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DATE: IN <u>01-10-89</u>	
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FILE OR REG. NO.	100-524
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PETITION OR EXP. NO.	
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DATE RECEIVED BY EFED	01-09-89
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RD ACTION CODE/TYPE OF REVIEW	660
TYPE PRODUCTS(S): I, D, H, F, N, R	, S <u>Insecticide</u>
DATA ACCESSION NO(S).	
PRODUCT MANAGER NO.	G. LaRocca (15)
PRODUCT NAME(S)	Diazinon
COMPANY NAME CIBA-G	EIGY Corporation
SUBMISSION PURPOSE Submissi	on of chronic aquatic
S	tudies for review
SHAUGHNESSEY NO. CHEMICAL AND	FORMULATION % A.I.
Diaz	inon
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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY WASHINGTON, D.C. 20460

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

TO:

George LaRocca (PM15)

Insecticide-Rodenticide Branch

Registration Division

FROM:

James Akerman, Chief

Ecological Effects Branch

Environmental Fate and Effects Division

SUBJECT: Review of Diazinon chronic aquatic studies

The Ecological Effects Branch has reviewed two studies submitted by CIBA-GEIGY. The results of these reviews are summarized below.

CITATION: Surprenant, D. C. 1988. The Chronic Toxicity of ¹⁴C-Diazinon Technical to <u>Daphnia magna</u> Under Flow-Through Conditions. Report No. 88-4-2644. Conducted by Springborn Life Sciences, Inc., Wareham, MA. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 407823-02.

CONCLUSIONS: This study is scientifically sound. However, it does not fulfill the guideline requirements for a <u>Daphnia magna</u> life-cycle test since statistical analyses on survival and length could not be verified. Based on the author's analyses, the MATC of ¹⁴C-Diazinon technical for <u>Daphnia magna</u> was >0.17 ug/L and <0.32 ug/L (geometric mean MATC = 0.23 ug/L) mean measured concentrations.

CITATION: Surprenant, D. C. 1988. The Toxicity of Diazinon Technical to Fathead Minnow (<u>Pimephales promelas</u>) Embryos and Larvae. Report No. 88-5-2702. Conducted by Springborn Life Sciences, Inc., Wareham, MA. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 407823-01.

CONCLUSIONS: This study is scientifically sound. However, it does not fulfill the guideline requirements for a fish early life stage test since the MATC value of Diazinon technical for Pimephales promelas could not be determined due to the adverse effects on growth observed at all test levels. Egg hatching and

larval survival were not affected by any test concentrations of Diazinon technical.

If you have questions regarding these reviews, please contact Skip Houseknecht at 557-4372.

DATA EVALUATION RECORD

1. CHEMICAL: Diazinon.

Shaughnessey No. 057801.

- 2. <u>TEST MATERIAL</u>: 1) ¹⁴C-Diazinon Technical, Lot #G-24480, an opaque liquid; 2) Diazinon Technical, Lot # FL-872049, an amber-colored liquid, 87.7% active ingredient.
- 3. STUDY TYPE: Daphnia magna Life-Cycle Flow-through Test.
- 4. <u>CITATION</u>: Surprenant, D. C. 1988. The Chronic Toxicity of ¹⁴C-Diazinon Technical to <u>Daphnia magna</u> Under Flow-Through Conditions. Report No. 88-4-2644. Conducted by Springborn Life Sciences, Inc., Wareham, MA. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 407823-02.
- 5. REVIEWED BY:

Prapimpan Kosalwat, Ph.D. Staff Toxicologist KBN Engineering and Applied Sciences, Inc.

signature: P. Kosalwat

Date: 2/23/89

6. APPROVED BY:

Isabel C. Johnson, M.S. Principal Scientist KBN Engineering and Applied Sciences, Inc.

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

Signature: Jabel C. Homon

Date: February 24,1989

Signature:

Date:

CLype Housekneth, Ph.O., M.P.H. Clyde R. Frueluck 9/13/89

7. CONCLUSIONS: This study is scientifically sound. However, it does not fulfill the guideline requirements for a <u>Daphnia magna</u> life-cycle test since statistical analyses on survival and length could not be verified. Based on the author's analyses, the MATC of ¹⁴C-Diazinon technical for <u>Daphnia magna</u> was >0.17 ug/L and <0.32 ug/L (geometric mean MATC = 0.23 ug/L) mean measured concentrations.

8. RECOMMENDATIONS: N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

A. <u>Test Animals</u>: <u>Daphnia magna</u> used in this test were obtained from populations cultured at Springborn Life Sciences, Inc. Offspring produced during the 24-hour period prior to test initiation were used.

Culture and test dilution water were fortified well water, filtered through an Amberlite XAD-7 resin column to remove any potential organic contaminants. The water generally had a total hardness and alkalinity as $CaCO_3$ of 160-180 and 110-130 mg/L, respectively; a pH of 7.9-8.3; a temperature of $21 \pm 1^{\circ}C$; a dissolved oxygen concentration of greater than 60% of saturation; and the specific conductivity range was 400-600 umhos/cm.

B. Test System: A 200-ml Mount and Brungs (1967) proportional diluter, calibrated to provide 50% dilutions between adjacent concentrations, delivered the dilution water and the ¹⁴C-Diazinon technical to the test vessels during the test. The diluter was constructed entirely of glass and silicone tubing, stoppers and sealant. The diluter system was equipped with a 30-ml gas-tight Hamilton syringe which delivered 0.0094 ml of the ¹⁴C-Diazinon technical stock solution (0.042 mg a.i./L) into 395 ml of dilution water in the mixing chamber of the system during each diluter cycle. This 395-ml solution (nominal concentration of 1.0 ug/L) served as the highest treatment level from which calibrated volumes were diluted to provide the 50% nominal concentration gradient.

Test vessels were glass battery jars having a volume capacity of 1.8 liters. Test solutions drained from the vessel through a 3.5 x 8.0 cm notch on the upper edge of the jars. The drains were covered with a Nitex 40-mesh screen to prevent loss of the daphnids. Five concentrations of ¹⁴C-Diazinon technical, a dilution water control, and a solvent control were maintained during the study. The solvent control contained the greatest amount of acetone present in any treatment level (i.e., 245 ul/L). All treatments and the controls were tested in quadruplicate. Test solutions were delivered to the vessels at an approximate rate of 6 aquarium volumes per 24-hour period. The test area was

illuminated with fluorescent lights at an intensity of 26-80 footcandles and a photoperiod of 16 hours light and 8 hours darkness. The study was conducted in an air-temperature controlled room designed to maintain the test solution temperature at 20 \pm 1°C.

- C. Dosage: Twenty-one day life-cycle flow-through test.
- Design: Based on a preliminary acute test, the nominal concentrations of ¹⁴C-Diazinon technical selected for the chronic test were 0.063, 0.13, 0.25, 0.50, and 1.0 ug/L. The test was initiated when forty daphnids (≤ 24-hour-old) were impartially selected and distributed to each test concentration and each control (10 per replicate). The test organisms were fed a diet consisting of a suspension of Fleischmann's yeast (5 mg/ml), a suspension of green algae (Ankistrodesmus falcatus) and Selco^R (a commercial mixture of proteins and fatty acid, 0.6 mg/ml). During the exposure, the food was introduced at a rate of 0.5 ml of yeast suspension, 3.0 ml of algal suspension, and 1.0 ml of Selco^R food supplement three times daily on weekdays and twice daily on weekends and holidays.

Adult survival and measurements of offspring production were made on days 1, 2, 4, and three times per week (Monday, Wednesday, and Friday) from day 7 through day 21. The offspring were removed, counted, and discarded. Test vessels were brushed to remove algal growth and the solution filtered through a fine mesh net at a minimum of twice per week. At test termination, the body length of all surviving daphnids in each treatment level and control was determined. Organism lengths were determined by measuring the daphnids from the top of the helmet to the base of the spine.

The test solution temperature was measured daily in one replicate vessel of each treatment level and each control solution throughout the 21-day exposure period. The dissolved oxygen concentration (DO) was measured every weekday in one replicate vessel and once a week in all replicate vessels of each treatment level and control. Total hardness, alkalinity, specific conductivity and pH were monitored weekly in one vessel of each treatment and control solutions. The pH and temperature were also measured once a week in all replicate vessels of each treatment level and control. Samples were removed from two replicate test solutions and the controls on test days 0, 7, 14, and 21, and analyzed for ¹⁴C-Diazinon technical. Additionally,

water samples were removed from two replicate vessels of the highest test concentration (1.0 ug/L, nominal) on days 0, 7, 14, and 21, and analyzed for nonlabeled Diazinon technical.

E. <u>Statistics</u>: One-way, single classification analyses of variance (ANOVA) were conducted on each endpoint to compare with the control and solvent control data. Since no statistically significant differences were found between control and solvent control data for any of the endpoints, the control and solvent control data were pooled for subsequent statistical analyses.

Significant differences of the ratio data (i.e., percentage survival) were determined after angular (arcsine square-root percentage) transformation of the data. Homogeneity of variances were determined using Williams' test coupled with Bartlett's test. If necessary, mean values were first transformed using square-root, arcsine square-root, or log conversion procedures. If, after appropriate transformation procedures had been applied to the data, Bartlett's test still failed to demonstrate homogeneity of variances, then non-parametric methods were used to compared sample means, such as Kruskal-Wallis and Steel's One-Many Rank tests.

The threshold concentration that produced statistically significant deleterious effects at the 95% level of certainty was expressed as the Maximum Acceptable Toxicant Concentration (MATC).

A computer program modified from the program of C. Stephen (Peltier, 1985) was used to calculate 1-, 2-, 4-, 7-, 14-, and 21-day median effect concentrations (EC50) and 95% confidence intervals.

12. REPORTED RESULTS: Dissolved oxygen, pH, specific conductivity, total hardness and alkalinity of the test solutions were not affected by the concentrations of ¹⁴C-Diazinon technical tested (Table 1, attached). Water quality conditions established for the test remained within acceptable ranges for the survival and reproduction of Daphnia magna. Throughout the 21-day exposure period, no visible sign of insoluble material was observed in any of the test solutions. Weekly analyses demonstrated that test concentrations in all aquaria were stable and generally consistent throughout the study (Table 2, attached). The mean measured concentrations of ¹⁴C-Diazinon technical

established in the test solutions during the exposure period were 0.027, 0.082, 0.17, 0.32, and 0.83 ug/L, representing 43, 63, 68, 64, and 83% of the nominal concentrations, respectively. Analysis of the highest test level for the presence of Diazinon technical during the study period resulted in measured concentrations which were consistent between sampling intervals and averaged 91% of the nominal concentration.

Weekly mean survival data for daphnids exposed to ¹⁴C-Diazinon technical is summarized in Table 5 (attached). All daphnids exposed to the two highest concentrations (0.32 and 0.83 ug/L) died within the initial 11 days of the study. At test termination, the mean survival of daphnids exposed to the remaining test levels (0.027, 0.082, and 0.17 ug/L) was statistically comparable to the survival of the pooled control organisms. Table 6 (attached) summarizes the 1-, 2-, 4-, 7-, 14-, and 21-day EC50 values and the corresponding 95% confidence intervals.

A summary of the mean cumulative number of offspring produced and the mean adult length is presented in Tables 7 and 8 (attached), respectively. Since no daphnids survived the 21-day exposure to ¹⁴C-Diazinon technical in the two highest test levels, the reproduction and growth of organisms at these levels were not statistically compared to control organism values. Daphnids exposed to ¹⁴C-Diazinon technical concentrations of 0.027, 0.082, and 0.17 ug/L reproduced and grew at rates which were statistically comparable to the reproduction and growth of the control organisms.

Based on these data, the Maximum Acceptable Toxicant Concentration (MATC) of $^{14}\text{C-Diazinon}$ technical to <u>Daphnia</u> magna was >0.17 ug/L and <0.32 ug/L (geometric mean MATC = 0.23 ug/L).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: No conclusions were made by the author. The raw data and the final report for this study were inspected by the Quality Assurance Unit of Springborn Life Sciences, Inc. to assure compliance with the study protocol, laboratory standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations.

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

- A. <u>Test Procedure</u>: An EPA's Standard Evaluation Procedure (SEP) for flow-through <u>Daphnia</u> chronic studies is not available at this time, thus ASTM recommended procedures were used in this data validation process. The test procedures were generally in accordance with the ASTM, except for the following deviations:
 - o Test vessels were brushed to remove algal growth at a minimum of twice each week. ASTM recommends the test vessels be brushed and rinsed with dilution water three times a week.
 - o The test solution temperature was measured daily in one replicate vessel of each test level and control, and weekly in all replicate vessels. ASTM states that temperature should be recorded at least hourly or the maximum and minimum temperatures must be recorded daily from at least one test vessel.
 - o The temperature in all test vessels was reported as being 16°C on day 21. The daily mean measured test temperature must be between 19 and 21°C. ASTM states that a test is unacceptable if any measured temperature was below 17°C or above 23°C.
- B. <u>Statistical Analysis</u>: The reviewer reanalyzed reproductive data (cumulative number of offspring produced per female) using one-way analysis of variance (see attached printouts). The results agree with those performed by the author, i.e., there was no significant difference between the pooled controls and any ¹⁴C-Diazinon technical test level analyzed.

There appears to be some discrepancies on the percentage survival values and transformed survival values (p.78 of the report). Therefore, statistical analysis on survival data could not be verified.

The author analyzed growth (i.e., length) data using the mean value of each replicate, thus ignoring the variation that existed within each replicate. Individual length should have been used in the statistical analysis. Since the raw data were not submitted with the report, statistical analysis on this parameter could not be verified.

C. <u>Discussion/Results</u>: All raw data for each endpoint and water quality parameters must always be submitted with the report. This study is scientifically sound. However, statistical analyses on survival and length could not be verified due to the lack of raw data.

According to the author's analyses, the MATC of ¹⁴C-Diazinon technical for <u>Daphnia magna</u> was >0.17 ug/L and <0.32 ug/L (geometric mean MATC = 0.23 ug/L) mean measured concentrations.

D. Adequacy of the Study:

- (1) Classification: Supplemental.
- (2) Rationale: Statistical analyses on survival and length could not be verified due to the lack of raw data.
- (3) Repairability: Yes, submit the raw data for survival and length.
- 15. COMPLETION OF ONE-LINER: Yes, February 23, 1989.

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File: reproont

Date: 02-23-1988

FILTER: None

N's, means and standard deviations based on dependent variable: REPROD

* Indicates statistics are collapsed over this factor * young/female

Factors: C N Mean S.D.

* 8 130.5000 11.2758

1 Control 4 127.0000 8.2865
2 Solvent Control 4 134.0000 13.9762

Number of variances= 2 df per variance= 3.

Source	df	SS (H)	MSS F	P
Between Subjects	7	890.0000		
C (CONC)	1	98.0000	98.0000 0.742	0.4214
Subj w Groups	6	792.0000	132.0000	

File: diazrepr

Date: 02-23-1988

FILTER: None

N's, means and standard deviations based on dependent variable: REPROD

* Indicates statistics are collapsed over this factor

Factors: C	Conc. (ng/L)	N	Mean	S.D.
*		20	123.9000	13.8750
1	Pooled Control	8	130.5000	11.2758
$\frac{1}{2}$	0.027	4	114.5000	13.3292
3	0.082	4	127.7500	7.5443
4	0.170	4	116.2500	18.8746

Source	df	SS (H)	MSS F	P
Between Subjects	19	3657.7998		
C (CONC)	3	995.2999	331.7666 1.994	0.1544
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Level	Mean	
1	130.500	
2	114.500	
3	127.750	
4	116.250	

Comparison	Tukey-A*	Newman -Keuls*	Bon- ferroni	Dunnett
1 > 2		•		
1 > 3				
1 > 4				
2 < 3				N.A.
2 < 4				N.A.
3 > 4				N.A.

* The only possible P-values are .01, .05 or .10 (up to 0.1000). A blank means the P-value is greater than 0.1000.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Study/Species/Lab/ Succession X Ac	ive	
Avian Reproduction,	Group Posse/	Reviewer/ Valida Date
Species:	Color Della Effected (Variation	Statu
Lab:	Creaming I	
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Field Study(Simulated/Actu Species:	al) Group Rate(ai/a) treacment Total : Mor (x	
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	Treatment I	
Lab:	Casamana II	•
Acc.	Treatment III	
	Crop/Site:	
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Chronic fish,	Concentrations Tested (pp_)=	
Species	MATE = >	•
Lab:	Congres Manager (a.)	•
Acc.	Comments:	
Chronic invertebrate	Concentrations Tourist	
Species <u>Daphnia magna</u> Lab Societal	Concentrations Tested (ppb) 0.027, 0.082, 0.17, 0.38 *** MATC =>0.17 (0.32 ppb. Effected Parameter(s) Sur	-, 0.83
Lab Springborn Life Seience Acc. Inc.	Ettected Parameter(s) Bur	vival
Acc. 407823-02 Ine.	Comment	Px/2-23-89 Supp
404843-02	* mean measured concentrations	· 1 sall

DATA EVALUATION RECORD

1. CHEMICAL: Diazinon.

Shaughnessey No. 057801.

- 2. TEST MATERIAL: Diazinon Technical, Lot #FL-872049, an amber-colored liquid, 87.7% active ingredient.
- 3. <u>STUDY TYPE</u>: Freshwater Fish Early Life Stage Test. Species Tested: Fathead minnow (<u>Pimephales promelas</u>).
- QUITATION: Surprenant, D. C. 1988. The Toxicity of Diazinon Technical to Fathead Minnow (Pimephales promelas) Embryos and Larvae. Report No. 88-5-2702. Conducted by Springborn Life Sciences, Inc., Wareham, MA. Submitted by CIBA-GEIGY Corporation, Greensboro, NC. EPA Accession No. 407823-01.
- 5. REVIEWED BY:

Prapimpan Kosalwat, Ph.D. Staff Toxicologist KBN Engineering and Applied Sciences, Inc.

signature: P. Kosalwat

Date: 2/1/89

6. APPROVED BY:

Isabel C. Johnson, M.S. Principal Scientist KBN Engineering and Applied Sciences, Inc.

signature: Joabel C. Tikn

Date: February 23,1989

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

Date:

7. CONCLUSIONS: This study is scientifically sound. However, it does not fulfill the guideline requirements for a fish early life stage test since the MATC value of Diazinon technical for <u>Pimephales promelas</u> could not be determined due to the adverse effects on growth observed at all test levels. Egg hatching and larval survival were not affected by any test concentrations of Diazinon technical.

8. RECOMMENDATIONS: N/A.

9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A.

11. MATERIALS AND METHODS:

- A. <u>Test Animals</u>: Fertilized eggs of fathead minnow (<u>Pimephales promelas</u>) were obtained from the fathead minnow culture unit maintained at Springborn Life Sciences, Inc.
- B. Test System: A modified proportional diluter, similar to that described by Mount and Brungs (1967) with a 0.50 dilution factor, was used to prepare and deliver the selected test concentration range of Diazinon Technical to the aquaria during the 34-day exposure. The dilution and control water source was well water which was pumped into an epoxy-coated concrete reservoir where it was supplemented with Town of Wareham untreated well water and aerated. Weekly characterization of the well water established the total hardness and alkalinity ranges of 27-34 and 24-28 mg/L as CaCO₃, respectively; the pH range of 7.0-7.6; and the specific conductivity range of 100-130 umhos/cm during the study period.

The diluter was calibrated to deliver five nominal concentrations of Diazinon technical, a dilution water control, and a solvent control to duplicate test aquaria. The solvent control solutions contained a concentration of acetone (i.e., 17.5 ul/L), which equaled the solvent level in the highest Diazinon Technical treatment level.

Each glass test aquarium measured 39 x 20 x 25 cm with a 19.5-cm high side drain that maintained a constant exposure solution volume of 15 L. The diluter delivered 0.5 L of solution to each aquarium at an average rate of 181 times per day. This delivery rate was equivalent to a flow rate of approximately 6.0 aquarium volumes per 24-hour period, with a 90% replacement time of approximately 9 hours. Embryo incubation cups were glass jars (5 cm O.D., 8 cm high) with 40-mesh Nitex screen bottoms. A rocker arm apparatus was used to gently oscillate the incubation cups in the test solutions. Sixteen hours of light at an intensity of 20-140 footcandles at the water surface were provided each day. The aquaria were impartially positioned in a water bath containing circulating water designed to maintain the test solution temperatures at 25 \pm 1°C.

- c. <u>Dosage</u>: Thirty-four day embryo, larval flow-through chronic test.
- Design: The nominal concentrations of Diazinon D. technical selected for the definitive early life stage exposure were 0.094, 0.19, 0.38, 0.75, and 1.5 mg a.i./L. Sixty embryos were impartially selected and distributed to each of 14 embryo incubation cups, one of which was then suspended in each duplicate test aquarium per exposure concentration and control. Dead embryos were counted daily until hatching was complete. Hatching was deemed complete (exposure day 4) when no more than 10% unhatched viable embryos remained in any egg incubation cup. Calculations of percentage survival of organisms at hatch were based on the number of live larvae and embryos per incubation cup after hatching was complete compared to the number of embryos per cup on test day 0.

To initiate the 30-day post-hatch larval exposure, 40 live larvae were impartially selected from the surviving larvae in each incubation cup on test day 4 and placed into their respective exposure aquaria. Larvae were fed live brine shrimp (Artemia salina) nauplii three times daily on weekdays and twice daily on weekends and holidays. Aquaria were brushed and siphoned when necessary to remove excess food and fecal matter.

Behavior and appearance of larvae were observed and recorded daily, and larval survival was estimated twice weekly. At 30 days post-hatch exposure (test termination), the percentage larval survival was determined. The larvae were measured and weighed individually to calculate the mean and standard deviation of total length and wet weight.

Dissolved oxygen concentration, pH and temperature were measured in each aquarium daily. Total hardness as CaCO₃ was measured on day 0 and weekly thereafter in alternating replicates of the high and low test concentrations, the dilution water control, and the solvent control. Samples were removed from all replicate test solutions and the controls on test days 0, 4, 11, 18, 25, 32, and 34, and analyzed for Diazinon technical.

E. <u>Statistics</u>: Statistical analyses were performed using the mean organism response of each replicate aquarium. All statistical conclusions were made at 95% level of certainty except in the case of the Chi-Square Goodness of Fit test and the Bartlett's test, in which 99% level of certainty was applied.

One-way, single classification analyses of variance were conducted on each endpoint to compare with the control and solvent control data. Since no statistically significant differences were found between control and solvent control data for any of the endpoints, the control and solvent control data were pooled for subsequent statistical analyses.

Significant differences in the percentage survival were determined after angular (arcsine square-root percentage) transformation of the data. Statistical comparison between results of pooled control and various dose levels of Diazinon technical for each endpoint was based on Williams', Dunnett's, or Kruskal-Wallis test. Larval survival data were analyzed before larval length and weight. Dose levels that caused significant survival effects were excluded from the analyses of larval growth.

The theoretical threshold concentration expected to produce no deleterious effects at the 95% level of certainty was estimated as the Maximum Acceptable Toxicant Concentration (MATC).

12. REPORTED RESULTS: At the concentrations of Diazinon technical tested, mean dissolved oxygen, pH, and total hardness varied minimally and were not affected by the established concentration gradient of Diazinon technical (Table 1, attached). Throughout the exposure period, no visible sign of insoluble material was observed in any of the solutions. Weekly analyses demonstrated that test concentrations in all aquaria were stable and generally consistent throughout the study. The mean measured concentrations of Diazinon technical established in the test solutions during the exposure period were 0.092, 0.17, 0.38, 0.76, and 1.60 mg a.i./L, representing 98, 89, 100, 101, and 107% of the nominal concentrations, respectively.

A summary of the biological results of the continuous 34-day exposure of fathead minnow embryos and larvae to measured concentrations of Diazinon technical is presented in Table 4 (attached). Fathead minnow survival at completion of the

hatching period (day 5) ranged between 80 and 93% and established no concentration-dependent relationship. Larval survival, after 30 days post-hatch exposure to all concentrations of Diazinon technical was statistically comparable to the control organism survival. Embryo hatchability and larval survival at test termination were not adversely affected by exposure to any of the concentrations of Diazinon technical tested.

The mean total length and weight of larvae exposed to the four highest test concentrations of Diazinon technical (i.e., 0.17, 0.38, 0.76, and 1.60 mg a.i./L) were significantly less than the length and weight of the pooled control larvae. Based on these results, the no-observed-effect concentration (NOEC) for fathead minnow survival and growth was determined to be 0.092 mg a.i./L.

Based on the significantly (P \leq 0.05) reduced larval length and weight at concentrations \geq 0.17 mg a.i./L Diazinon technical, the MATC for this material and fathead minnow was estimated to be > 0.092 and < 0.17 mg/L (geometric mean MATC = 0.13 mg a.i./L).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES: No conclusions were made by the author. The raw data and the final report for this study were inspected by the Quality Assurance Unit of Springborn Life Sciences, Inc. to assure compliance with the study protocol, laboratory standard operating procedures and the pertinent EPA Good Laboratory Practice Regulations.

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

- A. <u>Test Procedure</u>: The test procedure is generally in accordance with the SEP guidelines, except for the following deviations:
 - o The total hardness of dilution water used in the test was slightly lower than the recommended hardness of 40-48 mg/L as CaCO₃. Also, there was no report whether the water had been analyzed for pesticides, heavy metals, and other possible contaminants.
 - o The light intensity employed in this study was 215-1507 Lux (reported as 20-140 footcandles) at the surface of the test solutions. The SEP recommends the intensity of 400-800 Lux.

- o There was no information on how fertilized eggs were obtained from the culture.
- o Two replicate incubation cups with 60 embryos in each cup were used per treatment level and control. The SEP recommends a minimum of 20 embryos per replicate cup, with <u>four</u> replicates per concentration (80 embryos total).
- o Time to swim-up was not recorded.
- o The fish were fed until the end of the test. Fish should not be fed for at least 24 hours prior to test termination on day 32.
- B. Statistical Analysis: The reviewer reanalyzed the egg hatching, larval survival, length, and weight using analysis of variance (ANOVA) and multiple comparison tests. The arcsine square-root transformation was used to transform dichotomous data (i.e., hatching and survival data) before performing the ANOVA. For continuous data (i.e., length and weight data), raw data (individual measurements) were used in the analyses. All printouts are attached. A discussion of the reviewer's findings is presented in Section 14.C.
- c. <u>Discussion/Results</u>: Since no significant differences were found between the control and carrier control for any parameter tested, the pooled data of these two controls were used to compare with the data from each test concentration. The statistical analyses of egg hatching and larval survival performed by the reviewer agree with the author's results, i.e., no adverse effects were found on these two parameters.

The author analyzed the length and weight data using the mean value of each replicate, therefore ignoring the variation that existed within each replicate. Individual measurements of these two growth parameters (i.e., raw data) should have been used in the ANOVA and multiple comparison tests. In contrast to the author's findings which found no effects on length and weight at the lowest test concentration (0.092 mg a.i./L), the analyses performed by the reviewer showed adverse effects of Diazinon technical on growth at all concentrations tested when compared to the pooled controls. The reason for the difference was as explained earlier, i.e., the author analyzed the growth data using only the mean value of each replicate.

Therefore, the MATC value of Diazinon technical for fathead minnow could not be determined from this study due to the adverse effects on growth found at all test levels.

D. Adequacy of the Study:

- (1) Classification: Supplemental.
- (2) Rationale: The MATC value could not be determined due to the adverse effects on growth observed at all test levels.
- (3) Repairability: No.
- 15. COMPLETION OF ONE-LINER: Yes, February 21, 1989.

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Avian Reproduction, Species:	Group Dose(ppm) Effected/Parameters Mort.(%) ICSe Inh. Control Treatment I
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	Concentrations Tested (phy)= 0.092, 0.17, 0.38, 0.76, 1-60 MATE = > Could not be determined Effected Parameter = length, weight Conter. More. (1)= 4 Sol. Conter. More. (x)= 9 PK/2-21-89 Sup McComments: * Mean measured Concentrations
Chronic invertebrate Species Lab	Concentrations Tested (pp_)= MAIC =>

Analysis of Variance

File: diazcont

Date: 02-14-1988

FILTER: None

N's, means and standard deviations based on dependent variable: SURVHATC

Survival at hatch

* Indicates'statistics are collapsed over this factor

(Arcsine SORT)

S.D. Mean Factors: C 0.0685 1.1291 0.0725 1.1219 0.0928 1.1363

1.64

Fmax for testing homogeneity of between subjects variances:

Number of variances= 2 df per variance= Dependent variable: SURVHATC Analysis of Variance

Source	df	SS (H)	MSS	F	P
Between Subjects	3	0.0141			
C (CONC)	1	0.0002	0.0002	0.030	0.8786
Subj w Groups	2	0.0139	0.0069		

. Analysis of Variance Crunch pregram

Date: 02-14-1988

FILTER: None

Survival

N's, means and standard deviations based on dependent variable: LARVSURV

								Larval Survival
*	Indicates	`statistics	are	collapsed	over	this	factor	(Arcsine SORT)

Factors:	C		N N	Mean	S.D.
	*		4	1.3976	0.2100
	1	Control	2	1.4369	0.1894
	$\hat{2}$	Carrier Contr	d 2	1.3583	0.3005

Fmax for testing homogeneity of between subjects variances: Number of variances= 2 df per variance= 1.

Source	df	SS (H)	MSS	F	P
Between Subjects	3	0.1323			
C (CONC)	1	0.0062	0.0062	0.098	0.7839
Subj w Grouns	2	0.1262	0.0631		

Crunch program
File: diazcon1

Analysis of Variance

Date: 02-14-1988

FILTER: None

N's, means and standard deviations based on dependent variable: LENGTH

* Indicates statistics are collapsed over this factor

Factors:	C		N	Mean	S.D.
	*		150	24.7267	2.5981
	1	Control	, 73	24.9863	2.5954
	2	Carrier Contr	₹ 77	24.4805	2.5936

Fmax for testing homogeneity of between subjects variances:

Number of variances = 2 df per variance = 74.

Dependent variable: LENGTH

Analysis of Variance

Source	df	SS (H)	MSS	F	Р
Between Subjects	149	1005.7935			
C (CONC)	1	9.5863	9.5863	1.424	0.2346
Subj w Groups	148	996.2073	6.7311		

truck program

Analysis of Variance

File: diazcon2

Date: 02-14-1988

FILTER: None

N's, means and standard deviations based on dependent variable: WEIGHT

* Indicates.statistics are collapsed over this factor

Factors:	C		Ν	Mean	S.D.
	*	0 T D	150	0.1285	0.0419
	1	Central	73	0.1332	0.0445
	2	Carrier Control	77	0.1241	0.0391

Number of variances= 2 df per variance= 74.

Source	df	SS (H)	MSS	F	þ
Between Subjects	149	0.2613			
C (CONC)	1	0.0031	0.0031	1.778	0.1845
Subj w Groups	148	0.2582	0.0017		

Crunch program

Analysis of Variance

File: diazsurv

Date: 02-14-1988

FILTER: None

Hatching

N's,	means and	d standard	devia	tions	based	on	dependent	variable:	SURVHATI	2 0 4 hatch
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Factors:	c	Cone. (mga.i/L)	N	Mean	s.D.
,	*		14	1.1756	0.0846
	1	pooled control	4	1.1291	0.0685
	5	0.092	2	1.2665	0.0247
	3	0.17	2 100	1.1101	0.0890
	4	0.38	2	1.1279	0.0640
	5	0.76	2	1.2935	0.0134
	6	1.60	2	1.1731	0.0000

Source	df	SS (H)	MSS	F	P
Between Subjects	13	0.0930			
C (CONC)	5	0.0661	0.0132	3.935	0.0422
Subi w Groups	8	0.0269	0.0034		

Level	Mean	Level	Mean
1	1.129	6	1.173
2	1.267		
3	1.110		
4	1.128		
5	1.293		

• · · · · · · · · · · · · · · · · · · ·	m-b-ffm?	Tukey-A*	Newman	Bon- ferroni	Dunnett
Comparison	acuerie.	Tukey A*	redian	1611614	2 3 1 1 1 1 2 3 3
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2 > 6					N.A.
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3 < 5		0.1000	0.1000		N.A.
3 < 6					N.A.
4 < 5			0.1000		N.A.
4 < 6				•	N.A.
5 > 6					. N.A.

^{*} The only possible P-values are .01, .05 or .10 (up to 0.1000). A blank means the P-value is greater than 0.1000.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

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Analysis of Variance

File: diazsurv

1.0554

Date: 02-14-1988

FILTER: None

N's,	means and	d standard (deviations t	ased on d	ependent varia		
*	Indicates	statistics	are collaps	sed over t	his factor	harval Survival (Arcsine Sart)	
	Factors:	cone.	mg a.i./L)	N	Mean	S.D.	
		A2.	•	14	1.3428	0.2083	
		: Pooled	Control	4	1.3976	0.2100	
		pooled 2 0.092		2 2 B	1.3389	0.1272	
		3 0.17		2	1.4581	0.1595	
		4 0.38		2	1.3939	0.2501	
		5 2.76		2	1.3583	0.3005	

1.60 Fmax for testing homogeneity of between subjects variances:

Number of variances = 6 df per variance = 1.

0.76

Dependent variable: LARVSURV Analysis of Variance

Source	df	SS (H)	MSS	F P
Between Subjects	13	0.5640		
C (CONC)	5	0.2095	0.0419	0.946 0.5050
Subi w Groups	8	0.3545	0.0443	

Post-hoc tests for factor C (CONC)

Level	Mean	Level	Mean
1	1.398	6	1.055
.2	1.339		
. 3	1.458	*	
4	1.394		
5	1.358		

-			Newman	Bon-	
Comparison	Scheffe'	Tukey-A*	-Keuls*	ferroni	Dunnett
1 > 2					
1 < 3			d		
1 > 4					
1 > 5					•
1 > 6					
2 < 3					N.A.
2 < 4					N.A.
2 < 5					N.A.
$\overline{2} \rightarrow \overline{6}$					N.A.
3 > 4					N.A.
3 > 5					N.A.
3 > 6					N.A.
4 > 5					N.A.
4 > 6					N.A.
5 > 6					N.A.
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^{*} The only possible P-values are .01, .05 or .10 (up to 0.1000). A blank means the P-value is greater than 0.1000.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Crush program.

Analysis of Variance

File: diazleng

FILTER: None

N's, means and standard deviations based on dependent variable: LENGTH

* Indicates statistics are collapsed over this factor

Factors:	С	Conc. (mga.	i/L)	N	Mean	S.D.
	*			510	20.8941	4.6102
	1	Control (poole	d)	150	24.7267	2.5981
	2	0.092		75	23.6667	2.7130
	3	6 14		77	22.2597	2.5670
	4	0.38		75	19.1200	2.0266
	5	0.76		73	16.7260	2.4110
	6	1-60		60	13.3833	1.9838

Number of variances = 6 df per variance = 77.

Source	df	SS (H)	MSS	F	P
Between Subjects	509	10818.2832			
C (CONC)	5	7812.3906	1562.4782	261.982	0.0000
Cubi u Graupe	504	3005, 8926	5.9641		

Level	Mean	Level	Mean
1	24.727	6	13.383
2	23.667		
3	22.260		
4	19.120		
5	16.726		

Comparison 1 > 2 1 > 3 1 > 4 1 > 5 1 > 6 2 > 4 5 > 4 5 > 6 2 > 5 6 > 5 6 > 5 6 > 5 7 > 6 7 > 6 7 > 6 7 > 6 7 > 6 7 > 7 7	-Keuls* 0.0100 0.0100 0.0100 0.0100 0.0100 0.0100 0.0100 0.0100 0.0100 0.0100	ferroni 0.0341 0.0000 0.0000 0.0000 0.0005 0.0000 0.0000 0.0000 0.0000 0.0000	Dunnett 0.0500 0.0100 0.0100 0.0100 N.A. N.A. N.A. N.A. N.A.

^{*} The only possible P-values are .01, .05 or .10 (up to 0.1000). A blank means the P-value is greater than 0.1000.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

Crunch program

'Analysis of Variance

File: diaz

Date: 02-17-1988

FILTER: None

N's, means and standard deviations based on dependent variable: WEIGHT

* Indicates.statistics are collapsed over this factor

Factors:	Ċ	Cone (rug/2)	N	Mean	S.D.
	*		511	0.0921	0.0478
	1	Control (pooled)	150	0.1285	0.0419
	2	0.092	75	0.1110	0.0366
	3	0 17	78	0.1018	0.0346
	4	0.34	75	0.0802	0.0267
	5	0.76	73	0.0534	0.0231
	Ē	1 / 2	60	0.0266	0.0143

Fmax for testing homogeneity of between subjects variances:

Source	df	SS (H)	MSS	F	P
Between Subjects	510	1.1666			
C (CONC)	5	0.6109	0.1222	111.028	0.0000
Suhi w Grauns	505	0.5557	0.0011		

Level	Mean	Level	Mean
1	0.129	6	0.027
2	0.111		
3	0.102		
4	0.080		
5	0.053		

· .			-
	Newman	Bon-	
Comparison	-Keuls*	ferroni	Dunnett
1 > 2	0.0100	0.0032	0.0100
1 > 3	0.0100	0.0000	0.0100
1 > 4	0.0100	0.0000	0.0100
1 > 5	0.0100	0.0000	0.0100
1 > 6	0.0100	0.0000	0.0100
2 > 3	0.1000		N.A.
2 > 4	0.0100	0.0000	N.A.
2 > 5	0.0100	0.0000	N.A.
2 > 6	0.0100	0.0000	N.A.
3 > 4	0.0100	0.0011	N.A.
3 > 5	0.0100	0.0000	N.A.
3 > 6	0.0100	0.0000	N.A.
4 > 5	0.0100	0.0000	N. A.
4 > 6	0.0100	0.0000	N.A.
5 > 6	0.0100	0.0000	N.A.

^{*} The only possible P-values are .01, .05 or .10 (up to 0.1000). A blank means the P-value is greater than 0.1000.

For Dunnett's test only the P-values .05 and .01 are possible and only for comparisons with the control mean (level 1).

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NUTL: TAIS TEST CONTINUES THE TYPE I TAKE I ASTROLES SHARE RATE, ALPHA=0.05 DF=501 MSE=5.83773 CRITICAL VALUE OF STUDENTIZED HARREN. 148 11414UM SIGRIFICANT DIFFERENCE=1.1633 REGISTARS: CELL SIZES ARE NOT ESPAL.
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