

US EPA ARCHIVE DOCUMENT

File

DP Barcode : D181891, D181893,
D181894, D181895, D181896, D181832
PC Code No : 081901
EEB Out : JAN 12 1993

To: Walter Waldrop
Product Manager 71
Special Review and Reregistration Division (H7508W)

From: Douglas J. Urban, Acting 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 : Data Review for reregistration

Action Code : 627 Date Due : 11/22/92
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)	424338-06	Y	72-7(B)		
71-2(A)			72-3(A)			122-1(A)	424338-08 ✓	Y
71-2(B)			72-3(B)			122-1(B)	424338-09 ✓	Y
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	424328-01 ✓	Y
71-5(B)			72-4(A)			124-1		
72-1(A)			72-4(B)	424338-07	N	124-2		
72-1(B)	424338-04	Y	72-5			141-1		
72-1(C)			72-6			141-2		
72-1(D)	424338-05	N				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)

Jan



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

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

JAN 12 1993

MEMORANDUM

SUBJECT: Chlorothalonil: data review for reregistration.

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

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

As part of the reregistration process for the List A fungicide, chlorothalonil, the registrant, ISK Biotech Corporation, has submitted the following studies:

Hughes, J.S. and T.L. Williams. 1992. The Toxicity of Technical Chlorothalonil Fungicide to Selenastrum capricornutum. Laboratory Project ID No. B038-001-1. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. MRID No. 424328-01.

Machado, M.W. 1992. Bravo 720 - Acute Toxicity to Bluegill Sunfish (Lepomis macrochirus) Under Flow-Through Conditions. SLI Report No. 92-5-4248. Prepared by Springborn Laboratories, Inc., Wareham, MA. MRID No. 424338-04.

Wüthrich, V. 1990. Daconil 2787 Extra: 96-Hour Acute Toxicity Study (LC₅₀) in the Rainbow Trout. RCC Project 258052. Prepared by R C C Umweltchemie AG, Itingen/BL, Switzerland. MRID No. 424338-05.

Putt, A.E. 1992. Bravo 720 - Acute Toxicity to Daphnids (Daphnia magna) Under Flow-Through Conditions. SLI Report No. 92-4-4225. Prepared by Springborn Laboratories, Inc., Wareham, MA. MRID No. 424338-06.

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. MRID

MEMORANDUM

SUBJECT: Chlorothalonil: data review for reregistration.

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)

As part of the reregistration process for the List A fungicide, chlorothalonil, the registrant, ISK Biotech Corporation, has submitted the following studies:

Hughes, J.S. and T.L. Williams. 1992. The Toxicity of Technical Chlorothalonil Fungicide to Selenastrum capricornutum. Laboratory Project ID No. B038-001-1. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. MRID No. 424328-01.

Machado, M.W. 1992. Bravo 720 - Acute Toxicity to Bluegill Sunfish (Lepomis macrochirus) Under Flow-Through Conditions. SLI Report No. 92-5-4248. Prepared by Springborn Laboratories, Inc., Wareham, MA. MRID No. 424338-04.

Wüster, C. 1990. Daconil 2787 Extra: 96-Hour Acute Toxicity (LC₅₀) in the Rainbow Trout. RCC Project 250. Prepared by R C C Umweltchemie AG, Itingen/BL, Sw. MRID No. 424338-05.

Putt, A.E. 1992. Bravo 720 - Acute Toxicity to Daphnids (Daphnia magna) Under Flow-Through Conditions. SLI Report No. 92-4-4225. Prepared by Springborn Laboratories, Inc., Wareham, MA. MRID No. 424338-06.

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. MRID

SYMBOL	H7507C	H7507C	H7507C				
SURNAME	S. Perry	Chen	OK				
DATE	11/18/92	1/11/93	1-12-93				

No. 424338-07.

Backus, P. 1992. Effect of Chlorothalonil on Seed Germination/Seedling Emergence (Tier I). Laboratory Project ID No. 92-0119. Conducted by Ricerca, Inc., Painesville, OH. MRID No. 424338-08.

Backus, P. 1992. Effect of Chlorothalonil on Vegetative Vigor of Plants (Tier I). Laboratory Project ID No. 92-0120. Conducted by Ricerca, Inc., Painesville, OH. MRID No. 424338-09.

EEB has reviewed the above studies and classified them as follows:

GDLN No.	Species	% AI	Results	Classification	MRID No.
123-2	<i>Selenastrum capricornutum</i>	97.9	NOEC=0.05 ppm LOEC=0.1 ppm EC ₅₀ = 0.19 ppm	Core	424328-01
72-1 B	<i>Lepomis macrochirus</i>	54.0	96-hour LC ₅₀ = 26.3 ppb	Core	424338-04
72-1 D	<i>Oncorhynchus mykiss</i>	40.4	96-hour LC ₅₀ = 0.195 ppm	Invalid	424338-05
72-2 B	<i>Daphnia magna</i>	54.0	48-hour EC ₅₀ = 97 ppb	Core	424338-06
72-4 B	<i>Mysidopsis bahia</i>	100.0	not determined	Invalid	424338-07
122-1 A	see attached DER	97.9	see attached DER	Core	424338-08
122-1 B	see attached DER	97.9	see attached DER	Core	424338-09

Please see the attached DER's for justification of study classification. All applicable data requirements and their statuses can be found in the attached table. If you have any questions, please contact Tracy Perry at 305-6451 or Henry Craven at 305-5320.

Date: 11/09/92
Case No: 819269
Chemical No: 081501

PHASE IV
DATA REQUIREMENTS FOR
ECOLOGICAL EFFECTS BRANCH

Data Requirements	Composition ¹	Use Pattern ²	Does EPA Have Data To Satisfy This Requirement? (Yes, No)	Bibliographic Citation	Must Additional Data Be Submitted under FIFRA3(c)(2)(B)?
6 Basic Studies in Bold					
71-1(a) Acute Avian Oral, Quail/Duck	(TGAI)	A,B,C,E,F,J,K	YES	68753, 30395	NO
71-1(b) Acute Avian Oral, Quail/Duck	(TEP)	-	-	-	-
71-2(a) Acute Avian Diet, Quail	(TGAI)	A,B,C,E,F,J,K	YES	30388, 1151109	NO
71-2(b) Acute Avian Diet, Duck	(TGAI)	A,B,C,E,F,J,K	YES	39146, 30389	NO
71-3 Wild Mammal Toxicity	(TGAI)	-	-	-	-
71-4(a) Avian Reproduction Quail	(TGAI)	A,B,C,E,F,J,K	YES	40964104, 40729404	YES ³
71-4(b) Avian Reproduction Duck	(TGAI)	A,B,C,E,F,J,K	YES	40964102, 40729402	YES ³
71-5(a) Simulated Terrestrial Field Study	(TEP)	-	-	-	-
71-5(b) Actual Terrestrial Field Study	(TEP)	A,B,C,E,F,J,K	NO	-	NO ⁴
72-1(a) Acute Fish Toxicity Bluegill	(TGAI)	A,B,C,E,F,J,K	YES	41439, RIOCHL09, 29410, 30393	NO
72-1(b) Acute Fish Toxicity Bluegill	(TEP)	A	YES	424338-04	NO
72-1(c) Acute Fish Toxicity Rainbow Trout	(TGAI)	A,B,C,E,F,J,K	YES	56486	NO
72-1(d) Acute Fish Toxicity Rainbow Trout	(TEP)	A	NO	424338-05	YES ⁵
72-2(a) Acute Aquatic Invertebrate Toxicity	(TGAI)	A,B,C,E,F,J,K	YES	68754	NO
72-2(b) Acute Aquatic Invertebrate Toxicity	(TEP)	A	YES	424338-06	NO
72-3(a) Acute Estu/Mari Tox Fish	(TGAI)	A,B,C,E,F	YES	127863	NO
72-3(b) Acute Estu/Mari Tox Mollusk	(TGAI)	A,B,C,E,F	YES	138143	NO
72-3(c) Acute Estu/Mari Tox Shrimp	(TGAI)	A,B,C,E,F	YES	127864	NO

* In Bibliographic Citation column indicates study may be upgradeable

Date: 11/09/92
Case No: 819269
Chemical No: 081901

PHASE IV
DATA REQUIREMENTS FOR
ECOLOGICAL EFFECTS BRANCH

Data Requirements	Composition ¹	Use Pattern ²	Does EPA Have Data To Satisfy This Requirement? (Yes, No)	Bibliographic Citation	Must Additional Data Be Submitted under FIFRA3(c)(2)(B)?
72-3(d) Acute Estu/Mari Tox Fish	(TEP)	A	YES	-	YES ⁶
72-3(e) Acute Estu/Mari Tox Mollusk	(TEP)	A	NO	-	NO ⁷
72-3(f) Acute Estu/Mari Tox Shrimp	(TEP)	A	NO	-	NO ⁷
72-4(a) Early Life-Stage Fish	(TGAI)	A,B,C,E,F,J,K	NO	-	NO ⁸
72-4(b) Live-Cycle Aquatic Invertebrate	(TGAI)	A,B,C,E,F,J,K	YES	115107, 424338-07	YES ⁹
72-5 Life-Cycle Fish	(TGAI)	A,B,C,E,F,J,K	YES	30391	NO
72-6 Aquatic Org. Accumulation	(TGAI)	A,B,C,E,F,J,K	NO	-	YES ¹⁰
72-7(a) Simulated Aquatic Field Study	(TEP)	-	-	-	-
72-7(b) Actual Aquatic Field Study	(TEP)	A,B,C,E,F,J,K	PARTIALLY	00127862	YES ¹¹
122-1(a) Seed Germ./Seedling Emerg.	(TGAI)	A,B,C,E,F,J,K	YES	424338-08	NO
122-1(b) Vegetative Vigor	(TGAI)	A,B,C,E,F,J,K	YES	424338-09	NO
122-2 Aquatic Plant Growth	(TGAI)	-	-	-	-
123-1(a) Seed Germ./Seedling Emerg.	(TGAI)	-	-	-	-
123-1(b) Vegetative Vigor	(TGAI)	-	-	-	-
123-2 Aquatic Plant Growth	(TGAI)	A,B,C,E,F,J,K	YES	424328-01	NO
124-1 Terrestrial Field Study	(TEP)	-	-	-	-
124-2 Aquatic Field Study	(TEP)	-	-	-	-
141-1 Honey Bee Acute Contact	(TGAI)	A,B,C	YES	00036935, 00077759	NO
141-2 Honey Bee Residue on Foliage	(TEP)	-	-	-	-
141-5 Field Test for Pollinators	(TEP)	-	-	-	-

* In Bibliographic Citation column indicates study may be upgradeable

1. Composition: TGA= Technical grade of the active ingredient; PAIRA= Pure active ingredient, radiolabeled; TEP= Typical end-use product
2. Use Patterns: A= Terrestrial Food Crop; B= Terrestrial Feed Crop; C= Terrestrial Non-Food Crop; D= Aquatic Food Crop; E= Aquatic Non-Food Outdoor; F= Aquatic Non-Food Industrial; G= Aquatic Non-Food Residential; H= Greenhouse Food Crop; I= Greenhouse Non-Food Crop; J= Forestry; K= Outdoor Residential; L= Indoor Food; M= Indoor Non-Food; N= Indoor Medical; O= Indoor Residential; Z= Use Group for Site 00000
3. Special information (70-1-SS) is required of the registrant to explain the biological and ecological significance of the discoloration (yellowing) that occurred in both avian reproduction tests. This may be a thorough scientific explanation or may require further laboratory testing.
4. Based on new Agency policy, this study has been waived.
5. Required for the cranberry use only; 54% ai flowable concentrate.
6. Required for the cranberry use; 54% ai flowable concentrate. This study was initially reserved but is now required as the formulated product was more toxic to the bluegill than the technical.
7. This study was initially reserved pending the results of formulated product testing with freshwater species. As the formulated product was less toxic to *Daphnia magna* than the technical, this study is not required.
8. Full fish life cycle study suffices.
9. This data requirement has been fulfilled for the freshwater invertebrate life-cycle (115107); however, an estuarine invertebrate life-cycle study, with the mysid shrimp, is still outstanding.
10. This study is in reserve pending information from EFGWB.
11. A waiver request (DP Barcode D175643) for this requirement is currently in review in EEB.

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil.
Shaughnessey No. 081901.
2. **TEST MATERIAL:** Chlorothalonil (tetrachloroisophthalonitrile) technical; CAS No. 1897-45-6; Lot No. D5840923; 97.9% active ingredient; an off-white powder.
3. **STUDY TYPE:** 123-2. Growth and Reproduction of Aquatic Plants - Tier 2. Species Tested: *Selenastrum capricornutum*.
4. **CITATION:** Hughes, J.S. and T.L. Williams. 1992. The Toxicity of Technical Chlorothalonil Fungicide to *Selenastrum capricornutum*. Laboratory Project ID No. B038-001-1. Conducted by Malcolm Pirnie, Inc., Tarrytown, NY. Submitted by ISK Biotech Corporation, Mentor, OH. EPA MRID No. 424328-01.

5. **REVIEWED BY:**

Mark A. Mossler, M.S.
Agronomist
KBN Engineering and
Applied Sciences, Inc.

Signature: 

Date: 10/6/92

6. **APPROVED BY:**

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

Signature: 

Date: 10/6/92

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

Signature: 

Date: 11/20/92

Tracy & Perry 11/16/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. Based on nominal concentrations, the 5-day NOEC, LOEC, and EC₅₀ for *S. capricornutum* exposed to chlorothalonil technical were 0.05, 0.1, and 0.19 mg ai/l, respectively.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Species: The alga used in the test, *Selenastrum capricornutum*, came from laboratory stock cultures originally obtained from the University of Texas Culture Collection. Stock cultures were maintained in synthetic algal assay procedure nutrient medium (AAP) under 4306 lux illumination, and a temperature of $24 \pm 2^\circ\text{C}$. The cultures were continuously shaken at 100 rpm. Transfers were made regularly to provide logarithmically-growing cultures. The culture used as inoculum in this test had been transferred to fresh medium seven days before test initiation.
- B. Test System: All glassware was cleaned according to EPA methods and autoclaved before use. Test vessels used were 250-ml Erlenmeyer flasks fitted with foam stoppers which permitted gas exchange. The test medium was the same as that used for culturing with the pH adjusted to 7.5 ± 0.1 . The medium was filter sterilized ($0.22 \mu\text{m}$) prior to inoculation.

The test vessels were kept in an incubator with environmental conditions like those employed in culturing and continuous cool-white illumination ($4306 \pm 646 \text{ lux}$).

A 3.4 mg active ingredient (ai)/ml primary stock was prepared by diluting 87.5 mg of the test material to 25 ml with dimethylformamide (DMF). Secondary stock solutions were prepared from the primary stock. Test solutions were created by addition of appropriate volumes of the secondary stocks to nutrient medium. The solvent control contained 0.25 ml of DMF/l of nutrient medium.

- C. Dosage: Five-day growth and reproduction test. Based on the results of a preliminary test, five nominal concentrations of 0.025, 0.05, 0.1, 0.2, and 0.4 mg ai/l, and a solvent and medium control were selected for the definitive test.

The reported maximum concentration if applied to a 6-inch water column was 11.8 mg/l. Since this concentration is above the maximum water solubility of the test material (0.6-0.9 mg/l), the upper range of concentrations selected for the test was 3.5 mg/l (3 times the EEC assuming 1% runoff).

- D. **Test Design:** Fifty ml of the appropriate test or control solution were placed into each of three replicate flasks (3 per treatment level and the controls).

A 2-ml aliquot of a *Selenastrum capricornutum* culture was diluted with 18 ml of nutrient medium and the density was determined. An inoculum of cells calculated to provide 3,000 cells/ml was aseptically introduced into each flask. The inoculum volume was 0.241 ml per flask. The flasks were randomly repositioned each working day to minimize spatial differences in the incubator. Cell counts were performed using an electronic particle counter on test days 3, 4, and 5. Three counts per replicate were used on each counting day.

The pH was measured at test initiation and termination. Temperature was monitored manually daily and continuously with a recording device.

- E. **Statistics:** All calculations were based on nominal concentrations. The EC values and associated 95% confidence intervals (C.I.) were computed using weighted least squares non-linear regression of the cell counts (expressed as inhibition compared to the pooled control) at each concentration against the log of the test concentrations. The no-observed-effect concentration (NOEC) was estimated using analysis of variance (ANOVA) and Dunnett's test. The level of significance was $p \leq 0.05$.

12. **REPORTED RESULTS:** Cell counts and percent inhibition for each concentration after five days are given in Tables 3 and 4 (attached). Increasing concentrations of chlorothalonil resulted in increased cellular growth inhibition. Five-day responses ranged from 7% stimulation to 92.5% inhibition.

The five-day EC_{25} was 0.15 mg/l (95% C.I. = 0.13-0.18 mg/l) and the five-day EC_{50} was 0.21 mg/l (95% C.I. = 0.19-0.23 mg/l). The NOEC was determined to be 0.1 mg/l.

The pH ranged from 7.37 to 7.54 in all test solutions and the controls at test initiation. The pH values on day 5 ranged from 7.89 to 8.28.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the study authors.

Good Laboratory Practice and Quality Assurance statements were included in the report indicating compliance with EPA Good Laboratory Practice Standards, 40 CFR Part 160.

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

- A. Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

Cell growth measurements were not taken daily. Measurements were made on days 3, 4, and 5 only.

The results of the daily or continuous temperature measurements were not reported.

- B. Statistical Analysis:** The reviewer determined the EC₅₀ using EPA's Toxanal program. The lowest-observed-effect concentration (LOEC) and NOEC were determined using EPA's Dunnett's test program. The reviewer obtained a slightly more conservative value for the EC₅₀ using the moving average angle method. The EC₅₀ was determined to be 0.19 mg ai/l (95% C.I. = 0.18-0.21 mg ai/l). The NOEC and LOEC were determined to be 0.05 and 0.1 mg ai/l, respectively (see attached printouts).

- C. Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a Tier 2 non-target aquatic plant study. Based on nominal concentrations, the 5-day NOEC, LOEC, and EC₅₀ for S. capricornutum exposed to chlorothalonil technical were 0.05, 0.1, and 0.19 mg ai/l, respectively.

- D. Adequacy of the Study:**

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

- 15. COMPLETION OF ONE-LINER:** Yes, 9-30-92.

Page is not included in this copy.

Pages 12 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.
 - ☒ 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.

Selenastrum cell density

Summary Statistics and ANOVA

Transformation = None

Group	n	Mean	s.d.	cv%
Concentration (mg/l)				
1 = control	6	2923066.6667	239397.7332	8.2
2 0.025	3	3126600.0000	358748.5470	11.5
3 0.05	2	2906600.0000	51760.2164	1.8
4*0.1	3	2414333.3333	357018.8417	14.8
5*0.2	3	1656233.3333	254987.3396	15.4
6*0.4	3	220610.0000	45016.0982	20.4

NOEC = 0.05 mg/l*
LOEC = 0.1 mg/l*

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by a t - test with Bonferroni adjustment of alpha level

Minumum detectable difference for
t-tests with Bonferroni adjustment = -391810.330266
This difference corresponds to -13.40 percent of control

*
* Note - the above value for the minimum
* detectable difference is approximate as
* the sample sizes are not the same for all of
* the groups.
*

Between groups sum of squares =***** with 5 degrees of freedom.
Error mean square = ***** with 14 degrees of freedom.
Bartlett's test p-value for equality of variances = .217

MOSSLER CHLOROTHALONIL SELENASTRUM CAPRICORNUTUM 9-30-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.4	100	92	92	0
.2	100	43	43	0
.1	100	17	17	0
.05	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 .2179518

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
3	1.270005E-02	.1927294	.1760097 .2127247

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
3	.445905	3.419052	3.274346E-02

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

SLOPE = 4.026595
95 PERCENT CONFIDENCE LIMITS = 1.337792 AND 6.715399

LC50 = .1969003
95 PERCENT CONFIDENCE LIMITS = .1228307 AND .3441098

LC10 = 9.524528E-02
95 PERCENT CONFIDENCE LIMITS = 2.126412E-02 AND .1445151

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil.
Shaughnessey No. 081901.
2. **TEST MATERIAL:** Bravo 720; Lot No. 029249; 54% active ingredient; a grey viscous liquid.
3. **STUDY TYPE:** 72-1. Freshwater Fish Acute Flow-Through Toxicity Test. Species Tested: Bluegill Sunfish (*Lepomis macrochirus*).
4. **CITATION:** Machado, M.W. 1992. Bravo 720 - Acute Toxicity to Bluegill Sunfish (*Lepomis macrochirus*) Under Flow-Through Conditions. SLI Report No. 92-5-4248. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by ISK Biotech Corporation, Mentor, OH. EPA MRID No. 424338-04.

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:**

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

Signature:

Rosemary Graham Mora

Date:

10/5/92

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

Signature:

Henry T. Craven 11/24/92

Date:

Tracy A. Perry 11/4/92

7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a flow-through acute toxicity test using freshwater fish. The 96-hour LC_{50} value of Bravo 720 for bluegill sunfish was 26.3 $\mu\text{g a.i./l.}$ Therefore, Bravo 720 is classified as very highly toxic to bluegill sunfish. The NOEC, based on the lack of treatment-related mortality and sublethal effects, was 15 $\mu\text{g a.i./l.}$
8. **RECOMMENDATIONS:** N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. Test Animals: Bluegill sunfish (*Lepomis macrochirus*) were obtained from a commercial supplier in Ashford, CT. The fish were maintained in the laboratory in flowing well water and fed a commercially available pelleted fish food, *ad libitum*, daily. Water quality characteristics of the well water were a total hardness of 28-35 mg/l as CaCO_3 , an alkalinity of 20-28 mg/l as CaCO_3 , a conductivity of 100-130 $\mu\text{mhos/cm}$, and a temperature of 22-23°C. The laboratory was maintained on a 16-hour daylight photoperiod.

Feeding was discontinued 48 hours before the test. Mortality of 0.48% occurred during this period. Mean weight and length of a representative group from the test population were 0.39 (0.21-0.70) g and 30 (26-36) mm, respectively.

B. Test System: The test system consisted of 14 glass aquaria (39 x 20 x 25 cm), each containing approximately 11 l of test solution (solution depth of 14.5 cm). A constant-flow serial diluter delivered 50 ml/minute (or 6.5 volume replacements per day) of test solution or control water to each aquarium over the course of the study. The diluter was allowed to equilibrate for a minimum of 96 hours prior to test initiation.

The dilution water was from the same source as that used in holding.

The test aquaria were impartially placed in a water bath set to maintain 22 \pm 1°C. A 16-hour light/8-hour dark photoperiod (light intensity of 42-90 ft-candles) was used.

A stock solution (2000 $\mu\text{g/l}$) was prepared by dissolving approximately 0.10 g of test material in 50 l of dilution water. The solution was vigorously mixed for approximately 2 hours. The stock was delivered to the diluter mixing chamber using a mechanical pump. The resulting solution was equivalent to the highest nominal treatment level. This solution was diluted to provide the remaining nominal treatment levels.

- C. **Dosage:** Ninety-six-hour flow-through test. Based on preliminary testing, six nominal concentrations (16, 26, 43, 72, 120, and 200 µg/l) and a dilution water control were used.
- D. **Design:** Ten bluegill were impartially selected and distributed to each replicate aquarium (20 per treatment level). The fish were not fed during the test. The biomass loading was 0.054 g/l/day. Observations of mortality, sublethal responses, and test solution characteristics were made every 24 hours. Dead fish were removed at each observation.

The temperature, dissolved oxygen concentration (DO), and pH were measured once daily in each replicate of the exposure concentrations and the control. The temperature was also monitored continuously in one replicate of the dilution water control.

Water samples were removed from each replicate aquarium at test initiation and termination. The concentration of chlorothalonil in each sample was determined using gas chromatography and reported as µg formulation/l.

- E. **Statistics:** The median lethal concentration (LC₅₀) and associated 95% confidence interval (C.I.) for each 24-hour interval were calculated using a computer program developed by Stephan (1977, 1982).

- 12. **REPORTED RESULTS:** The mean measured concentrations were 7.2, 15, 28, 50, 94, and 120 µg/l which averaged 63% of nominal concentrations (Table 2, attached).

Based on mortality and mean measured concentrations of formulated product, the 96-hour LC₅₀ was 65 µg/l with a 95% C.I. of 50-94 µg/l (Table 3, attached). The slope of the 96-hour concentration-response curve was 4.1. The no-observed-effect concentration (NOEC) was 28 µg/l.

Dissolved oxygen ranged from 8.6 to 9.4 mg/l or 100 to 109% of saturation. The pH values ranged from 7.1 to 7.3. The temperature was 22-23°C throughout the test.

- 13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
"Bravo 720 is considered to be very highly toxic to bluegill sunfish."

Quality Assurance and Good Laboratory Practice Compliance Statements were included in the report, indicating that the study was conducted in accordance with EPA Good Laboratory

Practice Regulations (40 CFR, Part 160) except for the stability, characterization, and verification of the test substance identity. The dates of quality assurance inspections were also included.

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

- A. Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following:

The hardness of the dilution water was 30-36 mg/l as CaCO_3 . The SEP recommends that the hardness of dilution waters used in the testing of organic pesticides be 40-200 mg/l as CaCO_3 .

The test design for a formulated product study should include a control where the fish are exposed to just the carrier or inert ingredients. Such a control was not used in this test.

- B. Statistical Analysis:** The reviewer used EPA's Toxanal program to calculate the 96-hour LC_{50} value as 49 $\mu\text{g/l}$ (95% C.I. = 41-59 $\mu\text{g/l}$) using the moving average method (see attached printout 1). The mean measured concentrations of formulated product were converted to $\mu\text{g a.i./l}$ and the LC_{50} recomputed (see attached printout 2). The 96-hour LC_{50} was 26.3 $\mu\text{g a.i./l}$ (95% C.I. = 22.1-31.9 $\mu\text{g a.i./l}$).

- C. Discussion/Results:** This study is scientifically sound and meets the requirements for a flow-through toxicity test using freshwater fish. The 96-hour LC_{50} value of Bravo 720 for bluegill sunfish was 26.3 $\mu\text{g a.i./l}$. Therefore, Bravo 720 is classified as very highly toxic to bluegill sunfish. The NOEC, based on the lack of treatment-related mortality and sublethal effects, was 15 $\mu\text{g a.i./l}$.

- 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, 09-25-92.

Page _____ is not included in this copy.

Pages 21 through 22 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.
 - ☒ FIFRA registration data.
 - ☐ The document is a duplicate of page(s) _____.
 - ☐ The document is not responsive to the request.
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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.

RIFICI CHLOROTHALONIL LEPOMIS MACROCHIRUS 09-25-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
120	20	20	100	9.536742E-05
94	20	19	95	2.002716E-03
50	20	3	15	.1288414
28	20	0	0	9.536742E-05
15	20	1	5	2.002716E-03
7.2	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 50 AND 94 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 64.91872

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
5	3.395672E-02	48.68014	40.92956 - 58.94845

~~58.94845~~

LMR
9/25/98

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
8	7.713814	39.52426	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 = 3.839829
95 PERCENT CONFIDENCE LIMITS = -6.824817 AND 14.50447

LC50 = 55.04721
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 25.70345
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

#2

RIFICI CHLOROTHALONIL LEPOMIS MACROCHIRUS 09-25-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
65	20	20	100	9.536742E-05
51	20	19	95	2.002716E-03
27	20	3	15	.1288414
15	20	0	0	9.536742E-05
8	20	1	5	2.002716E-03
4	20	1	5	2.002716E-03

THE BINOMIAL TEST SHOWS THAT 27 AND 51 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 35.12458

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
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5	3.395672E-02	26.33621	22.14755 - 31.88434
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31.88434

LMR
9/25/98

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
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8	6.732898	34.66378	0
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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 = 3.848499
95 PERCENT CONFIDENCE LIMITS = -6.137521 AND 13.83452

LC50 = 29.72389
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 13.90295
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil.
Shaughnessey No. 081901.
2. **TEST MATERIAL:** Daconil 2787 Extra;
tetrachloroisophthalonitrile; Batch No. 10-89; 40.4% active
ingredient; a light grey liquid.
3. **STUDY TYPE:** 72-1. Freshwater Fish Static Acute Toxicity
Test. Species Tested: Rainbow Trout (*Oncorhynchus mykiss*).
4. **CITATION:** Wüthrich, V. 1990. Daconil 2787 Extra: 96-Hour
Acute Toxicity Study (LC₅₀) in the Rainbow Trout. RCC
Project 258052. Prepared by R C C Umweltchemie AG,
Itingen/BL, Switzerland. Submitted by ISK Biotech
Corporation, Mentor, OH. EPA MRID No. 424338-05.

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:**

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

Signature: *Rosemary Graham Mora*
Date: 10/5/92

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

Signature: *Henry T. Craven*
Date: Tracy L. Perry 11/4/92

7. **CONCLUSIONS:** This study is not scientifically sound. The
analytical results indicate that the actual concentrations
to which the fish were exposed are unknown. Based on
nominal concentrations, the 96-hour LC₅₀ was 0.195 mg
formulation/l. The NOEC could not be determined since
sublethal or lethal effects were observed in each exposure
level.

8. **RECOMMENDATIONS:** EEB recommends that subsequent tests be performed
under flow-through conditions as the test material is
9. **BACKGROUND:** very unstable.

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. Test Animals: Rainbow trout (*Salmo gairdneri*) were obtained from a commercial supplier in Zeiningen, Switzerland. The fish were maintained in a 250-l tank in filtered (5 μ m), aerated tap water for 17 days. The fish were fed a commercially available diet three times weekly. The temperature during holding was 12.5-14.0°C. Mean weight and length of 15 fish were 2.9 g and 65 (56-71) mm.

The fish were acclimated to reconstituted water at 13.0-15.0°C for seven days prior to test initiation. Feeding was discontinued one day before the test. There was no mortality in the population during acclimation.

- B. Test System: The test was conducted in an air-conditioned room maintained at "22 \pm 3°C" under 12 hours of light (500-1500 lux) per day.

The dilution water was reconstituted water prepared by dissolving NaHCO₃ (64.80 mg/l), CaCl₂·2H₂O (294.00 mg/l), MgSO₄·7H₂O (123.2 mg/l), and KCl (5.75 mg/l) in deionized water. The hardness was 14 dH°.

The test stock solution was prepared by diluting 500 mg of test "article" to 1000 ml with dilution water. A secondary stock was prepared by serial dilution. Fifteen liters of each test solution were made using dilution water and appropriate volumes of secondary stock.

- C. Dosage: Ninety-six-hour static test. Based on preliminary testing, five nominal concentrations (0.095, 0.171, 0.308, 0.556, and 1.0 mg/l) and a dilution water control were used.

- D. Design: Ten rainbow trout were used per concentration. The loading was 0.7-1.0 g/l. Observations of mortality were made daily. The temperature, dissolved oxygen concentration (DO), and pH were measured in each test container prior to test initiation and at 2, 24, 48, 72, and 96 hours. Any sublethal ("clinical") responses were monitored when water quality data were collected.

The concentration of the test material in the control, 0.095, 0.308, and 1.0 mg/l nominal test solutions were

measured using gas chromatography. The samples were collected at test initiation and at 2, 48, and 96 hours but not necessary analyzed.

- E. **Statistics:** The median lethal concentration (LC_{50}) and 95% confidence interval (C.I.) were calculated using the logit-model.

12. **REPORTED RESULTS:** The test solutions were turbid suspensions. The measured concentrations for the 0.095, 0.308, and 1.0 mg/l nominal concentrations were presented in Table 2 (attached).

The 96-hour LC_{50} was 0.195 mg/l (95% C.I. = 0.160-0.259 mg/l). The slope of the regression line was 5.177. The no-observed-effect concentration was <0.095 mg/l since sublethal effects were noted in all exposures (Tables 4 and 6, attached).

The DO ranged from 9.2 to 11.3 mg/l. The pH values ranged from 7.9 to 8.4 and the temperature was 13.0-14.0°C.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
"DACONIL 2787 EXTRA is considered to be highly toxic to the Rainbow trout."

A statement of compliance with GLP regulations was included in the report which noted that the range-finding tests were not conducted in compliance with GLP guidelines. The dates of quality assurance inspections were presented in the report.

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

- A. **Test Procedure:** This test was not scientifically sound. Deviations from the SEP are as follows:

The report did not state whether the nominal test concentrations were mg formulation/l or mg a.i./l. Based on the weights and volumes reported, the reviewer calculated that the concentrations were mg formulation/l.

The number of replicates used was not reported. From the results in Attachment Table 2 (attached), it appears that two replicates were used.

The loading was reported as 0.7-1.0 g/l. Based on an average fish weight of 2.9 g, five fish per vessel, and a solution volume of 15 l, the average loading was 0.95

g/l. Recommended loading for static coldwater tests is ≤ 0.8 g/l.

The test design for a formulated product study should include a control where the fish are exposed to just the carrier or inert ingredients. Such a control was not used in this test.

The test vessel construction materials and dimensions were not described in the report. Test vessels should be constructed of glass or stainless steel.

The test temperature ($13.0-14.0^{\circ}\text{C}$) was higher than recommended ($12 \pm 1^{\circ}\text{C}$).

The system used to control temperature was not described. Test solution temperature should have been measured at least every six hours if a water bath was used or continuously if ambient air temperature was used.

Each selected nominal concentration averaged 56% of the next highest concentration. Each concentration should be at least 60% of the next highest concentration.

The period between test solution preparation and the initiation of the test was not stated in the report. Tests should be initiated within 30 minutes of solution preparation.

The photoperiod used in the test was 12-hours light/12-hours dark; a 16/8 photoperiod should have been used.

A 15 to 30-minute transition period between light and dark is recommended in the SEP. A transition period was not used in the study.

- B. **Statistical Analysis:** The reviewer used EPA's Toxanal program to determine the 96-hour LC_{50} value as 0.21 mg formulation/l (95% C.I. = 0.095-0.308 mg formulation/l) using linear interpolation and binomial probability.
- C. **Discussion/Results:** Only 3 of the 5 test solutions were analyzed for the concentration of the test material. The results show that concentrations decreased an average of 18% during the first 2 hours of the test (Table 2, attached). After 48 hours, the measured concentration for test lowest test level had decreased by 91% from the initial measured concentration. The test material was not detected in

this level at the end of the test. It is likely that had the concentration of the test material remained fairly constant during the entire exposure, more mortality would have been observed and a more conservative LC_{50} calculated.

This study is not scientifically sound. Based on nominal concentrations, the 96-hour LC_{50} was 0.195 mg formulation/l. The NOEC could not be determined since sublethal or lethal effects were observed in each exposure level.

D. Adequacy of the Study:

- (1) **Classification:** Invalid.
- (2) **Rationale:** The analytical results indicate that the actual concentrations to which the fish were exposed are unknown.
- (3) **Repairability:** No.

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 09-25-92.

Page _____ is not included in this copy.

Pages 30 through 32 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.
 - ☒ FIFRA registration data.
 - ☐ The document is a duplicate of page(s) _____.
 - ☐ The document is not responsive to the request.
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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.

RIFICI CHLOROTHALONIL ONCORHYNCHUS MYKISS 09-25-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
1	10	10	100	9.765625E-02
.556	10	10	100	9.765625E-02
.308	10	10	100	9.765625E-02
.171	10	2	20	5.46875
.095	10	0	0	9.765625E-02

THE BINOMIAL TEST SHOWS THAT .095 AND .308 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 .2057936

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.

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil.
Shaughnessey No. 081901.
2. **TEST MATERIAL:** Bravo 720; Lot No. 029249; 54% active ingredient; a grey viscous liquid.
3. **STUDY TYPE:** 72-2. Freshwater Invertebrate Flow-Through Acute Toxicity Test. Species Tested: *Daphnia magna*.
4. **CITATION:** Putt, A.E. 1992. Bravo 720 - Acute Toxicity to Daphnids (*Daphnia magna*) Under Flow-Through Conditions. SLI Report No. 92-4-4225. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by ISK Biotech Corporation, Mentor, OH. EPA MRID No. 424338-06.
5. **REVIEWED BY:**

Louis M. Rifici, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Louis M. Rifici</i> Date: 10/5/92
---	--
6. **APPROVED BY:**

Rosemary Graham Mora, M.S. Associate Scientist KBN Engineering and Applied Sciences, Inc.	Signature: <i>Rosemary Graham Mora</i> Date: 10/5/92
Henry T. Craven, M.S. Supervisor, EEB/EFED USEPA	Signature: <i>H.T. Craven</i> Date: 11/20/92
7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a flow-through acute toxicity test using a freshwater invertebrate. Based on mean measured concentrations, the 48-hour EC₅₀ was 97 µg a.i./l therefore, Bravo 720 is classified as very highly toxic to daphnids. The NOEC was 49 µg a.i./l mean measured concentration.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. MATERIALS AND METHODS:

- A. **Test Animals:** *Daphnia magna* (≤ 24 hours old) were obtained from in-house cultures maintained under a 16-hour light photoperiod (light intensity = 32-44 ft-candles) at $20 \pm 2^\circ\text{C}$. The culture water was well water filtered through a resin column (Amberlite XAD-7) and a carbon filter to remove any organic contaminants then fortified to a hardness of 160-180 mg/l and an alkalinity of 110-130 mg/l as CaCO_3 . The pH was 7.9-8.3 and the conductivity was 400-600 $\mu\text{mhos/cm}$. The cultures were fed *Ankistrodesmus falcatus* and a trout food suspension once daily.
- B. **Test System:** An intermittent-flow proportional diluter used. The flow of test solution from the mixing/splitting chambers into the test chambers was restricted using glass capillary tubes (1 mm I.D.) to minimize turbulence in the chambers. Test solutions were delivered to each vessel at an approximate rate of 6.0 volume replacements per day.

The test vessels were made of glass and contained a constant solution volume of 1.8 l. The test solution depth was approximately 13 cm. Test temperature was controlled using a water bath set to $20 \pm 1^\circ\text{C}$. The test area was illuminated at an intensity of 34-55 ft-candles using fluorescent tubes on a 16-hour light/8-hour dark photoperiod.

A stock solution (500 $\mu\text{g/l}$) was prepared by dissolving 0.10 g of test material in 200 l of dilution water. The solution was vigorously mixed for approximately 2 hours. The stock was equivalent to the highest test concentration and was diluted to provide the remaining nominal treatment levels.

The dilution water was from the same source as that used in culturing. The water quality was described as a total hardness of 170 mg/l as CaCO_3 , an alkalinity of 110-120 mg/l as CaCO_3 , a pH of 8.1-8.3, and a conductivity of 500 $\mu\text{mhos/cm}$.

- C. **Dosage:** Forty-eight-hour, acute toxicity test. Based on preliminary testing, five nominal concentrations (65, 110, 180, 300, and 500 $\mu\text{g/l}$) and a dilution water control were selected for the test.
- D. **Design:** Two chambers were used for each concentration with ten impartially-selected daphnids per chamber.

The number of immobilized daphnids was recorded daily. Observations of sublethal effects and of the physical characteristics of the test solutions were made at test initiation and every 24 hours thereafter. The daphnids were not fed during the test.

Dissolved oxygen concentration, pH, and temperature were measured once daily in all replicates. At test initiation, hardness, alkalinity, and conductivity in one replicate vessel of each level were determined. The temperature of one control vessel was also monitored continuously using a minimum/maximum thermometer.

Water samples from both replicates of each concentration and the controls were taken at test initiation and termination. The concentration of chlorothalonil was determined using gas chromatography.

E. Statistics: The 48-hour median effective concentration (EC_{50}) and associated 95% confidence interval (C.I.) were calculated using a computer program developed by Stephan.

- 12. REPORTED RESULTS:** The mean measured concentrations were 50, 91, 160, 260, and 420 $\mu\text{g/l}$ and averaged 84% of nominal (Table 3, attached).

The 48-hour EC_{50} was 180 $\mu\text{g/l}$ (95% C.I. = 160-200 $\mu\text{g/l}$). The slope of the dose-response curve was 7.9. Sublethal and lethal effects were observed in the three highest test levels (Table 4, attached). The no-observed-effect concentration (NOEC) was 91 $\mu\text{g/l}$.

During the test, the dissolved oxygen concentration was 8.5-9.6 mg/l (93-105% of saturation) and the pH was 8.2-8.3. The results of continuous temperature monitoring established the test temperature as 19-21°C. The hardness, alkalinity, and conductivity of the exposure solutions were 170-180 mg/l as CaCO_3 , 110-120 mg/l as CaCO_3 , 500 $\mu\text{mhos/cm}$, respectively.

- 13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** Based on criteria established by US EPA (1985), Bravo 720 is classified as highly toxic to *Daphnia magna*.

Quality Assurance and GLP Compliance Statements were included in the report indicating adherence to US EPA GLP Regulations (40 CFR Part 160). The dates of study inspections were also included.

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

- A. Test Procedure:** The test procedures generally adhered to the SEP, except for the following:

A study performed using a formulated product should include a control containing an equivalent amount of the inert or carrier ingredients present in the formulation without the active ingredient. Such a control was not included in this test.

Observations of the daphnid cultures such as adult mortality, stress, and the presence of ephippia were not reported.

First instar *Daphnia magna* used in tests should be from the fourth or later broods of a given parent. The author did not indicate which brood was the source of the test animals.

- B. Statistical Analysis:** The reviewer used EPA's Toxanal program to verify the author's 48-hour EC_{50} and obtained similar results (printout 1, attached). The reported concentrations were converted to $\mu\text{g a.i./l}$ and the EC_{50} value determined to be $97 \mu\text{g a.i./l}$ (95% C.I. = $86-109 \mu\text{g a.i./l}$) using the probit analysis (printout 2, attached).
- C. Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a flow-through acute toxicity test using a freshwater invertebrate. Based on mean measured concentrations, the 48-hour EC_{50} was $97 \mu\text{g a.i./l}$ therefore, Bravo 720 is classified as very highly toxic to daphnids. The NOEC was $49 \mu\text{g a.i./l}$ mean measured concentration.
- 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, 09-28-92.

Page _____ is not included in this copy.

Pages 38 through 39 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.
 - ☒ FIFRA registration data.
 - ☐ The document is a duplicate of page(s) _____.
 - ☐ The document is not responsive to the request.
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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.

#1

RIFICI CHLOROTHALONIL DAPHNIA MAGNA 09-28-92

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
420	20	20	100	9.536742E-05
260	20	19	95	2.002716E-03
160	20	6	30	5.765915
91	20	0	0	9.536742E-05
50	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 91 AND 260 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 182.7639

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	5.135013E-02	175.8403	149.9065 - 209.3848

~~209.3848~~ *LR 9/28/97*

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
7	.2155924	1	.9989486

SLOPE = 10.44153
95 PERCENT CONFIDENCE LIMITS = 5.593328 AND 15.28974

LC50 = 180.1246
95 PERCENT CONFIDENCE LIMITS = 159.1661 AND 203.0837

LC10 = 136.1267
95 PERCENT CONFIDENCE LIMITS = 102.0844 AND 155.0415

RIFICI CHLOROTHALONIL DAPHNIA MAGNA 09-28-92

#2

CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
227	20	20	100	9.536742E-05
140	20	19	95	2.002716E-03
86	20	6	30	5.765915
49	20	0	0	9.536742E-05
27	20	0	0	9.536742E-05

THE BINOMIAL TEST SHOWS THAT 49 AND 140 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 98.28371

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS
4	5.135013E-02	94.72438	80.74865 - 112.8028

LR 9/29/92

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
13	.2147829	1	.9989508

SLOPE = 10.40969
95 PERCENT CONFIDENCE LIMITS = 5.585354 AND 15.23403

LC50 = 96.86479
95 PERCENT CONFIDENCE LIMITS = 85.5819 AND 109.2601

LC10 = 73.14155
95 PERCENT CONFIDENCE LIMITS = 54.86958 AND 83.32306

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 $25 \pm 2^{\circ}\text{C}$. 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 $\mu\text{g/l}$ 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 $\mu\text{g a.i./l}$),

a dilution water control, and a solvent control (23 μ l 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 F₀ 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 $\mu\text{g a.i./l}$ (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 $\geq 1.2 \mu\text{g a.i./l}$ 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 $<1.2 \mu\text{g a.i./l}$ and $>0.83 \mu\text{g a.i./l}$ (geometric mean MATC = $1.0 \mu\text{g a.i./l}$), 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.

Page 49 is not included in this copy.

Pages 49 through 50 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.
 - ☒ 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.

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

Study/Species/Lab/ Chemical
MRID # _____ % a.i. _____ Results _____ Reviewer/ Validation
Date _____ Status _____

Chronic Fish Concentrations Tested (ppm) - _____

Species: MATC - > _____ < _____ ppm _____

Lab: Effectuated Parameters - _____

MRID # Control Mortality (%) - _____ Solvent Control Mortality (%) - _____

Comments: _____

Chronic Invertebrate Concentrations Tested (ppm) - 0.65, 0.83, 1.2, 3.0, 5.1

Species: Mytilus edulis MATC - > 0.83 < 1.2 ppm 100 Invalid
9/30/91

Lab: Spungborn Labs. Inc. Effectuated Parameters - Reproductive but part

MRID # 424338-07 Control Mortality (%) - 23 Solvent Control Mortality (%) - 38

Comments: * mean measured concentrations

424338-07, chlorothalonil, 28-day survival
 File: a:42433807.dtl 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
 Bartlett's 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 CTRL) MEAN	=	0.9130	CALCULATED t VALUE	=	-1.0000
GRP2 (BLANK CTRL) 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	0	0	0	0	0
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.

Bartlett's 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 CTRL) MEAN =	0.3050	CALCULATED t VALUE =	-1.9106
GRP2 (BLANK CTRL) 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
				0 0 0 0 0 0
				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 \
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		2	0.270	0.270	0.290
3		2	0.310	0.310	0.290
4		2	0.110	0.110	0.110
5		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

55

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 ^x	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 ^x	1.50 ^x	1.20	0.27 ^o	1.40	0.56143	1.12286	1.45971
6	1.10	1.40	0.86	1.70 ^x	1.40	0.64161	1.28321	1.66818
7	1.00 ^x	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

^x higher

^o lower

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil.
Shaughnessey No. 081901.
2. **TEST MATERIAL:** Chlorothalonil (tetrachloroisophthalonitrile) technical; CAS No. 1897-45-6; Lot No. DS-2787-1002; 97.9% purity; a white powder.
3. **STUDY TYPE:** 122-1. Non-Target Plants: Seed Germination/Seedling Emergence Phytotoxicity Test - Tier 1. Species Tested: Soybean, Mustard, Radish, Tomato, Cucumber, Buckwheat, Oat, Sorghum, Corn, and Onion.
4. **CITATION:** Backus, P. 1992. Effect of Chlorothalonil on Seed Germination/Seedling Emergence (Tier I). Laboratory Project ID No. 92-0119. Conducted by Ricerca, Inc., Painesville, OH. Submitted by ISK Biotech Corporation, Mentor, OH. EPA MRID No. 424338-08.

5. **REVIEWED BY:**

Tracy L. Perry
Wildlife Biologist
Ecological Effects Branch

Signature: Tracy L. Perry
Date: 11/12/92

6. **APPROVED BY:**

Henry T. Craven
Head, Section 4
Ecological Effects Branch

Signature: Henry T. Craven
Date: 11/11/93

7. **CONCLUSIONS:**

Seed Germination: The most sensitive species in the germination test was cucumber (8% reduction in germination in comparison to either control).

Seedling Emergence:

Percent Emergence: Mustard had a 1% decrease in emergence in comparison to the pooled control. In all other cases, treatment emergence was equal to or greater than the control.

Plant Fresh Weight: The most sensitive species was onion (X(11% reduction in fresh weight in comparison to the pooled control)).

The seed germination and seedling emergence studies are scientifically sound and fulfill the guideline requirements

for Tier 1 tests using non-target plants. Tier 2 testing is not required as no adverse effects greater than 25% were seen with any of the plant species tested.

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

9. **BACKGROUND:**

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

11. **MATERIALS AND METHODS:**

A. **Test Plants:** Dicotyledon plants were represented by six species from five families (i.e., soybean, buckwheat, mustard, tomato, cucumber, and radish). Monocotyledon plants were represented by four species from two families (i.e., corn, oat, sorghum, and onion). Cultivars, lot numbers, germination ratings, and seed sources were provided in the report.

B. **Test System:**

Seed Germination: Two circles of filter paper were placed in the bottom of a glass petri plate (10 cm in diameter and 1.5 cm in height). The test solutions were prepared in acetone and 2 ml were applied to each plate. The acetone was allowed to evaporate under a fume hood for three hours.

After solvent evaporation, 2.5 to 7 ml of deionized water was added to each plate, depending on the seed species. Ten seeds of each crop were then added to each petri plate. The plates were sealed with Parafilm and randomly placed on an inclined rack (20° angle) in a darkened incubator. The temperature in the incubator was 24 ±3°C.

Seedling Emergence: Ten seeds of each species were planted in fiber pans (25.4 x 20.3 x 7.6 cm), filled with a sterilized soil/silica sand mix (pH of 5.9, organic matter content of 1.0%). Seeds were planted at a depth of 1.5 cm or less, depending on the species. The seedbed was lightly tamped and a thin covering of screened soil was placed on the top of each tray. Each treatment replicate was placed in an area measuring 12.3 ft².

All applications were performed with a track sprayer equipped with a single nozzle. A nozzle height of 16 inches and a nozzle pressure of 40 psi were used to cover the spray area. The test spray solution was

prepared by dissolving chlorothalonil in 100% acetone. The plants were sprayed at the equivalent of 1114 l/ha (119 gpa).

The pans were randomly placed in a greenhouse with an average temperature of 26°C (range of 24-29°C), an average humidity of 52% (range of 46-59%), and a 14-hour supplemented photoperiod (1000-8000 footcandles). The pans were watered by both overhead and bottom irrigation after treatment. The plants were fertilized with a 350 ppm solution of 20-20-20 fertilizer 7 days after treatment (DAT).

- C. **Dosage:** In both seed germination and seedling emergence tests, chlorothalonil was applied at a rate of 16 lb active ingredient (ai)/acre (A) to all plant species. A solvent and negative control were also prepared.

- D. **Design:**
Seed Germination: Each treatment/crop combination was replicated four times (i.e., 10 seeds/plate, 4 plates/treatment). After 7 days of incubation, the dishes were removed from the incubator and percent seed germination was calculated. Seeds were considered germinated if the radicle was greater than 5 mm long.

Seedling Emergence: Each crop/treatment combination was replicated four times (i.e., 10 seeds/pan, 4 pans/treatment level).

Non-quantified visual observations were recorded twice weekly. The percentage of the ten seeds planted in each pot which emerged was calculated for each treatment at 14 DAT. Seedling fresh weight was also recorded at 14 DAT (test termination).

- E. **Statistics:** Percentage values (germination and emergence) and fresh weight values were compared to the control, solvent control, and pooled control data to determine inhibition of 25% or greater.

12. **REPORTED RESULTS:**

Seed Germination: The effects of chlorothalonil on percent germination are shown in Tables 1 & 2 (attached). When compared to the three control groups, nine species exhibited percent effects equal to or greater than 100%. Cucumber germination appeared slightly reduced (92%).

Seedling Emergence: No phytotoxicity symptoms were observed throughout the course of the test. Plants were consistently observed to be healthy and vigorous.

Percent Emergence: Chlorothalonil elicited no effect on seedling emergence (Tables 4 & 5, attached). When compared with the three control groups, all species exhibited percent emergence equal to or greater than 97%.

Plant Fresh Weight: Responses of the ten test species to chlorothalonil ranged from 26% inhibition for onion to 28% stimulation for cucumber when compared to the control data (Tables 7 & 8, attached). In comparison to the solvent control data, all effects were greater than 90%, except for oat (85%).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**
No conclusions were made by the study author.

Statements of compliance to Quality Assurance and Good Laboratory Practice (GLP) Regulations were enclosed in the report indicating adherence to GLPs as specified by Title 40, Part 160 of the Code of Federal Regulations. The stability, homogeneity, and characterization of the test material are the responsibility of the sponsor.

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

A. **Test Procedure:** The test procedures followed the SEP and Subdivision J guidelines, except for the following:

In the seedling emergence test, seedling dry weights are preferable to fresh weights.

B. **Statistical Analysis:** Analysis of variance and Dunnett's test were conducted on cucumber germination and onion fresh weight. These two species and parameters were determined to be the most sensitive of those examined for the germination and emergence tests. No significant difference was observed between the negative control and solvent control or 16 lb ai/A treatment (see attached printouts).

C. **Discussion/Results:**

Seed Germination: The most sensitive species in the germination test was cucumber (8% reduction in germination in comparison to either control).

Seedling Emergence: (SE)

Percent Emergence: Mustard had a 1% decrease in emergence in comparison to the pooled control. In all other cases, treatment emergence was equal to or greater than the control.

Plant Fresh Weight: The most sensitive species was onion (11% reduction in fresh weight in comparison to the pooled control).

The seed germination and the seedling emergence studies are scientifically sound and fulfill the guideline requirements for Tier 1 tests using non-target plants. Tier 2 testing is not required as no adverse effects greater than 25% were seen with any of the plant species tested.

D. Adequacy of the Study:

(1) **Classification:** Core for both the seed germination and the seedling emergence studies.

(2) **Rationale:** N/A

(3) **Repairability:** N/A

15. **COMPLETION OF ONE-LINER:** Yes, 11/12/92.

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Pages 63 through 69 are not included.

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Cucumber germination

Summary Statistics and ANOVA

Transformation =		None		
Group	n	Mean	s.d.	cv%
1 = control	4	90.0000	8.1650	9.1
2 sub control	4	90.0000	.0000	.0
3 1/6 1/4 1/2	4	82.5000	20.6155	25.0

*) the mean for this group is significantly less than the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minumum detectable difference for Dunnett's test = -19.734051
This difference corresponds to -21.93 percent of control

Between groups sum of squares = 150.000000 with 2 degrees of freedom.

Error mean square = 163.888889 with 9 degrees of freedom.

*
* Warning - the test for equality of variances *
* could not be computed as 1 or more of the *
* variances is zero. *
*

onion fresh weight

Summary Statistics and ANOVA

		Transformation =		None	
Group	n	Mean	s.d.	cv%	
1 = control	4	.3525	.0665	18.9	
2 sat. control	4	.2250	.1360	60.5	
3 1/6 1/6 1/4 A	4	.2575	.0550	21.4	

*) the mean for this group is significantly less than
the control mean at alpha = 0.05 (1-sided) by Dunnett's test

Minumum detectable difference for Dunnett's test = -.143367
This difference corresponds to -40.67 percent of control

Between groups sum of squares = .035117 with 2 degrees of freedom.

Error mean square = .008650 with 9 degrees of freedom.

Bartlett's test p-value for equality of variances = .285

DATA EVALUATION RECORD

1. **CHEMICAL:** Chlorothalonil.
Shaughnessey No. 081901.
2. **TEST MATERIAL:** Chlorothalonil (tetrachloroisophthalonitrile) technical; CAS No. 1897-45-6; Lot No. DS-2787-1002; 97.9% purity; a white powder.
3. **STUDY TYPE:** 122-1. Non-Target Plants: Vegetative Vigor Nontarget Phytotoxicity Study - Tier 1. Species Tested: Buckwheat, Corn, Oat, Onion, Sorghum, Soybean, Tomato, Cucumber, Radish, Mustard.
4. **CITATION:** Backus, P. 1992. Effect of Chlorothalonil on Vegetative Vigor of Plants (Tier I). Laboratory Project ID No. 92-0120. Conducted by Ricerca, Inc., Painesville, OH. Submitted by ISK Biotech Corporation, Mentor, OH. EPA MRID No. 424338-09.
5. **REVIEWED BY:**

Tracy L. Perry Wildlife Biologist Ecological Effects Branch	Signature: Tracy L. Perry Date: 11/12/92
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6. **APPROVED BY:**

Henry T. Craven Head, Section 4 Ecological Effects Branch	Signature: Henry T. Craven Date: 11/11/93
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7. **CONCLUSIONS:** This study is scientifically sound and meets the requirements for a Tier 1 vegetative vigor test using non-target plants. In comparison to the pooled control data, the most sensitive species was soybean (9% inhibition of fresh weight). Tier 2 testing is not required as no adverse effects greater than 25% were seen with any of the plant species tested.
8. **RECOMMENDATIONS:** N/A.
9. **BACKGROUND:**
10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. MATERIALS AND METHODS:

- A. **Test Plants:** Monocotyledon plants were represented by four species from two families (i.e., sorghum, oat, corn, and onion). Dicotyledon plants were represented by six species from five families (i.e., soybean, buckwheat, mustard, radish, tomato, and cucumber). Cultivars, seed sources, lot numbers, and germination ratings were provided in the report.
- B. **Test System:** Seeds of each crop were planted in square plastic pots (9 cm) filled with a soilless commercial growing medium. Various amounts of seed of each species were planted at a depth from 0.5 to 1.5 cm. The plant species were allowed to grow for 7-14 days before treatment. After emergence, each pot was thinned to one plant per pot for corn, cucumber, soybean, and tomato. For the remaining species, plants were thinned to a uniform population to avoid crowding. Each treatment replicate was placed in a 12.3 ft² area.

All applications were performed with a sprayer equipped with a single nozzle. A nozzle height of 16 inches and a nozzle pressure of 40 psi were used. The test spray solution was prepared by dissolving chlorothalonil technical in 100% acetone. The plants were sprayed at the equivalent of 1114 l/ha (119 gpa).

The pots were watered overhead prior to test material application. After treatment, the pots were sub-irrigated or soil-watered only. Greenhouse conditions were as follows: a mean temperature of 26°C (range of 24-29°C), a relative humidity of 52% (range of 46-59%), and a 14 hour light/10 hour dark photoperiod with supplemental lighting (1000-8000 footcandles).

- C. **Dosage:** Chlorothalonil was applied at a rate of 16 lb active ingredient (ai)/acre (A) to all plant species. A solvent (100% acetone) and negative control were also prepared.
- D. **Design:** Each crop/treatment combination was replicated four times (i.e., 1 plant/pot, 5 pots/replicate, 4 replicates/treatment level for corn, cucumber, soybean, and tomato or a uniform stand of plants/pot, 1 pot/replicate, 4 replicates/treatment level for the remaining species). After treatment, the pots were randomly placed in an on-site greenhouse. The plants were fertilized with a 350 ppm solution of a 20-20-20 fertilizer 7 days after treatment.

Non-quantified visual assessments were made twice weekly and unusual growth was noted. At test termination (14 days), fresh weight of the above-ground portion of the plant was recorded.

- E. **Statistics:** Fresh weight values were compared to the control, solvent control, and pooled control data to determine inhibition of 25% or greater.

12. **REPORTED RESULTS:**

Phytotoxicity observations: At 4 days after treatment, necrotic spots were observed on the solvent control and treated cucumber plants. After this observation period, all plants appeared to be healthy and vigorous throughout the study.

Plant fresh weight: Cucumber demonstrated 26% inhibition in comparison to the negative control. However, when cucumber weight was compared to the solvent and pooled control, 6% stimulation and 13% inhibition were observed, respectively. Other species demonstrated inhibition as low as 13% and stimulation as great as 23% in comparison to the negative control (Tables 1 & 2, attached).

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:**

The author concluded that Tier 2 testing is not required.

Statements of compliance to Quality Assurance and Good Laboratory Practice (GLP) Regulations were enclosed in the report indicating adherence to GLPs as specified by Title 40, Part 160 of the Code of Federal Regulations. The stability, homogeneity, and characterization of the test material are the responsibility of the sponsor.

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

- A. **Test Procedure:** The test procedures followed the SEP and Subdivision J guidelines, except for the following:

For six of the plant species, it was not specified how many plants were present per pot. It appears that the pots did not contain an equal number of plants.

The stage of development of the plants at time of test substance application was not reported.

Although plant phytotoxicity was noted, a rating scale or explanations of a rating scale were not used or reported.

It is preferable to measure dry weights rather than fresh weights of plant material.

B. Statistical Analysis: The reviewer used a t-test to determine significant differences between control and treatment data. Since the solvent and negative control were significantly different for cucumber, a comparison was made between the solvent control and treatment data for this species. It is apparent that the effect was due to solvent rather than treatment (6% stimulation of cucumber growth in comparison to the solvent control). For the remaining nine species, the solvent and negative control data did not appear to be different, and the most sensitive species with respect to the pooled control was soybean (9% inhibition). The results of the t-test indicated that there was not a significant reduction in fresh weight for soybean (see attached printouts).

C. Discussion/Results: Although there was an unspecified amount of plants per pot for six of the ten test species, the reviewer believes that intra-specific competition was not a problem since the study was conducted for only two weeks.

Phytotoxicity: No phytotoxic effects were noted at test termination for all ten test species.

Plant fresh weight: In comparison to the pooled control data, the most sensitive species was soybean (9% inhibition).

This study is scientifically sound and meets the requirements for a Tier 1 vegetative vigor test using non-target plants.

D. Adequacy of the Study:

(1) **Classification:** Core.

(2) **Rationale:** N/A.

(3) **Repairability:** N/A.

15. COMPLETION OF ONE LINER: Yes, 11/12/92.

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Enter the name of the DATAFILE you wish to analyze: cucumbers
(Press RETURN if you wish to skip directly to T evaluation)

	1 'cont'	2 'sol cont'
Means =	80.225	56.1125
Variances =	61.45636	31.3115

p = 2.435625E-03

24.1125 +/- T(6) * 4.815847

78

