internal evidence of a derivation from simple leaves. One exception, the Quintas cean, has already been commented on. The only other major exception is the Leguminosae in which the evidence is consistent with the persistence of compound leaves since the glossopteral phase of evolution.

The fourth example, *Sagenopteris* from the Cretaceous is of a leaflet from a compound digitate leaf with a venation pattern representing an evolutionary stage between that of *Gangamopteris* and *Glossopteris*. The midrib is poorly developed, being a loose aggregation of parallel veins, and the principal vein islets are of the hydroid type which in the evolutionary sequence is one of the first to appear in *Gangamopteris*. Moreover, these vein islets run almost parallel with the midrib, whereas in the more advanced species of *Glossopteris* the principal vein islands along the midrib are of arcuate, angular or deltoid types and they make angles of about 70°–80° with a compact well formed midrib. The latter three types of vein island are the most advanced elements in the glossopteroid syndrome. They must carry considerable weight in assessing probable angiosperm affinities with the Glossopteridae, for they occur in several angiosperm families in a similar relationship to the midrib of a simple (not a compound) leaf. Unless it can be shown that the more advanced types of vein island occur in the same kind of relationship in the other four orders of gymnosperms, the claim of Alvin and Chaloner that "on venation pattern alone there is no closer similarity between *Glossopteris*-like angiosperms and *Glossopteris*" than between these and the other major taxa cannot be maintained.

R. MELVILLE

Royal Botanic Gardens,
Kew.

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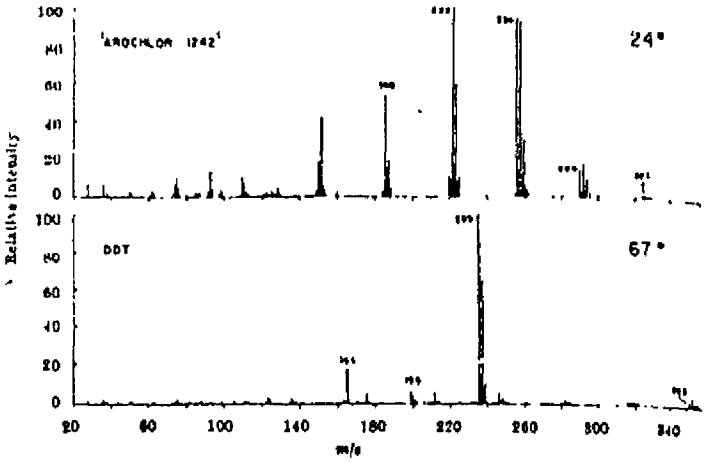

Fig. 1. 70 eV mass spectra (C.E.C. 21-110B instrument) of a 1 : 1 mixture of 'Arochlor 1242' and DDT at different source temperatures. Isotope clusters at *m/e* 222, 256, 291 and 324 result from individual components of 'Arochlor', the group of peaks at *m/e* 166 are mainly fragment ions from this substance.

Identification of Polychlorinated Biphenyls and DDT in Mixtures by Mass Spectrometry

RECENTLY it was recognized that certain chlorinated aromatic compounds, particularly polychlorinated biphenyls (PCBs), are found in natural samples as pollutants[1,2]. PCBs interfere with the analysis for chlorinated pesticides because some of the PCB components from the technical mixture have identical retention times and respond to the electron capture detector[3-6].

Although combined gas chromatography–mass spectrometry for detection of PCBs from natural samples has been described[3], the use of mass spectrometry, particularly high resolution techniques, for the unambiguous identification of PCBs in the presence of chlorinated pesticides without previous separation[3] has not been reported.

We have recorded the mass spectra of a commercial sample of PCB ('Arochlor 1242') in different conditions and obtained high resolution measurements to confirm the molecular formulae of major components. The peaks arising from the individual major components of the mixture were as follows. $C_{12}H_4Cl_6$: found, 322·8829; calc., 322·8830. (The experimental error is within ± 2 p.p.m. in all cases.) These can easily be distinguished from major peaks found in the most common chlorinated insecticides (Fig. 1 and refs. 6–13) by their position, peak pattern arising from the chlorine isotopes and particularly high resolution measurements. Furthermore, a useful spectrum of 'Arochlor 1242' can be obtained at low temperatures by direct introduction with the aid of a temperature controlled probe[14], and fractional sublimation from many chlorinated pesticides directly in the ion source seems possible (see Fig. 1).

Techniques for identification of trace quantities of PCBs and chlorinated insecticides in crude natural samples and their semiquantitative determination by mass spectrometry as well as the chemical characterization of major constituents of PCBs are being developed.

We thank D. J. Embree for recording the mass spectra.

O. HUTZINGER
W. D. JAMIESON

Atlantic Regional Laboratory,
National Research Council of Canada,
Halifax, Nova Scotia.

V. ZITKO

Water Pollution Research,
Biological Station,
Fisheries Research Board of Canada,
St Andrews, New Brunswick.

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MONS 204340

W-3, D-3

Sex Difference in the Number of Adipose Cells from Genetically Obese Rats

IT is well known that there is no increase in the number of adipose cells in many kinds of experimental obesity. In genetically obese mice, obob obese hyperglycaemic[1,2] or yellow obese[3] syndromes as well as in hypothalamic obesities produced by aurothioglucose in mice[4] or stereotaxic lesions in rats[5], there is only an enlargement of the adipose cells. The same results are obtained in rats made obese by a high fat diet[6]. There is only one known exception to this rule: obesity induced by a high fat diet in female mice[7], in which the number of cells is increased by a factor of 2.5. Because male animals were used in all experiments except the last, the question arises whether there may be a sex difference in the adipose tissue development in obese animals.

Genetically obese rats (fa/fa)[8] 2.5 months old and their litter mates, fed with my control diet, were used. Whole perigenital fat pads were weighed and treated as previously described[6]: portions of histological sections on slides, projected on the ground glass of a Projectina microscope, were counted for their adipose cells at a magnification of ×130. The mean volume was calculated and the number of adipose cells estimated by the ratio of fat pad weight to mean adipose cell volume ×0.91 (0.91 is the measured density of the pads).

Table 1 shows that the body weights of obese rats doubled in the females but increased less in the males. The weight of perigenital fat pads increased 3.2 and 9.7-fold in males and females respectively. Adipose cells enlarged 4.6 and 4.0-fold in obese males and females respectively. In male obese rats there were significantly fewer epididymal adipose cells than in their litter mates, but a 2.3-fold increase in their number was observed in the parametrial cells of the obese females.

Table 1 Weights of the Body and of the Two Perigenital Fat Pads of 2.5 Month Old Genetically Obese Male and Female Rats and Their Litter Mates

	Lean litter mates	Obese
Males		
Body weight (g)	276 ± 16.0	366 ± 17.3
Epididymal fat pad weight (g)	2.82 ± 0.260	9.00 ± 0.327
Adipose cell volume (×10³ μm³)	276 ± 25.5	1,275 ± 85.2
No. of adipose cells (millions)	9.34 ± 0.458	6.50 ± 0.256
Females		
Body weight (g)	165 ± 2.4	320 ± 13.6
Parametrial fat pad weight (g)	1.64 ± 0.189	15.9 ± 0.71
Adipose cell volume (×10³ μm³)	379 ± 46.6	1,536 ± 43.5
No. of adipose cells (millions)	4.08 ± 0.292	9.48 ± 0.460

The cell volume of the obese groups increased, but cell number increased only in the female group (2.3 times). All differences are highly significant ($P < 0.01$). Values are mean ± s.e. There were six animals in each of the four groups.

Hypertrophy of the adipose cells in the obese females could only account for a parametrial fat pad weight of 1.64 × 4.0 = 6.64 g—9.26 g less than the actual weight. And so about 70% of the weight of the pads was due to formation of new fat cells which were themselves hypertrophied.

These results clearly established a sex difference in the development of perigenital adipose tissue. They are in good agreement with the literature which records no increase in fat cell number in male obese rats or mice[1-6]. But there was a marked hyperplasia in the parametrial adipose cells in the females which was very similar to those observed in female mice made obese by a high fat diet[7].

Exceptions to the rule (that only hypertrophy is observed in male adult animals had been claimed: there is hyperplasia of adipose cells in rats after repeated injections of insulin[9, 10] or meal feeds[11]. In these animals there was no increase in the weight of the epididymal fat pad, and adipose cell volume seemed to be reduced; the differences, however, were very small. The principal effect, in the two cases, was a great increase in the total DNA content of the pads (+50% for meal fed rats[11] and +100% for rats treated with insulin[9]). Such differences cannot be the effect of a marked hyperplasia: the weight of fat pads was unchanged and cell volume very slightly smaller; so it can be concluded that there was an increased DNA content in stromal structures. Such an effect has been established in hypophysectomized rats after injections of somatotrophic hormone: the rate of DNA synthesis of supporting and vascular structures was enhanced, but there was no effect on division in adipose cells[12]. In my rats, the histological appearance of perigenital adipose tissue was the same as that described for rats made obese by a high fat diet[6]. There was no difference, except for the size of the cells, between fat tissues of control and obese rats, although there was a marked and similar hyperinsulinism in males as in females in the obese group (to be published later).

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DANIEL LEMONNIER

Laboratory of Biology and Human Nutrition, CNAM 292 Rue Saint Martin, 75 Paris 3

Received December 15, 1970.

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Polychlorinated Biphenyl absorbed from Sediments by Fiddler Crabs and Pink Shrimp

POLYCHLORINATED biphenyls (PCBs) are manufactured in the United States, Europe, and Japan and used as plasticizers, flame retardants, insulating and heat exchange fluids and in many other products[1]. Structurally related to DDT, soluble in lipid but relatively insoluble in water, and extremely persistent in the environment[1], they have been reported in many marine and estuarine organisms[2]. One PCB, 'Aroclor 1254' (Monsanto), was reported in water, sediments, and biota from Escambia Bay, Florida[3].

In August 1969, pink, white and brown shrimps (*Penaeus duorarum*, *P. setiferus*, and *P. aztecus*) from Escambia Bay were found to contain whole body residues of 'Aroclor 1254' at concentrations as high as 14.0 p.p.m. Most was concentrated in the hepatopancreas; residues in seven composite samples of at least five shrimps ranged from 0.6 to 120.0 p.p.m. In April 1970, fiddler crabs (*Uca minax*) collected at three stations along the lower Escambia River and upper Escambia Bay had individual whole body residues of 0.45 to 1.5 p.p.m.

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To determine the distribution of 'Aroclor 1254' in the estuary, forty samples of water were collected in glass bottles; twenty sediments were collected with a core or dredge sampler, and many estuarine organisms were captured by trawl and dredge. These were analysed by gas liquid chromatography*. The largest accumulations of 'Aroclor' were found in the sediments. In one survey (February 1970), concentrations ranged from 0.6 to 61.0 p.p.m. in a sample taken at the discharge point of the industrial plant that accidentally released the chemical and at eleven stations extending 16 km downstream (Fig. 1). 'Aroclor' was not detected in sediments collected above the plant, but soil samples from the bank near the mouth of the river 6.5 km downstream from the source had 1.4 to 1.7 p.p.m. of this chemical. Subsequent samplings from three stations in the Bay showed little change in the amounts of the chemical even after nine months. We therefore investigated whether shrimp and fiddler crabs could accumulate 'Aroclor 1254' from the sediments.

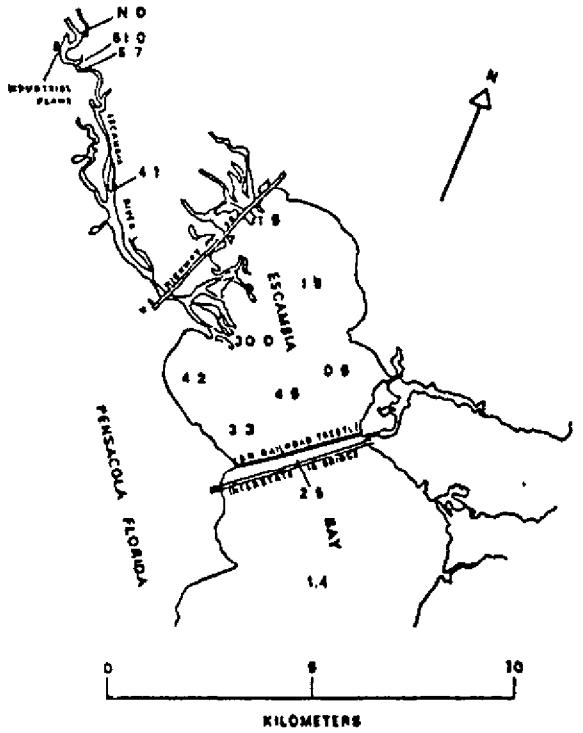

Fig. 1 Study area showing lower Escambia River, upper Escambia Bay and stations where sediment was collected on February 24, 1970. The amount of 'Aroclor 1254' found at each station is given in parts per million (p.p.m.). ND, Not detected.

Seven contaminated sediments (Table 1) were collected with a Petersen dredge from upper Escambia Bay and lower Escambia River at depths of 0.6 to 9 m. Beach sand and additional samples of sediment collected upstream from the contaminated section of the river served as uncontaminated controls. Four kilograms of each of the sediments and ten adult pink shrimp (*P. duorarum*) from a population which had previously shown no contamination with 'Aroclor' were placed in each of nine aquaria. Water at 17° C flowing at a rate of 12 l./h, with an average salinity of 27 parts per thousand (p.p.t.), covered the substratum to a depth of 10 cm.

Fiddler crabs (*U. pugilator*) were exposed to 'Aroclor' by placing them on five sediment substrates collected as described. Four kilograms of each of the sediments were placed in the upper end of five aquaria inclined 10 degrees so as to simulate a natural environment. Ten crabs from a population which had no contamination were placed in each aquarium with nine serving as a control. Water with an average salinity of 27 p.p.t. at 13° C flowed at 5 l./h through the lower end. Both shrimp and crabs were fed fish containing <0.03 p.p.m. 'Aroclor 1254' each day for 30 days. Five crabs were removed from each aquarium, killed and rinsed with a 50% mixture of acetone and water to remove 'Aroclor 1254' from material adhering to the exoskeleton. To ensure enough tissue for analyses, individual crabs and pooled samples of hepatopancreas from shrimp were mixed with anhydrous Na_2SO_4 and extracted with petroleum ether in a Soxhlet apparatus. The extract was purified with 'Florisil'[3]. 'Aroclor 1254' was quantitated by gas liquid chromatography[4]. The ratio of individual 'Aroclor' isomers maintained integrity in the sediments and tissues of test animals throughout the investigation.

Table 1 'Aroclor 1254' in Individual Fiddler Crabs and Hepatopancreas from Pink Shrimp

Sediment	'Aroclor 1254' in sediment (p.p.m. dry weight)	Average and range of 'Aroclor 1254' in five individual fiddler crabs (p.p.m. wet weight)	'Aroclor 1254' in combined hepatopancreas from four or more shrimp (p.p.m. wet weight)
Sandy silt*	61.0	80.0±25.0	240.0
Silt†	30.0	17.0±9.0	6.1
Sandy silt*	5.7	—	9.8
Silt†	4.9	3.6±0.9	1.3
Sandy silt*	4.1	—	6.7
Silt†	2.5	3.2±0.9	1.1
Silt†	1.4	—	0.2
Sand control*	ND	0.3±0.2	<0.2
Silt control†	ND	—	<0.2

The samples of crab and shrimp were kept for 30 days on naturally occurring sediments containing 'Aroclor'. Mortalities occurred in some groups of shrimp which could not be attributed to 'Aroclor 1254'.

* Sediment which contained particles of sand >354 μm in diameter.

† Sediment which contained particles of sand 354 μm or less.

—, No sample.

ND, Not detectable.

Fiddler crabs and shrimp exposed to the contaminated sediments accumulated 'Aroclor 1254' in their tissues by ingesting contaminated sediment particles or by absorbing the leached chemical from water. They sifted the sediments before ingesting the particles. In most cases, the amount of 'Aroclor' in individual crabs or in shrimp hepatopancreas was directly related to the amount in the sediments. We analysed the distribution of the chemical in the sediment in relation to particle size and found it was evenly distributed. Large concentrations of 'Aroclor' residues may also accumulate in the hepatopancreas of shrimp exposed to sandy silts because the chemical leaches from the sediments and is absorbed through the gills. Three and a half parts per billion (p.p.b.) were in the effluent water from the aquarium with sandy silt (61.0 p.p.m. 'Aroclor'); the water from the silt (30.0 p.p.m.) had only 0.5 p.p.b. Even though fiddler crabs were not continuously covered with water, they could have obtained 'Aroclor' from the water as they wetted their gills.

We have demonstrated experimentally that 'Aroclor 1254' can enter the estuarine food chain from sediments. Others[4] have identified this chemical in higher trophic life such as fish, and since PCBs are widespread in the world[5], we should

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become more alert to the presence, movement and effects of these chemicals in the environment

D. R. Nimmo
P. D. Wilson
R. R. Blackman
A. J. Wilson, jun.

US Environmental Protection Agency,
Gulf Breeze Laboratory,
Gulf Breeze, Florida 32561

Received September 17; revised December 10, 1970.

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ENVIRONMENTAL PROTECTION AGENCY
GULF BREEZE LABORATORY
PESTICIDE ANALYSIS REPORT

Chemical analyses conducted by:
Alfred J. Wilson Jr.
Gulf Breeze Laboratory
Gulf Breeze, Florida 32561

Agency submitting sample:

Verified by:
C. J. Wilson
Date: November 2, 1972

1972 ESCAMBIA BAY PCB SURVEY — Page 1 of 4 — Parts Per Billion (ng/l)

Sample Number	Date Collected (d-m-y)	Description	Station Location	Lab No.	DDE	DDD	DDT	Other Aroclor 1254
	13-11-72	Water	Above Monsanto Surface	406				ND*
	"	"	Above Monsanto Bottom	408				ND
	"	"	New Weir - Surface	410				ND
	"	"	New Weir - Bottom	412				ND
	"	"	Old Weir - Surface	414				ND
	"	"	Old Weir - Bottom	416				ND
	"	"	Hwy. 90 - Surface	418				ND
	"	"	Hwy. 90 - Bottom	420				ND
	"	"	Transect A - Surface	421				ND
	"	"	Transect A - Bottom	423				ND
	"	"	Transect B - Surface	426				ND
	"	"	Transect B - Bottom	428				ND

*ND = <0.03 PPB

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ENVIRONMENTAL PROTECTION AGENCY
GULF BREEZE LABORATORY
PESTICIDE ANALYSIS REPORT

Chemical Analyst: [illegible]
Alfred J. Wilson, Jr.
Gulf Breeze Laboratory
Gulf Breeze, Florida 32561

Agency submitting sample:

Verified by:

Date:

1972 ESCAMBIA BAY PCB SURVEY Page 2 of 4

Parts Per Million (µg/g)

Sample Number	Date Collected (d-m-y)	Description	Station Location	Lab No.	DDE	DDD	DDT	Other Aroclor 1254
	13-11-72	Sediment	Above Monsanto	429				ND*
	"	"	Above Monsanto	430				ND
	"	"	New Weir	431				ND
	"	"	New Weir	432				ND
	"	"	Old Weir	433				0.90
	"	"	Old Weir	434				2.0
	"	"	Hwy. 90	435				0.64
	"	"	Hwy. 90	436				0.83
	"	"	Transect A West	437				1.6
	"	"	Transect A Middle	438				1.6
	"	"	Transect A East	439				0.60
	"	"	Transect B East	440				0.25
	"	"	Transect B Middle	441				0.34
	"	"	Transect B West	442				0.18

MONS 204345

ENVIRONMENTAL PROTECTION AGENCY
GULF BREEZE LABORATORY
PESTICIDE ANALYSIS REPORT

Chemical analyst: [illegible]
Alfred J. Wilson, Jr.
Gulf Breeze Laboratory
Gulf Breeze, Florida 32561.

Agency submitting sample:

Verified by:

Date:

1972 ESCAMBIA BAY PCB SURVEY — Page 3 of 4

Parts Per Million (µg/g)

Sample Number	Date Collected (d-m-y)	Description	Station Location	Lab No.	DDE	DDD	DDT	Other Aroclor 1254
	13-11-72	Sediment (Core)	Above Monsanto 0-2"	443				ND*
	"	"	Above Monsanto 2-4"	444				ND
	"	"	Above Monsanto 4-7"	445				ND
	"	"	New Weir 0-2"	446				ND
	"	"	New Weir 2-4"	447				ND
	"	"	New Weir 4-6 1/4"	448				ND
	"	"	Old Weir 0-2"	449				0.97
	"	"	Old Weir 2-4"	450				5.8
	"	"	Old Weir 4-6 1/2"	451				250
	"	"	Hwy. 90 0-2"	452				0.15
	"	"	Hwy. 90 2-4"	453				0.21
	"	"	Hwy. 90 4-8"	454				0.97
	"	"	Hwy. 90 8-9"	455				3.1

*0.10 ppm

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ENVIRONMENTAL PROTECTION AGENCY
GULF BREEZE LABORATORY
PESTICIDE ANALYSIS REPORT

Chemical analyses performed by:
Alfred J. Wilson, Jr.
Gulf Breeze Laboratory
Gulf Breeze, Florida 32561.

Agency submitting sample:

Verified by:

Date:

1972 ESCAMBIA BAY PCB SURVEY — Page 4 of 4

Parts Per Million (µg/g)

Sample Number	Date Collected (d-m-y)	Description	Station Location	Lab No.	DDE	DDD	DDT	Other Aroclor 1254
	13-11-72	Sediment (Core)	Transect A - West 0-2"	456				0.14
	"	"	Transect A - West 2-4"	457				ND
	"	"	Transect A West 4-8"	458				ND
	"	"	Transect A - West 8-11"	459				ND
	"	"	Transect A - Middle 0-2"	460				5.7
	"	"	Transect A - Middle 2-4"	461				9.0
	"	"	Transect A - Middle 4-8"	462				8.9
	"	"	Transect A - Middle 8-12"	463				0.16
	"	"	Transect A - Middle 12-13"	464				1.6
	"	"	Transect A - East 0-2"	465				ND
	"	"	Transect A - East 2-4"	466				ND
	"	"	Transect A - East 4-8"	467				ND
	"	"	Transect A - East 8-12"	468				ND
	"	"	Transect A - East 12-16"	468				ND

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W-5
D-5

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Toxicity and distribution of Aroclor® 1254 in the pink shrimp *Penaeus duorarum**

D. R. Nimmo, R. R. Blackman, A. J. Wilson, Jr. and J. Forester

Environmental Protection Agency, Gulf Breeze Laboratory; Sabine Island, Gulf Breeze, Florida, USA

Abstract

The polychlorinated biphenyl Aroclor® 1254 was released in an accidental leakage of heat-exchange fluid from an industrial plant, into the Escambia River, near Pensacola, Florida, USA. This material was carried downstream, and is now found in the fauna of Escambia Bay and its contiguous waters, prime nursery areas for fishes and invertebrates such as penaeid shrimp. The significance of pollution by this chemical was assessed by establishing toxicity levels, determining routes of entry, and investigating its movement and distribution in various tissues of shrimp under controlled conditions in the laboratory. Aroclor 1254 added to the water was toxic to the juvenile pink shrimp *Penaeus duorarum* at a concentration of 1.0 part per billion within 15 days, but was less toxic to adult pink shrimp. Shrimp obtained the contaminant from water and food and concentrated it to 610.0 parts per million in the hepatopancreas. Aroclor 1254 residue data from shrimp collected in the estuary are included in the study.

Introduction

Since 1966, pesticide toxicologists and ecologists have become increasingly aware of polychlorinated biphenyls (PCBs). First discovered in fishes, feathers, and human hair (Jensen, 1966), residues have since been found in many organisms from diverse areas of the world. Structurally, PCBs resemble chlorinated hydrocarbon pesticides such as DDT, and are widely used in formulating plastics, resins for rubber-based lacquers, varnishes, paints, lubricants, heat-transfer fluids and electrical insulators. PCBs are relatively

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* Contribution No. 128, Gulf Breeze Laboratory.

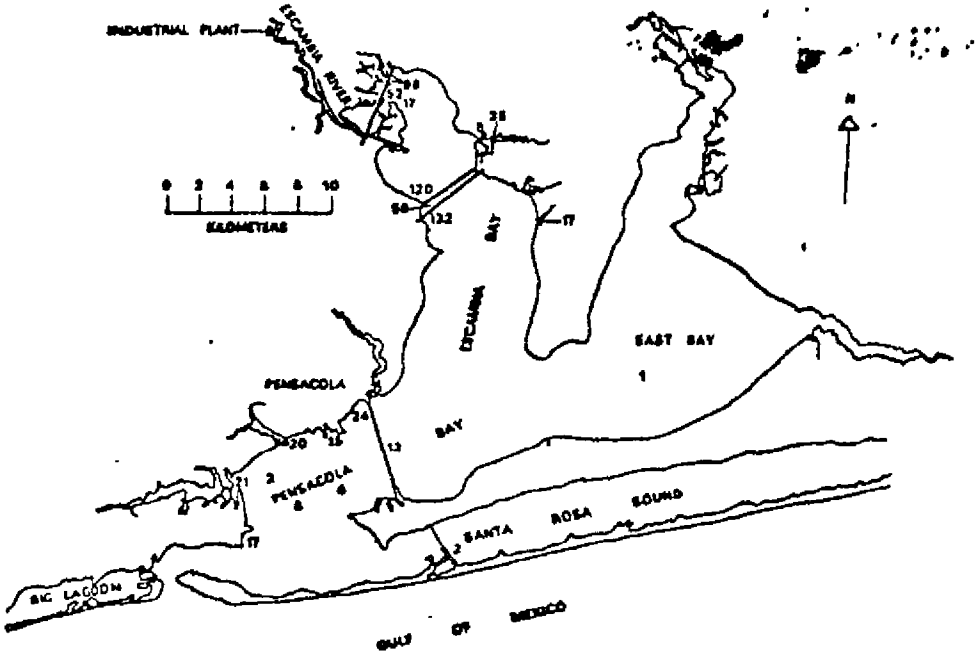

Fig. 1. Residues of Aroclor 1254 (in ppm) found in shrimp hepatopancreases from Escambia Bay and contiguous waters during 1969/1970. Each sample listed represents composite tissues of at least 5 individuals

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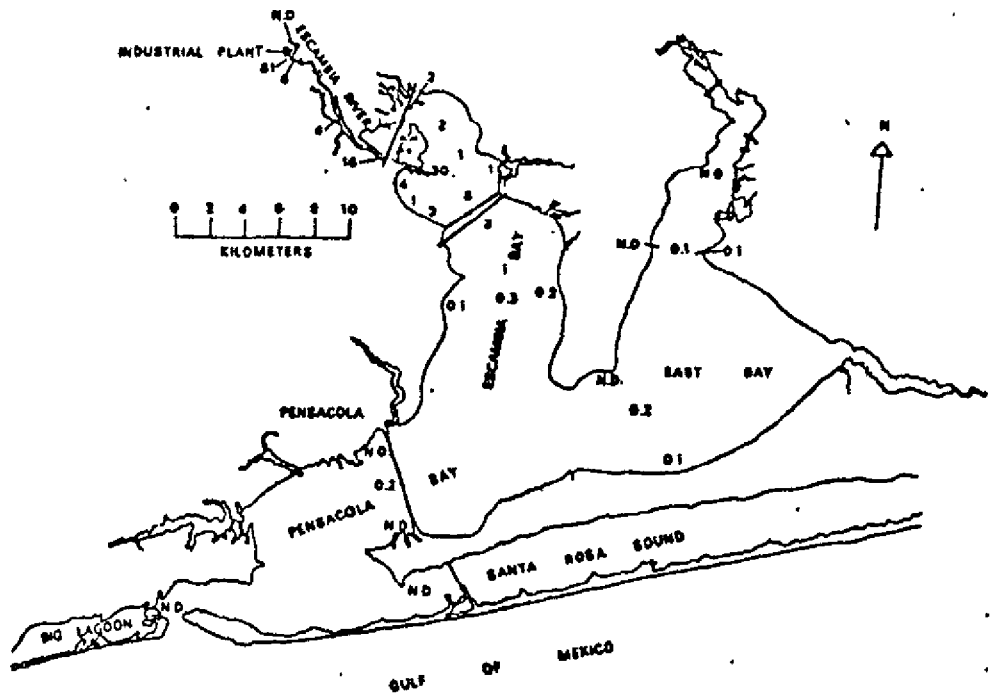

Fig. 2. Residues of Aroclor 1254 (in ppm) found in sediments from Escambia Bay and contiguous waters during 1969/1970. N.D.: less than 0.03 ppm

insoluble in water, but soluble in lipid and lipid solvents. In addition to their thermal stability they are also resistant to acid and base, and therefore, persist in the environment.

PCBs are toxic to trout and blue gill (spp. not given) — GUSTAFSON, 1970; shrimp (*Penaeus duorarum*) and oysters (*Crassostrea virginica*) — DUKE et al., 1970, and a fish (*Lagodon rhomboides*) — HANSEN et al., 1971. Also, a PCB used as a binder in epoxy paint was toxic to chickens (GUSTAFSON, 1970). Abnormally thin-shelled eggs of birds in Great Britain and North America were associated with residues of chlorinated hydrocarbons including the PCBs (RISEBROUGH et al., 1968). In 1969, a PCB (Aroclor 1254) was discovered as a contaminant in water, sediment and fauna of Escambia Bay, Florida (DUKE et al., 1970). One source of this material was traced to an accidental leak in a heat-exchange system of an industrial plant located several kilometers upstream in Escambia River. It is now present in estuarine organisms, including shrimp captured from Escambia Bay and contiguous waters (Fig. 1). Sediments from the river and upper bay appear to be a reservoir for the compound (Fig. 2). In earlier experiments, shrimp exposed to these sediments for 30 days accumulated the chemical (NIMMO et al., 1971). In this paper we report toxicity of Aroclor 1254 in water, rates of accumulation from food and water, and distribution of this PCB in the organs of the pink shrimp *Penaeus duorarum*.

Materials and methods

Shrimp for laboratory studies were obtained from two sources. Juvenile (2.5 to 3.8 cm) pink shrimp (*Penaeus duorarum*) were collected with a small net from Santa Rosa Sound at Pensacola Beach, Florida, in June through September. Adult pink shrimp from Tampa, Florida, were purchased from a live-bait dealer. Background concentrations of chlorinated hydrocarbon compounds in the hepatopancreases of all shrimp never exceeded 0.6 parts per million (ppm); whole-body residues were less than 0.01 ppm.

All shrimp were acclimated in flowing sea water for several days in the laboratory. Juveniles fed on detritus carried in by the flowing unfiltered sea water and adults were fed mullet (*Mugil cephalus*) muscle containing less than 0.03 ppm organochlorine compounds each day. Beach sand with no detectable organochlorine compounds was provided as a substrate for the shrimp.

Shrimp were exposed to Aroclor 1254 (hereafter called Aroclor) in flowing-water systems. The Aroclor was dissolved in polyethylene glycol 200, infused into the flowing water with syringe pumps, then mixed by

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a series of baffles as it flowed into aquaria. The volume of the aquaria varied from 18 to 160 l commensurate with the numbers and sizes of test animals and flow-rate of water. Animal to volume ratio was 1 animal per 2 l water. Concentrations of Aroclor were routinely measured by gas chromatography. No attempt was made to control salinities which ranged from 25 to 32‰, but electric aquarium heaters were used to maintain water temperatures between 20° and 30 °C.

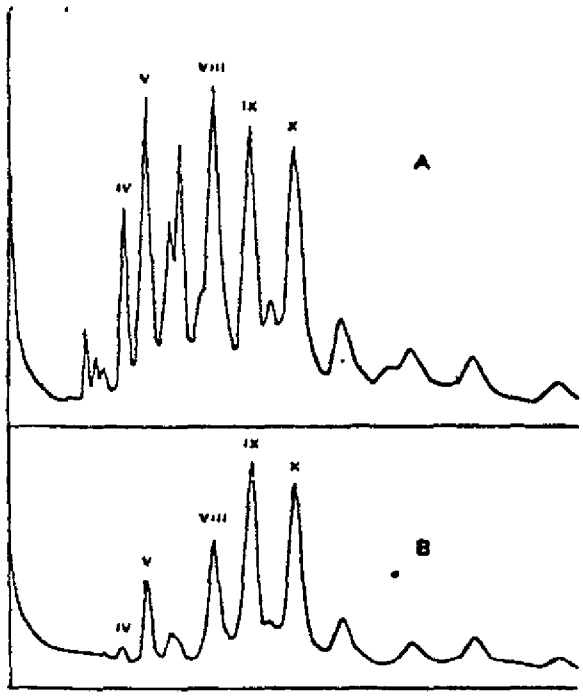

Fig. 3. Aroclor chromatograms (A) Aroclor 1254 standard, (B) Aroclor 1254, recovered from *Penaeus duorarum* hepatopancreas after shrimps had been fed fish containing material (see Table 4). Concentrations of DDT and its metabolites were negligible, therefore, no attempt was made to separate them from Aroclor isomers. Gas flow 25 ml/min, nitrogen; injection temperature 210 °C, oven temperature 190 °C, detector temperature 210 °C; H^3 electron capture detector; 152.4 × 0.317 cm glass-column packed with 2% OV-1 on 100/120 Gas Chrom Q

Concentrations of Aroclor in shrimp were determined from pooled samples by gas chromatography. When a group of 10 shrimp was exposed to a constant concentration of Aroclor in flowing-water, individual residues differed by a factor of 10. Consequently, laboratory analyses are from composite samples of at least 10 individuals, unless stated otherwise. All analyses on *Penaeus duorarum* from Escambia Bay and contiguous waters were on composite samples of at least 5 individuals.

Depending on type and weight of the samples, four methods of preparation were employed (1) Samples of shrimp or food items larger than 1 g were mixed with anhydrous sodium sulfate in a blender and extracted for 4 h with petroleum ether in a Soxhlet apparatus. Extracts were concentrated and eluted from a Florisil column with 6% ethyl ether in petroleum ether. (2) Samples less than 1 g were analyzed by a modification of the micro-method of Enos (private communication). Samples were weighed in Duall[1] tissue grinders and extracted with three 2.0 ml portions of acetonitrile. The acetonitrile extracts were combined and diluted with 6 ml of 2% Na_2SO_4 in distilled water, then agitated and extracted with three 2.0 ml portions of hexane. These extracts were combined and concentrated to about 0.5 ml, then transferred to a Size "B" Chromaflex[1] column containing 1.5 g of Florisil topped with 1.5 g of anhydrous sodium sulfate. The residue was eluted from the column with 20.0 ml of 1% ethyl ether in hexane. (3) Water samples were extracted with petroleum ether, then the extracts were dried with anhydrous sodium sulfate and reduced to an appropriate volume. (4) Sediments were analyzed by the method of Nimmo et al. (1971).

All eluates were adjusted to an appropriate volume for analysis by electron-capture gas chromatographs equipped with OV-1 columns. Quantitation of Aroclor 1254, a multiple-peaked compound, was made by averaging the heights of 5 major peaks which had retention times relative to aldrin of 1.31 (*IV*), 1.55 (*V*), 2.32 (*VIII*), 2.74 (*IX*) and 3.27 (*X*) (Fig. 3). Interference from DDT was negligible due to the relatively high residues of Aroclor 1254 in most samples. Laboratory tests indicated recovery rates above 80%, but data in this report do not include a correction factor for recovery. The presence of Aroclor 1254 in shrimp and sediments was verified by mass spectroscopy at the Environmental Protection Agency Laboratory, Athens, Georgia.

Results

Acute and chronic bioassays

Acute toxicity tests at this laboratory showed that Aroclor was about one tenth as toxic to juvenile *Penaeus duorarum* as DDT (Table 1). For example, 10.0 parts per billion (ppb) DDT in the water killed 100% of a population of shrimp in 96 h, whereas 100.0 ppb in the water was necessary to obtain the same results with Aroclor.

In chronic flowing-water bioassays, Aroclor at 0.94 ppb killed 51% of the juvenile shrimp (2.5 to 3.8 cm) within 15 days (Table 2). Juvenile shrimp were more sensitive to Aroclor than adults. Exposure to 3.5 ppb for 35 days resulted in a mortality of 50% in a group of adult shrimp (9.5 to 12.5 cm). The data

[1] Kontes Glass Co., Vineland, N. J., USA.

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show the need for challenging several stages in the life cycle of shrimp with chemicals such as Aroclor.

We have observed that symptoms of Aroclor poisoning in shrimp are different from those of most organochlorine insecticides. Pink shrimp which were exposed to 0.15 ppb or more DDT showed nervous impairments such as tremors, loss of equilibrium and, finally, paralysis as defined by cessation of locomotor movements. In tests with Aroclor, regardless of its concentration, shrimp showed delayed mortality and died at a rate of one or two per day with no apparent prior symptoms of poisoning. Like others (DUKE, et al., 1970; WILDISH, 1970), we suggest that crustaceans may be more susceptible to the chemical during molting.

Table 1. *Penaeus duorarum. Comparison of toxicities of p, p' – DDT and Aroclor 1254 to shrimp in flowing-water tests. Mean temperature and salinity of sea water in DDT experiment were 24 °C and 28 ‰, respectively; for Aroclor 1254, 19 °C and 31 ‰*

DDT[a]			Aroclor 1254[b]		
Test concentration (ppb)	Mortality 48 h (%)	96 h (%)	Test concentration (ppb)	Mortality 48 h (%)	96 h (%)
10.0	100	100	100.0	80	100
1.0	30	80	10.0	0	0
0.5	0	40	1.0	0	0
0.1	10	20	Control	0	0
Control	0	0			

[a] Personal communication, J. I. LOWE, Environmental Protection Agency, Gulf Breeze, Florida 32561, USA.
[b] DUKE et al., 1970.

Accumulation and transfer of Aroclor in tissues

The uptake of Aroclor from water by adult *Penaeus duorarum* (3.8 to 7.6 cm) and the translocation to the hepatopancreas, whole body and abdominal muscle was measured (Fig. 4). Accumulation was linear with time in the hepatopancreas ($r = 0.97$) and whole body ($r = 0.90$), but a plateau was reached

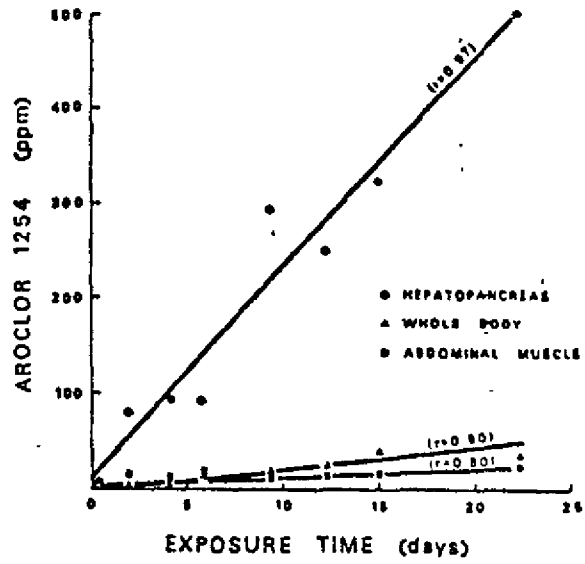

Fig. 4. *Penaeus duorarum*. Rates of absorption of Aroclor 1254, in various tissues of shrimp exposed to 2.5 ppb of the chemical in flowing water. Unexposed shrimp showed no detectable residue. r = correlation coefficient

Table 2. *Results of chronic bioassays with Aroclor 1254 and the pink shrimp Penaeus duorarum in flowing water*

Shrimp rostrum-telson length (cm)	Concentration[a] (ppb)	Average salinity (‰)	Average temperature (°C)	No. of test individuals	Replicates	Days exposed	Average mortality (%)	Level of significance
2.5 – 3.8	Control	32	29	65	5	15	12	—
2.5 – 3.8	0.57	32	29	20	2	15	30	0.10[b]
2.5 – 3.8	0.94	32	29	45	3	15	51	0.005[b]
2.5 – 3.8	9.4	32	29	20	2	15	90	0.001[b]
2.5 – 3.8	19.0	32	29	20	2	15	100	0.001[b]
4.2 – 7.2	Control	29	28	25	1	32	4	—
4.2 – 7.2	2.4	29	28	20	1	17	65	0.001[c]
4.2 – 7.2	3.1	29	28	25	1	32	60	0.001[c]
6.0 – 9.0	Control	29	20	43	1	53	26	—
6.0 – 9.0	4.3	29	20	46	1	53	83	0.001[c]
7.0 – 8.5	Control	31	29	60	1	18	0	—
7.0 – 8.5	4.0	31	29	60	1	18	41	0.001[c]
9.5 – 12.5	Control	28	20	50	1	35	8	—
9.5 – 12.5	3.5	28	20	50	1	35	50	0.001[c]

[a] Average of at least three determinations.
[b] Student's t-test.
[c] Chi-square.

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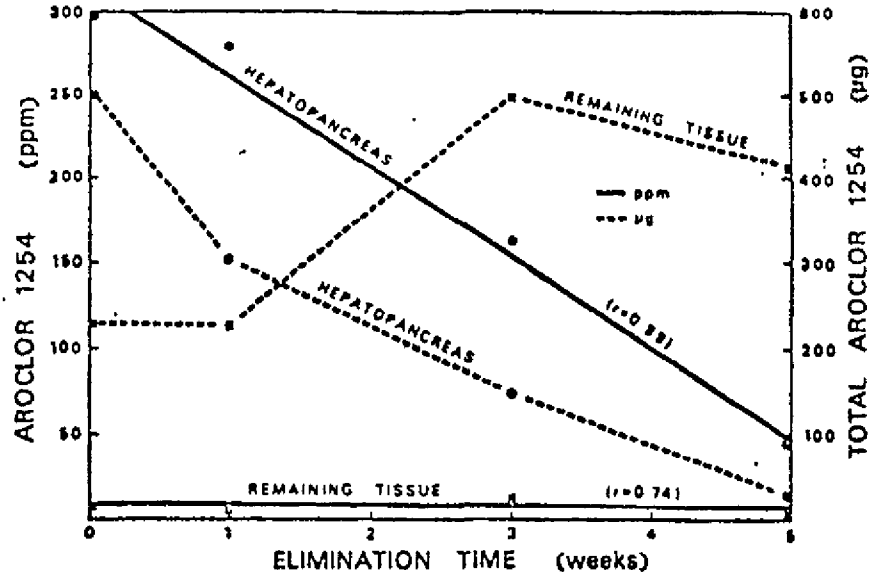

Fig. 5 *Penaeus duorarum*. Rates of elimination from hepatopancreas and subsequent increase in remaining tissue. Shrimp were exposed to 7.5 ppb Aroclor 1254 in flowing water for 16 days, then placed in Aroclor-free environment. Composite tissue samples from 8 individuals were analyzed for each determination. Unexposed shrimp showed no detectable residues. r = correlation coefficient

in the muscle within 2 days, with little increase thereafter. Residues in the hepatopancreas reached 510.0 ppm after 22 days and represented a 2.04×10^5 increase over the 2.5 ppb Aroclor in the test water. During this experiment, 50% of the exposed and 7% of the unexposed shrimp died.

In a subsequent experiment, most of the Aroclor was lost from the hepatopancreas and transferred to other tissues (Fig. 5). Adult shrimp (9.5 to 11.5 cm) were first exposed to 7.5 ppb Aroclor in the water for 16 days, then placed in an Aroclor-free environment for 5 weeks. Total weight (µg) in the hepatopancreas decreased by 80% in 5 weeks, but that in the remaining tissues almost doubled. Whole-body loss was from 731 to 466 µg or about 60% in 5 weeks.

A slightly different picture exists concerning the loss of Aroclor from the tissues if we express the amount in parts per million (Fig. 5). Aroclor (in ppm) showed little change in tissues other than the hepatopancreas during post exposure. In contrast, the rate of elimination from the hepatopancreas was constant and linear with time ($r = 0.99$), the biological halflife in this organ being 17 days. Aroclor is more persistent in the other tissues of shrimp than DDT, the insecticide being completely eliminated in 3 weeks (Nimmo et al., 1970). While the *Penaeus duorarum* were held in the Aroclor-free environment, 23% of the exposed shrimp died, with no loss of the unexposed.

Accumulation in body organs

Residues found in laboratory experiments are compared with those in natural populations of shrimps in Escambia and Pensacola Bays in Table 3. In all tests, the shrimp incorporated the chemical. The proportion of Aroclor in tissues of shrimp which were exposed to 0.2 ppb in the water for 50 days was nearest to that found in shrimps captured alive from the bays.

The distribution of Aroclor in the tissues of shrimp is much the same as DDT, maximum amounts occurring in the hepatopancreas and least in abdominal muscle or exoskeleton (Nimmo et al., 1970). Generally, the distribution of Aroclor in the tissues of *Penaeus duorarum* corresponded to the amount of lipid in the tissues.

We believe water and food are sources of Aroclor to shrimp, but we do not know which contributes more. Farfante (1969) summarized earlier work on the feeding habits of shrimp and reported that the three commercially-important penneid shrimps, pink *Penaeus duorarum*, white *P. setiferus*, and brown *P. aztecus*, are omnivorous. Some of the contents found in digestive tracts by other investigators include inorganic debris, detritus, and a variety of algae, including diatoms. Aroclor attached to detrital material in aquaria or in field substrates was probably ingested by the shrimp.

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Table 3. *Distribution of Aroclor 1254 in tissues of exposed Penaeus duorarum which had accumulated chemical from water and food and in penaeid shrimps from natural populations in the Pensacola estuary, Florida, USA*

Methods of exposure	Hepato-pancreas ppm	Ventral nerve	Digestive tract	Heart	Gills	Exo-skeleton	Abdominal muscle
Water							
Pink shrimp exposed to 3.5 ppb Aroclor in water for 35 days	108	120	32	77	59	14	15
Pink shrimp exposed to 0.2 ppb Aroclor in water for 50 days	30	3.3	3.2	2.8	1.4	0.8	0.7
Food							
Pink shrimp fed spot[a] (43.0 ppm whole body) for 16 days	146	89	16	25	38	6.5	67
Pink shrimp fed spot (field-captured, 0.2 ppm whole body) for 16 days	0.6	0.5	<0.1	0.3	0.3	0.2	<0.1
Pink shrimp fed croaker[b] (0.68 ppm in muscle) for 50 days	5.8	2.0	1.0	1.3	0.9	0.6	0.6
Natural populations							
Pink shrimp captured 10. I. 1970[c]	15	9.4	1.1	—	1.4	0.6	0.9
Pink shrimp captured 3. IV. 1970[c]	4.6	0.6	0.5	0.5	0.3	0.2	0.1
White shrimp captured 28. VIII. 1970[d]	17	4.0	1.5	1.0	0.8	0.8	1.3
Brown shrimp captured 28. VIII. 1970[d]	98	14	9.7	4.2	3.1	2.2	0.9

[a] The spot *Leiostomus xanthurus* were previously exposed to 5.0 ppb Aroclor 1254 in the water for 12 days.
[b] Atlantic croaker *Micropogon undulatus* were captured in Escambia Bay, Florida.
[c] Pensacola Bay.
[d] Escambia Bay.

Table 4. *Percentages[a] of 5 peaks in Aroclor 1254 recovered from water, fish and Penaeus duorarum*

Item	Peak (%)				
	IV	*V*	*VIII*	*IX*	*X*
Standard	10.0	18.5	25.0	22.2	20.0
Water (30 ‰ S)	8.2	15.6	30.0	22.8	23.1
Fish muscle[b]	5.1	10.8	27.4	27.9	28.4
Shrimp hepatopancreas[c]	2.8	10.9	23.9	31.1	31.1

[a] $\frac{\text{peak height}}{\text{sum of 5 peaks}} \times 100$.
[b] The Atlantic croaker *Micropogon undulatus* were captured from Escambia Bay and contained 0.68 ppm Aroclor 1254.
[c] The shrimp *Penaeus duorarum* were fed Atlantic croaker muscle.

We investigated differences in the proportion of Aroclor peaks with respect to the standard; the change is greatest in the hepatopancreas of shrimp (Fig. 3; Table 4). Heights of 5 peaks used for quantitation of the chemical show the greatest reduction in peaks *IV* and *V*, with some increase in *IX* and *X*. We do not know whether this reflects actual alterations in the molecules or differential solubilities in the various systems.

Results of field studies

Distribution of shrimp in the estuary in relation to salinity is a factor which regulates the amount of Aroclor in the body (Fig. 1). The brown shrimp *Penaeus aztecus* from upper Escambia Bay had the highest residues (132.0 ppm in the hepatopancreas). The white shrimp *P. setiferus* from the mouths of small streams emptying into Escambia Bay had a maximum residue of 59.0 ppm. The highest residue in the pink shrimp *P. duorarum* captured in Pensacola Bay was 15.0 ppm. *P. setiferus* is most abundant in low salinity waters of less than 10‰ and *P. aztecus* occurs mostly in waters of 10‰ or more; the abundance of *P. duorarum* is not as dependent on salinity (Farfante, 1969). Although this distribution may vary with locale, types of substrate and seasonal temperatures, shrimp with the highest residues in this study were those captured in the lower salinities. Because higher concentrations of Aroclor occur in the sediments of upper Escambia Bay (Fig. 2), it is possible that burrowing activities of brown shrimp in these sediments could have caused additional absorption of leached chemical through the gills (Nimmo et al., 1971) as well as ingestion of contaminated food.

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Discussion and conclusions

In our investigations, there has been no evidence that Aroclor in the water, sediments, or biota in Escambia Bay was toxic to shrimp. We found no dead or dying shrimp in areas of "fish kills" which occurred frequently during summer months of the past two years. Aroclor in water samples collected 48 cm above the sediments in the upper Bay was below that considered toxic to shrimp, but it was detectable (0.06 ppb in unfiltered water). It probably leached from the sediments or was attached to suspended particulate matter. Adult shrimp in live cages placed directly on sediments in upper Escambia Bay for 3 weeks did not die nor did the "controls" held 48 cm above in an uncontaminated substratum. Shrimp on lower sediments accumulated almost 3 times more Aroclor (6.7 ppm in the hepatopancreas) than did controls.

Nevertheless, our laboratory investigations show that Aroclor 1254 in solution is toxic in the 1 ppb range to shrimp. Therefore, the occurrence of this chemical in the water of Escambia Bay or in other estuarine areas is reason for concern. We also believe the residues found in shrimp from the Escambia Bay are high enough to be of importance, although we have not found a correlation between residues and mortality. If postlarval or juvenile shrimp were exposed directly to the sediments in upper Escambia Bay for a period of weeks, a threat could exist because of availability of PCB-laden detritus and also the leaching of the chemical at the water-substrate interface. We are now investigating this possibility.

Deaths of shrimp due to this or any other contaminant in natural environments would be difficult to observe. Except as larvae, shrimp are primarily benthic, secretive animals, and hide by burrowing in the sediment. If they are active at night, they usually remain below the substrate by day. Here, they may obtain higher concentrations of Aroclor than when swimming, and if they die, dead shrimp do not surface after dying as do fish, rather they decompose rapidly or are quickly eaten by predators.

Aroclor occurs in the tissues of shrimp which were captured several kilometers from the original sources of the material in Escambia Bay (Fig. 1). This suggests the potential of this material to be dispersed through the ecosystem, and it might be available to man through his seafood. We believe this contamination should serve as a warning to increase monitoring of the environment for Aroclor and related industrial materials.

Summary

1. A concentration of 1.0 part per billion of Aroclor in the water kills the juvenile pink shrimp *Penaeus duorarum* within 15 days in the laboratory. Adult shrimp are not as susceptible, but higher concentrations of 2.4 to 4.3 ppb kill within 17 to 53 days — depending on the maturity of test individuals.

2. In laboratory tests in which Aroclor was added to the water, *P. duorarum* absorbed the material and concentrated it in the hepatopancreas. Subsequently, it was transferred to other tissues, then lost. It was more persistent in the tissues of shrimp than the chemically-related pesticide, DDT.

3. Aroclor was absorbed from the water and its subsequent distribution in tissues was similar to that found in wild shrimp from contaminated areas. In the laboratory, shrimp also obtained the Aroclor from food, and we conclude that both sources are available to shrimp in the Pensacola estuary.

4. Concentrations of Aroclor in *P. duorarum* from the estuary appear to reflect distribution patterns in shrimp. Aroclor in shrimp captured at considerable distances (up to 24 km) from the original source demonstrate the potential for dispersion and concentration of this material in an ecosystem.

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First author's address: Dr. D. R. NIMMO
Environmental Protection Agency
Gulf Breeze Laboratory
Sabine Island
Gulf Breeze, Florida 32561
USA

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Aroclor 1254 (ppb) in Water of Escambia Bay System

DATE	ABOVE MONSANTO	NEW OUTFALL	OLD OUTFALL	DOWNSTREAM
8/15/69			71.0	
"			6.8	
9/ 2/69			3.5	
9/ 9/70			1.0	
9/18/70			3.0 surface	
"			0.6 bottom	
12/15/70			0.4 surface	
"			0.3 bottom	
"			ND 1/ filtered	
12/15/70			tr 2/ surface	ND Hwy 90
"			" "	" " "
"			" bottom	" " "
"			" "	" " "
"			" surface	
"			" "	
"			0.1 bottom	
"			0.08 "	
"			0.06 surface	
"			0.2 "	
"			ND bottom	
"			" "	
"			0.6 surface	
"			0.4 "	
"			ND bottom	
"			ND "	
"			0.2 surface	
"			" "	
"			" bottom	
"			" "	
"			0.4 surface	
"			0.3 bottom	
12/13/70			ND surface (filtered)	
"				ND Hwy 90
1/12/71			8.6 surface	
"			7.8 bottom	
"				ND White River
1/14/71			0.4 surface (filtered)	
1/23/71			2.0 surface	
4/16/71			1.3 "	
6/23/71			0.05 "	
6/23/71			"	0.03 3100 yd downstre from outfall
7/ 7/71			0.13 midstream at	
"			ND outfall	
7/ 8/71				ND
"				tr
"				0.37
"				—
"				—
"				—
8/10/71			<0.03 surface	
"			" bottom	
"		ND		
"		"		
"		"		
"		0.71		
9/10/71		0.11 surface	ND	
"		ND bottom	ND	
10/7/71		0.4 surface	ND	
"		ND bottom	ND	
10/26/71		tr surface	0.17 surface	tr
"		tr surface	0.15 "	
"		tr bottom	— bottom	
"		tr bottom	0.085 "	

1/ ND = <0.03 ppb

2/ Tr = 0.03 - 0.05 ppb

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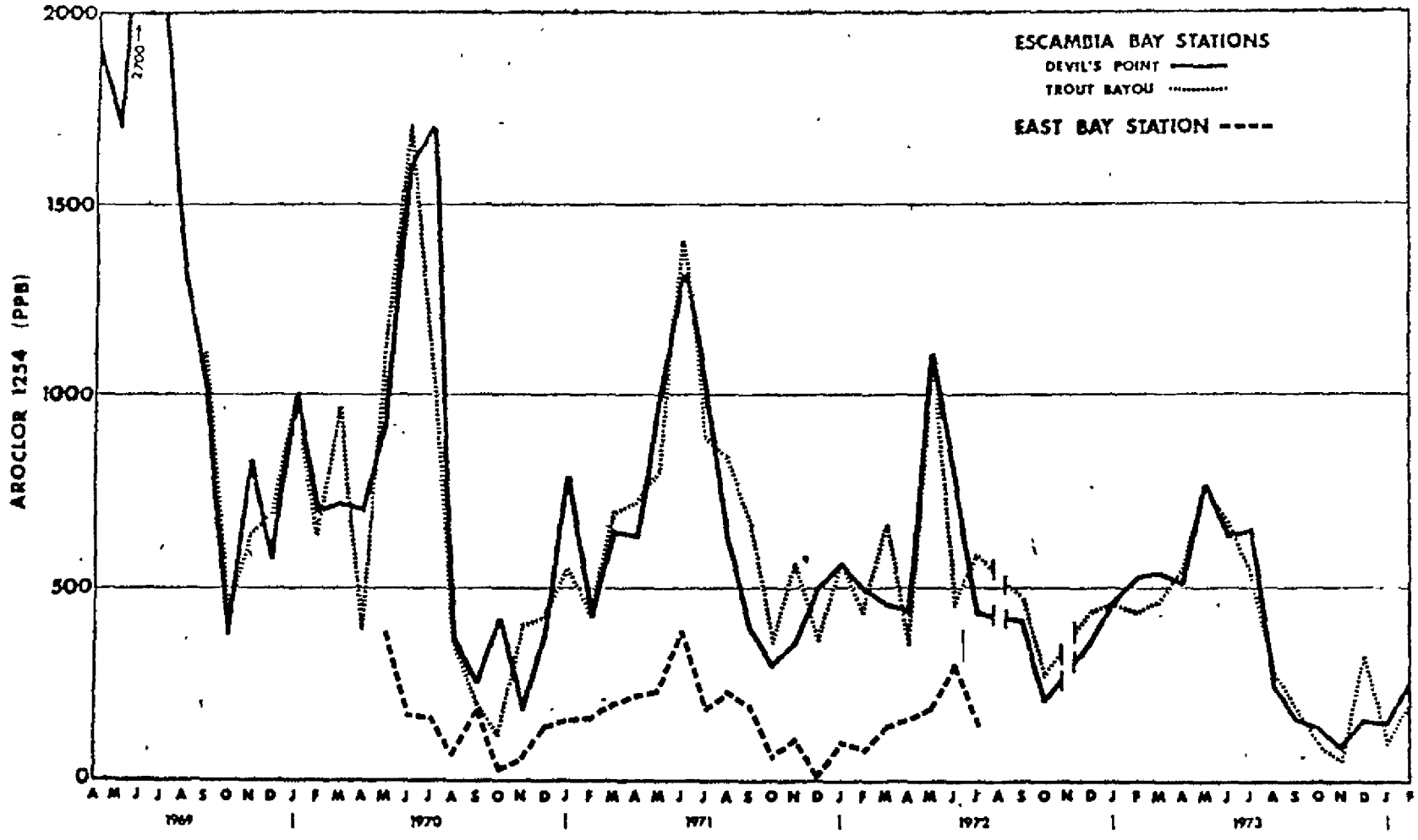
DATE	ABOVE MONSANTO	NEW OUTFALL	OLD OUTFALL	DOWNSTREAM
12/16/71		ND surface		
"		ND bottom	ND surface	
1/19/72	0.03 surface	0.10 surface	<0.03 surface	
2/17/72	" surface	0.11 surface	" "	
3/27/72	" "	0.09 "	" "	
5/16/72	ND; 0.03 "	<0.03 "	" "	
6/6/72	0.03 "	0.06 "	" "	
"		0.044 bottom		
6/7/72	0.03 "	0.03 surface	" "	
"		<0.03 "		
8/3/72	0.073; 0.074 surface	0.076; 0.17 surface	0.031; 0.058 surface	
"	0.058; 0.13 bottom	0.041; 0.032 bottom	0.064; 0.17 bottom	
8/7/72	0.036 surface	0.053 surface	0.048 surface	
"	0.055 bottom	0.033 bottom	0.080 bottom	
10/6/72	0.040 surface	0.20 surface	0.10 surface	
	0.076 bottom	0.089 bottom	0.18 bottom	
10/25/72	<0.03 surface	<0.03; 0.083 surface	<0.03; 0.044 surface	
11/13/72	ND surface	ND surface	ND surface	
12/11/72	<0.03 surface	0.081 surface	<0.03 surface	
"		<0.03 bottom		
1/16/73	<0.03 surface	<0.03 surface	<0.03 surface	
"			0.15 bottom	
2/6/73	<0.03 surface	0.076 surface	<0.03 surface	
"		<0.03 bottom		
3/15/73	<0.03 surface	<0.03 surface	<0.03 surface	
4/11/73	"	"	"	
5/24/73	"	"	"	
7/12/73	"	"	"	
9/20/73	"	0.1 surface	"	
"	"	<0.03 bottom	"	
11/ 1/73	"	0.077 surface	"	
"		<0.03 bottom	"	
1/23/74	"	0.11 surface	"	
[illegible]	"	<0.03 bottom	"	
3/2/74	ND	0.33; ND	ND	

1/ ND = < 0.03 ppb

2/ Tr = 0.03 - 0.05 ppb

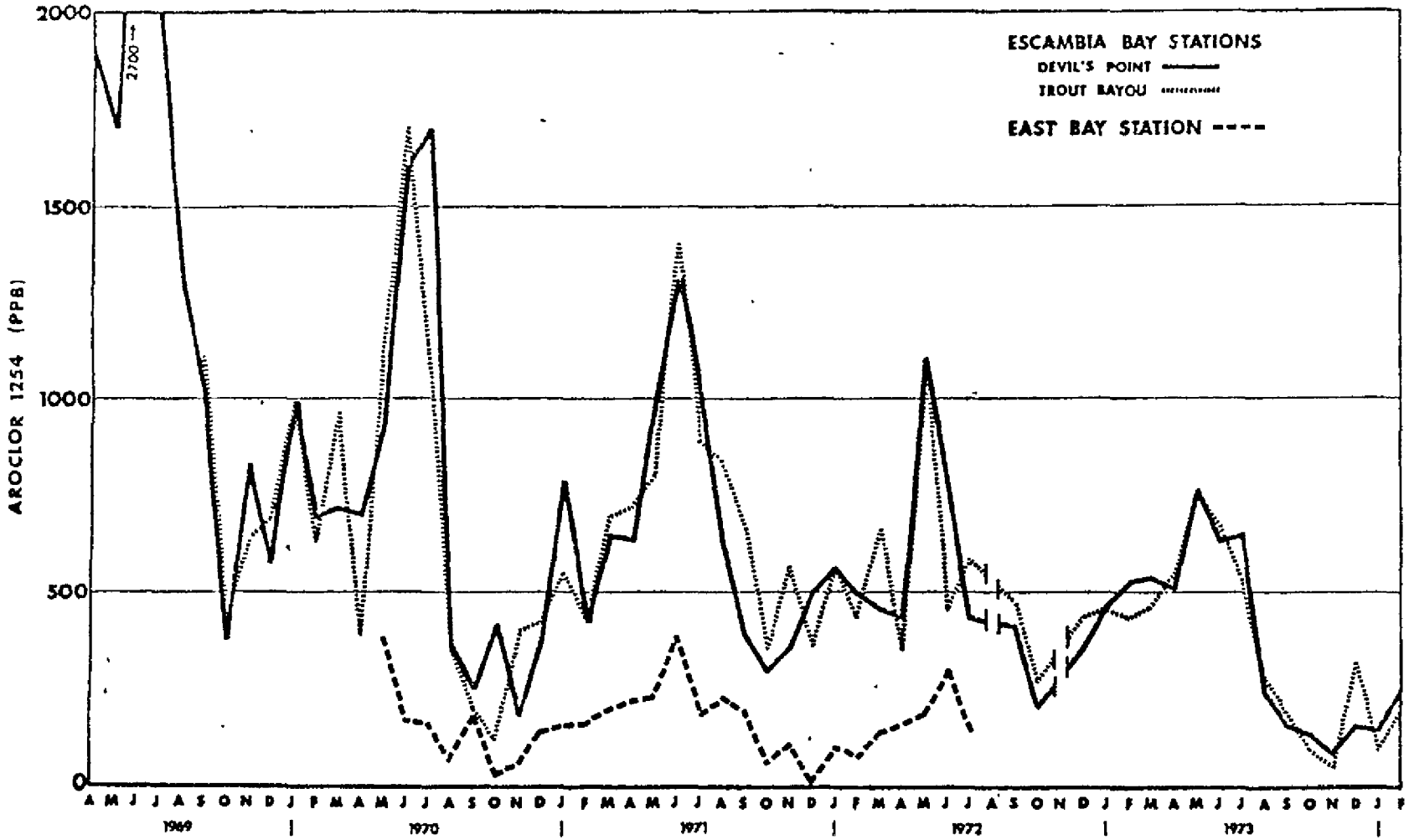
MONS 204356

RESIDUES OF AROCLOR 1254 IN OYSTERS



MONS 204357

RESIDUES OF AROCLOR 1254 IN OYSTERS



MONS 204358

GULF BREEZE, FLORIDA 32561

(12)

Bio-assay Screening Test

Chemical: Aroclor 1248
Use: Plasticizer for chlorinated rubber
Class: Chlorinated biphenyl
Grade: Technical

	Date d-m-y	*EC_{50} 24 hrs.	*EC_{50} 48 hrs.	Average Temp. °C.	Average Salinity ‰
Crustacea		PPM or mg/l			
Pink shrimp (Penaeus duorarum)	5-5-69	0.032	0.032	22	31
Mollusks					
Eastern oyster (Crassostrea virginica)	14-4-69	.017		20	29
Fish					
Pinfish (Lagodon rhomboides)	17-4-69	ir at 0.1	10% at 0.1	21	27

*Crustacea - 50% mortality or loss of equilibrium at end of indicated period. Mollusk - 50% decrease in shell deposition compared to controls after 96 hours exposure. Fish - 50% mortality at end of indicated period.

ne = no effect at 1.0 ppm or highest concentration test; ir = irritated only.

Copy verified by:

Date:

MONS 204359

BUREAU OF COMMERCIAL FISHERIES BIOLOGICAL FIELD STATION LABORATORY
GULF BREEZE, FLORIDA 32561

Bio-assay Screening Test

Date: May 5, 1969

Chemical: Aroclor 1248
Use: Plasticizer for chlorinated rubber
Class: Chlorinated biphenyl
Grade: Technical

CRUSTACEA	Test concentration ppm (mg./l.)	Paralysis or mortality - % 24 hrs.	48 hrs.	Average Temp. °C.	Salin %
Pink shrimp (Penaeus duorarum)	.1	100	100	22	31
	.01	0 (ir)	0 (ir)		
	.001	0	0		
EC_{50} (ppm)		0.032	0.032		

Copy verified by:

Date:

MONS 204360

BUREAU OF COMMERCIAL FISHERIES BIOLOGICAL FIELD STATION
GULF BREEZE, FLORIDA 32561

Bio-assay Screening Test

Date: April 17, 1969

Chemical: Aroclor 1248
Use: Plasticizer for chlorinated rubber
Class: Chlorinated biphenyl
Grade: Technical

FISH	Test concentration ppm (mg./l.)	Mortality - % 24 hrs.	48 hrs.	Average Temp. °C.	Average Salinity ‰
Pinfish (Lagodon rhomboides)	.1	0 (ir)	10	21	27
	.01	0	10		
	.001	0	0		
EC_{50} (ppm)		ir at 0.1	10% at 0.1		

Copy verified by:

Date:

MONS 204361

BUREAU OF COMMERCIAL FISHERIES BIOLOGICAL FIELD STATION
GULF BREEZE, FLORIDA 32561

Bio-assay Screening Test

Date: April 14, 1969

Chemical: Aroclor 1248
Use: Plasticizer for chlorinated rubber
Class: Chlorinated biphenyl
Grade: Technical

MOLLUSKS	Test concentration ppm (mg./l.)	Decrease in shell growth in 96 hours %	Number of weeks to recover	EC_{50} ppm	Average Temp. °C.	Average Salinity ‰
Eastern oyster (Crassostrea virginica)	.1	100 *	2	.017	20	29
	.01	36				
	.001	18				

* Fifty percent of the oysters dead after a 96-hour exposure to 0.1 ppm Aroclor 1248

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Date:

MONS 204362

12

BUREAU OF COMMERCIAL FISHERIES BIOLOGICAL ~~LABORATORY~~ FIELD STATION
GULF BREEZE, FLORIDA 32561

12

Bio-assay Screening Test

Chemical:	Aroclor 1260
Use:	Plasticizer for chlorinated rubber
Class:	Chlorinated biphenyl
Grade:	Technical

	Date d-m-y	*EC_{50} 24 hrs.	*EC_{50} 48 hrs.	Average Temp. °C.	Average Salinity ‰
		PPM or mg/ℓ			
Crustacea					
Pink shrimp (Penaeus duorarum)	14-5-69	ir at 0.1	10% at 0.1	23	28
Mollusks					
Eastern oyster (Crassostrea virginica)	12-5-69	.060		23	28
Fish					
Pinfish (Lagodon rhomboides)	13-5-69	ne at 0.1	ne at 0.1	23	29

*Crustacea - 50% mortality or loss of equilibrium at end of indicated period. Mollusk - 50% decrease in shell deposition compared to controls after 96 hours exposure. Fish - 50% mortality at end of indicated period.

ne = no effect at 1.0 ppm or highest concentration test; ir = irritated only.

Copy verified by: [signature]

Date: May 27, 1969

MONS 204363

BUREAU OF COMMERCIAL FISHERIES BIOLOGICAL LABORATORY FIELD STATION
GULF BREEZE, FLORIDA

Bio-assay Screening Test

Date: May 14, 1969

Chemical: Aroclor 1260
Use: Plasticizer for chlorinated rubber
Class: Chlorinated biphenyl
Grade: Technical

CRUSTACEA	Test concentration ppm - (mg./l.)	Paralysis or mortality - % 24 hrs.	48 hrs.	Average Temp. °C.	Salini ‰
Pink shrimp (Penaeus duorarum)	.1	ir	10	23	28
	.01	0	0		
	.001	0	0		
EC_{50} (ppm)		ir at 0.1	10% at 0.1		

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Date: May 27, 1969

MONS 204364

BUREAU OF COMMERCIAL FISHERIES BIOLOGICAL LABORATORY
FIELD STATION
GULF BREEZE, FLORIDA 32561

Bio-assay Screening Test

Date: May 12, 1969

Chemical: Aroclor 1260
Use: Plasticizer for chlorinated rubber
Class: Chlorinated biphenyl
Grade: Technical

MOLLUSKS	Test concentration ppm (mg./ℓ.)	Decrease in shell growth in 96 hours %	Number of weeks to recover	EC_{50} ppm	Average Temp. °C.	Average Salinity ‰
Eastern oyster (Crassostrea virginica)	.1	52	2	.060	23	28
	.01	44				
	.001	8				

Copy verified by:

Date: May 27, 1969

MONS 204365

BUREAU OF COMMERCIAL FISHERIES BIOLOGICAL FIELD STATION LABORATORY
GULF BREEZE, FLORIDA

Bio-assay Screening Test

Date: May 13, 1969

Chemical: Aroclor 1260
Use: Plasticizer for chlorinated rubber
Class: Chlorinated biphenyl
Grade: Technical

FISH	Test concentration ppm (mg./l.)	Mortality - % 24 hrs.	48 hrs.	Average Temp. °C.	Salinity ‰
Pinfish (Lagodon rhomboides)	.1	0	0		23 29
	.01	0	0		
	.001	0	0		
EC_{50} (ppm)		ne at 0.1	ne at 0.1		

Copy verified by:

Date: May 27, 1969

MONS 204366

(16)

GULF BREEZE ENVIRONMENTAL RESEARCH LABORATORY
GULF BREEZE, FLORIDA 32561

Bio-assay Screening Test

Chemical: Aroclor® 1242
Use: Plasticizer
Class: Chlorinated biphenyl
Grade: Technical

	Date d-m-y	96 hr. *EC_{50}	Average Temp. °C.	Average Salinity °/oo
Crustacea		PPM or mg/ℓ		
Brown shrimp (Penaeus aztecus)	7-8-72	0.009	32	28
Grass shrimp (Palaemonetes pugio)	21-8-72	0.016	31	29
Mollusk				
Eastern oyster (Crassostrea virginica)	18-9-72	0.032	31	30
Fish				
Pinfish (Lagodon rhomboides)	12-9-72	ne at 0.1	30	29

*Crustacea - 50% mortality or loss of equilibrium at end of 96 hours.
Mollusk - 50% decrease in shell deposition compared to controls after 96 hours expo
Fish - 50% mortality at end of 96 hours.
ne = no effect at 1.0 ppm or highest concentration test. Ir = irritated only.

Patricia R. Parrish

Copy verified by:

March 8, 1974
Date:

MONS 204367

GULF BREEZE ENVIRONMENTAL RESEARCH LABORATORY
GULF BREEZE, FLORIDA 32561

Bio-assay Screening Test

Date: August 1972

Chemical: Aroclor 1242 ®
Use: Plasticizer
Class: Chlorinated biphenyl
Grade: Technical

CRUSTACEA	Test Concentration ppm (mg /ℓ)	96 hrs. Paralysis or mortality (%)	Average Temp. °C.	Average Salinity °/oo
Brown shrimp (Penaeus aztecus)	0.1	100	32	28
	0.01	70		
	0.001	0		
EC_{50} (ppm)		0.009		
Grass shrimp (Palaemonetes pugio)	0.1	100	31	29
	0.01	20		
	0.001	20		
EC50 (ppm)		0.016		

Patrick E. Parrish
Copy verified by:

March 8, 1974
Date:

MONS 204368

GULF BREEZE ENVIRONMENTAL RESEARCH LAB
GULF BREEZE, FLORIDA 32561

Bioassay Screening Test

Date: September 18, 1972

Chemical: Aroclor 1254
Use: Plasticizer
Class: Chlorinated biphenyl
Grade: Technical

MOLLUSKS	Test concentration ppm (mg/ℓ)	Decrease in shell growth in 96 hours (%)	Average Temp. °C.	Average Salinity °/oo
Eastern oyster (Crassostrea virginica)	0.1	100	31	30
	0.01	0		
	0.001	3		

Patrick R. Parrish

Copy verified by:

Date: March 8, 1974

MONS 204369

GULF BREEZE ENVIRONMENTAL RESEARCH LABORATORY
GULF BREEZE, FLORIDA 32561

Bio-Assay Screening Test

Date: Sept. 12, 1972

Chemical: Aroclor® 1242
Use: Plasticizer
Class: Chlorinated biphenyl
Grade: Technical

FISH	Test Concentration ppm (mg/l)	Mortality (%) 96 hrs.	Average Temp. °C.	Average Salinity °/oo
Pinfish (Lagodon rhomboides)	0.1	0	30	29
	0.01	0		
	0.001	0		
EC_{50} (ppm)		ne at 0.1		

Patrick R. Parrish
Copy verified by:

March 8, 1974
Date:

MONS 204370

Exhibit 9

(17)

(17)

Growth Responses of Estuarine Bacteria to Mirex, Heptachlor and Polychlorinated Biphenyls[1/]

---

[1]Contribution No., Gulf Breeze Environmental Research Laboratory. Protection Agency, Gulf Breeze, Florida 32561. Associate Laboratory of the National Environmental Research Center, Corvallis, Oregon.

MATERIALS and METHODS

Organisms: Eighty-five bacterial isolates from various estuarine environments near Pensacola, Florida were screened for growth responsiveness to heptachlor, mirex and the PCBs, Aroclor® 1016 and 1242. The isolates were maintained and tested in a medium consisting of 1 g/l Difco yeast extract and 5 g/l Difco beef peptone in 20% artificial aged seawater (Rila marine mix), pH 7.4 at 28 C. Fisher agar (2%) was added for solid medium.

Preparation of sensitivity discs: Super absorbant sensitivity discs (Schleicher & Schuell) were saturated with each toxicant dissolved in acetone to give concentrations of 1.0, 2.5 or 5.0 mg/ml. Each saturated disc contained 0.1 ml solution. The discs were allowed to dry thoroughly for 24 hours at room temperature before use.

Screening procedures: The cultures were incubated in nutrient broth tubes for 18h on a rotary shaker at 28 C. One-tenth milliliter of a 1:1 dilution of the broth cultures in sterile 15°/oo seawater was spread on nutrient agar medium and allowed to dry. The toxicant treated, dry paper discs were arranged on the agar surface, four per plate. Discs treated with acetone, but no toxicant, were included as controls. Duplicates of each test were prepared and the cultures were incubated for 24h at 37 C before the results were observed. Isolates sensitive to 0.5 mg of either PCB were further tested at the 0.25 and 0.1 mg concentrations. Sensitivity was defined as a ring of inhibition surrounding the disc (Figure 1).

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Growth Responses of Estuarine Bacteria to

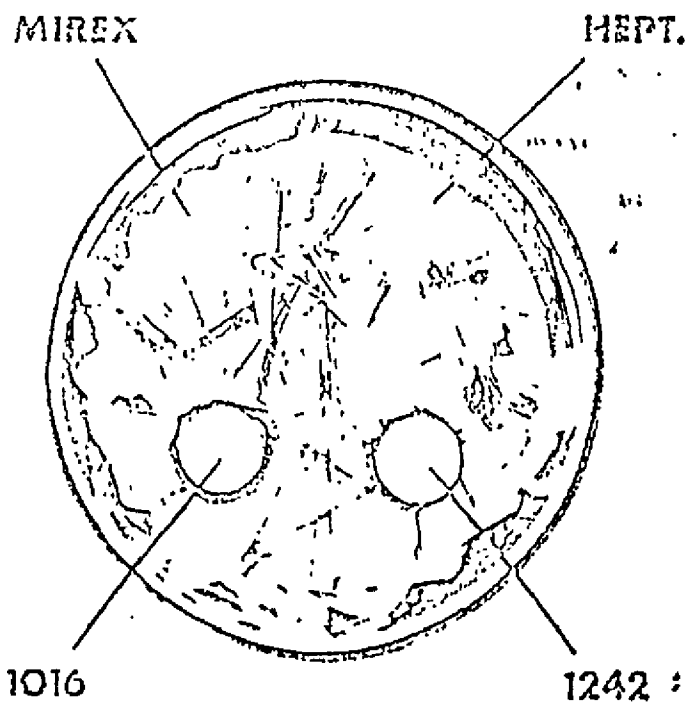
Mirex, Heptachlor and Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) have been shown to be stimulating to the in vitro growth of Escherichia coli, an indicator bacterium from human intestinal flora. (Keil, et al., 1972). The lesser chlorinated biphenyls have been shown to be oxidized by a bacterial isolate from sewage, Achromobacter pCB (Ahmed and Focht, 1973). Pesticides and other chemicals in aquatic and estuarine environments have produced various responses in microbial communities (Ware and Roan, 1970). The fate of many of these pollutants depends on their effect upon the total microbial community. Further, responses of individual organisms directly affected, either in a stimulatory or inhibitory fashion, can provide much insight into degradation or persistance of the toxicant in the system. However, the responses of aquatic and estuarine microorganisms to PCBs and organochlorine pesticides has not been established. (Paris and Lewis, 1973; Bourquin, 1973).

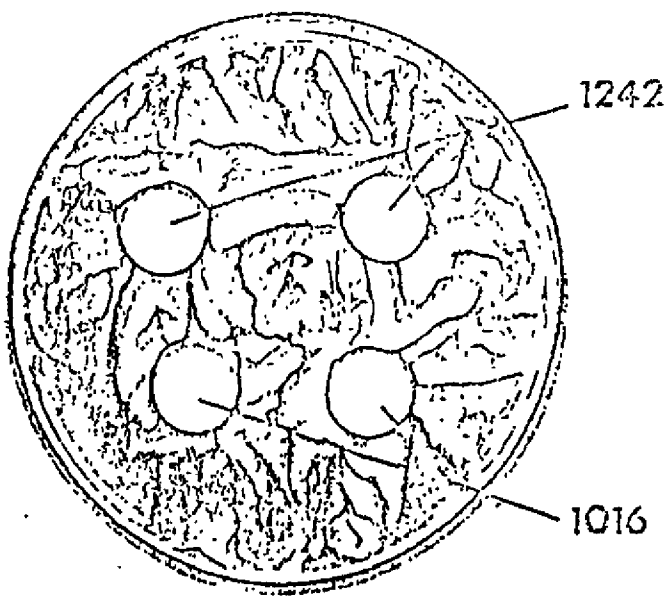
This investigation examines representative estuarine microorganisms responsive to PCBs and organochlorine pesticides using a rapid screeening procedure.

MONS 204373

GROWTH INHIBITION OF ESTUARINE BACTERIUM BY PCB's



0.5 mg



0.25 mg 0.1 mg

MONS 204374

(R) Registered trademark, Monsanto Company, St. Louis, MO. Mention of commercial products or trade names does not constitute endorsement by the Environmental Protection Agency.

Table 1

INHIBITION OF GROWTH OF ESTUARINE MICROORGANISMS BY PCB'S AND PESTICIDES a/

GBERL CULTURE NO.	GRAM REACTION & MORPHOLOGY	AROCLOR® 1242 (mg) 0.1	0.25	0.5	AROCLOR® 1016 (mg) 0.1	0.25	0.5	MIREX 0.5 mg	HEPTACHLOR 0.5 mg
3	+ ROD	++	++	+++ b/	++	++	+++	-	-
21	- COCCOID	++	++	+++	++	++	+++	-	-
35	- Cocci d	++	++	++	+	+++	+++	-	-
39	- COCCOID	++	+++	+++	++	+++	++++	-	-
53	- COCCOID	++	+++	+++	+++	+++	+++	-	-
54	+ ROD	++	+++	+++	+	+++	+++	-	-
7	+ ROD (S)	+	+	+	±	+	++	-	-
9	+ ROD	+	++	+++	+	++	+++	-	-
31	+ ROD	+	++	+++	+	++	+++	-	-
60	± ROD	+	++	++	++	++	++	-	-
86	- ROD	+	+	+	±	+	+	-	+
100	- ROD	+	++	++	+	++	++	-	-
8	+ ROD	±	+	++	±	+	++	-	-
11	- ROD	±	+	++	±	+	++	-	-
42	+ COCCUS	±	+	++	±	+	++	±	-
44	+ COCCUS	±	+	+	-	±		nt	nt
93	+ ROD	±	+	+	-	±	+	+	±
43	+ COCCUS	nt	nt c/	++	nt	nt	++	-	-
5	- COCCOID	-	-	++	-	-	++	-	-
13	- ROD	-	-	++	-	-	++	-	-
28	- ROD	-	-	++	-	-	++	-	-
32	+ COCCOID	-	+	+	-	±	+	-	-
41	- COCCOID	-	-	++	-	-	++	-	-
67	- ROD	nt	nt	+	nt	nt	+	±	±
69	- ROD	-	-	++	-	-	++	-	-

a. Growth inhibition on nutrient SW media, 85 cultures tested

b. (+) Degree of sensitivity, (±) Slightly sensitive, (-) Not sensitive

c. Not tested

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RESULTS

Preliminary tests proved techniques customarily employed in anti-biotic sensitivity testing, not applicable in the quantitation of the inhibitory response of mirex, heptachlor, and the PCBs. These toxicants are insoluble in water and the diffusion rates through the agar were variable, yielding non-quantitative zones of inhibition. A relative inhibitory effect, however, could be distinguished between the bacterial strains and toxicant levels tested by using the method described in Table 1.

Of the 85 bacteria tested, 26 demonstrated zones of inhibition to at least one of the test compounds (Table 1). The PCBs were markedly more inhibitory than the organochlorine pesticides. Only 3% of the strains screened showed inhibition at 0.5mg of mirex and heptachlor. This concentration of Aroclor® 1016 and 1242, however, inhibited 31% of the bacterial strains. Further, o.1mg of Aroclor® 1016 inhibited 69% of these strains and Aroclor® 1242 inhibited 62%.

We attempted to correlate PCB-sensitivity response with biochemical activity or cell composition (Table 2). PCB-sensitive bacteria were both Gram positive and negative and no morphological correlation was noted. However, a comparison of biochemical activities of sensitive and non-sensitive bacteria tested showed two distinctly different groupings. Amylase and gelatinase production was shown by 76% and 86% respectively, of the sensitive strains; however, of the total bacterial strains screened, only 33% and 42% of the populations showed these activities. No other biochemical activity differed

Table 2.

Comparison of Physiological Activities of TCB-sensitive and Non-sensitive Microorganisms

Bacteria Tested	Production of: Urease	Amylase	Lipase	Gelatinase	H_2S	Nitrate Reduction	Citrate Utilization	Gram Reaction
Sensitive	19*	76	33	86	5	29	48	50
Non-Sensitive	13	33	23	42	6	37	41	

*Percentage of cultures tested showing positive reaction, 100 cultures tested.

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distinctly from the non-sensitive bacterial populations. No characteristic gram reaction was shown by the PCB-sensitive bacteria.

DISCUSSION

The sensitivity disc testing procedure commonly used in clinical bacteriology has been successfully applied here as a rapid method for detecting PCB-sensitive estuarine microorganisms in pure culture. Although the procedure is qualitative only, it can serve to eliminate many hours of tedious experimentation involved in the isolation of suitable test organisms for more detailed examinations. This method has been used previously by investigators to study the effects of pesticides on growth of bacteria (Widdus, et al. 1971; Trudgill, et al. 1971). However, no significant response has been shown screening estuarine microbes and chlorinated hydrocarbon pesticides in this study. Conversely, PCB sensitivity in estuarine bacteria was quite dramatic (Figure 1) using this method. Keil et al. (1973) demonstrated no sensitivity to 0.02 mg PCB by E. coli using the same method on non-marine medium. These experiments indicated PCBs stimulate growth of E. coli. No explanation for this difference between stimulation in E. coli (Keil et al, 1973) and inhibiton in estuarine bacteria (this study) has been determined at this time.

The implications of this inhibition, if wide spread in the environment, are obvious. The sensitivity of a large number of heterotrophic bacteria to PCBs may be of considerable importance in an estuarine environment. PCBs have been shown to persist in estuarine sediments in relatively high concentration (9 ppm). Sediment cores taken in close proximity to another showed markedly different concentrations (0.06-9 ppm) indicating the PCBs are pro-

bably absorbed onto particulate organic matter during transport and deposition in estuarine sediments (Butler, 1974). The aforementioned concentrations, when considered absorbed onto distinct detrital particles, are not unlike those shown to be inhibitory to hetrotrophic growth in this study. Consequently, PCBs could inhibit the normal turnover of carbon in estuarine sediments by inhibition of specific heterotrophic bacteria attached to organic detritus.

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Paris, Doris and David L. Lewis. Residue Reviews 45, 95-124 (1973).

Trudgill, P. W., Widdus, and J. A. Rees, J. of Gen. Microbiol. 69, 1-13 (1971).

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Widdus, R., P. W. Trudgill, and D. C. Turnell, J. of Gen. Microbiol. 69, 22-31 (1971).

Studies on the Mechanism of Toxicity of DDT and Polychlorinated Biphenyls (PCBs): Disruption of Osmoregulation in Marine Fish

by W. B. Kinter,* L. S. Merkens,† R. H. Janicki,† and A. M. Guarino‡

In 1970 studies were initiated at the Mount Desert Island Biological Laboratory to explore mechanisms of toxicity underlying the high sensitivity of bony fish, teleosts, to organochlorine pollutants. The starting point was several reports that DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethane] inhibited Na,K-ATPase (Na^+, K^+-activated adenosine triphosphatase), as well as the knowledge that this enzyme appears to play a central role in osmoregulation by marine teleosts. In the face of a desiccative environment, these fish maintain body fluid hypotonicity (Fig. 1) by drinking sea water, absorbing water coupled with salts across the intestinal epithelium, and eventually secreting the NaCl across the gill epithelium while retaining the free water. The primary driving mechanism in both intestine and gill is the Na pump with which Na,K-ATPase appears to be intimately involved. For fuller discussion, see (2) and (3).

After demonstrating that DDT did, in fact, inhibit Na,K-ATPase activity in homogenates of intestinal mucosa and gill filaments from several marine teleosts (2), attention was focused on the sea water-adapted eel (*Anguilla rostrata*), in which osmoregulation has been well investigated. This eel can also adapt to fresh water and can be used for future DDT studies.

Materials and Methods

Eels, *Anguilla rostrata*, and killifish, *Fundulus heteroclitus*, were captured in estuaries along the Maine coast. They were adapted to and maintained in sea water for about three weeks before use. Non-radioactive puriss grade p,p'-DDT was procured from Aldrich Chemical Co., Milwaukee, Wis. ^{14}C-DDT was obtained from Amersham/Searle, Arlington Heights, Ill. and had a specific activity of 24 mCi/mM.

Aroclor 1221 was donated by Monsanto Co., St. Louis, Mo. Plasma milliosmolarity was determined by a standard freezing point depression technique, while Na^+ and K^+ were analyzed by means of a flame photometer. Levels of ^{14}C-DDT were determined as described previously (4). The fractional ^{22}Na space of isolated gills was measured according to the procedure of Kamiya (5) where eel gills are incubated for 1 hr at 15° in oxygenated sea water. Control gills were incubated with the solvent 0.5% N,N-dimethylformamide (DMF), and experimental gills were exposed to 50 ppm DDT in 0.5% DMF. Gill homogenates were assayed for Na,K-ATPase activity as described previously (2). Acute toxicity and osmoregulatory studies were conducted in killifish using a simple static system. For each experiment ten sea water-adapted fish (5 males and 5 females, each weighing about 5 g) were placed in an aluminum or enameled metal container holding 2 liters of sea water and maintained at 14–16° throughout the exposure time. In control containers 2 ml of ethanol were added to the sea water; in experimental containers Aroclor 1221 (mixture of PCBs

* Studies conducted at Mt. Desert Island Biological Laboratory, Salisbury Cove, Maine 04672.

† SUNY Upstate Medical Center, Syracuse, New York 13210.

‡ To whom reprint requests should be sent: Dr. A. M. Guarino, Bldg 10, Rm. 6N110, National Cancer Institute, Bethesda, Md 20014.

MONS 204382

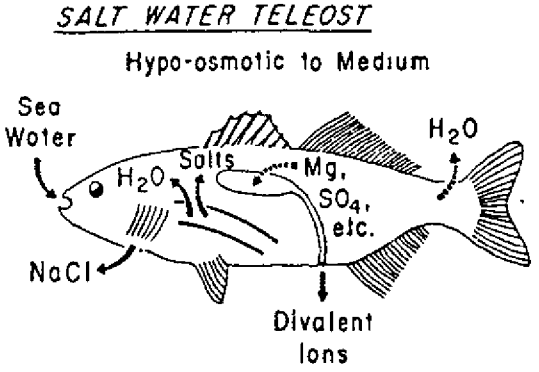

FIGURE 1. Water and salt transport in a salt water teleost. Typically the milliosmols/L are 1,000 for sea water and 350 for fish body fluids. Adapted from (1).

with 21% chlorine content) or p,p'-DDT was first dissolved in the ethanol. These solutions formed a cloudy suspension when added to the sea water, and in addition some separation of the Aroclor was noted, making the actual concentrations unknown (Aroclor 1248 was not tested due to much greater separation). Blood was drawn by cardiac puncture with lightly heparinized glass capillary tubes. Whole blood from 3 to 5 fish was pooled and centrifuged. The 0.05–0.1 ml of serum was diluted, and the osmolarity and concentration of Na and K ions were measured as for the eel.

Results

Ability to osmoregulate was followed in individual eels (about 100 g) placed in 15° sea water (2 liters) containing 0.1% ethanol and 1 ppm DDT, a level which is fatal by 10 hr. In Table 1 it is seen that, at 6 hr, plasma milliosmolarity of

Table 1. Effect of p,p'-DDT on plasma osmolarity, sodium and potassium in sea water adapted eels (*Anguilla rostrata*).*

Treatment	Osmolarity (mosmols /L)	Na^+ (mEq/L)	K^+ (mEq/L)
Control	358±10	163±2	2.4±0.3
p,p'-DDT	462±10†	192±3†	5.4±0.1†

* Mean±SE for 5–6 animals exposed to 1 ppm of DDT for 6 hr.

† Significantly different ($P<0.01$) from controls.

Table 2. ^{14}C-DDT levels in sea water adapted eels (*Anguilla rostrata*).*

Tissue	DDT levels, ppm
Plasma	17.2±3.0
Gill	22.2±2.2
Gut mucosa	6.8±0.8
Brain	11.2±1.0

* Mean±SE for 6 animals exposed to 1 ppm of ^{14}C-DDT for 6 hr.

Table 3. The effects of DDT on sodium space and Na,K-ATPase in gills from sea water adapted eels (*Anguilla rostrata*).*

Treatment	Na space†	ATPase activity‡
Control	0.112±0.010	0.37±0.07
DDT, 50 ppm	0.162±0.010§	0.23±0.03§

* Mean±SE for 15 gills in each group for Na space determination and 5 duplicate assays per group for ATPase assays.

† Fractional Na space after incubation at 15° for 1 hr expressed as ^{24}Na gill filament to media ratio.

‡ μmoles P_i/mg protein×hr.

§ Significantly different ($P<0.01$) from controls

Table 4. Summary of DDT effects on osmoregulation in sea water eels.

Study	Results: Gut	Results: Gill
In vivo at 6 hr (1 ppm in water)*	7 ppm DDT Plasma osmolarity	22 ppm DDT increased 29%
In vitro (50 ppm in media)	H_2O absorption from sacs decreased 47%†	Na content of isolated gills increased 45%
Homogenate (50 ppm)	Na,K-ATPase inhibited 63%†	Na,K-ATPase inhibited 38%

* DDT solubilized with ethanol (0.1% in sea water) or N,N-dimethylformamide (0.5% in media and 5% in homogenate).

† Calculated from ref. 3.

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Table 5. Acute toxicity of Aroclor 1221 and DDT in killifish.

Compound	Initial level* in sea water	Total number of fish	% Dead (cumulative)			
			Day 1	Day 2	Day 3	Day 4
Control (ethanol)		10	0	0	0	0
Aroclor 1221	7.5 ppm	10	0	0	0	0
	25 ppm	10	50	80	80	80
	75 ppm	50	88	96	98	100
DDT	0.025 ppm	10	0	0	0	0
	0.075 ppm	10	40	50	50	50
	0.25 ppm	10	60	80	90	90
	0.75 ppm	10	100	100	100	100

* See description of static system in text.

DDT-treated fish had increased about 30% compared to ethanol controls. Furthermore, in this table one can see that, while much of this increase was due to plasma Na, plasma K also doubled in these animals. In additional experiments with 1 ppm ^{14}C-DDT, the fish had taken up about half the radioactivity by 6 hr. The levels of radioactivity for some tissues are indicated in Table 2 and ranged from 7–22 ppm. Using isolated eel gills the fractional ^{22}Na space was found to be increased significantly (Table 3) by 50 ppm DDT. Data in Table 3 also shows that Na,K-ATPase activity was significantly inhibited (38%) by 50 ppm DDT.

Considered all together (Tables 1, 2, and 3 and summarized in Table 4), our eel data provide circumstantial evidence that disruption of osmoregulation is a primary toxic effect of DDT, *i.e.*, the DDT levels in gut and gill of fatally-dosed fish were high enough to have affected osmoregulatory Na transport under in vitro conditions. Both water absorption from gut sacs and Na content of isolated gills are directly dependent on Na pump activity and can be altered similarly by obtaining a highly specific Na,K-ATPase inhibitor. Moreover, from the published (3) dose-response curve for gut homogenate, 7 ppm DDT in mucosal tissue should still inhibit Na,K-ATPase activity

Table 6. Effects of Aroclor 1221 and DDT on serum osmolarity, Na and K in killifish.

Compound and initial level	*mosmols*/liter	Na^+ mEq/liter	K^+ mEq/liter
Untreated	352±2 (18)	173±2 (16)	3.6±0.2 (16)
6-Hour exposure			
Control	365±3 (6)*	171±2 (6)	4.3±0.2 (5)*
Aroclor 1221			
75 ppm	396±7 (9)†	173±3 (9)	4.4±0.3 (9)
DDT			
0.25 ppm	389±9 (8)†	178±4 (9)	4.4±0.3 (9)
24-Hour exposure			
Control	361±7 (6)	176±2 (6)	5.3±0.5 (6)*
Aroclor 1221			
25 ppm	374±7 (6)	179±4 (6)	4.5±0.3 (6)
75 ppm	407±10 (4)†	189±5 (4)†	5.0±0.4 (4)
DDT			
0.075 ppm	368±3 (7)	180±5 (5)	4.1±0.4 (6)

* Significantly different ($P < 0.05$) from untreated controls.
† Significantly different ($P < 0.05$) from ethanol-treated controls.

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by 45%, provided, of course, that tissue content on a wet weight basis is equivalent to homogenate content based on added DDT.

The data in this same curve also indicate that Mg-ATPase is less sensitive to DDT than Na,K-ATPase. Certainly, until more information is available, the present data would appear to provide a partial explanation as to why a whole body burden of less than 10 ppm DDT is often lethal to fish. It is also recognized that DDT may exert a primary toxic effect on the central nervous system; by 6 hr our fatally-dosed eels were hyperactive and easily stimulated to convulsions by touch. Such symptoms, however, could be secondary to alterations to osmolarity of plasma and cerebrospinal fluid.

Last summer, because of the chemical similarity between the insecticide DDT and the industrial PCBs (polychlorinated biphenyls) pollutants in the environment, acute toxicity and osmoregulatory effects were compared using killifish. The toxicity data, Table 5, show that, with respect to the acute lethal level in sea water, the Aroclor 1221 was less toxic than DDT by a factor of about 100. There was a "dose-response" in terms of the theoretical concentration in sea water and number of deaths for both Aroclor 1221 and DDT, but the actual dose which the fish received is unknown since the tissue levels were not measured.

The osmolarity and Na concentration of the serum (Table 6) consistently increased (toward sea water values) in fish exposed to lethal levels of either Aroclor 1221 or DDT. The Aroclor and DDT levels selected for these serum-composition studies were those that were potentially lethal to most of the fish, but low enough to have killed only some at the time (6 or 24 hr) samples were taken from those still surviving. With the higher levels tested, most of the increases in serum osmolarity and Na were statistically significant when compared to ethanol controls.

Also, serum osmolarity and K were elevated in all ethanol-treated controls ($P < 0.05$, except for 24-hr osmolarity); this elevation may have been due to the ethanol or to confinement of the fish in the experimental containers. Additional data (Table 7) show that in the extreme cases, when blood was sampled before the fish died but after they had completely lost their righting ability and remained sideways, the serum osmolarity and electrolyte concentrations were all increased well above normal. It should be noted that the osmolarity changes (Tables 6 and 7) cannot always be accounted for by changes in measured electrolytes.

Table 7. Osmolarity, Na and K in individual pooled samples of killifish serum.*

Compound and level	Exposure hr	mosmols / liter	Na^+ mEq/liter	K^+ mEq/liter
Aroclor 1221				
75 ppm	23–32	550	208	8.1
		428	210	7.3
DDT				
1 ppm	9–10	430	207	8.3
		440	210	8.9

* Each sample represents serum from about 3 fish.

Discussion

Overall, these results suggest that lethal levels of Aroclor 1221 and DDT both decrease the ability of killifish to osmoregulate to about the same degree. Also killifish and eels seem about equally sensitive to DDT. Thus, inhibition of Na,K-ATPase by appropriate levels of Aroclor 1221 was anticipated. Using a homogenate of King O'Norway (*Hemitripterus americanus*) intestinal mucosa 250 ppm of Aroclor 1221 inhibited the Na,K-ATPase by 51% and 22% in two experiments. Moreover, recently-published work by Yap et al. (6) showed that several of the Aroclor mixtures inhibited the enzyme from fish brain and kidney.

Concerning the molecular basis for Na,K-ATPase inhibition by DDT and PCBs, one may speculate that these lipophilic compounds interact with the phospholipid-activating component of this lipoprotein enzyme (7, 8). It is well known that many lipophilic compounds, e.g., cyclohexanone, inhibit the enzyme and evidence of proton interaction between DDT and a phospholipid, lecithin, recently has been reported (9). Moreover, other organochlorine insecticides, including endrin, also inhibit Na,K-ATPase from fish brain (10), and endrin has been observed to disrupt osmoregulation in both a marine and a fresh water teleost (11, 12). Thus, it seems reasonable to predict that many organochlorine

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pollutants will be found to disrupt osmoregulation in fish.

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Toxicity of Aroclors 1260, 1254, 1248, 1242, and 1016 to Salt Water Organisms in 48- and 96-Hour Bioassays.

Chemical	Organism	Type of Bioassay	Duration	Effect a)	Exhibit Number
Aroclor 1260	Ciliate	Static	96 hrs.	1000 µg/ℓ decreased growth	11
	Oyster	Flow through	"	60 µg/ℓ-EC 50	12
	Pink shrimp	"	48 hrs.	100 µg/ℓ-10% died	12
	Pinfish	"	"	100 µg/ℓ-n.e.	12
Aroclor 1254	Ciliate	Static	96 hrs.	1 µg/ℓ decreased growth	13
	Oyster	Flow through	"	14 µg/ℓ-EC 50 b)	1
	Pink shrimp	"	48 hrs.	32 µg/ℓ-LC 50 c)	1
	Pinfish	"	"	100 µg/ℓ-n.e. d)	1
Aroclor 1248	Ciliate	Static	96 hrs.	1000 µg/ℓ decreased growth	11
	Oyster	Flow through	"	17 µg/ℓ EC 50	12
	Pink shrimp	"	48 hrs.	32 µg/ℓ-LC 50	12
	Pinfish	"	"	100 µg/ℓ-10% died	12
Aroclor 1242	Oyster	"	96 hrs.	32 µg/ℓ-EC 50	16
	Brown shrimp	"	"	9 µg/ℓ-LC 50	16
	Grass shrimp	"	"	16 µg/ℓ-LC 50	16
	Pinfish	"	"	100 µg/ℓ-n.e.	16
Aroclor 1016	Oyster	"	"	10 µg/ℓ-EC 50	19
	Brown shrimp	"	"	10 µg/ℓ-LC 50	19
	Grass shrimp	"	"	12 µg/ℓ-LC 50	19
	Pinfish	"	"	100 µg/ℓ-n.e.	19

a) Concentrations are nominal.

b) EC 50--Effect is decreased shell growth in oysters.

c) LC 50--Concentration is lethal to 50% of test organisms.

d) n.e.--no observed effect at the stated concentration on growth or mortality only and is not to be interpreted in an absolute sense.

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

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Environmental Protection Agency, Gulf Breeze Laboratory; Sabine Island, Gulf Breeze, Florida, USA
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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 Jensen *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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* Contribution No. 146, Gulf Breeze Laboratory.

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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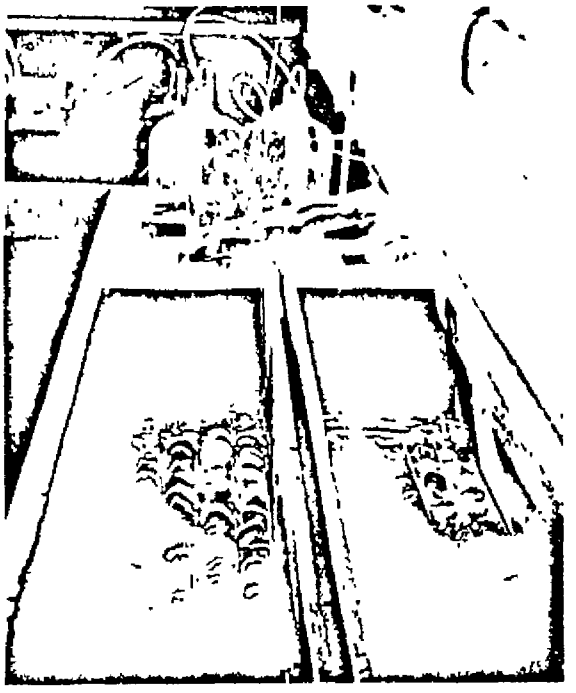

Fig. 1. Experimental and control tanks. Siphons transport water from constant-level reservoir (upper right) into mixing troughs and thus into tanks. Glass jars containing acetone-PCB stock solutions and pumps are in background

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

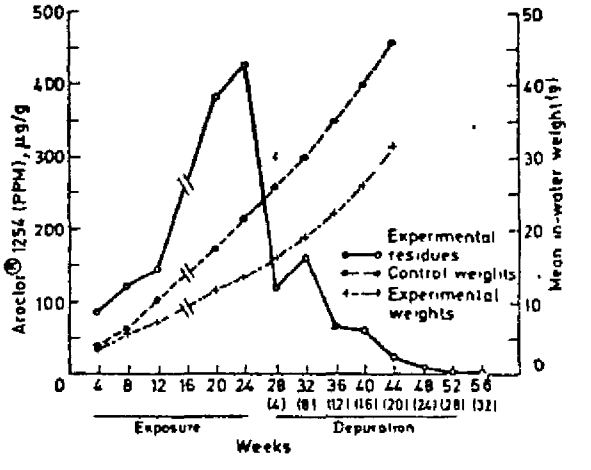

Fig. 2. *Crassostrea virginica*. Weight of control and experimental oysters and residue of Aroclor 1254 in experimental oysters after 24 weeks exposure to 5 ppb of this chemical

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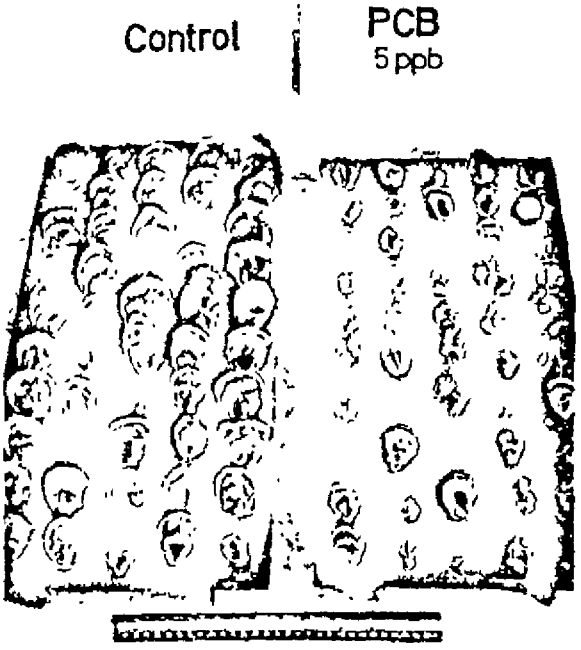

Fig. 3 *Crassostrea virginica* Control and experimental oysters after 14 weeks exposure to 5 ppb Aroclor 1254. Mean height and in-water weight of controls were 54 mm, 11.98 g; experimentals, 44 mm, 8.39 g

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 3.50 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 ($t = 0.21$; $df = 92$).

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, 20 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, 20 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 11° to 32 °C; salinity, 14 to 34 ‰ S

Date	Exposure (weeks)	Oysters (no.)	PPM (µg/g) of PCB (wet weight)	
			Control	Experimental
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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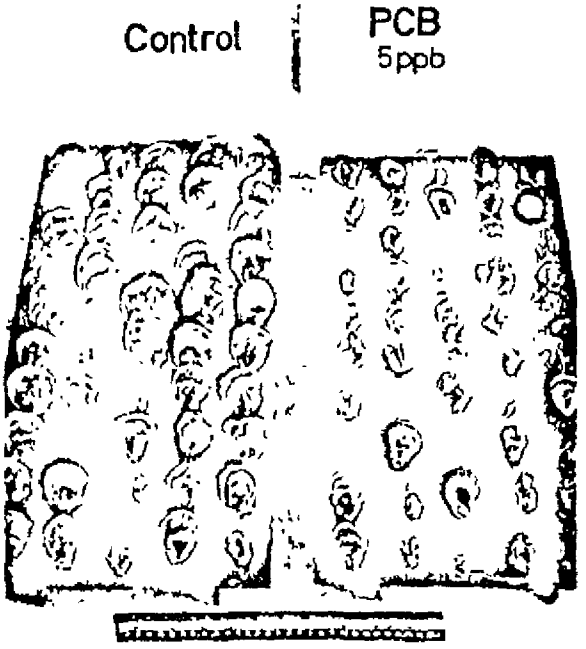

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Growth, 1 ppb Exposure

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.

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[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 (dilated) 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 40 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

[1] Dr. P. A. Butler, Environmental Protection Agency, Gulf Breeze, Florida 32561, USA.

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to starvation resulting from inhibition of digestive functions of the distal digestive diverticular epithelia (J. A Couch, personal communication[1])

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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[1] Dr. J. A. Couch, Environmental Protection Agency, Gulf Breeze, Florida 32561, USA.

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First author's address. Mr J. I. Lowe
Environmental Protection Agency
Gulf Breeze Laboratory
Sabine Island
Gulf Breeze, Florida 32561
USA

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D-25

The Toxity of Polychlorinated Biphenyls (PCB) in Sea Water to *Grammarus Oceanicus*

by D. J. Wilson
Fisheries Research Board of Canada Biological Station
St. Andrews, N. B.

This work was undertaken to investigate some lethal and sublethal effects of PCB following direct uptake from sea water. The dosing system devised by Zitko (1) has been employed. One of the commercially available PCB preparations, Aroclor 1254, was tested on *Gammarus oceanicus*.

Methods and Materials

Stock solutions of Aroclor 1254 and Corexit 7664 in various weight ratios were made up in distilled water. Test solutions were then prepared by pipetting known volumes of the stock into glass beakers and making up to 2 litres with sea water. Control beakers contained Corexit 7664 in sea water and sea water alone.

Five *Gammarus* were added to each beaker which was maintained at 5 ± 0.5 C and the survival time (± 6 hours) of each animal recorded. Bioassays were continued for 30 days and solutions changed weekly.

Results and Discussion

Apart from some deaths caused by cannibalism, no mortality occurred in sea water alone or Corexit 7664 in sea water in concentrations up to 1.9 g/ℓ. Colloidal solutions of Aroclor 1254 solubilized in Corexit 7664 (1:19 W/W) or emulsions (1:10, 1:1, and 1:0.1 W/W) in sea water were lethal at concentrations as low as 0.01 mg/ℓ and 0.1 mg/ℓ respectively. Observations suggested that moulting or freshly moulted animals were particularly vulnerable. Plotting the survival time of *G. oceanicus* against concentration (Fig. 1) showed a "critical point" of response below which there was, and above which there was no time/concentration effect. The lethal threshold is between 0.001 and 0.01 mg/ℓ for colloidal solution and between 0.01 and 0.1 mg/ℓ for emulsions of Aroclor 1254 solubilized in Corexit 7664 in sea water. After exposure to Aroclor 1254 in sea water for periods greater than 150 hours some *Gammarus* which died were found to have severely necrosed branchiae. Necrosis was indicated by a

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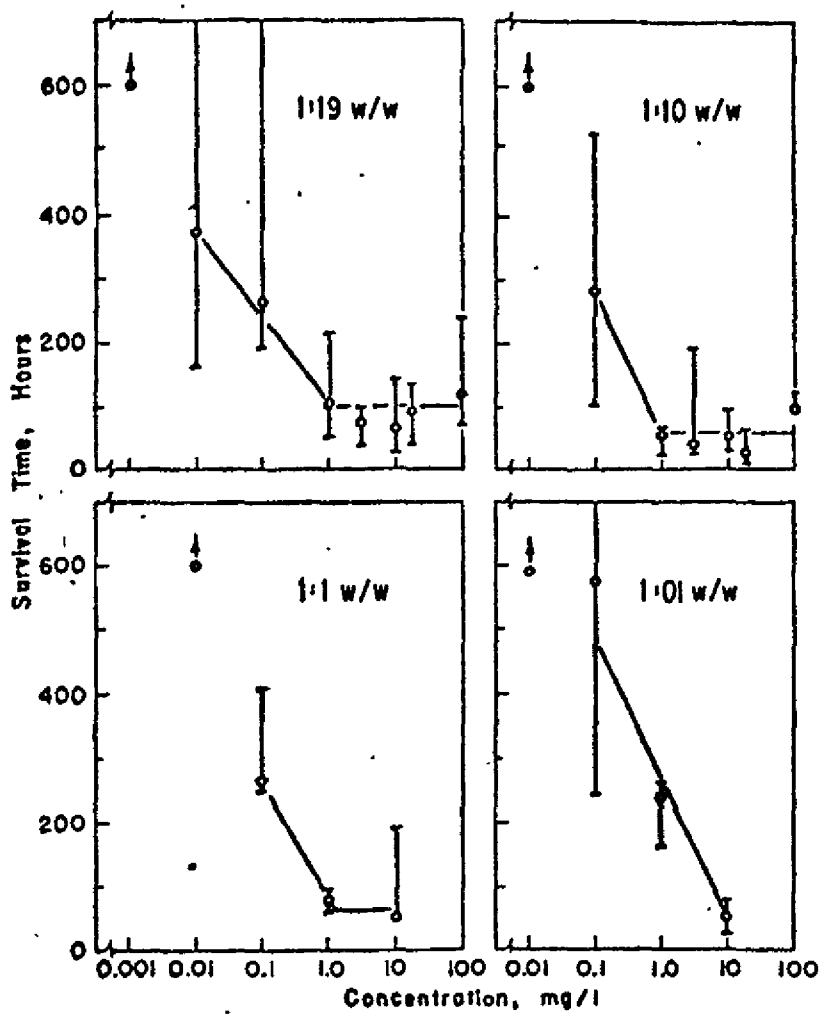

Figure 1. Median and range of survival time of *C. oceanicus* in Aroclor 1254:Corexit 7664 in sea water.

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chitinous wound callus which isolated the affected brownish tissue. A less extensive, sublethal necrosis was found in some animals down to 0.001 mg/ℓ of colloidal PCB in sea water. A sublethal branchial oedema was attributed to the effect of Corexit 7664 in sea water effective at a concentration down to 0.19 mg/ℓ.

Other data (2) show that the highest concentration of Corexit 7664 used (1.9 g/ℓ) to solubilize Aroclor 1254 is 15 times lower than the 48-hr LC50, and thus no mortality was caused directly by the emulsifier. Nevertheless it is possible that a synergistic effect occurred between the emulsifier and PCB.

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W-4
D-26

Chronic Toxicity, Uptake, and Retention of Aroclor® 1254 in Two Estuarine Fishes[1]

by D. J. Hansen, P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr., and P. D. Wilson

U.S. Environmental Protection Agency, Gulf Breeze Laboratory Sabine Island, Gulf Breeze, Florida

Evidence of the global ubiquity of polychlorinated biphenyls (PCB's) continues to mount. Since the first concern about these persistent industrial pollutants was voiced (1), PCB's have been found in fish-eating birds and their eggs in England (2), in seals and porpoises in Scotland (3), in fish-eating birds, their eggs, and fish in California (4), and in mussels, fish, and fish eating birds in the Netherlands (5). Workers at this laboratory found a PCB, Aroclor 1254, in the water, sediment, and biota of a local estuary, and reported its acute toxicity to some estuarine organisms (6). Because high residues of this PCB were found in five fishes in the estuary and it was not acutely toxic to juvenile pinfish (Lagodon rhomboides) in laboratory tests, we began chronic toxicity tests to determine effects of low levels of Aroclor 1254 on pinfish and spot (Leiostomus xanthurus), another estuarine fish. Concurrently, we measured uptake and retention of this PCB in six tissues of spot.

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[1] Gulf Breeze Contribution No. 120

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METHODS AND PROCEDURES

Juvenile pinfish and spot were exposed to Aroclor 1254 in a modification of flowing seawater bioassays routinely used in this laboratory (7). Fish were acclimated to test conditions prior to exposure. Each test was repeated at least once, and for each test, an equal number of fish were placed in two aquaria, one control and one experimental. Aroclor 1254 was dissolved in acetone or polyethylene glycol (molecular weight 200) and metered into the experimental aquaria. The same amount of solvent was added to control aquaria. Concentrations of this PCB in water samples taken periodically from experimental aquaria were within $\pm$ 20% of the desired concentration. All fish were regularly fed commercial fish food that contained no detectable PCB residue. (Limit of detectability was <0.02 parts per million, ppm.) Control fish contained no detectable PCB at the end of each experiment.

The same exposure techniques were used in determining the uptake and retention times of Aroclor 1254 in spot which were exposed for 56 days and then placed in flowing water free of the PCB for 84 days. Ten fish were removed at selected intervals for residue analyses of pooled samples of brain, gills, liver, muscle (all muscle above lateral line on left side of fish), and remaining tissues. Residues in all tissues were summed to compute levels of Aroclor 1254 in the whole fish.

Chemical analyses for residues of Aroclor 1254 in pooled samples of fish tissue were performed in the following manner:
A.) Samples that weighed more than one gram were mixed with sodium sulfate in a blender and extracted for four hours with petroleum ether in a Soxhlet apparatus. Extracts were concentrated and eluted from a Florisil column (8) with 6% ethyl ether in petroleum ether.
B.) Samples that weighed less than one gram were analyzed by a modification of the Enos (9) micro method. Samples were weighed in glass tissue grinders and extracted with three 2.0 milliliter (ml) portions of acetonitrile. The acetonitrile extracts were combined and diluted with 6.0 ml of distilled water. The mixture was agitated and extracted with three 2.0 ml portions of hexane. The extracts were combined and concentrated to about 0.5 ml. The extract was transferred to a size "B" Chromaflex[2] column (Kontes Glass Co.) containing 1.5 grams of Florisil topped with 1.5 grams of sodium sulfate.

[2] Use of trade names does not constitute endorsement by the U. S. Environmental Protection Agency.

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The extract was eluted from the column with 20.0 ml of 1% ethyl ether in hexane.

All eluates were adjusted to an appropriate volume for analysis by electron capture gas chromatographs equipped with DC-200 columns. The multiple-peaked Aroclor 1254 was quantitated by averaging the heights of five major peaks having a retention time relative to aldrin of 1.31 (peak IV), 1.55 (V), 2.32 (VIII) 2.74 (IX), and 3.27 (X). Interference from DDT and its metabolites was negligible due to the relatively high concentrations of Aroclor 1254 found in the samples. Recovery rates were above 80%. Data in this report were not corrected for percentage recovery.

RESULTS AND DISCUSSION

Mortality

Pinfish and spot died when exposed for 14 - 45 days to 5 parts per billion (ppb) of Aroclor 1254, but spot appeared unaffected by exposure to 1 ppb for periods up to 56 days (Table 1). Onset of death in both species exposed to 5 ppb was delayed. For example, mortality was less than 1% in the first week in 3 of 5 tests, and less than 15% in the other two tests. Further exposure to 5 ppb, however, resulted in mortalities ranging from 41 to 66%. Exposures were terminated after these mortalities occurred and live fish were either placed in water free of Aroclor 1254 or analyzed for this PCB.

Symptoms exhibited by fish prior to death were not those typical of poisoning by organochlorine pesticides (10). Instead, pinfish usually developed fungus-like lesions on the body, especially around the mouth, where hemorrhaging also occurred. Affected spot usually ceased feeding, became emaciated, and developed ragged fins and lesions on the body. Although the presence of one or more of these symptoms was typical, some fish appeared normal until they died. All control fish appeared healthy.

Some fish that survived exposure to 5 ppb of Aroclor 1254 became diseased and died even though they were placed in flowing water free of this PCB. For example, 61% (11 of 18) of the pinfish from a population exposed for 14 days died within two weeks, but there was evidence of healing in survivors. Forty-eight percent (48 of 100) of the spot from a population exposed for 26 days died within one week after being placed in PCB-free water.

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TABLE 1

Test conditions, mortality of fishes, and uptake of Aroclor 1254 in flowing water bioassays

Fish	SL (mm)	Number* Exposed	Concentration	Mortality** 25% Day	Mortality** 50% Day	Mortality** Terminal Percent	Days Exposed	Aroclor 1254 (ppm)*** in fish after exposure	Water Temperature, °C	Salinity, ‰
Spot	25	150	1 ppb	--	--	17	33	17	14 - 16	16 -32
Spot	40	150	1 ppb	--	--	1	56	27	23 - 32	10 -34
Spot	24	50	5 ppb	12	18	51	20	46	11 - 18	16 -32
Spot	24	300	5 ppb	23	26	53	26	120	8 - 10	20 -32
Spot	74	50	5 ppb	28	38	62	45	152	28 - 33	23 -34
Pinfish	30	50	5 ppb	9	12	66	14	14	16 - 22	20 -32
Pinfish	27	54	5 ppb	17	--	41	35	109	22 - 32	14 -34

* An equal number of fish were held in control aquaria. Control mortality never exceeded 7%.

** Mortality of spot exposed to 5 ppb Aroclor was significantly greater in all tests ($p < .001$) than that of control fish (χ^2 values were all greater than 10.83).

*** milligrams per kilogram

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Mortality did not appear directly related to the relative amount (ppm) of Aroclor 1254 stored by fish exposed to 5 ppb, rather the amount of PCB stored increased as duration of exposure increased (Table 1). For example, pinfish exposed for 35 days suffered half the mortality yet stored eight times more chemical than did pinfish exposed for only 14 days. Although mortality of spot (51 - 62%) was similar in three tests of different duration (20 - 45 days), the amount of Aroclor 1254 in the fish (46 - 152 ppm) increased as duration of exposure increased.

Uptake and Retention

Spot exposed to 1 ppb Aroclor 1254 for 56 days rapidly stored this chemical, maximum levels being attained in 14 to 28 days. Thereafter, the relative amount (ppm) was generally constant, whereas the absolute amount (micrograms, µg) continued to increase as the fish grew (Table 2). The liver concentrated the greatest relative amount of this PCB, followed in decreasing order by the gills, whole fish, heart, brain, and muscle. Maximum concentrations in whole spot was 3.7×10^4 times that in the test water. In earlier work with DDT at this laboratory, DDT content in pinfish and Atlantic croaker (Micropogon undulatus) exposed to 0.1 and 1.0 ppb p, p'-DDT in water increased for two weeks, then remained constant at 10^4 to 3.8×10^4 times the exposure concentration (11).

Aroclor 1254 was slowly lost from tissues of spot after they were placed in PCB-free flowing water (Table 2). After 84 days of flushing, the relative amount (ppm) in whole fish dropped 73% and the absolute amount (µg) decreased 61%.

The isomers of Aroclor 1254, with the exception of peak IV, maintained their integrity in spot. The height of peak IV in the standard was 18% of the total height of the five peaks used for quantitation. Regardless of the tissue analyzed, it decreased to 14% of the total in exposed fish and 7% in fish maintained in water free of PCB for retention studies.

CONCLUSIONS

Our results suggest that chronic exposure to Aroclor 1254 increased susceptibility of test pinfish and spot to disease, and also appeared to be toxic to these fish. This PCB is rapidly stored by pinfish and spot, and persists in tissues for approximately three months. Our findings emphasize the need for further study on the effect of chronic exposure of aquatic organisms to polychlorinated biphenyls.

MONS 204403

TABLE 2

Levels of Aroclor 1254 in ppm, wet weight, in spot exposed to 1 ppb of this chemical (each sample consisted of tissues from 10 fish). Total micrograms of Aroclor in parenthesis

	Number of days exposed							Number of days of flushing					
Tissue	0	3	7	14	28	42	56*	14	28	42	56	70	84*
Brain	.4 (.1)	3.6 (1.1)	6.0 (2.1)	8.6 (3.9)	8.4 (3.7)	12. (5.9)	8.3 (5.2)	7.6 (5.6)	5.8 (4.7)	5.0 (4.1)	3.5 (3.5)	3.4 (3.4)	2.9 (3.1)
Gills	.2 (.1)	9.0 (8.3)	11. (13.)	29. (41.)	40. (64.)	39. (82.)	46. (74.)	30. (75.)	30. (72.)	24. (65.)	12. (35.)	14. (46.)	12. (37.)
Heart	ND(<.1)	5.5 (.3)	6.8 (.5)	10. (1.0)	12. (.8)	17. (1.6)	13. (1.5)	10. (1.7)	7.8 (1.0)	6.1 (.8)	5.3 (1.0)	4.7 (.8)	2.5 (.6)
Liver	1.2 (.1)	34. (12.)	75. (42.)	96. (92.)	210. (126.)	107. (86.)	83. (72.)	65. (83.)	52. (60.)	28. (38.)	17. (30.)	29 (58.)	22. (35.)
Muscle	.1 (.1)**	1.0 (1.8)	1.8 (4.5)	3.6 (17.)	7.4 (24.)	7.6 (36.)	6.5 (45.)	6.9 (65.)	4.8 (45.)	3.4 (34.)	3.3 (44.)	2.5 (40.)	2.0 (32.)
Whole fish	.1 (1.5)	3.5 (72.)	7.2 (201.)	17. (661.)	37. (1417.)	30. (1553.)	27. (1983.)	25. (2452.)	19. (2041.)	12. (1373.)	9.3 (1309.)	9.3 (1486.)	7.2*** (782.)

* The same tissues analyzed from control spot contained no detectable Aroclor 1254 (<.1 to<1.0 ppm depending on sample weight).

** Total micrograms in sample. Sample approximately 26% of total amount of muscle.

*** Other tissues analyzed on day 84 were: Gall bladder 1.9 (.9), gonad .9 (.3), gut 2.5 (7.8), rest of muscle 4.5 (133.), and skin 12. (174.).

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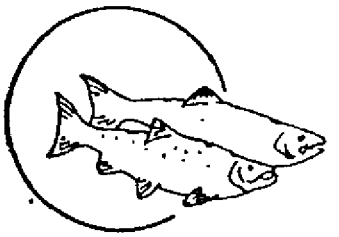
D-29



MONS 204406

PCB - Indications of effects on salmon

by

S. Jensen[1/], N. Johansson[2/] and M. Olsson[3/]

1/ Natl. Environment Protection Board.

2/ Salmon Research Institute.

3/ Swedish Museum of Natural History.

Information concerning the effect of PCB residues in fish, or the possible hazard posed by such residues to fish, is presently lacking in the literature. Studies of the effect of the chemical on salmon and salmon reproduction has recently started at the Salmon Research Institute.

MONS 204407

Effects on the liver in young salmon.

"Pale liver" is a frequently appearing pathological condition among young salmon in Swedish salmon rearing stations. The condition is related to a high degree of stainable fat in the organ, as revealed by the Sudan Black B technique (JOHANSSON, 1965). There are two commonly accepted theories about the cause of this type of "fatty livers": one is based on the probably existence of a toxic substance in the organism, the other postulates a degenerative action of imbalanced diets on hepatocytes.

MILLER (1944) exposed guinea pigs, rats and rabbits to chlorinated diphenyl by injection, applications to the skin and by feeding. Liver damage - fatty degeneration, atrophy of the centrolobular cells and perinuclear basophilic granulation were present in the test groups. Consequently the primary objectives of our investigations were to study the accumulation of PCB in salmon and whether chronic exposure to sublethal levels of the chemical has a direct pathological effect on the liver.

A group of 80 one-year old salmon (average weight 18 g), raised at the institute, were used for the first test. Forty of these were exposed to a constant flow of a commercial PCB product (average 5 Cl per molecule) for two month in a concentration of 1 ppb. The rest of the fish served as controls Both groups of fish were held in square tanks made of fiber glass (bottom area 0.9 m^2). The water flow was 10 lit/min and the water temperature 10° C $\pm$ 1. Samples were taken before the experiment began, and after 8, 12, 15, 18 and 50 days of exposure and were sent to the University of Stockholm (JENSEN) for residue analyses. At the same time samples were taken for histological examination. Livers were fixed in Bouin's Fixative mod. Hollande and 10 % Neutral Buffered Formalin pH 5.7. No mortality occured during exposure of fish to PCB.

Residue analyses showed, that the concentrations of PCB in fish from the test group and from the control group were of about the same size. (Befor the experiment began: 21.1 ppm in liver on fat basis. After 50 days of exposure: test group average 60.4 ppm, controls average 42.2 ppm in liver on fat basis).

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The histological examination of the material is not finished in all details, but hepatic fat occurred in practically all fishes in the test group and in the controls as well. However, in the controls the condition was possibly more slight or moderate in degree compared with the test group. Degenerating liver cells, some of them with vacuolated nuclei appeared more frequently in the test group than in the controls. Acid-fast pigment of ceroid type was noted for some fishes.

The concentrations of PCB residues in pretreatment samples and in the control group threw suspicion on occurrence of PCB-pollution of the aquatic environment. Later analyses of fish from the institute have confirmed this. To start new experiments it was necessary to get fish without basic levels of PCB-residues. After some efforts this have been done and a second test could start a few weeks ago.

Effects on salmon reproduction.

The young salmon reared at the Salmon Research Institute originates from ova taken from females grown up in the sea as well as from females reared up to maturity in fresh water at the institute. The latter females used for spawning usually have been kept in fresh water for three to eight years.

Since 1965 heavy losses of eggs originating from the females kept in fresh water have been noted, while eggs from females grown up in the sea have shown normal survival (Fig. 2A). All kinds of eggs have been incubated and handled in the same manner.

Eggs and fry have been examined for known diseases every year since 1966, but, with the exception of slight outbreaks of yolk-sac strangulation in some lots of fry in the years 1967 and 1968, we have never been able to make any true diagnosis. As most of the ova lost are supposed to be unfertilized, examinations of the gonads of parent males and females have been done. In the male gonads no structural abnormalities were found by studying stained sections in light microscope. The motility of sperm cells as well as the volume of the milt was normal. In the females, the maturing of the ovaries, expressed in egg diameter, egg number and weight of the ovary in percent of the total weight, seems to be normal compared with females grown up in the sea (JOHANSSON, to be published).

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In view of the differences in viability of ..a between the two types of parent females, and in the light of the fact that PCB pollution of the aquatic environment had been noticed, a study of the effect of PCB on reproduction in salmon was of immediate interest.

Primary it was necessary to find out whether high losses of eggs from certain females could be related to a high level of PCB residue. Nine females of different ages. (8+:1,[1] 7+:2, 6+:1, 5+:2, 4+:1 and 3+:2) and reared up to maturity in fresh water were selected for this study. Two females (ages 2.4+[2] and 2.3+), grown up in the sea served as controls (See table 1). One of these (W. 156/69) was from the river Lule älv, the other (W.D.) from the river Dalälven.

Eggs were stripped from the females on October 28 to November 10, and fertilized by milt from hatchery reared males. After water hardening, the eggs were incubated at the institute. The number of eggs in each lot varied between 2000 and 4000. The eggportion from female number 156/69 was divided into two equal lots. The one was incubated in the Heden station at the river Lule älv, the other one at the Salmon Research Institute at the river Dalälven.

Samples of eggs were taken two days after stripping and were sent to the Natl. Environment Protection Board-Special Laboratory, Uppsala for residue analyses. In some cases (see table 1) samples also were taken from eyed eggs.

The results of residue analyses are given in table 1. As the proportion of lipids present in salmonids and salmonid eggs is very variable, (the range for Salmon beeing 0.35-14 %) the concentrations are based on extractable fat.

a. Eggs from females grown up in the sea: PCB residues of 7.7 and 9.2 were noted for the samples taken two days after stripping. For female number 156/6 the concentration increased from 7.7 ppm to 14.0 ppm (6.3 ppm) during the period November to February in the lot of eggs which had been incubated at the river Lule älv, while the corresponding increase for the lot incubated at the river Dalälven were 7.7 to 19.8 ppm(12.2 ppm). The amount of fat in the ova was about the same in both lots.

1) 8+ indicates eight years and one summer in fresh water.

2) 2.4+ indicates two years in fresh water, four years in the sea and

b. Eggs from females reared in fresh water: The PCB residues found varied from 14.4 to 34.0 ppm when analyzed two days after stripping. Thus, the PCB residues were higher in eggs from females reared in fresh water than those in eggs from females grown up in the sea. On the other hand higher concentrations of DDT were noted for the "wild" females. The total DDT values in eggs from females number 61-13, 63-01 and 65-02 are mainly due to DDE.

As shown in fig. 1, the losses of ova during incubation varies from 13 to 100 % and are smallest in the lots from "wild" females. High losses correspond to high concentrations of PCB residues. The main losses are due to unfertilized eggs or to mortalities in an early stage of development. There are no extraordinary mortalities to be found in the yolk-sac stage.

In spite of the small material used, this preliminary study indicates a possible relationship between PCB in salmon eggs and egg loss. In order to find out if this relation is true, new experiments have been prepared for the season 1970/71. For these tests females exposed to known concentrations of PCB will be used for stripping of eggs.

Since there are pronounced variations in the viability of ova and fry in different Swedish salmon rearing stations and since increased losses of eggs have been noted for some stations in the last years, also a survey of the contamination of our east coast rivers would be of great value.

Female from family number	Female age	Organochlorine compounds in ova								% fat		% mortality
		Σ DDT fat basis		DDT fat basis		PCB fat basis		PCB fresh w. basis				
		1	2	1	2	1	2	1	2	1	2	
62-77	7+.	n.d.	n.a.	n.d.	n.a.	34.0	n.a.	1.90	n.a.	5.4	n.a.	100
65-310	4+.	n.d.	n.d.	n.d.	n.d.	27.6	30.0	1.9	1.6	6.9	5.3	99
64-10	5+.	n.d.	n.d.	n.d.	n.d.	20.2	25.0	1.2	1.0	5.9	4.2	98
63-17	6+.	n.d.	n.d.	n.d.	n.d.	26.9	34.0	1.6	1.9	5.9	5.5	82
61-52	8+.	n.d.	n.a.	n.d.	n.a.	31.7	n.a.	1.7	n.a.	5.4	n.a.	70
61-13	7-.	7.2	n.a.	n.d.	n.a.	17.5	n.a.	1.0	n.a.	5.7	n.a.	70
66-302	3+.	n.d.	n.d.	n.d.	n.d.	14.4	23.0	0.6	0.9	4.4	3.8	53
63-01	5+.	6.2	n.a.	n.d.	n.a.	15.8	n.a.	0.9	n.a.	5.8	n.a.	48
65-02	3+.	5.7	n.a.	n.d.	n.a.	15.2	n.a.	0.6	n.a.	4.2	n.a.	46
W. 156/69	2.4+	12.5	11.3	9.5	2.8	7.7	19.8	0.6	1.2	7.9	5.9	34
W. 156/69	2.4+	12.5	12.2	9.5	4.5	7.7	14.0	0.6	0.8	7.9	5.7	29
W.	2.3+	33.5	n.a.	11.6	n.a.	9.2	n.a.	0.4	n.a.	3.8	n.a.	16

Table 1. Concentrations of organchlorine compounds (ppm) in salmon ova and mortalities of eggs and sac fry from fertilization to the feeding stage; n.d., not detectable; n.a., not analysed; 1 = analysed two days after stripping; 2 = analysed from eyed eggs; W = wild female.

Σ DDT = DDT + 1.11 x (DDD + DDE).

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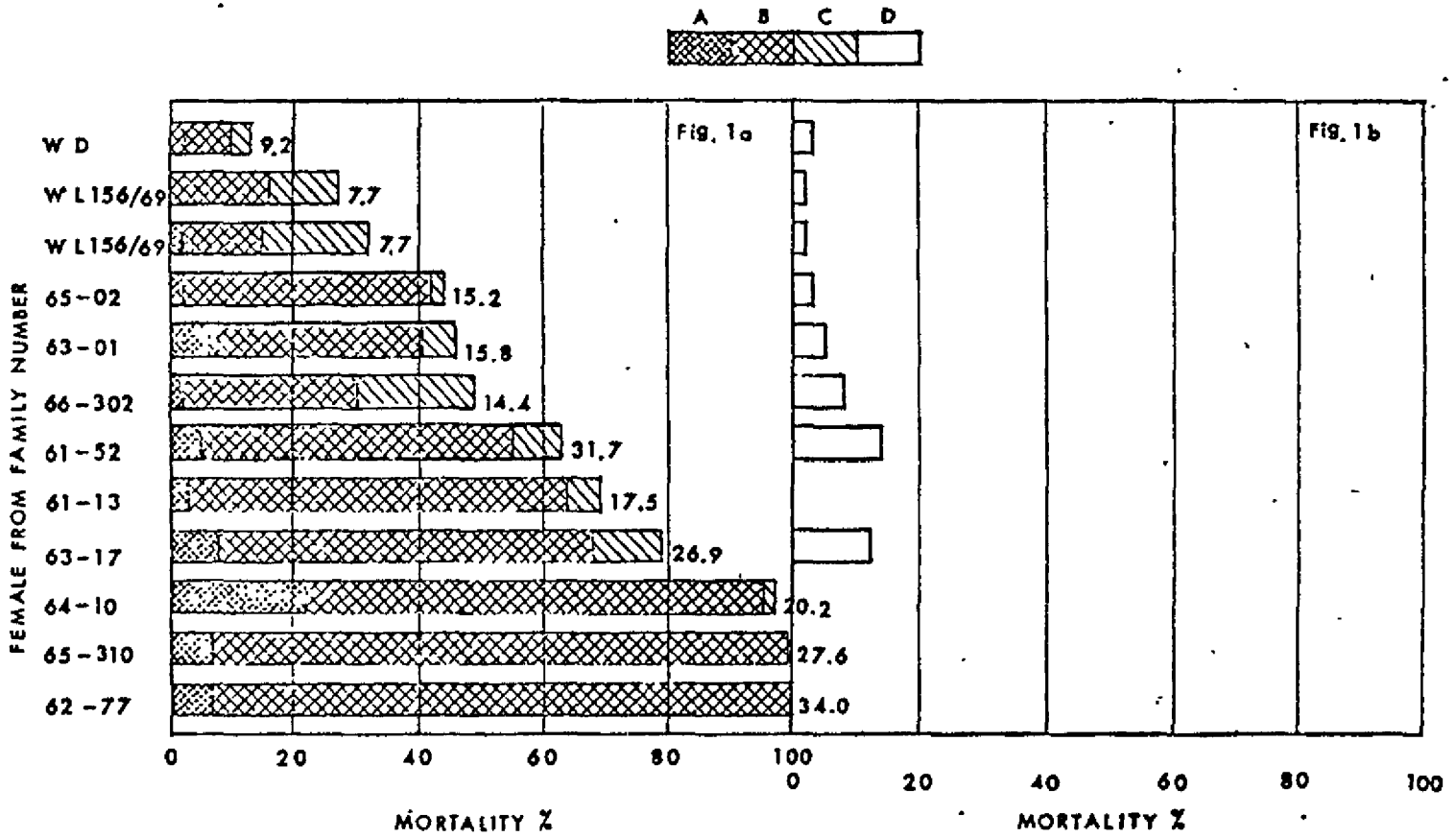

Fig. 1. Percentage of mortality during incubation (fig. 1 a) and in the yolk-sac stage (fig. 1 b); ova dead at the beginning of incubation (A); dead ova without developed embryos (B); dead ova with developed embryos (C); dead sac fry (D). W L and W D indicates females grown up in the sea. To the right of each bar is recorded the concentration of PCB residue in ova two days after stripping (ppm in fat).

MONS 204413

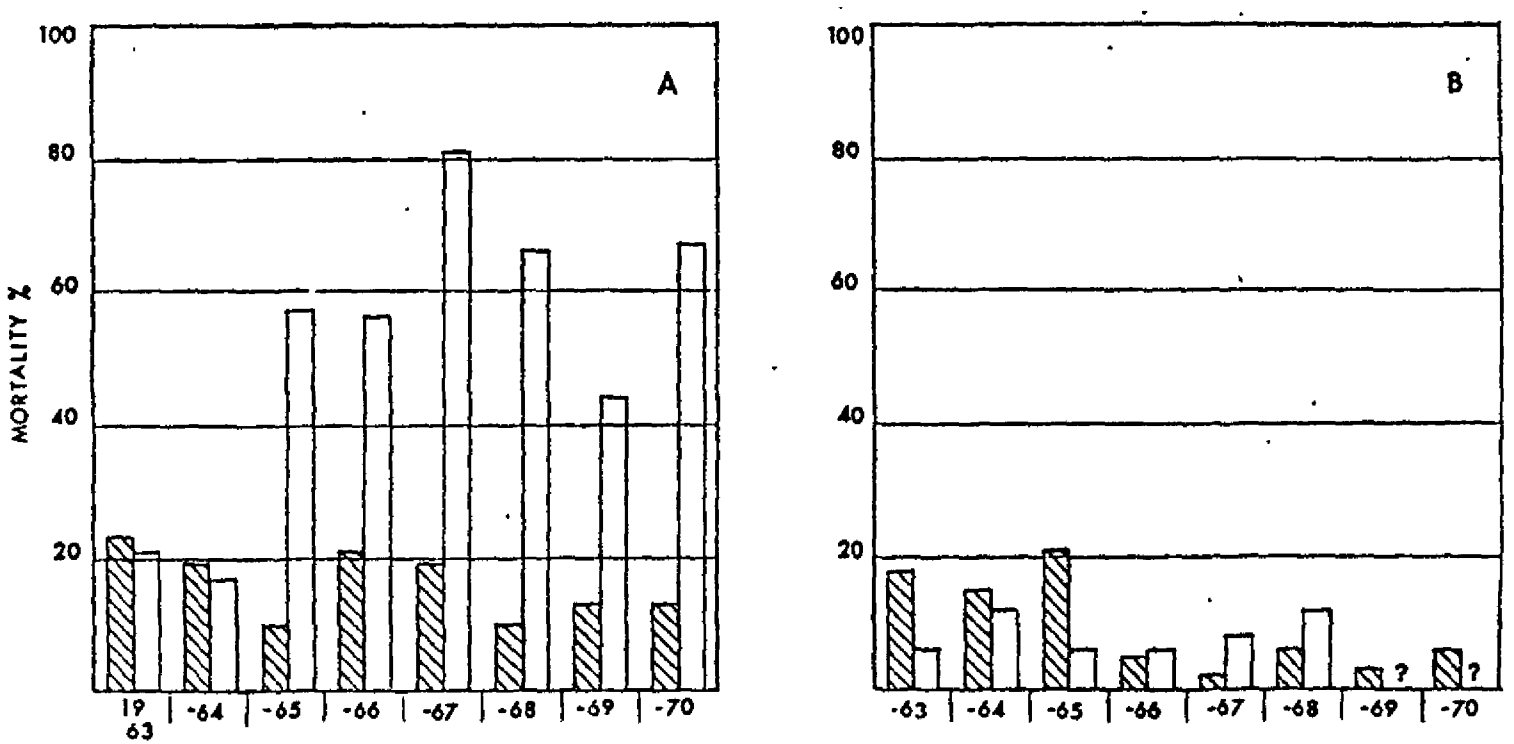

Fig. 2. Average losses of ova from fertilization to hatching (A.) and mortalities during the yolk-sac stage (B.) 1963 - 1970. Open bars = eggs and fry from females in fresh water; shaded bars = eggs and fry from females grown up in the sea.

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(31)

EFFECTS OF AROCLOR® 1016 ON EMBRYO, FRY, JUVENILE AND ADULT SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS)[1]

David J. Hansen, Steven C. Schimmel and Jerrold Forester

U. S. Environmental Protection Agency

Gulf Breeze Environmental Research Laboratory

Sabine Island, Gulf Breeze, Florida 32561

(Associate Laboratory of the

National Environmental Research Center,

Corvallis, Oregon)

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MONS 204416

EFFECTS OF AROCLOR® 1016 ON EMBRYO, FRY, JUVENILE AND ADULT SHEEPSHEAD MINNOWS (CYPRINODON VARIEGATUS)

David J. Hansen, Steven C. Schimmel and Jerrold Forester

ABSTRACT

We investigated the toxicity of Aroclor 1016 to, and its uptake by, fry, juvenile and adult sheepshead minnows (Cyprinodon variegatus) in intermittent-flow bioassays lasting 28 days. Fry, juvenile and adult fish apparently were not affected by 0.1, 0.32, 1.0, 3.2 or 10 µg/L, but died in 32 and 100 ppb µg/L of this polychlorinated biphenyl. Sheepshead minnows accumulated the chemical 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. As much as 77 ppm µg/g of Aroclor 1016 in eggs from exposed adults apparently did not affect survival of embryos and fry.

MONS 204417

INTRODUCTION

Polychlorinated biphenyls, PCB's, occur in estuarine environments in the United States (Butler, 1973; Nimmo and Bahner, 1974a). One PCB, Aroclor 1254, is acutely toxic (48 or 96 hours) to certain estuarine animals (Duke et al., 1970) and in exposures lasting 14 or more days it is toxic to some estuarine arthropods (Nimmo et al., 1971; Nimmo et al., 1974b), mollusks (Lowe et al., 1972) and fishes (Hansen et al., 1971; Schimmel et al., 1974). It is over 30 times more toxic to fry of sheepshead minnows (Cyprinodon variegatus) than to juveniles or adults (Schimmel et al., 1974). Fry from sheepshead minnow eggs containing Aroclor 1254 die within the first week following hatching (Hansen et al., 1974a).

A new PCB, Aroclor 1016, is now being manufactured to replace some of the PCB's that are no longer produced. Aroclor 1016 has not been found in oysters or fishes from estuaries in the United States sampled by the Environmental Protection Agency's National Estuarine Monitoring Program (P. A. Butler, personal communication[2/]). It is however, as acutely toxic to some juvenile and adult estuarine animals as are other PCB's and its delayed toxicity to pinfish (Lagodon rhomboides) in exposures lasting 14 or more days is similar to that found with another PCB (Hansen et al., 1974b).

2/ P.A. Butler, Ecological Monitoring Branch, Technical Services Division, Office of Pesticide Programs, U.S. Environmental Protection Agency, Gulf Breeze, Florida 32561.

This experiment was conducted to determine the toxicity of Aroclor 1016 in water to embryo, fry, juvenile and adult sheepshead minnows and its endogenous toxicity to embryos and fry reared from eggs from exposed adults. Also, chemical analyses were made to determine if this PCB accumulates in eggs, fry, juvenile and adult fish.

MATERIALS AND METHODS

Test Fish

Adult and juvenile fish were collected near the Gulf Breeze Laboratory and acclimated in 30 C seawater for at least 14 days before exposure. Fish were not used if mortality exceeded 1% or abnormal behavior was observed in the 48 hours immediately preceding a test. Adult fish averaged 41mm standard length (range 31-51mm) and juveniles averaged 20mm standard length (range 16-27mm). Fish were fed PCB-free dry commercial fish food daily and PCB-free frozen adult brine shrimp twice a week during acclimation and testing.

Eggs were obtained from acclimated adult females whose egg production was enhanced by hormonal injection using the techniques of Schimmel et al. (1974). Eggs, stripped manually from six or more females, were placed in 20-40ml of filtered 30 C seawater and fertilized with excised macerated testes from six or more males. Success of fertilization was determined by microscopic examination for cleavage 1.5 hours after mixing with macerated testes. Fry from the eggs were fed daily on PCB-free brine shrimp nauplii.

Aroclor 1016 exposure

Adult, juvenile, embryo and fry sheepshead minnows were exposed

simultaneously to Aroclor 1016 in two intermittent-flow bioassays, each lasting four weeks. In the first experiment, we exposed 25 adults (13 females and 12 males), 25 juveniles and 100 eggs in aquaria receiving 0, 0.1, 0.32, 1.0, 3.2, or 10 µg/ℓ of Aroclor 1016. In the second experiment exposure concentrations were 0, 1.0, 3.2, 10, 32, and 100 µg/ℓ. The dosing apparatus (Schimmel et al., 1974) was a modification of that of Brungs and Mount (1970). Each of approximately 150 daily cycles siphoned 1.5% of filtered 30 C seawater, 11 µg of carrier (polyethylene glycol 200) and appropriate amounts of PCB to each 80ℓ aquarium. Water and carrier without PCB were delivered to the control aquaria. Salinity of the water averaged 22.7‰ (12.0‰ to 32.0‰). Aquaria were checked daily to determine survival of embryo, fry, juvenile and adult fish.

Aroclor 1016 in eggs

The effect of Aroclor 1016 in eggs was determined during the second bioassay by enhancing egg production with hormonal injections of exposed adults, fertilizing the eggs artificially, then monitoring their development in flowing PCB-free seawater. Female sheepshead minnows were injected intraperitioneally with 50 I.U. human chorionic gonadotropic hormone on exposure days 25 and 27. On day 28 or 29, eggs were stripped manually from five females from each concentration and those from each female placed in individual beakers containing 20-40 ml of filtered 30 C seawater. Eggs from each female were fertilized with excised macerated testes from a male from the same concentration. Twenty-five eggs from each fish were placed in a 10 cm Petri dish to which a 9 cm-high collar of 300µ nylon

mesh was attached. Dishes were submerged 7 cm in the 80 % control aquarium which received approximately 225 % of filtered 30 C PCB-free seawater per day; average salinity was 24.0% (19.5% to 28.%). Success of fertilization was determined by microscopic examination for cleavage 1.5 hours after mixing with macerated testes. Thereafter, dishes were examined daily for 21 days to determine survival of embryos and fry. Fry were fed PCB-free live brine shrimp nauplii daily.

Chemical analyses

Concentrations of Aroclor 1016 in water and fish were determined by electron-capture gas chromatography. Unfiltered water samples from each concentration were obtained at least three times during each four-week exposure and analyzed. Concentrations of Aroclor were determined in fry, juvenile and adult fish surviving both bioassays and in eggs from adult fish from the second bioassay. All fish samples consisted of 14 or more individuals. Procedures used for water and fish samples are described by Hansen et al. (1974b). 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.

Statistical analyses

Concentrations of Aroclor 1016 lethal to 50% of the fish (95% confidence limits) were estimated using the method of Litchfield and Wilcoxon (1949). The χ^2 test for independent samples was used to compare the survival of control and PCB-exposed fish. Differences were considered significant at $\alpha = 0.05$.

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RESULTS AND DISCUSSION

Sheepshead minnow adults, juveniles, embryos and fry exposed to Aroclor 1016 for 28 days apparently were not affected by 0.1, 0.32, 1.0, 3.2 or 10 µg/ℓ, but died in 32 and 100 µg/ℓ, nominal concentrations, of this PCB (Table 1). Response to poisoning included darkened body coloration, uncoordinated swimming, cessation of feeding and lesions on the body of many juveniles and adults. Juvenile pinfish, another estuarine fish, also died in 32 µg/ℓ of Aroclor 1016, but survived lower concentrations (Hansen et al., 1974b); responses exhibited by both fishes were similar. Although fin was not observed in Aroclor 1016-exposed fish (present study; Hansen et al., 1974b) it was common in Aroclor 1254-exposed sheepshead minnows (Schimmel et al., 1974), spot (Leiostomus xanthurus) and pinfish (Hansen et al., 1971).

Sensitivities of embryos and fry, juvenile and adult sheepshead minnows to Aroclor 1016 were similar (Tables 1 and 2). Aroclor 1016 did not alter the survival of embryos to hatching or the hatching time. Eggs began to hatch on the fourth day of exposure, one-half hatched by day five and all hatched by day nine. Fry, juveniles and adults in 32 and 100 µg/ℓ began to die during the second week of exposure and over half died before the end of the second week. All fish in 100 µg/ℓ were dead after 3 weeks, but some fish of each life stage survived the four-week exposure to 32 µg/ℓ. Statistical analyses of weekly data show the similiarity of effects of Aroclor 1016 on each life stage (Table 2). In similar experiments, Schimmel et al. (1974) reported that Aroclor 1254 affected each of these life stages differently: Sheepshead minnow fry were much more sensitive than other stages.

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During three-weeks exposure, 0.32 µg/ℓ of Aroclor 1254 was lethal to fry, 10 µg/ℓ was lethal to juveniles and adults survived 10 µg/ℓ.

Sheepshead minnows exposed for 28 days to concentrations of Aroclor 1016 ranging from 0.1 and 100 µg/ℓ accumulated the chemical in proportion to the concentration in the test water (Table 3). In general, juvenile fish contained the greatest concentrations of the PCB, followed by adults and fry (fry were exposed 5 days as embryos followed by 28 days as fry). Concentration factors (concentration in whole-fish divided by nominal concentration in the test water) ranged from 2,500 to 8,100 for fry, 4,700 to 14,000 for adults and 10,000 to 34,000 for juveniles. Concentration factors in adult sheepshead minnows exposed identically to Aroclor 1254 were greater (15,000 to 30,000) (Hansen et al., 1974a). By comparison, concentration factors in fry and adult sheepshead minnows exposed for 3 weeks to 0.1, 0.32, 1.0, 3.2 or 10 µg/ℓ of Aroclor 1254 were similar, ranging from 11,000 to 32,000 (Schimmel et al., 1974).

Eggs from sheepshead minnows exposed to 1.0, 3.2 or 10 µg/ℓ of Aroclor 1016 for 4 weeks contained up to 77 µg/g of the PCB (Table 4). Fertilization success, survival of embryos to hatching and survival of fry for two weeks after hatching did not appear to be altered by this or lesser concentrations. Sheepshead minnow eggs that contained Aroclor 1254 in concentrations of 7 µg/g or more, however, exhibited decreased fry survival in the first week following hatching (Hansen et al., 1974a).

This study indicates that although Aroclor 1016 is readily accumulated by and is toxic to fry, juvenile and adult sheepshead minnows, it is about 100 times less toxic than Aroclor 1254 to sensitive fry. More important, concentrations of Aroclor 1016 in eggs as great as 77 µg/g apparently did not affect survival of embryos and fry for two weeks.

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MONS 204426

Table I. -- Toxicity of Aroclor® 1016 to embryos and fry, juvenile and adult sheepshead minnows (Cyprinodon variegatus) exposed for 28 days in two separate experiments.

CONCENTRATION (µg/ℓ)		PERCENTAGE SURVIVING FOR 28 DAYS		
NOMINAL	MEASURED	EMBRYOS AND FRY	JUVENILES	ADULTS
EXPERIMENT 1				
CONTROL	ND[a/]	76	100	96
0.10	ND	76	100	96
0.32	ND	73	100	100
1.0	0.46	90	100	100
3.2	1.4	75	100	96
10.	5.5	61	100	84
EXPERIMENT 2				
CONTROL	ND	77	100	92
1.0	0.29	78	96	88
3.2	1.0	67	100	88
10.	3.4	71	96	88
32.	15.	14	12	8
100.	42.	0	0	0

a/ ND = not detectable; <0.2 µg/ℓ

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Table II -- Nominal concentration (µg/ℓ) of Aroclor® 1016 lethal to 50 percent of the fry, juvenile, and adult sheepshead minnows (LC50) each week during a four-week bioassay. Ninety-five percent confidence limits are in parentheses.

STAGE	WEEK			
	1	2	3	4
Fry*	>100	31 (25-38)	24 (21-28)	21 (18-25)
Juveniles	>100	47 (35-62)	22 (17-28)	20 (15-26)
Adults	>100	42 (29-61)	28 (22-36)	19 (14-25)

*Bioassay began with eggs and embryos hatched from day four to day nine.

Table III -- Concentrations of Aroclor® 1016 in fry, juvenile and adult sheepshead minnows exposed for 28 days. Each sample consisted of a minimum of 14 fish. Concentration factors in parentheses.

CONCENTRATION IN WATER (µg/ℓ)		CONCENTRATION IN WHOLE FISH (µg/g wet weight)		
NOMINAL	MEASURED	FRY [a]	JUVENILES	ADULTS
EXPERIMENT 1				
CONTROL	ND[b]	ND[b]	ND	ND
0.10	ND	ND	2.3 (23,000)	0.84 (8,4
0.32	ND	0.81 (2,500)	8.9 (28,000)	1.5 (4,7
1.0	0.46	4.9 (4,900)	11. (11,000)	12. (12,0
3.2	1.4	22. (6,900)	79. (25,000)	46. (14,0
10.	5.5	38. (3,800)	230. (23,000)	100. (10,0
EXPERIMENT 2				
CONTROL	ND	ND	ND	ND
1.0	0.29	5.9 (5,900)	10. (10,000)	5.4 (5,4
3.2	1.0	26. (8,100)	54. (17,000)	22. (6,9
10.	3.4	57. (5,700)	220. (22,000)	110. (11,0
32.	15.	200. (6,200)	1100. (34,000)[c]	--
100.	42.	--	--	--

[a] Embryos and fry were exposed for 33 days.

[b] ND = not detectable; <0.2µg/ℓ in water, <0.2 µg/g in tissue.

[c] Sample consisted of three juvenile fish.

Table IV -- Success of fertilization of eggs from adult sheepshead minnows exposed to Aroclor® 1016 for four weeks, survival of embryos from fertile eggs until hatching and survival of hatched fry. Eggs are from five fish, 25 eggs each, per concentration. Percentages are in parentheses.

NOMINAL CONCENTRATION IN WATER (µg/ℓ)	CONCENTRATION IN EGGS (µg/g)	EGGS			FRY	
Adult Exposure	Average (range)	Tested	Fertile	Hatched	Week 1	Week 2
Control	ND[a]	125	119 (95)	82 (69)	81 (99)	60 (73)
1.0	4.2 (3.1-5.1)	124	120 (97)	76 (63)	71 (93)	64 (84)
3.2	17. (12-19)	125	122 (98)	102 (84)	99 (97)	91 (89)
10.	66. (51-77)	124	117 (94)	88 (75)	84 (96)	72 (82)

[a] ND = not detectable; 0.2 µg/g

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Toxicity of Aroclors 1254 and 1016 to Salt Water Organisms in Flow-through Bioassays Exceeding 96-hour Duration. (32)

Chemical	Organism	Duration (Days)	Measured Concentration (µg/ℓ) Significant Mortality	Measured Concentration (µg/ℓ) No Significant Mortality	Exhibit Number
Aroclor 1254	Blue crabs	20	_	3.5-4.2	1
	Pink shrimp	20	3.5-4.2	_	1
	Pink shrimp (juvenile)	15	0.94	0.57	5
	Pink shrimp	35	3.5	_	5
	Brown shrimp	30	3	_	24
	Grass shrimp	16	4.0	1.3	6
	Spot	20-45	5.0(±20%)	1.0(±20%)	26
	Pinfish	14-35	"	_	26
	Sheepshead minnows (embryos)	7	3.5	1.0	27
	" " (fry) a)	21	0.16	0.06	27
	" " (Juveniles)	21	3.5	1.0	27
	" " (adults)	21	_	3.5	27
	" " (adults)	28	5.6	1.1	28
	" " (reproduction)	28 b)	0.14	0.09	28
	" " (fry)	0	7.0 c)	5.4 c)	28
	Oysters d)	168-210	3.9	0.64	21
	Communities of Animals e)	120	0.6	<0.1	29
Aroclor 1016	Pinfish	42	13	7	19
	Sheepshead minnows (fry, juveniles and adults)	28	15	3.4	31

a) Fry exposed for about 7 days as embryos.

b) Adults exposed for 28 days, eggs fertilized and placed in "clean" H_2O.

c) Concentration in eggs µg/g wet weight.

d) Growth was slowed significantly by 3.9 µg/ℓ but not by 0.64 µg/ℓ.

e) Community composition was altered significantly by 0.6 µg/ℓ but not by <0.1 µg/ℓ.

STATEMENT OF ALFRED J. WILSON, JR.

My name is Alfred J. Wilson, Jr. My address is 3221 Rothschild Drive, Pensacola, Florida 32503. I am a research chemist at the Environmental Protection Agency's Gulf Breeze Environmental Research Laboratory, Gulf Breeze, Florida. I am in charge of the Analytical Services Program at this facility. I received my Bachelor of Science Degree from the University of New Hampshire in 1950.

I have been analyzing and/or supervising the analysis of pesticide residues in the marine environment since 1962. During that time, the Gulf Breeze Laboratory has analyzed over twenty thousand samples for the presence of pesticides and related organics in various substrates of the marine environment such as water, sediment, oysters, crabs, fish, birds, and mammals. I have utilized the methods which were current with the state of the art for the detection of these compounds. These techniques include paper and thin-layer chromatography, gas-liquid chromatography and mass spectrometry.

My statement will describe the analytical procedures used by the Gulf Breeze Environmental Research Laboratory for the detection of polychlorinated biphenyl compounds (PCB's) in the marine environment. In addition the statement will be supplement to, and verification of, data given in the following reports which have been submitted as exhibits. Appendix A

"A Polychlorinated Biphenyl (Aroclor 1254) in the Water, Sediment and Biota of Escambia Bay, Florida." T. W. Duke,

J. I. Lowe, and A. J. Wilson, Jr. Bulletin of Environmental Contamination and Toxicology 5(2):171-180. 1970

" Polychlorinated Biphenyls Absorbed from Sediments by Fiddler Crabs and Pink Shrimp." D. R. Nimmo, P. D. Wilson, R. R. Blackman, and A. J. Wilson, Jr. Nature, Vol. 231, pp 50-52, May 7, 1971

"Chronic Toxicity, Uptake, and Retention of Aroclor 1254 in Two Estuarine Fishes." D. J. Hansen, P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr., and P. D. Wilson. Bulletin of Environmental Contamination and Toxicology (2):113-119, 1971.

"Toxicity and Distribution of Aroclor 1254 in the Pink Shrimp Penaeus Duroarum. D. R. Nimmo, R. R. Blackman, A. J. Wilson, Jr. and J. Forester. Marine Biology 2(3): 191-197. 1971

"Effects of Aroclor 1254, a PCB, on Oysters, Crassostrea Virginica (Bivalvia: Protobranchia: Ostreidae). P. R. Parrish, J. I. Lowe, A. J. Wilson, Jr. and J. M. Patrick, Jr. The Association of Southeastern Biologists Bulletin (Abstract) Vol. 19, No. 2 p. 90, 1972.

The polychlorinated biphenyl compounds (PCB's) are industrially prepared by chlorination of biphenyl. Several fractions of different degrees of chlorination are manufactured by the Monsanto Company under the trademarks Aroclor 1221, 1232, 1248, 1254, 1260, 1262 and 1268. The last two digits designate the percent chlorine content of each

each formulation. The Aroclors are relatively inert, chemically resistant compounds which are practically insoluble in water but appear to be soluble in lipoid material. PCB's have been widely used for both consumer goods and industrial applications for more than 4 decades.

The methods used for the detection of PCB's in the marine environment are similar to those techniques used for the analysis of pesticides. The analytical procedures for the detection of PCB's vary with the type of substrate (water, sediment or biological material) and to some extent with the size of the sample. The usual technique, however, consists of extracting the substrate with a suitable organic solvent, purification (clean-up) of the resulting extract to remove lipoid and other interfering compounds, and finally, the identification and measuring of the compound.

PCB's are usually identified and quantified initially through gas liquid chromatography (GLC). A gas chromatograph equipped with an electron capture detector is used to identify and quantify chlorinated hydrocarbons in a cleaned-up extract. Sample components enter a detector, which in turn effects a response on a recorder; this results in peaks on a gas chromatogram for each component which is isolated. Pure materials of expected components of a sample have been previously injected into the chromatograph for a determination of the retention time, i.e., the time required for elution in the gas chromatograph. That retention time is than compared with those of the unknown components so as to identify, or qualify, them. Confirmation of results is sometimes undertsken by

using a different component (column) on the gas chromatograph to produce a different identifying retention time.

Quantiation is accomplished by measuring either the peak area or peak height and comparing it to previously determined known amounts of standards or reference materials. Peak area or peak height reflects the concentration of the component which elutes at a given time.

In addition to gas liquid chromatography, thin layer chromatography (TLC) and mass spectrometry are sometimes used to check GLC results. In thin layer chromatography, PCB's interact with an organic solvent to move up a glass sheet, with the amount of movement identifying the particular compound. In mass spectrometry, the compound is broken into its components and thereafter identified on an oscillograph or through computer analysis.

The identification and measurement of PCB's are complicated for the following reasons:

1. If PCB's are present in samples along with the commonly occuring organochlorine pesticides, i.e., DDE, DDD, and DDT, a separation of the PCB's from the pesticides must be performed before a reliable analysis can be done.

2. Each of the Aroclor series contain several compounds depending on the degree of chlorination and the position of the chlorine molecule on the biphenyl ring. Consequently, each of these compounds (isomers) elute from the gas chromatograph with different retention time producing a multiple peak chromatogram for each Aroclor. In addition

these isomers may be present in more than one Aroclor. For example, some of the isomers present in Aroclor 1254 are also present in Aroclors 1248, 1260 and 1262. Since environmental residues of PCB's are subject to varying degrees of alterations due to solubility, metabolism and other environmental factors, each Aroclor may not matintain its "integrity" in the field over long periods of time. Due to this multiplicity of peaks and isomers common to more than one Aroclor, it is difficult in some field samples to specifically identify what Aroclor is present and to accurately quantitate the amount present.

In laboratory PCB toxicity and bioaccumulation studies at the Gulf Breeze Laboratory these problems have not been encountered for the following reasons:

1. Test animals are analyzed prior to exposure to assure they are uncontaminated with organochlorine compounds. During exposure, water samples are analyzed to evaluate the validity of the exposure level and to assess water quality.

2. Since the identity of the Aroclor being evaluated is known and the exposure period is relatively short, all of the Aroclors tested to date can be identified and measured with reasonable accuracy. In some tissues we have observed differences in the proportion of Aroclor peaks with respect to a standard.

In samples where we have detected the presence of PCB's and interfering organochlorine pesticides we have separated these compounds by column chromatography with silicic acid as described by Armour-Burke (1). Currently we are quantitating the Aroclors by comparing the total

peak height of all discrete peaks with that of a standard. In addition, laboratory samples are fortified with an internal standard just prior to analyses. Samples are fortified with mirex, a compound which behaves analytically similar to PCB's. That is, it elutes from a Florisil column in the same fraction as PCB's and has similar, although not identical, recovery rates. This technique is valuable in assessing the validity of our results and is commonly practiced in analytical quality control. For example, if you only retrieve 50% of your fortification compound when normally over 90% is expectad, your results for the compound of interest is also low. This warrants a new sample or discarding the results. The analysis of control tissue (from animals not exposed to PCB's) offers an additional check on possible in-house laboratory contamination.

(1) Armour, J. A. and Burke, J. A., J. Assoc. Official Anal Chemists, 53, 761 (1970)

Alfred J. Hilsen Jr.

MAY 15 1974

N O T E

The exhibits for Alan V. Nebeker's statement will be mailed under separate cover.

May 13, 1974

MAY 3 1974

STATEMENT OF ALAN V. NEBEKER

My name is Alan V. Nebeker. I am now a Research Aquatic Biologist with the Western Fish Toxicology Station, Corvallis, Oregon, which is a field station of the National Water Quality Laboratory, EPA, Duluth, Minnesota. I was stationed at the Duluth laboratory from 1966 to 1971, under the direction of Dr. Donald I. Mount, Director, where I conducted research dealing with the effects of temperature, low pH, and low oxygen on survival and adult emergence of aquatic insects; and the effects of PCB's on survival and reproduction of Daphnia, Gammarus, Tanytarsus (fish food organisms), the flagfish and the fathead minnow.

I attended Utah State University, B.Y.U., the University of Montana, and the University of Utah. I received the B.S.,M.S., and Ph.D. Degrees at the University of Utah. My Master's Thesis study dealt with the effects of insecticides and herbicides on the survival of Gammarus, the freshwater scud or shrimp. My doctoral study dealt with the taxonomy and ecology of winter stoneflies, with special emphasis on the effects of stream temperature on adult emergence. I also served as Instructor in Biology for six years while at the University of Utah, and two summers at the University of Montana. I have published one book and thirty scientific papers in the field of aquatic biology and toxicology.

MONS 204439

My testimony is taken from the following exhibits:

Ex. 1. Nebeker, A. V., and F. A. Puglisi, 1974. Effect of Polychlorinated Biphenyls (PCB's) on Survival and Reproduction of Daphnia, Gammarus, and Tanytarsus. Trans. Amer. Fish. Soc. 103(4): Oct., 1974.

Ex. 2. Nebeker, A.V., F. A. Puglisi, and D. L. Defoe, 1974. Effect of Polychlorinated Biphenyl Compounds on Survival and Reproduction of the Fathead Minnow and Flagfish. Trans. Amer. Fish. Soc. 103(3): July, 1974.

Ex. 3. Defoe, D. L., R. W. Carlson, and G. D. Veith, 1973. Exposure of Fathead Minnows to Aroclor® 1248 and 1260. In: A Summary of NWQL Research Concerning the Effects of Polychlorinated Biphenyl on Water Quality and Aquatic Life. May, 1973. EPA, National Water Quality Laboratory, Duluth, Minnesota.

Ex. 4. Moorman, Ken, and K. E. Biesinger, 1973. Polychlorinated Biphenyl Research--a static bioassay using Aroclor® 1016 and related experiments. Unpublished Report. National Water Quality Laboratory, Duluth, Minnesota.

Ex. 5. Ringer, R. K., R. J. Aulerich, and M. Zabik, 1972. . Effect of Dietary Polychlorinated Biphenyls on Growth and Reproduction of Mink. Michigan State University, East Lansing, Michigan 48823. (a more complete citation will be here when I receive the paper from Gil Veith). Water and Waste Chemistry, 12(2):149-154.

Ex. 6. Platonow, N. S., and L. H. Karstad, 1973. Dietary Effects of Polychlorinated Biphenyls on Mink. Can. J. Comp. Med. 37: 391-400.

Ex. 7. Sanders, H.O. and J. H. Chandler, 1972. Biological Magnification of a Polychlorinated Biphenyl (Aroclor® 1254) from Water by Aquatic Invertebrates. Bull. Env. Cont. and Toxicol. 7(5): 257-263.

Ex. 8. Sanders, Herman O., 1972. Toxicity of Aroclors® to Aquatic Organisms: Invertebrates. Fish-Pesticide Laboratory, Columbia, Missouri. PCB Newsletter, 30 March, 1972, National Water Quality Laboratory, Duluth, Minnesota. pg. 18.

Ex. 9. Stalling, D. L., and F. L. Mayer, Jr., 1972. Toxicities of PCB's to Fish and Environmental Residues. Environmental Health Perspectives, April, 1972.

Ex. 10. Schoettger, R.A., B. Grant, H. D. Kennedy, F. L. Mayer, H. O. Sanders, and D. Sjedberg, 1970. Special Report on Polychlorinated Biphenyls (PCB's). In: Progress in Sport Fishery Research, Fish-Pesticide Research Laboratory, Division of Fisheries Research, Bureau of Sport Fishery and Wildlife, Washington, D.C.

Ex. 11. Mayer, Foster L., 1972. Chronic Toxicity of PCB's to Fish. Fish-Pesticide Laboratory, Columbia, Missouri. PCB Newsletter, March 30, 1972. National Water Quality Laboratory, Duluth, Minnesota.

Ex. 12. Morgan, J. R., 1972. Effect of Aroclor 1242® (a Polychlorinated Biphenyl) and DDT on cultures of an Alga, Protozoan, Daphnid, Ostracod, and Guppy. Bull. Env. Cont. and Toxicol. 8(3): 129-137.

Ex. 13. National Water Quality Laboratory, 1974. Unpublished reports on toxicity of isomers of Aroclor 1242 and 1254 to Daphnia magna. National Water Quality Laboratory, Duluth, Minnesota.

Ex. 14. Johnson, Waynon, 1973. Unpublished reports on toxicity of Aroclor 1016. Fish-Pesticide Laboratory, Columbia, Missouri.

Research on the effects of Polychlorinated Biphenyls on aquatic life was started at the National Water Quality Laboratory in 1970, when I began an initial survey of the eight Aroclors using the water flea Daphnia magna. We had to develop testing and analytical methodology before we could do any detailed bioassays. The initial Daphnia studies were static tests conducted in small covered beakers. Later Daphnia tests were conducted in continuous-flow systems, with survival and reproduction as measures of toxicity. Aroclor 1248 was the most toxic to Daphnia magna of the eight Aroclors tested in static tests (1221, 1232, 1242, 1248, 1254, 1260, 1262, 1268), the 3-week LC50 (where 50% die after 3-weeks) was 25 µg/liter. Aroclor 1254 was the most toxic PCB to Daphnia under continuous-flow conditions with a 3-week LC50 of 1.3 µg/liter (Exhibit 1). As better analytical techniques were developed it became apparent that significant losses of PCB occurred in both static and continuous-flow test systems, explaining the difference between static and continuous-flow LC50 values, due to sorption on food, glassware, and animal tissues, and loss to the air. The mid-range PCB's were most toxic to Daphnia in static tests. The toxicity of the PCB's decreased progressively from A-1248 to A-1221 and from A-1248 to A-1268 (Exhibit 1).

Two additional fish food animals were tested (Exhibit 1). Ninety-six hour LC50 values for A-1242 and A-1248 on Gammarus pseudolimnaeus in continuous-flow tests were 73 and 20 µg/liter. Survival after 60 days was 52% at 8.7 µg/liter A-1242 and 53% at 5.1 µg/liter A-1248. Reproduction and survival of young were good at 2.8 µg/liter A-1242 and 2.2 µg/liter A-1248. The midge Tanytarsus dissimilis, in continuous-flow tests, did

not emerge (as adults) in abundance above 5.1 µg/liter A-1248 or 3.5 µg/liter A-1254. The 3-week LC50 for A-1254 was 0.65 µg/liter for larvae and 0.45 µg/liter for pupae. Tissue residues in Gammarus ranged from 4.0 µg/gram A-1254 in control animals to 552 µg/gram A-1248 in animals held for 60 days in water containing 5.1 µg/liter A-1248 (Exhibit 1).

Several tests were completed with two fish species (Exhibit 2). Two 9-month continuous-flow bioassays and several intermediate length continuous-flow tests were conducted to determine safe levels of Aroclor 1242, A-1248, and A-1254 for the fathead minnow (Pimephales promelas) and A-1248 for the flagfish, Jordanella floridae. Calculated 96-hour LC50 values for newly hatched fathead minnows were 7.7 µg/liter for Aroclor 1254 and 15 µg/liter for A-1242. Three-month-old fatheads had a 96-hour LC50 of ca. 300 µg/liter for A-1242. Reproduction occurred at and below 1.8 µg/liter A-1254 and at and below 5.4 µg/liter A-1242. Newley hatched young were the most sensitive life stages. Eggs hatched better at higher PCB levels such as 15 µg/liter, due to apparent bacteriostatic properties of PCB's, though the young died rapidly after hatching. Growth of young fatheads was also affected above 2.2 µg/liter 1248, and none survived above 5.1 µg/liter after 30 days. Young flagfish did not survive at A-1248 concentrations above 5.1 µg/liter and did not grow well above 2.2 µg/liter. Tissue residues in fathead minnows ranged from 0.7 µg/gram A-1248 in control fish to 1036 µg/gram 1254 in fish held for 8 months in water containing 4.6 µg/liter A-1254.

PCB studies were continued at the National Water Quality Laboratory after I moved to Corvallis. David Defoe, et. al., conducted two complete fathead continuous-flow life cycle tests with A-1248 and A-1260 (Exhibit 3).

They showed that in 30-day acute exposures using A-1248 and fathead minnow fry (less than 12 hours old) 8 µg/l killed all the fry, 4.5 µg/l killed 50% of the fry and caused a 70% reduction in the weight of the fry, and 2.2 µg/l caused no mortality but a 20% reduction in weight. Similar studies with Aroclor 1260 showed that 9 µg/l killed all the fry and 4 µg/l caused 70% mortality and a 30% reduction in fry weight. Reproduction, measured as the number of eggs spawned per female, in females exposed to 1.1 µg/l A-1248 was only 60% of that of the controls. Aroclor 1260 did not affect reproduction at concentrations up to 2.1 µg/l (Exhibit 3).

Additional studies are presently continuing at NWQL under the direction of Dr. K. E. Biesinger and Dr. Gil Veith. Studies of individual isomers and Aroclor 1016 are being continued. Preliminary studies comparing the toxicity of A-1016 and A-1242 by Ken Moorman and Dr. Biesinger (Exhibit 4), NWQL, Duluth, have shown similar effects on Daphnia magna in static tests. No young survived after 3 weeks at 125 µg/l A-1016 or A-1242. At 100 µg/liter 100 young survived after 3 weeks in A-1016 and 36 young survived A-1242 (Exhibit 4).

Significant work with marine organisms under the direction of Dr. Tom Duke, Gulf Breeze, Florida, has been conducted but will not be discussed here. Also a great wealth of information has been published on PCB tissue levels in a wide variety of freshwater, marine, and terrestrial animals. This material also will not be discussed here, with the exception of a brief mention of the works of R. K. Ringer, R. J. Aulerich and M. Zabik, East Lansing, Michigan (Exhibit 5); and N. S. Platonow and L. H. Karstad, Guelph, Ontario (Exhibit 6). Very low levels of PCB's in Lake Michigan were magnified in salmon tissues, which were subsequently fed to mink by mink ranchers (Exhibit 5). Meat from cows fed PCBs were fed to mink to determine if mink were really susceptible (Exhibit 6).

Tissue levels of PCBs below the FDA recommended standard of 5 µg/gram in salmon caused reproductive failure and early kit mortality when fed to the mink.

Much excellent data on the toxicity of PCBs to freshwater animals has been produced by people at the Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Columbia Missouri, under the direction of Dr. Richard A. Schoettger. A wide range of fish and fish-food organisms have been tested with all the Aroclor series, including A-1016 and several individual isomers. Sanders and Chandler (Exhibit 7) demonstrated that after a 7-day exposure to 1.5 µg/liter A-1254 many late-instar mosquito larvae were unable to metamorphose into the adult form. Control organisms pupated with success. Sanders (Exhibit 8) presented data with individual isomers using Gammarus pseudolimnaeus as the test animal in static tests:

No. Cl	PCB Component	TL 50(µg/l) 24 hr.	96 hr.
2	2,4' - dichlorobiphenyl	0.15	0.12
2	4,4' - dichlorobiphenyl	0.16	0.10
3	2,3,4' - trichlorobiphenyl	0.10	0.07
5	2,4,5,2',5' pentachlorobiphenyl	0.43	0.21
6	2,4,6,2',4',6' hexachlorobiphenyl	0.20	0.15

Acute toxicity data for cutthroat trout, channel catfish and blue gills are presented by Stalling and Mayer (Exhibit 9). For Aroclor 1221-1268, 96 hr. LC50 values in static tests ranged from 1,170 to 50,000 µg/l for cutthroat trout. The acute oral toxicity of Aroclors 1242, 1248, 1254, and 1260 was greater than 1500 mg/kg in rainbow trout. Intermittent-flow bioassays of Aroclor 1242, 1248, and 1254 to blue gills resulted in

15-day LC50 values of 54, 76, and 204 µg/l, respectively. Exposures to channel catfish gave 15-day LC50 values of 107, 127, and 741 µg/l for the same Aroclors. All LC50 values decreased significantly with longer exposures. The 20-day LC50 values for Aroclor 1248, 1254, and 1260 were 10,135, and 245 µg/l for bluegills. The toxicity of the PCBs to fish was inversely related to percent chlorine (higher Aroclor number) (Exhibit 9).

The crayfish, Orconectes nais, were more susceptible to Aroclor 1242 (7-day LC50 = 30 µg/l) than they were to A-1254 (7 day LC50 = 80 µg/l). Palaemonetes Kadiakensis, the glass shrimp, were very sensitive to Aroclor 1254, having a 7-day LC50 value of 3 µg/l compared to a 5-day LC50 of 1 µg/liter for DDT (Exhibit 10). Growth of lake trout was retarded by Aroclor 1248 in the diet for 3 months. Concentrations of 1.2, 3.8, and 12 ppm decreased weight gain by 6, 10, and 28%, respectively, below that of controls. Growth of the high-exposure group was only about half that of the controls after 6 months. Conversely, thyroid activity, as indicated by 72-hr. radioiodine uptake, was stimulated by PCB exposure. Serum cortisol concentrations ranged from 33 to 46% below that of the controls. Depressed growth rates and elevated thyroid activity imply metabolic inefficiency producing a "loading" stress that can adversely affect viability and reproductive stress (Exhibit 10).

Foster L. Mayer (Exhibit 11) presented data on the chronic toxicology of PCBs to fish. Thirty-day TL50 values for 1242 were 84 µg/l for Blue gills and 87 µg/liter for channel catfish; 1248 values were 78 µg/l for Blue gills and 75 µg/l for catfish; 1254 values were 177 µg/l for Blue gills and 139 µg/l for catfish; 1260 values were 51 µg/l for rainbow trout, >400 µg/l for Blue gills and 433 µg/l for catfish.

The study by Morgan (Exhibit 12) on the effects of Aroclor 1242 on an alga, protozoan, daphnid, ostracod, and guppy presents data similar to that given above. Algal growth was inhibited at 20, 2, and .2 ppm in static tests, though they eventually reached the same cell count levels, due to loss of PCB from the static test system. There was no effect on the protozoan Tetrahymena vorax at 20ppm in the conditions it was tested at. Aroclor 1242 was toxic to Daphnia pulex however, as young daphnia died at levels as low as 0.02 ppm. At 20 ppm there was no survival of the ostracod Cypridopsis vidua. The guppy Poecilia reticulata had 100% mortality at 20 and 2 ppm, with 25% mortality at 0.2 ppm (Exhibit 12).

The National Water Quality Lab, Duluth, MN, is currently conducting PCB work. Exhibit 13 presents chronic toxicity data for isomers of Aroclor 1242 and 1254 (Exhibit 13). It will be seen that there is not much difference in toxicity between trichlorobiphenyl and tetrachlorobiphenyl isomers. The isomers were prepared and identified by Ron Webb, EPA, Athens, GA.

The table presented below illustrates a comparison of the toxicity of Aroclor 1242 and 1016, obtained from Waynon Johnson, Fish-Pesticide lab., Missouri (Exhibit 14). It is apparent that the toxicities are similar.

Comparative toxicity of Aroclor 1242 and 1016,
based on data from the Fish-Pesticide Lab. Columbia, MO.,
in continuous-flow exposures.

Test Organism	1242 (ug/l) 96 hr. LC50 values	1242 (ug/l) LC50 values after-	1016 (ug/l) 96 hr. LC50 values	1016 (ug/l) LC50 values after-
Rainbow Trout	67	10 days = 39	100 (sac fry)	17 days = 49
	109		440 (2.5 g)	23 days = 11
Bluegill	125	15 days - 54	46 (1.8 g)	35 days = 43
	154		420 (0.9 g)	22 days = 14

MONS 204448

Foster L. Mayer, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Chronic Toxicology of PCBs to Fish

TL50 values (5-30 days) from continuous flow exposures of Aroclors® 1242, 1248, 1254, and 1260 to three species of fish have been determined. Comparative toxicity data for DDT was also determined.

Compound	Species	TL50[1] (µg/l) for 5-30 days					
		5	10	15	20	25	30
DDT	RBT[2]	4.22	1.39	0.51	0.41	0.22	-
	BG[3]	1.18	1.43	1.63	1.27	0.59	0.35
	CCF[4]	>1.0	1.54	2.33	1.32	0.87	0.57
1242	RBT	67	48	18	10	12	-
	BG	125	125	164	125	120	84
	CCF	>100	125	219	150	132	87
1248	RBT	54	38	16	6.4	3.4	-
	BG	136	115	111	106	100	78
	CCF	>100	121	121	115	104	75
1254	RBT	142	160	64	39	27	-
	BG	>200	>200	303	260	239	177
	CCF	>200	303	286	293	181	139
1260	RBT	232	326	143	78	49	51
	BG	>400	>400	>400	>400	>400	>400
	CCF	>400	535	482	512	465	433

[1] temperature, 17°C

[2] Rainbow trout

[3] Bluegills

[4] Channel Catfish

MONS 204449

ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND MONITORING
NATIONAL WATER QUALITY LABORATORY

REPLY TO:
6201 Congdon Boulevard
Duluth, Minnesota 55804

30 March 1972

FIELD STATIONS:
Newtown, Cincinnati, Ohio
Corvallis, Oregon
Monticello, Minnesota

(Weber Field Abbotsford)

Dear Newsletter Subscribers:

Attached is the fourth issue of the PCB Newsletter. I have again tried to put your contributions in with as little change as possible. I have placed the news items into rather arbitrary categories, as Analytical Methods, Environmental Occurrence and Fate, Etc., to make finding the subject areas you are interested in more convenient.

Sincerely,

Ginny Snarski

Virginia M. Snarski
Research Aquatic Biologist

Attachment:
PCB Newsletter (fourth issue)

MONS 204450

Exhibit 13

Chronic toxicity of the isomers of Aroclor 1242 and 1254 to Daphnia magna tested in standing jars. Concentrations are based on the nominal concentration and expressed in µg/liter. (National Water Quality Lab. unpublished data)

Isomers	Aroclor (PCB)	100% Adult Mortality (µg/l)	Reproduction[a] No significant Impairment (µg/l)	Reproduction[a] Significant Impairment (µg/l)
Parent mixture	1242	< 50	25	50 µg/l
2,4,4' trichlorobiphenyl[b] 2,5,4' trichlorobiphenyl 3,4,2' trichlorobiphenyl	1242	< 50	25	50 µg/l
2,3,2',3' tetrachlor - 2,5,3',4' tetrachlor - 2,4,3',4' tetrachlor -	1242	< 50	12.5	25 µg/l
Parent mixture	1254	> 50	50	> 50 µg/l
2,5,2',5' tetrachlor - 2,4,2',5' tetrachlor - 2,3,2',5' tetrachlor -	1254	100	25	50
2,3,6,2',5' pentachlor - 2,4,5,2',5' pentachlor - 2,4,5,2',4' pentachlor - 2,4,5,2',3' pentachlor -	1254	100	50	100

a. As determined from an Analysis of Variance and Dunnett's Test($p=.05$)

b. Isomers prepared by Ron Webb, EPA, Athens, GA.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL WATER QUALITY LABORATORY
6201 CONGDON BOULEVARD
DULUTH, MINNESOTA 55804

April 2, 1974

Dear Dr. Nebeker:

Enclosed you will find the information on PCB's which you requested last week. All research work was done by LaVarre Walker while he was a presidential intern here last year, excluding isomers on 1242 which Leroy Anderson and I have recently started.

Since your call last week, I have reiewed LaVarre's records, and after doing so, have compiled all the raw data and performed statistical analysis on it and arranged it in table form. As I have typed the tables myself, you may want to change the table forms and/or have a secretary retype them if you find the information useful.

Sincerely yours,

Arlene Shelhon
Biological Lab. Technician

Enclosures:

MONS 204452

United States Department of the Interior
FISH AND WILDLIFE SERVICE
BURFAU OF SPORT FISHERIES AND WILDLIFE
Fish-Pesticide Research Laboratory
Route No. 1
Columbia, Missouri 65201

IN REPLY REFER TO:

March 29, 1974

Dr. Alan Nebeker
Western Fish Toxicology Laboratory
Environmental Protection Agency
1350 S. E. Goodnight
Corvallis, Oregon 97330

Dear Al:

Enclosed find the toxicity data we have available on Aroclor 1016. The work was accomplished both at this lab and at our LaCrosse, Wisconsin field station by Mr. Wilbur Mauck. As indicated previously, we have no specific isomer data nor any longer term, chronic toxicity test data.

Static and flow-through toxicity tests were conducted under conditions generally as outlined in APHA (1971), Lennon and Walker (1964), and Mount and Brungs (1967). Values for LC50 (lethal concentration estimate for 50% of the test organisms) were calculated according to the method of Litchfield and Wilcoxon (1949). Time independent LC50 and LC10 values (concentration estimate at which 50% (90%) of the test organisms can live for an indefinite period of exposure) were calculated by the method of Green (1965).

I am quite interested in following the progress of your action relative to Aroclor 1016 and would appreciate receiving copies of the information you accumulate on it. I would especially like to know the outcome of your efforts and what the future applications and production of 1016 is predicted to be.

If you have other questions concerning our work please feel free to call me.

Sincerely,

W. Waynon Johnson
Fishery Biologist

pm

Enc.

MONS 204453

Literature Cited

APHA, et al. (1971). Standard methods for the examination of water and wastewater. Amer. Public Health Assoc., Washington, D. C. 13th ed. 562-577.

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Mount, D. I. and W. A. Brungs. (1967). A simplified dosing apparatus for fish toxicology studies. Water Research, I:21-29.

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Table 1. Toxicity of Aroclor 1016 to selected species of freshwater fish by the static technique.

Test Organism		Test Conditions				LC Values (µg/l) and 95% Confidence Limits	
Species	Size (g)	Temp (°C)	pH	Alkal (mg/l)	Hard. (mg/l)	24 hr LC50	96 hr LC50
Bluegills	0.9	12	7.4	35	40	> 1200	1000 540-1550
Channel catfish	0.8	20	7.4	35	40	>100,000	>100,000
Chinook salmon	green eggs[1/]	12	7.4	35	40	> 1000	> 1000
Coho salmon	green eggs	12	7.4	35	40	> 1000	> 1000
Rainbow trout	0.5	12	7.4	35	40	800 470-1370	135 114-160
Yellow perch	0.8	12	7.4	35	40	-	240 153-375

1/ Fertilized eggs less than 48 hours old at initiation of test.

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Table 2. Toxicity of Aroclor 1016 to selected species of freshwater fish by the flow-through technique.

Test Organism		Test Conditions				LC Values (µg/l) and 95% Confidence Limits				
						96 hr.	Terminal Value		TI	TI
Species	Size (g)	Temp (°C)	pH	Alkal (mg/l)	Hard. (mg/l)	LC50	Day	LC50	LC10	LC50
Atlantic salmon	5.6	17	7.6	250	310	134 113-160	16	60 52-70	24	34
Bluegill	0.9	12	7.6	257	314	420 240-720	35	43 28-65	8.2	28
Bluegill	1.8	20	7.4	237	272	46 39-54	22	14 12-16	7	8
Brown trout	4.6	12	7.6	257	314	138 108-175	17	81 64-102	36	65
Rainbow trout	sac fry	12	7.6	257	314	440 366-527	17	49 33-72		
Rainbow trout	2.5	10	7.4	237	272	100 64-156	23	11 10-12		1.2

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PCB - Archive

Extra
N-5

EFFECT OF DIETARY POLYCHLORINATED BIPHENYLS ON GROWTH AND REPRODUCTION OF MINK

R. K. Ringer, R. J. Aulerich and M. Zabik
Departments of Poultry Science and Entomology
Michigan State University
East Lansing, Michigan 48823

The Great Lakes have played a prominent role in the development of the mink industry in nearby states as these lakes furnished the rancher with an inexpensive and abundant supply of fish deemed desirable for mink feed. During the middle 1960s, reports from mink ranchers stating that reduced reproduction was a consequence of feeding Great Lakes fish were called to the attention of university researchers (Hartsough, 1965). Coho salmon taken from the tributaries of Lake Michigan during the spawning run in 1967 were made available to commercial ranchers as food for mink and resulted in a precipitous increase in newborn mink (kit) mortality. The extent of mortality appeared as a time-dosage response to coho salmon in the diet. Adult breeders were reported to be unaffected. Rancidity, chlorinated hydrocarbon pesticide contamination and mercury poisoning were each prominent suspects as the causative agent responsible for the high kit mortality. Because of the disastrous effect of feeding coho salmon, mink ranchers discontinued use of Great Lakes fish in mink diets.

In 1969 our laboratory confirmed the mink industry's observation that Lake Michigan coho salmon, when fed to breeder mink, resulted in either total cessation of mink reproduction (embryo toxicity) or kit mortality, the degree of complication being dependent upon the percentage coho salmon fed and the duration of feeding (Aulerich et al., 1971). Coho salmon per se were not implicated since west coast coho salmon yielded totally acceptable reproductive parameters. Subsequent reproduction studies yielded data indicating that the causative agent(s) was not confined solely to the salmon from Lake Michigan, but was apparently present, although to a lesser extent, in other fish from the Great Lakes (Aulerich et al., 1971).

Rancidity and mercury contamination data for the various fish did not correlate with mink toxicity data and each was discounted as a contributor to the problem; however, hydrocarbon pesticide contamination (total DDT and isomers or dieldrin) of the fish fed was inversely proportional to number of mink offspring born per female. This relationship appears spurious and may reflect, in part, the fat content of the fish. Feeding either DDT and isomers or dieldrin in levels far in excess of that found analytically in the fish failed to simulate the effect on mink of feeding diets containing coho salmon (Aulerich and Ringer, 1970; Aulerich et al., 1971).

This study was undertaken to investigate the effects of polychlorinated biphenyls, known to contaminate Lake Michigan and its fish, on growth and reproduction of ranch mink.

MONS 204457

MATERIALS AND METHODS

This study consisted of three feeding trials encompassing polychlorinated biphenyls (PCB) supplementation to mink. PCB supplementation was by incorporating the Aroclor® into a cereal grain premix and then into the diet. The same basal (control) diet, except as specified, was fed in all experiments. The diet composition was cereal, 15%; tripe, 20%; beef liver, 5%; horsemeat, 15%; chicken, 15%; and fish, 30%. Standard ranch procedures of animal care and breeding were used throughout the course of the experiments.

RESULTS

Experiment I

A total of 45 mink were divided into 3 groups (12 females and 3 males per dietary treatment group). The treatments were a basal diet containing ocean fish, substitution of coho salmon for the ocean fish, and the basal diet supplemented with 10 ppm each of Aroclors 1242, 1248, and 1254.

No female mink receiving dietary coho salmon bore young (whelped) nor did those receiving 30 ppm PCB supplementation (Table 1). All adult mink fed supplemental PCB's died by the end of the normal whelping period. Those females, on PCB's, that survived sufficient time to produce offspring showed no signs of whelping. Six mink fed coho salmon died during the experimental period of January 1 to June 30. No adult control mink died.

Table 1

Effect of Dietary PCB Supplementation(1) on Reproduction of Ranch Mink (January 1 to June 30)

	Females mated	Females whelped	Kits born		Kits alive (4 wks.)
			Alive	Dead	
Basal (control)	12	11	35	19	29
30% coho salmon	12	0	0	0	0
Basal + 30 ppm PCB's	11(2)	0	0	0	0

(1) 10 ppm each of Aroclors 1242, 1248, and 1254.
(2) One female died prior to the mating season.

Tissue analysis for PCB residues from several mink that died while on experimentation are shown in Table 2. Control mink were sacrificed for tissue analysis. The similarity in the tissue residues of the PCB

Aroclor® is the trade name of polychlorinated biphenyls by Monsanto Company, St. Louis, Mo.

treatment and the coho treatment group is striking. Organ weights expressed as a percentage of brain weight from those mink that died and from sacrificed controls were not significantly different ($P > 0.05$).

Table 2

PCB Tissue Residues (in ppm) of Mink that Died While Receiving Dietary Coho Salmon or Supplemental PCB's[1] and from Sacrificed Controls (Mean ± S.E.)

	Basal (control)	30% coho salmon	Basal + 30 ppm PCB's
No. mink	4	3	12
Brain	< 0.01	11.07 ± 0.78	11.00 ± 1.43
Liver	< 0.01	5.21 ± 1.66	4.18 ± 0.58
Kidney	< 0.01	6.37 ± 0.25	4.47 ± 0.42
Spleen	< 0.01	6.19 ± 0.09	4.79 ± 0.39
Lung	< 0.01	5.15 ± 0.22	4.78 ± 0.53
Muscle	< 0.01	4.73 ± 1.80	4.88 ± 0.54
Heart	< 0.01	2.84 ± 1.21	3.26 ± 0.49

(1) 10 ppm each of Aroclors 1242, 1248, and 1254.

Experiment II

Thirty-five mink were divided into five groups of six females and one male per group. Dietary treatments were initiated on August 25 to measure growth rate of young, growing mink and will continue until June 30 when the reproductive phase is complete. Treatments consisted of a basal diet with ocean fish, basal plus 5 ppm Aroclor 1254, basal plus 10 ppm Aroclor 1254, basal plus 10 ppm Aroclor 1254 and 10 ppm DDT, and basal plus 10 ppm Aroclor 1254 and 0.5 ppm dieldrin.

Results of body weight gains for the growth phase from August 25 to December 22 are shown in Table 3. Results for the reproduction phase are included in Table 4.

Table 3

Effect of Aroclor 1254, Alone or in Combination with Pesticides, on Body Weight Gains of Female Mink

Dietary treatment	No. Mink	Initial body wt. (gm) (Aug. 25)	Body weight gain (gm) ± S.E. from initial wt. 1 mo. (Sept. 24)	2 mo. (Oct. 25)	3 mo. (Nov. 25)	4 mo. (Dec. 22)
Basal diet (30% ocean fish mix; control)	6	1020	43 ± 17.4	203 ± 25.4	187 ± 46.2	210 ± 35.1
Basal diet plus 5 ppm PCB(1)	6	940	62 ± 22.4	153 ± 44.2	138 ± 42.7	128 ± 43.7
Basal diet plus 10 ppm PCB	6	923	43 ± 20.4	148 ± 32.0	132 ± 26.3	92 ± 35.1*
Basal diet plus 10 ppm PCB and 10 ppm DDT	6	1052	43 ± 14.5	57 ± 14.6**	28 ± 21.5**	-15 ± 35.4**
Basal diet plus 10 ppm PCB and 0.5 ppm dieldrin	6	940	108 ± 25.1	133 ± 26.7	115 ± 18.9	57 ± 24.4**

(1) Aroclor 1254.
*Significantly different ($P < 0.05$) from controls.
**Significantly different ($P < 0.01$) from controls.

Table 4

Effect of Aroclor 1254, Fed Alone or in Combination with Pesticides, on Mortality and Reproduction of Mink (Aug. 25 to May 10) (6 females and 1 male/group)

	% mortality	Females alive	Females whelped	Kits born Alive	Kits born Dead	Kits/female alive
Control	14	5	3	17	8	5.0
5 ppm PCB(1)	29	4	0	0	0	0
10 ppm PCB	71	1	0	0	0	0
10 ppm PCB + 10 ppm DDT	43	4	0	0	0	0
10 ppm PCB + 0.5 ppm dieldrin	100	0	0	0	0	0

(1) Aroclor 1254.

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th Pesticides,

± S.E. from initial wt.

3 mo. (Nov. 25)	4 mo (Dec. 2
187 ± 46.2	210 ± 35.1
138 ± 42.7	128 ± 43.1
132 ± 26.3	92 ± 35.1
88 ± 21.5**	-15 ± 35.4
115 ± 18.9	57 ± 24.4

th Pesticides,
• May 10)

born Dead	Kits/female alive
8	3.0
0	0
0	0
0	0
0	0

Data from this experiment demonstrate that 10 ppm Aroclor 1254 depressed growth gains, when fed continuously to growing mink. A dose-response curve for data on-0, 5, and 10 ppm Aroclor 1254 at 4 months treatment yielded a regresaion equation of $\hat{Y} = 207.5 - 107.4X$ where $X = \ln(\frac{ppm}{5} + 1)$. This regresaion equation gives a significant dose-response ($P < .05$).

All mink fed PCB supplemented diets failed to produce offspring. Those breeders that died during the gestation period exhibited various degrees of embryo toxicity when the uterus was opened for examination.

Experiment III

One hundred twenty-eight mink were divided into eight groups of twelve females and four males per group to evaluate the effect of Aroclor 1254 on reproduction. The dietary treatments were initiated on January 1 and will continue until June 30. Included in the treatments (Table 5) was a group given 5 ppm Aroclor 1254 plus 6 ppm DDT and 0.2 ppm dieldrin. These levels approximate the residue values found in diets where 30 percent raw Lake Michigan coho salmon constituted the fish portion of the diet. Cooked coho salmon (120° C at 1.05 kg/sq cm for 30 min) was included to test for the heat stability of the factor(s) causing the reproductive problem.

Table 5

Effect of Aroclor 1254 Fed Singly and in Combination with Hydrocarbon on Reproductive Parameters (Jan. 1 to May 10) (12 females and 4 males/group)

	% mortality	Females alive	Females whelped	Kits born Alive	Kits born Dead	Kits/female alive
Control (ocean fish)	6	11	11	56	10	6.0
Raw coho salmon	19	11	0	0	0	0
Cooked coho salmon	6	12	0	0	0	0
1 ppm PCB(1)	6*	10	8	35	8	4.3
5 ppm PCB	0	12	3	3	6	0.8
15 ppm PCB	31	8	0	0	0	0
5 ppm PCB + 6 ppm DDT	0	12	4	5	6	0.9
5 ppm PCB + 6 ppm DDT + 0.2 ppm dieldrin	19	10	5	15	7	2.2

(1) Aroclor 1254.
* One female escaped.

Data in Table 5 indicate that 5 ppm Aroclor 1254 markedly reduced reproduction when fed only 4 months, 1 ppm Aroclor 1254 caused only slight reduction and 15 ppm PCB totally inhibited reproduction. The factor(s) present in Lake Michigan coho salmon was heat stable and duplication, in

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part, of several factors known to be present in the salmon did not totally reproduce the coho salmon effect.

The results of these experiments clearly point out the sensitivity of mink to polychlorinated biphenyls and cast suspicion on PCB's as a possible factor involved in the poor reproduction following Lake Michigan coho salmon feeding.

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Aulerich, R. J., R. K. Ringer, H. L. Seagren and W. G. Youatt, 1971. Effect of feeding coho salmon and other Great Lakes fish on mink reproduction. Can. J. Zool. 49: 611-616.

Hartsough, G. R., 1965. Great Lakes fish now suspect as mink food. Amer. Fur Breeder 38: 25.

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N-7

Biological Magnification of a Polychlorinated Biphenyl (Aroclor® 1254) from Water by Aquatic Invertebrates

by Herman O. Sanders and Jack H. Chandler

Fish-Pesticide Research Laboratory
Bureau of Sport Fisheries and Wildlife
United States Department of the Interior
Columbia, Mo.

Polychlorinated biphenyls (PCBs) are chemical mixtures of chlorinated biphenyl isomers. These mixtures have a wide range of industrial application and are marketed in the United States as the Aroclor®-1200 series. A recent report describes the widespread occurrence of PCBs in the environment and their potential detrimental effects on organisms in aquatic ecosystems (1). Their insecticidal properties are well documented in literature (2,3). Only a few studies report the toxicity of PCBs to aquatic invertebrates (4,5). Some excellent reviews by Peakall and Lincer (6); Gustafson (7); and Veith and Lee (8) summarize and evaluate our current knowledge concerning contamination of the environment by PCBs.

Results of previous bioassay experiments with aquatic invertebrates indicated that crustacea and immature insects rapidly accumulate total body concentrations of DDT or aldrin many thousands of times that of surrounding water (9). Although concentrations of PCBs entering the aquatic environment are generally below levels acutely toxic to aquatic invertebrates, these pollutants can be accumulated to appreciable concentrations by aquatic invertebrates. Thus, organisms at higher trophic levels may be exposed to significant amounts of PCBs via the food chain.

The purpose of this study was to determine the rate of accumulation and biological magnification of ^{36}Cl-labeled Aroclor® 1254 from water by eight species of aquatic invertebrates. The invertebrates were exposed to Aroclor® 1254 at concentrations less than 3 ppb, which is similar to levels detected in the water of Escambia Bay, Florida (4). In addition to measurements of accumulation, acud exposed to Aroclor® 1254 were analyzed by gas-liquid chromatography to investigate potential shifts in residue composition which may have occurred as a result of metabolism.

Materials and Methods

The organisms tested were four species of crustacea; scud, Gammarus pseudolimnaeus Bousfield; glass shrimp, Palaemonetes kadiakensis Rathbun; crayfish, Orconectes nais Faxon; a daphnid, Daphnia magna Strauss, and four species of immature aquatic insects, stonefly (naiad), Pteronarcys dorsata Say; dobsonfly (larvae) Corydalus cornutus Linnaeus; mosquito (larvae), Culex tarsalis Coquillett; and the phantom midge, Chaoborus punctipennis (Say). Daphnids were obtained from laboratory-reared cultures and all other

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TABLE I

Biological magnification of ^{36}Cl-labeled Aroclor® 1254 by aquatic invertebrates

Organism	Organisms per sample[1]	Water concentration ppb $\bar{x}$ ± SE[2]	Organism concentration (4 day exposure) ppm $\bar{x}$ ± SE	Magnification factor[3] 1-day	4-day	7-day	14-day	21-day
Daphnid *Daphnia magna*	60	1.1 ± 0.2	52 ± 2.0	24,700	47,000	-	-	-
Phantom midge *Chaoborus punctipennis*	5	1.3 ± 0.1	30 ± 1.6	22,000	23,000	23,800	24,800	-
Scud *Gammarus pseudolimnaeus*	6	1.6 ± 0.1	39 ± 3.0	17,000	24,000	26,000	27,500	27,000
Mosquito larvae *Culex tarsalis*	10	1.5 ± 0.3	27 ± 2.0	12,600	18,000	20,000	-	-
Glass shrimp *Palaemonetes kadiakensis*	3	1.3 ± 0.1	16 ± 2.6	10,300	12,300	13,700	14,200	16,600
Stonefly *Pteronarcys dorsata*	3	2.8 ± 0.8	7.0 ± 0.30	2,100	2,500	2,800	2,900	2,800
Dobsonfly *Corydalus cornutus*	3	1.1 ± 0.1	5.1 ± 0.23	1,400	4,600	5,700	6,600	6,800
Crayfish *Orconectes nais*	2	1.2 ± 0.1	0.2 ± 0.20	570	1,700	3,400	4,500	5,100

[1] Samples were taken in triplicate.

[2] Samples were taken in triplicate and expressed as mean value ± standard error (P=.05).

[3] Concentration in organism/concentration in water.

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organisms were collected from streams and ponds near the Fish-Pesticide Research Laboratory, Columbia, Missouri.

The ^{36}Cl-labeled Aroclor® 1254 used in these experiments was prepared by neutron irradiation in a nuclear reactor (10). This irradiated material was purified by silicic acid chromatography (10,11). The specific activity of this compound was 90.2 cpm/μg (disintegrations/min per microgram).

The experiments were conducted in a continuous-flow bioassay system designed for exposing small invertebrates to constant concentrations of a toxicant over extended periods. Test water came from a deep well and had a pH of 7.4; alkalinity and hardness were 260 and 270 mg/l as $CaCO_3$, respectively. The desired concentration of ^{36}Cl-labeled Aroclor 1254 was prepared in a reservoir and pumped through glass tubing into exposure vessels by a metering unit, at approximately 5 ml/min. Exposure vessels were 2-l glass aquaria containing one liter of water maintained at 21 ± 1 C°. To allow for concentration equilibrium, the bioassay system was operated for at least 24 hr prior to addition of organisms. All invertebrates were allowed to adjust to the test environment for 48 hr prior to exposure. The organisms were not fed during the experiments and mortality in the test population and controls were less than 10%. Previous studies (12,13) have shown that aquatic insect controls can live for several weeks without feeding.

Invertebrate samples for radiometric analysis were taken in triplicate. Sample size ranged from three crayfish to sixty daphnids. Individual samples were prepared directly for analysis by homogenizing the whole organism in a tissue grinder. The homogenate was obtained by adding 6 ml of Triton X-100®: toluene (2:3 v/v) emulsifier to each sample during grinding (9). The homogenate was then transferred to a glass vial for counting and a toluene-fluor mixture was used to bring the total volume of the scintillation vial up to 15 ml. The radioactivity of the sample was measured in a Beckman 200-L liquid scintillation counter. The concentration of Aroclor® 1254 in water was monitored radiometrically by extracting triplicate 100-ml water samples with redistilled hexane and by preparing the sample as previously described for the liquid scintillation method.

Concentrations of PCBs in the samples were obtained by converting cpm (counts/min) to dpm (disintegrations/min) after determining the efficiency of liquid scintillation counting. All samples were corrected for quench by use of external and internal standards. Residue concentrations presented in the text and tables were computed on whole-body dry-weight basis. Dry-weight conversion factors were obtained by drying the organisms to a constant weight at 50° C. Magnification was expressed as the ratio of concentration in organism to concentration in water.

Results and Discussion

PCB uptake kinetics

The rate of uptake and biological magnification of Aroclor® 1254 by some aquatic invertebrates was very rapid (Table 1). For example, when *D. magna* were exposed for 4 days to water containing 1.1 ± 0.2 ppb of 1254, they accumulated total body concentrations

[1] Samples were taken in triplicate and expressed as mean value ± standard error ($P = .05$).
[2] Concentration in organism/concentration in water.

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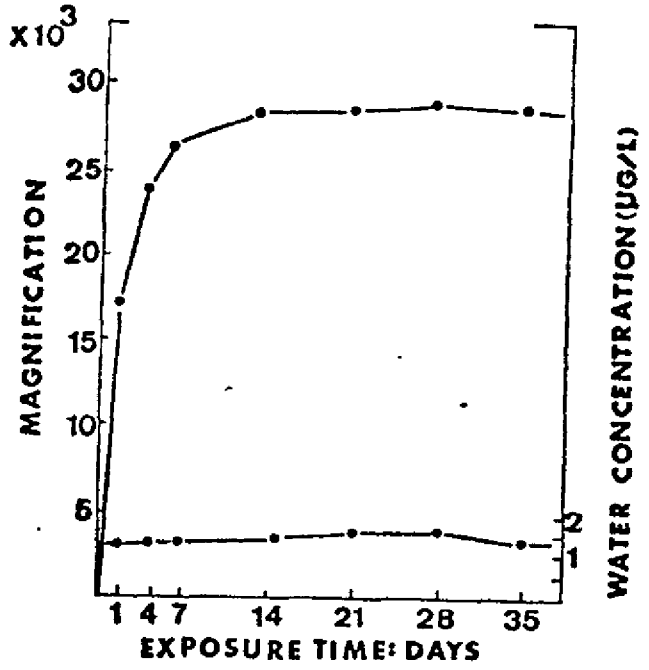

Figure 1. Biological magnification of ^{36}Cl-labeled Aroclor^{R} 1254 from water by scud Gammarus pseudolimneaus during 35 days of exposure. -·- = concentration in water; -o- = magnification in organism (total body residue/water concentration).

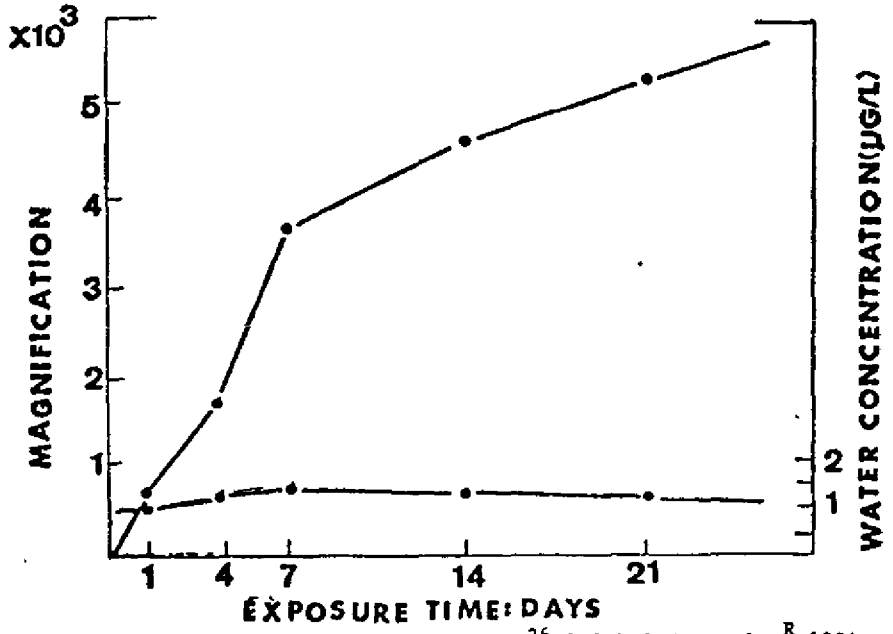

Figure 2. Biological magnification of ^{36}Cl-labeled Aroclor^{R} 1254 from water by crayfish Orconectes nais during 21 days of exposure. -·- = concentration in water; -o- = magnification in organism (total body residue/water concentration).

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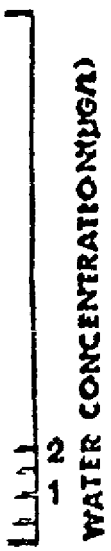
48,000 times greater than those in water.

In similar experiments, late-instar mosquito larvae exposed to water containing 1.5 ± 0.3 ppb of 1254 accumulated 19 ppm of this compound within 24 hr, which represents a 12,600-fold magnification. This accumulation continued until the seventh day of exposure when the mosquito larvae metamorphosed into the pupal stage. The pupae showed no apparent symptoms of toxicosis after this 7-day exposure. However, many of the pupae were unable to metamorphose into the adult form. Control organisms pupated with success. Moriarty (3) reported that the acute toxicity of Aroclor® 1254 to grasshoppers is not a significant factor. However, he suggested that a possible latent toxicity of this compound to grasshopper nymphs occurred at ecdysis, resulting in higher mortality for moulted individuals.

In some experiments, we were able to continue the exposure until an equilibrium concentration was reached within the organism. For example, scud exposed continuously to 1.6 ± 0.1 ppb of Aroclor® 1254 in water reached an equilibrium concentration in 14 days. Total body residues at this time were 44 ppm, a concentration 27,500 times that in water (Figure 1). Once this equilibrium was reached within the organism, no further magnification of residue was detected after an additional 21 days of exposure. Stonefly naiads, exposed to water containing 2.8 ppb of 1254, accumulated 8 ppm after a 7-day exposure; residues remained at 8-9 ppm after an additional 14 days of exposure. Early instar crayfish apparently reached an equilibrium concentration somewhat more slowly than the other invertebrates investigated. Crayfish accumulated 1254 at a linear rate until the experiment was terminated after 21 days of exposure (Figure 2).



Isomer changes of PCB residues in scud

To further elucidate the uptake of PCBs by scud, component analysis of the PCB residues were made by gas-liquid chromatography (GLC). Scud were exposed to 1.6 ppb of Aroclor® 1254 for 14 days. This exposure was previously shown to result in maximal residue accumulation in the organism. After sample extraction and cleanup of the extracts, the extracts were analyzed by GLC (14). The GLC peak area of each PCB component was measured and each component area expressed as a relative peak area (RPA). The PCB component RPA was determined by dividing the individual component area by the sum of all PCB component areas. The component relative peak areas were also calculated for Aroclor® 1254. To express the change in PCB residue composition in scud a concentration factor for each sample component was calculated by determining the ratio of the sample components RPA to the Aroclor® component RPA (Table 2).

The isomer uptake of Aroclor® 1254 by scud was characterized by progressively greater uptake of the lower chlorinated PCB isomers. Significant shifts in the ratios of the individual components occurred. Concentration factors ranged from 2.00 for a trichloro biphenyl isomer; 1.00-1.31 for pentachloro isomers; 0.49-0.80 for hexachloro isomers; and 0.25-0.50 for heptachloro isomers. No lower chlorinated components were observed in the chromatograms of the PCB residues in scud indicating that in these exposures reductive dechlorination of Aroclor® 1254 did not occur.

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We believe that ratios of the various components should be determined in examining the residues of PCBs from environmental samples. Knowledge of changes in the relative ratios of the PCB isomers may be important in evaluating the toxicological effects of PCBs.

Acknowledgements

The authors gratefully acknowledge the contributions of Dr. David Stalling and Mr. James Johnson of our staff for GLC residue analysis of scud samples.

TABLE 2

Changes in isomer ratios of Aroclor® 1254 residues in scud

Peak No.	Rel. Ret. Time[1]	No. Cl's[2]	Concentration Factor[3] Sample 1	Sample 2	Average	Aroclor® 1254 R.P.A.
1	0.27	3	2.00	2.00	2.00	0.03
2	0.37	4	1.69	1.72	1.71	0.29
3	0.45	4	1.81	1.81	1.81	0.11
4	0.50	4	1.80	2.00	1.90	0.05
5	0.63	5	1.31	1.31	1.31	0.51
6	0.77	5	1.00	1.00	1.00	1.00
7	0.98	5	1.13	1.16	1.15	0.38
8	1.07	5	1.07	1.16	1.12	0.57
9	1.19	6	0.33	0.66	0.49	0.03
10	1.31	6	0.84	0.85	0.85	0.65
11	1.59	6	0.55	0.55	0.55	0.33
12	1.67	6	0.80	0.80	0.80	0.30
13	1.79	7	0.43	0.57	0.50	0.07
14	2.04	6	0.56	0.60	0.58	0.45
15	2.61	6	0.50	0.62	0.56	0.08
16	3.22	7	0.25	0.50	0.37	0.04
17	3.68	7	0.25	0.25	0.25	0.04

[1] Retention time relative to p,p' DDE on GLC column of 2 mm i.d. x 1.8 m 0.3% w/w OV-7 on 80-100 mesh Corning® 110 glass beads; 15 ml/min. flow of nitrogen carrier gas; column operated at 155° C.

[2] Number of chlorine atoms substituted on biphenyl ring determined by gas chromatography-mass spectrometry (9).

[3] Concentration factor = Relative Peak area of sample ÷ Relative Peak areas as Aroclor® 1254.

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esidue

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ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF RESEARCH AND MONITORING
NATIONAL WATER QUALITY LABORATORY

REPLY TO
6201 Congdon Boulevard
Duluth, Minnesota 55804

FIELD STATIONS
Newtown Cincinnati Ohio
Corvallis Oregon
Monticello, Minnesota

30 March 1972

Dear Newsletter Subscribers:

Attached is the fourth issue of the PCB Newsletter. I have again tried to put your contributions in with as little change as possible. I have placed the news items into rather arbitrary categories, as Analytical Methods, Environmental Occurrence and Fate, Etc., to make finding the subject areas you are interested in more convenient.

Sincerely,

Ginny Snarski

Virginia M. Snarski
Research Aquatic Biologist

Attachment:
PCB Newsletter (fourth issue)

P C B N E W S L E T T E R

30 March 1972

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ANALYTICAL METHODS

AND

CHEMICAL CHARACTERISTICS

MONS 204472

David L. Stalling and James N. Huckins, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Silicic Acid PCB-Pesticide Separation Method

Using the Armour and Burke Silicic Acid-Celite® Column procedure for separation of PCBs from DDT and its analogs, some components of Aroclors® 1242, 1232, and 1221 elute in the pesticide fraction. To date, there has been little information available on the component distribution of the mentioned Aroclors in the PCB-pesticide fractions. In our analyses, Celite has often been found to be contaminated with a compound of unknown origin, which has a GC retention time near p,p'-DDT. Celite does not appear to enhance PCB-pesticide separation nor does it seem necessary in order to maintain an adequate column flow rate if a pressure head is used.

To overcome these problems inherent in the Armour and Burke Silicic Acid-Celite System, we have modified the method. Celite was eliminated and only 2% water (98g silicic acid + 2 ml water) is added to deactivate the silicic acid. In addition, because small amounts of benzene contaminants in the solvents affect elution, the PCB phase eluting solvent was changed by adding 0.1% benzene (v/v) to petroleum ether. The pesticide phase solvent was replaced with 20% (v-v) diethyl ether in benzene and we selected the optimum elution volumes for both phases. These modifications have permitted PCB-Pesticide separations having the maximum number of components of Aroclors® 1242, 1232, and 1221 in the PCB fraction without eluting DDT and analogs and other similar pesticides in the PCB fraction.

Experimental

Reagents

(a) Silicic acid - Mallinckrodt, 100 mesh powder; Analytical Reagent No. 2848. Prepare as described by the Armour and Burke method with the following exceptions: activate for a minimum of 12 hrs at 130° and add 2% water (98g silicic acid + 2 ml water) with a pipette.

(b) Solvents used - petroleum ether, benzene, and diethyl ether suitable for pesticide analysis.

(c) Eluting mixture - PCB fraction (0-250 ml): 0.1% benzene, 99.9% petroleum ether (v/v). Pesticide fraction (250-450 ml): 20% diethyl ether, 80% benzene (v/v).

Apparatus

(a) Chromtographic column - 22 mm I.D. x 300 mm glass column with removable teflon stopcock, joined at the top with a 250 ml glass reservoir having a 24/40 standard female ground glass joint. A 24/40 male joint joined to a 1/2" short piece of heavy walled tubing is attached to an air outlet. This adapter is held in place on the column with a standard taper clamp (Thomas), and will maintain a pressure of about 5 lbs.

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Procedure

Weigh 20 g of 2% silicic acid into a 250 ml beaker. Immediately slurry with 70 ml of pet ether and pour into chromatographic column with stopcock open. Rinse the silicic acid remaining in the beaker into the column with additional pet ether and rinse down sides of column. Allow the silicic acid to settle but do not allow the column to drain dry at any time. Next add 2 g of Na_2SO_4 to the top of the silicic acid.

Apply air pressure (2-3 psi) until the pet ether level in the column is about even with the top of the Na_2SO_4 layer. Add sample cleaned up with Florisil or gel permeation in not more than 5 ml of pet ether or iso-octane and apply pressure until sample level is even with Na_2SO_4. Wash down sides of the column with 5 ml of pet ether and bring solvent level even with Na_2SO_4 again. Place a 150 ml graduated beaker under the column and collect next rinse of 5 ml 0.1% benzene in pet ether. Then add about 275 ml of 0.1% benzene in pet ether to the column reservoir and apply air pressure until a flow rate of approximately 4 ml/min is achieved.

Collect the 0-100 ml eluant fraction and quickly place a graduated 400 ml beaker under the column while still eluting. Collect the next 100-250 ml fraction and stop elution. The solvent level should be close to the top of the Na_2SO_4 layer. Place another 400 ml graduated beaker under the column and add 200 ml of 20% diethyl ether in benzene to the column reservoir. Continue elution until the 200 ml mark is reached. This completes the separation.

Results and Discussion

The elution pattern using the method described above is as follows. The 0-100 ml PCB fraction will contain the first three components of technical chlordane, hexachlorobenzene (C_6Cl_6), mirex and some Aroclor® 1260. The 100-250 ml PCB fraction contains components of Aroclors® 1260, 1254, 1248, and most 1242 components, and some late GC eluting components of 1232 and 1221. Pesticides found in the PCB fraction (100-250 ml) are aldrin, heptachlor (also a component of technical chlordane), and some p,p'-DDE. The pesticide fraction (250-450 ml) will contain most of the components of Aroclor® 1221 and the first few early GC eluting components of Aroclors® 1232 and 1242. Using a mixture of Aroclors® 1260, 1242, and 1221 as a standard, the actual PCB component split centers around the fifth, sixth, or seventh PCB peak from the solvent peak using an OV-7 GC column. If PCB components with shorter GC retention time than peak #5 from the solvent peak elute in the PCB fraction (0-250 ml), most of the p,p'-DDE, o,p'-DDE, p,p'-DDT and o,p'-DDT will elute in the PCB fraction. (If the quantative separation of mirex from Aroclor® 1260 is desired, another 2% silicic acid column, using pet ether only as an eluting solvent, is necessary. Mirex elutes in the 0-100 ml fraction.)

Because silicic acid may vary in adsorptive capacity from one batch to another, it is advisable to run a mixture of Aroclor standards (1260, 1242, and 1221) along with the samples to be processed. This enables good definition of the Aroclor component split between PCB and pesticide fractions for a specific lot of silicic acid.

It was determined experimentally that additional small amounts of benzene which might be present as contamination in pet ether have little effect on the PCB elution pattern. We also found that 20% diethyl ether in benzene (v/v) as the pesticide eluting solvent gave recoveries similar to the pesticide eluting solvent described by Armour and Burke.

Reverse Phase Thin Layer Chromatography (RPTLC) of Aroclors® (PCBs), Halowaxes® and Pesticides

Reverse phase TLC has been used to resolve components of Phenochlor Dp6 or Aroclor® 1260 (R.H. DeVos and E. W. Peet, Bull. Environ. Contam. Toxicol., 6, 164-170, 1971). RPTLC permits component separation by partition between a non-polar stationary phase and a polar mobile phase. Kieselguhr G plates (layer thickness 0.25 mm) were impregnated with liquid paraffin as the stationary phase. The components of Aroclor's® 1232, 1248, 1254, 1260; Halowaxes® 1099, 1013, 1014, and several chlorinated pesticides were resolved with one of four solvent systems (mobile phases); I-water, acetonitrile, and methanol (20:40:40); II-water, acetonitrile and methanol (15:40:45); III-water, acetonitrile and methanol (10:45:45); IV-water, acetone, acetonitrile and methanol (5:15:40:40). The R_f values for these materials were determined in solvent systems II and IV. Components of (RPTLC) spots were characterized by GC and/or GC-MS. In general, GC peaks with higher retention times corresponded to spots with lower (RPTLC) R_f values. Certain GC peaks were resolved into more than one component by RPTLC. RPTLC did not resolve aldrin and DDE from some components of PCB's and Halowaxes with similar R_f values. However, when the PCB components are recovered from the plate, they are easily separated from aldrin and DDE by GC. Research is presently underway on the application of RPTLC principles to column chromatography.

Experimental data presented here was collected by: Judith Armour, Bernadette McMahon, John Roach, Winfred Wiencke, and Susan Young of the Division of Chemical Technology; Herbert Masumoto and Milton Luke of the Los Angeles District; and compiled by Bernadette McMahon, Food and Drug Administration

New information on the composition of Aroclor references and revised data on the behavior of Aroclors in analytical procedures has been obtained in a series of short term studies in laboratories of the FDA. A GLC system capable of determining the polychlorinated terphenyl (PCT) components of certain Aroclors was developed and subsequently used to obtain the new and revised information on PCT.

Aroclor 1232 Reference Material

New information on the manufacturing process for Aroclor 1232 sheds light on the reason for the differences among the chromatograms of several samples of 1232 obtained from Monsanto at different times. It has been revealed that when Aroclor 1232 was being manufactured for sale (it is no longer available), it was made by combining amounts of Aroclors 1221 and 1242 until the mixture contained 32% chlorine by weight. This information explains how it is possible that the gas chromatograms of some lots of Aroclor 1232 reference materials have resembled chromatograms of 1242, and others have more closely resembled chromatograms of 1221.

It is probable that the manufactured lots of Aroclor 1232 varied in composition from one to another. For this reason, residues of Aroclor 1232 that may be found would be unlikely to match any arbitarily chosen 1232 reference material. It is therefore recommended that residues suspected of being "Aroclor 1232," but resembling Aroclor 1221 and/or 1242, be quantitated by comparison to Aroclor 1221 and/or 1242 reference materials, rather than by comparison to an Aroclor 1232 reference.

From now on, Aroclor 1232 will not be considered a separate entity by our laboratories, and lists of data on the behavior of Aroclors in this correspondence will not include Aroclor 1232.

Significance: Little Aroclor 1232 was ever sold (according to Monsanto) and none is available now, so residues of "1232" are not likely. The suggested means of quantitation is available only in case such a residue is found. Toxicological and ecological reasons for identifying the residue will not be jeopardized by reporting the residue as 1221 and/or 1242 rather than as 1232.

An EC-GLC System for the Determination of Polychlorinated Terphenyls and/or Polychlorinated Biphenyls

Winfred Wiencke

A two-column EC-GLC system has been devised to permit determination of both PCB and polychlorinated terphenyls (PCT) using only one dual-column oven. The use of the GLC columns commonly used for pesticide and PCB residue determination is impractical for PCT determination because of the extended elution times (up to several hours) and consequent poor sensitivity to the PCT with such columns.

A low load (1%) OV-101 column at 240°C permits elution of the PCT in Aroclor 5460 in less than 15 min and improves sensitivity so that one half scale response for 20 ng is readily attainable. A second column of 3% Dexsil, maintained in the same 240°C column oven, permits elution of the PCB in Aroclor 1260 in less than 10 min. Response to 1260 is greater than response to the same amount of 5460 when the two columns are tee'd into the same detector. It is also possible to utilize two EC detectors, one for each column.

Specific parameters of the system are:

OV-101 Column for PCT

Column: 6' x 1/4" glass, on column injection
Liquid phase: 1% w/w OV-101
Support: 100/120 mesh Gas Chrom Q or equivalent
Column oven temp: 240°C
N_2 flow rate: 120 ml/min
Injector temp: 250°C
Conditioning: 24 hours @ 270°C with a final hour at 300°C; N_2 purge at 10-30 ml/min
EC detector temp: 200°C

Dexsil 300 Column for PCB

Column: 6' x 1/4 " glass, on column injection
Liquid phase: 3% w/w Dexsil 300
Support: 100/120 mesh Gas Chrom Q or equivalent
Column oven temp: 240°C
N_2 flow rate: 60 ml/min
Injector temp: 250°C
Conditioning: 48 hrs. @ 270°C with a final 2 hrs. @ 300°C; N_2 purge at 10-30 ml/min
EC detector temp: 200°C

Electronics

Electrometer: 1 x 10^{-9} Amperes for full scale deflection (AFS)
Chart speed: 1/2" per min
EC Detector: Concentric type (Packard), H^3 foil

Aroclors 4465, 5442, and 5460 Reference Materials

By definition of the manufacturer, the Aroclors named in the title are composed of mixtures of the following:

4465 - chlorinated biphenyls and terphenyls, with 65% chlorine by weight

5442 - chlorinated terphenyls, with 42% chlorine by weight

5460 - chlorinated terphenyls, with 60% chlorine by weight

It is now apparent that when Aroclors 4465, 5442, and 5460 were chromatographed at conditions normally used for the chromatography of the mixtures of chlorinated biphenyls, the chlorinated terphenyls present in these mixtures were seen, at best, only as very late eluting peaks. In turn, this failure of the chromatographic system to visualize the terphenyls led to the reporting of some inaccurate data on these mixtures.

Recent investigations from several sources have revealed new information about the Aroclors in question. In part, the evidence has been obtained by use of the 1% OV-101 GLC column, which permits terphenyls to elute quickly enough to be easily seen and measured (see above).

New findings on the Aroclors of interest follow:

4465. The reference material available in our laboratories appears to be mostly biphenyl compounds, despite Monsanto's description of this mixture as 60:40 terphenyl: biphenyl. Chromatography of 4465 on a GLC column which permits early elution of terphenyls does not reveal the presence of additional significant compounds.

Because the 4465 reference material obtained from Monsanto by FDA does not match Monsanto's description of the product, it is suspected that the terphenyl: biphenyl proportion must vary among different manufactured lots. The description of the material as a combination of terphenyl and biphenyl also suggests that 4465 may have been made by combining amounts of other Aroclors until 65% chlorination was obtained. Therefore, it may be possible to handle residues of "4465" by comparing them to other Aroclor references which they resemble.

Monsanto no longer manufactures Aroclor 4465, so no additional samples of reference material are available. For this reason, data presented in other sections of this communication were obtained using the reference material containing mostly chlorobiphenyls.

5442. The reference material of 5442 available in our laboratories contains some contamination with chlorinated biphenyls, as evidenced by the early eluting peaks in the gas chromatograms obtained using GLC parameters "normal" for PCB determination. The contamination is relatively small, and the data for 5442 presented elsewhere in this communication was obtained by measuring the recovery of the terphenyls using the 1% OV-101 column (see above).

5460. The 5460 available in our laboratories has now been shown to contain a relatively large (about 5%) contamination of chlorinated biphenyls. It is now apparent that only the biphenyl contaminant was readily

visualized by the "normal" GLC systems, which explains why chromatograms of 5460 resembled 1260 so closely. The chlorinated terphenyl components of 5460 can be seen and measured when the 1% OV-101 GLC column is used (see above). Data on the behavior of 5460 presented elsewhere in this correspondence were obtained using the OV-101 column to visualize the terphenyl components of 5460 for measurement.

Significance: Residues of chlorinated terphenyls are presumably of as much interest and importance as those of chlorinated biphenyls. Until recently, the capability for detecting and measuring chloroterphenyl residues was limited by the very long retention times and consequent poor sensitivity for the compounds on the GLC columns (PAM 311.2) normally used for analysis of chlorinated biphenyls.

The chlorinated terphenyls are recovered, at least partially, through the analytical procedures used for organochlorine pesticides and PCB (see other sections of this correspondence for details). It is now possible, by means of the 1% OV-101 GLC column described by Wiencke (see above), to visualize residues of chlorinated terphenyls and to estimate their quantity against a known amount of reference material. This is a recommended procedure for detection and measurement of residues in samples suspected of contamination with chlorinated terphenyls. In particular, samples whose preliminary GLC analysis shows peaks eluting beyond the retention times of chlorinated biphenyls should be injected on the alternate column to visualize any terphenyls present. Chloroterphenyl residues too low to be recognized in ordinary sample examination can be discovered by injections of the sample on the 1% OV-101 column.

Variations in Partitioning Among the PCB/PCT

Susan Young, Bernadette McMahon

It has been determined that the efficiency of transfer of the PCB from petroleum ether to acetonitrile decreases with increasing chlorination of the compounds. In particular, the following recoveries of Aroclor standards were obtained when a known amount of each Aroclor in 15 ml petroleum ether was partitioned into four successive 30 ml portions acetonitrile, each acetonitrile portion being backwashed with an additional 15 ml petroleum ether (FDA Pesticide Analytical Manual I (PAM I), 211.14b):

Aroclor	µg in 15 ml PE	% recovery
1221	40	94
1242	40	97
1248	40	93
1254	40	87
1260	40	78
1262	40	74
4465	20	71

Among the PCB components of any given Aroclor, those whose peaks eluted first from a DC-200 gas chromatographic column showed the highest recoveries. Percentage recovery decreased with increasing retention times (which generally increase with increasing chlorination), again suggesting that the more highly chlorinated the isomer, the poorer its transfer from petr ether to acetonitrile.

The two Aroclors made up of PCT display a similar pattern. When Aroclors 5442 and 5460 were partitioned as described above, 92 and 77% of the original 20 µg were recovered, respectively. Recoveries of individual PCT in the Aroclors were not determined, because the OV-101 GLC column for PCT does not resolve the components well enough for measurement of each.

Significance: The experiments performed in gathering this recovery data were designed to duplicate the partitioning step most often used in the analysis of fatty foods for organochlorine pesticides and/or PCB. The residue analyst should be aware that recovery through PAM I method 211 (fatty foods) will be different for different Aroclors and should avoid attributing losses completely to the Florisil or silicic acid column chromatographic steps. In addition, the pattern of PCB isomers in a given Aroclor is altered to some degree by this phenomenom, and may cause confusion to the inexperienced analyst.

Recovery of Aroclors Through Florisil Column Chromatography

New information on the behavior of Aroclors in other parts of the methodology has prompted reassessment of the elution of Aroclors from the Florisil column (PAM I 211.14d). Frequent use of pesticide residue methodology for the analysis for PCB has assured us that the chlorinated biphenyls elute in the 6% ethyl ether/petroleum ether eluate. Additional experiments have shown that Aroclors 4465, 5442, and 5460 also elute in the 6% eluate.

Recovery of Aroclors Through Silicic Acid Column Chromatography

Judith Armour, Herbert Masumoto

Masumoto (in press, JAOAC) has pointed out that some of the chlorobiphenyl isomers present in Aroclors are not eluted from the silicic acid column by petroleum ether as originally reported. Instead, the chlorobiphenyl isomers with early GLC retention times (which, generally, are those with lowest chlorination) are likely to be held on the silicic acid column and eluted from it only by the second, polar eluant. Aroclors consisting mostly of lower chlorobiphenyl isomers will therefore show low recoveries in the petroleum ether eluate.

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Recoveries of PCB in the following Aroclors were obtained from a column made of silicic acid and Celite (JAOAC 53, 701 (1970)) which had been previously standardized by addition of enough H_2O to effect separation of pp'DDE and Aroclor 1254:

Aroclor	Recovery
1221	essentially all in polar eluate
1242	20-35% in polar eluate
1248	less than 20% in polar eluate
1254	completely in petroleum ether
1260	completely in petroleum ether
1262	completely in petroleum ether
4465	completely in petroleum ether

The two Aroclors consisting of PCT show a similar relationship between chlorine content and elution from silicic acid. All the Aroclor 5442 added to a silicic acid column eluted in the polar eluate, while the more highly chlorinated 5460 appeared mostly in the polar eluate, but began to elute in the petroleum ether eluate.

Significance: Knowledge of the elution characteristics of the Aroclors from the silicic acid column will aid the analyst in his choice of the appropriate analytical treatment of samples containing both pesticides and PCB. If examination of the original GLC chromatogram shows the PCB to resemble an Aroclor known to be incompletely separated from pesticides by silicic acid, alternate treatment(s) can be chosen to permit quantitation of the residues.

Response of GLC Detectors to the Chlorine Content in Aroclors

Winfred Wiencke, John Roach

Experiments were performed to determine whether the responses of the electron capture and microcoulometric detectors were proportional to the chlorine content of Aroclors regardless of the nature of the compounds comprising the Aroclor. Results indicate that the response of the halogen-specific microcoulometric detector to the same amount of chlorine in different Aroclors was essentially the same. In contrast, the response of the electron capture detector to the more highly chlorinated Aroclors was disproportionately high when compared to the response of the same detector to Aroclors with lower chlorine content.

The electron capture detector that was used was first shown to give reproducible results and to be linear in response to amounts of heptachlor epoxide injected. Then, injectionsof six Aroclors were made and the total area of response measured for each. Calculations were performed so that responses to the different Aroclors could be compared to one another, on the basis of response/weight chlorine. The following ratios were obtained in this way:

Aroclor	Relative Response/Weight Chlorine
1221	1
1242	3
1248	4.6
1254	9.6
1260	12.7
1262	10.4

It is obvious from these data that response of the EC detector is not proportional to the amount of chlorine present in the chlorobiphenyl molecule.

In contrast, the response of the microcoulometric detector to injections of the same amounts of chlorine in each of four different Aroclors was essentially the same for each Aroclor. Because detector sensitivity changed with time, ratios of $\frac{\text{response to Aroclor X}}{\text{response to Aroclor 1242}}$ are compared, where the injection of Aroclor 1242 and the injection of the other Aroclor were made within an hour of one another. Other special precautions were also utilized to improve reproducibility; e.g., the first two injections made after cell flushing and equilibration were not considered.

The following ratios were obtained:

Relative Response

(for same weight chlorine)

Aroclor	Mean	Range (number of injections)
1242	1.00	(used as standard)
1246	0.97	0.90-1.03 (3)
1254	1.00	0.92-1.23 (5)
1262	1.10	0.86-1.26 (6)

Despite the problems encountered in dealing with the microcoulometric system, these data do present evidence that microcoulometric response is essentially proportional to the amount of chlorine present, regardless of the structure of the compounds.

Significance: The choice of Aroclor reference material against which to measure an apparent PCB residue is not always obvious. Alteration of the original Aroclor can occur in several ways and the residue may not closely resemble any of the Aroclors. The choice of reference material can significantly affect the quantitative result of the analysis when the electron capture detector is used, because of its different response to different Aroclors. The analyst should be aware of this possibility and, if possible, quantitate by microcoulometric detection when the residue identity is questionable.

H. D. Gesser, A. Chow and F. C. Davis, Chemistry Department, University of Manitoba, Winnipeg, Canada, and J. F. Uthe and J. Reinke, Fresh Water Institute, 501 University Crescent, Winnipeg, Canada

The Extraction and Recovery of Polychlorinated Biphenyls (PCB) Using Porous Polyurethane Foam

Key Words: PCB, extraction, water, polyurethane foam, pollution.

Abstract

A fast and efficient method has been developed for the extraction and concentration of PCB from water. The method uses porous polyurethane foam as the absorption column for the PCB which is eluted with acetone and hexane for G.C. analysis.

Introduction

The analysis of chlorinated pesticide residues[1] and PCB[2] in water continue to present problems. The extraction of chlorinated pesticides from water with organic solvents[3] is time consuming and can only be done with 95-100% efficiency in a laboratory. The absorption of PCB on activated carbon[4] is complete but its recovery is variable, inefficient (25-30%) and the recovered material is significantly different from the initial PCB. The reversed liquid-liquid partition filter developed by Ahling and Jensen[5] gave excellent recovery for PCB (93-100%) but is limited by relatively low flow rates ($\leq$ 65 ml/min) and requires elaborate preparation as well as vacuum or suction to achieve these filtration speeds. A recent review of the subject has been presented by Thornburg[6].

The recent paper by Bowen[7] on the absorption properties of polyurethane foams presented a new and simple method of concentrating metallic ions from dilute aqueous solution[8]. The results suggested to us that the foams have a possible absorption capability for trace organic contaminants in water and we undertook to test this for the analysis of PCB in water.

Experimental and Results

Preliminary experiments showed that polyurethane foam does extract PCB quantitatively from water. PCB was removed from the foam by Soxhlet extraction with hexane. PCB-hexane solutions were concentrated by slow evaporation to 2 ml and samples analyzed by electron capture gas chromatography. Water spiked with known quantities of PCB gave about 50% recovery for the overall method. The loss was suspected to occur through the open condenser of the Soxhlet extractor. This was verified when a second clean foam was placed in the open end of the condenser and was shown to contain a major fraction of the PCB lost.

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A simpler and more efficient method therefore was developed using polyurethane foam plugs designed as culture tube stoppers (diSPo stoppers from Canlab, Winnipeg). These have a higher density (0.027 gm/cm^3) but are more porous than the commercial sheet polyurethane foam (0.022 gm/cm^3) used in preliminary work. The foam plugs (22 mm diameter and 38 mm long) were cleaned by shaking in a 1:1 hexane-acetone solution in a steel tube with a steel ball. The plugs were then pushed down a 20 mm I.D. glass chromatographic column and washed with 25 ml of ethyl alcohol to remove residual solvent and air. This was followed by careful washing with 200 ml of distilled water. The aqueous PCB solution was then passed through the column with a flow rate of about 250 ml/min without the need of pressure or vacuum. It was found that 95% of the PCB was retained by the first plug and a few percentages of PCB was retained by a second plug, thus making two plugs a necessary and sufficient column. Water was squeezed from the foam column prior to the extraction of PCB. The PCB was eluted from the polyurethane foam column with 20 ml of acetone followed by 100 ml of hexane, care being taken to wash down the walls of the glass column. It should be noted that at this stage if a significant quantity of H_2O (> 1 ml) has been co-extracted with the hexane then a separatory funnel separation of the two phases is required before the elutant is dried with about 20 g of anhydrous sodium sulfate. The dried elutant was concentrated on a rotary evaporator to 10 ml and a 5 µl sample was analyzed by G.C. The G.C. used was a Hewlet Packard Model 5750 equipped with a ^{63}Ni electron capture detector operated at 260°C using a helium carrier gas and a 90% argon-10% methane purge gas. Pulse time was 50 microsec., range and attenuation was 1 x 128. The 6' x 1/4" glass column was packed with 2% SE-30, 3% QF-1 on 80 - 100 mesh Chromosorb W HMDS treated at a temperature of 200°C. Injection port temperature was 230°C.

The results of extraction and recovery of PCB from water by the urethane foam column are summarized in Table 1 where quantitative calculations are based on the peak height of 13 resolved peaks, with the average and mean deviation for each analysis given.

TABLE 1

The Extraction and Recovery of PCB From 1 Liter of Water

PCB added	PCB analyzed	% recovery
20 µg.	19.6	98±6
20 µg.	19.0	95±4
20 µg.	18.6	93±14
20 µg.	19.0	95±3
20 µg.	18.2	91±7

Similar results were obtained with 2.0 µg/liter of PCB in water. It was also shown that PCB is not washed out of the foam by water. A

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complete analysis can be done within one hour. The results clearly indicate the method to be both fast and efficient. The extraction can be performed at the water sampling site and the foam plug (if column wall interference is avoided) or the acetone-hexane solution can be returned to the laboratory for concentration and analysis.

Discussion

The limits of detection and analysis of PCB in water is presumably governed by the amount of water chromatographed through the foam column. The polyurethane foam is not specific to PCB and it will absorb organochlorinated pesticides as well and a complex mixture would require some separation[9,10] prior to G.C. analysis.

The application of this method to inorganic ions will be reported shortly.

Acknowledgements

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Ronald G. Webb, Research Chemist, EPA, Southeast Water Laboratory, Athens, Georgia 30601

Chemical Identification of PCB Isomers

A paper on "Identities of Polychlorinated Biphenyl (PCB) Isomers in Aroclors" by R. G. Webb and Ann C. McCall has been accepted for publication in the July issue of the Journal of the Association of Official Analytical Chemists. Preprints are available.

Another excellent paper in this area is by D. Sissons and D. Welti, Journal of Chromatography, 60, 15 (1971).

Two pure PCB isomers found in Aroclors 1242-1254 are available as 10 mg samples. The isomers are 2,5,2',5'-tetrachlorobiphenyl and 2,4,5,2',5'-pentachlorobiphenyl.

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We are working on the preparation of a series of Aroclor solutions to be used as quantitative standards, particularly for electron-capture GC analysis. We hope to provide solutions in which the amount in each major GC peak is known as well as the total amount present together with the response factor of each peak relative to p,p'-DDE. Our target date is May 1.

ENVIRONMENTAL OCCURRENCE AND FATE

George R. Harvey, H. P. Miklas, V. T. Bowen, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Chlorinated Hydrocarbon Pollutants in the Atlantic Ocean

Summary of Investigations, 1971

We are engaged, in close cooperation with the Department of Biology, in determining the concentrations of polychlorinated biphenyls (PCB) and members of the DDT family in organisms collected from the open Atlantic Ocean, North and South. This is the first study of its kind in the high seas and has furnished us with new insights into transport mechanisms and food chains.

The data strongly indicate that the atmosphere provides the major path for delivery of both DDT and PCB to the ocean. The North Atlantic plankton show almost uniformly high levels (100-900 ppm) in their fat. Thus, the quantities of these pollutants delivered to the ocean by the atmosphere obliterate any concentration gradient from continental run-off. Secondly, there is very little difference in concentrations of these pollutants between the North and South Atlantic. Considering the smaller land surface of the southern hemisphere, the low level of industrialization and the lack of North to South circulation of Atlantic surface waters, the South Atlantic pollution must be transported there by global winds from the northern hemisphere.

Our data appear to offer no support for the widely held view that concentrations of chlorinated hydrocarbons are magnified along marine food chains. Many prey-predator couples (flying fish-plankton, Arctic Cod - Atlantic Cod, etc.) reveal concentrations just the reverse of what would be expected from biomagnification. Different species which have similar diets and collected in the same nets show widely different concentrations of DDT and PCB. We believe that the levels found within an organism are a result of the partitioning of the DDT and PCB between the water and the body lipids. The lipids of each species will have a definite capacity for these chemicals regardless of its position in the food chain. Air breathing animals must depend on excretion and metabolism, which are apparently less efficient than equilibration with water, and accumulate these pollutants. This discovery would have been extremely difficult to make in coastal waters where the organisms are subjected to sporadic and high doses of pollutants from spills, floods and dumping operations. The open-ocean is remote enough so as not to be affected by daily and local inputs.

Finally, PCB formulations must be transported to the ocean by discreet aerosol masses which do not mix with similar formulations during their atmospheric history. This phenomenon is not shared by DDT which is largely transported on atmospheric particulate matter. This must be a consequence of the different modes of injection into the atmosphere: DDT by slow evaporation from soil and PCB by distilling from incinerators, dump fires and explosions (aircraft crashes).

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We are continuing to explore these phenomena and will soon study the mechanisms of uptake and transfer of chlorinated hydrocarbons in the open-sea.

These studies are being supported by the National Science Foundation under Grant GX 28334 and by the U. S. Atomic Energy Commission under Contract AT(30-1)-3563.

John L. Hesse, Michigan Water Resources Commission, Lansing, Michigan, December 17, 1971

PCB's in Michigan Waters

The following is a brief summary of the Michigan Water Resources Commission PCB monitoring results during 1971.

Analyses of 98 water samples (30-hour composites) from 30 major tributaries to the Great Lakes indicate that PCB contamination of surface waters is widespread with 71 percent of all samples having detectable (greater than 10 ppt) concentrations. Concentrations greater than 100 ppt have been detected in seven streams, all located in the industrialized southeastern portion of the lower peninsula. The Saginaw River has consistently had the highest concentrations with as much as 2,900 ppt on one date.

Sampling of Great Lakes water intakes has shown much lower PCB concentrations than in tributary streams. Only 3 of 36 samples contained detectable concentrations with a high of 300 ppt.

Limited sampling of groundwater supplies in the southern lower peninsula has indicated that groundwater has not been significantly contaminated with PCB's. One of 4 samples contained 19 ppt while the other 3 were less than 10 ppt.

Analyses of surface water runoff from sanitary and industrial landfills in Michigan suggest that these are a minor source of environmental contamination. Five of the 9 samples collected had PCB concentrations ranging from 40 to 300 ppt while the others contained less than 10 ppt.

Discharges from municipal wastewater treatment facilities appear to be a very significant source of PCB losses to the waters of the state. Twenty-eight of 32 WWTP discharges sampled contained greater than 100 ppt PCB's. Eight had concentrations in excess of 1,000 ppt with a high of 336,000 ppt. Investigations have been initiated which should identify the major sources of input to the municipal systems with the greatest contamination.

Discharges from a cross-section of industries throughout Michigan were sampled for PCB concentrations. PCB losses from industries is apparently very widespread. Thirty of 58 industrial discharges tested had PCB concentrations greater than 100 ppt. Eleven industries had discharges containing PCB's in excess of 1,000 ppt.

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Because the Saginaw River was identified as having the highest PCB concentrations of any major tributary to the Great Lakes within Michigan, an intensive survey effort was conducted to isolate the major sources of PCB contamination in that basin. Water samples collected at key locations ranging from each of the four major tributaries of the Saginaw down to the mouth of the river indicated that the primary increase in PCB concentrations occurs in a short zone in the immediate vicinity of Bay City and Essexville. More intensive sampling in that zone revealed two major sources of PCB losses to the river. Actions were taken toward elimination of these sources of PCB contamination.

James J. Lichtenberg, Group Leader Pesticides Identification, Environmental Protection Agency, National Environmental Research Center, Cincinnati, Ohio 45268

In anticipation of a possible probe into the historical occurrence of PCB's in surface waters, stored carbon adsorption sample extracts (CCE) from three selected sites were analyzed. The samples were cleaned up and separated on a microcolumn of Davison 950 silica gel according to the procedure of Leoni [Journal of Chromatography, 62, 63 (1971)] prior to gas chromatographic determination. The results show an estimated 1 to 2 µg/l of PCB's in one sample. The chromatogram suggested a probable mixture of Aroclors 1242, 1248, and 1254. A trace of what appeared to be Aroclor 1232 was noted in another sample. The third sample showed no Aroclor characteristics. These positive occurrences are significant in light of the fact that they occurred in samples collected in mid-1966.

Effective procedures for clean-up and separation of the Aroclors from pesticides and other interfering materials are required for adequate analysis of CCE. Of the published procedures that we have tested, the Leoni procedure has proven the most useful for this purpose.

Ronald Parejko, Department of Biology, Northern Michigan University, Marquette, Michigan 49855

At Northern Michigan University, Marquette, Michigan, I am establishing a chlorinated hydrocarbon pesticides and PCB analysis laboratory. This laboratory will have its center of interest, at least initially, focused upon the quantification of PCB'a in lake trout taken from Lake Superior.

The lab is currently in the primary stage of setting-up and as yet no data has been accumulated. Funding for the installation of the laboratory has been provided by the Office of Water Resources Research, U. S. Department of the Interior.

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TOXICITY AND OTHER PHYSIOLOGICAL EFFECTS

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Pete Benville, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Toxicity of Aroclors® to Aquatic Organisms

Fish: Tests to determine the chronic toxicity of Aroclors® to fish are continuing. Aroclors® 1242, 1248, and 1254 were about equally toxic to yellow perch (30-day TL50 = 72 to 92 µg/l), while Aroclor® 1260 was less than half as toxic.

Herman O. Sanders, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Invertebrates

Preliminary static bioassays with individual PCB isomers were begun using scud (Gammarus pseudolimnaeus). These organisms were selected as the amounts of pure isomers available did not permit bioassays using larger organisms.

No. Cl	PCB Component	TL50 (mg/l) 24 hr	96 hr
2	2,4'-dichlorobiphenyl	0.15	0.12
2	4,4'-dichlorobiphenyl	0.16	0.10
3	2,3,4'-trichlorobiphenyl	0.10	0.07
5	2,4,5,2',5'-pentachlorobiphenyl	0.43	0.21
6	2,4,6,2',4',6'-hexachlorobiphenyl	0.20	0.15
4	mixture of 3, decachlorobiphenyls	0.30	0.11

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Foster L. Mayer, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Route #1, Columbia, Missouri 65201

Chronic Toxicology of PCBs to Fish

TL50 values (5-30 days) from continuous flow exposures of Aroclors® 1242, 1248, 1254, and 1260 to three species of fish have been determined. Comparative toxicity data for DDT was also determined.

Compound	Species	TL50[1] (ug/l) for 5-30 days					
		5	10	15	20	25	30
DDT	RBT[2]	4.22	1.39	0.51	0.41	0.22	-
	BG[3]	1.18	1.43	1.63	1.27	0.59	0.35
	CCF[4]	>1.0	1.54	2.33	1.32	0.87	0.57
1242	RBT	67	48	18	10	12	-
	BG	125	125	164	125	120	84
	CCF	>100	125	219	150	132	87
1248	RBT	54	38	16	6.4	3.4	-
	BG	136	115	111	106	100	78
	CCF	>100	121	121	115	104	75
1254	RBT	142	160	64	39	27	-
	BG	>200	>200	303	260	239	177
	CCF	>200	303	286	293	181	139
1260	RBT	232	326	143	78	49	51
	BG	>400	>400	>400	>400	>400	>400
	CCF	>400	535	482	512	465	433

[1] temperature, 17°C

[2] Rainbow trout

[3] Bluegills

[4] Channel Catfish

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These data compare favorably with our previously determined TL50 values at a comparable exposure period. However, these TL50 values are somewhat higher than the previous values. Dietary protein levels were increased during the two test periods and may have decreased the PCB toxicity during this extended exposure. Toxicity appears to be inveresely related to chlorine content and at 30 days had not become independent of exposure time.

George Yip and James D. Link, U. S. Food and Drug Administration, Washington, D.C. 20204

PCB Metabolism

Aroclor 1254 residues were determined on organs of rats which had been given single daily doses of 300 mg/kg Aroclor 1254 for 5 days or 15 mg/kg for 14 days. Livers, kidneys, fat, blood, and urine were analyzed by electron-capture GC. Except for differences in amounts found, all samples had similar type chromatograms showing major losses of early eluters and more significantly, a complete loss of one major peak. Using a standard supplied by Dr. R. G. Webb, this peak has been tentatively identified as 2,3,6,3',4'-pentachlorobiphenyl by GLC and IR. Quantities of this component will be obtained for further in vivo and in vitro studies to determine its fate in biological systems.

S. Bailey, P. J. Bunyan, and J. M. J. Page, Ministry of Agriculture, Fisheries and Food, Pest Infestation Control Laboratory, Hook Rise South, Tolworth, Surbiton, Surrey

Investigations are now under way into the effects produced by individual polychlorinated biphenyl isomers following low level chronic feeding to Japanese quail. Various parameters of microsomal activity are being measured and an attempt will be made to correlate these with tissue residue levels. Excreta are being monitored for metabolic transformation products. It is hoped that an overall picture will emerge relating these measurements to the extent and pattern of chlorination.

L. S. Merkens and W. B. Kinter, Departments of Pharmacology and Physiology, Upstate Medical Center, SUNY, Syracuse, New York 13210

Two types of studies were done with DDT and Aroclor 1221 (provided by Monsanto Co.) in marine adapted killifish (Fundulus heteroclitus). Acute toxicity and changes in serum osmolality were measured. Both tests were done using a simple static system. In each experiment 10 fish, each 5 g, were maintained in 2 liters of sea water at 15 C with 0.1% ethanol. The experimental compounds were first dissolved in 2 ml of ethanol before added to the sea water. Aroclor 1221 at 75 ppm (theoretical concentration in the sea water) was less toxic than DDT by a factor of about 100. It

demonstrated roughly equivalent lethality (24 hours) as 0.25 ppm DDT. Exposure of these same levels (75 ppm Aroclor 1221 and 0.25 ppm DDT) for 6 hours increased serum milliosmolality (determined by freezing point depression) from 365 ± 3 (SE) (6 samples each from 3-5 ethanol control fish) to 396 ± 7 (9) and 389 ± 9 (8), respectively ($p<0.05$). A more complete description of methods and data will appear in Environmental Health Perspectives.

D. R. Nimmo, D. J. Hansen, N. R. Cooley, and J. A. Couch, Environmental Protection Agency, Gulf Breeze Laboratory, Sabine Island, Gulf Breeze, Florida 32561

The Environmental Protection Agency Laboratory at Gulf Breeze, Florida, an associate laboratory of the National Environmental Research Center, Corvallis, Oregon, is continuing to study the effects of polychlorinated biphenyls on estuarine organisms. We are now completing many experiments reported in the last newsletter and beginning new studies.

Studies have been completed on the effect of Aroclor 1248 on growth of populations of the ciliate protozoan, Tetrahymena pyriformis W, employing as criteria of effect the alteration of growth rate during exponential growth and of population density at 96 hours, when control populations are maximal. The ability of this ciliate to accumulate PCB from the medium and concentrate it was investigated. Similar studies of Aroclor 1260 are nearing completion.

The relationship of salinity of test water and toxicity of Aroclor 1254 to longnose killifish, Fundulus similis, is being investigated. As fish die, they are dissected and samples of individual tissues as well as composite samples of whole fish are analyzed for Aroclor 1254. After half of the fish die, the survivors are also dissected forresidue analysis. Results are incomplete.

We have studied the effects of 0.1, 1.0, and 10.0 ppb of Aroclor 1254 on setting and growth of animals in sand-filled aquaria during a four-month exposure. Most animals have been identified and enumerated. Species diversity indices have been computed, but statistical analysis of the data is incomplete.

The ability of animals to avoid Aroclor is being tested by allowing them to move into water containing Aroclor or into Aroclor-free-water. Experiments using pink shrimp, Penaeus duorarum, and mosquito fish, Gambusia affinis, have been completed. Use of other crustaceans and fishes is being considered.

A crystalloidal material has been found in the nuclei of hepatopancreatic cells of shrimp exposed to 5 ppb Aroclor 1254 for long periods of time. Also, liver necrosis has been found in spot, Leiostomus xanthurus, similarly exposed to this PCB.

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We have found the tolerance limit of 50% of a population of estuarine grass shrimp, Palaemonetes pugio, to be between 4.0 and 12.5 ppb Aroclor 1254. In this 16-day experiment, we found correlations between concentration of this material in the water and mortality. Preliminary tests showed that Palaemonetes kadiakensis, the fresh-water grass shrimp found in this area, was much less susceptible to this PCB than P. pugio, the local estuarine grass shrimp.

Dave Hansen reported that a newly identified source of contamination of Aroclor 1254 in bioassay water to be Teel submerisble pumps, No. 1P597, that were used to supply water to a constant-head box. At times, concentrations in the water exceeded 0.2 ppb (µg/l). Residue analyses performed on various portions of the pump revealed that the grease used to lubricate the shaft was about 0.37 percent polychlorinated biphenyl.

Therefore, this pump should be used only if the grease is replaced or in experiments in which presence of this contaminant would not alter results.

Alan V. Nebeker, Research Aquatic Biologist, Western Fish Toxicology Laboratory, NWQL, U. S. Environmental Protection Agency, 200 S.W. 35th Street, Corvallis, Oregon 97330

Toxicity of Polychlorinated Biphenyls (PCB's) to Fish and Other Aquatic Life

Final Report

Summary of Findings (Abstract)

Aroclor 1248 was the most toxic of the eight Aroclors tested to Daphnia magna in static tests, with 16 percent impairment of reproduction occurring at 16 µg/liter. Aroclor 1254 was most toxic under continuous-flow conditions with 16 percent reproductive impairment at 0.48 µg/liter. The Aroclors were much more toxic under continuous-flow than static conditions, with 16 percent impairment of reproduction by Aroclor 1248 occurring at 1.0 µg/liter.

Calculated 96-hour LC50 values for newly-hatched fathead minnows were 7.7 µg/l for Aroclor 1254 and 15 µg/l for 1242. Three-month-old fatheads had a 96-hour LC50 of ca. 300 µg/l for 1242. Reproduction occurred at and below 1.8 µg/l 1254 and at and below 5.4 µg/l 1242. Polychlorinated biphenyls were acutely toxic but exhibited much greater chronic toxicity at very low levels, due to their cumulative nature. Newly hatched young were the most sensitive life stage. Young fathead growth was also affected above 2.2 µg/l 1248 and none survived above 5.1 µg/l after 30 days.

Young flagfish, Jordanella floridae, did not survive above 5.1 µg/l 1248 and did not grow well above 2.2 µg/l.

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Ninety-six hour LC50 values for Aroclor 1242 and 1248 with Gammarus pseudolimnaeus were 73 and 29 µg/liter. Survival after 30 days was 53 percent at 8.7 µg/l 1242 and 52 percent at 5.1 µg/l 1248. Threshold values were unobtainable as deaths continued to occur until end of test, indicating the cumulative chronic toxicity of PCB's. Good reproduction and survival of young occurred at 2.8 µg/l 1242 and 2.2 µg/l 1248.

Abundant adult emergence of the midge Tanytarsus dissimilis did not occur above 5 µg/l 1248 or 3.5 µg/l 1254. The calculated 3-week LC50 (50% reduction based on control as 100%) for Aroclor 1254 was .65 µg/l for larvae and .45 µg/l for pupae.

Application factors of 0.10 for 1242 and 0.15 for 1248 were calculated for the PCB's and Gammarus. Application factors of 0.2 for 1254 and 0.16 for 1242 were calculated for fathead minnows using newly hatched fish to obtain the 96-hour LC50.

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Toxicities of PCBs to Fish and Environmental Residues

by David L. Stalling and Foster Lee Mayer, Jr.*

Polychlorinated biphenyls (PCBs) have been found in fish and wildlife from many parts of the world at levels which may adversely affect aquatic organisms and interfere with pesticide residue analyses (1, 2, 3, 4). The environmental occurrence, uses, and present toxicological aspects of PCBs were recently reviewed by Peakall and Lincer (5), Gustaffson (6), and Risebrough (7). PCBs have gas chromatographic characteristics similar to many organochlorine insecticides. Jensen (8) first identified unknown gas chromatographic peaks as PCBs in extracts of pike and an eagle which were analyzed for organochlorine insecticides.

The Monsanto Company is the sole manufacturer of PCBs in the United States (6) and markets eight formulations of chlorinated biphenyls under the trademarks Aroclor 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268. The latter two digits designate the percent chlorine of each formulation. Aroclor 1248 and 1254 are the materials produced in greatest quantities and are used as a dielectric fluid in capacitors and in closed-system heat exchangers (9). Aroclor 1242 is used as a hydraulic fluid, and Aroclor 1260, as a plasticizer. Chlorinated terphenyls are marketed under the trademark Aroclor 5442 and 5460. A mixture of bi- and terphenyls is designated Aroclor 4465. The Monsanto Company has restricted the sale of PCBs for uses in which disposal of the end products cannot be controlled, such as the use of PCBs as plasticizers (6).

* Chemist and Fishery Biologist, respectively, Fish-Pesticide Research Laboratory, Bureau of Sport Fisheries and Wildlife, Columbia, Missouri 65201.

Chemical Composition and Analysis

PCBs occur in the aquatic environment as mixtures of chlorinated biphenyl isomers (10, 11). The biphenyl structure may be substituted with one to ten chlorine atoms, and over 200 compounds are possible (6). The isomer composition and chromatographic characteristics of each formulation have been described by Stalling and Huckins (12) and Bagley (13). The PCBs can cause serious interference in gas chromatographic (GC) determination of chlorinated insecticides (4). Analysis of PCBs is best accomplished by GC after separation of PCBs and pesticides during sample cleanup using silicic acid column cleanup (14). No standardized GLC method has been proposed for the analysis of mixtures of PCB formulations in environmental samples. The solubility of these formulations in water have not been precisely determined but is reported in the range of 100–1000 μg/l (9). Considerable research remains to be done on the structure of the isomers of each technical material and how environmental residues of various Aroclors are to be reported.

Environmental PCB Residues

Analyses of 40 fish (cross-check sample) from the 1970 National Pesticide Monitoring Program were made using sample preparation techniques which separated PCBs from pesticides and other industrial residues (Table 1). These analyses employed GC and gas chromatography-mass spectrometry (GC-MS) for confirmation The samples were prepared using gel permeation chromatography (23), and PCBs were separated from the pesticides with silicic acid (14) Only

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Table I. Composition of PCB residues in selected fish samples from the 1970 National Pesticide Residue Monitoring Program.

River	Location	Species	PCB residue as Aroclor type (μg/g whole body)				
			1232	1248	1254	1260	total
Ohio	Cincinnati, O.	Carp	10.2	75	42	6.0	133
Ohio	Cincinnati, O.	White crappie	15.9	17	27	5 6	66
Ohio	Marietta, O.	Channel catfish	38	23	11	4.9	77
Ohio	Marietta, O.	Channel catfish	15.0	5 2	12.6	4 6	38
Yazoo	Redwood, Miss.	Smallmouth buffalo	72	—	1 4	—	7.5
Hudson	Poughkeepsie, N.Y.	Goldfish	8.6	173	32	—	213
Allegheny	Natrona, Pa.	Walleye	—	5.2	25	4 6	35
Delaware	Camden, N.J.	White perch	—	8 0	6.8	3 9	19
Cape Fear	Elizabeth Town, N.C.	Gizzard shad	18 9	—	2.6	1.1	23
Lake Ontario	Port Ontario, N.Y.	White perch	12 9	—	4.6	1.2	19
Mississippi	Memphis, Tenn.	Drum	11 2	—	4 5	3.4	19
Merrimac	Lowell, Mass.		13.8	75	6.1	3.2	98

one of the forty samples contained less than 1 μg/g PCB residue. The composition of PCB residues in a lake trout and coho salmon were examined by GC-MS and found to be a mixture of Aroclor 1248 and 1254 (Figure 1), and residues of PCBs were in excess of the "DDT" residue levels (Table 2).

Widespread pollution of the major waterways has occurred, and appreciable PCB residues exist in fish. In view of the PCB bioconcentration in fish, PCB levels as high as 5 μg/l may exist in the waters of the Hudson River. These levels are certain to have adverse effects on aquatic organisms

Toxicity and Bioaccumulation

Fish

Chronic and acute toxicity studies on aquatic organisms are quite limited Due to the low solubility of PCBs, tests to obtain 96-hr LC_{50} values do not adequately reflect their toxicities to fish (Table 3). For Aroclor 1221-1268, 96-hr LC_{50} values ranged from 1,170 to 50,000 μg/l for cutthroat trout (15). The acute oral toxicity of Aroclors 1242, 1248, 1254, and 1260 was greater than 1500 mg/kg in rainbow trout.

Intermittent-flow bioassays of Aroclor 1242, 1248, and 1254 to bluegills resulted in 15-day

Table 2. Pesticide and PCB Residues in a Coho Salmon and a Lake Trout from Lake Michigan.

	Residues in μg/g whole fish									
	Dieldrin	p,p′ isomers			o,p′ isomers		Total "DDT"	PCB (Aroclor)[1]		
		DDE	DDD	DDT	DDE	DDT		1248	1254	Total
Lake trout[2]	0.10	13.0	1.8	5.2	0 9	1.8	22 7	12 0	16 0	28 0
Coho salmon[3]	0 10	4.3	0.8	2.3	0 4	0 5	8.3	6.7	6 7	13.4

[1] Calculated from ratios of characteristic 1248 and 1254 peaks.

[2] Lake trout (J3 L)—taken 10-14-69. 690 mm, 3590 gm, immature M. Taken in 2 5 fathoms in Little Bay de Noc Michigan

[3] Coho salmon (7Cs)—taken 9-3-69. 675 mm, 5718 gm F. Taken in 17-19 fathoms 4 miles NW of Manistee, Michigan

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LC_{50} values of 54, 76 and 204 µg/1, respectively (Table 4). Exposures to channel catfish gave 15-day LC_{50} values of 107, 127, and 741 µg/1 for the same Aroclors. All LC_{50} values decreased significantly with longer exposures. The 20-day LC_{50} values for Aroclors 1248, 1254, and 1260 were 10, 135, and 245 µg/1 for bluegills and 300, and 296 µg/1 for Aroclors 1254 and 1260 with channel catfish at 20°C. The toxicity of Aroclor 1248 to bluegills and channel catfish increased two-fold at 27°C. The toxicity of the PCBs to fish is inversely related to percent chlorine (higher Aroclor number).

In chronic tests to determine the effects of Aroclor 1242 and 1254 on fathead minnow reproduction, all fish exposed to greater than 8.3 µg/1

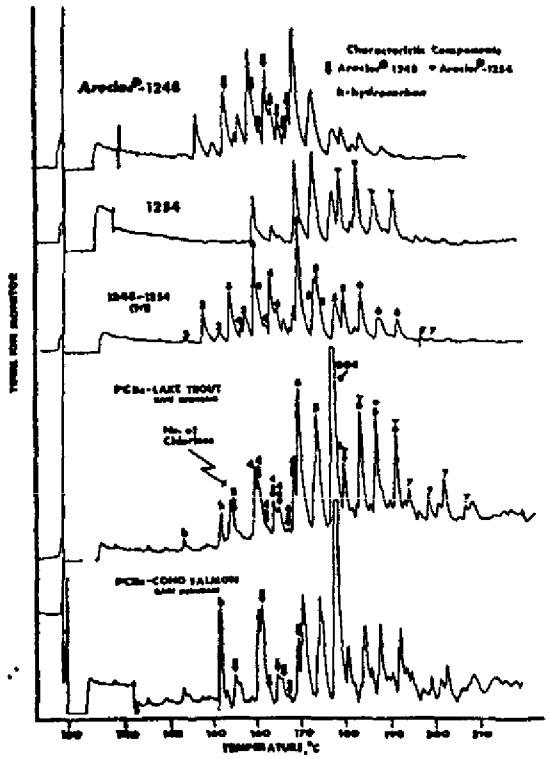

FIGURE 1. Composition of PCB residues in fish. Arrows and triangles designate the most characteristic peaks of the Aroclor. All numbered peaks in chromatograms of fish extracts are PCBs. These numbers designate the number of chlorine atoms in the compounds as determined by mass spectrometry. Temperature program rate, 4°C/min, He 9 psig, 50'×0.020" i.d. SE-30 SCOT column, PE-2708 GC-MS.

TABLE 3. Acute toxicity[1] of several Aroclors to selected fishes.

Aroclor	Species	Temperature (C)	96-hr LC_{50} (µg/1)
1221	Cutthroat trout	8.9[2]	1,170
1232	Cutthroat trout	8.9	2,500
1242	Cutthroat trout	8.9	5,430
1248	Cutthroat trout	8.9	5,750
1254	Cutthroat trout	8.9	42,500
1260	Cutthroat trout	8.9	60,900
1262	Cutthroat trout	8.9	50,000
1268	Cutthroat trout	8.9	50,000
1248	Channel catfish	18.3[3]	0,000
1254	Channel catfish	18.3	12,000
1248	Bluegills	18.3[3]	278
1254	Bluegills	18.3	2,740

[1] Static bioassay.
[2] Alkalinity, 159 ppm; pH 7.6
[3] Alkalinity, 35 ppm; pH 7.1.

of each Aroclor died. Reproduction occurred at and below 8.3 µg/1 Aroclor 1242 and at and below 2.8 µg/1 Aroclor 1254 (17).

Accumulation of Aroclor 1248 and 1254 by bluegills chronically exposed to 2–10 µg/1 was from 26,300 to 71,400 times the exposure levels for both PCBs. Concentration factors were not strongly dependent upon water exposure levels, but a direct correlation of water concentrations and whole-body residues may exist. No major modification of the PCB isomer ratios was observed in the tissue residues, and no new components were identified (18).

Coho salmon fed Aroclor 1254 for 240 days at concentrations of 14.5 to 14,500 µg/kg body weight per day (dietary concentrations of 0.4 to 580 µg/g) accumulated whole-body residues which were 0.9 to 0.5 times the exposure levels (300 mg/kg highest residue value). Growth rates were not affected; however, all fish exposed to the highest treatment died after 240 days' exposure, and thyroid activity was stimulated in all but the lowest treated group (19)

Direct water exposures appear to represent a greater hazard to fish than dietary exposures, although both contribute to tissue residues However, in the environment the kinetics of residue uptake from dietary sources could be more important, since PCBs have a high affinity for

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Table 4. Intermittent-flow bioassays of Aroclor against three fishes.[1]

Aroclor	Species	LC_{50} (μg/1)					
		5 days	10 days	15 days	20 days	25 days	30 days
1254	Rainbow trout	156	8	—	—	—	—
1260		—	240	94	21	—	—
DDT		2.26	0.87	0.26	—	—	—
1242	Bluegills	154	72	54	—	—	—
1248		307	160	76	10	—	—
1254		—	443	204	135	54	—
1260		—	—	—	245	212	151
1242	Channel catfish	—	174	107	—	—	—
1248		—	225	127	—	—	—
1254		—	—	741	300	113	—
1260		—	—	—	296	166	137
1248[2]	Bluegills	137	76	—	—	—	—
1248[2]	Channel catfish	—	94	57	—	—	—

[1] Temperature, 20°C; alkalinity, 260; pH, 7.4.
[2] Temperature, 27°C.

sediments and PCBs can readily enter food chains (20, 21).

Aquatic Invertebrates

Daphnia magna: Aroclor 1248 is the most toxic of the Aroclor series to *Daphnia*, and preliminary studies indicate that levels above 5 μg/1 are not safe for reproduction (17). After 48 hours' exposure to 300 μg/1, *Daphnia* concentrated Aroclor 1254 by 48,000 times (22).

Gammarus pseudolimnaeus: The level of Aroclor 1248 that did not affect reproduction was comparable to that for daphnids, ca 5 μg/1 (17). The 96-hr LC_{50} values for Aroclor 1248 and 1254 were 52 μg/1 and 2,400 μg/1, respectively (Table 5). Scud were more sensitive to Aroclor 1242 (96-hr LC_{50} = 10 μg/1). After exposing another species of scud (*Gammarus fasciatus*) to 1.6 μg/1 Aroclor 1254 for 14 days, the PCBs were concentrated 27,000 times the exposure level. No further increase in PCB residue resulted after an additional 21 days of exposure (22).

Orconectes nais: Crayfish were more susceptible to Aroclor 1242 (7-day LC_{50} = 30 μg/1) than they were to Aroclor 1254 (7 day LC_{50} = 80 μg/1). PCB residues in crayfish did not reach equilibrium after a 28-day exposure to Aroclor 1254, and the uptake was linear during this period (22).

Palaemonetes kadiakensis: Glass shrimp were very sensitive to Aroclor 1254, having a 7-day LC_{50} value of 3 μg/1 compared to a 5-day LC_{50} of 1 μg/1 for DDT (22).

Marine Organisms

Immature pink shrimp were extremely sensitive to Aroclor 1254 exposures. Fifty-one percent of the shrimp died within 15 days when continuously exposed to 0.94 μg/1 Aroclor 1254 (25). Mortalities in two estuarine fishes (*Lagodon rhomboides* and *Leiostomus xanthurus*) exposed for 14 to 45 days to Aroclor 1254 were observed at 5 μg/1. Fish mortalities were not observed at 1 μg/1 during the same exposure time (24). PCB concentration factors in these fish were similar to uptake by freshwater fish ($1–5 \times 10^4$ times the exposure levels).

Correlation of Reproduction and PCB Residues

In preliminary investigations, Jensen et al (25) reported a possible relationship between PCB residues in salmon eggs and egg mortality in Sweden. On a fat basis, PCB residues in eggs ranged from 7.7 to 34 μg/g and mortality ranged from 16 to 100 percent. A regression analysis of their data resulted in a coefficient of correlation of 0.85, and the correlation was significant at $P < .001$.

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Table 5. Aroclor and DDT toxicity to invertebrates.

Compound	Organism[1]	Bioassay type[2]	Exposure (days)	LC_{50} (μg/l)
Aroclor 1242	Crayfish	static	7	30
Aroclor 1254	Crayfish	static	7	100
Aroclor 1254	Crayfish	continuous-flow	7	80
DDT	Crayfish	static	4	100
Aroclor 1242	Scud	continuous-flow	4	10
Aroclor 1242	Scud	continuous-flow	10	5.0
Aroclor 1248	Scud	static	4	52
Aroclor 1254	Scud	static	4	2,400
DDT	Scud	static	4	3.2
DDT	Scud	continuous-flow	5	0.6
Aroclor 1254	Glass shrimp	continuous-flow	7	3.0
DDT	Glass shrimp	static	5	1.0
DDT	Glass shrimp	continuous-flow	5	1.3
Aroclor 1242	Dragonfly	static	7	800
Aroclor 1254	Dragonfly	static	7	1,000
Aroclor 1242	Damselfly	continuous-flow	4	400
Aroclor 1254	Damselfly	continuous-flow	4	200
DDT	Damselfly	static	4	56

[1] Crayfish (*Orconectes nais*), Scud (*Gammarus fasciatus*), Glass shrimp (*Palaemonetes kadiakensis*), Dragonfly (*Macromia sp.*), Damselfly (*Ischnura verticalis*).

[2] Temperature, 15.6°C; alkalinity, 35 ppm; pH, 7.1.

Summary

The occurrence of high levels of PCB residues in aquatic organisms (fish and invertebrates) as complex mixtures of Aroclor formulations has been determined by GC analysis and confirmed by GC-MS. Suitable methods for sample cleanup include gel permeation and silicic acid column chromatography. Agreement on methods for reporting PCB residues is lacking.

The highest concentrations of PCB residues in freshwater fish occur in rivers which are associated with industrialized areas, i.e. Hudson River (ca 200 μg/g whole body residue) and the Ohio and Allegheny Rivers (ca 100 μg/g). Residue levels associated with these rivers approach PCB residues which were associated with fish mortalities in chronic continuous-flow exposures (500–600 μg/g).

Concentration of PCBs by fish is greater than 40,000 times the exposure levels. Similar concentration factors have been observed for invertebrates. Adverse effects on reproduction of aquatic organisms may occur at PCB concentrations of 5 μg/l or less. Further studies are required to determine more definitely the exposure levels which do not adversely effect aquatic organisms.

The problem of PCB residues in industralized areas can be greatly improved by close monitoring of effluents and by identification of those PCB uses which contribute to effluent contamination. Elimination of widespread PCB residues in fish at concentrations of 1–20 μg/g will require greater efforts, such as the control of PCBs from sewage treatment plants and other as yet unidentified sources of PCBs into the aquatic environment.

Related Materials

Chlorinated napthalenes are recommended for uses similar to those of the PCBs (27). These materials are recovered by the same analytical procedures as the PCBs (28) and may be recognized by their gas chromatographic elution patterns if PCBs are not present. The materials are designated Halowax 1000, 1031, 1099, 1013, 1014, and 1051 and are listed in an order of increasing chlorine content (27). Aquatic toxicity information is not presently available, nor has

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the persistence and fate of these materials been examined in the aquatic environment.

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IV-12

Effects of Aroclor 1242* (a Polychlorinated Biphenyl) and DDT on Cultures of an Alga, Protozoan, Daphnid, Ostracod, and Guppy[1]

by JANET R. MORGAN
College of Fisheries, University of Washington, Seattle, Wash

The polychlorinated biphenyls (PCB's) are a group of chemicals having physical and chemical properties sufficiently similar to those of DDT to warrant investigation (10). The Monsanto Company, the sole producer of PCB's in the United States, markets them under the trade name of Aroclor. Aroclor 1242 is a mixture of PCB compounds containing approximately 42% chlorine by weight. Diverse in their commercial applications, but most commonly employed as plasticizers, PCB's have increased in usage dramatically since the forties (6). Jensen first reported finding PCB's in pike, other fish, eagle feathers and human hair from Sweden in 1966 (1). Subsequently, scientists from around the world began analyzing wildlife tissues for the presence of PCB's. Chlorinated biphenyls were detected in fish, mussels, and birds from the Rhine River and the coast of the Netherlands (7), in sparrowhawks, kestrels and kittiwakes from Great Britain (4) and peregrine falcons from the United States (5). Cultures of the marine diatom Cylindrotheca closterium have been shown capable of absorbing Aroclor 1242 from sea water containing 0.1 ppm and concentrating it 900 to 1000 times (6). This concentration caused, however, a marked reduction in growth, chlorophyll index, and RNA synthesis of the marine diatom, but no noticeable effect on DNA synthesis.

The aim of the present study was to investigate some of the effects of Aroclor 1242 and DDT on a variety of aquatic organisms. For a more detailed report, please refer to the Master's thesis (9).

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[1] Fisheries contribution No. 350

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Methods

Five different organisms were used in the present investigation: the alga Chlamydomonas reinhardtii, the ciliate protozoan Tetrahymena vorax, the cladoceran Daphnia pulex, the ostracod Cypridopsis vidua, and the guppy Poecilia reticulata. Media 63 (12) was the experimental medium in which the effects of the toxicants upon algae, cladocerans, and ostracods were studied. Bioassay media, modified from Fernell and Rosen (3) to contain 1/100 of the organic components and 1/10 of the organic salts, was used for the Tetrahymena studies. Coarsely-filtered pond water was used in the guppy toxicity tests. Since both DDT and PCB have a tendency to adhere to surfaces (2, 14), disposable glassware was used to prevent residues of the chlorinated hydrocarbons from affecting the results of subsequent experiments. The culture vessels were either quart or half-gallon mason jars with half of a disposable plastic Petri plate for a lid or disposable glass test tubes for the protozoan studies. At the start of an experiment all culture vessels and media were autoclaved at 250° C. for 25 minutes to insure sterility (except the guppy experiments in which pond water was used). One hundred percent p,p'-DDT, designated as the "ESA pesticide reference standard" was used in all DDT experiments and the Aroclor 1242 used was obtained directly from Monsanto in St. Louis, Missouri. The test chemical was generally added to the growth media prior to the introduction of the organisms. In order to distribute the test chemical throughout the media, and to ease in making dilutions, acetone was used as a vehicle for DDT, and 95% ethyl alcohol was used for the PCB. A concentration of 0.2 ml solvent/liter media was used, which did not appear to adversely affect the tested organisms as shown by preliminary experiments and usually demonstrated by the solvent controls. Once algal density was determined, a known volume of inoculum was added to each culture so that the initial concentration in the culture vessel would be 0.1 x 10^5 cells/ml. Adult Daphnia, ostracods, and fish were removed from their stock cultures, randomized, and counted before placing them in the test cultures. Experimental cultures containing algae and/or Daphnia were grown at 28.5° C. ± 1°C. under a bank of fluorescent lights which delivered 900 foot-candles

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(as measured by a GE model 213 lightmeter with a blue filter). Tetrahymena vorax cultures, grown in test tubes, were placed on a roller tube apparatus which rotated the tubes at 1/5 rpm. Protozoan, ostracod, and guppy bioassays were conducted under the conditions of 23.5° C±1°C. and 75 footcandles of light.

During the course of an experiment, population counts of the organisms present in each culture vessel were recorded. The Chlamydomonas cells were killed and

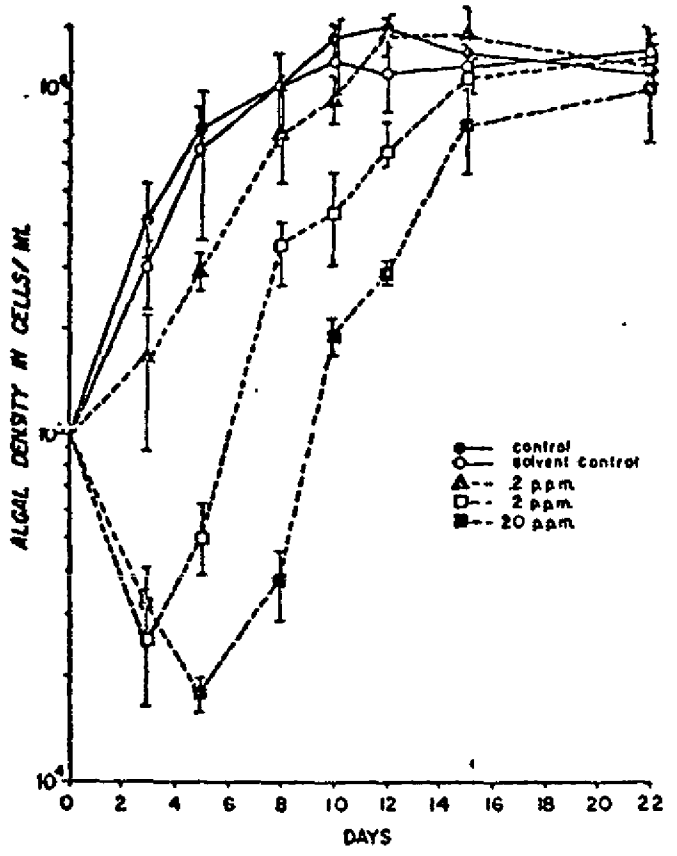

Fig. 1. Effect of the addition of 0.2, 2, and 20 ppm Aroclor 1242 on the growth of Chlamydomonas reinhardtii. Each point represents the mean of two observations whose individual values are indicated at the range.

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stained by adding a drop of I_2-KI solution, then counted using a haemacytometer at 100X. The Tetrahymena were stained in the same manner, then ten 0.01 ml drops of the culture were distributed on a Falcon counting plate and the cells in each counted at 50X. Carbon-14 uptake by algal cells subjected to various concentrations of both DDT and PCB was determined by incubating 5 ml samples with $NaH^{14}CO_3$ and using a standard filtration-planchet method. Gas chromatography analysis was performed by Dr. James Saddler of the Fisheries Research Institute on five algal cultures--a control, a solvent control, and three cultures to which 20, 2, and 0.2 ppm Aroclor had been added. The algal pellets and supernatant samples were extracted with acetonitrile-petroleum ether; purified by elution on a Florisil column; and injected into a gas chromatograph equipped with an electron capture detector.

Observations--Discussion

Figure 1 illustrates the effect of three different doses (0.2, 2, and 20 ppm) of Aroclor 1242-alcohol on the density of Chlamydomonas over a period of 22 days. Both the control and the solvent control followed the typical parabolic growth curve with no initial lag period. There was a period of rapid growth (the log phase) followed by a leveling off of the population size as the culture approached the stationary phase. Aroclor-containing cultures did not follow this pattern, however, but took a much longer time to approach the cell density of the controls. In the Chlamydomonas cultures to which the two highest dosages of Aroclor were added there appeared to be not only a delay before exponential growth began, but an initial drop in the algal density below the original inoculum size. The severity of the response was dose related. After day 5, the growth rate of these inhibited cultures increased so that by day 22 they had cell densities similar to the controls. By day 22, the standing crop of Chlamydomonas in all of the cultures appeared to be about the same. This experiment was repeated several times with similar results, although the degree of inhibition from equal additions of PCB varied somewhat. This variation may be due to heterogeneity of the PCB globular suspensions (14), or to differences in other physical factors from experiment to experiment.

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In order to determine whether ^{14}C uptake by Chlamydomonas cells was also affected by the addition of Aroclor 1242, the experiment was repeated using five different doses of PCB (0.002, 0.02, 0.2, 2, and 20 ppm), incubating $NaH^{14}CO_3$ with samples taken from the cultures at three different points in the growth period. On day 4, the control had the greatest ^{14}C uptake and the PCB containing cultures had uptakes per cell and per ml in inverse relationship to their PCB concentration. By days 11 and 15, the ^{14}C uptake per cell were all markedly reduced and showed no dose-related response. These results were undoubtedly influenced by the varying amounts of nutrients still available. The ^{14}C uptake per ml tended to increase during the duration of the experiment, the values generally in inverse relation to the dose.

A possible reason for the apparent recovery of Chlamydomonas from the initial effects of PCB-containing media is that the PCB was degraded or otherwise made less effective during the course of the experiment. Since an experiment conducted by Risebrough (11) indicated that PCB compounds could be broken down by ultraviolet light, the possibility that the fluorescent lights used in growing the algae cultures were causing degradation of the added PCB was investigated. Mason jars of Media 63 containing 2 ppm Aroclor 1242 were placed under the bank of fluorescent lights for 7, 5, 2 or 0 days prior to their inoculation with Chlamydomonas. All of the PCB-containing cultures exhibited delayed algal growth and there did not appear to be any correlation between degree of inhibition and the length of time the media was under the lights after the PCB was added. This seemed to indicate that neither degradation by light nor adsorption of PCB on the glass were significant factors in the delayed growth and subsequent recovery of the Chlamydomonas cultures. Another possibility is that the PCB may have been metabolized either by the algae or by contaminating bacteria. No difference, however, was noted in the chromatograms between contaminated and axenic cultures, nor in degree of algal growth inhibition, hence bacterial degradation of Aroclor 1242 was probably not a significant factor.

Data from the gas chromatography analysis seem to indicate that the Chlamydomonas was concentrating the PCB, for a much higher concentration was found in the

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algal pellet (which took up about 0.05 ml in volume) than in the one liter of supernatant. For example, in cultures to which 20 ppm (mg/l) PCB had been added, 0.52 mg were detected in the algae, and only 0.04 mg in the supernatant. A large percentage of PCB that was added was not recovered from either the media or the algae. This may have been lost because of adherence to the glass sides of the mason jars, sinking of small PCB globules to the bottom, vaporization, codistillation with water, metabolism by the algae, or loss in the extraction, Florisil clean-up, or injection processes.

The effect of DDT on the growth and ^{14}C uptake of Chlamydomonas cultures was studied. In the tested concentration range of 0.2 ppm to 20 ppm DDT, the algal growth curves did not differ appreciably from those of the controls. While both Wurster (13) and Menzel et al. (8) were able to demonstrate that DDT reduced ^{14}C uptake in various species of marine phytoplankton, in the present study no significant effect of DDT on ^{14}C uptake by Chlamydomonas was noted. Mensel et al. (8) did observe that Dunaliella tertiolecta, a green flagellate, did not appear to be affected by concentrations of DDT as high as 1 ppm. It is thus possible that Chlamydomonas, like Dunaliella, is relatively DDT resistant, and yet may be effected by polychlorinated biphenyls which may restrict ^{14}C uptake by a different mechanism.

The toxicity of PCB and DDT to organisms other than algae was then investigated. The growth curves of the protozoan Tetrahymena vorax grown in the presence of Aroclor 1242 (0.02 to 20 ppm) and in the presence of DDT (0.01 to 100 ppm) seem to indicate that neither chemical has any effect upon the growth rate or population size. Aroclor 1242 appeared to be fairly toxic to Daphnia pulex, however. Young Daphnia died at levels as low as 0.02 ppm. Mortality may be linked with other stresses as well, however, for when the Daphnia were supplied with algal culture, the death rate was cut to one-third. A comparison of the relative toxicity of PCB and DDT to ostracods revealed a greater mortality of Cypridopsis vidua at 20 ppm PCB than at the same concentration of DDT. At 20 ppm there were no survivors out of 20 ostracods in the Aroclor-containing cultures while there were 13 survivors in the DDT cultures. At lower concentrations, however, DDT appeared to have more of an effect than

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the PCB. The guppy Poecilia reticulata had 100% mortality at 20 and 2 ppm, but only 25% mortality at 0.2 ppm. The behavior of the fish just prior to death in PCB-containing cultures was quite different from the normal symptoms of hydrocarbon pesticide poisoning. In some cases behavior included rapid pivoting of the body with the nose pressed close to the bottom of the jar; fungus-like growths were also often associated with dying or recently-dead fish.

To explore the passage of PCB through the food chain, Daphnia in PCB-free media were supplied with algal cells that had been grown in Aroclor-containing media, centrifuged, and resuspended in PCB-free media. All of the three-day old Daphnia fed algae cells grown in media containing 20 ppm were dead by the fourth day, and there were only three survivors from among the thirty fed algae from the culture containing 2 ppm. The mortality rate was higher among control Daphnia that were starved than among those that were given PCB-free algae.

Summary

Aroclor 1242 appeared to temporarily inhibit the growth rate of Chlamydomonas as evidenced by cell counts and ^{14}C uptake, while DDT did not appear to have any effect. A greater concentration of PCB was found in the algal cells than in the media, indicating that concentration and passage of the biphenyl compounds through the food chain may take place. Neither Aroclor 1242 nor DDT in the amounts tested appeared to have any effect on the growth or cell density of Tetrahymena vorax cultures. A summary of the results of the toxicity tests on Daphnia, ostracods, and guppies is presented in Table 1. According to the table, Daphnia pulex were quite sensitive to additions of Aroclor 1242 as low as 0.02 ppm. The toxicity of PCB and DDT to the ostracod Cypridopsis vidua appeared to be approximately the same. Young guppies died in concentrations of 2 ppm Aroclor 1242. Aroclor 1242 appears to have a much lower toxicity to cladocerans and guppies than p,p'-DDT. Although the effect of PCB on algal growth appeared to be temporary, in view of its evident capability to be passed through the food chain and its selective

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TABLE 1

Summary of PCB toxicity studies. +++ = many survivors; ++ = most survivors; + few survivors; 0 = no survivors.

Test Situation	Table #	age	dose of PCB (ppm) 0.0002	0.002	0.02	0.2	2	20	observation day
Daphnia									
fed PCB-containing cells*	2	young	---	---	---	+++	+	0	4
direct addition of PCB-alcohol									
non-fed	3	young	+++	+++	+	0	0	0	4
fed	3	young	+++	+++	++	0	0	0	4
PCB-containing algal cultures	4	adult	---	---	---	---	0	0	3
	5	adult	---	+++	+++	++	0	0	2
	5	adult	---	+++	+++	0	0	0	9
Ostracods									
PCB-containing algal cultures	6	adult	---	+++	+++	+++	++	0	3
Guppies									
direct addition of PCB-alcohol	7	young	---	---	---	---	0	0	7
	8	young	---	+++	+++	++	0	0	1

* Daphnia in PCB-free media were fed washed Chlamydomonas cells grown in PCB suspensions of the indicated doses.

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toxicity to certain zooplankton, it would be unwise to attempt to predict its effect on the ecosystem without further study.

Acknowledgements

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