REPORT TO

MONSANTO COMPANY

FOUR-DAY FISH TOXICITY STUDIES WITH AROCLOR 1242, AROCLOR 1254, AND AROCLOR 1260 IN BLUEGILLS AND CHANNEL CATFISH

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I. Introduction

At the request of Monsanto Company, four-day fish toxicity studies using bluegills (Lepomis macrochirus) and channel catfish (Ictalurus punctatus) were conducted with the following three materials:

1. Aroclor 1242, Lot No. AK-255
2. Aroclor 1254, Lot No. AK-38
3. Aroclor 1260, Lot No. AK-3

Water samples were collected under the following three bioassay vessel conditions:

1. Aroclor and fish exposure
2. Blank exposure (Aroclor concentration under Aroclor and fish exposure, but without fish).
3. Control exposure (fish but no Aroclor).

The water collection schedule is presented in Table I.

TABLE I

TEST MATERIALS: Aroclor 1242, Aroclor 1254, and Aroclor 1260

Four-Day Fish Toxicity Studies

Bioassay Vessel Conditions	Water Collection Intervals (Hours) 0	1	2	3	6	12	24	48	72	96
Aroclor-Fish Exposure (Aroclor plus Fish)										
No Survival Concentration	X									
Partial Survival Concentration	X									
Total Survival Concentration	X	X	X	X	X	X	X	X	X	X
Blank Exposure (Aroclor but no Fish)										
No Survival Concentration	X	X	X	X	X	X	X	X	X	X
Partial Survival Concentration	X	X	X	X	X	X	X	X	X	X
Total Survival Concentration	X	X	X	X	X	X	X	X	X	X
Control Exposure (Fish but no Aroclor)	X	X	X	X	X	X	X	X	X	X

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II. Summary

The results of the four-day fish toxicity studies are summarized below.

Test Material	Four-Day TL_{50} (ppm) Bluegills	Channel Catfish
Aroclor 1242	0.24	0.13
Aroclor 1254	> 100.0	≈ 0.20
Aroclor 1260	> 100.0	> 100.0

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

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III. Procedure

The procedure was generally the same for each sample and species of fish.

Healthy, young fish with an average length of 35 to 75 millimeters were used as test animals. All fish were kept under observation for a period of not less than ten days prior to experimental use. During this period, the fish were observed for general health and suitability as test animals. The fish were held in large aerated stock tanks and were fed frozen brine shrimp or Purina Trout Chow #2 until three days immediately prior to testing. The channel catfish and bluegills were maintained at a temperature of 18° C. All stock tanks and bioassay vessels contained reconstituted, deionized water. The following compounds were added in the amounts stated per liter of deionized water:

30 mg calcium sulfate
30 mg magnesium sulfate
48 mg sodium bicarbonate
2 mg potassium chloride

Preliminary screening was conducted in order to find the general levels of toxicity of Aroclor 1242, Aroclor 1254 and Aroclor 1260. Once these levels had been determined, three to ten concentrations of each test material were selected for the respective bioassays. Bioassay vessels were filled with 12.5 liters of well aerated reconstituted water. Ten fish were tested at each concentration.

After a 24-hour acclimatization period, calculated amounts of each Aroclor were dispersed into the respective vessels. The test dilutions were prepared within one hour of the bioassay.

Following the introduction of the test material, the fish were observed for 96 hours, during which time all mortalities and/or untoward behavioral reactions were recorded. The concentration of dissolved oxygen was determined for all test solutions in which mortalities occurred to determine that adequate oxygen was available to the test specimens.* Concentrations of dissolved oxygen of 4 mg/l (4 ppm) for bluegills and channel catfish were considered to be minimal for valid testing. The pH of the test solution was also determined when mortalities were recorded. All fish were collected, rinsed with deionized water, and preserved by freezing for future residue analysis.

At the end of the observation period, the four-day median tolerance limits (TL_{50}'s) for each Aroclor were calculated employing the technique of Litchfield and Wilcoxon**.

In addition, water samples were collected at selected time intervals as listed in Table I.

* Hach OX-4-P Dissolved Oxygen Test Kit, Hach Chemical Company, Ames, Iowa.

** J. T. Litchfield, Jr. and F. Wilcoxon, "A Simplified Method of Evaluating Dose-Effect Experiments," J. Pharm. & Exp. Ther. 96, 99 (1949).

IV. Results

A. Survival, DO, and pH Data

The survival, dissolved oxygen content, and pH data for bluegills and channel catfish are presented in Tables II through VII.

TABLE II

TEST MATERIAL: Aroclor 1242

Four-Day Fish Toxicity Study - Bluegills

Survival, DO, and pH Data

Concentration* (ppm)	Number of Survivors (Hours) 1-6	24	48	72	96	Percent Survival	Dissolved Oxygen (ppm) and pH 1-6 Hrs. DO	pH	24 Hrs. DO	pH	48 Hrs. DO	pH	72 Hrs. DO	pH	96 Hrs. DO	pH
0.01	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
0.10	10	10	10	10	7	70	-	-	-	-	-	-	-	-	5	7
0.18	10	10	10	9	7	70	-	-	-	-	-	-	5	7	5	7
0.32	10	10	10	8	6	60	-	-	-	-	-	-	5	7	5	7
0.56	10	9	5	3	1	10	-	-	5	7	4	7	4	7	4	7
1.0	10	4	0	-	-	0	-	-	5	7	5	7	-	-	-	-

Four-Day TL_{50} = 0.24 ppm

Note: The test material was dispensed into the bioassay vessels in the form of a 0.1 percent (w/v) dilution in acetone.

- = Not determined.

* Concentrations are expressed in terms of Aroclor 1242.

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TABLE III

TEST MATERIAL: Aroclor 1254

Four-Day Fish Toxicity Study - Bluegills

Survival, DO, and pH Data

Concentration* (ppm)	Number of Survivors (Hours) 1-6	24	48	72	96	Percent Survival	Dissolved Oxygen (ppm) and pH 1-6 Hrs. DO	pH	24 Hrs. DO	pH	48 Hrs. DO	pH	72 Hrs. DO	pH	96 Hrs. DO	pH
0.01	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
0.18	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
0.32	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
0.56	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
1.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
1.8	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
3.2	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
5.6	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
10.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
100.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-

Four-Day TL_{50} > 100.0 ppm

Note: The test material was dispensed into the bioassay vessels in the form of 0.1, 1.0, and 10.0 percent (w/v) dilutions in acetone for levels 0.01-0.56 ppm, 1.0-10.0 ppm, and 100.0 ppm, respectively.

- = Not determined.

* Concentrations are expressed in terms of Aroclor 1254.

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TABLE IV

TEST MATERIAL: Aroclor 1260

Four-Day Fish Toxicity Study - Bluegills

Survival, DO, and pH Data

Concentration* (ppm)	Number of Survivors (Hours) 1-6	24	48	72	96	Percent Survival	Dissolved Oxygen (ppm) and pH 1-6 Hrs. DO	pH	24 Hrs. DO	pH	48 Hrs. DO	pH	72 Hrs. DO	pH	96 Hrs. DO	pH
10.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
100.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-

Four-Day TL_{50} > 100.0 ppm

Note: The test material was dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) dilution in acetone.

- = Not determined.

* Concentrations are expressed in terms of Aroclor 1260.

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TABLE V

TEST MATERIAL: Aroclor 1242

Four-Day Fish Toxicity Study - Channel Catfish

Survival, DO, and pH Data

Concentration* (ppm)	Number of Survivors (Hours) 1-6	24	48	72	96	Percent Survival	Dissolved Oxygen (ppm) and pH 1-6 Hrs. DO	pH	24 Hrs. DO	pH	48 Hrs. DO	pH	72 Hrs. DO	pH	96 Hrs. DO	pH
0.032	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
0.056	10	10	10	10	8	80	-	-	-	-	-	-	-	-	5	7
0.10	10	10	8	7	6	60	-	-	-	-	6	7	6	7	5	7
0.18	10	10	2	1	0	0	-	-	-	-	6	7	6	7	5	7
0.32	10	7	2	1	1	10	-	-	7	7	6	7	6	7	-	-
0.56	10	2	0	-	-	0	-	-	7	7	6	7	-	-	-	-
1.0	10	0	-	-	-	0	-	-	7	7	-	-	-	-	-	-

Four-Day TL_{50} = 0.13 ppm

Note: The test material was dispensed into the bioassay vessels in the form of a 0.1 percent (w/v) dilution in acetone.

- = Not determined.

* Concentrations are expressed in terms of Aroclor 1242.

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TABLE VI

TEST MATERIAL: Aroclor 1254

Four-Day Fish Toxicity Study - Channel Catfish

Survival, DO, and pH Data

Concentration* (ppm)	Number of Survivors (Hours) 1-6	24	48	72	96	Percent Survival	Dissolved Oxygen (ppm) and pH 1-6 Hrs. DO	pH	24 Hrs. DO	pH	48 Hrs. DO	pH	72 Hrs. DO	pH	96 Hrs. DO	pH
0.10	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
0.18	10	10	10	9	6	60	-	-	-	-	-	-	6	7	5	7
0.32	10	10	10	9	3	30	-	-	-	-	-	-	7	7	6	7
0.56	10	9	9	6	1	10	-	-	8	7	7	7	7	7	7	7
1.0	10	9	8	2	1	10	-	-	8	7	7	7	7	7	7	7
1.8	10	10	10	2	2	20	-	-	-	-	-	-	6	7	-	-
3.2	10	10	8	0	-	0	-	-	-	-	6	7	6	7	-	-
5.6	10	10	7	2	2	20	-	-	-	-	6	7	6	7	-	-
10.0	10	10	5	1	1	10	-	-	-	-	6	7	6	7	-	-

Four-Day TL_{50}=0.20 ppm

Note: The test material was dispensed into the bioassay vessels in the form of 0.1 and 1.0 percent (w/v) dilutions in acetone for levels 0.1 ppm through 0.56 ppm and 1.0 ppm through 10.0 ppm, respectively.

- = Not determined.

* Concentrations are expressed in terms of Aroclor 1254.

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TABLE VII

TEST MATERIAL: Aroclor 1260

Four-Day Fish Toxicity Study - Channel Catfish

Survival, DO, and pH Data

Concentration* (ppm)	Number of Survivors (Hours) 1-6	24	48	72	96	Percent Survival	Dissolved Oxygen (ppm) and pH 1-6 Hrs. DO	pH	24 Hrs. DO	pH	48 Hrs. DO	pH	72 Hrs. DO	pH	96 Hrs. DO	pH
10.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
100.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-

Four-Day TL_{50} > 100.0 ppm

Note: The test material was dispensed into the bioassay vessels in the form of a 10.0% (w/v) dilution in acetone.

- = Not determined.

* Concentrations are expressed in terms of Aroclor 1260.

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B. Reactions

The reactions noted following introduction of Aroclor 1242, Aroclor 1254, and Aroclor 1260 are presented for bluegills and channel catfish in Tables VIII through XIII, respectively.

TABLE VIII

TEST MATERIAL: Aroclor 1242

Four-Day Fish Toxicity Study-Bluegills

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
0.01	None	-	-	-
0.10	Hypoactivity	96	No recovery noted	96
0.18	Hypoactivity	72	No recovery noted	72 - 96
0.32	Hypoactivity	12	No recovery noted	72 - 96
0.56	Hypoactivity	1 - 6	No recovery noted	24 - 96
	Loss of equilibrium	12	36	
1.0	Hypoactivity	1 - 6	Until	24 - 48
	Loss of equilibrium	1 - 6	death	
	Prostration	12		

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TABLE IX

TEST MATERIAL: Aroclor 1254

Four-Day Fish Toxicity Study-Bluegills

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
0.01	None	-	-	-
0.18	None	-	-	-
0.32	None	-	-	-
0.56	None	-	-	-
1.0	None	-	-	-
1.8	None	-	-	-
3.2	None	-	-	-
5.6	None	-	-	-
10.0	None	-	-	-
100.0	None	-	-	-

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TABLE X

TEST MATERIAL: Aroclor 1260

Four-Day Fish Toxicity Study-Bluegills

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
10.0	Hypoactivity	72	No recovery noted	-
100.0	Hypoactivity	72	No recovery noted	-

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TABLE XI

TEST MATERIAL: Aroclor 1242

Four-Day Fish Toxicity Study-Channel Catfish

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
0.032	None	-	-	-
0.056	Hypoactivity	96	No recovery noted	96
	Loss of equilibrium	96		
0.10	Hypoactivity	48	No recovery noted	48 - 96
	Loss of equilibrium	48	24	
0.18	Hypoactivity	48	Until death	48 - 96
	Prostration	72		
0.32	Hypoactivity	1 - 6	No recovery noted	
	Prostration	24	24	24 - 72
	Surfacing	48	24	
	Vertical swimming	48	24	
0.56	Hypoactivity	1 - 6	Until death	24 - 48
	Prostration	24		
	Vertical swimming	24		
1.0	Hypoactivity	1 - 6	Until death	24
	Prostration	1 - 6		
	Vertical swimming	1 - 6		

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TABLE XII

TEST MATERIAL: Aroclor 1254

Four-Day Fish Toxicity Study-Channel Catfish

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
0.10	None	-	-	-
0.18	Hypoactivity	72	No recovery noted	72 - 96
0.32	Hypoactivity	72	No recovery noted	72 - 96
0.56	Hypoactivity	24	No recovery noted	24 - 96
1.0	Hypoactivity	24	No recovery noted	24 - 96
	Prostration	24	48	
1.8	Hypoactivity	24	No recovery noted	72
	Prostration	24	48	
3.2	Hypoactivity	24	Until death	48 - 72
	Prostration	24	48	
5.6	Hypoactivity	24	No recovery noted	48 - 72
	Prostration	24	48	
10.0	Hypoactivity	24	No recovery noted	48 - 72
	Prostration	24	48	

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TABLE XIII

TEST MATERIAL: Aroclor 1260

Four-Day Fish Toxicity Study-Channel Catfish

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
10.0	Hypoactivity	72	No recovery noted	-
100.0	Hypoactivity	72	No recovery noted	-

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