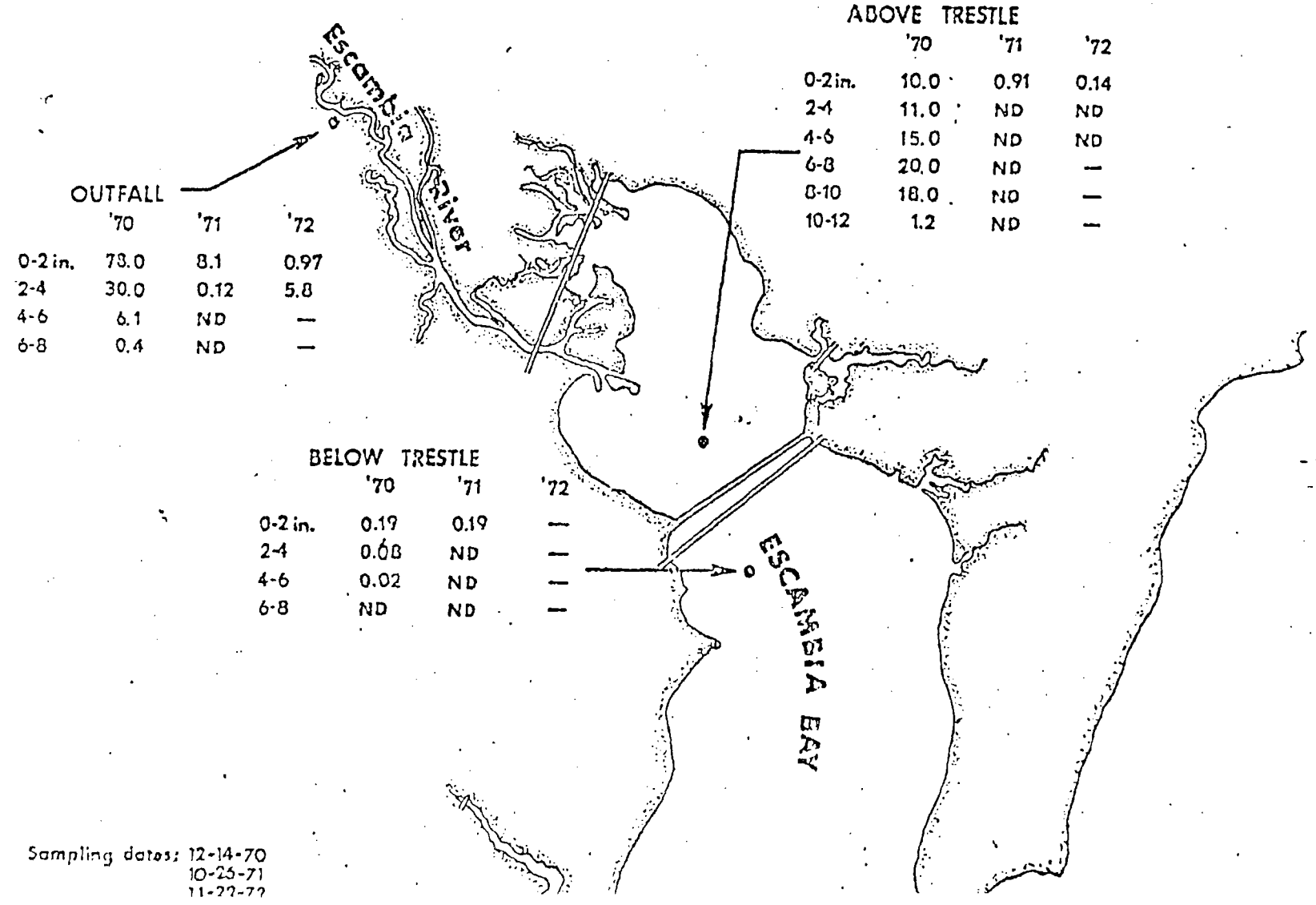
SEDIMENT CORE CONCENTRATIONS (ppm) OF AROCLOR



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	0.68	ND	—
4-6	0.02	ND	—
6-8	ND	ND	—

Sampling dates: 12-14-70
10-25-71
11-22-72

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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.

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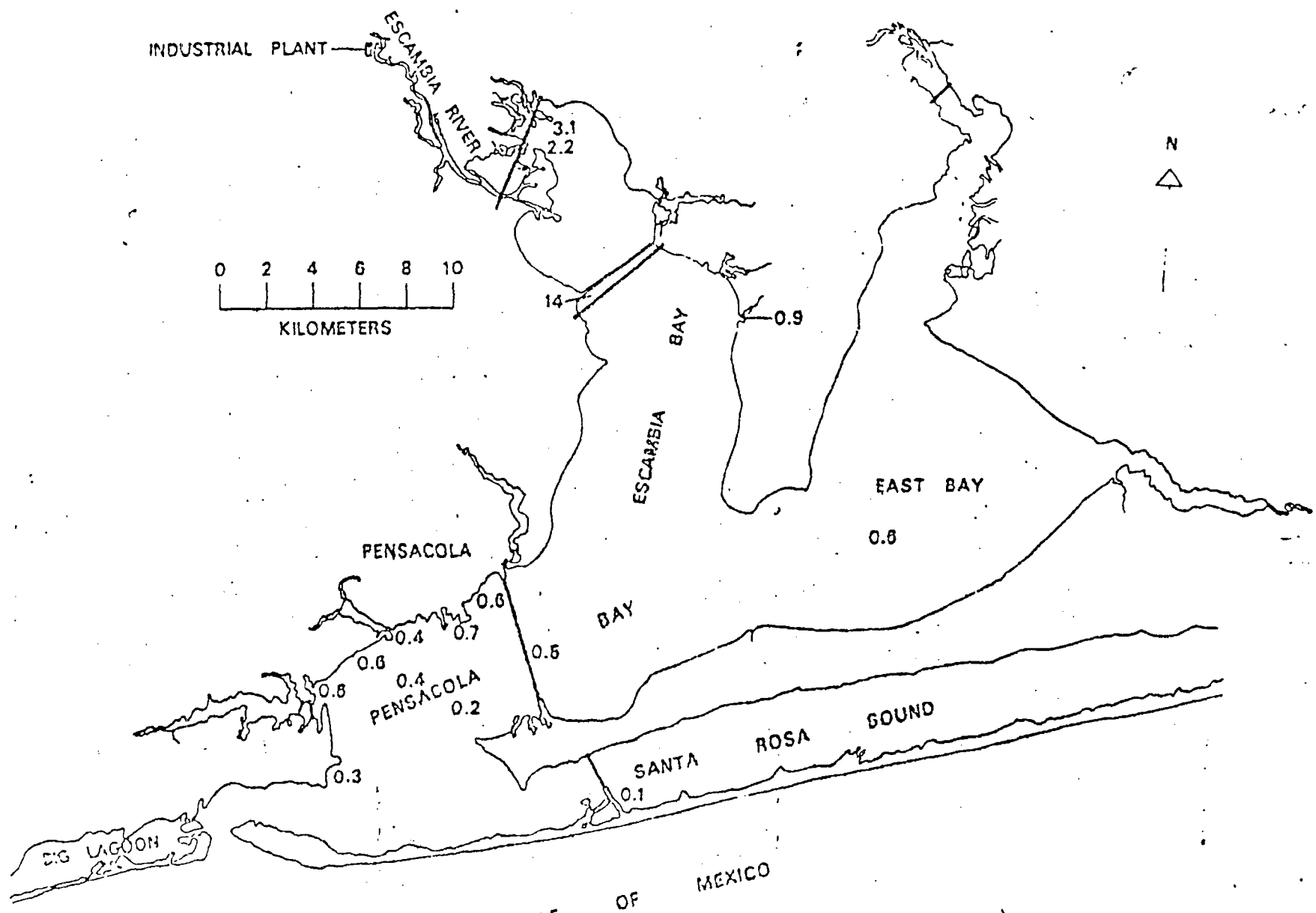

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Figure 5. Comparison of concentrations of Aroclor 1254 found in species collected in Escambia (left side) and East Bays (right side): seagrasses, Spartina sp. and Zostera marina; Rangia clams, Rangia cuneata; olive nerite, Neritina reclivata; brown 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; and Atlantic cutlassfish, Trichiurus lepturus.

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AROCLOR® 1254

(ppm) ND	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

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

Toxicant (µg/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.

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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.

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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.

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DISTRIBUTION OF AROCLOR 1254 IN TISSUES OF SHRIMP

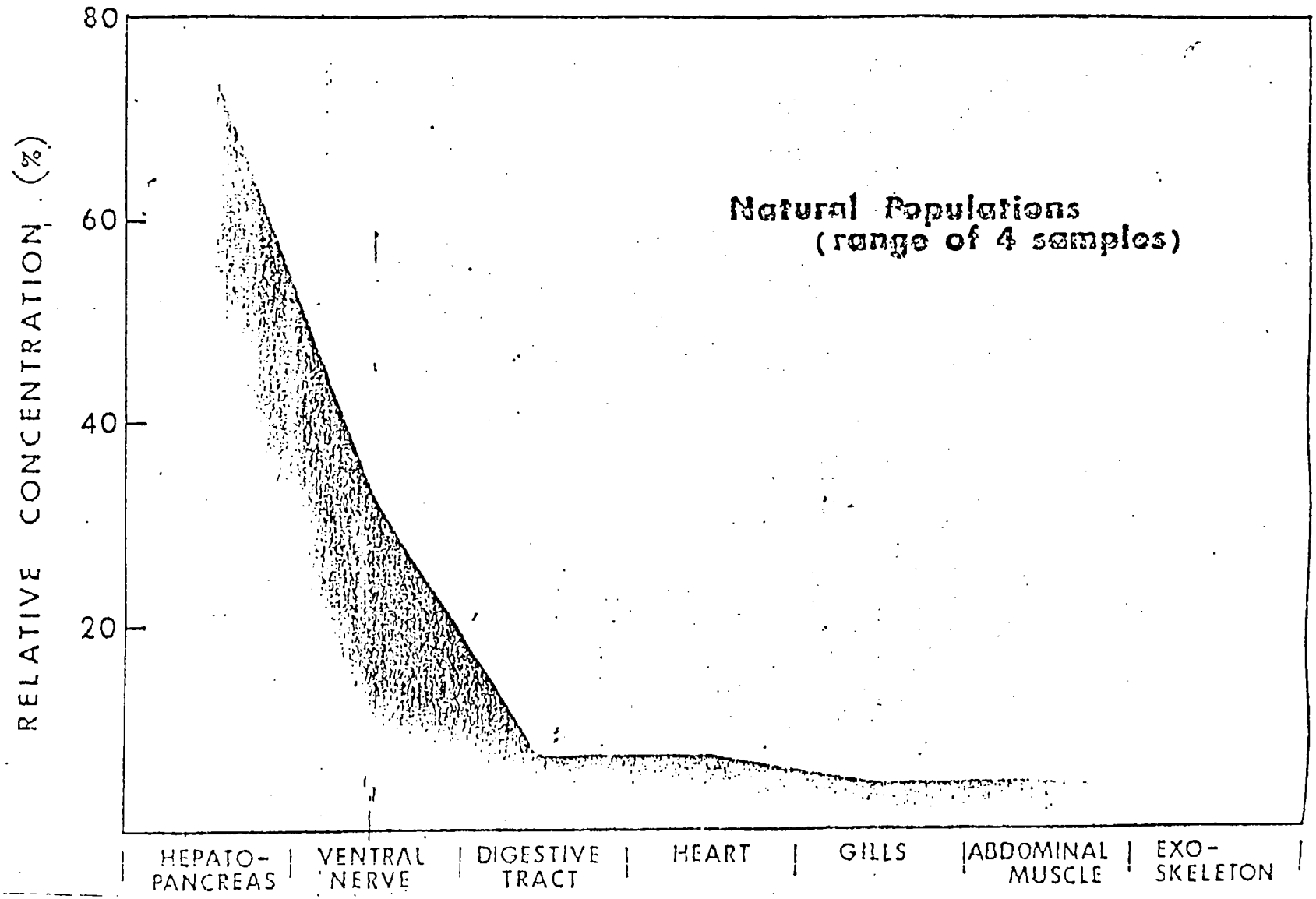

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Figure 7. Distribution of Aroclor 1254 in tissues of shrimp fed Aroclor 1254-contaminated diets.

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DISTRIBUTION OF AROCLOR 1254 IN TISSUES OF SHRIMP

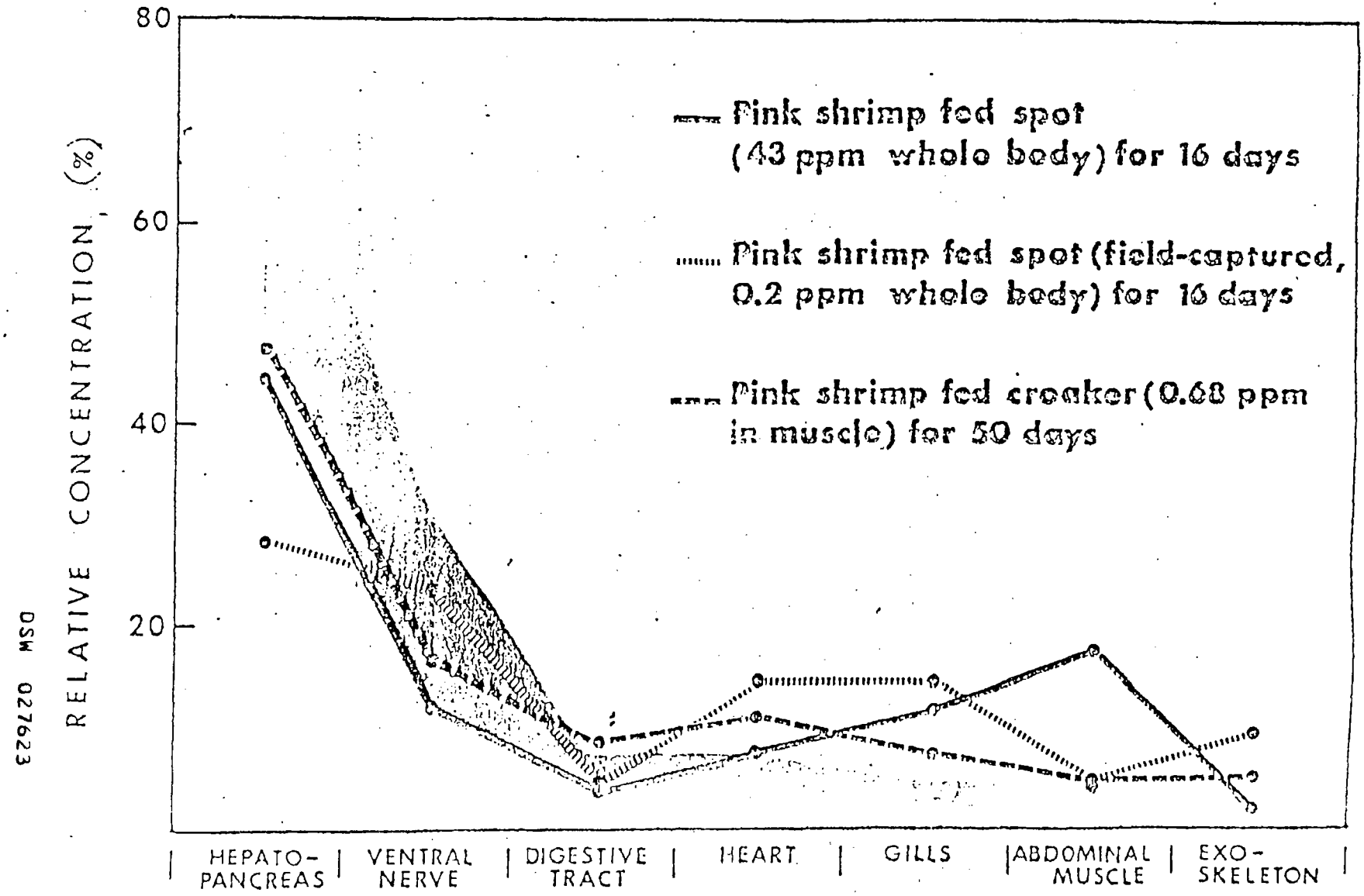

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Figure 8. Distribution of Aroclor 1254 in tissues of shrimp exposed to Aroclor 1254 in unfiltered and unfiltered seawater.

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DISTRIBUTION OF AROCLOR 1254 IN TISSUES OF SHRIMP

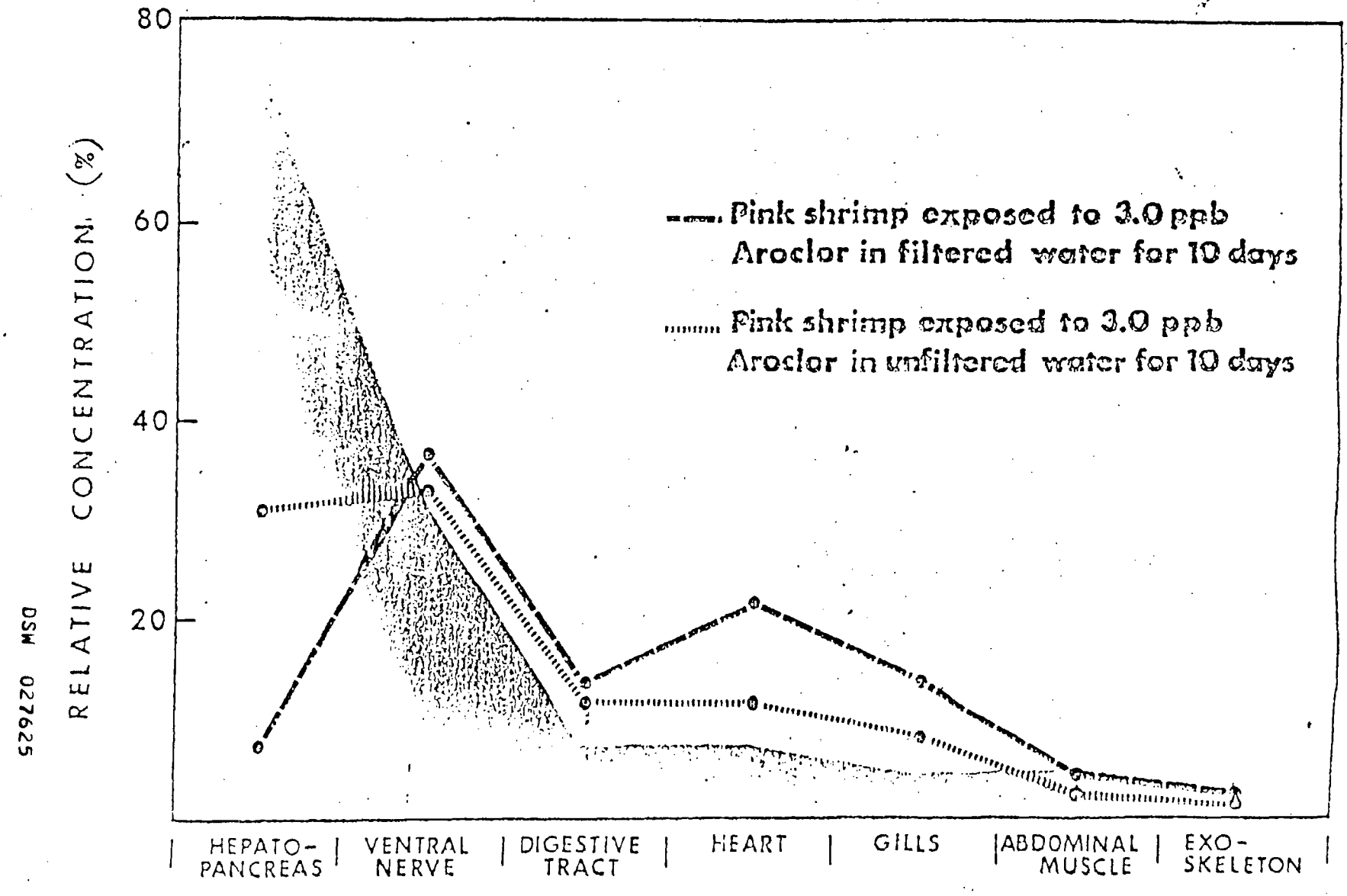

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Figure 9. Distribution of Aroclor 1254 in tissues of shrimp exposed to the chemical in flowing-water aquaria.

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DISTRIBUTION OF AROCLOR 1254 IN TISSUES OF SHRIMP

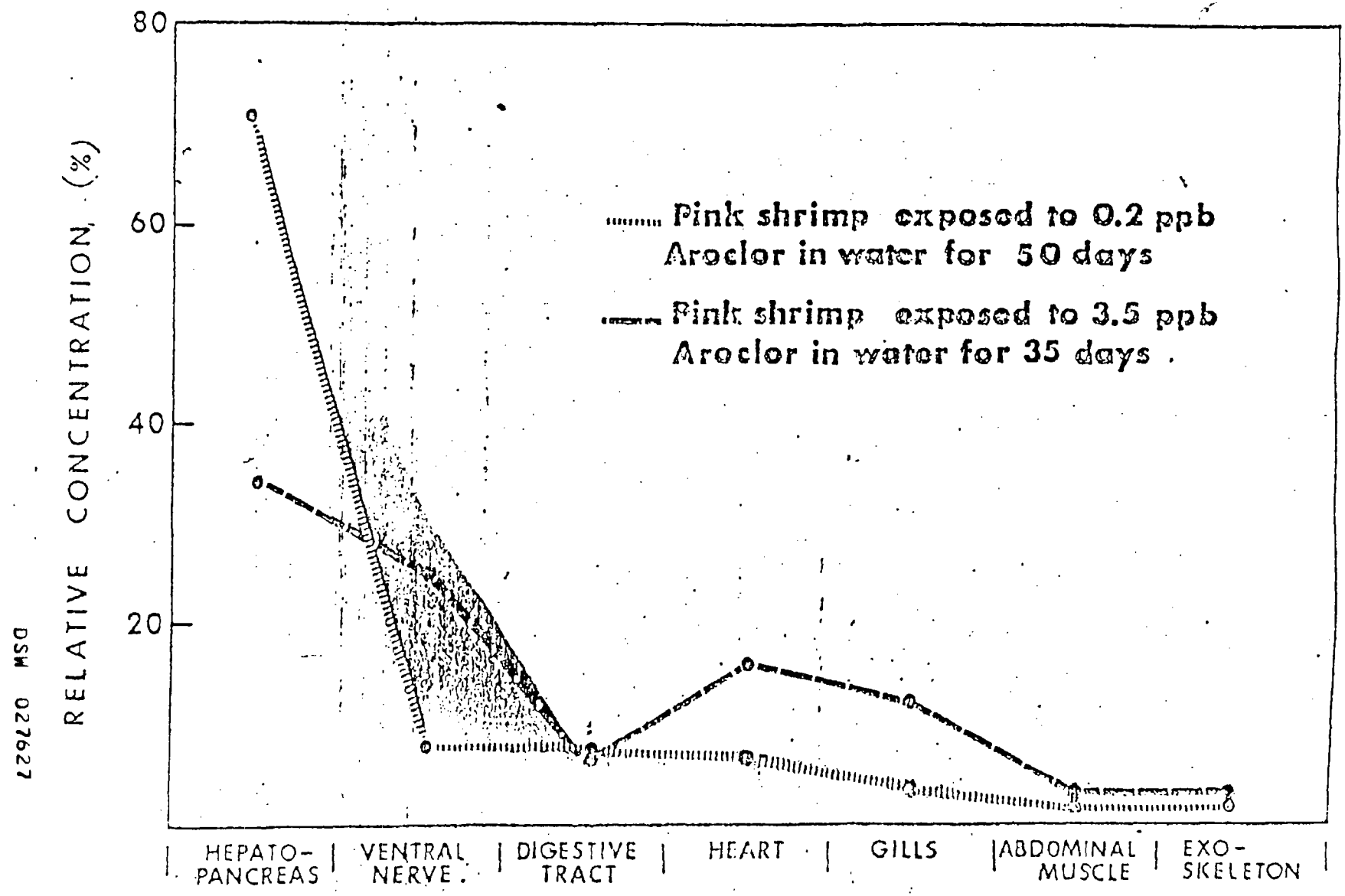

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Figure 10. Aroclor 1254: Uptake and depuration in grass shrimp exposed to 0.04, 0.09 and 0.62 ppb in water.

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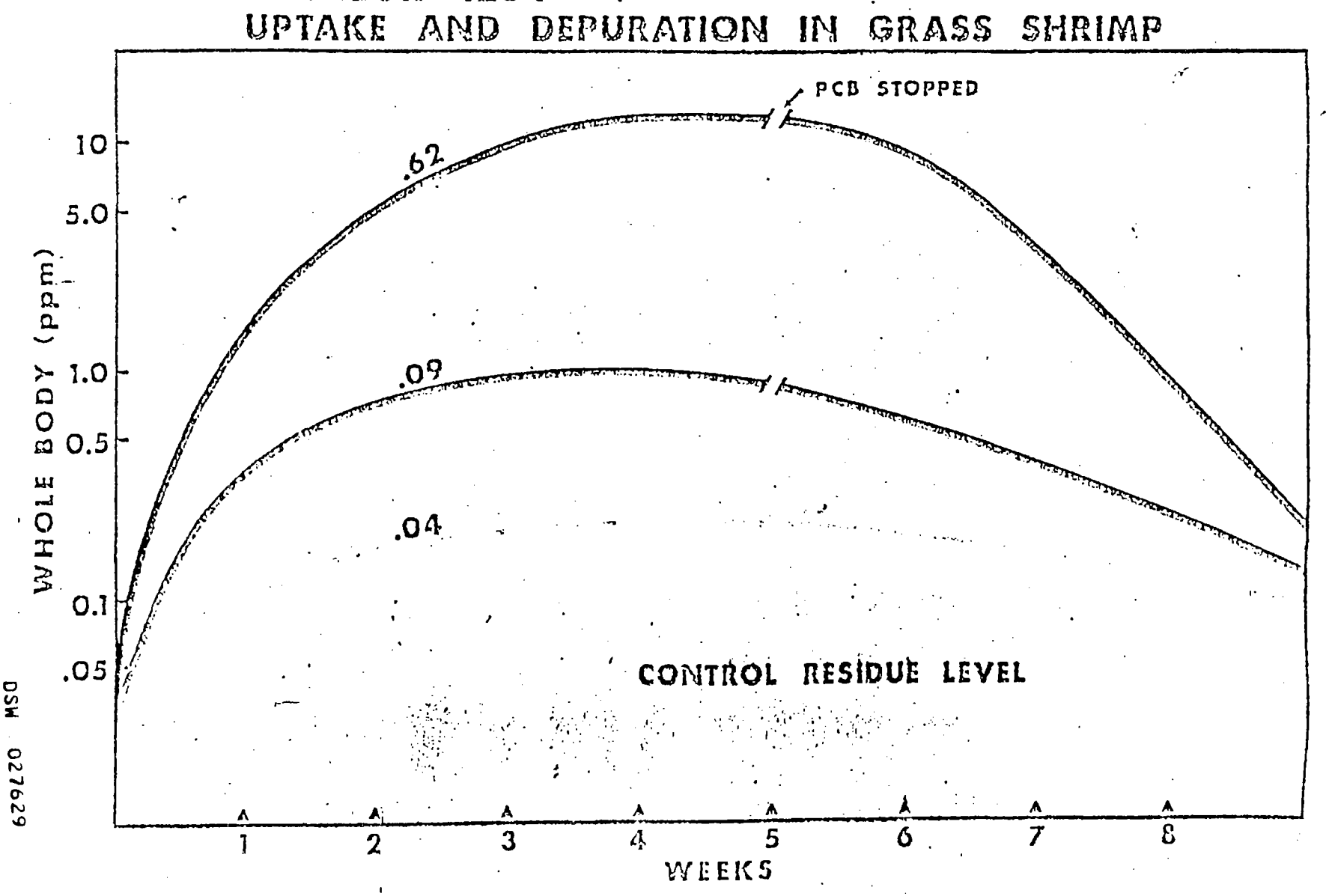

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Figure 11. Normal vesicular connective tissue (parenchyma) from control oyster. Note uniform cell distribution and paucity of leukocytes. X100.

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.

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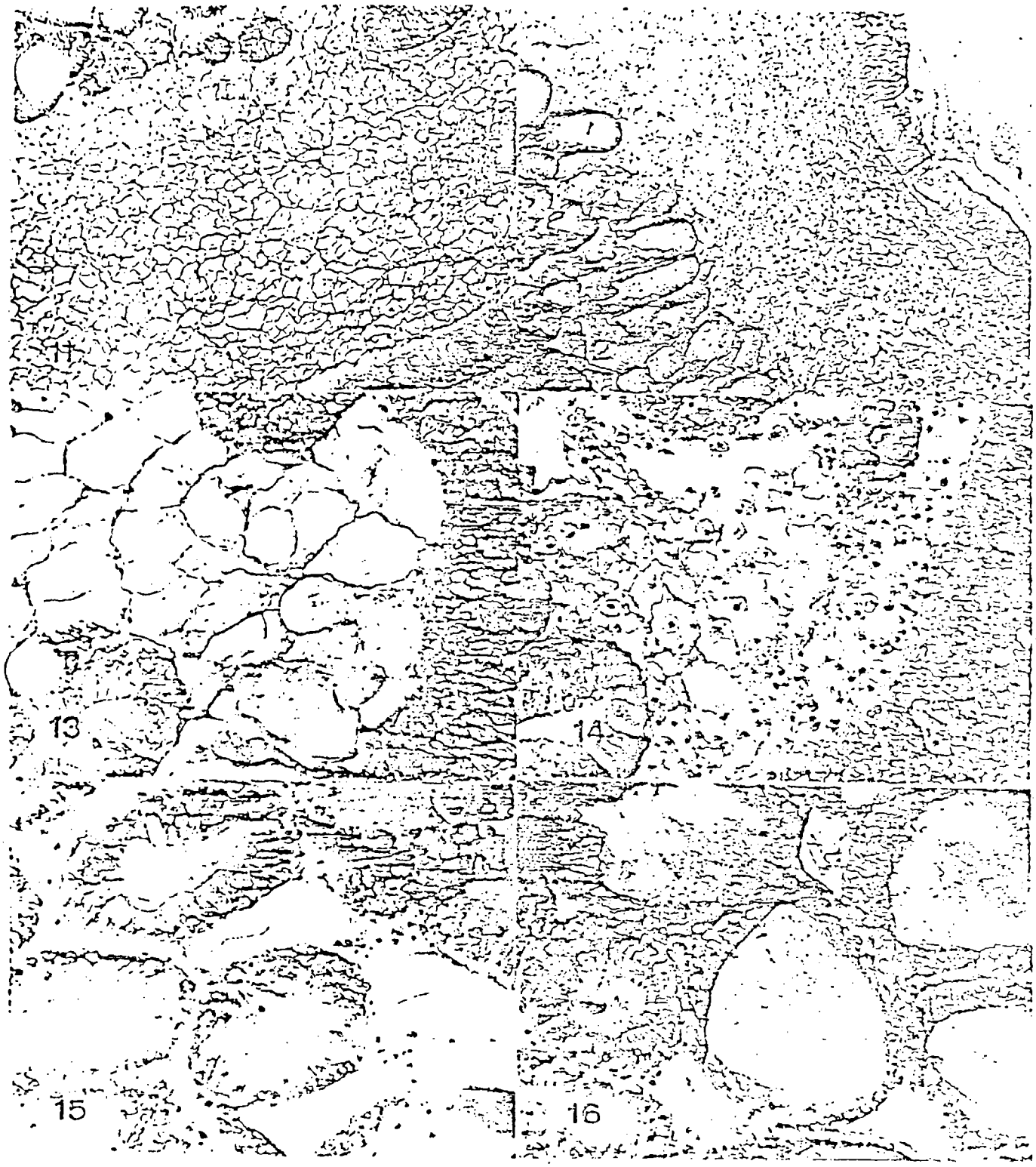

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Figure 17. Liver parenchyma of normal spot (Lagodon rhomboides) from control tank. Note uniform orientation of hepatocytes. X1000.

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.

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DSW 027633

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.

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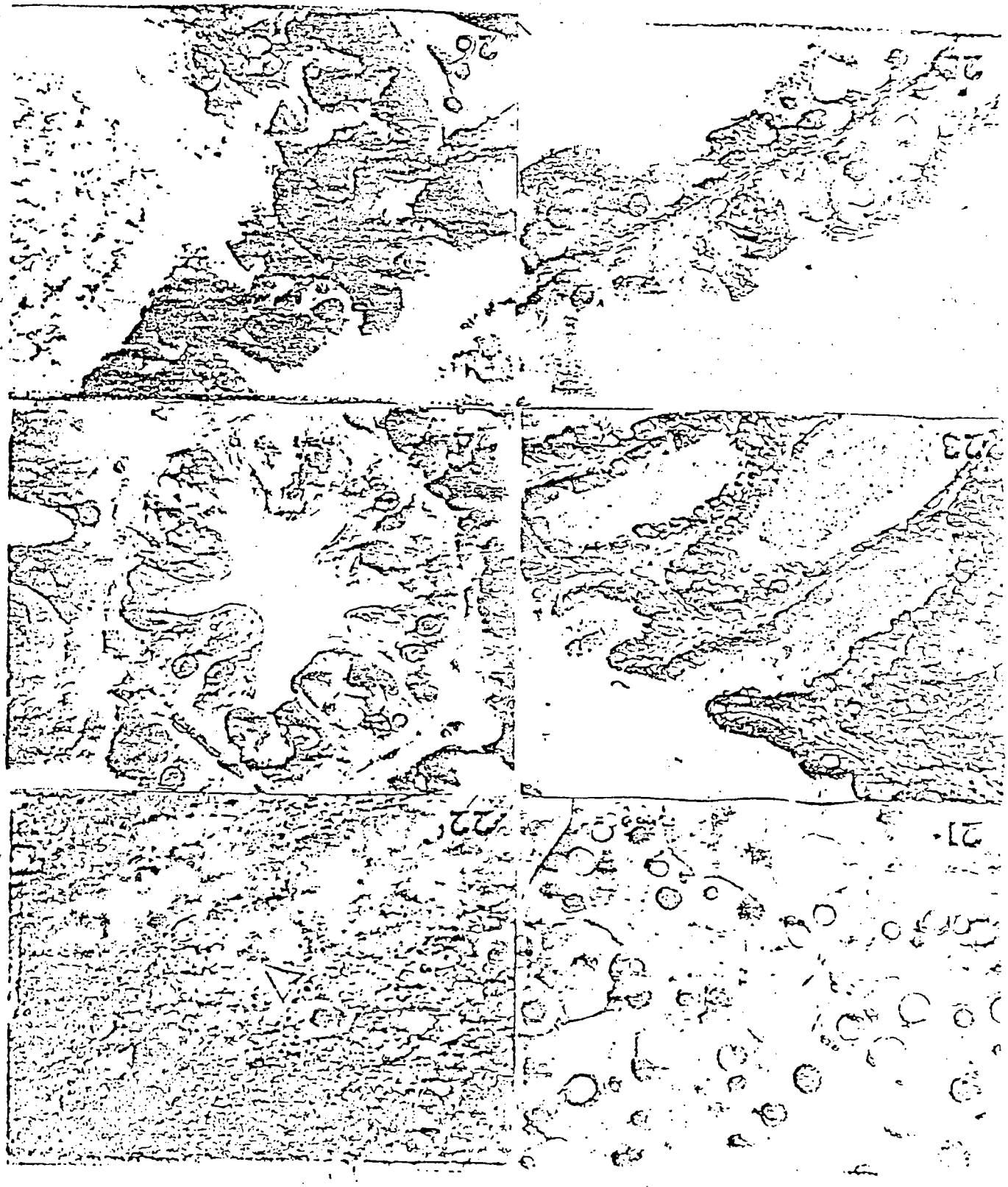

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