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REPORT TO

MONSANTO COMPANY

FOUR-DAY FISH TOXICITY STUDIES ON SEVEN MATERIALS

IBT NO. A7264

DSW 035823

STLCOPCB4019647

REPORT TO

MONSANTO COMPANY

FOUR-DAY FISH TOXICITY STUDIES ON
SEVEN MATERIALS

IBT NO. A7204

I. Introduction

At the request of Monsanto Company, four-day fish toxicity studies in Rainbow Trout (Salmo gairdneri) and Bluegills (Lepomis macrochirus) were conducted on the following seven materials:

1) Aroclor 1242, Lot #AK-255
2) Aroclor 1254, Lot #AK-38
3) Aroclor 1260, Lot #AK-3
4) Aroclor 5460, Lot #AK-390
5) Toxaphene
6) Technical DDT (2-1/2#), B0903604
7) Halowax 1099, 52 percent Cl

STLCOPCB4019648

II. Summary

The results of the four-day fish toxicity studies are summarized below. Due to the paucity of one of the seven test materials (Halowax 1099), complete data are not available and the figures in parentheses represent only range-finding data.

Test Material	Four-Day LC_{50} (ppm) Rainbow Trout	Bluegill
Aroclor 1242	> 100.0	0.66
Aroclor 1254	> 100.0	0.66
Aroclor 1260	> 100.0	> 100.0
Aroclor 5460	> 100.0	> 100.0
Toxaphene	0.0080	0.0056
Technical DDT	0.0052	0.0076
Halowax 1099	(10-100)	(> 100.0)

Respectfully submitted,

INDUSTRIAL BIO-TEST LABORATORIES, INC.

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III. Procedure

The procedure was generally the same for both bluegills and rainbow trout with each test material.

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 until three days immediately prior to testing. The rainbow trout were maintained at a temperature of 13°C and the bluegills at a temperature of 24°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 level of toxicity before beginning the actual bioassay. Once this level had been determined one to five concentrations were selected for the actual bioassay. Bioassay vessels lined with disposable polyethylene bags were filled with ten liters of well aerated reconstituted water. To evaluate the selected concentrations, ten trout were placed in each bioassay vessel. In the case of the bluegills only five fish were placed in each bioassay vessel in order to insure adequate dissolved oxygen concentration (D.O.)

in the warmer water; therefore two groups of five bluegills were tested at each selected concentration. An untreated control group consisting of 10 fish was also employed.

After a 24-hour acclimatization period, calculated amounts of test material necessary to produce the desired one to five concentration levels were dispensed into the respective vessels. The test solutions 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 insure that adequate oxygen was available to the test specimens*. Concentrations of dissolved oxygen of 4 mg/l (4 ppm) for warm water fish (bluegill) and 5 mg/l (5 ppm) for cold water fish (trout) were considered to be minimal for valid testing. The pH of the test solution was also determined when mortalities were recorded.

At the end of the observation period all data were collected and arrangements were made to calculate the 96-hour LC_{50} employing the technique of Litchfield and Wilcoxon**.

* Standard Methods for the Examination of Water and Wastewaters, Twelfth Edition, American Public Health Association, Inc., 1790 Broadway, New York, N. Y.: Part III, pp. 406-410.

** J. T. Litchfield, Jr. and F. Wilcoxon, "A Simplified Method of Evaluating Dose-Effect Experiments," J. Pharm. & Exp. Ther., Vol. 96, No. 2, pp. 99-113.

STLCOPCB4019651

IV. Results

No deaths or untoward behavioral reactions were noted among any of the animals in the untreated control groups.

A. Rainbow Trout

1. Aroclor 1242, Aroclor 1254, Aroclor 1250, Aroclor 5460, Toxaphene, Technical DDT, Halowax 1099*

a. Mortality, DO, and pH Data

The mortality, dissolved oxygen content, and pH data are presented in Tables I through IV.

* Range finding mortality only.

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TABLE I

TEST MATERIAL: Aroclor 1242, 1260, ~~5460~~, 1254

Four-Day Fish Toxicity Study - Rainbow Trout

Mortality, DO, and pH Data

Test Material	Concentration* (ppm)	Number Dead Hours: 1-6	24	48	72	96	Total Number Dead	Number Tested	Per Cent Dead	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
Aroclor 1242	100.0	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
Aroclor 1254	100.0	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
Aroclor 1260	100.0	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
~~Aroclor 5460~~	100.0	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-

Four-Day LC_{50}

Aroclor 1242 > 100.0 ppm
Aroclor 1254 > 100.0 ppm
Aroclor 1260 > 100.0 ppm
~~Aroclor 5460~~ > 100.0 ppm

* The test materials were dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) solution in acetone. Concentrations are expressed in terms of actual test material as received.

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STLCOPCB4019653

TABLE II

TEST MATERIAL: Toxaphene

Four-Day Fish Toxicity Study - Rainbow Trout

Mortality, DO, and pH Data

Concentration (ppm)	Number Dead Hours: 1-6	24	48	72	96	Total Number Dead	Number Tested	Per Cent Dead	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
0.0056	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.010	0	9	0	0	0	9	10	90	-	-	8.2	7.4	-	-	-	-	-	-
0.018	0	9	1	-	-	10	10	100	-	-	8.2	7.6	8.0	7.4	-	-	-	-
0.032	0	4	6	-	-	10	10	100	-	-	7.8	6.9	8.1	7.0	-	-	-	-
0.056	0	10	-	-	-	10	10	100	-	-	8.1	7.2	-	-	-	-	-	-

96-Hour LC_{50} = 0.008 ppm

The test material was dispensed into the bioassay vessels in the form of a 0.01 percent (w/v) solution in acetone. Concentrations are expressed in terms of actual Toxaphene as received.

DSW 035830

STLCOPCB4019654

TABLE III

TEST MATERIAL: Technical DDT

Four-Day Fish Toxicity Study - Rainbow Trout

Mortality, DO, and pH Data

Concentration* (ppm)	Number Dead Hours: 1-6	24	48	72	96	Total Number Dead	Number Tested	Per Cent Dead	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
0.001	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.0018	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.0032	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.0056	0	0	4	1	2	7	10	70	-	-	-	-	8.0	7.3	7.8	7.2	8.4	7.2
0.01	0	6	3	1	-	10	10	100	-	-	8.2	7.3	8.0	7.2	7.9	7.2	-	-

Four-Day LC_{50} = 0.0052 ppm

* The test material was dispensed into the bioassay vessels in the form of a 0.001 percent (w/v) solution in acetone. Concentrations are expressed in terms of actual Technical DDT as received.

DSW 035831

STLCOPCB4019655

TABLE IV

TEST MATERIAL: Halowax 1099

Range Finding Fish Toxicity Study - Rainbow Trout

Mortality, DO, and pH Data

Concentration* (ppm)	Number Dead Hours: 1-6	24	48	72	96	Total Number Dead	Number Tested	Per Cent Dead	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
100.0	0	0	0	0	2	2	2	100	-	-	-	-	-	-	-	-	-	-
10.0	0	0	0	0	0	0	2	0	-	-	-	-	-	-	-	-	-	-

Four-Day LC_{50} = 10-100 ppm

* The test material was dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) solution in acetone. Concentrations are expressed in terms of actual Halowax 1099 as received.

DSW 035832

STLCOPCB4019656

b. Reactions

The reactions noted following introduction of the Aroclor 1242, Aroclor 1254, Aroclor 1260, Aroclor 5460, Toxaphene, and Technical DDT are presented in Tables V through X respectively.

STLCOPCB4019657

TABLE V

TEST MATERIAL: Aroclor - 1242

Four-Day Fish Toxicity Study - Rainbow Trout

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
100.0	Hypoactivity	24	No Recovery Noted	-
	Inverted Swimming	72	No Recovery Noted	-

DSM 035834

STLCOPCB4019658

TABLE VI

TEST MATERIAL: Aroclor - 1254

Four-Day Fish Toxicity Study - Rainbow Trout

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
100.0	No Reactions Noted	-	-	-

DSW 035835

STLCOPCB4019659

TABLE VII

TEST MATERIAL: Aroclor - 1260

Four-Day Fish Toxicity Study - Rainbow Trout

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
100.0	Hypoactivity	72	No Recovery Noted	-

DSW 035836

STLCOPCB4019660

TABLE VIII

TEST MATERIAL: Aroclor - 5460

Four-Day Fish Toxicity Study - Rainbow Trout

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
100.0	No Reactions Noted	-	-	-

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DSM 035837

STLCOPCB4019661

TABLE IX

TEST MATERIAL: Toxaphene

Four-Day Fish Toxicity Study - Rainbow Trout

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
0.0056	No Reactions Noted	-	-	-
0.010	Hypoactivity	24	24	24
0.018	Hyperactivity	24	Until Death	24-48
0.032	Hyperactivity	24	Until Death	24-48
0.056	Hyperactivity	1-6	Until Death	24

DSM 035838

STLCOPCB4019662

TABLE X

TEST MATERIAL: Technical DDT

Four-Day Fish Toxicity Study - Rainbow Trout

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
0.001	No Reactions Noted	-	-	-
0.0018	No Reactions Noted	-	-	-
0.0032	No Reactions Noted	-	-	-
0.0056	Hyperactivity	1-6	No Recovery Noted	48-72-96
0.01	Hyperactivity Prostration	1-6	Until Death	24-48-72

DSM 035839

STLCOPCB4019663

B. Bluegills

1. Aroclor 1242, Aroclor 1254, Aroclor 1260, Aroclor 5460 Toxaphene, Technical DDT and Halowax 1099*

a. Mortality, DO, and pH Data

The mortality, dissolved oxygen content, and pH data are presented in Table XI through XVII respectively.

* Range finding mortality only.

DSW 035840

STLCOPCB4019664

TABLE XI

TEST MATERIAL: Aroclor 1242

Four-Day Fish Toxicity Study - Bluegill

Mortality, DO, and pH Data

Concentration* (ppm)	Number Dead Hours: 1-6	24	48	72	96	Total Number Dead	Number Tested	Per Cent Dead	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	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.18	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.32	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.56	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
1.0	0	10	-	-	-	10	10	100	-	-	7.6	7.2	-	-	-	-	-	-

Four-Day LC_{50} = 0.66 ppm

* The test material was dispensed into the bioassay vessels in the form of a 1.0 percent (w/v) solution in acetone. Concentrations are expressed in terms of actual Aroclor 1242 as received.

DSM 035841

STLCOPCB4019665

TABLE XII

TEST MATERIAL: Aroclor 1254

Four-Day Fish Toxicity Study - Bluegill

Mortality, DO, and pH Data

Concentration* (ppm)	Number Dead Hours: 1-6	24	48	72	96	Total Number Dead	Number Tested	Per Cent Dead	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	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.18	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.32	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.56	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
1.00	0	0	6	3	1	10	10	100	-	-	-	-	7.8	7.2	6.5	7.2	6.2	7.1

Four-Day LC_{50} = 0.66 ppm

* The test material was dispensed into the bioassay vessels in the form of a 1.0 percent (w/v) solution in acetone. Concentrations are expressed in terms of actual Aroclor 1254 as received.

DSM 035842

STLCOPCB4019666

TABLE XIII

TEST MATERIAL: Aroclor 1260

Four-Day Fish Toxicity Study - Bluegill

Mortality, DO, and pH Data

Concentration* (ppm)	Number Dead Hours: 1-6	24	48	72	96	Total Number Dead	Number Tested	Per Cent Dead	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
100.0	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-

96-Hour LC_{50} > 100.0 ppm

* The test material was dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) solution in acetone. Concentrations are expressed in terms of actual Aroclor 1260 as received.

DSW 035843

STLCOPCB4019667

TABLE XIV

TEST MATERIAL: Aroclor 5460

Four-Day Fish Toxicity Study - Bluegill

Mortality, DO, and pH Data

Concentration* (ppm)	Number Dead Hours: 1-6	24	48	72	96	Total Number Dead	Number Tested	Per Cent Dead	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
100.0	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-

Four-Day LC_{50} > 100.0 ppm

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

DSM 035844

STLCOPCB4019668

TABLE XV

TEST MATERIAL: Toxaphene

Four-Day Fish Toxicity Study - Bluegill

Mortality, DO, and pH Data

Concentration[a] (ppm)	Number Dead Hours: 1-6	24	48	72	96	Total Number Dead	Number Tested	Per Cent Dead	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
0.0010	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.0018	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.0032	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.0056	0	0	5	0	0	5	10	50	-	-	-	-	7.3	7.2	-	-	-	-
0.01	0	0	7	0	1	8	10	80	-	-	-	-	7.3	7.2	-	-	6.1	7.2

[a] 48-Hour LC_{50} = 0.0056

95 percent Confidence Limits of LC_{50} = 0.0036 - 0.0087 ppm

[a] The test material was dispensed into the bioassay vessels in the form of a 0.01 percent (w/v) solution in acetone. Concentrations are expressed in terms of actual Toxaphene as received.

DSM 035845

STLCOPCB4019669

TABLE XVI

TEST MATERIAL: Technical DDT

Four-Day Fish Toxicity Study - Bluegill

Mortality, DO, and pH Data

Concentration* (ppm)	Number Dead Hours: 1-6	24	48	72	96	Total Number Dead	Number Tested	Per Cent Dead	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
0.001	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.0018	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.0032	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.0056	0	0	0	0	0	0	10	0	-	-	-	-	-	-	-	-	-	-
0.01	0	10	-	-	-	-	10	100	-	-	7.6	7.1	-	-	-	-	-	-

Four-Day LC_{50} = 0.0078 ppm

* The test material was dispensed into the bioassay vessels in the form of a 0.001 percent (w/v) solution in acetone. Concentrations are expressed in terms of actual Technical DDT as received.

DSM 035846

STLCOPCB4019670

TABLE XVII

TEST MATERIAL: Halowax 1099

Range Finding Fish Toxicity Study - Bluegill

Mortality, DO, and pH Data

Concentration* (ppm)	Number Dead Hours: 1-6	24	48	72	96	Total Number Dead	Number Tested	Per Cent Dead	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
100.0	0	0	0	0	0	0	2	0	-	-	-	-	-	-	-	-	-	-
10.0	0	0	0	0	0	0	2	0	-	-	-	-	-	-	-	-	-	-

96-Hour LC_{50} > 100.0 ppm

* The test material was dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) solution in acetone. Concentrations are expressed in terms of actual Halowax 1099 as received.

DSM 035847

STLCOPCB4019671

b. Reaction

The reactions noted following introduction of Aroclor 1242, Aroclor 1254, Aroclor 1260, Aroclor 5460, Toxaphene, and Technical DDT are presented in Tables XVIII through XXIII respectively.

DSW 035848

STLCOPCB4019672

TABLE XVIII

TEST MATERIAL: Aroclor - 1242

Four-Day Fish Toxicity Study - Bluegill

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
0.1	No Reactions Noted	-	-	-
0.18	No Reactions Noted	-	-	-
0.32	Hyperactivity	1-6	24	-
0.56	Hyperactivity	1-6	24	-
1.0	Hyperactivity	1-6	Until Death	24

DSM 035849

STLCOPCB4019673

TABLE XIX

TEST MATERIAL: Aroclor - 1254

Four-Day Fish Toxicity Study - Bluegill

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
0.1	No Reactions Noted	-	-	-
0.18	No Reactions Noted	-	-	-
0.32	No Reactions Noted	-	-	-
0.56	Hyperactivity	1-6	24	-
	Hypoactivity	24	48	-
1.0	Hypoactivity	24	Until Death	48-72-96

DSM 035850

STLCOPCB4019674

TABLE XX

TEST MATERIAL: Aroclor - 1260

Four-Day Fish Toxicity Study - Bluegill

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
100.0	Hypoactivity	48	No Recovery Noted	-

DSM 035851

STLCOPCB4019675

TABLE XXI

TEST MATERIAL: Aroclor - 5460

Four-Day Fish Toxicity Study - Bluegill

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
100.0	Hypoactivity	72	No Recovery Noted	-

DSW 035852

STLCOPCB4019676

TABLE XXII

TEST MATERIAL: Toxaphene

Four-Day Fish Toxicity Study - Bluegill

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
0.001	No Reactions Noted	-	-	-
0.0018	No Reactions Noted	-	-	-
0.0032	No Reactions Noted	-	-	-
0.0056	Hypoactivity	48	24	48
0.01	Hypoactivity	24	Until Death	48-96
	Prostration	24		
	Loss of equilibrium	48		

DSW 035853

STLCOPCB4019677

TABLE XXIII

TEST MATERIAL: Technical DDT

Four-Day Fish Toxicity Study - Bluegill

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
0.001	No Reactions Noted	-	-	-
0.0018	No Reactions Noted	-	-	-
0.0032	No Reactions Noted	-	-	-
0.0056	No Reactions Noted	-	-	-
0.01	Hyperactivity	1-6	Until Death	24

DSM 035854

STLCOPCB4019678