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 unpublished data)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.

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

MONS 073926

Exhibit 4

(4)

POLYCHLORINATED BIPHENYL RESEARCH
a Static Bioassay using Arochlor® 1016 and Related Experiments

by Ken Moorman
Bemidji State College
Bemidji, Minnesota

Intern at
National Water Quality Laboratory
Duluth, Minnesota

MONS 058494

1st wk count Nov. 14
2nd wk count Nov. 21
3rd wk count Nov. 23

9101	1			10ppb	2			25ppb	3			50ppb	4			75ppb
1st wk	5	5	5	5	5	5	5	5	5	4	5	5	5	5	5	5
2nd wk	4	3	0	4	4	5	5	5	3	4	1	5	5	5	5	5
3rd wk	12	23	0	6	17	21	22	21	0	21	0	43	0	10	18	10
	4	3	0	4	3	3	5	5	3	4	1	5	5	5	5	5
	82	72	0	78	60	65	83	90	33	55	35	79	38	67	73	65
T. Al	11				16				13				20			
T. Y	773				376				268				271			

9101	5			100ppb	6			125ppb	7			150ppb	8			200ppb
1st wk	5	5	5	5	0	0	0	0	0	0	0	0	0	0	0	0
2nd wk	5	3	3	4												
	9	2	1	0												
3rd wk	5	3	3	3												
	64	29	8	41												
T. Al	14				0				0				0			
T. Y	154				0				0				0			

17

Asclent Cont.					
1st wk	5	5	5	5	
2nd wk	2	4	4	0	
	7	0	22	0	
3rd wk	1	3	4	0	
	19	66	77	0	
T. Al	8				
T. Y	195				

9101	9			10ppb	10			25ppb	11			50ppb	12			75ppb
1st wk	5	5	4	1	5	5	4	5	5	5	4	5	5	4	4	5
2nd wk	4	2	0	0	3	0	1	0	5	5	4	4	4	0	4	4
	1	0	0	0	0	0	0	0	0	33	2	0	12	0	0	0
3rd wk	4	2	0	0	3	0	0	0	5	5	4	3	4	0	4	3
	38	11	0	0	11	0	17	0	38	66	33	2	61	0	62	2
T. Al	6				4				17				11			
T. Y	50				28				179				127			

18

Water Cont.					
1st wk	5	5	5	5	
2nd wk	4	5	5	5	
	14	28	14	29	
3rd wk	4	5	5	5	
	84	87	109	85	
T. Al	19				
T. Y	451				

9101	13			100ppb	14			125ppb	15			150ppb	16			200ppb
1st wk	5	5	5	5	0	4	3	0	0	0	0	0	0	0	0	0
2nd wk	5	4	5	5	0	2	3	0								
	8	0	0	0	0	0	0	0								
3rd wk	1	4	5	4	0	2	0	0								
	9	4	13	2	0	0	0	0								
T. Al	16				2				0				0			
T. Y	36				0				0				0			

Total Yeast

MDNS 058495

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.

MCNS 073927

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	
5/ 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 May 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	
12/28/90			ND surface (filtered)	ND May 7
"				"
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 "	
8/23/71			"	0.03 100 yd downstrm from outfall
7/ 7/71			0.13 midstream at outfall	
"			ND	
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

MONS 083394

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	"	
2/12/74	ND	0.33; ND	ND	

1/ ND = ∠0.03 ppb

2/ Tr = 0.03 - 0.05 ppb

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

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.	48 hrs.	Average Temp. °C.	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:

Date: May 27, 1969

MONS 088394

BUREAU OF COMMERCIAL FISHERIES BIOLOGICAL 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.	Salinity ‰
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		

Copy verified by:

Date: May 27, 1969

MONS 088395

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: [signature]

Date: May 27, 1969

MONS 088396

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 088397

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).

MONS 066140

Growth Responses of Estuarine Bacteria to

Mirex, Heptachlor and Polychlorinated Biphenyls

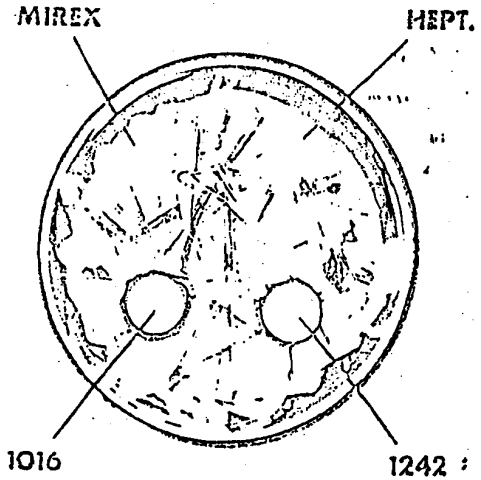
Polychlorinated biphenyls (PCBs) have been shown to be stimulating to the in vitro growth of Escherichia coli, an indicator bacterium from human intestinal flora. (Keil, et al., 1972). The lesser chlorinated biphenyls have been shown to be oxidized by a bacterial isolate from sewage, Achromobacter pCB (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.

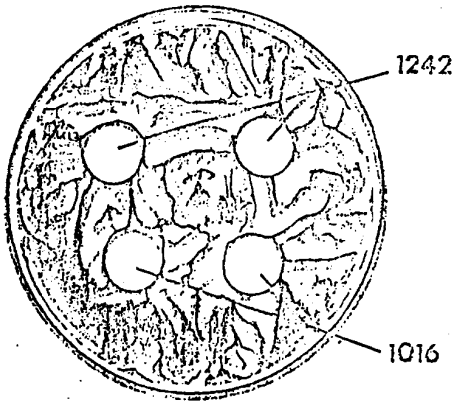
MONS 066141

GROWTH INHIBITION OF ESTUARINE BACTERIUM BY PCB's

MONS 066142



0.5 mg



0.25 mg 0.1 mg

Ⓡ 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^R 1242 (mg) 0.1	0.25	0.5	AROCLOR^R 1016 (mg) 0.1	0.25	0.5	MIREX 0.5 mg	HEPTACHLOR 0.5 mg
3	+ ROD	++	++	+++ b/	++	++	+++	-	-
21	- COCCOID	++	++	+++	++	++	+++	-	-
35	- Coccid	++	++	++	+	+++	+++	-	-
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

MONS 066144

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.lmg 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~~ produced 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

MONS 066146

Table 2.

Comparison of Physiological Activities of PCB-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.

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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MONS 066149

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.

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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 purity 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

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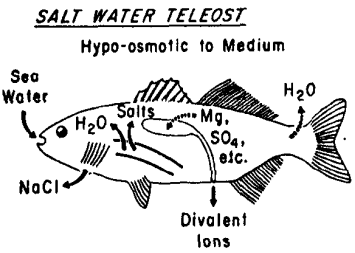

FIGURE 1. Water and salt transport in a salt water teleost. Typically the milliosmols/L are 1,000 for sea water and 350 for fish body fluids. Adapted from (1).

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 Pi/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	*mosmole*/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 (0 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	Osmolarity mosmols/liter	Na^+ mEq/liter	K^+ mEq/liter
Aroclor 1221				
75 ppm	23–32	550	208	8.1
		428	216	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.

PCB-Articles

Extra

N-5

1.40	1.61	1.81
10	11	12
6	6	6
30	30	34
27	31	31
28	33	33
28	22	39
16	12	48
16	14	86

1

EFFECT OF DIETARY POLYCHLORINATED BIPHENYLS ON GROWTH AND REPRODUCTION OF MINK

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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.

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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.

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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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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 regression equation of $\hat{Y} = 207.5 - 107.4X$ where $X = \ln(\frac{ppm}{5} + 1)$. This regression 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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N-7

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

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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, scud exposed to Aroclor® 1254 were analysed 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), Pteronarcya 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

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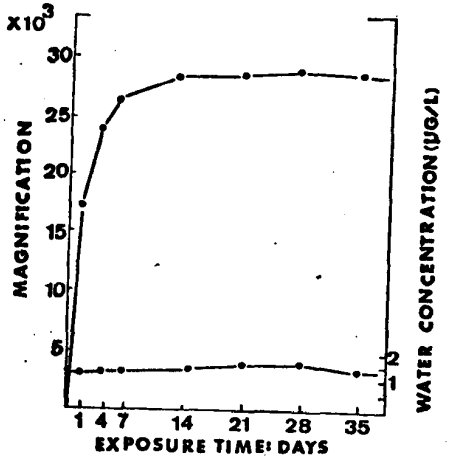

Figure 1. Biological magnification of ^{36}Cl-labeled Aroclor^{R} 1254 from water by acud Gammarus pseudolimneaus during 35 days of exposure. -·- = concentration in water; -o- = magnification in organism (total body residue/water concentration).

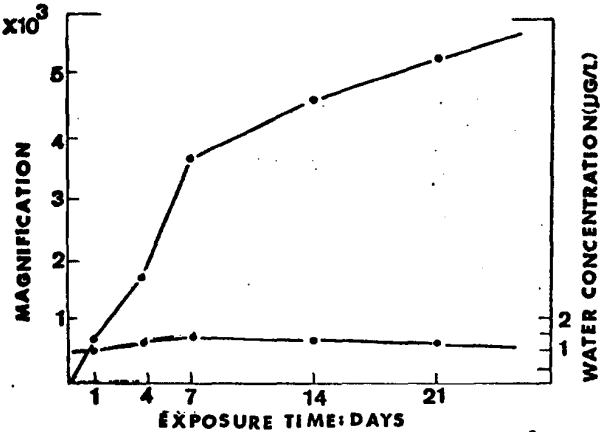

Figure 2. Biological magnification of ^{36}Cl-labeled Aroclor^{R} 1254 from water by crayfish Orconectes nais during 21 days of exposure. -·- = concentration in water; -o- = magnification in organism (total body residue/water concentration).

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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 characterised 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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IV-12

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

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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 10^6 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

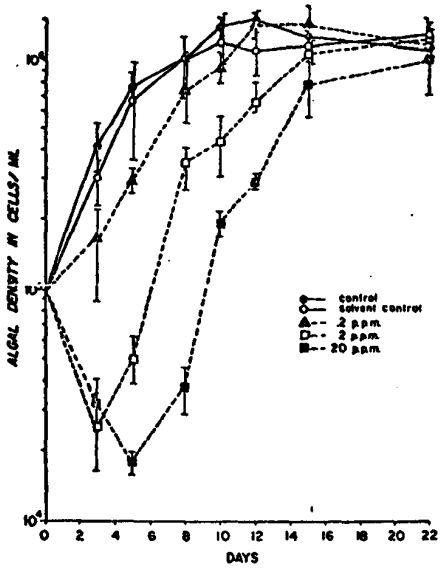

Fig. 1. Effect of the addition of 0.2, 2, and 20 ppm Aroclor 1242 on the growth of Chlamydomonas reinhardtii. Each point represents the mean of two observations whose individual values are indicated or the range.

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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	7	young	---	---	---	---	0	0	7
of PCB-alcohol	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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MONS 083409

Exhibit 6

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

by

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MONS 047000

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

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

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

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

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

MONS 047001

METHODS AND MATERIALS

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

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

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

P. pugio were exposed to Aroclor 1254-contaminated sediments in upper Escambia Bay from November 1971 to February 1972. Grass shrimp in specially-constructed cages (5) were exposed directly to the sediments. Average concentration of Aroclor 1254 in the uppermost two inches of sediment in November 1971 was 5 ppm (dry weight).

RESULTS OF LABORATORY EXPOSURES

Tests conducted in flowing water showed P. pugio was susceptible to Aroclor 1254 (Table 1). In a 7-day exposure, 50% died at 9.1 µg/l, but significant mortality did not occur at 0.62 and 0.62 µg/l. In the second series of tests lasting 4 days, 4.8 and 12.5 µg/l were toxic, but significant mortality did

TABLE 1.

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

Test Conc. (ug/l)	Days Exposed	Average Mortality (%)**	Body Conc. (mg/kg)	Concentration Factor
CONTROL	7	4(0 - 20)	0.1	———
0.17	7	8(0 - 40)	1.3	7600
0.62	7	4(0 - 20)	5.4	8700
9.1	7	60(20 - 80)***	65.0	7100
CONTROL	16	25(0 - 50)	<0.1	———
1.3	16	40(0 - 100)	18.0	14000
4.0	16	45(25 - 50)***	27.0	6700
12.2	16	55(50 - 75)***	46.0	3700

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

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

***Significant at P >0.05.

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TABLE 2.

ACCUMULATION OF AROCLOR 1254 BY
*Palaemonetes pugio**

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

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

occur in 1.3 µg/l.

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

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

RESULTS OF FIELD EXPOSURES

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

DISCUSSION

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

TABLE 3.

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

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

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TABLE 3 (Continued)

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

* Magnification factor not calculated.

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

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

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

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

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

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MONS 047011

Aroclor 1016: Toxicity to Estuarine Animals

U. S. Environmental Protection Agency

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MONS 047012

Aroclor 1016: Toxicity to Estuarine Animals

Hansen

ABSTRACT

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

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

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

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

MATERIALS AND METHODS

Test animals

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

Acute and chronic tests

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

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

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

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

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

Histopathological examination

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

Chemical analyses

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

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

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

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

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

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

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

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

RESULTS AND DISCUSSION

Acute (96-hr) exposure

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

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

3/ P. R. Parrish, unpublished data.

Chronic (42-day) exposure

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

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

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

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

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

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

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

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

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

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

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

Chronic (56-day) exposure

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

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

Aroclor 1016 was lost from the tissues after pinfish were placed

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

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

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

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

ACKNOWLEDGEMENTS

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

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MONS 047027

Aroclor 1016: Toxicity to Estuarine Animals

Hansen

TABLE 1

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

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

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

TABLE 2

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

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

TABLE 3

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

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

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

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

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

TABLE 4

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

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

1/ ND - not detectable; <0.1 µg/g.

TABLE 5

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

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

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

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LEGEND FOR FIGURES

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

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

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

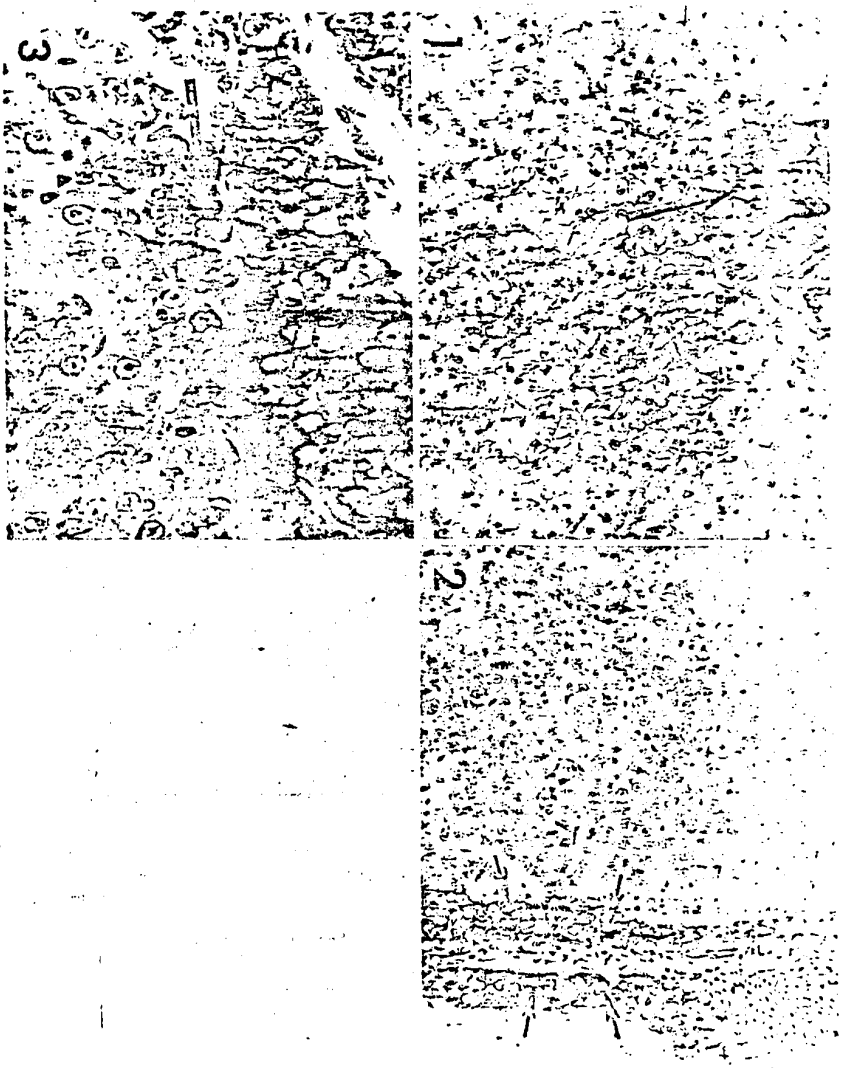

FIGURE 4

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

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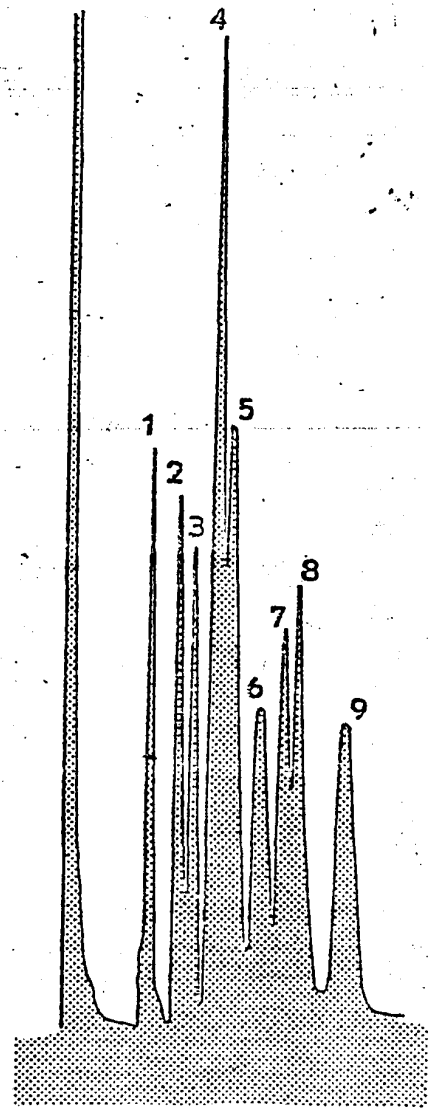

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(22)

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

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

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

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

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ABSTRACT

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

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

MDNS 047040

INTRODUCTION

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

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

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

MONS 047041

METHODS AND MATERIALS

Temperature and Salinity Studies

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

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

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

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

Aroclor 1254 Exposure

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

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

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

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

Chemical Methods

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

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

Statistical Methods

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

MONS 047045

RESULTS AND DISCUSSION

Temperature Study

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

Salinity Study

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

Aroclor 1254 Study

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

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

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

MONS 047047

ACKNOWLEDGEMENTS

We thank Gary Cook and Dennis Knight for chemical analyses of water samples from the Aroclor 1254 test and Steve Foss for preparing the illustrations.

MONS 047048

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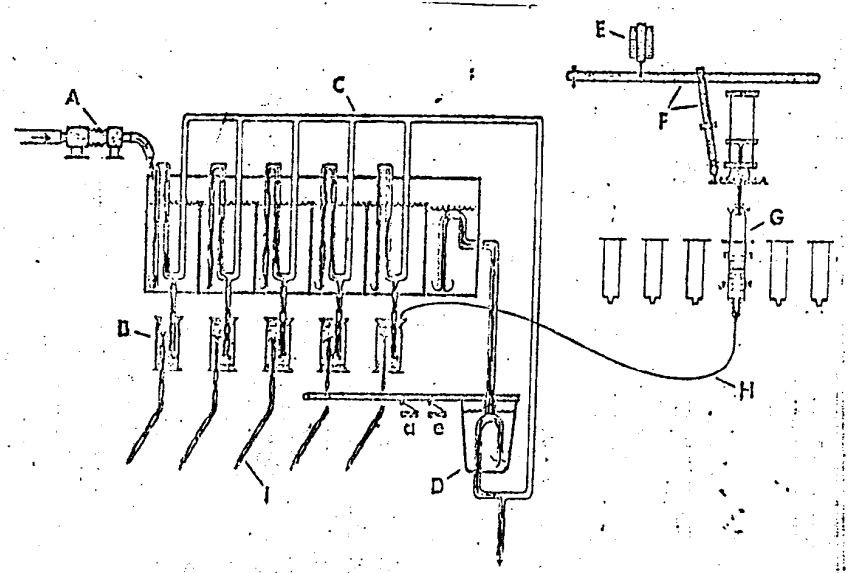

FIGURE 1. Dosing apparatus: A-oscillating pump; a-pump micro-switch; B-mixing bottles; C-vacuum lines; D-receiving bucket; E-solenoid; e-solenoid micro-switch; F-injector lever apparatus; G-50cc. syringe; H-toxicant delivery tubing; I-water delivery tube.

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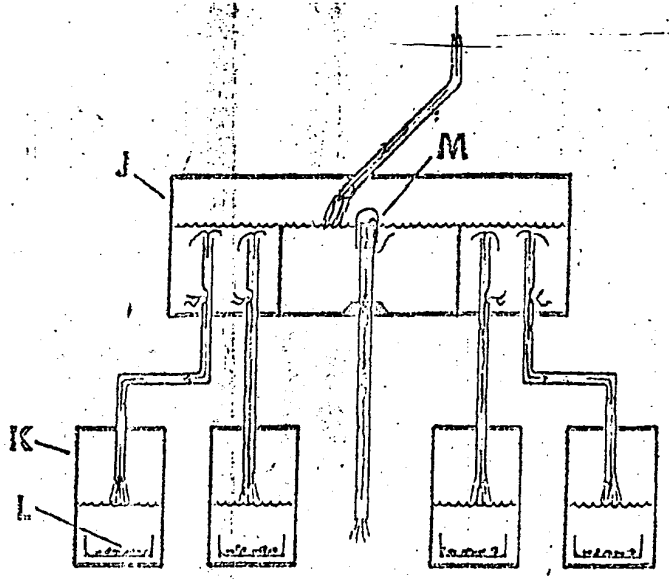

FIGURE 2. Distribution apparatus: J-distribution box; K-egg/larvae trays; L-Petri dishes; M-siphon.

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

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

* Lose of 25 eggs due to spillage.

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

MONS 042052

MONS 047053

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

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

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

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

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

* Significantly different from control fish (χ^2, $\alpha = 0.01$).

MONS 047054

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

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

* 24-hour static test.

MONS 047055

Prog. in Sport Fishery Res.

Biological Studies on PCBs

JAN 28 1976

Acute toxicity to fish The toxicity of the Aroclor® series was determined using the standard 96-hr static bioassay and cutthroat trout. Additional tests were also made with bluegills and channel catfish (Table 1). The solubilities of the Aroclor® formulations in water are reported in the range of 0.1-0.3 µg/l.

The results of these tests indicate a decrease in acute toxicity for Aroclor® 1254 and higher. The PCB toxicity indicated by these tests is misleading in that most fish mortalities occurred in the last 24 hours of the test and the concentrations of the test solutions were considerably above the true solubilities. The toxicities of these compounds became more apparent in long term, intermittent-flow bioassays.

The acute oral toxicity of Aroclor® 1242 and 1260 to rainbow trout was greater than 1500 mg/kg (given per os for 5 days at 300 mg/kg/day).

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MONS 041403

Table 1. Acute toxicity[1] of several Aroclors® to selected fishes.

Aroclor®	Species	Temperature (C)	96-hr TL_{50} (ug/l)
1221	Cutthroat trout	8.9[2]	1,170
1232	" "	8.9	2,500
1242	" "	8.9	5,430
1248	" "	8.9	5,750
1254	" "	8.9	42,500
1260	" "	8.9	60,900
1262	" "	8.9	>50,000
1268	" "	8.9	>50,000
4465	" "	8.9	>50,000
5442	" "	8.9	> 50,000
5460	" "	8.9	> 50,000
1248	Channel catfish	18.3[3]	6,000
1254	" "	18.3	12,000
1248	Bluegills	18.3[3]	278
1254	"	18.3	2,740

1/ Static bioassay

2/ Alkalinity, 159 ppm; pH 7.6

3/ Alkalinity, 35 ppm; pH 7.1

Chronic toxicity to fish Intermittent-flow tests were conducted with rainbow trout, channel catfish, bluegills, and yellow perch and Aroclors® 1242, 1248, 1254 and 1260. Rainbow trout were the most susceptible to the Aroclor® compounds, then yellow perch, bluegills, and channel catfish (Table 2). Aroclor® 1248 was the most toxic to all species tested. Aroclor® 1242 was equal or perhaps slightly more toxic than 1254. The test with yellow perch and 1248 serves as a model for estimating time-independent toxicity (Figure 1).

MONS 041405

Table 2. Toxicity of four Aroclors® and DDT to four species of fish in dynamic exposures.

Species	Compound	TL_{50}s (ppb) after 5 to 35 days						
		5	10	15	20	25	30	35
Rainbow trout	DDT	4.2	1.4	0.5 (0.3-0.9)	0.4	0.2	-	-
	Aroclor 1242	67	48	18 (11-31)	10	12	-	-
	Aroclor 1248	54	38	16 (9-31)	6.4	3.4	-	-
	Aroclor 1254	-	160	64 (29-140)	39	27	-	-
	Aroclor 1260	232	326	143 (33-610)	78	49	51	-
Channel catfish	DDT	-	-	2.3	1.3	0.86	0.57 (0.28-1.1)	-
	Aroclor 1242	-	125	219	150	132	87 (46-164)	-
	Aroclor 1248	-	121	121	115	104	75 (29-197)	-
	Aroclor 1254	-	303	286	293	181	139 (94-349)	-
	Aroclor 1260	-	535	482	512	465	433 (185-1010)	-
ucgills	DDT	1.2	1.4	1.6	1.3	0.59 (0.39-0.89)	0.35 (0.21-0.58)	-
	Aroclor 1242	-	-	164	125	120	84 (48-150)	-
	Aroclor 1248	136	115	111	106	100	78 (42-143)	-
	Aroclor 1254	-	-	303	260	239	177 (97-321)	-
	Aroclor 1260	-	-	-	-	-	-	-

MONS 041406

Table 2. Continued

Species	Compound	TL_{50}s (ppb) after 5 to 35 days						
		5	10	15	20	25	30	35
Yellow perch	Aroclor 1242	-	-	-	135	94 (78-114)	92	-
	Aroclor 1248	-	-	110	90	78	72 (58-88)	-
	Aroclor 1254	-	-	128	92	92	92 (76-110)	-
	Aroclor 1260	-	-	-	-	-	240	185 (139-247)

Water: pH = 7.4, alkalinity=237 ppm, hardness=272 ppm, and 17° C.
No value means insufficient data for computation of TL_{50}.

MONS 041407

AROCLOR® 1248 - YELLOW PERCH

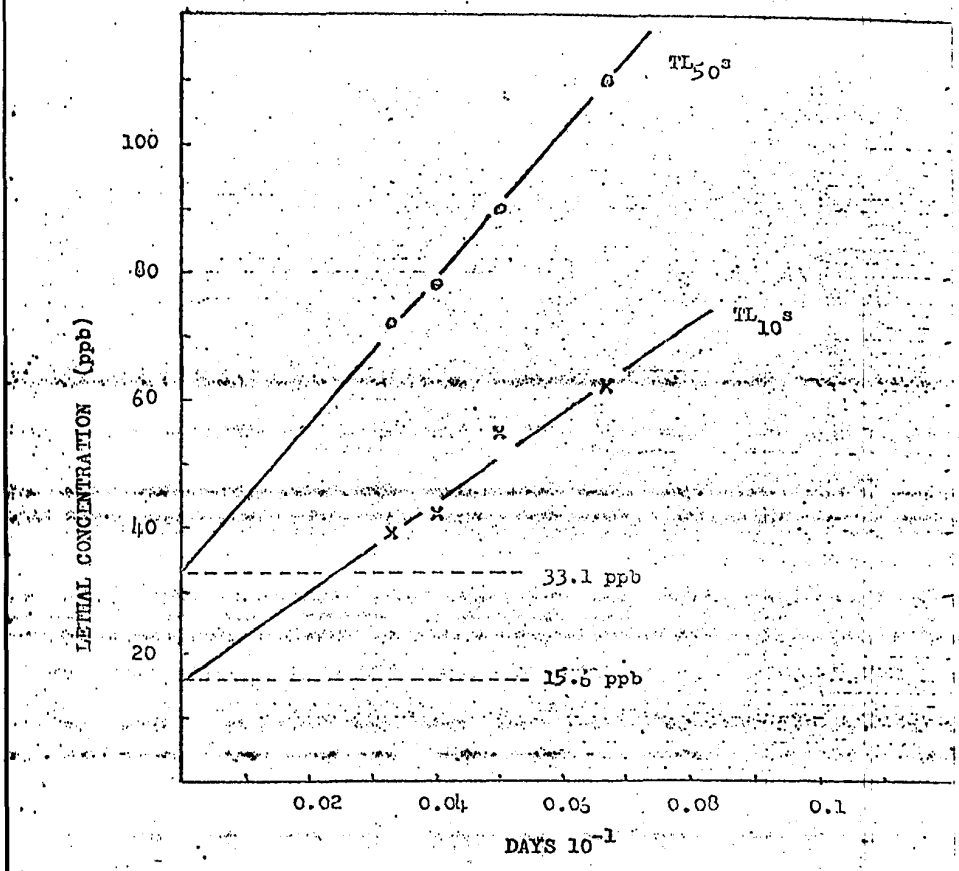

Figure 1. Estimation of time-independent toxicity by intermittent-flow bioassay.

MONS 041408

Toxicity to Invertebrates Static and continuous-flow tests with invertebrates did not differ as much as those with fish. In general, insects were much more resistant to the Aroclors® than were crustacea (Table 3). Scud (Gammarus fasciatus) were also exposed to Aroclor® 1254 for 7 days in an intermittent-flow diluter at 17° C and the TL_{50} value was 34 µg/l (24-45). Homologues having lower chlorine numbers were more toxic to scud than those having higher chlorine numbers (Table 4).

Bioconcentration by aquatic microorganisms Aroclors®, like some of the organochlorine insecticides, appear to be rapidly and greatly concentrated by various organisms. For example, preliminary experiments showed that Daphnia magna concentrated ^{36}Cl-Aroclor® 1254 by 45,000 times after only 48 hours of exposure to a concentration of 300 ng/l. These data closely parallel that obtained in DDT and aldrin investigations with Daphnia magna.

Bioconcentration by aquatic invertebrates The potential of 1254 to accumulate in fish-food organisms was investigated by exposing several species of aquatic invertebrates to a ^{36}Cl-labeled formulation of this chemical in a continuous-flow system. All invertebrates exposed to 1254 showed an initial period of rapid uptake and residue magnification many times greater than the concentrations in water. For example, late instar mosquito larvae Culex tarsalis exposed to 1.5 µg/l of 1254 accumulated a residue of 19 mg/kg within 24 hours. This represents a 12,600-fold increase over the residues in water. The tissue residues continued to accumulate until the seventh day when the mosquito larvae transformed into pupae.

Table 3. Aroclor® and DDT toxicity to invertebrates.

Compound	Organism[1]	Bioassay type[2]	Exposure (days)	TL_{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 (Nacromia sp.), Damselfly (Ischnura verticalis).

2/ Temperature, 15.6° C; alkalinity, 35 ppm; pH, 7.1

Table 4. Toxicity[1/] of five PCB homologues to scud[2/] at 21 C.

PCB isomer	TL_{50} (mg/l) and 95% confidence intervals at -- 24 hr	96 hr
2,3,4' trichlorobiphenyl	0.10 (0.08-0.14)	0.07 (0.05-0.12)
4,4' dichlorobiphenyl	0.16 (0.11-0.23)	0.10 (0.06-0.15)
2,4' dichlorobiphenyl	0.15 (0.10-0.20)	0.12 (0.08-0.18)
2,4,6,2', 4', 5' hexachlorobiphenyl	0.20 (0.16-0.26)	0.15 (0.09-0.21)
2,4,5,2', 5' pentachlorobiphenyl	0.43 (0.38-0.50)	0.21 (0.12-0.36)

[1/] Static bioassay.

[2/] Gammarus pseudolimnaeus.

MONS 04411

In some tests, we were able to continue the exposure until an apparent plateau or equilibrium concentration was reached within the organism. For example, scud were exposed continuously to 1.6 µg/l of 1254 in water until residues reached an apparent equilibrium concentration at 14 days; total body residues at this time were 38 mg/kg, a concentration 27,000 times that in water. Once this equilibrium was reached within the organism, no further residue magnification was recorded after an additional 21 days of exposure (Figure 2). Waterfleas accumulated a residue 30,000 times the water concentration (1.1 µg/l) in 3 days. In a similar study early instar crayfish Orconectes nais exposed to 1.2 µg/l of 1254 accumulated residues at a slower rate. The crayfish were accumulating 1254 at a linear rate after 28 days of exposure when the experiment was terminated (Figure 3).

Scud exposed to 1254 were analyzed by gas-liquid chromatography to investigate potential shifts in the residue composition which may have occurred as a result of metabolism. However, we found no significant alterations in the chemical, but some isomers were accumulated more than others. The most notable shift of residue components was a two-fold increase in concentrations of the lower chlorine isomers (tri- and tetrachlorobiphenyl isomers). The relative concentration of higher chlorinated isomers (penta- and hexachloro biphenyls) were decreased by one half.

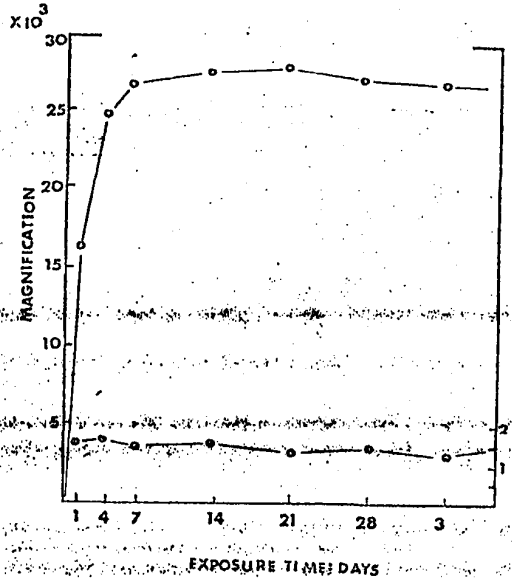

Figure 2. Biological magnification by scud, Gammarus pseudolimnaeus of ^{36}Cl-Aroclor® 1254 from water during 35 days exposure.

MONS 041413

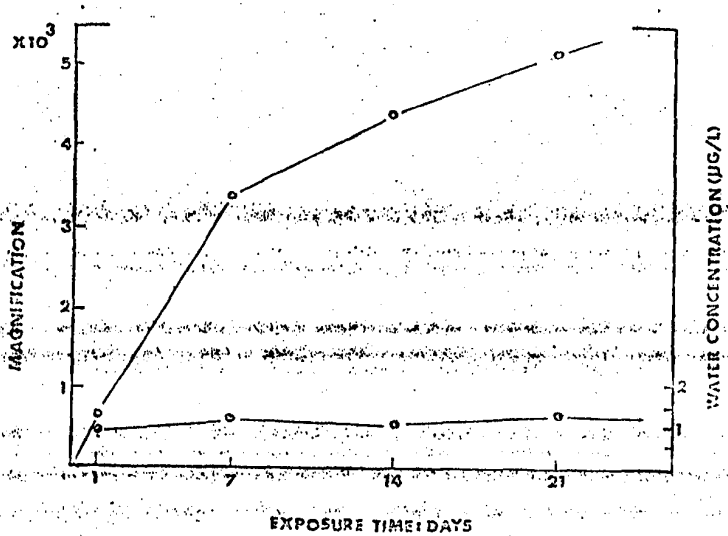

Figure 3. Biological magnification by crayfish Orconectes nais of ^{36}Cl-Aroclor® 1254 from water during 21 days of exposure.

Physiologic effects of PCBs Because the thyroid plays an integral role in reproduction, osmoregulation, and oxidative metabolism, we studied its activity in five species of fish exposed to organochlorine chemicals by diet or bath. Aroclors® 1248 and 1254 had significant effects on thyroid activity (72-hr ^{125}I-uptake) (Table 5). The lowest dietary concentrations that affected thyroid activity were 0.48 µg/gm (ppm in dry food) of Aroclor® 1254. These concentrations are comparable to those found in natural fish foods.

Table 5. Thyroid activities in five species of fish exposed to polychlorinated biphenyls (PCBs) or DDT.

Chemical	Dosage (µg/kg bw/day)	Concentration (ppm)	Species	Exposure (days)	Changes in thyroid activity[1]	Whole body residue[2]
Aroclor 1232	40	1.2	Channel Catfish	160	NC	3.7
Aroclor 1232	400	12	Channel Catfish	160	NC	8.3
Aroclor 1248	40	1.2	" "	160	NC	4.2
Aroclor 1248	400	12	" "	160	NC	14.0
Aroclor 1254	40	1.2	" "	160	+56	5.4
Aroclor 1254	400	12	" "	160	+91	21.0
Aroclor 1260	40	1.2	" "	160	NC	5.5
Aroclor 1260	400	12	" "	160	NC	45.0
Aroclor 1254	1.45	0.05	Coho salmon	260	NC	0.40
Aroclor 1254	14.5	0.48	" "	260	+52	0.59
Aroclor 1254	145	4.8	" "	260	+35	3.87
Aroclor 1254	1450	48	" "	260	+87	54.5
Aroclor 1254	14,500	480	" "	260	+119	646.0

MONS 041416

Table 5. Continued.

Chemical	Dosage (ug/kg bw/day)	Concentration (ppm)	Species	Exposure (days)	Changes in thyroid activity[1]	Whole body residue[2]
Aroclor 1248	-	4	Bluegills	74	NC	NA
Aroclor 1248	-	7.3	" "	74	-29	NA
Aroclor 1254	-	4.6	" "	74	NC	NA
Aroclor 1254	-	7.5	" "	74	+54	NA
Aroclor 1248	-	6.7	Channel catfish	77	+50	NA
Aroclor 1248	-	11.8	" "	77	+50	NA
Aroclor 1254	-	3.5	" "	77	+44	NA
Aroclor 1254	-	5.3	" "	77	+105	NA

1/ Percent change in 72-hr ^{125}I-uptake from controls; NC = no significant difference.

2/ NA = not analyzed; residue concentration = ug/g of wet body weight.

MONS 041417

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 (Figure 4). 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 success.

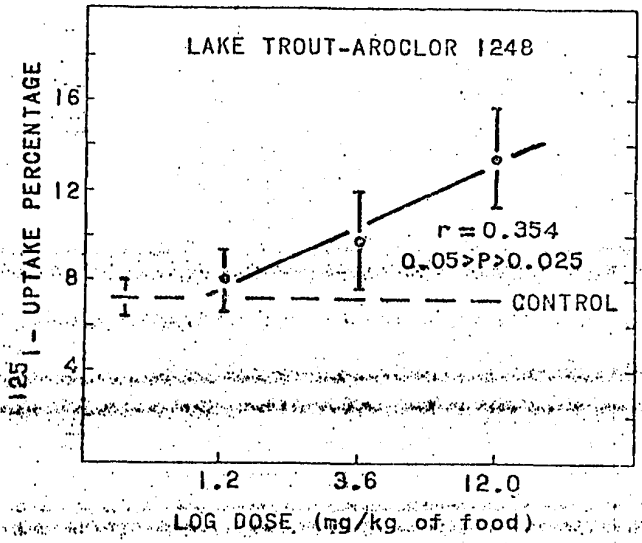

Figure 4. Thyroid stimulation by 6 month dietary exposure to Aroclor® 1248.

MONS 041419

Chronic dietary toxicity of PCBs

Aroclor® 1248 was incorporated in diets of juvenile lake trout at concentrations of 0.1, 1.8 and 6.0 mg/kg of food (concentrations comparable to those found in natural foods). These concentrations depressed growth and serum cortisol levels, but stimulated thyroid activity during the first 160 days of the experiment (Table 6a). After 320 days, however, there were few differences between control and treated fish.

Dose-dependent pathologic changes were observed in the gills and livers of PCB-dosed fish. Gill lesions consisted of hyperplasia and hypertrophy of the respiratory epithelium and edema and intercapillary congestion of the lamellae. Changes in the liver included parenchymal degeneration, cytoplasmic vacuolization of the hepatic cells, and pleomorphism of the liver cells.

Whole-body residues increased during the exposure (Table 6b), but after the trout were fed uncontaminated food for 60 days, residues declined an average of 31%, 15%, and 12% in the 0.6-, 1.8-, and 6.0-mg/kg lots, respectively.

Also, lake trout growth was retarded by dietary Aroclor® 1248 and chlordane in combination. Fish that consumed food containing 0.94 µg/g of Aroclor® 1248 and 0.26 µg/g chlordane for 80 days grew 15% less than the controls.

Table 6a. Growth, serum cortisol concentrations, and thyroid activity in juvenile lake trout exposed by feed to Aroclor® 1248.

	Percent growth			Serum cortisol		Thyroid activity	
	160 days	310 days	total growth	160 days	320 days	160 days	320 days
Control	887	2,930	3,817	6.5±0.8	10.3±0.6	3.6±0.4	1.2±0.2
0.6 mg/kg	708	3,017	3,724	3.9±0.4	7.8±1.4	4.0±0.7	1.3±0.1
1.8 mg/kg	777	3,032	3,810	4.3±0.8	11.2±1.4	4.9±1.1	1.2±0.1
6.0 mg/kg	522	3,262	3,783	3.5±0.2	13.7±1.3	6.7±1.1	1.0±0.1

Table 6b. Whole body residues (µg/g) in juvenile lake trout fed Aroclor® 1248.

Exposure (days)	Doses (mg/kg of dry food)			
	0	0.6	1.8	6.0
0	0.17	0.17	0.17	0.17
70	0.11	0.26	0.59	2.00
145	0.09	0.42	1.20	3.70
246	0.15	0.81	2.40	6.80
320	0.13	1.60	2.70	9.70
Recovery[1/]				
60	0.12	1.10	2.30	8.50

1/ Fed non-contaminated food.

MONS 041421

Uptake of dietary PCBs

Dietary doses of 1.45, 14.5, 145, 1,450, and 14,500 µg Aroclor® 1254/kg body wt. per day to coho salmon had no effect on growth, nor produced observable toxicosis until death occurred in the highest dosage group after 260 days. The two highest dosage groups achieved extremely high concentrations of residues (Table 7). Fish fed 14,500 µg/kg began dying on the 260th day, and 5 days later all fish had died. Thyroid activity was increased significantly after 260 days of treatment (Table 5). A detectable stimulation of thyroid activity occurred in salmon dosed with 1/1000th of that causing mortality.

Dietary doses of Aroclors® 1232, 1248, 1254, and 1260 to channel catfish at concentrations of 2.4 and 24 mg/kg (dry wt) caused no mortality nor had any effect on growth. Thyroid activity was altered significantly only by Aroclor® 1254. The low dose increased uptake by 56%, whereas a 91% increase was induced by the high dose. Residue accumulation was greatest in the groups exposed to the high dose of Aroclor® 1260; i.e., as the percent chlorine content of the PCB molecule increased, residue accumulation increased (Table 8).

MONS 041423

Table 7. Whole body residues of Aroclor® 1254 (PCB) in coho salmon exposed to dietary concentrations of Aroclor® 1254 at 18° C.

Exposure (days)	PCB residues[1] (µg/g) after exposure to theoretical dietary dosages[2] (µg/kg body wt per day) of --					
	0	1.45	14.5	145	1,450	14,500
14	0.15	0.19	0.28	0.96	8.9	81.7
28	0.15	0.23	0.42	1.32	11.4	114.5
56	0.12	0.18	0.69	2.90	21.8	300.0
112	0.17	0.47	0.50	3.83	21.6	384.0
142	0.16	0.35	0.61	3.52	34.18	361.0
200	0.14	0.36	0.68	2.48	57.14	659.0
260	0.17	0.40	0.59	3.87	54.5	645.5

[1] Pooled samples of four fish.

[2] Dosage corresponds to 0.19 (background in feed), 0.25, 0.64, 6.4, 64, and 640 µg/gm food dry wt., respectively.

Table 8. Whole body residues of Aroclors® 1232, 1248, 1254, and 1260 in channel catfish after 193 days exposure to dietary concentrations of the PCBs at 25° C.

Aroclor	Dosage[1] (µg/kg bw/day)	Exposure (Days) 17	45	87	150	193
1232	480	9.0[2]	5.4	5.2	11.3	14.5
1232	48	0.8	1.9	1.7	4.5	3.2
1248	480	13.0	2.3	4.3	NA	13.0
1248	48	0.6	0.9	1.3	NA	1.9
1254	480	10.0	0.8	0.8	14.2	21.0
1254	48	0.8	0.03	0.2	2.3	4.8
1260	480	6.2	5.0	7.5	17.0	32.0
1260	48	0.7	1.2	1.7	2.3	3.5

1/ Dosages of 480 and 48 µg/kg body wt. per day corresponds to 24 and 2.4 µg/gm food (dry wt.), respectively.

2/ Mean of three samples (µg/gm).

Uptake of PCBs from water by fish

After 77 days, bluegills and channel catfish exposed to two concentrations of Aroclors® 1248 and 1254 in intermittent-flow diluters, magnified the Aroclors® by a factor of 20,000 to 60,000. Although the mean Aroclor® residue in the fish seemed dependent on water concentration, the magnification factor from water did not. For example, bluegills exposed to 2.6 and 7.3 µg/l of Aroclor® 1254 in water magnified those concentrations by 26,560- and 26,300-times and contained mean residues of 68.3 and 192 µg/g of whole fish, respectively.

Chromatograms of Aroclor® residues in bluegills and catfish showed a difference in storage rates for various Aroclor® components. Uptake appeared biased toward the later eluting components from the GC. After Aroclor® exposure was terminated, PCB elimination by fish was more rapid for the early eluting components.

MONS 041425

POLYCHLORINATED BIPHENYLS IN MICHIGAN WATERS

Report to WRC Comm

June 1973

MAY 28 1974

The Bureau of Water Management's last progress report to the Commission on the PCB situation was in December, 1971. Some of you have requested an updating. That is the purpose of this report today.

Since the last briefing to you, EPA has come out with a recommendation that receiving waters should not contain PCB's in excess of 0.01 ppb. Such a low level was deemed necessary in view of the fact that research has demonstrated biomagnification of PCB's in fish can be as high as 200,000 times the concentration in water to which they are exposed. There is an indication now that EPA may even lower their recommended level by adopting the recommendation of 0.002 ppb made in the upcoming edition of Water Quality Criteria (NAS, 1972), commonly referred to as the "Blue Book". Anyway, the EPA recommendation of 0.01 ppb for receiving waters has provided us with a guideline to use in reference to our monitoring programs.

As you may recall, Michigan instituted a monitoring program for polychlorinated biphenyls (PCB's) early in 1971. The initial program consisted of a state-wide water sampling survey for determination of concentrations in inland waters and tributaries to the Great Lakes. Supplementary sampling has since been conducted on stream sediments, municipal raw water intakes, groundwater drinking supplies, sanitary landfill runoffs, municipal wastewater treatment plants, industrial discharges, and fish from both inland and Great Lakes waters. Sampling is being continued in tributary streams, wastewater treatment plants, and in fish on a periodic basis as a means of following the effectiveness of control and reduction programs.

Water sampling in thirty Michigan tributaries during 1971 and 1972 showed that about 60 percent of the samples exceeded the 0.01 ppb guideline with one stream, the Saginaw River, having a high concentration of 2.9 ppb (Table 1). It is interesting to note that fish from the Saginaw River were found to have PCB concentrations as high as 165 ppm (Table 2). Five Michigan streams had mean concentrations in water samples over 0.10 ppb, 10 times higher than the EPA guideline. These were the Saginaw, Tittebawassee, Clinton, Raisin and Rouge Rivers, all located in the populous southeastern lower peninsula which drains into lower Lake Huron, Lake St. Clair and Lake Erie. Of the Lake Michigan streams, the Grand and Kalamazoo rivers consistently showed the highest concentrations with means of 0.041 and 0.065 ppb, respectively.

In May, 1973 (just last month) we sampled the water in the Lower Pennisula tributaries again and found just 8 (36 percent) exceeding the 0.01 ppb guideline (Table 3). This is an apparent improvement over previous years which we hope is the beginning of a downward trend. Improvements were especially noted in the Saginaw, Tittabawassee, and Raisin Rivers.

Sampling of municipal wastewater treatment plant (WWTP) effluents throughout Michigan in 1971 and 1972 indicated that many WWTP's represent major point sources of PCB loss to surface waters (Table 4). The average PCB concentration of 60 effluents sampled was 2.55 ppb. While only seven of the WWTP effluents exceeded 1 ppb, the Bay City WWTP located on the Saginaw River had an average effluent concentration of 120 ppb (high of 340 ppb), skewing the over-all average for all plants sharply upward. The average concentration excluding the Bay

MONS 041426

were, with one exception, located on rivers already identified as having levels of PCB contamination. One would tend to believe that if industrial has been decreased, it would be reflected first in reduced concentrations charges from wastewater treatment plants. Sampling of 58 WWTP effluents , however, continues to show high PCB levels with an average concentration ppb (Table 5), including the Bay City plant. Although still high, clear that the PCB discharge from the Bay City WWTP has been greatly reduced.

Sampling of sewage sludge from 57 of the 58 WWTP's tested in 1973 showed City to have the highest concentration of PCB's in the sludge being removed treatment process. The PCB concentration in the sludge from the Bay plant was 352 ppm (dry weight basis) compared to a state-wide average of ppm (Table 6).

In an attempt to identify major contributors of PCB's within municipal systems, sampling at key points in the interceptor systems has been started within the municipalities of Bay City, Mt. Clemens, Saginaw, Adrian, Flint and Kalamazoo. This program has been very successful in that it has identified numerous PCB sources. In most cases, our agency has contacted the industries identified and have asked for corrective action. The City of Detroit, however, has taken its own initiative and is getting good cooperation. Two of the major sources identified in the Detroit system have completed a changeover to non-PCB products and the other two are underway.

A broad scale sampling of industrial discharges throughout Michigan has shown results indicative of the widespread usage of PCB's. Nearly half of the industrial discharges sampled have contained more than 0.1 ppb with many in excess of 1 ppb. The highest concentration detected in an industrial discharge in Michigan has been 7,200 ppb and this was just found recently. Automotive plants, chemical companies, and paper product manufacturers are among the types of industries with the most concentrations. Also industries and building complexes, including some schools and hospitals, with heat transfer systems appear to be another major source. Industries identified as sources of PCB pollution have been encouraged to change to replacement compounds or to clean up their operation so that discharges of PCB are eliminated. Our sampling of industries generally has shown that even though Monsanto Company (the only U.S. manufacturer of PCB's) has stopped the sale of PCB's for all uses except electrical transformers and capacitors, many industries apparently have large enough stock piles of PCB for replacement that have not yet been forced into switching to substitute compounds. We view continuing search for sources as a necessity so that we can speed up the changeover process. There are some indications that even after an industry stops the use of PCB's a residual is going to continue in their effluent for considerable length of time until the sewers can be completely flushed out.

We are encouraged that some rivers are starting to show reductions in PCB concentrations. An example of one location in Michigan where monitoring and subsequent control measures have resulted in a marked reduction in PCB contamination is the Saginaw River basin. Monitoring of major Great Lakes tributaries during 1972 identified the Saginaw River as having the highest concentrations of PCB of any tributary within Michigan boundaries. Intensive sampling throughout the river basin in search of point sources successfully isolated ten major points of PCB discharge to the river system. Each of the industrial sources were asked to identify the cause of their losses and to take corrective action. The following is a listing of the point sources and corrective actions taken to this date:

Chevrolet Bay City Parts Plant - PCB's had been used as hydraulic fluids in the die-cast machines. Losses were occurring both in the cooling

Our last report from General Motors showed conversion to a phosphate-ester based compound about 50 percent completed.

Bay City WWTP - Interceptor sampling identified the Chevrolet Parts Plant as the major contributor to this sytem. Since the initial steps of the General Motors plant conversion away from PCB fluids, the PCB levels in the WWTP effluent have dropped to about 1 percent of the previous highs.

General Motors Grey Iron and Nodular Iron Foundries, Saginaw - The problem here was also related to losses of hydraulic fluids. Conversion to phosphate ester fluids is reported to be complete.

Saginaw WWTP - Concentrations of PCB's in the effluent were not nearly as high as in the Bay City Plant. The Grey Iron and Nodular Iron Foundries were identified as the major contributors to the system. Concentrations in the WWTP effluent have declined to about 50 percent of their previous levels.

Buick Motors, Flint - This plant was using PCB's as hydraulic fluids in 13 die cast machines. Conversion to a phosphate ester-glycol product was completed November 1, 1972.

Chevrolet Van Slyke Engine Plant, Flint - A coolant containing PCB's has been replaced with a substitute product.

Flint WWTP - Concentrations have steadily declined since our 1st sampling period in the fall of 1971 and now are considered very low.

Dow Corning Company, Midland - PCB's have been used in the heat transfer systems at this plant. Conversion to a substitute fluid is 50 percent complete.

Dow Corning Semiconductor Products Plant, Hemlock - Conversion of heat transfer fluids was completed in January, 1973.

Voluntary conversion to non-PCB containing products is proving to be very effective toward lowering the PCB concentrations in the Saginaw River. If you will refer to Table 7 of your written report you will note a sharp decline at our monitoring site near the river mouth in October, 1972. Concentrations have remained fairly low ever since that date with the exception of the sample from last month which indicated an increase back up to 0.2 ppb. Overall the situation appears to have improved considerably and we hope that a corresponding decline in PCB concentrations in fish from the river and the Saginaw Bay will soon be observed.

You may recall that in May, 1972 we reported to you about a PCB situation in the Kalamazoo River. During our intensive water quality and biological survey of the Kalamazoo watershed we discovered sharp increases in PCB's in fish below the municipalities of Battle Creek and Kalamazoo. Follow up surveys were unsuccessful at identifying any major sources in Battle Creek but in the Kalamazoo vicinity surveys have pointed the finger at both past and present wastepaper recycling operations by the paper industry. Certain sediment deposits in an impoundment on Portage Creek, a tributary to the Kalamazoo River, were found to contain PCB's at concentrations exceeding 350 ppm (dry weight basis). Leaching

from these PCB enriched sediments appears to be significant. During a drawdown of the impoundment last summer, scouring of the bottom sediments occurred and a sharp rise in PCB levels in river water was observed as the sediments moved downstream. As a result of sampling within the WWTP interceptor system, it is apparent to us that the remaining de-inking operations are still contributing to the PCB contamination problem. We are not sure what the solution to this problem might be because it would obviously involve a policy decision at the National level about the merits of recycling paper.

In 1972 Michigan developed a cooperative State-Federal study of contaminant levels in Great Lakes fish species including lake trout, salmon, whitefish, chubs, menominee, yellow perch, alewife, carp, and suckers. Fish from Lakes Michigan, Huron, Erie, and Superior are systematically being sampled on an annual basis to obtain information on areas where contaminants approach or exceed FDA guidelines for consumer protection. The project, developed by the State agencies in cooperation with the U.S. Food and Drug Administration and the Bureau of Sport Fisheries and Wildlife Great Lakes Laboratory, will establish patterns of residues of mercury, PCB's, DDT and dieldrin in Great Lakes fish. Table 8 shows the concentrations of PCB's detected in fish from Lake Michigan during the first year of this study. PCB's in Salmonids (lake trout, steelhead, coho salmon, and chinook salmon) and chubs were shown to exceed the FDA guideline of 5.0 ppm while concentrations in other species did not exceed this guideline. Sampling will be continued on an annual basis.

One area in which we have very little information so far is the potential for loss of PCB's from electrical transformers (one of the two remaining uses) through leakage, servicing, and replacement of worn-out or damaged units. We hear rumors that many of the small transformer maintenance companies, apparently unaware of the potential hazards of PCB's, are dumping PCB-based transformer fluids down the nearest drain or giving them to waste oil haulers rather than returning them for incineration. We intend to make a questionnaire survey followed up by an educational type letter with guidelines for safe disposal.

In summary, we think we have made considerable progress in the past 2 years toward getting rid of the PCB problem. On the other hand, while we have some indications of environmental reductions, we are afraid the contamination problems are not going to disappear as rapidly as we would like because of the slow transition to substitute products and the tremendous capacity for biological communities to magnify this type of persistent compound.

John L. Hesse
Aquatic Biologist
Water Quality Appraisal Section
Bureau of Water Management
Department of Natural Resources

Basin	River	PCB's (ppb as 1254)	Range (ppb as 1254)
igan	St. Joseph	0.013	<0.010-0.039
	Kalamazoo	0.065	0.019-0.097
	Grand	0.041	0.011-0.080
	Muskegon	0.010	<0.010-0.037
	Manistee	0.014	<0.010-0.039
	Boardman	0.017	<0.010-0.044
	Elk	0.012	<0.010-0.039
n	Saginaw	1.100	0.450-2.900
	Cass[1]	0.014	<0.010-0.048
	Flint[1]	0.078	0.010-0.150
	Shiawassee[1]	0.029	<0.010-0.073
	Tittabawassee[1]	0.140	<0.022-0.230
	Au Sable	<0.010	<0.010-0.010
	Thunder Bay	0.023	<0.010-0.037
	Cheboygan	0.032	<0.010-0.053
Clair	Clinton	0.150	<0.039-0.430
	Black	0.043	<0.010-0.110
	St. Clair	0.010	<0.010-0.029
	Raisin	0.210	<0.068-0.500
	Huron	0.012	<0.010-0.039
	Rouge[2]	0.470	0.058-1.600
	Detroit	0.020	<0.010-0.053

utary to Saginaw River
tary to Detroit River downstream from Detroit River sampling station

Table 2. Concentrations of poly-chlorinated biphenyls (PCB's) in fish from Saginaw River, December 7, 1971. (ppm, wet weight).

Species	Location	Length	Percent fat	PCB's based upon 1242 standard
Perch	Karn-Weadock Discharge Channel	9.0	1.3	16.3
Carp	" " "	21.0	0.9	8.8
Catfish	" " "	8.0	7.0	37.1
Catfish	" " "	7.5	4.9	47.9
Carp	U.S. Coast Guard Station	23.0	---	48.0
Carp	" " " "	24.0	10.5	20.4
Carp	" " " "	20.0	6.5	15.5
Carp	" " " "	24.0	5.9	45.8
Carp	" " " "	21.0	4.6	30.2
Pike	" " " "	27.0	1.6	16.6
Pike	" " " "	23.0	1.1	6.9
Gizzard Shad	" " " "	14.0	17.5	165.3
Gizzard Shad*	" " " "	6.0	15.3	32.5
Gizzard Shad*	" " " "	4.5	15.0	161.9
Gizzard Shad*	" " " "	5.0	17.0	77.0
Gizzard Shad*	Zilwaukee Bridge	4.5	15.7	24.1
Gizzard Shad*	" "	5.0	9.6	52.0
Gizzard Shad*	" "	5.0	8.7	10.4

* Concentration based upon analysis of whole fish. All others based upon edible portion only.

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Table 3. PCB Concentrations in Michigan Tributaries to the Great Lakes, May - June, 1973. Concentrations in parts per billion.

Lake Basin	River	Collection Date	PCB's as Aroclor 1254
Michigan	St. Joseph	5-11-73	< 0.010
	Kalamazoo	5-11-73	0.040
	Grand	5-11-73	0.033
	Muskegon	5-11-73	< 0.010
	Manistee	5-11-73	0.037
	Boardman	5-17-73	0.018
	Elk	5-17-73	< 0.010
Huron	Saginaw	5-24-73	0.210*
	Cass	5-24-73	< 0.010
	Flint	5-24-73	< 0.010
	Shiawassee	5-24-73	< 0.010
	Tittabawassee	5-24-73	< 0.010
	AuSable	5-17-73	0.013
	Thunder Bay	5-17-73	< 0.010
	Cheboygan	5-17-73	< 0.010
St. Clair	Clinton	6-8-73	0.200*
	Black	6-8-73	0.052
	St. Clair	6-8-73	< 0.010
Erie	Raisin	6-8-73	< 0.010
	Huron	6-8-73	< 0.010
	Rouge	6-8-73	0.460*
	Detroit	6-8-73	< 0.010

*Aroclor 1242

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of Municipal Wastewater Treatment Plants throughout Michigan 1971-1972. Unless specified, all samples are 8 hr. composites.

CITY	DATE	PCB's as Aroclor 1254 (ppb)	Mean Conc. (ppb)
[illegible]ian	3-30-72	1.80	4.60
	6-13-72	14.00	
	6-14-72	6.90	
	9-6-72	0.41	
[illegible]bion	3-28-72	0.44	0.44
[illegible]n Arbor	10-14-71	0.14	0.12
	3-30-72	<0.10	
[illegible]tle Creek	10-5-71	0.39	0.39
	3-28-72	0.16	
	2-21-72	0.92	
	6-6-72	0.28	
	6-7-72	0.21	
[illegible]y City	11-4-71	340.00	120.00
	11-22-71	210.00	
	11-23-71	60.00	
	3-2-72[a]	200.00	
	4-4-72	19.00	
	6-13-72	11.00	
	6-14-72	25.00	
	9-14-72	5.70*	
[illegible]on Harbor - [illegible]. Joseph	10-23-71	0.99	0.65
	4-4-72	0.31	
[illegible]ighton	10-5-71	0.38	0.38
[illegible]rlotte	7-20-72	0.61	0.61
[illegible]nstantine	4-4-71	0.85	0.85
[illegible]roit	10-14-71	2.20	1.95
	12-20-72[c]	3.00	
	1-20-72	0.92	
	3-9-72	2.40	
	4-17-72[b]	1.80	
	6-1-72	0.88	
	10-19-72	2.50**	
[illegible]ter	10-14-71	<0.10	0.10

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CITY	DATE	PCB's Aroclor 1254 (ppb)	Mean Conc. (ppb)
. Lansing	11-15-71	0.69	0.51
	4-3-71	0.35	
scanaba	12-16-71	0.29	0.29
sexville	11-4-71	0.21	0.24
	3-31-72	0.28	
lint	10-5-71	1.30	0.68
	3-31-72	0.92	
	6-7-72	0.47	
	6-8-72	0.30	
	10-5-72	< 0.10	
lushing	10-5-72	0.52	0.52
ladstone	7-21-72	0.19	0.19
rand Haven	4-4-72	< 0.50	< 0.50
rand Rapids	11-15-71	0.37	0.52
	3-30-72	0.68	
olland	10-20-71	0.79	0.60
	3-29-72	0.42	
oughton- ncock	3-21-72	< 0.10	0.10
on Mountain- ingsford	3-21-72	0.55	0.87
	4-4-72	1.20	
ronwood	3-20-72	0.16	0.16
ackson	11-16-71	<0.10	<0.10
	2-21-72	<0.10	
	3-28-72	<0.10	
alamazoo	10-5-71	1.30	0.66
	3-28-72	0.83	
	6-6-72	0.48	
	6-7-72	0.53	
	12-6-72	0.19	
Anse	4-21-72	<0.10	<0.10
nsing	10-7-71	0.23	0.18
	4-4-72	0.13	

ITY	DATE	PCB/s Aroclor 1254 (ppb)	Mean Conc. (ppb)
anistique	12-15-72	<0.20	<0.20
arquette	12-14-71	0.35	0.35
arshall	3-28-72	<0.10	<0.10
enominee	12-15-71	0.35	0.35
idland	2-2-72	0 29	0.23
	3-31-72	C 40	
	4-14-72	0 13	
	[d]10-2-72	0 13	
ilford	10-14-71	<0.10	<0.10
onroe	11-15-71	0.60	0.46
	3-30-72	0.33	
t. Clemens	11-15-71	1.40	5.21
	3-30-72	2.90	
	6-7-72[c]	4.50	
	6-8-72[c]	3.10	
	7-26-72	9.40	
	8-2-72	10.00	
. Pleasant	3-31-72	<0.10	<0.10
uskegon	11-15-71[c]	0.28	0.28
skegon Heights	11-15-71[c]	0.37	0.37
iles	4-4-72	0.68	0.68
orway	4-5-72	0.40	0.40
ntonagon	4-21-72	<0.10	<0.10
osso	9-14-72	<0.10	<0.10
rchment	4-20-72	<0.10	<0.10
ntiac (Auburn Rd.)	11-17-71	<0.10	0.35
	3-30-72	0.61	
ntiac (E. Blvd.)	11-17-71	0.20	0.45
	3-30-72	1.30	
	6-6-72	0.15	
	6-7-72	0.16	
rtage	11-15-71[d]	1.90	1.90

ry	DATE	PCB's as Aroclor 1254 (ppb)	Mean Conc. (ppb)
rt Huron	11-15-71	0.28	0.40
	3-30-72	0.52	
ginaw	10-6-71	1.10	1.80
	3-31-72	3.80	
	6-7-72	2.00	
	6-8-72	1.50	
	10-4-72[d]	0.74	
. Ignace	12-15-72	<0.20	<0.20
uth Haven	4-4-72	<0.10	<0.10
rtz Creek	10-5-72	<0.10	<0.10
ree Rivers	4-4-72	<0.30	<0.30
enton	11-16-71	0.14	0.53
	3-30-72	1.10	
	6-6-72	0.47	
	6-7-72	0.91[e]	
rren	11-15-71	0.16	0.13
	3-30-72	0.10	
yne County yandotte)	11-16-71	0.64	0.40
	3-30-72	0.17	
ite Pine	4-21-72	0.2	0.20
oming	11-30-72[c]	0.44	0.49
	3-30-72	0.55	
silanti	11-16-71	0.22	0.21
	4-2-72	0.21	
silanti Twp #1	11-16-71	0.12	0.11
	4-2-72	<0.10	
silanti Twp #2	11-16-71	0.19	0.17
	4-2-72	0.16	

roclor 1242 Standard
Aroclor 1242 and 1254 in 1:1 ratio

[a] hr [b]10 hr [c]24 hr [d]Grab [e]hexane lost · GRAND MEAN 2.55

TECHNICAL REPORTS

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
Health Services and Mental Health Administration

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Public Health Reports

Yusho, a poisoning caused by rice oil contaminated with polychlorinated biphenyls

MASANORI KURATSUNE, M.D., TAKESUMI YOSHIMURA, M.D., JUNICHI MATSUZAKA, M.D., and ATSUKO YAMAGUCHI, M.D.

SPORADIC outbreaks of a peculiar skin disease were reported in Fukuoka-Ken (Fukuoka Prefecture), Japan, in early October 1968. It was characterized by such symptoms as follicular accentuation, acneform eruption, pigmentation of the skin and nails, and hypersecretion of the Meibomian gland. Staff members of the department of dermatology of the Faculty of Medicine, Kyushu University, Fukuoka, who examined some of the early patients suspected they had chloracne. A possible causal relationship between the disease and ingestion of a certain brand of rice oil was also suspected in view of the distinct familial aggregation of the patients and their common use of the oil (*1*). This relationship was subsequently proved, and the disease was called Yusho, or rice oil disease.

To clarify the cause of the epidemic, a study group headed by Prof. S. Katsuki of the Kyushu University Faculty of Medicine was organized by the staff of that university, the School of Medicine of Kurume University, and local health departments. An epidemiologic study subgroup was set up within the study group on October 19, 1968, which was directed by Kuratsune and also included the other three authors. This subgroup immediately designed and carried out a series of extensive surveys in close cooperation with the department of hygiene of our faculty of medicine and the departments of public health of the Prefecture of Fukuoka and of the cities of Kitakyushu, Fukuoka, and Ohmuta.

The epidemic spread not only over Fukuoka-Ken but also over 20 other prefectures in the western part of Japan. It produced 1,001 patients —502 males and 499 females—according to the latest tabulation by the section of food hygiene, Ministry of Welfare. Although our study of the epidemic was confined to Fukuoka-Ken, and the epidemic proved to be caused by accidentally contaminated oil, we believe that publication of our results will help prevent similar food poisonings. These incidents can occur anywhere in the world if sufficient care is not exercised.

The authors are with the department of public health, Faculty of Medicine, Kyushu University, Fukuoka, Japan. Dr. Kuratsune is a professor of public health, Dr. Yoshimura and Dr. Matsuzaka are working toward doctoral degrees in public health, and Dr. Yamaguchi is a research student. *The study on Yusho was supported by a special research grant from the Japanese Government.* *Tearsheet requests to Masanori Kuratsune, M.D., Faculty of Medicine, Kyushu University, Fukuoka, Japan.*

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Most of the results of our study have already been published in Japanese (*2–5*). We refer also, when necessary, to some important observations made by a clinical study subgroup headed by Prof. K. Higuchi of the department of dermatology at our university and also to studies by a chemical study subgroup headed by Prof. H. Tsukamoto of the Faculty of Pharmaceutical Sciences.

Clinical Symptoms

The subjective symptoms of the 89 male and the 100 female patients whose cases of Yusho had been diagnosed by the end of October 1968 are listed in table 1. Most of these symptoms related to the skin and eyes. Detailed clinical examinations of these 189 persons by the clinical study subgroup revealed many of the clinical characteristics of Yusho (*1,6–9*).

Dermatological observations. Acneform eruption—marked enlargement and elevation of the follicular opening; pigmentation of the skin, lips, gingiva, the mucous membrane of the oral cavity, and nails; formation of hyperkeratotic plaque on the soles of the feet and palms of the hands; hyperhidrosis; hypertrichosis; swelling of Montgomery's gland in the breast; cyst formation of the sebaceous gland in the genital region; dry skin; and, in children, milia near the joints.

Table 1. Percent distribution of symptoms of Yusho reported by 189 patients examined before October 31, 1968

Symptoms	Males (N=89)	Females (N=100)
Dark brown pigmentation of nails	83.1	75.0
Distinctive hair follicles	64.0	56.0
Increased sweating at palms	50.6	55.0
Acnelike skin eruptions	87.6	82.0
Red plaques on limbs	20.2	16.0
Itching	42.7	52.0
Pigmentation of skin	75.3	72.0
Swelling of limbs	20.2	41.0
Stiffened soles in feet and palms of hands	24.7	29.0
Pigmented mucous membrane	56.2	47.0
Increased eye discharge	88.8	83.0
Hyperemia of conjunctiva	70.8	71.0
Transient visual disturbance	56.2	55.0
Jaundice	11.2	11.0
Swelling of upper eyelids	71.9	74.0
Feeling of weakness	58.4	52.0
Numbness in limbs	32.6	39.0
Fever	16.9	19.0
Hearing difficulties	18.0	19.0
Spasm of limbs	7.9	8.0
Headache	30.3	39.0
Vomiting	23.6	28.0
Diarrhea	19.1	17.0

Ophthalmic observations. Hypersecretion of the meibomian gland and abnormal pigmentation of the conjunctiva.

Neurological observations. Numbness, pain, hypoesthesia, and lowered sensory conduction by the radial and sural nerves.

Other observations. Elevated serum triglyceride.

Epidemiologic Data Available

When the epidemiologic study subgroup began to act, it had several pieces of information that appeared to be important but whose implications were uncertain, such as the following:

1. The familial aggregation of patients was notable, and the disease showed no distinct age and sex preference.

2. All the patients seemed to have used one brand of rice oil (abbreviated as K rice oil because it was produced by K Company). An emergency survey undertaken by the health department of the city of Fukuoka had disclosed that some of the patients in a certain area had, as a group, purchased a can of K rice oil and divided it among the group members. Those who did not buy the oil seemed to be unaffected even though they lived next door to the patients.

3. The patients seemed to be broadly distributed throughout Fukuoka-Ken.

These data of course were valuable, but we knew that if we relied on them alone, they might mislead us. We exercised particular caution, therefore, to avoid such a risk, always seeking possible antitheses when interpreting any result.

Descriptive Epidemiologic Studies

The epidemiologic study subgroup first tried to learn how the 325 patients were distributed by place of residence and time of onset of the disease (*2*). Those persons who thought they were suffering from Yusho were asked to report to their local health departments. From October 1968 until the epidemic practically terminated in January 1969, a total of 6,611 persons in Fukuoka-Ken had reported that they were affected. Among them, 325 cases of Yusho were diagnosed by the clinical study subgroup. An early analysis of a portion of the 325 persons with confirmed cases, carried out in late October 1968, revealed several peculiar features in the geographic distribution of cases, features which did not basically change during the epidemic. Therefore, only the results of

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analysis of these 32 cases will be presented to illustrate these peculiarities.

Familial aggregation. The 325 patients belonged to 112 families, a result indicating a distinct familial aggregation, which is considered one of the most important characteristics of Yusho.

Time of occurrence. Ninety-nine percent of the patients were affected during 1968, and 55 percent of the cases were concentrated in the 3 months from June to August (table 2). The monthly distribution of the cases was alike for both sexes. These results indicated that exposure to the causal agent or agents was probably temporal and indifferent to sex.

Sex and age. Distribution of the 325 patients by sex and age revealed no significant sex difference (table 3). Age-specific incidence rates per 100,000, calculated by using the census population of 1965, indicated lower risks for both males and females in the age group over 60 years, but again no marked sex difference (table 4).

Geograp distribution. We calculated crude incidence rates of Yusho for three large cities (Kitakyushu, Fukuoka, and Ohmuta) and for the jurisdictional areas of the 22 local health departments of Fukuoka-Ken. A very marked difference was noted in their rates. Ten health departments reported no cases of Yusho while extremely high incidence rates per 100,000 were noted for the area covered by the health department of Soeda (58.9) and that covered by the health department of Tagawa (44.5); intermediately high rates, from 7 to 14, were observed for the three large cities and the area of the Kasuya health department.

There are many coal mines, most of which have recently been abandoned, in the jurisdictional areas of the Soeda and Tagawa health departments. The city of Kitakyushu has big steel and chemical industries, Fukuoka is a seat of commerce, and Ohmuta has big coal mines and chemical industries. In contrast, the Kasuya health de-

Table 2. Distribution of 325 patients with Yusho diagnosed by January 20, 1969, by sex and month when symptoms appeared

Month	Males		Females		Total	
	Number	Percent	Number	Percent	Number	Percent
Before 1968	2	1.3	2	1.2	4	1.2
1968						
January	0	.0	0	.0	0	.0
February	4	2.5	6	3.6	10	3.1
March	8	5.1	12	7.2	20	6.2
April	15	9.5	10	6.0	25	7.7
May	18	11.4	15	9.0	33	10.2
June	23	14.6	33	19.8	56	17.2
July	27	17.1	32	19.2	59	18.1
August	34	21.5	30	18.0	64	19.7
September	17	10.8	12	7.2	29	8.9
October	8	5.1	14	8.4	22	6.8
November	1	.6	1	.6	2	.6
December	1	.6	0	.0	1	.3
Total	158	100.0	167	100.0	325	100.0

Table 3. Distribution of 325 patients with Yusho diagnosed by January 20, 1969, by sex and age group

Age group (years)	Males		Females		Total	
	Number	Percent	Number	Percent	Number	Percent
0-9	37	23.4	27	16.2	64	19.7
10-19	38	24.1	28	16.8	66	20.3
20-29	28	17.7	36	21.6	64	19.7
30-39	30	19.0	39	23.4	69	21.2
40-49	11	7.0	23	13.8	34	10.5
50-59	9	5.7	11	6.6	20	6.2
60-69	4	2.5	3	1.8	7	2.2
70-79	1	.5	0	.0	1	.3
Total	158	100.0	167	100.0	325	100.0

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partment covers an area that is primarily agricultural. Most areas where few Yusho patients were seen were agricultural. No other common socioeconomic or environmental factor was observed that might explain the geographic distribution of patients.

Oil Used by Patients

As stated, K rice oil had been suspected as the possible cause of the epidemic. K rice oil is a high-quality salad oil, made from rice bran by the procedure shown in the chart. It was popular in Fukuoka-Ken, even though it was slightly more expensive than other oils on the market. A thorough investigation was undertaken to determine whether the patients with Yusho had actually used this brand of oil before they were affected and if so, whether there were any common characteristics in regard to the container of the oil (bottle or can), date of production, date of purchase, or route of marketing. To seek information on these points, an investigation was conducted in cooperation with the local health departments. All patients and their wives were visited and interviewed in their homes. In addition, all the available shipping records of K Company and all available records of purchases and sales of K rice oil at wholesale oil dealers' offices and at retail stores were carefully examined.

These followup surveys soon disclosed an astonishing fact. All the persons with Yusho had used, for varying periods, K rice oil which came either from 16.5 kg. cans or 1.65 kg. bottles. One hundred seventy of the 325 patients—49 households—had used only canned oil, while the remaining 155 patients—63 households—had used only bottled oil (table 5). We were able to confirm the shipping dates for the oil used by all but four of these 170 patients. No matter where the persons lived or what their age was, 166 of the 170 had apparently used rice oil that was shipped by the K Company on February 5 and 6, 1968, according to the lot numbers on the few remaining cans. Even the four patients for whom we could not confirm shipping dates of the oil had also undoubtedly used a canned rice oil produced by the K company.

The shipping or production dates could not be confirmed, however, for the bottled K oil that 155 patients had used exclusively, because they had no old bottles at home. Purchasers of oil in bottles return the empty ones to retail stores when they buy new oil. Nevertheless, all the shipping, purchase, or selling records at K Company, at the oil dealers, and at the retail stores were examined to learn whether any bottled K rice oil had been shipped to, and had reached, the retail stores from which the 155 patients bought their oil. All shipments of oil to the stores used by the patients were further investigated to determine whether any of them had been made on February 5 or 6, 1968, or immediately before or after these dates.

By this method, we discovered that 143 of the 155 patients might have bought the bottled oil shipped from the K Company in the period February 6–15, 1968. Whether the remaining 12 patients might have also purchased oil shipped in this period remains in doubt because of lack of records. Thus, nearly all of the patients had a distinguishing characteristic—they all had a common experience of use, or possible use, of rice oil shipped by one company in a specific period. Such a coincidence could hardly be expected to happen by chance.

Table 4. Age-specific incidence rates for Yusho, by sex of patient

Age group (years)	Incidence rates per 100,000	
	Males	Females
0–9	11.4	8.6
10–19	8.9	6.6
20–29	9.1	10.3
30–39	9.6	11.9
40–49	5.4	9.3
50–59	5.4	7.2
60–69	3.5	2.4
70 and over	1.7	.0
All ages	8.3	8.1

Table 5. Distribution of 325 patients and 112 households that used K rice oil, by type of oil container and shipping dates of oil

Container and shipping dates	Patients		Households	
	Number	Percent	Number	Percent
Users of canned oil	170	52.3	49	43.8
Shipped February 5 and 6	166	51.1	47	42.0
Shipping dates unknown	4	1.2	2	1.8
Users of bottled oil	155	47.7	63	56.3
Probably used oil shipped February 5–15	143	44.0	56	50.0
Shipping dates unknown	12	3.7	7	6.3
Total	325	100.0	112	[1] 100.1

[1] Does not add to 100.0 percent because of rounding.

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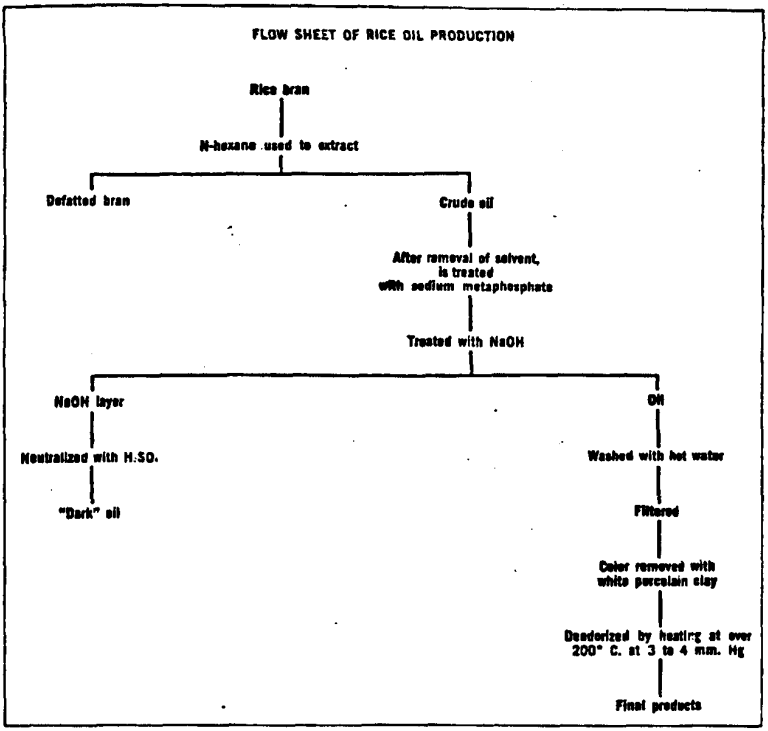

Our survey also demonstrated a high attack rate for all persons who consumed the oil. Altogether, 266 persons in 64 households had consumed canned K rice oil shipped in the period in question, and 170 had confirmed cases of Yusho. Thus, the attack rate was as high as 63.9 percent (*2*).

K Oil From Other Shipments

An additional survey seemed necessary to determine whether those persons who regularly used K rice oil but had not used any from shipments made in the period in question were free of the disease. For this purpose, a group of persons was needed who had reliable records of their health status and of their purchases of K rice oil. A group of 113 persons of 29 households living in one apartment house seemed to be appropriate subjects for this study.

According to the records of this group and the oil dealer who supplied them, these 113 persons had purchased canned K rice oil as a unit from one dealer and divided it among themselves fairly regularly from December 1967 to September 1968 except for the period from January to April 1968, when this bulk purchasing was not carried out. Their disease experience, particularly any skin disease that occurred during 1968, was carefully explored through interviews and, if necessary, through investigation of their medical records at hospitals. No cases of Yusho were found among

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members of this group. Thus, we demonstrated that persons who had used K rice oil regularly for more than a half year but did not use the oil from the specific shipments did not suffer from Yusho (2).

Retrospective Case-Control Studies

All these results suggested that Yusho was causally connected with use of the K rice oil that was shipped from K Company on February 5 and 6, 1968, or soon thereafter. Nevertheless, some other factors or agents might have been the primary or secondary cause of the epidemic. Therefore two case-control studies were done. In one, the personal backgrounds of the patients and matched controls were compared. In the other, the use of oil and fats in the households of the patients and the controls were compared.

To discover whether persons with Yusho cases and those without had different personal backgrounds, home interviews were conducted with all persons whose cases had been reported by October 31, 1968, and with healthy controls matched by age, sex, and place of residence. One hundred twenty-one patients and 121 controls (53 males and 68 females) were selected in this manner and asked 60 questions concerning their occupations,

Table 6. Percent of 121 patients and 121 controls having various allergies and dietary and personal habits, based on case-control study

Items investigated	Patients	Controls
Allergic to fish	5.0	7.5
Allergic to aspirin	.0	4.2
Allergic to drugs other than aspirin	7.5	6.6
Bathing facilities at home	84.7	85.5
Bath every day	73.0	70.6
Pets at home	[1] 18.3	[1] 36.5
Floor space of house smaller than 66 square meters	66.9	66.1
Handling agricultural chemicals	2.5	6.6
Taking codliver oil	10.8	8.3
Taking vitamin pills	23.2	18.3
Taking other restorative drugs	9.1	7.5
Water supply available at home	81.3	74.7
Dining out occasionally	28.1	30.6
Taking all meals with the family	88.8	89.6
Green vegetables daily	63.1	58.9
Milk nearly every day	49.0	39.0
Butter nearly every day	22.4	24.9
Eggs nearly every day	64.7	59.8
Fried foods or tempura nearly every day	[1] 22.4	[1] 11.6
Foods prepared with oil nearly every day	45.7	35.7
Fish nearly every day	21.6	29.1
Mayonnaise nearly every day	10.8	10.8
Instant "rahmen" or Chinese noodles nearly every day	10.8	10.0

[1] $P < 0.05$.

Table 7. Distribution of 69 households with patients and 207 control households, by use of various fats or oils, based on case-control study

Fat or oil	Households with patients		Control households	
	Number	Percent	Number	Percent
Butter	35	50.7	105	50.7
Margarine	44	63.8	127	61.4
Sesame oil	21	30.5	85	41.1
Rape-seed oil	10	14.5	77	37.2
Rice-bran oil	66	95.7	64	30.9
Lard	12	17.4	38	18.4
Other oils	13	18.8	117	56.5

medical background, general health status, habits, customs, diet, pets, and other characteristics of their lives.

The patients and the controls differed significantly in respect to only two of the personal factors tested (table 6). Persons who ate fried foods or tempura nearly every day were more numerous among the patients than among the controls, while more of the controls than the patients had pets.

To compare the use of oil and fats, 69 households with Yusho patients were matched by place of residence with 207 households without such patients—three control households for each household with cases.

As to regular use of fats and oils, a distinct difference was noted between the two groups only for rice-bran oil. About 96 percent of the patients' households and 31 percent of the controls' households reportedly used rice-bran oil regularly (table 7).

When asked about the use of rice oil from the K Company, all of the households with patients affirmed its use in comparison with only 32.4 percent of the control households; 61.3 percent of the control households denied using it; the remaining 6.3 percent were uncertain. Thus, the case-control studies clearly indicated that none of the factors tested except use of K rice oil could account for the disease (2).

Dose-Response Relationship

To prove that a causal relationship existed, a distinct dose-response relationship needed to be demonstrated. Estimating the quantity of contaminated rice oil consumed by each patient and members of his family was not feasible. Nevertheless, if we disregarded age and sex, we could estimate roughly the average amount of oil each person had consumed by dividing the amount used in a

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household by the number of persons in it, taking into account the frequency with which these persons ate at home, but not the possible loss of oil during and after cooking, nor the varying tastes and food intake of the family members.

Dr. Masayasu Goto, a dermatologist, with no knowledge of the amount of oil used by the 146 consumers of the contaminated oil, examined these persons and then graded the cases of Yusho he diagnosed according to their clinical severity. Only later did we link the severity of the cases, as determined by the dermatologist, to the amount of contaminated oil used by the patient.

Eighty of the 146 users of the contaminated oil were believed to have consumed, individually, less than 720 ml. (table 8). Of these 80 light users, 12 percent were not affected, 49 percent had light cases, and 39 percent had severe cases; the attack rate was 88 percent. The 45 persons who had used 720–1,440 ml. were all affected; 69 percent of this group had severe cases. A still larger proportion of the severe cases was found among the persons who had used more than 1,440 ml. of the contaminated oil; this group had an attack rate of 100 percent.

Since the clinical severity of the disease was not found to differ significantly between the sexes but differed considerably according to age, a standardization for age seemed necessary to achieve a more accurate interpretation of the observed relationship between use of the oil and the clinical severity of the disease. With the age composition of the 146 users of the oil as the standard, the factor of age was standardized for each of the three levels of oil intake. The figures, however, hardly changed from those shown in table 8. Thus a very clear dose-response relationship could be demonstrated, even though the estimates of the dose were inaccurate (*3*).

Toxic Agent

In view of all these epidemiologic observations, we concluded that K rice oil from specific shipments had caused the Yusho. Now the question arose as to why the oil was toxic. To answer this question, we of course had to rely on other approaches than epidemiology. In mid-November 1968, the chemical study subgroup discovered, mainly by gas chromatographic analysis, that the canned K rice oil shipped on February 5, 1968, portions of which had been collected from some of the patients, was contaminated with about 2,000 ppm of Kanechlor. Kanechlor is a commercial brand of polychlorinated biphenyls containing 48 percent chlorine. Skin fat collected from the patients also contained Kanechlor (*10*).

In this discovery, Prof. K. Inagami of the department of food technology, Faculty of Agriculture, Kyushu University, and his associates played a leading role. A technological inspection of the K Company plant revealed that Kanechlor was used as an agent to transfer heat to the processed oil at a reduced pressure so that odorous substances could be removed (see chart). The Kanechlor must have leaked from the heating pipe in which it was being conveyed and contaminated the oil, since small openings were discovered in the old pipe.

To discover whether only the K rice oil shipped in February 1968 was contaminated with Kanechlor, the chemical study subgroup analyzed 109 samples of the bottled K rice oil which, according to the lot numbers, had been shipped between October 1967 and October 1968. This analysis revealed that only the samples shipped on February 7, 10, 12, 15, 17, 18, and 19 and on March 11, 18, and 21, 1968, were contaminated with Kanechlor. This contamination was most marked for the oil shipped on February 7; the contamination was rapidly reduced to nil after February 10 (*10*). No analysis could be made of bottled K rice oil shipped February 5 and 6 because of the lack of samples.

Similarly, 479 random samples of bottled rice oil were analyzed for chlorine content by using the X-ray fluorescence method with a count meter from a U.S. instrument manufacturer (the Applied Research Laboratory). Again, only those samples shipped from February 7 to 10 contained a large amount of chlorine (maximum 462 ppm). None of the oils shipped in other months were contaminated with more than a trace amount of chlorine. Thus, the results of chemical analysis coincided with those achieved by epidemiologic approaches.

Other Epidemiologic Results

Babies born to patients. In late October 1968 a pregnant patient with Yusho had a stillbirth in which the fetus was unusual; the skin, nails, and gingiva were a peculiar dark brown and stained. Similar fetuses were delivered by other patients with Yusho. Since no such birth or stillbirth was known to have been reported previously in Japan, the study group investigated these occurrences with particular care. The epidemiologic study

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subgroup questioned all female patients with Yusho and all unaffected women in the households of patients with Yusho about any pregnancies and deliveries in the period from February 15, 1968, to the end of the year.

Thirteen women—10 with Yusho, two without, and one with status unspecified—had given birth to 11 live born and two stillborn infants during the specified period. (Eight of the live born infants were males and three were females; one of the stillborn infants was a female and the sex of the other was unspecified.) Among these infants and fetuses, 10 had the characteristic grayish dark-brown stained skin at birth, five the dark-colored nails and gingiva, and nine an increased eye discharge. Women with Yusho accounted for all these deliveries, except for the delivery of one of the nine babies with eye symptoms.

The high incidence of these unusual phenomena among pregnant women with Yusho strongly suggested that the phenomena were caused by ingestion of the contaminated oil. The fact that nothing unusual was seen in the women's medical histories, health conditions, or use of drugs during pregnancy also supported such an interpretation.

All these facts, together with the results of chemical assays which showed that the placenta and the subcutaneous adipose tissue of the stillborn fetuses contained components of Kanechlor, prove that babies and fetuses were affected because of the women's ingestion of this particular complex compound (*4*).

The majority of the babies were small for their periods of gestation. As they grew older, their stained skin gradually faded. Postnatal growth curves for the affected babies were found to be similar in shape to the national standards when both sexes combined were compared. When only males were compared, the curves for the affected male infants were plainly lower than the national standard curves. A clear comparison between the curves for girls could not be made because of the limited number of female infants who were affected by Yusho.

The amount of contaminated rice oil that nine of the 13 mothers consumed during pregnancy was estimated to be 0.3 to 2.6 liters. No distinct dose-effect relationship could be demonstrated between oil consumption and the babies' symptoms because of the limited number of cases available for analysis. Nor could evidence be obtained in regard to any possible physical and mental retardation of the babies (*4*).

Growth of affected children. To discover whether Yusho disturbs children's growth, the affected school children, 23 boys and 19 girls, were compared in 1967, 1968, and 1969 with 719 healthy children matched in height and body weight. The gain in height or in body weight which each sick child showed before the poisoning, namely from 1967 to 1968, and the gain after the poisoning, from 1968 to 1969, were compared with the distribution of the corresponding gains seen in the corresponding control group.

The gains of the affected boys in both height and weight decreased significantly after the poisoning, while the affected girls showed no definite change in this respect. Because of the limited number of cases available for analysis, no significant relationship could be found between the clinical severity of the cases and the decrease in gains in height and weight.

Discussion

This unfortunate episode of rice oil poisoning taught us some important lessons. In the food industries of many countries, no effective administrative control and supervision is apparently exercised over the use of toxic substances like Kanechlor. These products do not contaminate the final products in normal operations, but they do when the operation is accidentally disturbed or carelessly conducted. In contrast to the case with food additives, too little care has been exercised to avoid the hidden risks in the use of such substances. A greatly increased effort is needed to improve this situation.

The polychlorinated biphenyls seem to be particularly hazardous in this respect because their use is still increasing in many industries, including food industries. If these substances are absorbed by the body, they will easily accumulate, gradually reaching a level high enough to exert adverse effects, since they have an extremely slow rate of metabolization or excretion. Many patients with Yusho are still tortured by the disease even after more than 2 years of treatment. A 1971 report has indicated that the subcutaneous fat of the patients still contains a large amount of polychlorinated biphenyls although their dermal conditions are slowly improving (*12*).

Recently, also, it has been demonstrated that wide environmental pollution with polychlorinated biphenyls is quietly growing in many countries (*11*). To prevent another epidemic of Yusho,

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which will break out on a global scale if the pollution worsens, radical efforts are urgently needed.

Our success in identifying the cause of the epidemic seems to have been due to many factors: (*a*) common epidemiologic principles were honestly followed, (*b*) local health departments and the patients themselves cooperated fully, (*c*) the causal agent was not multiple, but single, (*d*) contamination of the oil with Kanechlor was temporary and marked, (*e*) the latent period of the epidemic was several months, and the epidemic was relatively short, (*f*) Yusho had characteristic symptoms, allowing easy differentiation from other diseases, (*g*) some of the oil used by the patients, along with the original containers, were still available, and (*h*) some persons who bought the oil as a group had contracted the disease. All these factors facilitated our study.

Epidemiologic study of an illness or of adverse effects which were more chronic, less peculiar, which progressed less noticeably than Yusho, and were caused by multiple agents would present greater difficulties.

We need to develop more sensitive and more efficient epidemiologic methods. We must, moreover, exert constant efforts to make our epidemiologic information more accurate, quantitatively and qualitatively, by using the methodology of other disciplines when it seems necessary for our purposes.

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Yusho, a poisoning caused by rice oil contaminated with polychlorinated biphenyls. HSMHA Health Reports, Vol. 86, December 1971, pp. 1083–1091.

A peculiar skin disease, Yusho, was observed in residents of Fukuoka-Ken, Japan, in October 1968, which later spread over the western part of the country. By use of basic epidemiologic methods, the cause of the disease was shown to be a specific brand of rice oil that had become contaminated with a heat transfer agent during processing. This agent, Kanechlor, is a complex mixture of polychlorinated biphenyls.

Women who consumed the contaminated oil delivered babies or fetuses with peculiar dark-brown stained skin, nails, and gingiva. The poisoning significantly decreased the growth of the boys who were affected, while the girls who were affected showed no such change. Many of the persons who contracted the disease are still tortured by it even 2 or more years later, despite all possible treatment methods.

In view of the slow recovery from the poisoning and the increasing pollution of the environment with these compounds on a global scale, radical efforts must be made to avoid the hidden risks of contamination.

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An Epidemiologic Study of Amputees in the East Harlem Community

COURTNEY B. WOOD, M.D., M.P.H., ROBERTA S. VOLANTE, R.N., M.A., and ROBERT BERENSON, M.M.S.

MASS screening for one or a number of diseases is classic public health methodology. The effectiveness of screening programs depends on several factors; notably, their success in reaching high-risk groups, the extent to which the diseases identified are subject to therapeutic attack, and the availability and accessibility of followup care. To the extent that mass screening or any like programs fulfill these criteria, they perform a valuable health service within a community; that is, they have the potential of leading to reductions in morbidity, mortality, or disability.

The problem often is, however, that existing programs for one reason or another are weak, especially in reaching the desired population and in the area of followup care. The question arises as to whether there is any way of using the valuable experience of traditional public health methodology to reach the community with an approach that would be more responsive to its health needs and more effective in meeting these needs.

To that end we proposed and executed a pilot study that was based on the resolve of the department of community medicine, Mount Sinai School of Medicine, "to identify and solve health problems in the community." We decided to select a disease that was easily identifiable by the general population and for which there was a relatively simple and highly visible solution. The approach was to be one of search and service, with a view to developing a network of indigenous epidemiologic intelligence within a given community; namely, East Harlem.

Amputation of an extremity was selected as the most recognizable disease entity for which followup services are accessible, available, and effective. In this study an amputee is defined as a person lacking part or all of one or more extremities.

Objective and Method

The project had the following objectives:

1. To identify all amputees in the East Harlem community and to review their medical and socioeconomic characteristics
2. To assess the current need

Dr. Wood is associate professor of community medicine. Mrs. Volante is community nursing consultant and instructor in community medicine, and Mr. Berenson is a fourth year medical student, Mount Sinai School of Medicine of the City University of New York. *The New York Community Trust provided funds for the study.* *Tearsheet requests to Courtney B. Wood, M.D., Mount Sinai School of Medicine, City University of New York, 19 E. 98th St., New York City 10029.*

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analysis of these 32 cases will be presented to illustrate these peculiarities.

Familial aggregation. The 325 patients belonged to 112 families, a result indicating a distinct familial aggregation, which is considered one of the most important characteristics of Yusho.

Time of occurrence. Ninety-nine percent of the patients were affected during 1968, and 55 percent of the cases were concentrated in the 3 months from June to August (table 2). The monthly distribution of the cases was alike for both sexes. These results indicated that exposure to the causal agent or agents was probably temporal and indifferent to sex.

Sex and age. Distribution of the 325 patients by sex and age revealed no significant sex difference (table 3). Age-specific incidence rates per 100,000, calculated by using the census population of 1965, indicated lower risks for both males and females in the age group over 60 years, but again no marked sex difference (table 4).

Geograp distribution. We calculated crude incidence rates of Yusho for three large cities (Kitakyushu, Fukuoka, and Ohmuta) and for the jurisdictional areas of the 22 local health departments of Fukuoka-Ken. A very marked difference was noted in their rates. Ten health departments reported no cases of Yusho while extremely high incidence rates per 100,000 were noted for the area covered by the health department of Soeda (58.9) and that covered by the health department of Tagawa (44.5); intermediately high rates, from 7 to 14, were observed for the three large cities and the area of the Kasuya health department.

There are many coal mines, most of which have recently been abandoned, in the jurisdictional areas of the Soeda and Tagawa health departments. The city of Kitakyushu has big steel and chemical industries, Fukuoka is a seat of commerce, and Ohmuta has big coal mines and chemical industries. In contrast, the Kasuya health de-

Table 2. Distribution of 325 patients with Yusho diagnosed by January 20, 1969, by sex and month when symptoms appeared

Month	Males		Females		Total	
	Number	Percent	Number	Percent	Number	Percent
Before 1968	2	1.3	2	1.2	4	1.2
1968						
January	0	.0	0	.0	0	.0
February	4	2.5	6	3.6	10	3.1
March	8	5.1	12	7.2	20	6.2
April	15	9.5	10	6.0	25	7.7
May	18	11.4	15	9.0	33	10.2
June	23	14.6	33	19.8	56	17.2
July	27	17.1	32	19.2	59	18.1
August	34	21.5	30	18.0	64	19.7
September	17	10.8	12	7.2	29	8.9
October	8	5.1	14	8.4	22	6.8
November	1	.6	1	.6	2	.6
December	1	.6	0	.0	1	.3
Total	158	100.0	167	100.0	325	100.0

Table 3. Distribution of 325 patients with Yusho diagnosed by January 20, 1969, by sex and age group

Age group (years)	Males		Females		Total	
	Number	Percent	Number	Percent	Number	Percent
0–9	37	23.4	27	16.2	64	19.7
10–19	38	24.1	28	16.8	66	20.3
20–29	28	17.7	36	21.6	64	19.7
30–39	30	19.0	39	23.4	69	21.2
40–49	11	7.0	23	13.8	34	10.5
50–59	9	5.7	11	6.6	20	6.2
60–69	4	2.5	3	1.8	7	2.2
70–79	1	.5	0	.0	1	.3
Total	158	100.0	167	100.0	325	100.0

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