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REPORT TO

ETHYL CORPORATION

FOUR-DAY STATIC FISH TOXICITY STUDIES WITH METHYL CHLORIDE ETHYL CHLORIDE VINYL CHLORIDE AND ETHYLENE DICHLORIDE IN BLUEGILLS AND LARGEMOUTH BASS

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III. Procedure

The procedure was generally the same for both bluegills and largemouth bass. Healthy fingerlings with an average length of 35 to 75 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 ten days prior to experimental use. The fish were held in large, aerated, stock tanks and fed brine shrimp or small minnows until three days prior to testing. The bluegills were maintained at a temperature of 18°C and the largemouth bass at a temperature of 13°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 of each test material. Once this level had been determined, five concentrations of each test material were selected for the respective bioassays. Bioassay vessels were lined with disposable polyethylene bags and filled with 12.5 liters of well aerated, reconstituted water. Ten fish were tested at each concentration.

After a 24-hour acclimatization period, each gaseous test material was bubbled through an air stone at a constant rate of flow for five determined time periods in the reconstituted water. Water samples were taken at one minute and one, 6, 24, 48, 72, and 96 hours after bubbling*. Concentrations of the test materials were determined by using a gas chromatograph**.

Following the introduction of the test materials, 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 measured in each solution in which mortalities occurred to determine if an adequate supply of oxygen was available to the test specimens***. Concentrations of dissolved oxygen of 4 mg/L (4 ppm) were considered to be minimal for testing. The pH of the test solution was also determined when mortalities were recorded.

As a quality check, each lot of experimental fish was challenged with a reference pesticide, Toxaphene. Toxaphene was dispensed into the bioassay vessels in the form of a 0.01 percent (w/v) solution in acetone.

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

* Ethylene dichloride was dispensed into the vessels in the form of a 10.0 percent (w/v) solution in ethanol.

** The final concentration values are the mean values for each time period.

*** Hach OX-2-P Dissolved Oxygen Test Kit, Hach Chemical Co., Ames, Iowa.

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

D. Ethylene Dichloride

1. Survival, DO, and pH Data

The survival, dissolved oxygen content, and pH data for bluegills and bass are presented in Tables XIII and XIV.

TABLE XIII

TEST MATERIAL: Ethylene Dichloride

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
56.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
75.0	10	10	10	9	7	70	-	-	-	-	-	-	6.2	7	6.1	7
100.0	10	10	10	7	4	40	-	-	-	-	-	-	6.5	7	6.5	7
135.0	10	9	7	2	2	20	-	-	7.2	7	7.5	7	6.4	7	-	-
180.0	10	5	5	2	0	0	-	-	7.6	7	-	-	6.2	7	6.7	7

Four-Day TL_{50} = 94.0 ppm.
95% Confidence Limits of TL_{50} = 79.7 - 110.9 ppm.

* The test material was dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) solution in ethanol. Concentrations are expressed in terms of Ethylene Dichloride.

- = Not determined.

TABLE XIV

TEST MATERIAL: Ethylene Dichloride

Four-Day Static Fish Toxicity Study - Largemouth Bass

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
32.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
42.0	10	10	10	10	10	100	-	-	-	-	-	-	-	-	-	-
56.0	10	9	9	9	5	50	-	-	6.2	7	-	-	-	-	4.2	7
100.0	10	10	10	10	2	20	-	-	-	-	-	-	-	-	3.9	7
180.0	10	10	10	5	0	0	-	-	-	-	-	-	4.0	7	4.0	7

Four-Day TL_{50} = 66.0 ppm.
95% Confidence Limits of TL_{50} = 48.9 - 89.1 ppm.

* The test material was dispensed into the bioassay vessels in the form of a 10.0 percent (w/v) solution in ethanol.
Concentrations are expressed in terms of Ethylene Dichloride.

- = Not determined.

2. Reactions

The reactions noted following introduction of the Ethylene Dichloride are presented in Tables XV and XVI.

TABLE XV

TEST MATERIAL: Ethylene Dichloride

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)
56.0	Quiescent	24	No recovery noted	-
75.0	Quiescent	1-6	No recovery noted	72, 96
100.0	Quiescent	1-6	No recovery noted	72, 96
135.0	Quiescent	1-6	72	24, 48, 72
	Excitable	72	No recovery noted	
	Surfacing	72	No recovery noted	
	Sounding	72	No recovery noted	
	Swimming: Gyrating	72	No recovery noted	
	Inverted	72	No recovery noted	
	On side	72	No recovery noted	
	Against tank sides on bottom	72	No recovery noted	
180.0	Excitable	1	1-6	24, 72, 96
	Surfacing	1	1-6	
	Sounding	1	1-6	
	Swimming: Gyrating	1	1-6	
	Inverted	1	1-6	
	On side	1	1-6	
	Against tank sides on bottom	1	1-6	
	Quiescent	24	Until death	

TABLE XVI

TEST MATERIAL: Ethylene Dichloride

Four-Day Static Fish Toxicity Study - Largemouth Bass

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
32.0	Quiescent	24	48	-
	Motionless: Flaccidity	24	24	
42.0	Quiescent	24	72	-
	Motionless: Flaccidity	48	24	
	Respiratory rate: Slow	48	24	
56.0	Quiescent	1-6	24	24, 96
	Motionless: Flaccidity	24	48	
	Respiratory rate: Slow	24	48	
100.0	Quiescent	1-6	24	96
	Swimming: Gyrating	24	72	
	Motionless: Flaccidity	24	72	
	Respiratory rate: Slow	24	72	
	Surfacing	96	No recovery noted	

TABLE XVI continued

TEST MATERIAL: Ethylene Dichloride

Four-Day Static Fish Toxicity Study - Largemouth Bass

Summary of Reactions

Concentration (ppm)	Reaction	Time of Onset Following Dose Administration (hours)	Duration of Reaction (hours)	Time of Death Following Dose Administration (hours)
180.0	Quiescent	1-6	Until death	72, 96
	Motionless: Flaccidity	1-6	Until death	
	Swimming: Inverted	24	24	
	Gyrating	48	Until death	
	Respiratory rate: Slow	24	24	