File Copy

253374
RECORD NO.

128897
SHAUGHNESSEY NO

REVIEW NO.

EEB REVIEW

DATE: IN 10-14-89 OUT 5/14/90

FILE OR REG. NO. 10182-96

PETITION OR EXP. NO.

DATE OF SUBMISSION 9-28-89

DATE RECEIVED BY HED 10-13-89

RD REQUESTED COMPLETION DATE 1-15-90

EEB ESTIMATED COMPLETION DATE 1-15-90

RD ACTION CODE/TYPE OF REVIEW 570

TYPE PRODUCT(S) Synthetic Pyrethroid

DATA ACCESSION NO(S)

PRODUCT MANAGER, NO. G. LaRocca(15)

PRODUCT NAME(S) Karate (PP321)

COMPANY NAME ICI Americas, Inc.

SUBMISSION PURPOSE Registrant response to EEB review of Mescosm study (dated 4-11-89)

SHAUGHNESSEY NO.	CHEMICAL	% A.I.
128897	lambda-cyhalothrin	

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

File Copy

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

MAY 14 1990

SUBJECT: EPA's Review of the August 21, 1989 ICI, America Response of the PP321 Mesocosm Data Evaluation Record

FROM: James W. Akerman, Chief
Ecological Effects Branch
Environmental Fate and Effects Division (H-7507-C)

TO: George La Rocca, PM 25
Insecticide and Rodenticide Branch
Registration Division (H-7505-C)

The Ecological Effects Branch (EEB) within the Office of Pesticide Programs, of the Environmental Protection Agency (EPA) received a response from ICI Americas Inc. to the Karate (PP321) Mesocosm Review on October 13, 1989.

1. Introduction-

The following is EPA's review of ICI's October 13, 1989 response. EPA has summarized both our original comments and ICI's comments, following the same sequence and section designations of the ICI comments.

2. Study Reclassification-

As concluded in the previous study evaluation of August 22, 1989, this study demonstrates that PP321 adversely affects aquatic environments. However, there are certain deficiences in the study design and execution that were noted.

For example, ICI acknowledged that the number of fish at study initiation is indeterminate. In addition, the residue samples were not reported as proposed in the original protocol between ICI and EPA.

Even with the unresolved problems in the study, however, the data are sufficient for EPA to conclude that PP321 adversely affects aquatic environments. These data are sufficient to fulfill the § 72-7 data requirement imposed in June 1986.

In spite of the study deficiencies, ICI reported statistically significant differences between each treatment and the control for the young of the year and total fish, each representing an adverse treatment effect. EPA notes that the observed average treatment response roughly corresponds to approximately a one-third decrease in biomass for the young of the year fish. The significant decrease at the lowest treatment level is of particular importance since this level is only 0.01 of that considered as a typical expected exposure with a 5% spray drift.

EPA is not requiring additional studies because it is clear that this chemical has demonstrated serious adverse effects at or below the typical expected exposure on cotton.

2.1 Residue Monitoring Program

2.1.1. Sampling Program

Originally, EPA pointed out that the residue sampling program was not consistent with the approved protocol, and therefore, the protocol not satisfied.

ICI responded by claiming that all samples were taken as described in the protocol, but "not all of the samples collected were analyzed".

Therefore, even though all the scheduled residue samples were sampled according to the protocol, ICI acknowledges that many of the samples were not analyzed for residues.

Based on the data provided to EPA, the replicate ponds were not reproducible with respect to measured concentrations of chemical within each pond. There was a chance of even more variability between the ponds but the residues for the ponds were not reported. The residue analyses for both water and sediment were reviewed once again and the comments are as follows:

<u>Water Residue Analyses</u>

ICI may have sampled more ponds but reported only 14 measured concentrations of the 19 scheduled water samplings for the high-dose ponds. In addition, only 2 replicates, not 4 as required, were measured giving a total of only 28 samples of 76 expected samples; e.g., only 36 % of the expected samplings were actually measured for the high-dose ponds.

One issue is the considerable variability between ponds within the same treatment level, as well as variability within the individual zones. At times, the average within pond residues varied by as much as 3 fold.

For example, on September 23, 1986, the residues in the two replicate high-dose ponds, 2B and 3B, were 3 pptr and 8 pptr, respectively, in the shallow/middle zones and 24 pptr and 2 pptr in the deep/bottom zones. There was a 12 fold difference in the residues collected from the deep zone of the two ponds. If each replicate received the same concentration of PP321, one would have expected the residue profiles for each sampling date in each replicate pond to be more similar. This observed variability could have been reduced if more samples had been analyzed.

A second issue is the failure to report residues for large periods of time during the application phase of the study. For example, the residues for the high treatment ponds were not reported for 26 days, from June 30, 1986 to July 25, 1986. During this period of time, there were 4 drift applications and 2 runoff applications to the ponds. Pond 2B was reported to have residues as high as 90 pptr in the water on July 25. However, we do not know how typical this concentration is for the 26-day period; they may have been higher following the applications.

This incident was also observed for the reported residues for the mid-dose ponds. Only 15% of the water samples per replicate were analyzed (6 of 76 samples, as proposed in the approved protocol). The mid-dose ponds were not measured from June 16, 1986 to July 26, 1986. Therefore, the residues in the water were not measured while the ponds were undergoing 6 drift applications and 3 runoff applications. The last reported measured concentrations were for July 31, 1986, although the study continued until November 6, 1986. There was a two-fold difference in measured concentrations for the limited time reported.

The investigators believed that the chemical was rapidly adsorbed by the bottom sediments. However, the data do not fully confirm this, For example, the residues in the water of pond 2B were 3 pptr one day post treatment but were as high as 24 pptr in the same sampling zone 14 days later . The investigators needed to report more residue data to support their hypothesis.

Hydrosoil Residue Analyses

ICI measured less than 50% of the collected hydrosoil samples for residues. The measured concentrations varied by two-fold.

The study authors reported less than a two-fold difference for the mid-dose pond hydrosoil samples for the 11 samples measured for each replicate. Based on the submitted data, at least 2 more sampling dates should have been included in the hydrosoil residue sampling schedule. All 16 ponds should have been analyzed. However, the study authors reported the measured concentrations in the sediment until 10/27/86 even though reported sampling in the water was discontinued 3 months earlier.

Summary of Residue Analyses

ICI measured only two of the high-dose ponds and two of the mid-dose ponds. No measured concentrations were reported for the low-dose ponds, even though there was a significant effect on fish biomass and other aquatic organisms, i.e., macroinvertebrates, zooplankton, etc.in the ponds. Only one control pond was analyzed for residues. Therefore, of the 304 expected sampling days, only 54 of these (i.e. 18%) were actually measured for residues.

The mean values for each of the two high-dose ponds and the mid-dose ponds were approximately the same, with a five-fold difference between the high dose and mid-dose . Because of the week-to-week variability in the reported residues for these ponds a clear distinction between treatment levels cannot be substantiated (page 14, sections i and ii of ICI response).

The investigators had difficulties analyzing residues in the water and sediment. They should have contacted EPA to discuss these problems when it became apparent they could not complete the analyses within the agreed time frame (page 14, first paragraph of ICI response).

ICI contends that it was unnecessary to follow through with the originally agreed residue analyses schedule since the concentrations were so similar between ponds and within ponds. This statement is based on their belief that the chemical was evenly applied to each replicate pond, that the replicate ponds were similar with regard to distribution of the chemical in the water column and that the chemical concentrations were expected to rapidly decrease in the water column due to adsorption to organic matter and deposition in bottom sediments. However, EPA has effectively shown that there was enough variability within and between ponds to question the basis for these assumptions.

2.1.2. Pond Cross-Contamination

The concern for pond cross-contamination has not been adequately resolved since the residue monitoring regime was so deficient. ICI responded by contending that the method of application was designed to substantially limit but not prevent cross-contamination. Not all of the control ponds were analyzed for residue, nor were any of the low treatment ponds analyzed for residue. Therefore, based on the submitted data, EPA can not conclude if there was or was not cross-contamination between the ponds.

2.2 Stocking of Adult Bluegills into Mesocosms

2.2.1 Size of Fish

EPA commented that the smallest size of the bluegills added to the ponds was less than what was reported in the text of the final report. ICI's response was that the total wet weight of fish added to each pond ranged between 910 g and 1360 g, (note* a 450 g difference), the average wet weight ranged from 40-50 g per fish, and actual measurements per fish were not done, since handling of the fish was not advised by the supplier.

EPA accepts that the replacement fish may have been smaller, and that indeed the total weight of fish introduced into any of the ponds was changed very little by the replacements.

Ultimately, the variability in fish size was not expected to severely impact the study, since indeed the data indicate there was a significant impact on fish biomass even at the lowest dose.

2.2.2 Number of Fish

EPA commented that there appeared to be either an error in stocking or an inadvertent intrusion of bluegills into the test ponds because the number of mature fish ($\geq$ 11 cm) recovered at the end of the study did not coincide with the number of bluegills stocked and replaced in each pond (25/mesocosm). The number of mature fish per pond ranged from 23 to 47 per pond.

ICI accepted that there is an apparent anomaly when the numbers of fish collected at the end of the study are compared with the numbers stocked. ICI believes that larval fish and/or eggs could have been introduced during the filling and invertebrate stocking period, especially since the stock pond contained a bluegill population. The ponds had 2.5mm screen placed over the inflow pipes.

According to Carlander (1977), larval fish or eggs would most likely not have reached maturation or grown to adult size by the time the fish were harvested in November of 1986. Therefore, it is unlikely that the larval fish or eggs from the stock pond would have caused an increase in the >11 cm size group, as seen in the ponds. The point is that no matter how the adult fish were inadvertently loaded, the reliability of the study is reduced. If the number of fish is unknown at test initiation, then the exact impact of the chemical is unknown at test termination.

2.3 Data Discrepancies in Tables 127 and 128.

During the initial review of the study, EPA determined that there were discrepancies within the raw data presented in Tables ICI 127 and 128. ICI acknowledged the data discrepancies and corrected the data points, and added five other corrections. Since there were eight corrections within two tables, the quality assurance is highly questionable in this study.

2.4 Fish Harvest Data for Pond 5B.

EPA observed that regardless of a recording error, the study authors should have presented the weight and number data that were available for one of the low-dose ponds, 5B, specifically for the 3 cm size class.

ICI submitted the raw data sheet which provides the weight data for the 3 cm size class (Appendix I). EPA does not agree that it appears as though the 4 g should read as 41 g on the page. In addition, EPA does not understand how the number of fish were recorded or calculated. It is not clear from the raw data sheet how many fish were collected per size group. ICI should report the calculation method used to determine fish numbers per size group.

2.5 Recording Error for Pond 7B Hydrosoil Residue

EPA accepts the correction for Table ICI 55d.

3.0 Test Procedures

3.1 PP321 Application Rates and EEC's

EPA expects "that any effects seen in this study at the high-dose are effects we expect to see under normal aerial spray application conditions."

ICI does not believe that a spray-drift entry of 5% and run-off entry of 15% represent typical exposure during application. ICI also believes that EPA is being inconsistent with both its past agreement with ICI and the present approach with other pyrethroids.

EPA accepted a spray-drift entry rate of 3.15% for the cypermethrin mesocosm study and is an average value obtained from a study conducted by ICI. This spray-drift is based on actual field data collected in the previous cypermethrin pond study in Alabama. Therefore, actual residue monitoring data were available to support that drift rate used in that study to represent a specific circumstance.

EPA recommends that a spray drift entry rate of 5% be used as the medium drift loading in the mesocosm studies for pesticides that are applied aerially. It has been determined that 5% represents a typical rate of spray drift, and is used in the absence of actual spray drift data. If ICI wants EPA to consider using a spray drift rate lower than 5%, then actual spray drift data for PP321 are required to support ICI's expected spray-drift. The spray-drift monitoring would be required to include field data with a 5 miles an hour wind velocity. On another note, since effects were evident at 0.05% drift rate, the argument for a lower drift rate may be moot.

Since the exposure of concern is expected to come primarily from spray drift, the run-off rate of 15% (1.5%/ Acre X 10 Acre Drainage Basin) in the high-dose when compared to the expected 1% (0.1%/Acre X 10 Acre Drainage Basin) in the medium-dose is not expected to substantially contribute to the total exposure of PP321 in the aquatic ecosystem. The run-off entry estimates add little to the total water column exposure since PP321 so readily binds to the sediment. It is estimated that the residue in the water column is 0.275 pptr with a 15% runoff loading versus a 0.18 pptr from 1% runoff loading (Appendix II).

EPA believes that the high-dose ponds represent the typical loadings that is expected when this chemical is aerially applied to cotton. Spray-drift and run-off entry data, collected during an actual field situation, would be needed to negate EPA's presumption of adverse effects using this chemical under an actual field situation.

3.2 Sampling and Treatment Program

EPA agrees that varied application to the mesocosms was agreed upon in the protocol, but nevertheless weakens certain interpretations.

3.3 Pond Assignment and Cross- Contamination

Please refer to section 2.1.2. of this document.

3.4 Residue Monitoring

Please refer to section 2.1.1. of this document.

3.4.2 Hydrosoil Residue Depths

Because the sediment had been sampled only in 5 cm increments, and had not been analyzed in smaller increments, EPA estimated the potential residues in the top 0-2.5 cm in the mid-dose ponds by multiplying the residues by 2. ICI has agreed to the method of calculation and has provided Table ICI R7 and/Table ICI R8 listing the residues in the mid-dose ponds. ICI agrees that "the values are high and may exceed 6,000 pptr. After dosing, values ranged from >700 to 2,000 pptr. The residues remained as high as 3,800 pptr up to six weeks post-application in the upper 2.5 cm."

EPA understands that residue analyses are cost and labor intensive, however the goal is to properly analyze the samples so that the data can be used for risk assessment purposes. The hydrosoil cores should have been sampled at 1-2 cm as per amended protocol (See Attachment B, EPA review signed on June 26, 1986). The study authors failed to report the data in increments less than 5 cm for the mid-dose ponds, and failed to report any residues in the low-dose ponds.

3.4.3. Hydrosoil Residue Concentrations

EPA understands why ICI feels that there is a "very clear correlation between the PP321 residues in ponds at these two concentrations" (high and mid-dose), "The applications were 10-fold different and the residues correspond closely to this".

However, EPA does not fully agree. These conclusions are based on mean values and do not show the overlap in concentrations that occurred. For an earlier sampling date (June 30, 1986) the differences ranged from 2 to 19 fold within the same zone, as the actual residues in the soil ranged from <0.2 ppb to 52.5 ppb in the high-dose ponds. The residues in the mid-dose ponds ranged from <0.2 ppb to 4.8 ppb during the application period. During the post application period the residue concentrations in the high-dose ponds averaged approximately 15 fold higher (October 28, 1986). This difference may be due in part to the higher rate of application, to spray drift and to adsorption of the chemical to the sediments as they dropped through the water column.

EPA noticed in this review that the residues reported in Appendix V part 4, do not correspond with the recently submitted ICI R9. ICI should explain these discrepancies in data reporting.

3.5 **Zooplankton and Macroinvertebrate Biomass**

EPA agrees that biomass measurements were not requested in the accepted protocol. However, the April 1986 mesocosm workshop concluded that biomass for invertebrates was an essential parameter and ICI had two participants at this meeting. EPA has since determined from the review that biomass data would have been useful to evaluate secondary effects.

3.6 **Stocking of Adult Bluegills**

Please refer to section 2.2 of this document.

3.7 **Period of Baseline Study**

EPA does not challenge that the length of time for maturation of the ponds was mutually agreed upon by both parties. EPA did express concern that the baseline year may not have been adequate for colonization by aquatic organisms. Based on this study and a review of new information, EPA believes a longer colonization period may be warranted. Even ICI agreed that the ponds would have matured further if left another year, but they also stated that divergence may have also resulted, giving much more variation in population parameters. As future mesocosm data become available, EPA will be evaluating what time frames are needed to properly colonize these test systems.

3.8 **Residues in Fish and Invertebrates**

EPA agrees that the final protocol did not require residue analysis on these organisms. However, residue analysis of the fish are now required for all the mesocosms being conducted on synthetic pyrethroids, since this information is useful in assessing the potential bioaccumulation in the environment.

3.9 Data Discrepancies and Recording Error.

Please refer to sections 2.3, 2.4, and 2.5 of this document.

4.0 Interpretation of the Data

EPA argues that even though many differences noted for various parameters were not statistically significant, they were in most cases, consistently lower in the treated ponds. A preponderance of evidence emerges when one considers that the treated ponds generally exhibit one-sided (e.g. reduced) deviations from control ponds, often dose related. In general, while variability in different parameters may have precluded achieving statistical significance, EPA contends that the consistent differences between control and treated ponds cannot be ignored.

In addition to these trends, there were many endpoints that did show a statistically significant effect when compared to the control ponds, such as, length of fish and fish biomass. Considering the low power of either a directional or non-directional t-test to detect a "large effect", EPA considers the observed significant differences to be biologically important.

In these instances, EPA contends that the action of PP321 on the components of the ecosystem is a contributing factor affecting the dynamics of the entire system and this culminates in the significant reductions in the top of the food chain (fish).

4.1 Statistical Analyses

EPA had originally validated the statistical analysis by using ANOVA and appropriate hypothesis tests (Duncan's, Dunnetts, and Williams). The population means had been transformed where needed by natural log or ln (x+1).

ICI believes that it was justified in using the two-sided t-test for the pairwise comparisons of each PP321 treatment mean against the control mean.

Under the advisement of Dr. Clayton Stunkard, Office of Policy, Planning and Evaluation, EPA does not take issue with the choice of the multiple comparison tests. But EPA does believe that a one sided t-test is better than a two-sided t-test for the data analysis of the PP321 mesocosm study. EPA conducted an analysis of variance on the revised data to determine whether there were differences in the interpretation of the data; the results were the same (please refer to Tables 1 through 3).

4.2 Residue Analysis

4.2.1 Water

The study authors believe that the report summary initially submitted was appropriate and useful.

EPA determined that water residues were higher than reported in the summary. According to the raw data, the residues were as high as 99 pptr in the high-dose ponds, and three weeks after the last application, the residues were recorded to be 24 pptr.

EPA reviewed the last ICI response; again, based on the raw data, the residues were as high as 24 pptr 3 weeks after application in one of the high-dose ponds. There is no evidence to negate these numbers. Another detail that EPA failed to point out in the first review, was that the study authors failed to report residues for a 26 day time frame in the high-dose ponds. During this time there were 4 drift applications and 2 runoff applications. Therefore the two high-dose ponds could have had residues as high as 99 pptr and 94 pptr or higher for the entire 26 days.

4.2.2 Hydrosoil

EPA indicated that the residues may accumulate in the hydrosoil, since the average residues were 24 ppb at test termination for high-dose ponds. The residues in the hydrosoil were as high as 58.8 ppb one month after the final application in the high-dose ponds and remained as high as 35.3 ppb two months post-application in the high-dose ponds.

EPA continues to believe that the high concentrations of PP321 in the hydrosoil adversely affect substrate associated organisms. EPA did not imply that accumulation of PP321 by fish would only occur from eating benthic organisms. EPA stated that high body burdens will <u>likely</u> occur from fish feeding on substrate-associated food organisms. One could also interpret this to mean that because bluegills also ingest soil when feeding, this is another potential source of entry into the fish.

4.3 Phytoplankton

4.3.1 through 4.3.4

EPA concluded that there were possible reductions in phytoplankton number and biomass, alterations in species dominance, reductions in chlorophyll a, and reductions in P/R ratio, which may be correlated with certain physicochemical changes, all of which may be attributed to exposure to PP321.

ICI contends that no consistent statistically significant differences are observed for any of the above parameters and, therefore, PP321 did not significantly affect phytoplankton in the mesocosm test.

Once again, as stated in section 4.0 , the wide variability for most of the parameters identified renders statistical significance difficult to demonstrate in a study this size. That is, one cannot contend with any reasonable statistical confidence that PP321 caused or did not cause perturbations. EPA did not suggest that its conclusions were based on observations of statistical significance, although a few statistically significant differences were identified and conceded to by ICI. The few differences so noted may or may not be attributable to "multiplicity-of-tests" phenomena. EPA based its conclusions of the phytoplankton parameters primarily on qualitative analyses.

The argument that EPA makes is that even though differences noted were not significant in most cases they were consistent. For example, chlorophyll a in all treatment ponds was reduced from the control. A preponderance of evidence emerges when one considers why the treated ponds generally exhibit one-sided (e.g. reduced) deviations from control ponds, often dose related, even if these deviations cannot be statistically verified. In these instances, EPA contends that the action of PP321 in the system cannot be categorically ruled out as a contributing influence.

4.4 Zooplankton

4.4.1 Broad Categories

EPA maintains that lumping of zooplankton by broad taxa may mask potential effects to specific taxa. EPA maintains that isolation of cosmopolitan and dominant taxa enhances its understanding of the effects of toxicants in complicated ecosystems. Loss of cosmopolitan and dominant taxa may be considered an adverse effect.

EPA agrees with ICI that the effects to some zooplankters, especially between weeks 0 and 2, may in part be due to other factors including fish predation and sediment loading. EPA also suggested that high temperatures may have accounted for some of the impact to plankton because temperatures exceeded 29°C during this time.

4.4.2 Individual Taxa

ICI believes that there was no effect of PP321 on the overall rotifer, or zooplankton protozoan populations. EPA disagrees with ICI; EPA contends that there were potential effects of PP321 to zooplankters, especially the crustaceans. Changes in these populations corroborate ICI's and EPA's conclusion that treatment of ponds with PP321 caused reductions in length of fish and fish biomass.

EPA also maintains that seasonal cycles of ecodominant zooplankters, e.g., Polyarthra, should be similar among all ponds regardless of treatment if there is no effect of the chemical on the populations or communities. When they are not similar, a potential effect of the chemical treatment is indicated.

EPA maintains that while the variability in the numbers of zooplankton populations may preclude obtaining statistical significance, the differences observed between control and treatment ponds should not be ignored.

EPA does not agree with ICI's conclusion that PP321 had no effect on the overall rotifer population or on its component taxa; ICI's original report (Figure ICI 35c) indicates very dramatic effects after week 8 even though the differences were not statistically significant.

EPA agrees that PP321 may not have had an effect on the protozoan populations.

EPA does not agree that no effects to crustacea were noted in the mid- and low-dose ponds. EPA also does not agree that the high-dose pond did not affect ostracods or cladocerans, but only substantially reduced the copepod populations. EPA notes that while treatment did not affect the numbers of ostracods, omnivorous scavengers (ICI R20), treatment definitely affected the proportion of ostracods in the relation to the crustacean community (ICI R8). Copepod nauplii became less and less of an ecodominant after commencement of treatment (ICI R8); in fact, the proportion of copepodites in the mid-dose ponds were also much lower than those observed in the low-dose and control ponds. High-dose treatment also caused a shift in the dominant zooplankters from limnetic to littoral forms (e.g., Alona, Acroperus and Pleuroxus) (ICI R8).

EPA does not accept ICI's contention that PP321 bound to the sediments does not cause toxicity. Observations from studies conducted by Dr. Frank Stay (EPA-Duluth) suggest very long term effects due to synthetic pyrethroids probably bound to organic particles.

4.4.3 Recovery in high dose ponds

EPA agrees with ICI's statement that zooplankton "recovery is a delicate and dynamic balance between reproduction, predation and community competition". EPA also agrees that there are many factors that support and affect zooplankton populations. Most importantly, all organisms must have an appropriate food supply. However, EPA contends that if PP321 did not affect specific zooplankters, and if appropriate food were available, then cosmopolitan populations should exhibit similar seasonal cycles in both the treated and control ponds. In this case, the treated ponds did not exhibit similar trends when compared to the control ponds.

4.4.4 Reasons for effects

EPA contends that there may have been both direct and indirect effects on zooplankton populations. EPA's review of phytoplankton effects is noted above in Section 4.3.

EPA does not deny that fish predation and sediment may have had a contributing influence on zooplankton populations during the early portion of the experiment (Weeks 0 to 2). What EPA contends is that, after week 2, PP321 also contributed to adverse effects for reasons previously listed.

4.4.5 Collection Mesh Size

EPA agrees with ICI in that smaller organisms will obviously be lost through a mesh size of 60 microns.

4.4.6 Significance to Fish

EPA commented that there was significant impact on the food sources of the bluegill. EPA's position is that these reductions could have an undesirable effect on fish production the following year. Numbers of certain zooplankton (viz. small cladocerans and cyclopoid adults) were reduced in treated ponds but not in control ponds.

ICI's response was to disagree with EPA's interpretation of data published by Siefert (1972). EPA stated that preferred food were Polyarthra and cyclopoid nauplii not Hexarthra. EPA desires to maintain focus on the data submitted by ICI and the impacts observed in this study, rather than debate the significance of Siefert's (1972) results.

ICI suggested that alternative food sources were utilized by the 5 mm size bluegill. This claim must be documented with valid data. Further, the argument that numbers of bluegill at the 6-25 mm life stage were comparable in all the treatments supports the premise that a reduction in number of preferred food sources represents an impact. It appears that all treatment ponds when exposed to PP321 showed the same level of effect. EPA believes it is because the ponds were not tested at a no-observable-effect level. In other words, there was an adverse impact at the lowest dose tested.

In addition, the significant effects of PP321 on fish biomass at all treatment levels strengthens the argument that the absence of preferred food items impeded the optimal growth and development of bluegill. During the period of gonadal recrudescence, an adequate diet is essential for building liver reserves which are integral for the production of plasma proteins vital to the transport of reproductive metabolites. Therefore EPA contends that zooplankton-related effects on young fish can have an impact in subsequent years.

4.5 Macroinvertebrates

4.5.1 General Effects

Changes in these populations corroborate EPA's conclusion that treatment of ponds with PP321 caused adverse effects to fish populations due to direct impact on macroinvertebrates. EPA maintains that some of the effects observed on macroinvertebrates were due to a diversity of causes such as fish predation, sediment loading, (EPA, not ICI, even suggests the possibility of high summer temperatures) and PP321. Even though the differences were not always statistically significant, the treatment totals generally were lower than the control totals.

4.5.2 Effects on Specific Taxa

Changes in these populations corroborates EPA's conclusion that treatment of ponds with PP321 caused adverse effects to fish populations. Even though the differences were not statistically significant, ICI Figure 37b indicates that the snail populations in the low-dose ponds were much lower than the snail populations in the controls and other treatment levels. No dose-related effect was implied.

Even though the differences were not statistically significant, ICI Figure 38g indicates that chironomid emergence was lower in the treated ponds between weeks 10 to 16. No dose related effect was implied.

Even though the differences were not statistically significant, treatment with PP321 caused a depression in quantitative visual observations of Veliidae and Notonectidae (Figures ICI 39d and 39e). A comparison of the patterns illustrated in these figures indicates that effects began at the low-dose and were most apparent at the mid- and high-rates of treatment.

EPA agrees with ICI that application of PP321 at the high-rate had an impact on Haliplidae (ICI 39g). Even though the differences were not statistically significant, ponds treated with PP321 had lower numbers of ceratopogonids on substrates (ICI 37j). No early seasonal peak noted for the control and low-dose ponds occurred in the mid-and high-dose ponds.

EPA agrees with ICI that PP321 caused a reduction in Tanypodinae (Figure ICI 38e). After treatment began, there appears to be a dose related effect; i.e., the sequence from higher to lower numbers are control < low-dose < mid-dose <high-dose. EPA does not agree that the lowered numbers at the end of the sampling regime indicate complete population recovery.

EPA feels that the early fluctuations of Chaoboridae numbers are similar among treatments. After treatment commenced, population pulses were virtually non-existent in the mid-dose and high-dose ponds. After treatment ceased only the control ponds exhibited a major peak, i.e., the low-dose pond also did not exhibit a major peak. All data indicate a potential effect of PP321 on chaoborids.

EPA agrees with ICI that the Gerridae and Veliidae were affected by treatment with PP321 (Figures ICI 39c and ICI 39d).

EPA agrees with ICI that the mayflies, especially the Baetidae and Caenidae, were affected by treatment with PP321 (Figures ICI 37e, ICI 37f, and ICI 38a). EPA agrees that fish predation may account for some of the decline in these populations during weeks 2 through 6, but fish predation <u>per se</u> does not account for the reductions and timing of decreases noted in the mid- and high-dose ponds. Declines in these treatments may be due to toxicity or enhanced predation by impairing the behavior of the mayflies.

EPA agrees that the high-dose with PP321 virtually eliminated the Leptoceridae (Figures ICI 37i and ICI 38b). EPA also agrees with ICI that populations of Belostomatidae and Notonectidae (Figures ICI 39b and ICI 39e), Zygotperans (Figure ICI 37h) and Coleopterans (Figure ICI 39i) were affected by PP321 in the high-dose ponds. EPA also agrees with ICI that coleopterans were affected in the mid-dose ponds.

In summary, EPA contends that macroinvertebrate populations were affected by PP321. ICI provides information that supports EPA's conclusions regarding the sensitivity of many organisms to synthetic pyrethroids.

ICI concluded that "the groups living within the hydrosoil (Oligochaeta and Chironominae) were clearly unaffected by the chemical at any rate which suggests that concentrations in the hydrosoil were probably not responsible for the toxic effects seen on the other organisms." EPA does not agree. Observations from microcosm studies conducted by Dr. Frank Stay (EPA-Duluth) suggest very long term effects due to synthetic pyrethroids bound to organic particles.

ICI concluded that "the quantity of organisms available as food for the fish was more than sufficient to maintain the bluegill populations in all ponds (fish approximately 5cm and larger feed on macroinvertebrates). This is possible if their opportunistic feeding habits are considered and also their undeniable preference for hydrosoil-living chironomids (Gerking, 1962) which were unaffected by the chemical." Given the problems described by EPA in evaluating the adult fish population size/biomass (Figure EPA.17), and the differences in the proportion of fish in the 3 to 6 cm size classes (Figure EPA.15) and fish biomass (Figure EPA.18), among the treatment levels, EPA strongly believes that PP321 directly and indirectly affects the fish populations.

4.6 Fish

4.6.1 Statistical Analysis of Fish

EPA has again summarized the results of the impact on fish. In this analysis we examined the data in three categories. The first category is <11 cm size group (Young of Year or YOY), the second category is ≥11cm size group (adults) and the third category, total fish (both YOY or first year class and the adults together).

Since it was clear from the response that the study authors were unsure of the number of adults at study initiation, EPA did not analyze the data by sexes, since it would be impossible to assume the number of each sex at test initiation.

4.6.2 Adult Fish Stocked and Recovered at Study End

ICI concurs with EPA that numbers of adult fish collected at the end of the study were markedly different from numbers supposedly stocked and that the extra adults present could not have been due to rapid growth of fish spawned during the mesocosm study. ICI contends that the discrepancy is due to fish introduced as eggs or larvae at stocking in 1985 and that nothing (including tagging) could have been done to prevent the situation.

EPA suggested that ICI investigate tagging techniques that cause minimum stress. Tagging would allow for determination of fish origin. Secondly, perhaps electroshocking, seining, or the use of traps in the supply ponds could prevent the accidental delivery of stray fish into the test system. EPA disagrees with ICI's assessment and believes that certain precautions and procedures could have been taken to prevent the problem.

4.6.3 Recording Error for Fish in Pond 5B

EPA accepts the more complete data submitted by ICI for 3 cm fish in pond 5B.

4.6.4 Biomass and Numbers of Young-of-Year Fish

ICI agrees with EPA that there is a significant difference between control and all three treatment rates, in numbers and biomass of some size groups of YOY fish. EPA's analysis of variance and subsequent comparisons of the revised data from ICI lead to several observations. Contrasts were specified to compare each treatment with the control as well as the mean of all three treatments with the control mean. Specifically, for numbers of fish no overall difference was detected among all four treatments for all fish, young-of-year fish (<11 cm), or adults (≥11 cm). In general, however, each treatment group showed somewhat larger numbers of fish than the control for all fish and for the young-of-year fish.

For fish biomass significant differences were found among all four treatments for all fish and for young-of-year fish, and for each treatment mean versus control for all fish and for young-of-year fish. In general, the treated ponds yielded fish biomass ranging from 70-80% of the control when all fish were examined and yielded approximately 60-70% of control when young-of-year fish were examined separately (Figures EPA. R1, and R2).

With regard to fish lengths, significant differences existed among all four treatments for all fish and for young-of-year fish, with each of the three treatment groups being significantly lower than the control (approximately 80% of control level) for all fish and for fish <11cm.

4.6.5 Growth Rates of Young-of-year Fish

ICI responses are as follows:

1. Available data do not allow for calculation of growth rates.

2. Good growth rates occurred in all mesocosms.

3. Modes and medians for total numbers and biomass are not sensitive enough to detect differences between control and treatment pond groups.

EPA has recalculated and re-analyzed all data. In spite of the inadequacies in the execution of the study, significant effects continue to be seen (See Figures EPA. R3 and 4). EPA feels that the data available clearly demonstrate adverse effects attributable to PP321. Further, mode, not median was used to estimate growth rates.

EPA disagrees with ICI that the growth rates were good in all mesocosms. In control ponds 2 cm fish were <15% of the total and 4 cm fish were >40% of the total. If growth rates were comparable in all mesocosms, size class distribution in treatment ponds would have mimicked those seen in the control ponds. EPA observed the opposite, and consequently concludes that the growth rates were slower among treated pond groups.

4.6.6 Growth Reduction in Adult Fish

ICI states there is no evidence of a reduction in the growth of the adult fish in the PP321-treated ponds. ICI contends further that EPA's observation of a minimum size adult fish present in the control ponds of 14 cm was incorrect (the presence of a single 12 cm fish in control pond 9A was not indicated in the original report). ICI has also stated that "to partly base the hypothesis, that there was an adult fish growth reduction, on only five minimum-sized fish from 16 ponds is tenuous". Finally, ICI disagrees with the use of weight-length relationships of the fish at the end of the study to predict mean weights of fish stocked at the beginning of the study. Therefore ICI concludes that there is no difference in the rates of growth of the adult fish.

Even if the control minimum size was changed to 12 cm, subtle growth effects would be evident at the high-dose, which is lower than what fish may experience in a worst case situation during an actual treatment.

ICI's argument that it is illogical or impossible to use weight-length relationships to back-calculate size is unsubstantiated. Weight-length relationships are commonly used in fisheries management for predicting growth or estimating past growth at desired size classes. Further, ICI ran regression analyses and found curvilinear relationships which were determined to be not significantly different. EPA contends that due to the lack of key statistical data (i.e. regression equations, R^2 values, etc.) it is not possible to adequately evaluate ICI's regression analysis. Again, in spite of the dicrepancies in the study execution, EPA still concludes that there were differences in the rates of growth of the adult fish.

4.6.7. Size of Stocked Adult Fish

ICI concludes that there is no evidence for an effect of PP321 treatment upon growth of the adult fish at any of the rates. EPA stated that the proportion of 17, 18, and 19 cm fish in control ponds should have been greater when compared to treatment ponds if growth was impaired by PP321. If there was no effect by PP321, then proportions of the larger sized fish should have been comparable between the control ponds and the treatment ponds. Neither of the above scenarios were evident. Therefore EPA concludes that there were problems associated with the stocking of the mesocosm ponds.

4.6.8 Chemical Stress

ICI has responded that the data demonstrate no significant stress effect from exposure to PP321 on young fish. Greater numbers of young fish seen in the PP321 treatments ponds from the quadrate visual observations indicate the possibility of chemical stress. The data indicate that the fish surfaced more frequently when exposed to PP321. ICI contends that the number of statistical differences is small. EPA suggests that perhaps more than 40 time points are required to show greater significance. In addition, EPA does not think that the basic responses of fish exposed to pyrethroids are chemical specific. Therefore observances of stress responses by fish to other pyrethroids are valid and help substantiate a chemical stress effect.

4.6.9 Reasons for Control/Treatment Differences in Fish

ICI agrees that with all PP321 treatments, when compared to controls, there were significant differences in both numbers and biomass of some size groups of young-of-year fish. However, ICI contends that treatment differences are not likely to be due to PP321.

4.6.9.1 Direct effects of PP321 on fish

ICI stated that at the high-rate treatments, direct effects of PP321 on fish were very unlikely. At the mid- and low-rate, direct effects would not have occurred. Furthermore, ICI concluded that PP321 had no direct effects on fish in the mesocosm study.

ICI feels that on the basis of EPA's suggestion that fish nested about June 1 or 2, the proposal of delayed breeding is unlikely. Regardless of the fact that initial nest times may have preceded the commencement of treatment, subsequent nestings certainly could have been affected by PP321 yielding the same results. ICI has not provided sound data to the contrary.

ICI contends that it is possible to compare laboratory data to field data and that levels of PP321 in the mesocosms were markedly less than was used in laboratory toxicity tests. EPA believes this argument is irrelevant since it is clear there is a dynamic situation occurring in the mesocosms which is not present in laboratory tests. The data show that there were marked effects on fish biomass and fish lengths in ponds treated with PP321 when compared to the control.

4.6.9.2. Indirect effects of PP321 on fish

ICI believes that it has demonstrated that the overall food supply for young or adult fish was unaffected at any of the three rates applied to the mesocosms, although EPA disputes these arguments. ICI in fact agrees that there were effects of PP321 on some zooplankton and macroinvertebrates at the high-rate. However ICI does not think that this had an indirect effect on fish growth because of opportunistic feeding, but ICI has presented no data to substantiate this claim. ICI argues that a dose response curve would have occurred if there had been an effect. EPA believes that the "lack" of a DOSE RESPONSE does not necessarily correlate with a "NO EFFECT" particularly in a dynamic environment. EPA contends that there were some indirect effects on fish exposed to PP321.

4.6.9.3. Numbers of adult male and female fish

ICI concluded that the difference seen in specific size groups in all treatment ponds was probably not due to the variations in the number of male and female fish in each pond. EPA believes that the differences in numbers of reproductively mature fish in the mesocosm ponds will obscure the interpretation of data from this aspect of the study.

4.6.9.4 Tadpole numbers

EPA gave a cursory review of the tadpole data. Though the data were interesting, the study was not designed to assess the effects from exposure to PP321 on tadpoles. There was no correlation between the reductions of fish lengths or fish biomass with the number of tadpoles. ICI agreed with this data analysis (ICI R20) since there was no significant difference between the ponds with and without tadpoles.

4.6.9.5 Aberrant control results

ICI proposes that the differences between the control and PP321 treated ponds could easily have arisen from minor changes in adult fish breeding times in individual ponds, which were not related to PP321 usage.

EPA has shown in the previous figures EPA 14, 15, 16 and 17 that there were adverse effects on the fish populations in the treated ponds versus the control ponds. It is clear from the figures and the statistical analyses that there was an impact on fish biomass at all doses tested. The reason there was no clear dose response curve as discussed by ICI, is that the ponds were not exposed to a low enough dose, therefore, a no-observable-effect level was not attained. In addition, since the high dose is actually the expected typical application rate, the high-dose ponds were not treated with a realistic "high" dose again, resulting in a similar impact in all treated ponds. When reviewing the proportion of young fish it is clear that there was an effect on growth, therefore, a decrease in numbers of fish in the 4 through 6 cm size class. This may ultimately affect the next year's reproductive class, since the treated ponds may be delayed in maturation.

5. Discussion and Conclusions

EPA is aware of the time, energy and expense required to conduct and complete a field study of this magnitude. EPA is also aware of the problems that may arise in the field when carrying out an aquatic field study. ICI and EPA have corresponded back and forth on a number of occasions explaining the interpretations of the data, and explaining any discrepancies.

ICI believes that PP321 when used as recommended for agricultural purposes is unlikely to cause adverse effects on populations or productivity in aquatic ecosystems. ICI admits that some minor effects may occasionally be observed but these will be transient or inconsequential to the biology of the ecosystem.

After reviewing the last ICI response and the previous communications, EPA believes that this study clearly shows adverse impacts to the lower tiered aquatic organisms, which include, phytoplankton, zooplankton, macroinvertebrates, as well as the higher tiered organism, the bluegill sunfish, Lepomis macrochirus.

EPA has shown that there was a significant reduction in growth and biomass of the fish in the treated ponds when compared to the control ponds at all doses tested. From the data submitted, EPA expects that effects seen in this study may be observed under field conditions with the use of this chemical. EPA believes that even with an application rate of 0.01 of the accepted label for cotton, the detrimental effects would be evident in the field.

In conclusion, the results from this study fails to negate the presumption of adverse effects and clearly demonstrates that lambda-cyhalothrin when used under typical conditions, is expected to cause serious adverse effects on the environment.

Citations

Carlander, K.D. 1977. Handbook of Freshwater Fishery Biology. The Iowa State University Press, Ames, Iowa.

Gerking, S.D. 1962. Production and Food Utilization in a Population of Bluegill Sunfish. Ecol. Monogr. 32: 31-78.

Siefert, R. E. 1972. First Food of Larval Yellow Perch, White Sucker, Bluegill, Emerald Shiner, and Rainbow Smelt. Trans. Amer. Fish. Soc. Vol. 101. No.2, April 1972., pp. 219-225.

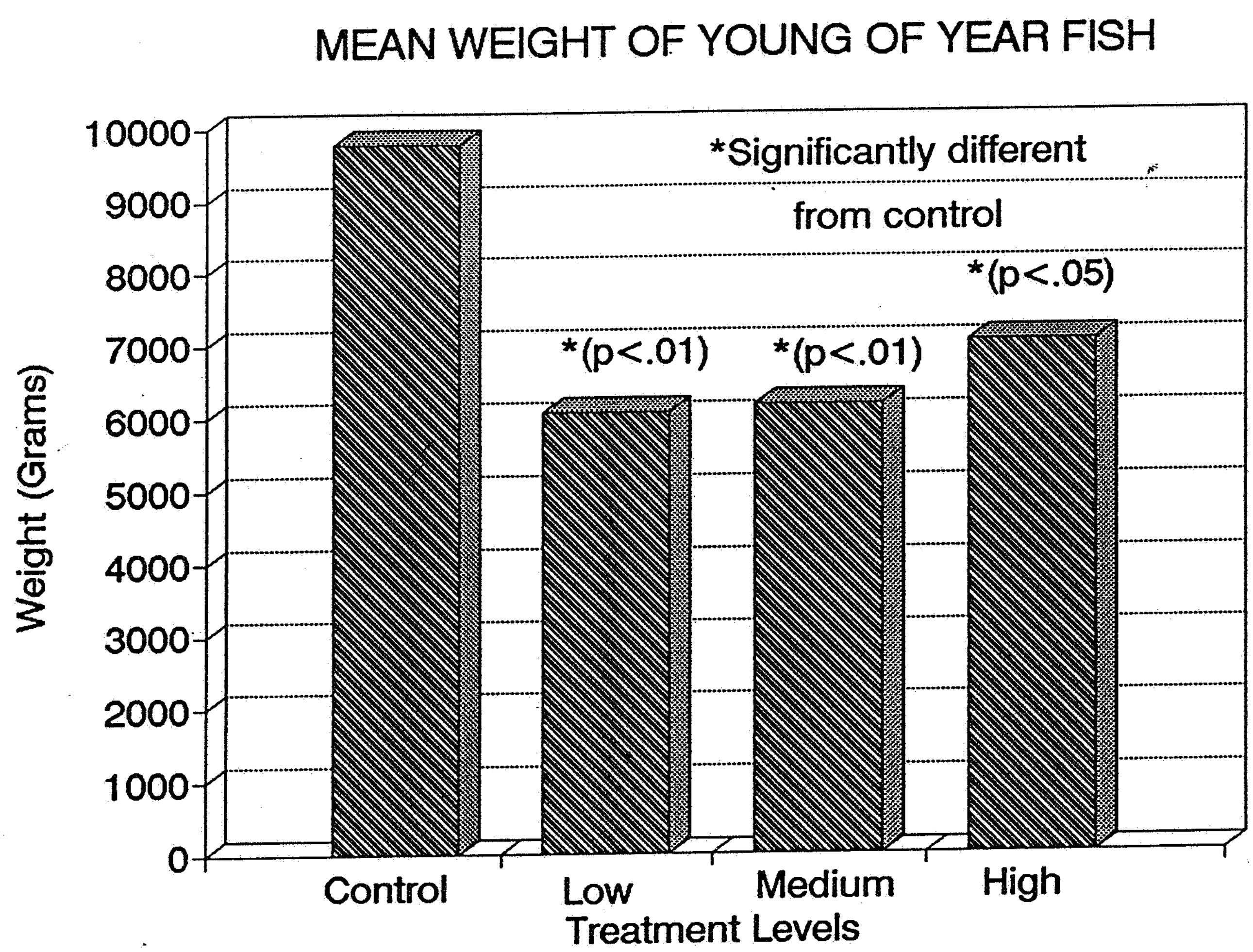


Figure EPA R1

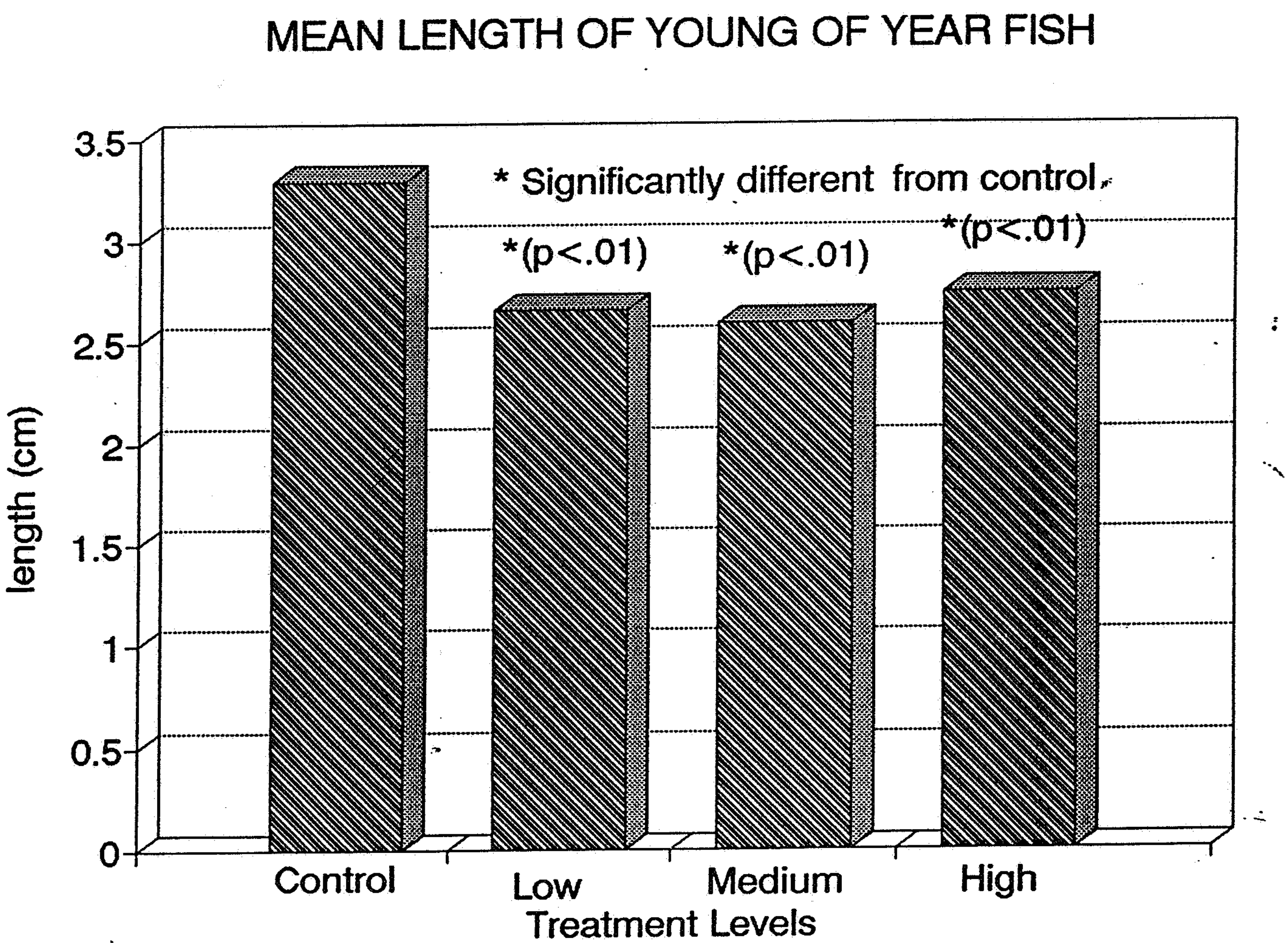


Figure EPA R3

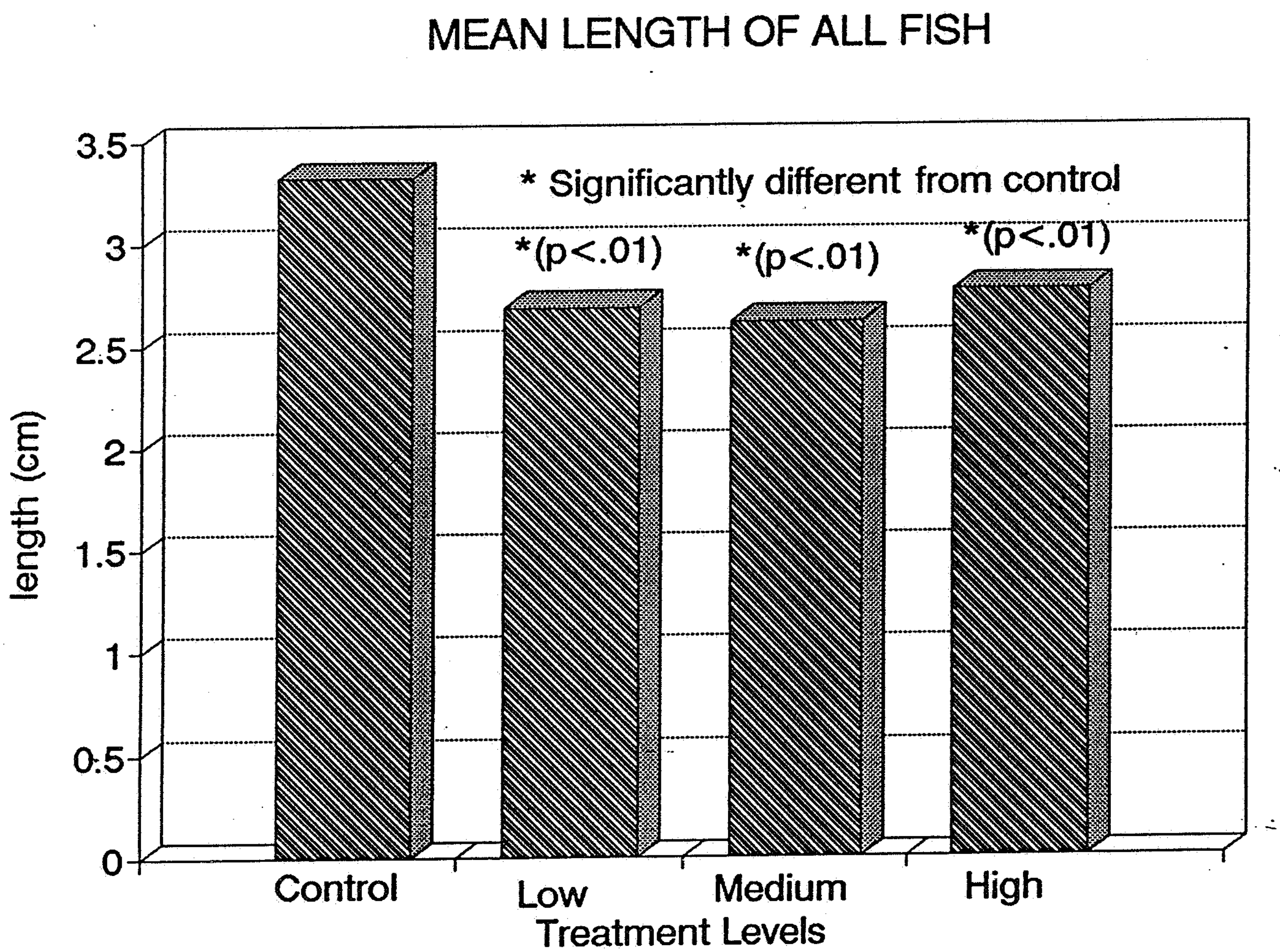


Figure EPA R4

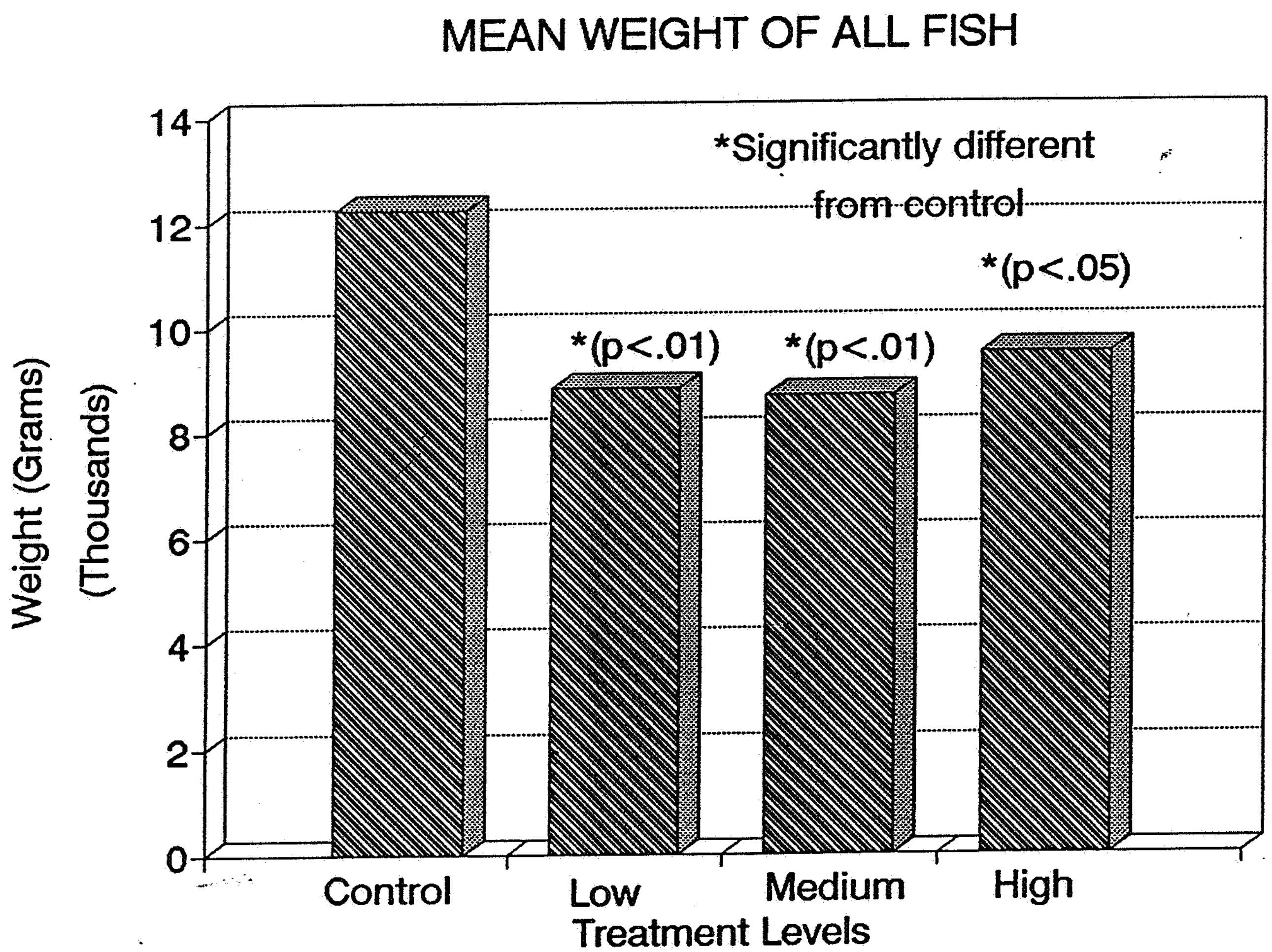


Figure EPA R2

TABLE 1

Weight of Fish

Trt		< 11 cm	≥ 11 cm	All Fish
1	Mean	9759.5000	2474.5000	12234.0000
2	Mean	6049.2500	2781.7500	8831.0000
3	Mean	6154.7500	2544.0000	8698.7500
4	Mean	7026.7500	2470.5000	9497.2500
All	Mean	7247.5625	2567.6875	9815.2500
2+3+4	Mean	6410.2500	2598.7500	9009.0000
F	Among 1,2,3,4	4.233	0.760	3.904
F	.99,3,12	5.953	5.953	5.953
F	.95,3,12	3.490	3.490	3.490
F	Among 2,3,4	0.813	1.870	0.525
F	.99,2,12	5.096	5.096	5.096
F	.95,2,12	3.885	3.885	3.885
t	(2+3+4) - 1	-3.448	0.640	-3.344
t	2 - 1	-3.118	1.291	-2.881
t	3 - 1	-3.030	0.292	-2.993
t	4 - 1	-2.297	-0.017	-2.317
t	.01,12	-2.681	-2.681	-2.681
t	.05,12	-1.782	-1.782	-1.782

TABLE 2

Number of Fish

Trt		< 11 cm	≥ 11 cm	All Fish
1	Mean	16112.7500	29.0000	16141.7500
2	Mean	18781.2500	30.7500	18812.0000
3	Mean	19337.7500	30.2500	19368.0000
4	Mean	18918.5000	29.0000	18947.5000
All	Mean	18287.5625	29.7500	18317.3125
2+3+4	Mean	19012.5000	30.0000	19042.5000
F	Among 1,2,3,4	0.941	0.064	0.943
F	.99,3,12	5.953	5.953	5.953
F	.95,3,12	3.490	3.490	3.490
F	Among 2,3,4	0.073	0.132	0.073
F	.99,2,12	5.096	5.096	5.096
F	.95,2,12	3.885	3.885	3.885
t	(2+3+4) - 1	1.658	0.247	1.660
t	2 - 1	1.246	0.353	1.247
t	3 - 1	1.506	0.252	1.507
t	4 - 1	1.310	0.000	1.311
t	.99,12	2.681	2.681	2.681
t	.95,12	1.782	1.782	1.782

TABLE 3

Mean Length of Fish

Trt		< 11 cm	≥ 11 cm	All Fish
1	Mean	3.291	16.731	3.317
2	Mean	2.657	17.097	2.681
3	Mean	2.587	16.737	2.609
4	Mean	2.739	16.630	2.760
All	Mean	2.819	16.799	2.842
2+3+4	Mean	2.661	16.821	2.683
F	Among 1,2,3,4	6.444	0.356	6.473
F	.99,3,12	5.953	5.953	5.953
F	.95,3,12	3.490	3.490	3.490
F	Among 2,3,4	0.717	1.016	0.708
F	.99,2,12	5.096	5.096	5.096
F	.95,2,12	3.885	3.885	3.885
t	(2+3+4) - 1	-4.315	0.228	-4.326
t	2 - 1	-3.547	0.754	-3.547
t	3 - 1	-3.934	0.013	-3.945
t	4 - 1	-3.088	-0.208	-3.104
t	.01,12	-2.681	-2.681	-2.681
t	.05,12	-1.782	-1.782	-1.782

Karate ecological effect review

Page ______ is not included in this copy.

Pages 33 through 34 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) ______

___ The document is not responsive to the request

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

APPENDIX II

DISTRIBUTION IN WATER SEDIMENT

		SED x 99.9%	WATER COLUMN x Ø.1 %
15%	Ø.Ø3 lb x Ø.15 = Ø.ØØ45 lb	Ø.ØØ44955 lb (247.226 ppt)	Ø.ØØØØØ45 lb (Ø.275 ppt)
1%	Ø.Ø3 lb x Ø.Ø1 = Ø.ØØØ3 lb	Ø.ØØØ2997 lb (18.282 ppt)	Ø.ØØØØØØ3 lb (Ø.Ø18 ppt)

AVERAGE FISH WEIGHT BY SIZE

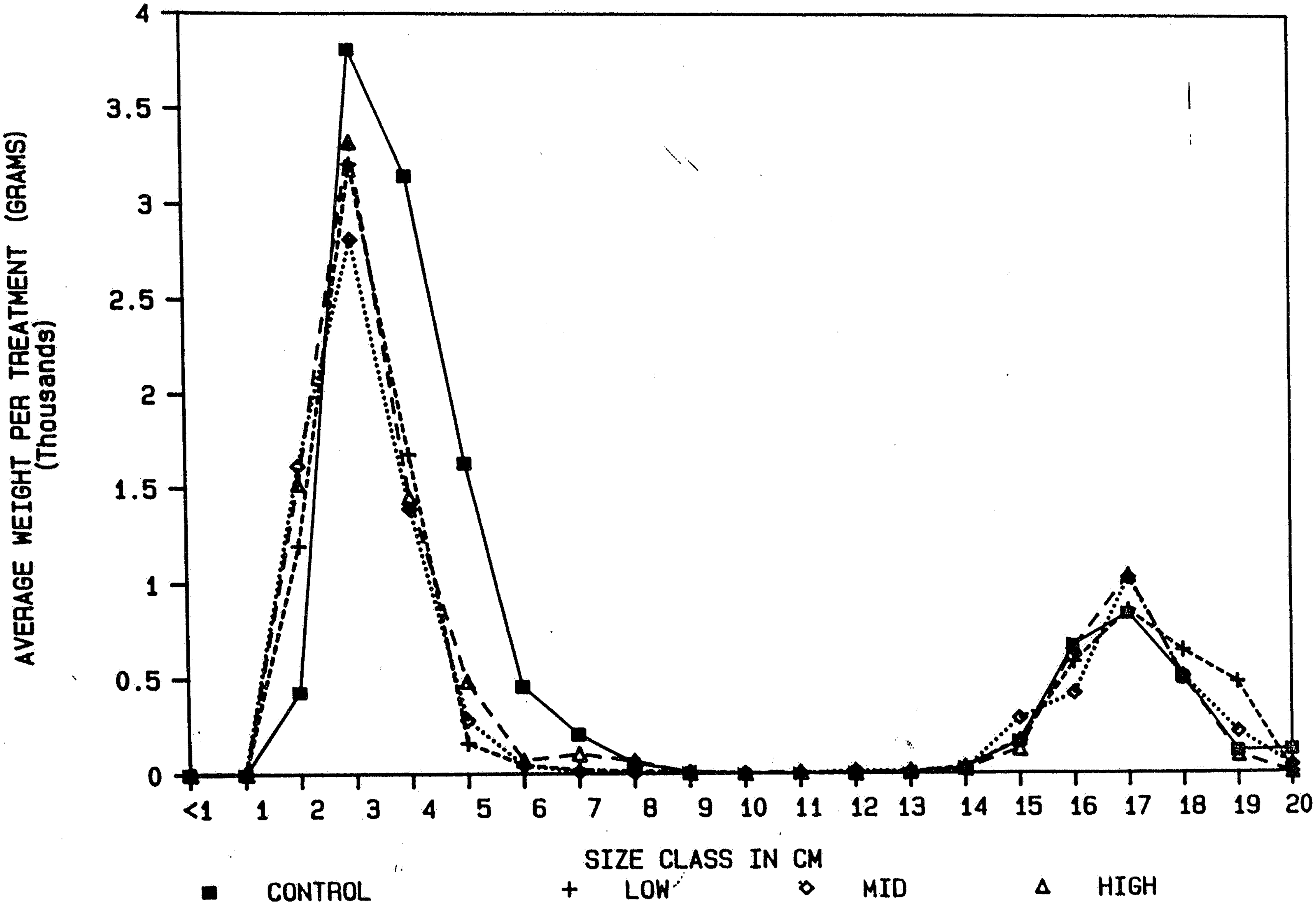


Figure EPA. 15

PROPORTION OF YOUNG FISH

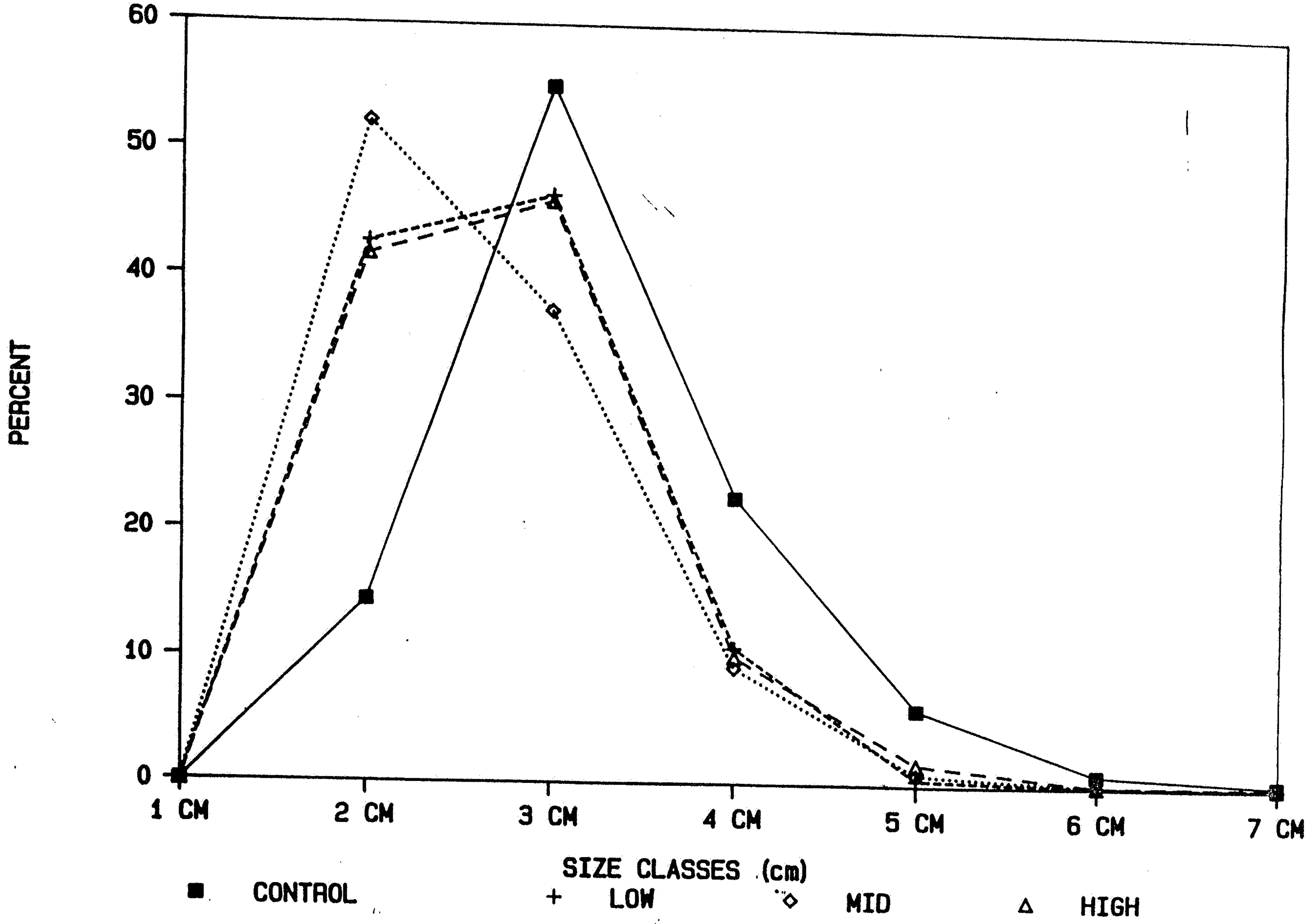
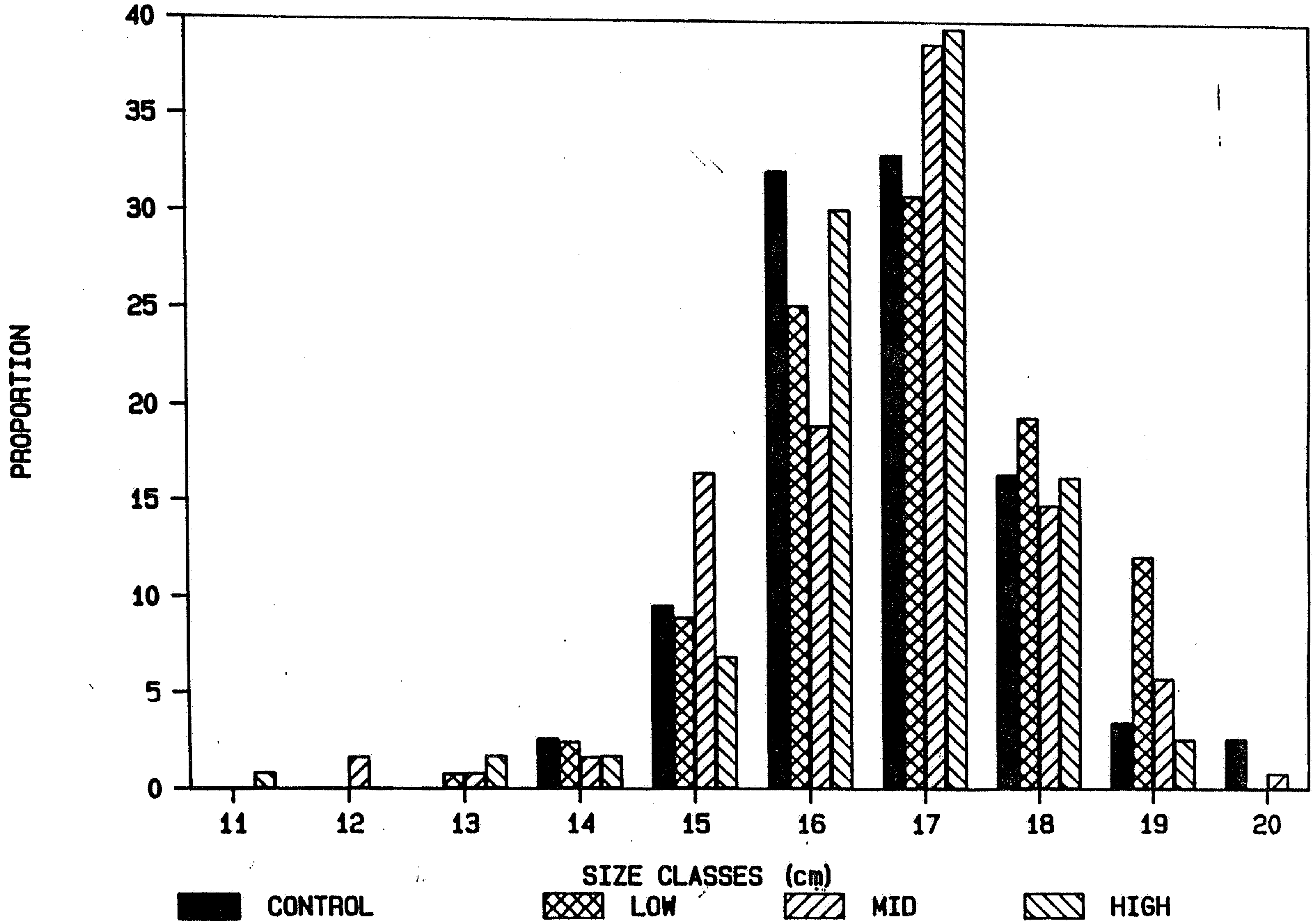


Figure EPA.16

PROPORTION OF ADULT FISH



FISH OBSERVATIONS DURING APPLICATION

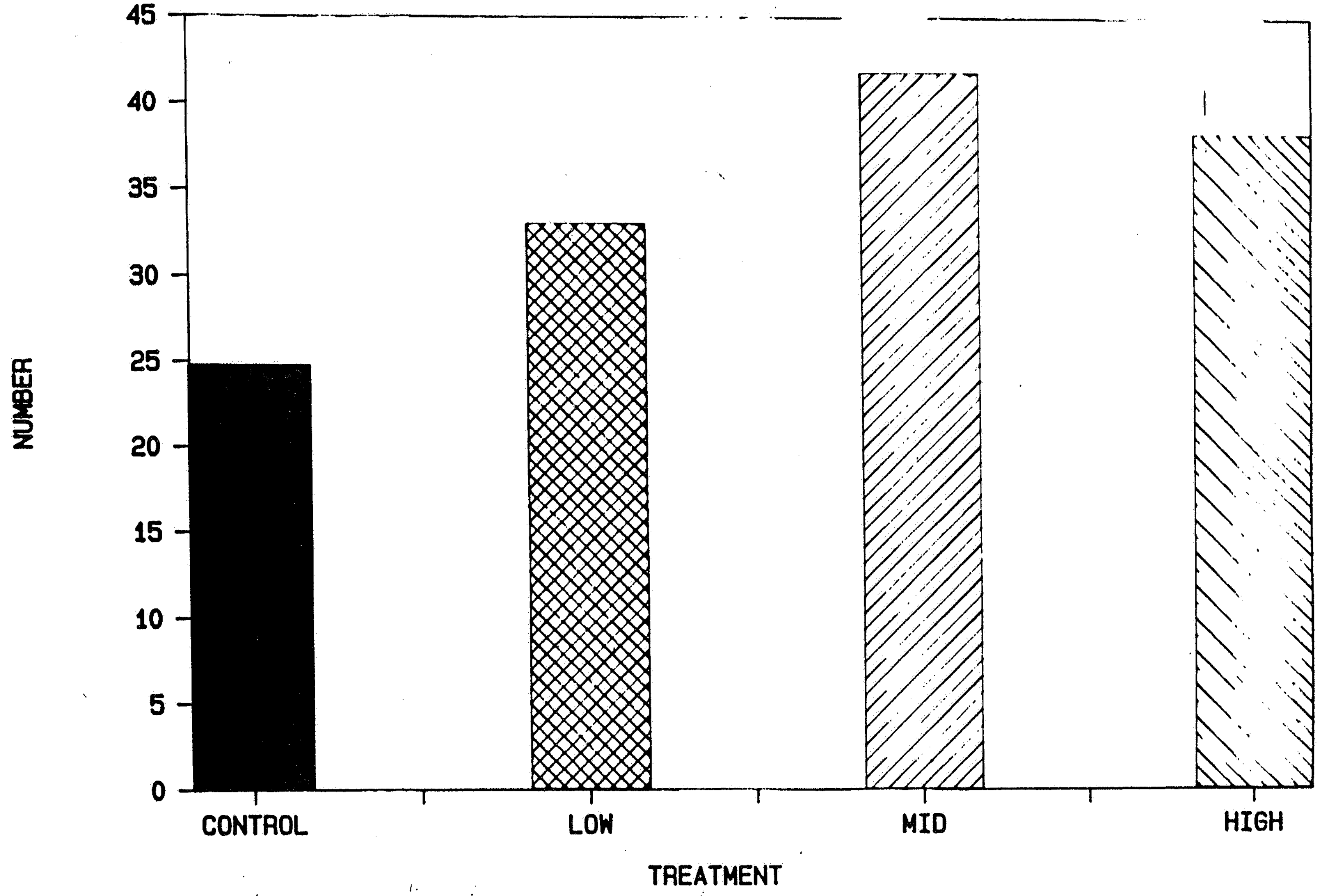


Figure EPA.18

Karate ecological effect review

Page ______ is not included in this copy.

Pages 40 through 57 are not included in this copy.

The material not included contains the following type of information:

___ Identity of product inert ingredients

___ Identity of product impurities

___ Description of the product manufacturing process

___ Description of product quality control procedures

___ Identity of the source of product ingredients

___ Sales or other commercial/financial information

___ A draft product label

___ The product confidential statement of formula

___ Information about a pending registration action

X FIFRA registration data

___ The document is a duplicate of page(s) ______

___ The document is not responsive to the request

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