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Shaughnessey No.

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MRID NO(S). 415190-1

PRODUCT MANAGER NO. 15

PRODUCT NAME(S) Karate

COMPANY NAME ICI Americas, Inc.

SUBMISSION PURPOSE Review of Data/full life cycle study

SHAUGHNESSEY NO.	CHEMICAL AND FORMULATION	% A.I.
128897	lambda-cyhalothrin	97

7/26/1990

DATA EVALUATION RECORD

1. Chemical: Lambda-cyhalothrin (Karate)(PP 321)

2. Test Material: Technical grade - 96.7% (non-radioactive material) w/w purity, mean measured (active material) >97.9% (using hexane-dichlormethane -acetonitrile TLC solvent system).

IUPAC Chemical Name:

(R) a-cyano-3-phenoxybenzyl (1S)-cis-3-(Z-2-chloro 3,3,3-trifluoroprop-1-enyl)-2,2-dimethyltyclo-propanecarboxylate

(S) a-cyano-3-phenoxybenzyl (1R)-cis-3-(Z-2-chloro 3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclo-propanecarboxylate

(lambda-cyhalothrin is the racemic mixture containing the above two isomers in a 1:1 ratio)

3. Study/Action Type: Fish Full Life Cycle Study

Species Tested: Fathead Minnow (Pimephales promelas)

4. Study ID: Lambda-cyhalothrin (Karate PP 321): Determination of chronic toxicity to fathead minnow (Pimephales promelas full life cycle 1989, performed by Imperial Chemical Industries PLC, Brixham Lab, UK. Study authors were J. F. Tapp, B. G. Maddock, B. J. Gillings. Submitted by ICI Americas, Inc. June 1990. MRID No. 415190-01.

5. Reviewed by:

Candace Brassard (Signature) Candace Brassard
Biologist
EEB/EFED (Date) 7/25/90

6. Approved by:

Ann Stavola (Signature) Ann Stavola
Acting Head - Section III
EEB/EFED (Date) 7/26/90

7. Conclusions: Based on the data that was submitted, EEB is unable to ascertain if this study is scientifically sound. There are significant discrepancies that need to be

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addressed. The study authors should refer to section 14 A for a detailed listing of concerns.

Specifically, the study authors reported there were residue levels of lambda-cyhalothrin in the Dilution Water Control and the Solvent Control. The study authors should clarify if indeed these reported levels were from chemical contamination. In fact, in some cases, the levels in the controls overlapped the range of residues reported in the lowest treatment level.

There is also a concern that the level of quality assurance was adequate, since there was inconsistencies with data reporting.

8. Recommendations: The study authors should address concerns identified in section 14 A of this document.

9. Background:

10. Discussion of Individual Tests or Studies:

11. Materials and Methods:

A. Test Animals: The fish were originally purchased from Sea Plantations Engineering Technology, Salem, Mass. and held in the lab since Nov. 1987. Brood stock was maintained at the Brixham Laboratory.

Collection of Eggs: Six batches of eggs from the spawnings of six females were pooled in a dish filled with dilution water. Each batch was less than 24 hours old. All eggs were at the morula or blastula stage of development. Sets of five eggs were randomly selected and randomly assigned to incubating cups until each of the 28 cups contained 20 randomly selected eggs.

After the eggs were distributed they were treated for 15 seconds to 60 mg/l concentration of malachite green(to prevent fungal infection), then they were rinsed with freshwater.

B. Dosage: Five nominal concentrations solvent control and dilution water control were used in the study.

Dilution Water: Dechlorinated tap water was used. Water passed through activated carbon filtered to 1 um to remove particulate material and preheated to 25°C in a header tank on the test rig. Residual chlorine was measured.

Stock Solution: New stock solutions were made up once a week throughout the study. Two stock concentrates of lambda-cyhalothrin in triethylene glycol were prepared. The nominal concentration of stock solution was 2,500 mg/l.

A concentrate of active lambda-cyhalothrin was prepared by pipetting nominally 7.6 MBq (1.98 mg) of the test material in hexane solution into a vial and evaporating the solvent under nitrogen.

Nominal Concentrations: 0.03, 0.06, 0.12, 0.25 and .50 ug/l lambda-cyhalothrin, solvent control, and control.

Analytical Determination of Test Concentrations:

The principal method used to determine concentrations of lambda-cyhalothrin was liquid scintillation counting (LSC). The accuracy of the results were confirmed by gas-chromatography (GC).

For LSC analyses samples were taken in both replicate tanks (A & B) every day from - 6 to 3 days, then both replicates were analyzed twice per week until day 97. Then samples were taken once a week in each tank until study termination.

For GC analyses, samples were taken days -4, 0, 2, 8, then weekly for the next 13 weeks, and thereafter every two weeks until study termination. No correction was made for the purity of the material.

Tissue Analysis: Residues in eggs, larvae and adult fish were measured by LSC following combustion and recorded. Residue analysis was carried out based on number of eggs.

C. Study Design/System Design

Duplicate glass spawning tanks (A+B) were used for each test concentration. These tanks measured 595 x 300 x 305 mm and had a capacity of 42.5 litre, with a water depth of 240 mm. Duplicate progeny tanks measured 250 x 200 x 255 mm with a water depth of 180 mm and a 9 litre capacity.

Incubation cups were made from 80 mm lengths of 50 mm glass tubing with nylon mesh (0.47 mm) cemented to the bottom of each cup using silicone sealant. The cups were suspended in progeny test chambers and oscillated

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vertically over a distance of 25 mm at a rate of 2 oscillations per minute.

Spawning tiles were constructed of 10 cm lengths of a semicircular section of UPVC gutter.

Photoperiod - 16 Hours Light: 8 Hours Dark photoperiod per day-with white fluorescent lights above tanks; photoperiod did not follow Evansville, Indiana schedule.

Delivery System - The dilution water was fed from an aerated temperature controlled head tank via control mixing chamber. The flows were regulated by "capillary" flow control devices fitted with siphon breaks- the nominal flow rate of the dilution was 800 ml/minute to each mixing chamber. The toxicant flow rate was normally 0.020 ml/minute, giving a nominal dilution of 80,000 times in the mixing chambers. The test solutions passed through mixing chambers into flow splitting chambers from which separate lines supplied at least 6 tank volumes per day to the duplicate spawning and progeny tanks. The flow rates to these two types of tanks were set at 230 +/-20 ml/minute and 50 +/-4 ml/minutes, respectively.

Daily checks were made to ensure correct operation of dosing system. Calibration of the dosing system was made by direct measurement of flow rates into and out of the mixing and flow splitting chambers three times per week throughout the test period. On day 32 and 60, surviving fish were photographed to determine lengths.

Then fish were transferred to spawning tanks on exposure day 60 (post-hatch day 56).

Adults

On day 90, the spawning tiles were placed into adult tanks. On exposure day 134, perforated stainless steel dividers were placed in adult tanks, so that there were four equal sized breeding compartments.

Adult fish exposure terminated on day 300 of the study. Each fish was sexed, weighed and measured and frozen for residue analysis. In addition to the full life cycle study, embryo-larval and hatchability studies were conducted until day 56 post-hatch.

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Feeding Regime -

On days 0-2, fish larvae were fed uncoiled powdered pruteen once per day. On days 3-4 post-hatch, the fish larvae were fed once with Pruteen and twice with Artemia (brine shrimp) larvae, at a rate of 400 shrimp larvae per minnow larva. From day 5 to 15 post-hatch, the fish larvae were fed on Artemia larvae, 3 times per weekday, and 2 times/a day on weekends. From day 16 post-hatch onwards Artemia or Artemia with promin was fed at various different stages and various amounts depending on the stage and size of the test organism.

Statistical Analysis-

Relative standard deviation (RSD) was defined as the standard deviation expressed as a percentage of the mean (this is equivalent to the coefficient of variation). The RSDs of the lengths of the larvae at 28 and 56 days post-hatch for control replicates were calculated to determine the acceptability of the data according to the EPA Environmental Effects Guidelines.

Hatch and Survival

The percent hatch and survival were tested using an exact 2x2 contingency table test to compare the treatments with the controls. Significant differences were identified at P=0.05 level.

Larval

The larval length and weight data from solvent and dilution water controls were compared using Dunnett's t-tests at the P=0.05 level. If no significant differences were found, all control data were pooled. If significant differences were found, the solvent control data were used for further analysis.

The length and weight data from each replicate of each treatment were compared using a t-test and, if no significant differences were found (P=0.05), the replicates were pooled for further analysis. Otherwise individual replicates were treated separately.

One-way and two-way analysis of variance was carried out on the length and weight data. If a significant F-value was obtained, Dunnett's t-tests were used to compare each treatment with the control (Ref 4) (P=0.05 and P=0.01).

The F_1 generation data were analyzed using the same methods as described above.

Egg Production

Egg production data included: number of batches of eggs, number of eggs per batch, total eggs, mean eggs per batch and range of egg numbers for each treatment. From day 150 to 300, individual egg production therefore "female-day" was recorded, since the pairs were in individual chambers.

12. Reported Results

Analytical Results

Overall the mean measured concentrations were 68% of the nominal concentrations. A total of 707 samples were measured over the duration of the study. The measured concentrations for the various chambers per test concentration are listed in Tables 8- 15. Lambda-cyhalothrin(cis B) eperimizes rapidly in water to the cis A enantiomer pair. Results from the GC analysis indicated that the total of the cis A and cis B isomer pairs was a total of 60% of the nominal concentration. (The LSC determinations do not discriminate between the cis A and cis B isomer pairs).

Lambda-cyhalothrin consists of the cis B isomer pair only, and as this comprises 77% of the total as determined by the confirmatory analysis, the LSC results have been corrected by this factor to estimate the true content of lambda-cyhalothrin in the test solutions (Tables 11, 15, and 16).

The mean measured residues (by LSC) for the various test concentrations were as follows:

Nominal Conc. ug/l	Mean Measured Conc. ug/l Cis A and B	Cis B*	% of Nominal
DWC	-	-	-
SC	-	-	-
0.03	0.019	0.015	50
0.06	0.040	0.031	52
0.12	0.081	0.062	52
0.25	0.179	0.139	56
0.50	0.354	0.273	55

* Correction factor applied = 77% (46/60)

Statistical Validation

The relative standard deviations(RSD) for F_0 generation larvae at 28 and 56 days post-hatch and at exposure day 300 were all less than 40%. The RSD for the F_1 generation at 56 days post-hatch was also less than 40%. (Tables 22, 24, 28 and 42).

The study authors reported the data for the control pooled and unpooled so that individual comparisons could be made.

Results from Measured Parameters

All of the following parameters are reported in measured concentrations(corrected cis B) unless otherwise noted.

Hatchability of F_0 Generation Embryos

The overall percent hatch was 87.3%. Hatch day was on exposure day 4. The study authors reported that there was no significant difference in any of the test concentrations when compared to the controls, therefore, the LOEL is greater than 0.273 ug/l (measured concentrations). See Table 18, 19, 20.

Survival of F_0 Generation Larvae to 28 days Post-Hatch.

No larvae survived to 28 days at the highest test concentration (0.273 ug/l). The survival was only 52% at the next test concentration, therefore, the mean LOEL was determined to be 0.139 ug/l (measured) concentrations. See Table 48.

Survival of F_0 Generation to 56 days Post-Hatch

There was significant reduction in survival between 28 and 56 days post-hatch when compared to the pooled controls.(See Tables 19 and 20). The LOEL for this parameter was determined to be 0.139. (Table 48).

Length of F_0 Generation Larvae at 28 and 56 days Post-Hatch

The average length was greater in all the test concentrations when compared to the control. In fact, the length was significantly greater in the 0.139 ug/L test level, since there was such high mortality in these test vessels, there was more room, or a lower loading factor. So the fish grew faster. The LOEL was 0.139 ug/l. See Tables 21-24 and 48.

Survival of F_0 Generation Fish to 300 Days

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Mortality after pairing had no dose response relationship. The NOEL was 0.139 ug/l. (Tables 25, 26, and 48). The top surviving concentration (0.139 ug/l) had 100 % survival over the 151 day period to the end of the exposure.

Length and Weight of F_0 Generation Fish at 300 Days

The LOEL is reported to be greater than 0.139 ug/l, since there was no effect at any of the treatment levels when compared to the controls.

Egg Production

A total of 86,633 eggs were produced in 1,048 batches. Egg production was divided into two phases.

The first phase was day 87 to 149, which was prior to pairing of the adults (as the sex could not be determined until full maturity or day 150). During this period, 22,280 eggs were recorded in 255 separate batches. During this period the actual number of eggs per female could not be ascertained since the females were not separated.

The second phase was from day 150 to day 300, the fish were paired and separated into four replicate breeding chambers in each adult tank. A total of 64,353 eggs were collected from 793 batches. See Tables 32-35.

Twelve females and one male died during the second phase of the egg production period (Tables 45 and 46) so the number of available female breeding days was reduced in some of the chambers. See Table 33. A total of 39 females and 44 males survived until termination.* The number of eggs per available day and the average number of batches per day per chamber have been calculated. See Table 33.

The study authors reported that the two lowest concentration showed an increase in egg production and the highest test concentration showed less, but not significantly so; P=0.05. The LOEL was therefore determined to be greater than 0.139 ug/l measured concentrations. See Table 36 and 48.

* Note: The number of males and females do not total to 48 of each, as would be expected. This is because at the highest concentration, three of the fish that were thought to have been males were actually females.

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Hatchability of F_1 (Second)Generation Embryos

When compared to the pooled controls, there was a significant reduction in hatching success at 0.139 ug/l. Therefore, the NOEL= 0.062 ug/l and the LOEL= 0.139 ug/l measured concentration. (See Table 37, 39, 40, and 48).

Survival of F_1 Generation Larvae to 56 days Post-Hatch

The average survival was reported to be 80% at 56 days post-hatch for pooled control data. The NOEL was determined to be 0.031 ug/l and the LOEL= 0.062 ug/l measured concentrations. See Tables 38-40 and 48.

Length of F_1 Generation Larvae at 56 days Post-Hatch

Length had increased in the top two concentrations when compared to the control. This was not considered an adverse effect, since there was an increase in mortality in these treatment levels, there was more room for the remaining fish. In other words, a lower loading factor, and therefore, the fish could grow faster. Therefore, the LOEL was greater than 0.139 ug/l.(Tables 41, 42 and 48).

Weight of F_1 Generation Larvae at 56 days Post-Hatch

Weight had increased in the top three concentrations. This is believed to due from a lower loading factor, see comments in above section. The LOEL was greater than 0.139 ug/l measured concentrations. See Tables 43, 44 and 48.

Adult Mortalities- Exposure Days 60-300

A total of 27 deaths were reported in the F_1 generation fish after day 60. The study authors reported these deaths not to be dose related. In addition, there were 3 culls, were a total of 63 males, 78 females and 2 fish with indeterminate sex were removed. See Tables 44 and 45.

Deformities

During the entire study, only five fish were reported to be deformed. All five were F_0 generation males, with four sacrificed during the first cull (on exposure day 154). These deformities were noted in the control, and the two lowest concentrations, therefore, there was not a dose response relationship. See Table 47.

LC50 values- F_0 and F_1 Generation Survival

The following results are based on the survival of the F_0 generation larvae to 96 hours in the separate acute study with larvae from the same batch of eggs that was used to start the main study and the survival of the first and second generation larvae to 56 days post hatch within the main study.

F_0 Generation(separate study)	96 hour LC50 = 0.360 ug/l
F_0 Generation	56 day(post-hatch)= 0.108 ug/l
F_1 Generation	56 day(post-hatch)= 0.059 ug/l

See Tables 19, 39, and 65-68,69.

Overall NOEL, LOEL and MATC

The overall NOEL was determined to be 0.031 ug/l measured concentration (corrected cis B). The most sensitive parameter was F_1 survival to 56 days post-hatch. The overall LOEL was 0.062 ug/l mean measured (corrected cis B). See Table 48.

With the range being from 0.031 ug/l to 0.62 ug/l, the geometric mean MATC was determined to be 0.044 ug/l. This MATC is one order of magnitude lower than the geometric mean derived from an fish early life stage study that was conducted on sheepshead minnow, with a MATC range of 0.38 ug/l to 0.25 ug/l mean measured concentrations.

Bioaccumulation of lambda-cyhalothrin

In all of the following instances, the lambda-cyhalothrin residue content of the adult F_0 fish, F_1 eggs, or F_1 larvae in the dilution water control and the solvent control was not significantly different from the background levels using radiolabeled material. All the following BCF's were calculated using measured residues in test vessels.

F_0 Generation adults

After 300 days exposure to the test solutions the lambda-cyhalothrin content of the adult F_0 fish in the dilution water control and the solvent control was not significantly different from the background levels. The BCF ranged from 3952 (in the 0.062 ug/l) to 6691 which was at the 0.139 ug/l. The overall mean BCF was 4982. See Table 51.

F_1 Generation Eggs

The exposure concentrations of 0.03, 0.06, 0.12, and 0.25 ug/l (nominal) produced BCFs of 1313, 1132, 1440 and

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1360, respectively in the F_1 generation eggs. See Tables 49, 50 and 52.

F_1 Generation Larvae

The exposure concentrations of 0.03, 0.06, 0.12, and
0.25 ug/l (nominal) produced BCFs of 3853, 3452, 5258 and 4633 respectively, with an overall mean of 4299. See Table 53.

Water Quality Observations

Dilution Water

The pH of the dilution water ranged from 7.0 to 8.1
with a mean of 7.4. See Table 54. The conductivity ranged from 100-160 uS/cm, with a mean of 125 uS/cm. The alkalinity or hardness of the dilution water ranged from 18.6 to 33.9 and from 32.6 to 57.0 mg/l as Ca CO3 respectively.

Residual chlorine was also measured for a total of 221 occasions. On three occasions, the residual chlorine level exceeded 4 ug/l. These were 9, 9 and 72 ug/l, respectively. The study authors reported that the 72 ug/l was suspect, since another sample taken prior to carbon filtration indicated that the residual chlorine was 18 ug/l.

A total of 89 measurements were made for ammonia. The levels ranged from less than 0.05 mg/l to 0.12 mg/l ammoniacal nitrogen.

The dissolved organic carbon content of the dilution water ranged from 0.001-2.1 mg/l with a mean of 0.84 mg/l.

The dilution water was periodically screened for heavy metals and pesticides. See Table 55.

Measurements in Exposure Vessels

The overall DO was 7.06 mg/l. The minimum recorded was 5.2 mg/l. The overall pH was 7.2 with a range of 6.09 to 8.36 (see Table 58). The overall mean temperature was 24.87oC, with a range of 24.0 to 26.2oC with a total of 1625 measurements-see Table 60 and 61.

Light Intensity of Test System

The light intensity varied from 1100 to 1806 lux at
study initiation to 1000 to 1800 lux at study termination.

Flow Rates and Toxicant Dilution Factors

The flow rate to the progeny tanks was nominally 55+/- 4 ml/minute. The recorded mean values ranged from 50 to 51 ml/minute. The flow rate to the adult tanks ranged from 228 to 234 ml/min with an average of 231 ml/min.

The mean toxicant flow rate ranged from 0.0100 to 0.0101 ml/min within an average of 0.0101 ml/min compared to nominal value of 0.01 ml/minute. The total measured flow averaged 813 ml/min. The resulting mean dilution factor was 80508 overall. This was equivalent to 98% nominal.

Protocol Used to Conduct Study

The study authors reported that the study was conducted in accordance with the EPA SEP Guidelines for a Fish Life Cycle Study EPA 540/9-86-137, with the exception of the following:

-The study was initiated with 80 eggs instead of the recommended 200 in the SEP. The study authors reported that an increase in number of eggs, increases the chance for fungal infection. The eggs chosen for study initiation were carefully selected for viability before placing into the individual chambers.

-The study authors interpreted the SEP to require two replicates for each concentration. The test was run with two incubation cups per replicate, but the larvae were then pooled to give two replicates per concentration.

13. Study Author's Conclusions / Quality Assurance Measures:

Based on all the various parameters measured, the most sensitive endpoint was F_1 survival. The NOEL was determined to be 0.031 ug/l and the LOEL was 0.062 ug/l. All the values are based on the corrected mean measured concentrations of lambda-cyhalothrin (ug/L) measured by liquid scintillation counting (correction factor 77% to allow for Cis B content).

The conduct of this study has been inspected /audited in accordance with Imperial Chemical Industries policies and procedures for Good Laboratory Practice.

14. Reviewer's Interpretation and Evaluation of the Study

A. Test Procedures:

The following discrepancies were noted:

- As pointed out by the study authors, the study was initiated with 80 eggs instead of the recommended 200 eggs. According to the study authors, these eggs were carefully selected for viability.

- The larvae were pooled where as the SEP guidelines recommends pooling when the adults are spawning. Therefore, the number of larval tanks are reduced by one-half.

-The percent hatch in one of the four incubation cups in the DWC was only 68.4 or 6/19 eggs failed to hatch. The average percent hatch for that replicate was 79.5.

- It appears that the study authors did not randomly select the larvae that were used for the remaining of the study. In addition, the study authors reported that 4 of the 5 fish that were deformed on day 154 and were sacrificed. Another instance where it appears the fish were not randomly selected for the continuation of the study.

-Table 32 indicates that in the two lowest concentrations, spawning took place earlier, and the numbers of eggs produced per batch at the beginning (exposure day 90 of the study) showed that this chemical cause a hermetic effect.

-The study authors kept the photoperiod constant at 16 hours daylight. Consequently, the females after pairing continued to produce eggs. Therefore, the study never was terminated after 1 week post-egg production as stated in the SEP. The study authors should be aware that the SEP is not a protocol, but a set of guidelines by which EEB evaluates that particular study. The Study authors should refer to the citations which lists the protocols (within the SEP) in designing that particular study.

-There is a concern that the Dilution Water Control (DWC) and the Solvent Control (SC) tanks may have been contaminated with test material. According to Table 10 (page 46) and Table 13 (page 51), the DWC and SC was reported to have levels of material as high as 0.007 ug/l Cis-A, and 0.010 ug/l of Cis-B. Both of the reported levels (for Cis-A and Cis -B) overlapped the lowest test concentration. The study authors should explain why the levels were as high, and confirm if indeed there was or was not contamination in the controls.

If indeed these levels were the lowest level of detection for that day, then the study authors should address why the solvent control on day 189 reported levels of Cis-A higher than the two lowest test concentrations. And if indeed the levels reported are the actual level of detection, then the baseline level of detection appears to be unstable.

- The dilution water used during this study was dechlorinated water. The ASTM (1980) states that dechlorinated water should not be used as dilution water because removal of chlorine is rarely complete and residual chlorine can be quite toxic to aquatic organisms. If used, it must be shown that the concentration of residual chlorine is <3 µg/L in fresh samples of the dilution water. However, the dechlorinated water used in this test is considered acceptable due to the following reasons: 1) the water had been dechlorinated using sodium thiosulfate and then passed through activated carbon; 2) the residual chlorine in the dilution water was measured frequently during the test; 3) percent survival of larvae and adult fish was acceptable in both controls.

- The eggs used in this test were from six females; the SEP recommends ten females.

- The ASTM states that measured concentration of test material in any chamber should be no more than 30% higher or lower than the nominal concentration. If the concentration of the test chamber is too low, the stock solution of test solution may have been prepared incorrectly, the metering system may not have been calibrated correctly, or volatility and degradation may have occurred. During this study, most measured concentrations were >30% lower than the nominal concentrations. However, since the concentrations of the test solutions were measured frequently and the values obtained were consistent, this deviation did not affect the test validity.

- The mortality in the both the solvent controls and the dilution water controls was higher than would have been expected in a life cycle study. Unfortunately, since the photoperiod did not follow the Evansville, Indiana times, the females did not stop spawning. EEB did evaluate the control data to include the first seven spawns, and determine that the egg production as well as survival was within the range of other historical control data that are available to EEB.

- The study authors should report the historical control data for the fish life cycle studies that have been conducted at this facility. EEB questions the early spawning, on day 87, since the data available to EEB

indicates spawning typically starts much later- closer to day 120 of the study. In addition, the study authors should report why there was such a delay in separating the fish into pairs since spawning started so early. It seems that the sexes of the fish should have been discernible earlier in the study. For example, based on territorality or spawning behavior. Or perhaps the fish were spawning too early.

- The study authors should explain why the 0.012 ug/l (nominal concentration) treatment level did not include a second batch of F1 eggs for replicate B. Please refer to Table 39 (page 150). Especially since it was clear from that same table but (page 147), that there were three batches of eggs for that replicate. Therefore, fewer F1 eggs/larvae were evaluated for percent hatch, 28 and 56 day survival at that treatment level.

- There appears to be data discrepancies, or poor quality assurance of the data reporting. Please refer to the following:

  Table 38- 0.12 ug/l treatment level - Date of spawning-26.8.88 and date of hatch 31.8.88. It should only take 5 days to hatch.

  Table 39 indicates that again a hatch date was 31.10.88 for 0.12 ug/l treatment level, and page 147 shows no spawning close that date.

B. Statistical Analysis:

The Ecological Effects Branch confirmed all statistical analysis by using the ANOVA arc-sine transformation, with Duncan Grouping, for percent hatchability for both the F0 and F1 generations, as well as survival for F0 and F1 generation, at the various intervals (28 days, 56 days, and at study termination). The ANOVA , with Duncan Grouping, was used to determine the level of significance for the length and weight of the F0 and the F1 generation (28 day, 56 days and 300 days). The 300 day data was also broken down into groups of males and females. The egg production data were also analyzed using the ANOVA and the Duncan Grouping. Specifically the egg production data were broken down by numbers of spawns per female/treatment level and total number of eggs per female/treatment level from day 149 to 300. The egg production data (total number of eggs per female was also logged, in order to determine if logging the data changed the results.

Ultimately, EEB found a few parameters where the statistical results were different, in all cases, EEB's results were more sensitive. These include: F0 length at 28 and 56 days, and F0

length and weight at 300 days. The pooling of the control data may have affected ICI's conclusions.

C. Results/Discussion- All of the numbers below are based on measured concentrations (Corrected for cis B).

1) Hatchability of F0 Embryos- Our analysis indicated there was no statistical differences among the treatment and the control eggs regarding the cumulative number of larvae that hatched by day 5 of the study. The percent hatched per cup ranged from 78.75 % for the 0.062 to 90.1 % for the 0.139 ug/l. There was not a dose response relationship. The hatching values for DWC and the SC are, 84.5% and 89.0%, respectively. The NOEC was greater than 0.273 ug/l.

2) Survival of F0 Larvae at 28 days and 56 days post-hatch- At this point in the study, the highest test concentration (0.273 ug/l) had no surviving larvae by day 7. By day 28 of the study- only 52 % of the larvae survived at the next highest concentration (0.139 measured conc.) The 28 day F0 larval survival of DWC and the SC were 98 % and 88%, respectively. The NOEL was 0.062 ug/l and the LOEL was 0.139 ug/l.

Again by day 56 of the study the treatment level which showed signs of adverse effects was 0.139 ug/l. The DWC and the SC were 94 % and 88%, respectively. The NOEL was 0.062 ug/l and the LOEL was 0.139 ug/l for 56 day F0 larval survival.

3) Length of larvae at 28 and 56 days post-hatch- Our analysis indicated that the length of the larvae significantly increased after 28 days post-hatch at the 0.139 ug/l treatment level. These results are different from what the study authors reported, which indicated that the NOEL was >0.139 ug/l. Therefore, the NOEL was 0.062 ug/l and the LOEL was 0.139 ug/l.

In addition, EEB conducted the statistical analysis and determined the larvae increased in weight at 56 days post-hatch at the 0.031 ug/l treatment level. Therefore, the NOEL was 0.031 ug/l and the LOEL was 0.062 ug/l. EEB believes that the reason for the increase in growth may be a hormetic effect, which is commonly seen when fish are exposed to low sublethal concentrations of toxicants*, or due to a reduction in the number in the test vessel, thereby lowering the loading factor. *Note- This may also explain why at the two lowest doses the egg production was considerably higher than even the controls- again a hometic affect.

4) Survival of F0 fish at 300 days. Our statistical analysis indicated that survival was not affected up to 0.139 ug/l. The mortality was high from day 150 to 300 in the DWC and the Solvent control when comparing to historical control data. Specifically for the DWC and SC the percent survival were 81.25% and 87.5%,

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respectively. However, these values do fall within the range of acceptability of the study. When reviewing the data, it appeared the mortality was primarily females, and all except one female had at least 5 spawnings prior to death. Therefore, the NOEL was determined to be $\geq$ 0.139 ug/l.

5) Length and Weight of F0 Fish at 300 Days- Since there is such a wide variability in growth in males and females by day 300, we separated the males from the females when conducting the statistical analysis. For the females, both the weight and the length revealed a NOEL of 0.139 ug/l.

When we analyzed the male weight data, the results from the statistical analysis indicated that the weight increased at the 0.062 ug/l treatment group. However, at the higher dose (0.139 ug/L), the increase was not as great, so that there was not a clear dose response with regards to weight and concentration. With regards to length of the males, the males increased in length at 0.062 ug/l when compared to the controls. Again, EEB attributes the increase in weight and length at the lower concentration to hormesis. Therefore, the length and weight of the males were affected at 0.062 ug/l, indicating a NOEL of 0.031 ug/l for both parameters.

The results of the length /weight data are as follows:

Treatment Level (measured conc.) ug/l	Mean Weight(gm) Male	Female	Mean Length (mm) Male	Female
DWC	4313.8	1474	57.9	44.6
SC	3966.3	1841.7	55.6	47.5
0.015	4047.5	2006.7	56.8	47.6
0.031	4007.1	1714.3	55.1	46.4
0.062	5221.3	1597.5	61.4	45.4
0.139	4686.0	1760.9	61.7	46.8

6) Egg Production - Our statistical analysis included several parameters. Specifically, Al Jarvinen, (personal communications, EPA Lab, Duluth, MN) suggested the egg production data be analyzed from day 150 to 300 since that is when the adults were paired. The study authors reported all the necessary data to evaluate this data properly. According to the statistical analysis, none of the treatment levels showed a decrease in egg production when compared to the control data. However, while reviewing the data, it was determined that the variation was so great that a statistical difference would be difficult to ascertain. The following is a summary of the egg production data from day 150 to 300.

Treatment Level measured conc. ug/l	Total No.Eggs (mean)	Total No. Batches Eggs (mean)
DWC	1552	21
SC	1407	18.8
0.015	2155	21.0
0.031	1809	22.1
0.062	940	13.5
0.139	288	4.2

The above mean total number of eggs shows that there is a biological effect when exposed to the chemical even though there was no statistically significant difference when compared to the controls.

The total number of batches of eggs showed that there was indeed a statistical significant difference at the 0.139 ug/l. Therefore, the NOEL was 0.062 ug/l and the LOEL was 0.139 ug/l for the total number spawns/treatment level.

In addition, we analyzed the egg production data using log transformation, since there was such a wide variability of the data, and we determined that using log numbers, the NOEL was 0.062 ug/l and the LOEL was 0.139 ug/l.

Since there was mortality and since the study authors reported that in three of the replicate chambers only females were loaded into the tanks, therefore no egg production took place in those chambers, EEB also analyzed the data by the number of eggs/female/breeding day. The NOEL was determined to be 0.062 ug/l and the LOEL was determined to be 0.139 ug/l.

7) Effects on F1 Embryos and Larvae- The results from the statistical analysis indicated that effects to the F1 generation to be the most sensitive endpoint. The lowest NOEL showing clear adverse effects were seen for the F1 generation. The following is a mean of the percent hatch and survival summary of the F1 generation:

Treatment Level ug/l measured conc.	Percent Hatch	Survival (%) 28 days	56 days
DWC	97.2	89	84
SC	94.9	85	75
0.015	92.4	90	87
0.031	95.1	94	85
0.062	92.3	55	50
0.139	82.2	41	29

Results from our statistical analysis indicate that the 28 day survival had a NOEL of 0.031 ug/l and a LOEL of 0.062 ug/l. The 56 day survival was not significantly different at the 0.062 ug/l treatment level (NOEL), but is significantly different at the 0.139 ug/l (LOEL).

In addition to the survival data, the 56 day larvae were measured for length and weight. The results are as follows:

Treatment Level (measured conc. ug/l)	Length (mm)	Weight (gm)
DWC	24.9	231.5
SC	25.0	250.5
0.015	25.1	248.6
0.031	25.8	274.7
0.062	27.6	300.5
0.139	28.5	345.2

Our statistical analysis of the 56 day larvae weight and length data indicate that the growth was stimulated in the treatment levels when compared to the controls. Specifically the NOEL for both parameters was determined to be 0.031 ug/l and the LOEL was 0.062 ug/l.

8) Effects on Adult F0 Fish- During the course of the study only five fish were noted to have any physical deformity. All five were F0 generation males and four were sacrificed during the cull of excess fish on day 154. The fifth was only recorded as deformed on two occasions(once on day 21 and once on day 300). At the end of the study only this one fish remained with any degree of deformity. The deformities were noted in both controls and the two lowest exposure concentrations and were therefore considered not to be related to the test compound.

9) Bioaccumulation of lambda-cyhalothrin- For all of the following parameters measured for bioaccumulation, the content of lambda-cyhalothrin was not different in the DWC and the SC from the background levels. The results of the data are as follows:

Treatment Level ug/l (measured conc.)	Bioconcentration Factors (BCF) F0 Adult Fish (300 days)	F1 Eggs	F1 Larvae
0.015	5060	1313	3853
0.031	4226	1132	3452
0.062	3952	1440	5258
0.139	6691	1360	4633

10) LC50 values- The study authors also conducted LC50 studies on F0 generation larvae for 96 hours with the same larvae from the same batch of eggs that were used to start the main study. The survival of F0 and F1 generation larvae to 56 days post-hatch within the main study was also assessed. The following values were determined:

		measured concentrations
F0 generation	96 hour LC50	= 0.360 ug/l
F0 generation	56 day (post-hatch) LC50	= 0.108 ug/l
F1 generation	56 day (post-hatch) LC50	= 0.059 ug/l

The summary of the NOEL and LOEL values are as follows:

RESULTS FROM STATISTICAL ANALYSIS

PARAMETER	NOEL	LOEL
Percent hatch (FO)	≥0.273 ug/l	-
FO Survival to 28 days	0.062 ug/L	0.139 ug/L
FO Survival to 56 days	0.062 ug/l	0.0139 ug/l
FO Survival to 300 days	≥0.139 ug/l	-
F1 percent hatch	0.062 ug/l	0.139 ug/l
F1 Survival to 28 days	0.031 ug/l	0.062 ug/l
F1 Survival to 56 days	0.062 ug/l	0.139 ug/l
FO length at 28 days	0.062 ug/l	0.139 ug/l
FO length at 56 days	0.031 ug/l	0.062 ug/l
FO length at 300 days		
Females	≥0.139 ug/l	-
Males	0.031 ug/l	0.062 ug/l
FO Weight at 300 days		
Females	≥0.139 ug/l	-
Males	0.031 ug/l	0.062 ug/l
F_1 weight at 56 days	0.031 ug/l	0.062 ug/l
F_1 length aT 56 days	0.031 ug/l	0.062 ug/l
Total eggs (days 87-300)	0.062 ug/l	0.139 ug/l
Number of eggs/female/ breeding day	0.062 ug/l	0.139 ug/l
Total eggs/female	0.139 ug/l	-
Total eggs from days 150-300	>0.139 ug/l	
Number of batches eggs 150-300	≥0.139 ug/l	
Egg Production- log- 150-300	0.062 ug/l	0.139 ug/l

D. Adequacy of the Study

1) Classification- Not classified

2) Rationale- EEB was unable to classify this study at this time since there are discrepancies that need to be addressed by the study authors in order for EEB to determine if this study is scientifically sound and satisfies data requirements. Please refer to section 14.A. for a review of the discrepancies.

3) Repairability- The study authors must address the discrepancies noted in section 14. A. One particular concern, it appears that there was contamination in the DWC and SC tanks by the levels of lambda -cyhalothrin reported by the study authors (GC analysis). If the study authors provide information to negate these concerns then the classification will be reconsidered. Another concern is the adequacy of the quality assurance. EEB has found discrepancies in the data reporting that need to be addressed.