DP Barcode : D190323
PC Code No : 109701
EEB Out : JUL 22 1993

JUL 22 1993

To: Jay Ellenberger
Product Manager 50
Special Review and Reregistration Division (H7508W)

From: Anthony F. Maciorowski, Chief
Ecological Effects Branch/EFED (H7507C)

Attached, please find the EEB review of...

Reg./File # : 109701
Chemical Name : Permethrin, mixed cis,trans
Type Product : Insecticide
Product Name : Permethrin products
Company Name : Zeneca Limited
Purpose : Submission of data for reregistration in support of Case No. 2510.

Action Code : 627 Date Due : 07/15/93
Reviewer : C. Laird Date In : 04/22/93

EEB Guideline/MRID Summary Table: The review in this package contains an evaluation of the following:

GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT	GDLN NO	MRID NO	CAT
71-1(A)			72-2(A)			72-7(A)		
71-1(B)			72-2(B)			72-7(B)		
71-2(A)			72-3(A)			122-1(A)		
71-2(B)			72-3(B)			122-1(B)		
71-3			72-3(C)			122-2		
71-4(A)			72-3(D)			123-1(A)		
71-4(B)			72-3(E)	427233-01	Y	123-1(B)		
71-5(A)			72-3(F)			123-2		
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)			124-2		
72-1(B)			72-5			141-1		
72-1(C)			72-6			141-2		
72-1(D)						141-5		

Y=Acceptable (Study satisfied Guideline)/Concur
P=Partial (Study partially fulfilled Guideline but additional information is needed
S=Supplemental (Study provided useful information but Guideline was not satisfied)
N=Unacceptable (Study was rejected)/Nonconcur

DP BARCODE: D190323 REREG CASE # 2510

CASE: 819432 DATA PACKAGE RECORD DATE: 04/16/93
SUBMISSION: S438931 BEAN SHEET Page 1 of 1

* * * CASE/SUBMISSION INFORMATION * * *

CASE TYPE: REREGISTRATION ACTION: 627 GENERIC DATA SUBMISSION
CHEMICALS: 109701 Permethrin, mixed cis,trans (ANSI) 100.00 %

ID#: 109701
COMPANY:
PRODUCT MANAGER: 50 JAY ELLENBERGER 703-308-8085 ROOM: CS1 4J1
PM TEAM REVIEWER: LINDA DELUISE 703-308-8065 ROOM: CS1 4N6
RECEIVED DATE: 04/06/93 DUE OUT DATE: 07/05/93

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 190323 EXPEDITE: N DATE SENT: 04/16/93 DATE RET.: / /
CHEMICAL: 109701 Permethrin, mixed cis,trans (ANSI)
DP TYPE: 999 Miscellaneous Data Package
ADMIN DUE DATE: 07/15/93 CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT
DIV : EFED	4/22/93	08/03/93
BRAN: EEB	4/22/93	/ /
SECT:	/ /	/ /
REVR :	/ /	/ /
CONTR:	/ /	/ /

* * * DATA REVIEW INSTRUCTIONS * * *

PLEASE REVIEW MRID 42723301 FOR 72-3(E)

* * * ADDITIONAL DATA PACKAGES FOR THIS SUBMISSION * * *

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL
190324	TB-2	04/16/93	07/15/93	Y	N	N

100.0 Pesticide Name: Permethrin

100.3 Submission Purpose:

Submission of an 48-hour Embryo-Larval study.

101.0 Chemical and Physical Properties:

101.1 Common Name: Permethrin

101.2 Chemical Name:

(3-phenoxybenzyl (1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate)

103.0 Toxicological Properties:

48-hour Embryo-Larval for Pacific Oyster (Crassostrea gigas).

105.0 Conclusions:

Since measured test concentrations decreased substantically from test initiation (0 hour) to test termination (48 hour), it is believed that permethrin absorbed to the test vessel walls. However, this study does fulfill the guideline requirement for an acute toxicity study for a formulated product using mollusc embryos and larvae. Based on percentage of normal larvae and mean measured concentrations, the 48-hour EC_{50} was 6.5 mg of formulation/l or 0.65 mg ai/l which classifies 10% EC formulation of permethrin as highly toxic to Crassostrea gigas. The NOEC was 2.7 mg ai/l.

Curtis E. Laird 7-19-93
Curtis E. Laird, Fishery Biologist
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

for
Norman J. Cook, Head-Section 2
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

Anthony F. Maciorowski, Chief 7/22/93
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

DATA EVALUATION RECORD

1. **CHEMICAL:** Permethrin. Shaughnessey Number: 109701.

2. **TEST MATERIAL:** 10% EC formulation of permethrin (3-phenoxybenzyl (1*RS*)-*cis*-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate); CAS No. 52645-53-1; 10.06% active ingredient w/w; a clear yellow liquid.

3. **STUDY TYPE:** 72-3. Mollusc 48-hour Embryo-Larval Study. Species Tested: Pacific Oyster (*Crassostrea gigas*).

4. **CITATION:** Thompson, R.S. and S.A. Sankey. 1992. Permethrin: Acute Toxicity of a 10% EC Formulation to Larvae of the Pacific Oyster (*Crassostrea gigas*). Laboratory Project ID No. BL4689/B. Study performed by ZENECA Limited, Brixham Environmental Laboratory, Freshwater Quarry, Brixham, Devon, U.K. Submitted by ZENECA Inc., Wilmington, DE. EPA MRID No. 427233-01.

5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Rosemary Graham Mora
Date: 14 June 93

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
Senior Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: P. Kosalwat
Date: 6/14/93

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: C.E.T. 7-19-93 H.T. Craven
Date: 7/20/93

7. **CONCLUSIONS:** Although measured test concentrations decreased substantially from test initiation to test termination, it is not feasible for this test to be conducted under flow-through or static-renewal conditions. Therefore, this study is scientifically sound and meets the guideline requirements for an acute toxicity study using mollusc embryos and larvae. Based on percentage of normal larvae and mean measured concentrations, the 48-hour EC_{50} was 6.5 mg of formulation/l or 0.65 mg a.i./l which classifies 10% EC formulation of permethrin as highly toxic to *Crassostrea gigas*. The NOEC was 2.7 mg/l mean measured concentration or 0.27 mg a.i./l.

8. **RECOMMENDATIONS:** N/A

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Embryos of the Pacific oyster (*Crassostrea gigas*) were obtained by inducing oysters to spawn. Adult oysters, with a shell length of 92-104 mm, were obtained on the day of test initiation from Guernsey Sea Farms, Guernsey, where they had been held in flowing seawater (23 ±1°C) for more than four weeks.

Individual oysters were cleaned of debris and fouling organisms and transferred to beakers containing filtered (1 µm) seawater. Oysters were induced to spawn by briefly raising the water temperature to 25-28°C, followed by the addition of heat-deactivated sperm suspension to the water. The egg suspension from a single female was fertilized with sperm from one induced male to provide the embryo suspension. The suspension was maintained at 20 ±1°C with gentle agitation by an orbital shaker, until used to inoculate the test solutions. Embryos (1.9 hours post-fertilization) were used to initiate the test.

B. **Test System:** The study was conducted in 250-ml glass beakers with loose fitting lids, each containing 200 ml of test solution. Four replicates of the control and two replicates of each test concentration were prepared. The test temperature was maintained at 20 ±1°C by controlling the room temperature. A photoperiod of 8 hours of light/16 hours of darkness with a 15-minute gradual transition period was provided.

The dilution water was natural seawater collected from Tor Bay, Devon. The dilution water was adjusted to a salinity of 32 ±2 parts per thousand (ppt) with distilled water, and filtered (0.2 µm) before use.

A stock solution (100 mg/l) was prepared by dissolving 100 mg of test substance in the dilution water to a final volume of 1 l. Appropriate aliquots of this stock were added to dilution water to prepare the test solutions.

C. **Dosage:** Forty-eight-hour, static test. Six nominal concentrations (1.0, 1.8, 3.2, 5.6, 10, and 18 mg/l) were used in this study. In addition, a dilution water control was included.

D. **Design:** At the start of the test, an inoculum of embryo suspension (0.84 ml) was randomly added to each vessel. Three additional vessels containing control water were inoculated and sampled immediately for definitive determination of the inoculum density which was 24.0 embryos/ml.

After 48 hours, each vessel was mixed with a perforated plunger, and 20 ml of solution removed and fixed with 1 ml of buffered formalin. Once the larvae had settled, the sample volume was reduced (larval density increased) by a factor of 2. Subsequently, the number of normal and abnormal larvae were counted in replicate 1 ml subsamples from each sample, in ring cells mounted on Sedgewick-Rafter grid slides under a microscope. D-veliger larvae were defined as normal if the bivalve (Prodissoconch I) shell was fully formed. All larvae observed, other than empty shells, were recorded. For the inoculum samples, all dividing embryos were counted.

The temperature was measured daily in one replicate of each treatment, and hourly in an extra vessel containing dilution water. The salinity of the control and the highest test concentration and the pH and dissolved oxygen concentration (DO) of each test solution were measured at the start of the test, using the excess solution remaining after filling the test vessels. At test termination, the pH and DO in one replicate of each solution were measured.

Each test solution was sampled for determination of the test substance concentrations at test initiation and termination of the test using gas chromatography. Samples were taken from the excess solutions at the start of the test, and from one replicate of each solution at the end of the test.

E. **Statistics:** Results of the toxicity test were used to calculate the percentage reduction of normal oyster larvae of each test concentration when compared to the control. The percentage reduction of normal 48-hour embryos was determined as follows:

$$\% \text{ Reduction} = \frac{\% \text{ normal in control minus } \% \text{ normal in treatment}}{\% \text{ normal in control}} \times 100$$

These treatment percentage reductions were used to calculate the median effective concentration (EC_{50}) and its 95% confidence limits, defined as the concentration resulting in a 50% reduction in normal development of the larvae. This was calculated by moving average angle analysis using a computer program (Stephan, 1977).

12. **REPORTED RESULTS:** Mean measured concentrations were 0.52, 0.94, 1.6, 2.7, 5.9, and 11.0 mg of formulation/l which represent 48-61% of nominal concentrations (Table 1, attached). The reduction in measured concentrations over the test period was attributed to the "adsorption of permethrin onto surfaces with which it came into contact." The 5.9 and 11.0 mg/l test solutions were slightly opaque, "suggesting that the solubility of the test substance had been exceeded."

The data showing the counts of normal, abnormal, and total larvae in subsamples of the test solutions, and of embryos in the inoculum samples, are presented in Table 2 (attached). It should be noted that these values (and subsequent derived values) are not corrected for dilution by fixative (x 1.05) and concentration (x 2) by sedimentation of the samples.

Table 3 (attached) shows the number and percentage of normally developed larvae. Mean normal development in the control was 102%. Table 3 also shows the percentage reduction in normal development compared with the control.

The 48-hour EC_{50} was 6.5 mg of formulation/l with a 95% confidence interval of 6.1-6.9 mg/l. The no-observed effect concentration (NOEC) was 2.7 mg/l, since reduction in normal development $\leq$2.7 mg/l "did not exceed 3% which was considered to be not significant without the need for statistical analysis."

During the test, the test solutions had a DO of 7.0-7.4 mg/l and a pH of 8.04-8.24. The temperature ranged from 19.8-21.0°C. The salinity was 31.8 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The authors made no conclusions in the report.

A GLP compliance statement was included in the report indicating that the study conducted in accordance with the principles of Good Laboratory Practice of the United Kingdom Department of Health Compliance Programme (1989). This statement also indicates that the study satisfies the requirements of 40 CFR 160. A quality assurance statement was also included.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with the SEP, but deviated as follows:

Test concentrations decreased substantially during the test. Measured concentrations at test termination were 8.4-34.1% of initial measured concentrations (Table 1, attached).

The test was conducted using a formulated product and the test design did not include a control(s) using the inert ingredient(s) as recommended.

The authors did not report percentage of mortality or the EC_{50} based on mortality of oyster embryos and larvae.

The test vessels used in this test were 250-ml glass beakers; the SEP recommends 1-l test vessels.

The SEP states that embryos should be tested within one hour of spawning and after fertilization. This test used embryos 1.9 hours after fertilization.

B. Statistical Analysis: The reviewer used EPA's Toxanal computer program to calculate the 48-hour EC_{50} for the normal development of oyster larvae (printout, attached). The result was less conservative than that of the authors.

Percentage mortality based on the initial and final number of embryos at each treatment level was less than 50%, therefore, no statistical analysis was necessary.

C. Discussion/Results: Although measured test concentrations decreased substantially from test initiation to test termination, it is not feasible for this test to be conducted under flow-through or static-renewal conditions. Based on percentage of normal larvae and mean measured concentrations, the 48-hour EC_{50} was 6.5 mg of formulation/l or 0.65 mg a.i./l

which classifies 10% EC formulation of permethrin as highly toxic to *Crassostrea gigas*. The NOEC was 2.7 mg/l mean measured concentration or 0.27 mg a.i./l.

D. **Adequacy of the Study:**

(1) Classification: Core for a formulated product.

(2) Rationale: N/A.

(3) Repairability: N/A.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes; 4 June 1993.

PERMETHRIN

Page_____ is not included in this copy.

Pages 11 through 14 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

____ Identity of product impurities.

____ Description of the product manufacturing process.

____ Description of quality control procedures.

____ Identity of the source of product ingredients.

____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

____ The document is a duplicate of page(s) _____.

____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Rosemary Graham Mora Permethrin Crassostrea gigas

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
11	100	100	100	0
5.9	100	20	20	0
2.7	100	0	0	0
1.6	100	0	0	0
.94	100	0	0	0
.52	100	0	0	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 7.120715

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

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