079401
SHAUGHNESSE NO.

8
REVIEW NO.

EEB BRANCH REVIEW

DATE: IN 1-10-84 OUT 2-27-84

FILE OR REG. NO. 8340-13

PETITION OR EXP. PERMIT NO.

DATE OF SUBMISSION 12-23-84

DATE RECIEVED B HED 1-6-84

RD REQUESTED COMPLETION DATE 3-6-84

EEB ESTIMATED COMPLETION DATE 3-6-84

RD ACTION CODE/T E OF REVIEW 660/Reg. Std.

T E PRODUCT(S): I, D, H, F, N, R, S Insecticide

DATA ACCESSION NO (S). 252043

PRODUCT MANAGER NO. G. LaRocca (15)

PRODUCT NAME(S) Thiodan

COMPAN NAME American Hoescht Corporation

SUBMISSION PURPOSE Submission of data plus extension request on certain studies

SHAUGHNESSE NO.	CHEMICAL, & FORMULATION	% A.I.
079401	Endosulfan (Tech)	

BWQ MALLARD > LD50
RBT LC50

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

29 FEB 1984

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO: George LaRocca, PM 15
Registration Division, TS-767c

THRU: Dave Coppage, Head Sec. 3
Ecological Effects Branch
Hazard Evaluation Division, TS-769c

THRU: Clayton Bushong, Chief
Ecological Effects Branch
Hazard Evaluation Division, TS-769c

SUBJECT: EEB Review of Endosulfan (Reg No. 8340-13)
Data submitted in response to Registration Standard data requests. Accession No. 252043.

EEB received and reviewed three (3) data submissions under Reg. NO. 8340-13. These data were submitted in response to the Agency's Registration Standard for Endosulfan. We make the following conclusions regarding acceptability of the studies:

Roberts, N.L. and C.N. Phillips. 1983.
- Acute toxicity to Bobwhite quail. The study is scientifically sound. The guidelines requirement is satisfied. Technical endosulfan is "highly toxic" to Bobwhite quail; LD_{50} = 42 (25-56) mg/kg, single oral dose.

Roberts, N.L. and C.N. Phillips. 1983
- Acute toxicity to mallard duck. The study is scientifically sound. The guidelines requirement is satisfied. Technical endosulfan is "highly toxic" to mallard duck; LD_{50} = 28 (22-36) mg/kg, single oral dose.

Fischer, R. 1983.
- Acute toxicity to Rainbow Trout. The study is scientically sound. The guidelines requirement is satisfied, but the author's conclusions are not accepted. The 96-Hr. LC_{50} for Rainbow Trout is recalculated by EEB to 0.83 (0.54-1.18) ug/L. Technical endosulfan is "very highly toxic" to Rainbow Trout.

Regarding the registrant's request for an extension of the deadlines for submitting the avian reproduction studies, since the deadline of Nov., 1983 is well past, there is little point in EEB commenting on this request.

The avian reproduction study protocols submitted appear adequate to perform a guidelines study.

John J. Bascietto
Wildlife Biologist, Sec. 3
Ecological Effects Branch
Hazard Evaluation Division, TS-769c

DATA EVALUATION RECORD

AL: Endosulfan

ATION: Technical (97.2% a.i.)

N: Roberts, N.L. and C.N. Phillips. 1983. The acute xicity (LD_{50}) of Endosulfan - Technical to the Mallard performed by Huntingdon Research Center, England, ted by American Hoechst Corp, Somerville, New Jersey, port of Reg. No. 8340-13. Accession No. 252043.

D BY: John J. Bascietto
Wildlife Biologist
EEB/HED

VIEWED: 2/22/84

PE: Avian acute oral toxicity (LD_{50})

A) species - mallard duck (Anas platyrynchos)

D RESULTS:

LD_{50} = 28 mg/kg (22-36 mg/kg)

R'S CONCLUSIONS: The study is scientifically sound. LD_{50} = 28(22-36) mg/kg, endosulfan technical is toxic" to mallard ducks. The study fulfills the of the guidlines requirement.

4

... and Methods

...re: Procedures were those recommended by protocols ...to in EPA guidelines. A range finding study was ...ed to establish the definitive test concentrations. ...initive study, testing ten (10) birds per level at ...) endosulfan concentrations plus one(1) vehicle ...rol (corn oil) concentration, was performed. This study ... 14-day acclimation period, followed by a single dose ...al gavage, followed by a 14-day observation period. ... diet were available ad libitum. Body weight and ... consumption measurements were as per guidelines.

...tical Analysis - treatment group mortality was ...ed by Finney probit analysis.

...

...llowing mortatilies were reported:

	Treatment level	# Dead/10
...le control)	0 mg/kg endosulfan	0
...ent)	5 " "	0
")	10 " "	0
")	20 " "	1
")	40 " "	9
").	80 " "	10

... groups 4, 5 and 6 were "unsteady" after dosing. ... in groups 4 and 5 remained so for several hours, ... normal by the end of Day 1. All birds that died ...thin 4 hours of dosing.

...'s Evaluation

...ures: guidelines protocols were followed. The birds ...signed to treatment groups on the basis of bodyweight ... the aim of all treatment groups having similar initial ...ight means". Generally, this would not be acceptable ... mean body weights were significantly different as ...ed by comparison of group mean weights; but in this ... group mean weights at initiation of the study were not ...antly different.

...ical Analysis - analyzed and verified by EEB's computer ... (Stephan's) for calculating median lethal doses and ...idence intervals. See attached verification sheet.

C. Results -

The results, as calculated by EEB, are generally in agreement with the authors'. There are no remarkable bodyweight nor feed consumption data.

Gross post-mortem examinations revealed no remarkable abnormalities but the results were not presented in the report.

D. Conclusions

1. Category: Core

2. Rationale: guidelines

3. Repair: N/A

BASCIETTO ENDOSULFAN MALLARD ACUTE ORAL LD50
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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
80	10	10	100	.0976563
40	10	9	90	1.07422
20	10	1	10	1.07422
10	10	0	0	.0976563
5	10	0	0	.0976563

THE BINOMIAL TEST SHOWS THAT 20 AND 40 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 28.2843

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	.114405	28.2843	20.737	42.1922

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	.332745	1	.999987

SLOPE = 8.52835
95 PERCENT CONFIDENCE LIMITS = 3.60885 AND 13.4478

LC50 = 28.2843
95 PERCENT CONFIDENCE LIMITS = 22.0823 AND 36.2281

LC10 = 20.0738
95 PERCENT CONFIDENCE LIMITS = 11.4987 AND 24.8936
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079401

DATA EVALUATION RECORD

1. CHEMICAL: Endosulfan

2. FORMULATION: Technical (97.2% a.i.)

3. CITATION: Roberts, N.L. and C.N. Phillips. 1983. The acute oral toxicity (LD_{50}) of Endosulfan - technical to the Bobwhite quail. Performed by Huntingdon Research Center, England; submitted by American Hoechst Corp. in support Reg. No. 8340-13. Accession No. 252043.

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

5. DATE REVIEWED: 2/22/84

6. TEST TYPE: Avian acute oral toxicity (LD_{50})

A) Species: Bobwhite quail (Colinus virginianus)

7. REPORTED RESULTS:

LD_{50} = 42 mg/kg (35-56 mg/kg).

8. REVIEWER'S CONCLUSIONS: The study is scientifically sound. With an LD_{50} = 42 (35-56) mg/kg endosulfan technical is "highly toxic" to bobwhite quail. The guidelines requirement is satisfied.

8

9. Materials/Methods

A) Procedure: The protocol used is basically that recommended by the EPA guidelines. A range finder used 3 groups of 2 birds (one male; one female) was used to establish range for definitive study. The definitive study tested a corn oil vehicle control group of 10 birds plus five(5) treatment groups of 10 birds each. The birds were allocated to treatment groups on the basis of bodyweight with equal numbers of males & females per group. A 14-day acclimation period was followed by single dose by oral gavage, followed by A 14-day observation period. Water and diet were ad libitum. Bodyweight and feed consumption measured as per guidelines.

B) Statistics - treatment group mortality was analyzed by Finney probit analysis.

10. Results

The following mortalities were reported:

Group	Treatment Level	# Dead/10
1 (control-Corn oil)	0 mg/kg endosulfan	0
2 (Treatment)	10 " "	0
3 (")	15 " "	0
4 (")	23	0
5 (")	34 " "	3
6 (")	51 " "	7

Birds in groups 4 and 5 were "subdued" after treatment. Those at the highest treatment level were "unsteady" after dosing. Groups 5 & 6 remained "subdued" through the fifth day of the observation period.

11. Reviewer's Evaluation

A. Procedures: guidelines procedures were used. The birds were assigned to treatment groups on the basis of bodyweight "with the aim of all treatment groups having similar initial bodyweight means" Generally, this would not be acceptable if the mean bodyweights were significantly different as reflected by comparison of group mean weights. but in this case, group mean weights at initiation of the study were not significantly different, however the male and female mean weights were different with the males at about 205 g/bird and females at about 188 g/bird. This is acceptable.

B. Statistical Analysis - Analyzed and verified by EEB's computer program (Stephan's) for calculation of median lethal doses and 95% confidence intervals. See attached verification sheet.

C. Results

The results, as calculated by EEB, are generally inagreement with the authors'. There are no remarkable bodyweight or feed consumption data. Most birds that died, did so within 48 hours, although 1 male at 34 mg/kg died 7 days after dosing. No birds died after 7 days post-dosing. Gross post-mortems revealed no remarkable abnormalities but the results were not presented in the report.

D. Conclusions

1) Category: Core

2) Rationale: Guidelines

3) Repair: N/A

TRANSCRIPTION ENVIRONMENTAL RESEARCH LABORATORY ACUTE ORAL LD50

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
51	10	7	70	17.1875
34	10	3	30	17.1875
23	10	0	0	.0976563
15	10	0	0	.0976563
10	10	0	0	.0976563

THE BINOMIAL TEST SHOWS THAT 23 AND +INFINITY 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 41.6413

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
2	.272537	41.6413	34.772	57.89

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
9	.393508	1	.916451

SLOPE = 7.60772
95 PERCENT CONFIDENCE LIMITS = 2.83538 AND 12.3801

LC50 = 42.2735
95 PERCENT CONFIDENCE LIMITS = 35.2096 AND 56.1018

LC10 = 28.7828
95 PERCENT CONFIDENCE LIMITS = 16.0333 AND 34.6831

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079401

DATA EVALUATION RECORD

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}.

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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.

ASSOCIATED 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 2D96 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.	IS	48 hours Mort.	IS	72 hours Mort.	IS	96 hours Mort.	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 computerised 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

I-4