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Effects of the Polychlorinated Biphenyl Aroclor® 1254 on the American Oyster *Crassostrea virginica**

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Abstract

Young oysters (Crassostrea virginica) were continuously exposed to Aroclor® 1254, a polychlorinated biphenyl (PCB), in flowing, unfiltered seawater. Growth rate (height and in-water weight) was significantly reduced ($\alpha = 0.05$) in oysters exposed to 5 µg/l (ppb) for 24 weeks. Growth rate was not affected in oysters exposed to 1 ppb for 30 weeks. Mortality was not significant in exposed and control groups. In oysters exposed to 5 ppb, greatest PCB residue (whole body) was 425 mg/kg (ppm), 85,000 × the concentration in the water, and less than 0.3 ppm was retained after 28 weeks depuration in PCB-free water. In oysters exposed to 1 ppb, greatest residue was 101 ppm, 101,000 × the concentration in the water, and less than 0.2 ppm was retained after 12 weeks depuration. *Examination of oysters* exposed to 5 ppb of this PCB for pathogenesis revealed atrophy of digestive diverticular epithelium and degeneration of vesicular connective tissues concomitant with leukocytic infiltration, but tissue recovery seemed excellent after 12 weeks depuration.

Introduction

The presence of polychlorinated biphenyls in the marine environment and in individual marine organisms was documented by Jenson *et al.* (1969), Koeman *et al.* (1969), Nimmo *et al.* (1971), and Zitko (1971). One PCB, Aroclor 1254, was found in oysters in a local estuary, Escambia Bay, Florida (USA), and subsequent sampling of water, sediment and fauna revealed its widespread occurrence in this bay (Duke *et al.*, 1970). Laboratory bioassays showed that shell growth of young oysters was significantly inhibited by a 96 h exposure to 10 ppb or more of this PCB, but its chronic effects were unknown.

To study physiological and pathological effects of long-term exposure to Aroclor 1254, we reared oysters from spat to adults in flowing seawater which contained low concentrations of this PCB. One group of oysters was exposed to 5 ppb for 24 weeks, and another group was exposed to 1 ppb for 30 weeks.

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Materials and Methods

Experimental Techniques

The experimental design used in these exposures was essentially that of Lowe *et al.* (1971). *Crassostrea virginica* were held in tanks of flowing, unfiltered seawater, to which stock solutions of Aroclor 1254 in acetone were added continuously to give the desired concentration. No attempt was made to change the temperature or salinity of the seawater pumped into the laboratory from Santa Rosa Sound.

In both 5 and 1 ppb exposures, Milton Roy Instrument Mini-pumps® continuously metered 60 ml/h of acetone-PCB stock solution into an experimental tank, and the same amount of acetone without PCB into a control tank (Fig. 1). Each tank received 400 l/h seawater. Weekly gas chromatographic analyses of the test water verified the concentration of PCB. Average measured concentration for the 5 ppb exposure was 3.9 ppb (range 2.6 to 5.6); average for the 1 ppb exposure was 0.64 ppb (range 0.37 to 0.91).

The young oysters (31 mm mean height) used in the 5 ppb exposure were removed from an experimental tank of flowing seawater in which fish had been exposed to 1 ppb of Aroclor 1254. These oysters were carefully loosened from the surface of the tank, where they had set as free-swimming larvae, and placed in another tank for the 5 ppb exposure. Analysis of a pooled sample of 10 oysters when the 5 ppb exposure began revealed 17 ppm whole body residue of Aroclor 1254. A similar group of oysters (31 mm mean height) removed from a PCB-free tank from the same fish experiment, used as controls, contained no detectable PCB residue.

The young oysters used in the 1 ppb exposure set on cultch suspended from a dock near our laboratory; they contained no PCB residues.

For both exposures, 50 experimental and 50 control oysters were cleaned, weighed and measured individually at 2 week intervals. The in-water weight of each oyster was measured to the nearest 0.01 g on a top-loading balance with suspension attachments (An-

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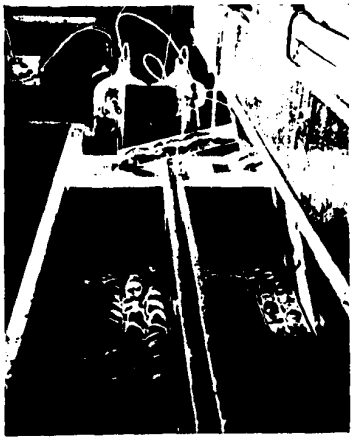

Fig. 1. Experimental and control tanks. Siphons transport water from constant-level reservoir (upper right) into mixing troughs and thus into tanks. Glass jars containing acetone-PCB stock solutions and pumps are in background

drews, 1961). The height of each oyster was measured to the nearest millimeter. Weights and heights of control and PCB-exposed oysters were compared statistically every two weeks using Student's t test (Snedecor and Cochran, 1967). Differences were considered significant at $\alpha = 0.05$. Additional oysters were held in each tank and sampled periodically for residue analysis or histopathological examination.

Chemical Analyses

Oyster tissue and test water were prepared for analysis by electron-capture gas chromatography as follows:

(1) Oyster tissue was carefully removed from the shell and mixed with anhydrous sodium sulfate in a blender. The mixture was then extracted in a Soxhlet apparatus for 4 h with petroleum ether. Extracts were concentrated to approximately 10 ml and transferred in 3- to 4-ml portions to a 400 mm × 20 mm chromatographic column that contained 76 mm of unactivated Florisil. After each portion settled in the column, vacuum was applied to evaporate the solvent. This was repeated after each addition and after three 5-ml petroleum ether rinses of the extraction flask. Vacuum was disconnected after all solvent had evaporated, and the residue was eluted from the column with 70 ml of 9:1 mixture of acetonitrile and distilled water. The eluate was evaporated just to dryness, and the residue transferred to a Florisil column with petroleum ether (Mills *et al.*, 1963). Aroclor 1254 was eluted from the column with 6% ethyl ether in petroleum ether, and adjusted to an appropriate volume for analysis.

(2) Water samples were extracted with petroleum ether. The petroleum ether solution was dried by passing it through a column of anhydrous sodium sulfate and then adjusted to an appropriate volume for analysis.

The samples were analyzed with a Varian Aerograph Model 1400 gas chromatograph equipped with a 182.8 cm × 3.2 mm (internal diameter) glass column packed with 2% OV-101 on Gas Chrom Q and an electron capture detector. The carrier gas was prepurified nitrogen. Operating conditions were: injector, 210 °C; column, 195 °C; detector, 215 °C; gas flow, 25 ml min^{-1}.

The multiple-peaked compound Aroclor 1254 was quantitated by averaging the peak height of 5 major peaks on the chromatograph. Residue data in this report do not include a correction factor for PCB not recovered. Recovery rates, however, were above 80% in control oyster and water samples spiked with different concentrations of Aroclor 1254.

Results

Growth, 5 ppb Exposure

In 44 weeks (31 July, 1970/3 June, 1971), control oysters grew from a mean weight and height of 1.28 g, 31 mm, to 45.84 g, 83 mm. Oysters (which contained

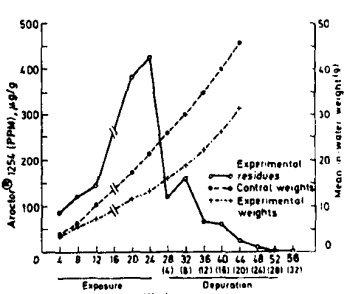

Fig. 2. *Crassostrea virginica*. Weight of control and experimental oysters and residue of Aroclor 1254 in experimental oysters after 24 weeks exposure to 5 ppb of this chemical

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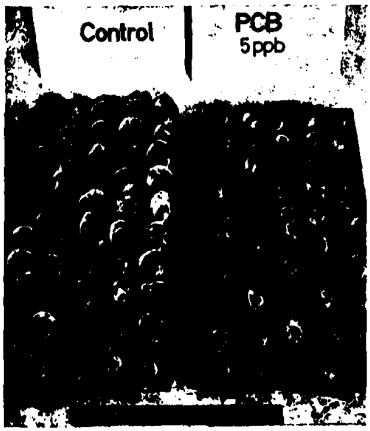

Fig. 3. *Crassostrea virginica*. Control and experimental oysters after 14 weeks exposure to 5 ppb Aroclor 1254. Mean height and in-water weight of controls were 54 mm, 11.98 g; experimentals, 44 mm, 8.39 g

17 ppm whole body residue of Aroclor 1254) exposed to 5 ppb PCB grew from a mean weight and height of 1.32 g, 31 mm, to 31.31 g, 72 mm during the same period. These oysters were maintained in PCB-polluted water for 24 weeks and in PCB-free water for 28 weeks. Biweekly weights for 44 weeks are plotted in Fig. 2. The mean weight and height of the control oysters were significantly greater than those of the PCB-exposed oysters after 6 weeks and, thereafter, throughout the experiment. After 14 weeks, the mean weight and height of the controls exceeded the experimentals by 3.59 g and 10 mm, respectively (Fig. 3). At the end of 24 weeks exposure, the mean weight and height of the control oysters exceeded those of the experimentals by 8.08 g and 13 mm ($t = 9.21$; $df = 92$).

Growth, 1 ppb Exposure

Growth of oysters reared in 1 ppb PCB was not affected. In 30 weeks (16 November, 1970/15 June, 1971), control oysters grew from a mean weight and height of 1.13 g, 26 mm, to 18.70 g, 57 mm. In the same period, oysters exposed to 1 ppb PCB grew from a mean weight and height of 1.15 g, 26 mm, to 20.08 g, 59 mm. These oysters were in the PCB-polluted water throughout the 30 week period.

Mortality

Mortality was not significant in exposed and control groups in either experiment. In the 5 ppb experiment, 3 exposed and 2 control oysters died. No death occurred in the 1 ppb experiment.

Accumulation and Retention, 5 ppb Exposure

The oysters reared in 5 ppb PCB accumulated the chemical throughout the exposure, reaching 425 ppm at the end of 24 weeks. The oysters purged themselves of most of the PCB after being held in PCB-free seawater for 28 weeks (Table 1).

Accumulation and Retention, 1 ppb Exposure

The oysters reared in 1 ppb PCB were sampled only three times for residue analyses:

(1) Twelve oysters were randomly selected after 25 weeks of exposure and analyzed as three groups of 4 oysters. They contained 85, 99, and 120 ppm (aver-

Table 1. *Crassostrea virginica. Accumulation of Aroclor 1254 by oysters reared in flowing seawater that contained 5 ppb of the chemical, and depuration in PCB-free seawater. Average daily temperature and salinity (July, 1970/September, 1971) was 24 °C, 27 ‰ S. Temperature range was 17° to 32 °C; salinity, 14 to 34 ‰ S*

Date	Exposure (weeks)	Oysters (no.)	PPM (µg/g) of PCB (wet weight)	
			Control	Experimentals
July 31, 1970	0	10	ND[a]	17[b]
Aug. 27, 1970	4	10	ND	87
Sept. 24, 1970	8	10	ND	119
Oct. 22, 1970	12	10	ND	145
Dec. 17, 1970	20	10	Trace[c]	381
Jan. 14, 1971	24	5	Trace	425
	Depuration (weeks)			
Feb. 11, 1971	4	5	Trace	119
March 11, 1971	8	5	Trace	137
April 8, 1971	12	5	Trace	42
May 6, 1971	16	5	Trace	37
June 3, 1971	20	10	Trace	21[d]
July 6, 1971	24	10	Trace	2.9[e]
Aug. 9, 1971	28	5	ND	Trace
Sept. 8, 1971	32	5	ND	ND

[a] Not detectable; less than 0.2 ppm.
[b] Young oysters contained 17 ppm Aroclor 1254 when 5 ppb exposure started.
[c] Trace; 0.2 to 0.3 ppm.
[d] Oysters analyzed individually; range 4 to 36 ppm; standard deviation = 10.1.
[e] Oysters analyzed individually; range 0.6 to 8.9 ppm; standard deviation = 2.6.

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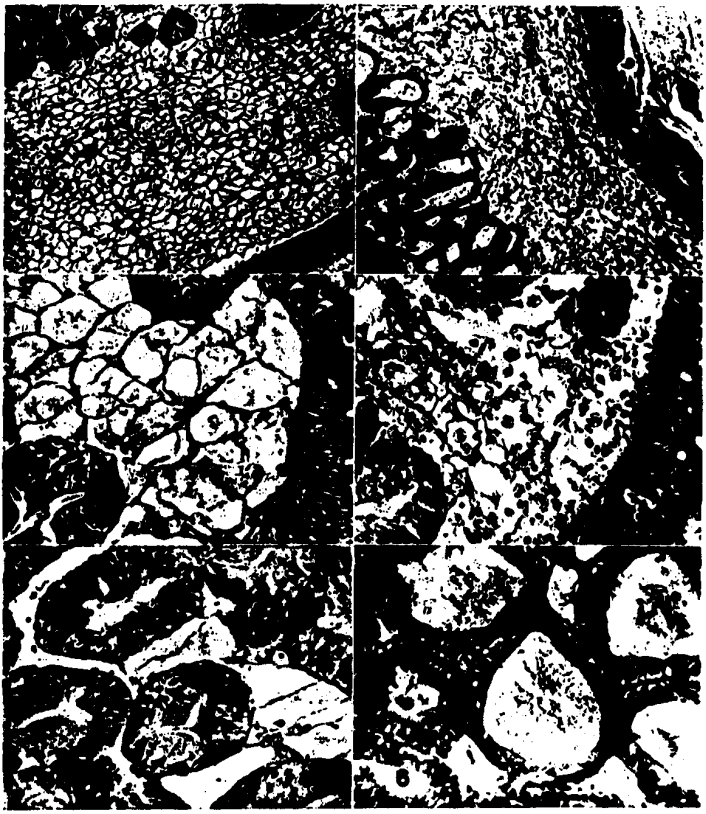

Fig. 4. *Crassostrea virginica*. 1: Section of normal oyster, note compact vesicular connective tissue with minimum number of leukocytes in intercellular position, 100 ×; 2: section of oyster from 5 ppb PCB exposure, note extensive leukocytic infiltration in region of vesicular connective tissue and around gut basement membrane, also note dilated appearance of digestive diverticula, 100 ×; 3: detail of vesicular connective tissue of normal oyster, note compact, consistent pattern of vesicular cells and relatively few leukocytes in intercellular spaces, 1000 ×; 4: detail of heavy infiltration of leukocytes from oyster showing vesicular connective tissue degeneration from 5 ppb exposure, note absence of compact pattern as in section 3, 1000 ×; 5: detail of one proximal duct (ciliated) and several distal digestive diverticula from normal oyster, note normal thick epithelium and tri-radiate lumina, 1000 ×; 6: detail of diverticula from oyster exposed to 5 ppb, note extremely atrophied epithelia and appearance of dilation of diverticula, 1000 ×

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age = 101) of the PCB. Twelve control oysters, analyzed as two groups of 6, contained 0.38 and 0.31 ppm PCB, indicating an extremely low concentration of PCB in the control water. However, the chemical was never detected (sensitivity less than 0.03 ppb) in control water samples.

(2) A pooled sample of 5 oysters analyzed at the end of the 30 week exposure contained only 46 ppm PCB. Five pooled control oysters contained no detectable PCB residue (less than 0.2 ppm). The most likely explanation for the approximate 50% decrease in PCB residue during the last 5 weeks of the exposure (May/June) is that the chemical was lost through spawning. Records of natural spatfall kept at this laboratory show that oysters were spawning during this period (P. A. Butler, personal communication[1]).

(3) After 12 weeks in PCB-free water, a pooled sample of 5 oysters contained no detectable residue.

Histopathological Examination

Dr. J. A. Couch, pathobiologist at this laboratory, examined tissues of oysters from exposed and control groups. One to 2 cm transverse sections from the labial palp region of each randomly selected oyster was fixed in Davidson's fixative, embedded in paraffin, sectioned at about 7 μ, and stained with Harris' hematoxylin.

Oysters Exposed to 5 ppb for 24 Weeks

These oysters displayed general tissue alterations in the vesicular connective tissue (parenchyma) around the digestive diverticula of the hepatopancreas. Tissues were altered in 8 of 10 oysters examined. The normal compact vesicular pattern (Fig. 4:3) was lost and actual replacement of vesicular cells by heavy infiltration of leukocytes occurred (Fig. 4:4). In most oysters, these degenerative changes were extensive and involved much of the vesicular connective tissue.

The most specific tissue change occurred in the epithelia of the unciliated, distal segments of the digestive diverticula. The thick, deep epithelia which form tri- or quadri-radiate lumina in a normal oyster (Fig. 4:5) were greatly altered in exposed oysters. Eight of 10 oysters displayed distinct atrophy of the distal diverticular epithelium (Fig. 4:6). This gave the diverticula a dilated appearance (Fig. 4:2, 6), which resulted from the thinner epithelium and increased luminal area. Two of the 8 oysters that had atrophy of diverticular epithelia also had extensive vacuolization of these epithelia.

Six oysters had heavy leukocytic infiltration of the gonads. Three oysters were without significant gonadal development, a normal condition for time of sampling (January), whereas 1 (a male) had ripe gonads.

Ten oysters were examined after 12 weeks depuration in PCB-free water. Recovery of vesicular connective tissue and digestive diverticula seemed excellent.

Oysters Exposed to 1 ppb for 30 Weeks

The 10 oysters examined showed only slight departure from normal. One had a heavy leukocytic infiltration around the gut and adjacent vesicular connective tissue, whereas 3 had light, general leukocytic infiltration around the gut. Eight oysters were gravid.

One oyster was lightly infected by *Labyrinthomyxa marina* (*Dermocystidium marinum*), and the gills of another were lightly infected by *Nematopsis* sp.

Discussion and Conclusions

Oysters do not appear to be as susceptible to PCB poisoning as are marine crustaceans and fishes. Other research at this laboratory showed that 1 ppb of Aroclor 1254 is lethal to the juvenile pink shrimp *Penaeus duorarum* within 15 days (Nimmo *et al.*, 1971) and 5 ppb is lethal to two estuarine fishes, *Lagodon rhomboides* and *Leiostomus xanthurus*, within 14 to 45 days (Hansen *et al.*, 1971). In our study, growth was significantly inhibited and tissues were altered (without significant mortality) in oysters reared in seawater which contained 5 ppb Aroclor 1254, but these changes did not occur in oysters reared in 1 ppb. The fact that some of the oysters used in this study set and grew in 1 ppb Aroclor 1254 indicates that free-swimming oyster larvae and subsequent spat can tolerate low concentrations of this PCB.

Direct comparison of the toxicities of PCBs and chemically-related pesticides to oysters cannot be made because of insufficient data, but some contrasts can be made. Mean in-water weight of oysters grown in seawater which contained 3 ppb of insecticides (1 ppb each of DDT, toxaphene and parathion) was 2.8 g less than that of control oysters after 9 months exposure (Lowe *et al.*, 1971), whereas a 2.8 g difference between control and experimental oysters occurred after only 3 months exposure to 5 ppb Aroclor 1254. Non-specific and reversible tissue alterations, such as atrophy of digestive tubules and leukocytic infiltration of various tissues, occurred in oysters from the pesticide exposure described above and in oysters exposed to 5 ppb Aroclor 1254 for 24 weeks. Degeneration of vesicular connective tissue concomitant with heavy leukocytic infiltration can be a sign of non-specific pathogenesis in oysters and may occur in oysters suffering from adverse conditions such as starvation or infectious diseases. In the presence of an irritant and potential poison (such as Aroclor 1254), and concurrent with the more specific response of atrophy of the distal diverticular epithelia, it is possible that the connective tissue degeneration is related

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to starvation resulting from inhibition of digestive functions of the distal digestive diverticular epithelia (J. A. Couch, personal communication[3]).

Oysters concentrate and store PCBs in their tissues. *Crassostrea virginica* reared in 5 ppb Aroclor 1254 for 24 weeks concentrated the chemical 85,000 × the concentration in the water; those reared in 1 ppb concentrated it 101,000 ×. Oysters exposed to 1 ppb DDT for 24 weeks, however, concentrated the insecticide 76,000 × (Lowe *et al.*, 1971). Although commercial oyster beds may seldom be chronically exposed to concentrations of PCBs as high as those used in this study, shellfish such as oysters could accumulate sufficient residue of the chemical to prevent them from being marketed for human food (through the enforcement of "safe tolerances" set by governmental agencies). Oysters from a natural reef in Escambia Bay, Florida (USA), concentrated up to 3 ppm of Aroclor 1254 following an accidental leakage of the chemical from an industrial plant about 6 miles away (Duke *et al.*, 1970). Oysters that contained 2.8 ppm PCB were recently taken from the Elizabeth River, Virginia (USA) (P. A. Butler, personal communication).

This study showed some effects of chronic PCB-exposure on oysters. Our results suggest that the well-being of natural populations of oysters would not be affected by the levels of PCB which, to date, have been found in open estuarine and marine waters. This is not to say that PCB-contaminated oysters could not be a problem in the concentration and movement of this chemical in estuarine and marine ecosystems. Increased contamination of estuaries by PCBs could result in adverse effects on growth of oysters. If direct PCB contamination of an estuary occurs rather than contamination from river discharge as in Escambia Bay, natural oyster populations could be adversely affected. Possible synergistic or additive actions of disease, other pollutants, and PCBs might also affect growth and survival of oysters.

Summary

1. Growth rate (height and in-water weight) of *Crassostrea virginica* was significantly reduced during 24 weeks of continuous exposure to 5 ppb Aroclor 1254, a polychlorinated biphenyl, but oysters exposed to 1 ppb for 30 weeks were not so affected.

2. Mortality was not significant in any exposed or control group.

3. Oysters exposed to 5 ppb accumulated the chemical 85,000 × the concentration in the test water, and those exposed to 1 ppb accumulated it 101,000 ×. Oysters exposed to 5 ppb purged themselves of most of the PCB after 28 weeks in PCB-free water, and those exposed to 1 ppb purged themselves of the chemical after 12 weeks depuration.

4. Tissue alterations were greatest in oysters exposed to 5 ppb, and were characterized by atrophy of digestive diverticular epithelium and degeneration of vesicular connective tissue concomitant with leukocytic infiltration. Tissue recovery seemed excellent after 12 weeks depuration.

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