MRID 136995

079401

DATA EVALUATION RECORD

72-1

1. CHEMICAL: Endosulfan

2. FORMULATION: Technical (95.9% a.i.)

3. CITATION: Fischer, R. 1983. The Effect of HOE002671 OI ZD96 0002 (Endosulfan, active ingredient 95.9%) on Salmo gairdneri (Rainbow Trout) in a Static Test. Performed and submitted by American Hoechst AG verk. Pharma/Landwirt schaft, 1 Frankfurt, Federal Republic of Germany; Reg. No. 8340-13. Acc. No. 252043.

4. REVIEWED B : John J. Bascietto
Wildlife Biologist
EEB/HED

5. DATE REVIEWED: 2/22/84

6. TEST T PE: Freshwater fish LC_{50} (coldwater species)

A) Species - Rainbow Trout (Salmo gairdneri)

7. REPORTED RESULTS: (Nominal concentrations)

24-Hr.	LC_{50} = 3.67	ug/l	
48-Hr.	" = 1.60	"	
72-Hr.	" = 1-1.35	"	
96-Hr.	" = 0.93	:	().81-1.08) ug/l

8. REVIEWER'S CONCLUSIONS: The study is scientically sound but the statistical anlysis neglected some pertinent mortality. EEB recalculated the 96-hr. LC50 as 0.83 (0.54-1.18) ug/L, indicating that technical endosulfan is "very highly toxic" to freshwater fish. The study fulfills the guidelines requirement for a coldwater fish LC_{50}.

9. Materials/Methods

A. Procedures

The guidelines protocol for 96-Hr freshwater fish LC_{50} tests were followed except that 300 l (approx 75 gallon) aquaria were used as the test vessels, with 200 l of test solution. Biological loading was 0.19 g/l (10 fish per aquaria). Water and solvent (acetone) controls were tested. "Hard" reconstituted water was used as the diluent (the report states that "soft" water was used - see sec. 10 below). Calculations of the aqueous concentrations of test substance were based on 100% purity of the test material (the test material was actually only 95.5% endosulfan). Results are reported as "nominal" concentrations. Analytical determinations of chemical concentrations were not performed. Test temperatures were 11.3 - 12.5°C.

B. Statistical Anlaysis

LC_5, LC_{50}, and LC_{95} were calculated at 24, 48 and 96-hour exposures by use of probit analysis by computer program (SAS Institute Inc., Cary, N.C.)

10. Results

Percent mortality and abnormal behavioral responses are listed in Table 1.

The authors reported several signs of intoxication at 0.42 ug/L and above. These are specified as to time of observation and number of fish in Table 1. These behavioral responses included: erratic swimming, surface swimming, cramped swimming, nervousness, slow reactions, swimming at bottom of test vessel, "head-down" swimming, horizontal turns.

The authors state that they deleted from LC_{50} calculations the mortalities at 0.42 ug/L - 24 Hrs. and at 0.075 ug/L - 96 hrs. because "as endosulfan is known to cause behavioral effects before mortality, those mortalites were recognized as not toxicant related and were not taken into the calculation" (report, p.5). They claim the deletion of these two mortalities do not significantly alter the LC_{50} values.

D.O., p.H. and temperature values were reported at 0, 24, 48, and 96 hours for the 0.75 and 0.075 ug/L vessels, as well as for 5.6 ug/L at 0 hr. only and at all times for the controls. D.O. ranged from 8.27 - 9.98 ppm in both water and acetone controls and from 8.50 -9.97 ppm in the experimentals. pH of the control ranged from 7.35 - 8.29; 7.20 - 8.19 in experimentals. Temperature of test water ranged from 11.3 - 12.3 in control and test vessels.

Physical and chemical Parameters of original dilution water:

total hardness mg/L as $CaCO_3$	0 hours: 61.8	96 Hrs:	48.1
total Alkalinity mg/L as $CaCO_3$	0 hours: 40.3	96 Hrs:	33.8
No_2 mg/L	0 hours: 0.045	96 Hrs:	0.04
Conductivity umhos/cm	0 hours: 140	96 HRS:	151

11. Reviewer's Evaluation

A. Procedures: EPA guidelines procedures were followed except that 300 L stainless steel vessels with 200 L of test water were used instead of the recommended 19L/15L glass vessels. This in itself does not invalidate the effort.

B. Statistical Analysis -

EEB's verification of the statistical analysis (see attached verification sheet) indicates the results using the probit method should probably not be used. EEB rejects the author's deletion of the mortalities at 0.42 ug/L and 0.075 ug/L and included these in our statistical analysis. We do consider behaviorally related deaths as significant to the determination of the toxicant's LC_{50}. We used the "moving average" method.

C. Results

The 96-hour LC_{50} as recalculated by the "moving average" method is:

96-hr LC_{50} = 0.83 (0.54 - 1.18) ug/L
(95% confidence interval)

This indicates the material is "very highly toxic" to freshwater fish.

There is an apparent severe effect on the swimming behavior prior to death. These deleterious effects could contribute to increased susceptibility to predation, as well as causing direct mortality through intoxication or inability to swim (move water over gill surface) or to eat.

D. Conclusions

1. Category: CORE as recalculated by EEB. (Registrant must accept the recalculated LC_{50} or study is classified "supplemental")

2. Rationale: guidelines, with EEB recalculation of LC_{50}.

3. Repair: N/A.

BASCIETTO ENDOSULFAN RAINBOW TROUT LC50

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
5.6	10	10	100	.0976563
4.2	10	10	100	.0976563
3.2	10	10	100	.0976563
2.4	10	10	100	.0976563
1.8	10	10	100	.0976563
1.35	10	10	100	.0976563
1	10	5	50	62.3047
.75	10	1	10	1.07422
.56	10	1	10	1.07422
.42	10	1	10	1.07422
.32	10	0	0	.0976563
.24	10	0	0	.0976563
.18	10	0	0	.0976563
.135	10	0	0	.0976563
.1	10	0	0	.0976563
.075	10	1	10	1.07422

THE BINOMIAL TEST SHOWS THAT .75 AND 1.35 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 1

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
13	.362002	.83827	.541844	1.18331

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
6	11.2875	118.166	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.64268
95 PERCENT CONFIDENCE LIMITS =-8.5956 AND 15.881

LC50 = .851194
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = .381403
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

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TABLE 1 Percent Mortalities and Intoxication Symptoms of Hoe 002671 01 ZD96 0002 (Endosulfan, active ingredient 95.9 percent) on Salmo gairdneri (Rainbow trout)

Intoxication Symptoms (IS): type and number of affected fish
Behaviour: B1 erratic swimming, B2 surface swimming, B3 cramped swimming, B4 nervousness, B5 slow reaction, B6 swimming at the bottom of the test vessel, B7 head down swimming, B8 horizontal turns

Test grp No	Concentr. µg/l	24 hours Mort.	24 hours IS	48 hours Mort.	48 hours IS	72 hours Mort.	72 hours IS	96 hours Mort.	96 hours IS
Contr.	-	0	--	0	--	0	--	0	--
Solv.	Acetone	-	--	-	--	-	--	-	--
Contr.	0.04 ml/l	0	--	0	--	0	--	0	--
I	5.6	100	--	100	--	100	--	100	--
II	4.2	80	B2/1 B7/1 B8/1	100	--	100	--	100	--
III	3.2	20	B1/8 B2/4 B3/4	100	--	100	--	100	--
IV	2.4	0	B4/10 B3/3	100	--	100	--	100	--
V	1.8	0	--	50	B3/5 B2/2	100	--	100	--
VI	1.35	0	--	40	B3/6 B2/3	100	--	100	--
VII	1.0	0	--	0	B3/10 B2/1	10	B3/9 B2/5	50	B3/4 B6/2 B2/1
VIII	0.75	0	--	0	B3/10	0	B5/10 B3/2 B2/1	10	B3/6 B6/1
IX	0.56	0	--	0	B3/3	0	B5/10 B3/2 B2/1	10	B3/2
X	0.42	10 *	--	10 *	--	10 *	B2/2 B3/2	10 *	B3/1

* refer results

TABLE 1 - continued

Test grp No	Concentr. µg/l	24 hours Mort.	24 hours IS	48 hours Mort.	48 hours IS	72 hours Mort.	72 hours IS	96 hours Mort.	96 hours IS
XI	0.32	0	--	0	--	0	--	0	B3/1
XII	0.24	0	--	0	--	0	--	0	--
XIII	0.18	0	--	0	--	0	--	0	--
XIV	0.135	0	--	0	--	0	--	0	--
XV	0.1	0	--	0	--	0	--	0	--
XVI	0.075	0	--	0	--	0	--	*10	--

* refer results

TABLE 2 LC - values determined by the computerized probit analysis

	24 hours	48 hours	72 hours	96 hours
LC_{05} µg/l 95 % Conf. lim.	2.86 1.15 - 3.22	1.07 0.72 - 1.26	# range 0.75 - 1.00	0.61 0.42 - 0.72
LC_{50} µg/l 95 % Conf. lim.	3.67 3.26 - 4.12	1.60 1.39 - 1.84	# range 1.00 - 1.35	0.93 0.81 - 1.08
LC_{95} µg/l 95 % Conf. lim.	4.71 4.17 - 6.89	2.39 2.02 - 3.58	# range 1.00 - 1.35	1.42 1.19 - 2.13

could not be calculated by the probit analysis