REPORT TO

MONSANTO COMPANY

FOUR-DAY STATIC FISH TOXICITY STUDIES WITH AROCLOR 1221, AROCLOR 5432, AROCLOR 5442, AROCLOR 5460, AND MCS 1016 IN BLUEGILLS AND CHANNEL CATFISH

JANUARY 12, 1972

BBT NO. A9580

I. Introduction

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

1. Aroclor 1221
2. Aroclor 5432
3. Aroclor 5442
4. Aroclor 5460
5. MCS 1016

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

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

The water collection schedule is presented in Table I.

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TABLE I

TEST MATERIALS: Aroclor 1221, Aroclor 5432, lor 5442, Aroclor 5460 and MCS 1016

Four-Day Static Fish Toxicity Studies

Water Collection Intervals

Bioassay Vessel Conditions	Hours 0	1	2	3	6	12	24	48	72	96
Test Material Fish Exposure (Test Material 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 (Test Material 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 Test Material)	X	X	X	X	X	X	X	X	X	X

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II. Summary

The result of the four-day static fish toxicity studies using bluegills and channel catfish are summarized below.

Test Material	Four-Day Static TL50 (ppm) Bluegills	Channel Catfish
Aroclor 1221	0.23 ppm	3.34 ppm
Aroclor 5432	>100.0 ppm	>100.0 ppm
Aroclor 5442	>100.0 ppm	>100.0 ppm
Aroclor 5460	>100.0 ppm	>100.0 ppm
MCS 1016	8.65 ppm	0.75 ppm

Respectfully submitted,

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jg:bp

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III. Investigational Procedure

Healthy fingerlings with an average length of 13 to 73 millimeters were used as test animals. All fish were kept under observation for general health and suitability as test animals for a period of not less than 14 days prior to experimental use. The fish were held in stock tanks and were fed frozen brine shrimp or Purina Trout Chow #2 until three days prior to testing. The fish were maintained at a temperature of 18°C.

All stock tanks and bioassay vessels contained reconstituted 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 toxic level of each test material. Once this level had been determined, three to five concentrations of each test material were selected for the respective bioassays. Bioassay vessels were filled with 12.5 liters of reconstituted water. Ten fish were tested at each concentration.

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

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behavioral reactions were recorded. The concentration of dissolved oxygen was measured for all solutions in which mortalities occurred in order to determine if the test water contained adequate oxygen.* Concentrations of dissolved oxygen of 4 mg/L (4 ppm) for warm water fish (bluegills and channel catfish) were considered adequate for 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) of each test material were calculated, if possible, employing the technique of Litchfield and Wilcoxon**.

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

* Hach CK-2-P Dissolved Oxygen Test Kit, Hach Chemical Company, Ames, Iowa.

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

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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 U through X1.

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TABLE II

TEST MATERIAL: Aroclor 1221

Four-Day Static 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 Hr. DO	pH	24 Hr. DO	pH	48 Hr. DO	pH	72 Hr. DO	pH	96 Hr. DO	pH
0.10	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
0.18	10	9	6	6	6	0	-	-	5.6	6.5	5.8	6.8	-	-	-	-
0.32	10	9	4	3	3	30	-	-	6.2	6.8	5.4	6.9	3.6	6.6	-	-
0.56	10	5	1	0	0	0	-	-	7.2	7.0	6.8	7.0	5.3	7.0	-	-
1.00	10	0	0	0	0	0	-	-	6.8	6.8	-	-	3.1	6.9	-	-

Four-Day TL_{50} = 0.25 ppm
95% Confidence Limits of TL_{50} = 0.16 - 0.32 ppm

- = Not determined.

* The test material was dispensed into the bioassay vessels in the form of a 0.1 percent (v/v) dilution in acetone. Concentrations are expressed in terms of Aroclor 1221.

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TABLE III

TEST MATERIAL: Aroclor 5432

Four-Day Static 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 Hr. DO	pH	24 Hr. DO	pH	48 Hr. DO	pH	72 Hr. DO	pH	96 Hr. DO	pH
1.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
10.0	10	10	10	10	9	90	-	-	-	-	-	-	-	-	1.6	6.8
100.0	10	10	10	10	9	90	-	-	-	-	-	-	-	-	1.8	6.8

Four-Day TL_{50} > 100.0 ppm

- = Not determined.

* The test material was dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) dilution in acetone. Concentrations are expressed in terms of Aroclor 5432.

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TABLE IV

TEST MATERIAL: Aroclor 5442

Four-Day Static Fish Toxicity Study - Bluegills

Survival, DO, and pH Data

Concentration[a] (ppm)	Number of Survivors Hours: 1-6	24	48	72	96	Percent Survival	Dissolved Oxygen (ppm) and pH 1-6 Hr. DO	pH	24 Hr. DO	pH	48 Hr. DO	pH	72 Hr. DO	pH	96 Hr. DO	pH
1.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
10.0	10	10	10	10	9	90	-	-	-	-	-	-	-	-	8.9	6.8
100.0	10	10	10	10	9	90	-	-	-	-	-	-	-	-	2.3	6.7

Four-Day TL_{50} > 100.0 ppm

- = Not determined.

a The test material was dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) dilution in acetone. Concentrations are expressed in terms of Aroclor 5442.

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TABLE V

TEST MATERIAL: Aroclor 5460

Four-Day Static Fish Toxicity Study - Bluegills

Survival, DO, and pH Data

Concentrations[a] (ppm)	Number of Survivors Hours: 1-6	24	48	72	96	Percent Survival	Dissolved Oxygen (ppm) and pH 1-6 Hr. DO	pH	24 Hr. DO	pH	48 Hr. DO	pH	72 Hr. DO	pH	96 Hr. DO	pH
1.0	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

- = Not determined.

a The test material was dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) dilution in acetone. Concentrations are expressed in terms of Aroclor 5460.

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TABLE VI

TEST MATERIAL: MCS 1016

Four-Day Static Fish Toxicity Study - Bluegills

Survival, DO, and pH Data

Concentration[a] (ppm)	Number of Survivors Hours: 1-6	24	48	72	96	Percent Survival	Dissolved Oxygen (ppm) and pH 1-6 Hr. DO	pH	24 Hr. DO	pH	48 Hr. DO	pH	72 Hr. DO	pH	96 Hr. DO	pH
0.10	10	10	9	9	9	90	-	-	-	-	5.7	7.6	-	-	-	-
0.18	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
0.32	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
0.56	10	9	9	9	9	90	-	-	6.9	7.4	-	-	-	-	-	-
1.00	10	2	0	0	0	0	-	-	5.5	7.7	5.5	7.6	-	-	-	-

Four-Day TL_{50} = 0.65 ppm

- = Not determined.

[a] The test material was dispensed into the bioassay vessels in the form of a 0.1 percent (w/v) dilution in acetone. Concentrations are expressed in terms of MCS 1016.

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TABLE VII

TEST MATERIAL: Aroclor 1221

Four-Day Static 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 Hr. DO	pH	24 Hr. DO	pH	48 Hr. DO	pH	72 Hr. DO	pH	96 Hr. DO	pH
0.56	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
1.00	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
1.80	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
3.20	10	6	6	6	6	60	-	-	4.7	6.5	-	-	-	-	-	-
5.60	0	3	2	0	0	0	7.8	7.8	7.0	6.9	6.8	6.7	5.4	6.9	-	-

Four-Day TL_{50} = 3.54 ppm

- = Not determined.

* The test material was dispensed into the bioassay vessels in the form of a 1.0 percent (w/v) dilution in acetone. Concentrations are expressed in terms of Aroclor 1221.

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TABLE VIII

TEST MATERIAL: Aroclor 5432

Four-Day Static Fish Toxicity Study - Channel Catfish

Survival, DO, and pH Data

Concentrations* (ppm)	Number of Survivors Hours: 1-6	24	48	72	96	Percent Survival	Dissolved Oxygen (ppm) and pH 1-6 Hr. DO	pH	24 Hr. DO	pH	48 Hr. DO	pH	72 Hr. DO	pH	96 Hr. DO	pH
1.0	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

- = Not determined.

* The test material was dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) dilution in acetone. Concentrations are expressed in terms of Aroclor 5432.

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TABLE IX

TEST MATERIAL: Aroclor 5442

Four-Day Static 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 Hr. DO	pH	24 Hr. DO	pH	48 Hr. DO	pH	72 Hr. DO	pH	96 Hr. DO	pH
1.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
10.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
100.0	10	10	10	10	8	80	-	-	-	-	-	-	-	-	1.3	6.8

Four-Day TL_{50} > 100.0 ppm

- Not determined.

* The test material was dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) dilution in acetone. Concentrations are expressed in terms of Aroclor 5442.

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TABLE X

TEST MATERIAL: Aroclor 5460

Four-Day Static Fish Toxicity Study - Channel Catfish

Survival, DO, and pH Data

Concentration[a] (ppm)	Number of Survivors Hours: 1-6	24	48	72	96	Percent Survival	Dissolved Oxygen (ppm) and pH 1-6 Hr. DO	pH	24 Hr. DO	pH	48 Hr. DO	pH	72 Hr. DO	pH	96 Hr. DO	pH
1.0	10	10	10	9	9	90	-	-	-	-	-	-	2.6	7.0	-	-
10.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
100.0	10	10	9	9	7	70	-	-	-	-	3.7	7.2	-	-	1.0	6.9

Four-Day TL_{50} > 100.0 ppm

- = Not determined.

a The test material was dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) dilution in acetone. Concentrations are expressed in terms of Aroclor 5460.

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TABLE XI

TEST MATERIAL, MCS 1016

Four-Day Static Fish Toxicity Study - Channel Catfish

Survival, DO, and pH Data

Concentration[a] (ppm)	Number of Survivors Hours: 1-6	24	48	72	96	Percent Survival	Dissolved Oxygen (ppm) and pH 1-6 Hr. DO	pH	24 Hr DO	pH	48 Hr. DO	pH	72 Hr. DO	pH	96 Hr. DO	pH
0.10	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	-	-	-	-	-	-	-	-	-	-
1.00	10	7	0	0	0	0	-	-	5.3	7.2	3.0	7.4	-	-	-	-

Four-Day TL_{50} = 0.75 ppm

- = Not determined.

[a] The test material was dispensed into the bioassay vessels in the form of a 0.1 percent (w/v) dilution in acetone. Concentrations are expressed in terms of MCS 1016.

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B. Reactions

The reactions noted following introduction of the test materials are presented in Tables XII through XXI.

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TABLE XII

TEST MATERIAL: Aroclor 1221

Four-Day Static 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)
0.10	None	-	-	-
0.18	Swimming: Gyrating	12	24	24, 48
	Quiescent	48	24	
0.32	Quiescent	48	24	24-72
0.56	Surfacing	24	No recovery noted	24-72
	Twitching	24	24	
	Quiescent	48	No recovery noted	
1.00	Motionless: Flaccidity	1-6	24	24, 72
	Surfacing	1-6	24	
	Swimming: Erratic	12	Until	
	Gyrating	48	death	
	Respiratory rate: Rapid	24		

DSM 004022

M 30164

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TABLE XIII

TEST MATERIAL: Aroclor 5432

Four-Day Static 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)
1.0	None	-	-	-
10.0	Quiescent	72	No recovery noted	96
	Surfacing	96		
100.0	Quiescent	72	No recovery noted	96
	Surfacing	96		

DSM 004023

M 30165

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TABLE XIV

TEST MATERIAL: Aroclor 5442

Four-Day Static 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)
1.0	Quiescent	96	No recovery noted	-
10.0	Quiescent	72	No recovery noted	96
	Surfacing	96		
100.0	Quiescent	72	No recovery noted	96
	Motionless; Flaccidity	96		

DSM 004024

M 30166

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TABLE XV

TEST MATERIAL: Aroclor 5442

Four-Day Static Fish Toxicity Study - Pimephales

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
1.0	None	-	-	-
10.0	Quiescent	72	No recovery noted	-
100.0	Quiescent	24	No recovery noted	-

DSM 004025

M 30167

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TABLE XVI

TEST MATERIAL: MCS 1016

Four-Day Static 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)
0.18	None	-	-	48
0.18	None	-	-	-
0.32	Quiescent	96	No recovery noted	-
0.56	Quiescent	48	No recovery noted	24
1.00	Twitching	24	[illegible]	24, 48
	Swimming: On side	24	death	

DSM 004026

M 30168

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TABLE XVII

TEST MATERIAL: Aroclor 1221

Four-Day Static 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)
0.56	None	-	-	-
1.00	None	-	-	-
1.80	Quiescent	1-6	24	-
	Surfacing	1-6		
	Swimming; Gyrating	1-6		
3.20	Quiescent	1-6	No recovery noted	24
	Motionless; Flaccidity	1-6		
	Swimming; On side	48		
5.60	Quiescent	1-6	Until death	1-72
	Motionless; Flaccidity	1-6		
	Surfacing	1-6		

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TABLE XVIII

TEST MATERIAL. Aroclor 5432

Four-Day Static 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)
1.0	Quiescent	96	No recovery noted	-
	Surfacing	96		
10.0	Quiescent	72	No recovery noted	-
	Surfacing	96		
100.0	Quiescent	72	No recovery noted	-
	Surfacing	96		

DSM 004028

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TABLE XIX

TEST MATERIAL: Aroclor 5432

Four-Day Static 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)
1.0	Quiescent	96	No recovery noted	-
	Surfacing	96		
10.0	Quiescent	72	No recovery noted	-
	Surfacing	96		
100.0	Quiescent	72	No recovery noted	96
	Surfacing	96		

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TABLE XX

TEST MATERIAL: Aroclor 5460

Four-Day Static 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)
1.0	Quiescent	72	No recovery noted	72
10.0	Quiescent	48	No recovery noted	-
100.0	Quiescent	24	No recovery noted	48, 96
	Surfacing	96		

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TABLE XXI

TEST MATERIAL: MCS 1016

Four-Day Static Fish Toxicity Study - Channel Catfish

Summary of Reactions

Concentration (ppm)	Reaction		Time of Onset Following Dose Administration (hours)	Duration of [illegible]tion (hours)	Time of Death Following Dose Administration (hours)
0.10	None		-	-	-
0.18	None		-	-	-
0.32	Quiescent		96	No recovery noted	-
0.56	Quiescent		72	No recovery noted	-
1.00	Quiescent		1-6	Until death	24, 48
	Swimming:	Inverted	1-6		
		On side	1-6		

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