File

DP Barcode : D191593
PC Code No : 081901
EEB Out : JUL 22 1993

To: Walter Waldrop
Product Manager 71
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 # : 081901
Chemical Name : Chlorothalonil
Type Product : Fungicide
Product Name :
Company Name : ISK Biotech Corporation
Purpose : Rebuttal to EEB review of mysid life-cycle (72-4b) study.

Action Code : 627 Date Due : 7/23/93
Reviewer : Tracy L. Perry

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)			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)	42433807	P	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

1/26

DP BARCODE: **D191593** REREG CASE # 009

CASE: 819269 DATA PACKAGE RECORD DATE: 05/24/9
SUBMISSION: S441229 BEAN SHEET Page 1 of 1

* * * SUBMISSION INFORMATION * * *

CASE TYPE: REREGISTRATIO ACTION: 627 GENERIC DATA SUBMISSION
CHEMICALS: 081901 Chloro nil 100.00 %

ID#: 081901
COMPANY:
PRODUCT MANAGER: 71 WALTER WALDROP 703-308-8062 ROOM: CS1 3B3
PM TEAM REVIEWER: ANDREW W ERTMAN 703-308-8063 ROOM: CS1 32B5
RECEIVED DATE: 05/24/93 DUE OUT DATE: 08/22/93

* * * DATA PACKAGE INFORMATION * * *

DP BARCODE: 191593 EXPEDITE: N DATE SENT: 05/24/93 DATE RET.: / /
CHEMICAL: 081901 Chlorothalonil
DP TYPE: 001 Submission Related Data Package
ADMIN DUE DATE: 07/23/93 CSF: N LABEL: N

ASSIGNED TO	DATE IN	DATE OUT
DIV : **EFED**	5/26/93	/ /
BRAN: **EEB**	5-26-93	/ /
SECT:	/ /	/ /
REVR :	/ /	/ /
CONTR:	/ /	/ /

* * * DATA REVIEW INSTRUCTIONS * * *

Please evaluate the enclosed **rebuttal to an Agency review** dated 1/12/93. The review was for a **Life Cycle Invertebrate** study on **chlorothalonil** (MRID #42433807) that was found to be "Invalid." If this information is not enough to upgrade the study, ISK is requesting a meeting in the next 60 days or so. Thanks for your help.

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

DP BC	BRANCH/SECTION	DATE OUT	DUE BACK	INS	CSF	LABEL

2

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

JUL 22 1993

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Chlorothalonil: rebuttal to EEB review of mysid shrimp life-cycle study (MRID No. 424338-07).

FROM: Anthony Maciorowski, Branch Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H7507C)

TO: Walter Waldrop, PM 71
Reregistration Branch
Special Review and Reregistration Division (H7508W)

ISK Biotech has submitted a rebuttal to EEB's review of the mysid shrimp life-cycle study conducted with technical chlorothalonil (MRID No. 424338-07). EEB originally classified the study as invalid due to three major reasons: 1) Test concentrations of several replicates were variable and did not meet ASTM requirements; 2) The average solvent control survival was only 62%; and, 3) The NOEC and MATC could not be determined.

After reevaluation of the study, EEB has reached the following conclusions: 1) Although test concentrations of several replicates were variable, test concentrations overall were consistent enough to determine the toxicity of chlorothalonil to mysid shrimp; 2) The low survival rate (47%) found in one of the solvent replicates was an isolated occurrence and not indicative of the health of the test population as a whole. The other solvent replicate and the dilution water replicates all had survival rates of 77%. 3) When the solvent and dilution water controls are pooled (as they should be if not statistically different), an NOEC of 0.83 ug/L (mean measured concentration) is established based on reproductive effects (see attached). The LOEC is 1.2 ug/L (mean measured concentration).

Therefore, the mysid shrimp life-cycle study may be upgraded from invalid to supplemental. This study does not fully satisfy guideline requirements as the individual growth data have not been submitted for first generation male and female shrimp. Once these data have been submitted and analyzed, this study may be upgraded to core. If you have any questions, please contact Tracy Perry at 305-6451 or Henry Craven at 305-5320.

chlorothalonil: repro success
File: C:\STATS\TOXSTAT\CHLMYS2.REP Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	pooled control	4	0.520	0.520	0.520
2	0.65 ppb	2	0.270	0.270	0.290
3	0.83 ppb	2	0.310	0.310	0.290
4	1.2 ppb	2	0.110	0.110	0.110
5	3.0 ppb	2	0.075	0.075	0.075
6	5.7 ppb	2	0.000	0.000	0.000

chlorothalonil: repro success
File: C:\STATS\TOXSTAT\CHLMYS2.REP Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
pooled control	0.520				
0.65 ppb	0.290	1.356		1.86	k= 1, v= 8
0.83 ppb	0.290	1.356		1.96	k= 2, v= 8
1.2 ppb	0.110	2.417	*	2.00	k= 3, v= 8
3.0 ppb	0.075	2.624	*	2.01	k= 4, v= 8
5.7 ppb	0.000	3.066	*	2.02	k= 5, v= 8

s = 0.196
Note: df used for table values are approximate when v > 20.

NOEL = 0.83 ppb (mean measured conc.)

LOEL = 1-2 ppb (mean measur. conc.)

ISK·BIOTECH

May 17, 1993

Andy Ertman
Office of Pesticide Programs (H7508W)
Special Review and Reregistration Division
U.S. Environmental Protection Agency
Document Processing Desk (RS-0097)
Room 266A, Crystal Mall No. 2
1921 Jefferson Davis Highway
Arlington, VA 22202

Dear Mr. Ertman:

SUBJECT: CHLOROTHALONIL DATA CALL-IN (RS-0097)
MYSID SHRIMP STUDY (MRID NO. 424338-07)
REQUEST FOR RECONSIDERATION

REQUEST FOR A MEETING IN 60 DAYS

Enclosed are letters from Springborn Laboratories, Inc. and Ricerca, Inc., dated April 19, 1993 and April 23, 1993, respectively, which address the Agency's review and reasons for rejection of the chlorothalonil mysid shrimp life cycle study (MRID No. 424338-07).

The Springborn letter provides a response to each of the Agency's reasons for determining that the study was invalid. It is Springborn's conclusion that the study was conducted in general accordance with the recommended procedures, is scientifically sound and accurately defines the chronic toxicity of chlorothalonil to mysid shrimp. Ricerca's toxicologists agree with Springborn and both laboratories feel that the study should be re-reviewed in light of information provided in the Springborn letter.

Thus, ISK Biotech hereby requests that the Agency consider the responses made in both of the attached letters and consider upgrading the classification of this study to core. If there are additional questions regarding this study, and if the Agency still does not agree that this is a valid "core" study, ISK Biotech hereby requests a meeting approximately 60 days from submission of this response.

Sincerely yours,

Ralph P. Burton

Ralph P. Burton
Manager, Product Registrations

cc: S. K. Shults

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ISK Biotech Corporation, 5966 Heisley Road, P.O. Box 8000, Mentor, Ohio, U.S.A. 44061-8000
216/357-4100 • FAX: 216/354-9506

Ricerca, Inc.

April 23, 1993

J. R. Lucietta
ISK Biotech Corporation
5966 Heisley Road
P. O. Box 8000
Mentor, OH 55061-8000

Dear Mr. Lucietta:

SUBJECT: **DEFENSE OF THE CHRONIC MYSID SHRIMP STUDY WITH TECHNICAL CHLOROTHALONIL (BATCH T-117-12)**

Ricerca Document Number: 3228-89-0043-TX-002
Springborn Report Number: 90-05-3330
EPA MRID Number: 424338-07

The Manager of Environmental Toxicology at the laboratory which conducted the Chronic Mysid shrimp study, Springborn Laboratories, Environmental Sciences Division, has prepared a document (enclosed) which comments and offers Springborn's interpretations on each of three comments which the Environmental Protection Agency (EPA) considers the reasons the study should be rejected. As we have discussed, it is my opinion that the Chronic Mysid shrimp study with technical chlorothalonil should not be rejected but rather should be re-reviewed in light of the comments from Springborn. This re-review should result in a classification as Core for this study.

If you would like to set up a meeting with EPA after they have had a month or so to read the Springborn document, representative(s) from Springborn and representative(s) from Ricerca could meet with you and EPA representatives to discuss this study.

Sincerely,

Steven K. Shults

Steven K. Shults
Research Toxicologist

al/41

Enclosure

c: J. Laveglia
J. C. Killeen, Jr.
D. M. Serrone

7528 Auburn Road • P.O. Box 1000 • Painesville, Ohio 44077-1000
Telex: 196212RICERCA UT • Fax: 216/354-4415 • 216/357-3300

Springborn Laboratories, Inc.
Environmental Sciences Division
790 Main Street • Wareham, Massachusetts 02571 • (508) 295-2550 • Telex 4436041 • Facsimile (508) 295-8107

19 April 1993

Steven K. Shults
Research Toxicologist
Toxicology and Animal Metabolism
Ricerca, Inc.
7528 Auburn Road
PO Box 1000
Painseville, Ohio 44077-1000

RE: Response to EPA Review of the Study Report for the Chronic Toxicity of T-117-12 to Mysid Shrimp (*Mysidopsis bahia*)

Dear Steve:

As we discussed, I have completed the review of EPA's comments on the submitted study report entitled "(T-117-12) - Chronic Toxicity to Mysid Shrimp (*Mysidopsis bahia*)",SLI Report# 90-05-3330, EPA MRID No. 424338-07. The Agency has classified the study as invalid for the following reasons (1) Survival in the solvent control was 62%, which is considered unacceptable control survival by ASTM; (2) The concentrations of several replicates were highly variable during the test. (3) Based on reproductive data, mysids at all chlorothalonil concentrations tested were significantly affected and therefore, the NOEC and MATC could not be determined. Based on my review of these comments and the referenced ASTM guideline I have prepared the following responses to each of the Agency's concerns.

Comment# 1. The survival of mysids in the solvent control was 62%, which is considered unacceptable based on criterion established by ASTM guidelines.

SLI Response - The reported 62% survival of organisms in the solvent control is based on the average of the percent organism survival in two replicate vessels. Organisms in one replicate vessel survived at a rate of 77% which was consistent with the survival in the dilution water controls (i.e., 77%) and exceeded the ASTM guidelines (≥70%), while the survival in the other replicate vessel was 47%. Although Springborn acknowledges that the survival in one replicate vessel of the solvent control was lower than standard expectations, the survival in the remaining solvent control vessel and in the dilution water control solutions established that the lower than expected survival rate was associated with a condition isolated to the replicate exposure vessel and not indicative of poor health of the test population or unacceptable system conditions.

The ASTM guidelines state that the survival of mysids in the dilution water control and in the solvent control are to be statistically analyzed to establish if a difference in survival rates occurred between the two groups. If no statistical difference is observed, the data from both controls should be used for meeting the requirements for acceptability and for calculation of results. The

Springborn Laboratories

ASTM guideline states that a test is considered unacceptable if more than 30% of the first generation control mysids died between pairing and the end of the test. Since during this study, statistical analyses of the control and solvent control data established that no significant difference existed between the performance of the two groups, the data from both controls were combined (i.e., pooled) for use in the calculation of the test results and in determining test acceptability based on the guideline criteria. This pooled data established that 30% of the exposed control (dilution water and solvent control) mysids died between test initiation and test termination. Based on these data, it was determined that the performance of the control organisms during this study met the acceptance criteria. Obviously, the method of analyzing data can be interpreted differently. For example, during this study, Springborn Laboratories interpreted the guidelines to state that the data from the control groups are to be combined or pooled if no statistical difference exists between the two groups.The acceptability criteria was then applied to the pooled control data. If each control group is compared individually a different conclusion may result. Although Springborn Laboratories believes that our interpretation of the guidelines is accurate, we understand and appreciate that it is difficult to write guidelines that avoid the possibility of several correct assumptions. In many cases, the use of different interpretations regarding the treatment of data can result in significantly different conclusions regarding the determination of NOEC and MATC values. Review of the data for the life-cycle test with mysids and T-117-12 established that regardless of the statistical treatment of the F_0 survival data, the conclusion that the survival of the F_0 mysids was not adversely affected by exposure to 5.7 μg /L of T-117-12 would remain the same. In other words, comparison of the survival of the organisms in the highest treatment level (i.e., survival of 77% @ 5.7 μg/L) to the survival of the organisms in the dilution water control (77%), to the survival of the solvent control organisms (62%) and to the survival data for the pooled dilution water and solvent control (70%) result in the same conclusion. Statistical comparisons using all three control values established that organism survival was not adversely affected by exposure to 5.7 μg/L T-117-12 (the highest treatment level tested). The data generated during this chronic exposure clearly established that survival was unaffected by exposure to all concentrations $\leq$5.7 μg/L T-117-12 and that survival was not the most sensitive parameter in evaluating the toxicity of the test article. The NOEC established for this test was determined based on adverse effects on organism reproduction and was established at 0.83 μg/L.

Comment# 2. The concentrations of several replicates were highly variable during the test.

SLI Response - ASTM guidelines state that if either of the following occurs, the test will be considered unacceptable: (1) The measured concentration of test material in any treatment was less than 50% of the time weighted average measured concentration for more than 10% of the duration of the test or (2) The measured concentration of test material was more than 30% of the time-weighted average measured test concentration for more than 5% of the duration of the test. Although the agency reviewer indicates that the criteria for acceptability was violated at several treatment levels, my review of the data establishes that with the exception of one treatment level, the range of measured concentrations observed within each exposure level was within the acceptable range. The only exception was observed at the 2.5 μg/L nominal treatment level. During the 28-day test, the average measured concentration for this treatment level was 1.2 μg/L, therefore based on ASTM guidelines, the acceptable range of measured concentrations for this treatment level was 0.60 μg/L to 1.6 μg/L. On two occasions (i.e., day 0 and 23) the measured concentration in <u>one</u> replicate vessel of the 2.5 μg/L treatment level was determined to be 1.7 μg /L which <u>slightly</u> exceeded the upper limit of the acceptable range (i.e., 1.6 μg/L). A third deviation from the acceptable range was recorded on day 23, when the measured concentration in one replicate vessel was reported as 0.27 μg/L. Although the above mentioned measured

concentrations deviated from the ASTM guidelines for acceptability, it should be recognized that these deviations were isolated to one treatment level and represented only 3 of 50 measurements made during the life cycle test and two of the outliers deviated from the acceptable range by 0.1 µg/L. The Agency reviewer states that additional deviations occurred, however, based on my understanding of the ASTM guidelines these additional deviations can not be verified. For example, the reviewer has indicated that the measured concentration of 7.1 µg/L reported on day 23 (replicate B of the highest treatment level) deviated from the acceptable range established by ASTM. The time weighted average measured for this treatment level was 5.7 µg/L. Thirty percent of the time weighted average is 1.71, therefore the upper limit of the acceptable range is 7.4 µg/L. Based on the information provided by the Agency, I suspect that the reviewer applied the acceptance criteria to each individual replicate treatment. That is, replicate A was evaluated separately from replicate B of the same treatment level. This type of analysis, due to the limited number of data points, can result in a rejection analysis which is too sensitive. In addition, evaluation of each individual replicate treatment appears to deviate from the ASTM guidelines which states that the acceptance criteria is calculated and used on a treatment level basis. Considering the relatively low exposure levels and only one which measurement significantly deviated from the acceptability criteria established by ASTM, Springborn Laboratories believes that sufficient control and consistency in exposure conditions were maintained to accurately evaluate the toxicity of T-117-12 to mysid shrimp.

Comment# 3. Based on reproduction data, mysid shrimp exposed to all concentrations of chlorothalonil, were significantly affected during the chronic test.

SLI Response - ASTM guidelines (section 9.2.4.3) states "If a test contains both a dilution - water control and a solvent control, the survival, growth and reproduction of the mysids in the two controls should be compared. If a statistically significant difference in either survival, growth or reproduction is detected between the two controls, only the solvent control may be used for meeting the requirements of 13.1.9 and 13.1.10 and as the basis for calculation of the results. Analysis of the data for the life cycle test with mysids and T-117-12 established that no significant difference existed between the survival, growth and reproduction of the two control groups. Therefore, as stated in the ASTM guidelines, analysis to determine treatment level effects, all comparisons were performed using the pooled data from both control groups. The Agency reviewer has stated that the solvent control group was adversely affected by the solvent used during this study and suggests that comparisons to establish treatment level effects be performed using the dilution - water control data. Using the dilution-water control data, the reviewer has determined that all exposure levels maintained during the life-cycle test adversely affected the reproductive performance of the exposed mysids. The reviewer justifies the use of the dilution-water control data since all treatment levels did not contain the same concentration of solvent. Springborn Laboratories does not agree that the use of the dilution water control data is the most appropriate method for determining the effect / no-effect concentrations for the completed life-cycle exposure with T-117-12. As previously stated, statistical analyses of the control groups determined that no significant differences existed between the performance of the dilution water and solvent control organisms. Therefore, as recommended by ASTM, both controls (pooled) are to be use for determining treatment effects. If a significant difference was observed between the performance of the two control groups, the use of the dilution-water control data, as suggested by the reviewer, also deviates from the procedure stated in the ASTM guidelines. In addition, the guidelines do not assume consistent solvent concentrations at all treatment levels and state that the solvent control data must be used to evaluate effects when differences are observed between the two control groups. I assume, if the performance of the solvent control groups were superior

to the dilution water control organisms, the reviewer may have suggested to use the solvent control data for determining treatment effects and would not have been concerned that the solvent concentration was not consistent among all treatment levels. Springborn Laboratories analyzed the data as stated in the referenced guidelines and therefore believes that the review for acceptability of the study should be conducted using the recommended procedures. The use of pooled data, is consistent with the recommended guidelines and provides the most accurate comparison. Although the reviewer is correct in stating that the solvent concentration is not consistent between all treatment levels, the use of the dilution water control data assumes that no solvent was in any of the treatment levels. The use of the solvent control data would assume that all treatment levels were equally affected by the solvent concentration. Either of these choices could bias the results and alter the conclusions of the toxicity estimations. The use of the pooled control data provides a comparison standard which is a compromise between the two above mentioned choices and what we believe to be the most accurate comparison. Based on the comparison using the pooled control data, adverse effects on organism reproduction were determined at the three highest exposure levels (i.e., 5.7, 3.0 and 1.2 µg/L). The No Observed Effect Concentration was determined to be 0.83 µg/L of T-117-12.

In addition to the comments which resulted in the invalid classification of the life cycle test, the reviewer listed several other comments which deviated from recommended ASTM practices. Since these additional comments were not listed as reasons for the invalid classification, these issues will be reviewed and incorporated during future testing programs as appropriate, however a response was not prepared for this study.

Based on our understanding and interpretation of the ASTM guidelines we believe that the study was conducted in general accordance with the recommended procedures, is scientifically sound and accurately defines the chronic toxicity of T-117-12 to mysid shrimp. Therefore, the study should be re-evaluated and considered for acceptance as Core. Repeat of the study would not provide any additional information which would alter a risk assessment.

Sincerely,
SPRINGBORN LABORATORIES, INC.

DC Surprenant

Donald C. Surprenant
Program Manager
Environmental Toxicology

cc: K. Grandy

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil.
Shaughnessey No. 081901.

2. **TEST MATERIAL:** T-117-12 (chlorothalonil technical); 100% active ingredient; a light tan powder.

3. **STUDY TYPE:** 72-4. Saltwater Mysid Life-Cycle Toxicity Test. Species Tested: *Mysidopsis bahia*.

4. **CITATION:** Hoberg, J.R. 1991. (T-117-12) - Chronic Toxicity to Mysid Shrimp (*Mysidopsis bahia*). SLI Report No. 90-05-3330. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by ISK Biotech Corporation, Mentor, OH. EPA MRID No. 424338-07.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: Louis M Rifici
Date: 10/5/92

6. **APPROVED BY:**

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

Signature: P. Kosalwat
Date: 10/5/92

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

Signature: Henry T. Craven
Date: Tracy L. Perry 11/16/92

7. **CONCLUSIONS:** This study is not scientifically sound. Survival in the solvent control was 62% which is considered unacceptable control survival by ASTM. The concentrations of several replicates were highly variable during the test. Based on reproductive data, mysids at all chlorothalonil concentrations tested were significantly affected. The NOEC and MATC could not be determined.

8. **RECOMMENDATIONS:** N/A.

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Animals:** Mysids (*Mysidopsis bahia*; ≤24 hours old) were obtained from in-house cultures maintained on a 16-hour light (30-100 ft-candles) photoperiod. The culture water was from the same source as the water used in the test. The temperature during culture was 25°C and the salinity of the culture water was approximately 32 parts per thousand (ppt). The mysids were fed brine shrimp nauplii.

B. **Test System:** An intermittent-flow proportional diluter delivered test solution or control water to individual glass aquaria (39 x 20 x 25 cm). The aquaria were fitted with self starting siphons and the solution volume fluctuated between 4 and 7 l to ensure solution exchange. The volume of each aquarium was replaced an average of 13 times every 24 hours. The diluter was operated for approximately 30 days prior to test initiation.

The test aquaria were impartially positioned in a temperature-controlled water bath maintained at 25 ±2°C. Light was provided on a 16-hour light/8-hour dark photoperiod using fluorescent tubes with an intensity of 30-100 ft-candles.

Unpaired mysids were held in retention chambers constructed of glass petri dishes (10-cm in diameter) with 15-cm high nylon screen (363-µm mesh) collars. Pairing chambers held sexually mature male and female pairs and were constructed of cylindrical glass jars (5.1 cm diameter, 10 cm high) containing two 1.9-cm holes covered with nylon screen.

A 0.44 mg a.i./ml stock solution was prepared by dissolving 0.1108 g of test material in acetone to volume in a 250-ml volumetric flask. An appropriate volume of the stock (43.5 µl) was delivered to the diluter mixing chamber resulting in a high nominal exposure of 10 µg/l which was diluted (50%) to provide the lower nominal concentrations.

The test dilution water was filtered (20 and 5 µm) natural seawater collected from the Cape Cod Canal, Bourne, MA.

C. **Dosage:** Twenty-eight-day life-cycle toxicity test. Based on a preliminary testing, five nominal concentrations (0.63, 1.3, 2.5, 5.0, and 10 µg a.i./l),

a dilution water control, and a solvent control (23 µl acetone/l) were used.

D. **Design:** Mysids were impartially selected and distributed to 28 retention chambers until each contained 15 mysids. Two retention chambers were placed in each aquarium, yielding 30 mysids per replicate aquarium and 60 organisms per test level.

The mysids were fed 24 hour old brine shrimp nauplii twice daily.

To facilitate counting, the retention chambers were removed from the aquaria and placed on a black background. The number of live and dead mysids was determined daily and the chambers were gently brushed and siphoned to remove detritus. Any abnormal appearance or behavior was noted.

When the mysids reached sexual maturity (day 17), they were paired and transferred to isolation jars (10 per replicate). Mysids not used for reproduction were housed in a single retention chamber per replicate. Any paired males that died during the reproduction portion of the study were replaced. Dead females were not replaced. Reproductive output (number of offspring per female per reproductive day) was determined daily. "If the development of brood pouches used in distinguishing female organisms from males; was delayed due to toxicant exposure, those organisms were maintained in clean retention chambers until maturity was observed or until test termination."

At termination, the F_o mysids (males and females were recorded separately) were blotted dry, dried at 60°C for 24 hours, cooled in a desiccator, and weighed to the nearest 0.01 mg. Before drying, brine shrimp nauplii were removed from the female brood sacs when observed, but eggs and juveniles were not removed.

The dissolved oxygen concentration (DO) and pH were measured daily in each aquarium. The temperature and salinity in both replicates of the dilution water control were measured daily. Temperature of a solvent control chamber was continuously monitored using a minimum/maximum thermometer.

Water samples were collected from each replicate aquarium on days 0, 7, 14, 23, and 28 for chemical analysis. The highest test concentration was also

sampled on day 3 (but the results were not reported). The concentration of T-117-12 was determined using gas chromatography.

E. **Statistics:** The endpoints analyzed were survival, dry body weight by sex, and reproduction. The responses of the dilution water control and solvent control mysids were compared using t-tests. The survival, reproduction, and growth of the solvent and dilution water controls were not significantly different. All statistical comparisons of treatment response were made to the pooled control data. The survival data were arcsine square root transformed prior to analysis. Homogeneity of variance and normality for each data set were checked using Bartlett's test and the chi-square test, respectively. All data sets were analyzed using William's test and a 95% level of certainty.

12. **REPORTED RESULTS:** No undissolved test material was observed in the exposure solutions. The mean measured concentrations were 0.65, 0.83, 1.2, 3.0, and 5.7 µg a.i./l (Table 2, attached).

The survival of adult mysids was reported in Table 3 (attached). After 28 days, there was no significant difference between pooled control and exposed mysid survival.

The number of offspring/female/reproductive day at concentrations ≥1.2 µg a.i./l was significantly reduced when compared to the pooled control (Table 3, attached).

Mean body weight at test termination (day 28) was not significantly affected by exposure to T-117-12 at the concentrations tested (Table 4, attached).

During the test, the DO was maintained between 79 and 117% of saturation. The pH was 7.7-8.0 and the temperature was 23-26°C. The salinity ranged from 31 to 33 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** The maximum acceptable toxicant concentration (MATC) was <1.2 µg a.i./l and >0.83 µg a.i./l (geometric mean MATC = 1.0 µg a.i./l), based on the most sensitive parameter, mysid reproduction.

Good Laboratory Practice statements were included in the report, indicating that the study was conducted in accordance with EPA Good Laboratory Practice Standards set forth in 40 CFR Part 160. The stability, characterization,

and verification of the test substance identity was the responsibility of the test sponsor. The dates of quality assurance inspections were included in the report.

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

A. **Test Procedure:** ASTM guidelines (1990) were used to evaluate this study. The test was not scientifically sound. Deviations from the ASTM were the following:

On test days 0, 7, and 23, several replicates had measured concentrations which were more than 30% higher than the time-weighted average concentrations (TWAC) for those replicates (Table 2, attached). Replicate A of the 2.5 µg/l level (1.2 µg/l mean measured concentration) was more than 30% higher than the TWAC on days 0 and 7 and less than 50% of the TWAC on day 23.

Survival in the solvent control replicate A was 47% (Table 3, attached). Survival in replicate B was 77% giving a combined survival for the solvent control of 62%. Control survival of at least 70% is required.

The test material was not identified by a batch or lot number.

Mysids were dried for only 24 hours; 72-96 hours or to a constant weight is recommended. In addition, the mysids were weighed to the nearest 0.01 mg; 0.001 mg is recommended.

The method used for transferring mysids to the test vessels was not described in the report or the study protocol. Mysids must be handled gently using nylon screen or wide-bore glass pipettes.

The temperature during the test (23-26°C) was lower than recommended (27°C).

No raw water quality values and survival, reproduction, or individual weight measurements were presented in the report.

B. **Statistical Analysis:** Survival data did not meet the assumption of homogeneity of variances due to zero variance in the dilution water control data. The data were analyzed using one-way analysis of variance (ANOVA) and Dunnett's and Kruskal-Wallis tests (Toxstat Version 3.3). Survival of the mysids was not

significantly affected by exposure to the test material (see attached printout 1-3).

The reproduction data (except for the highest concentration where there was no reproduction) were analyzed using one-way ANOVA and various parametric multiple comparisons. Compared to the solvent control, there was no effect on reproduction (see attached printout 4). However, compared to the dilution water control, all exposed mysids had significantly reduced reproductive output. In this test, the solvent appears to adversely affect the mysids. Since the solvent concentration was not the same in all test concentrations (and the solvent control contained the highest solvent concentration used in the test), it would be best to compare the treatments to the dilution water control data.

Growth data were not analyzed since only the average growth by replicate data were included in the report.

C. **Discussion/Results:** This study is not scientifically sound. Survival in the solvent control was 62% which is considered unacceptable control survival by ASTM. The concentrations of several replicates were highly variable during the test. The NOEC and MATC could not be determined.

D. **Adequacy of the Study:**

(1) **Classification:** Invalid.

(2) **Rationale:** The test concentrations were variable and did not meet ASTM requirements. In addition, the average solvent control survival was only 62%.

(3) **Repairability:** No.

15. **COMPLETION OF ONE-LINER FOR STUDY:** Yes, 09-30-92.

REFERENCES:

ASTM. 1990. Standard Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids. E1191 - 90.

CHLOROTHALONIL

Page _____ is not included in this copy.

Pages 17 through 19 are not included in this copy.

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) _____.

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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.

Shaughnessey # 081901 Chemical Name chlorothalonil Chemical Class _____ Page 1 of 1

Study/Species/Lab/ MRID #	Chemical % a.i.	Results	Reviewer/ Date	Validation Status
Chronic Fish Species: Lab: MRID #	_____	Concentrations Tested (pp___) = _____ MATC = >_____<_____ pp___. Effected Parameters = _____ Control Mortality (%) = Solvent Control Mortality (%) = Comments:	_____	_____
Chronic Invertebrate Species: Mysidopsis bahia Lab: Springborn Labs. Inc. MRID # 424338-07 (20)	100	Concentrations Tested (pp b*) = 0.65, 0.83, 1.2, 3.0, 5.7 MATC = > 0.83 < 1.2 pp b*. Effected Parameters = Reproductive output Control Mortality (%) = 23 Solvent Control Mortality (%) = 38 Comments: * mean measured concentrations	LAR 9/30/92	Invalid

424338-07, chlorothalonil, 28-day survival
File: a:42433807.dt1 Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance
These two tests can not be performed because at least one group has zero variance.
Data FAIL to meet homogeneity of variance assumption.

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	0.9130	CALCULATED t VALUE =	-1.0000
GRP2 (BLANK CRTL) MEAN =	1.0706	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	-0.1576		

TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	6	0.099	0.017	0.955
Within (Error)	7	0.121	0.017	
Total	13	0.221		

Critical F value = 3.87 (0.05,6,7)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	solvent control	0.913	0.620		
2	water control	1.071	0.770	-1.197	
3	0.65	1.097	0.785	-1.394	
4	0.83	1.066	0.765	-1.160	
5	1.2	1.178	0.850	-2.013	
6	3.0	0.943	0.650	-0.228	
7	5.7	1.066	0.765	-1.160	

Dunnett table value = 2.82 (1 Tailed Value, P=0.05, df=7,6)

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	solvent control	2			
2	water control	2	0.360	58.1	-0.150
3	0.65	2	0.360	58.1	-0.165
4	0.83	2	0.360	58.1	-0.145
5	1.2	2	0.360	58.1	-0.230
6	3.0	2	0.360	58.1	-0.030
7	5.7	2	0.360	58.1	-0.145

424338-07, chlorothalonil, 28-day survival
File: a:42433807.dt2 Transform: ARC SINE(SQUARE ROOT(Y))

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	solvent control	0.913	0.620	8.500
2	water control	1.071	0.770	15.000
3	0.65	1.097	0.785	16.000
4	0.83	1.066	0.765	15.500
5	1.2	1.178	0.850	25.000
6	3.0	0.943	0.650	9.500
7	5.7	1.066	0.765	15.500

Calculated H Value = 5.141 Critical H Value Table = 12.590
Since Calc H < Crit H FAIL TO REJECT Ho:All groups are equal.

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2 (p=0.05)

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 1	0 6	0 4	0 7	0 2	0 3	0 5
1	solvent control	0.913	0.620	\						
6	3.0	0.943	0.650	.	\					
4	0.83	1.066	0.765	.	.	\				
7	5.7	1.066	0.765	.	.	.	\			
2	water control	1.071	0.770	.	.	.	.	\		
3	0.65	1.097	0.785	.	.	.	.	.	\	
5	1.2	1.178	0.850	.	.	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,7) = 3.038 SE = 4.114

data compared to dilution water control data only

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.057	0.011	0.956
Within (Error)	6	0.072	0.012	
Total	11	0.129		

Critical F value = 4.39 (0.05,5,6)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	water control	1.071	0.770		
2	5.7	1.066	0.765	0.044	
3	0.65	1.097	0.785	-0.237	
4	0.83	1.066	0.765	0.044	
5	1.2	1.178	0.850	-0.983	
6	3.0	0.943	0.650	1.167	

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

424338-07, chlorothalonil, 28-day survival
File: a:42433807.dt2 Transform: ARC SINE(SQUARE ROOT(Y))

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	water control	2			
2	5.7	2	0.294	38.2	0.005
3	0.65	2	0.294	38.2	-0.015
4	0.83	2	0.294	38.2	0.005
5	1.2	2	0.294	38.2	-0.080
6	3.0	2	0.294	38.2	0.120

424338-07, chlorothalonil, young/reproductive day
File: a:42433807.dt3 Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
Data PASS normality test at P=0.01 level. Continue analysis.

Bartletts test for homogeneity of variance
Data PASS homogeneity test at 0.01 level. Continue analysis.

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	0.3050	CALCULATED t VALUE =	-1.9106
GRP2 (BLANK CRTL) MEAN =	0.7350	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	-0.4300		

TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at alpha=0.05
TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.554	0.111	5.451
Within (Error)	6	0.122	0.020	
Total	11	0.676		

Critical F value = 4.39 (0.05,5,6)
Since F > Critical F REJECT Ho:All groups equal

TUKEY method of multiple comparisons

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 6 5 3 1 4 2
6	3.0	0.075	0.075	\
5	1.2	0.110	0.110	. \
3	0.65	0.270	0.270	. . \
1	solvent control	0.305	0.305	. . . \
4	0.83	0.310	0.310	. . . . \
2	water control	0.735	0.735	* * . . . \

* = significant difference (p=0.05) . = no significant difference
Tukey value (6,6) = 5.63 s = 0.020

data compared to dilution water control only
WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	water control	2	0.735	0.735	0.735
2	0.65	2	0.270	0.270	0.290
3	0.83	2	0.310	0.310	0.290
4	1.2	2	0.110	0.110	0.110
5	3.0	2	0.075	0.075	0.075

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
water control	0.735				
0.65	0.290	2.850	*	2.02	k= 1, v= 5
0.83	0.290	2.850	*	2.14	k= 2, v= 5
1.2	0.110	4.003	*	2.19	k= 3, v= 5
3.0	0.075	4.227	*	2.21	k= 4, v= 5

s = 0.156

TITLE: 424338-07, chlorothalonil, 28-day survival
FILE: a:42433807.dt1
TRANSFORM: ARC SINE(SQUARE ROOT(Y)) NUMBER OF GROUPS: 7

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	0.4700	0.7554
1	solvent control	2	0.7700	1.0706
2	water control	1	0.7700	1.0706
2	water control	2	0.7700	1.0706
3	0.65	1	0.7000	0.9912
3	0.65	2	0.8700	1.2019
4	0.83	1	0.8000	1.1071
4	0.83	2	0.7300	1.0244
5	1.2	1	0.8000	1.1071
5	1.2	2	0.9000	1.2490
6	3.0	1	0.7700	1.0706
6	3.0	2	0.5300	0.8154
7	5.7	1	0.8000	1.1071
7	5.7	2	0.7300	1.0244

TITLE: 424338-07, chlorothalonil, young/reproductive day
FILE: a:42433807.dt3
TRANSFORM: NO TRANSFORMATION NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	solvent control	1	0.3000	0.3000
1	solvent control	2	0.3100	0.3100
2	water control	1	0.9600	0.9600
2	water control	2	0.5100	0.5100
3	0.65	1	0.3100	0.3100
3	0.65	2	0.2300	0.2300
4	0.83	1	0.3800	0.3800
4	0.83	2	0.2400	0.2400
5	1.2	1	0.1700	0.1700
5	1.2	2	0.0500	0.0500
6	3.0	1	0.0600	0.0600
6	3.0	2	0.0900	0.0900

concentration data

ROW	day0	day7	day14	day23	day28	min	twa	max
1	5.40	5.50	5.50	6.20	6.40	2.87143	5.74286	7.46571
2	5.80	5.60	3.10	7.10✕	6.10	2.66518	5.33036	6.92946
3	3.50	3.00	2.00	3.00	2.90	1.38393	2.76786	3.59821
4	3.60	3.10	2.20	3.50	3.20	1.50714	3.01429	3.91857
5	1.70✕	1.50✕	1.20	0.27•	1.40	0.56143	1.12286	1.45971
6	1.10	1.40	0.86	1.70✕	1.40	0.64161	1.28321	1.66818
7	1.00✕	0.78	0.70	0.74	0.67	0.38241	0.76482	0.99427
8	1.00	0.88	0.82	0.78	0.88	0.42643	0.85286	1.10871
9	0.83	0.67	0.60	0.70	0.55	0.33339	0.66679	0.86682
10	0.75	0.60	0.64	0.66	0.52	0.31902	0.63804	0.82945

✕ higher

• Lower